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EFFECTS OF INTRACEREBRAL MICROINJECTION OF SYMPATHOMIMETIC
AMINES, CHOLINOMIMETICS AND CHLORPROMAZINE

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Thomas Alan Rudy, B.S., M.S.

The Ohio State University
1970

Approved by

Harold W. Wolf
Adviser
College of Pharmacy
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VITA

August 16, 1940...... Born ---- Columbus, Ohio

1963............... B.S. (cum laude), College of Pharmacy, The Ohio State University, Columbus, Ohio

1963-1965........... Research Assistant, College of Pharmacy, The Ohio State University, Columbus, Ohio

1966............... M.S., College of Pharmacy, The Ohio State University, Columbus, Ohio

1965-1967........... American Foundation for Pharmaceutical Education Fellow, The Ohio State University, College of Pharmacy, Columbus, Ohio

1967-1970........... National Institutes of Health Pre-Doctoral Fellow, The Ohio State University, College of Pharmacy, Columbus, Ohio

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CHAPTER I
GENERAL INTRODUCTION AND REVIEW OF THE LITERATURE

Hypothalamic Control of Thermoregulation—Anatomical and Functional Considerations

Introduction

The experiments discussed in this dissertation deal with the effects of certain chemical agents upon thermoregulation when such substances are injected directly into the hypothalamus of the cat. An overview of current concepts of hypothalamic anatomy and function as they relate to the central control of body temperature is provided in the following pages in order to establish a background for discussion and interpretation of the experimental data.

According to present concepts (Hammel, 1968; Benzinger, 1969), the hypothalamus contains neural mechanisms for 1) detection of its own temperature 2) reception of thermal information detected at extrahypothalamic sites 3) comparison of hypothalamic temperature to a virtual setpoint, and 4) generation of impulses which initiate effector responses (shivering, piloerection, nonshivering thermogenesis, postural changes, vasomotor changes, sweating, fur wetting and panting) which tend to reduce the difference between hypothalamic temperature and the virtual setpoint.

Although data derived from electrophysiological recordings, thermal and electrical stimulation, ablation and transection experiments have shown that these functions reside in the hypothalamus, our understanding of the neural organization underlying each function is rudimentary,
as is our knowledge of the hypothalamic loci subserving them. However, there is no paucity of speculation concerning these questions. Of particular interest are data relating to hypothalamic control of panting, shivering and cutaneous vasomotor tone, the three parameters measured in the experiments to be presented later. A major portion of this overview will be devoted to discussion of these phenomena. The sensitivity of the hypothalamus to its own temperature and the influence of extra-hypothalamic thermal and non-thermal input will also be considered.

Thermodetection Within the Hypothalamus

The first suggestion that the brain might contain thermosensitive structures was made by Bergman in 1845. As a possible explanation for the thermal vasomotor changes he had been observing, he suggested that the brain might contain thermal receptors which activate vasodilation when blood temperature increases above the normal level. Experimental evidence supporting this view was provided 67 years later by Barbour (1912), who heated and cooled the basal ganglia of anesthetized dogs using water-perfused thermodes. Heating caused a decrease in body temperature and cooling, an increase. Barbour thus thought that the central thermoreceptors were located in the striatum. These results were confirmed by Prince and Hahn (1918) who repeated the experiment in unanesthetized cats. However, in both experiments it was necessary to heat or cool the water perfusing the thermodes to levels outside the physiological range. This suggested that some structure distal to the striatum was actually responsible for the observed effects. Indeed, Moore (1918) determined that the corpus striatum was not responsible, but he did not establish the location of the actual thermosensitive area. Although the
importance of the hypothalamus for thermoregulation had been demonstrated by Isenschmid and Krehl (1912), it fell to Hasama (1929) to demonstrate that central thermoreceptors reside within this structure. By direct warming of the base of the hypothalamus and the preoptic regions to elevated but not unphysiological levels, this investigator was able to elicit sweating of the footpads and a fall in rectal temperature in the cat.

Much more specific localization of the thermosensitive area was provided by Magoun et al. (1938). These workers explored the responses of anesthetized cats to diathermic heating of sites distributed throughout a large part of the brain. These sites included

... a large portion of the cerebral cortex, the subcortical white matter, the ventral telencephalon rostral to the reactive area, the caudate and lentiform nuclei of the corpus striatum, the diencephalon surrounding the responsive field and the midbrain behind it as far caudal as the anterior end of the pons.

The "responsive field" was an area which, when heated, produced open mouth panting and occasionally, sweating on the footpads. The most responsive site lay between the optic chiasm and the anterior commissure and extended about 1 to 2 millimeters anterior and posterior to a line drawn between the two. The lateral limits appeared to be about 4 millimeters from the midline. A less responsive region continues

... backward through the diencephalon in the dorsal part of the hypothalamus and the ventral part of the thalamus and at more caudal levels occupies a progressively more dorsal location. At the transition to the midbrain, it is located in the vicinity of the central grey matter surrounding the anterior end of the cerebral aqueduct.

Responsivity decreased greatly the more caudal the location of the stimulated site.
Beaton et al. (1941) performed a similar experiment using monkeys. Their exploration of the brain was somewhat more limited than that of Magoun et al., the most caudal site tested lying in the tuberal region. As in cats, the most responsive region was found to lie between the anterior commissure and the optic chiasm and to extend 4 to 6 millimeters laterally. However, in contrast to the cat, the region was found to be highly localized and did not extend more than a few millimeters caudally. Local heating of this reactive region produced sweating, increased respiratory rate and, possibly, cutaneous vasodilation.

In order to avoid the hypothalamic damage inherent in the bi-electrode diathermic heating technic, Hemingway (1940) heated the anterior and posterior hypothalamus by diathermy using, as the active electrode, gold foil placed between the ventral surface of the brain and the cranium. Heating the anterior hypothalamus of unanesthetized dogs produced immediate vasodilation of the ears and cessation of shivering but did not produce panting. Heating the posterior hypothalamus elicited only a slight reduction in shivering and no vasodilation. Since the heating field extended only 2 millimeters from the electrode, Hemingway suggested that no panting was observed upon heating the anterior hypothalamus because the thermal receptors which activate panting were located more dorsally in the hypothalamus than those activating vasodilation or inhibiting shivering. Alternatively, it was suggested that elicitation of panting may require greater heating than that necessary to activate the latter two responses.

Folkow et al. (1949) repeated the experiments of Magoun et al. using conductive heating of the hypothalamus (by means of water perfused thermodes) as well as diathermic heating. Both technics produced cutaneous
vasodilation, but not panting, in anesthetized cats and dogs. Since conductive heating was equally as effective as diathermic heating, it was concluded that the responses seen were due to thermal stimulation rather than capacitive discharge or some other electrical phenomenon associated with diathermy. In the cat, the location of the reactive field producing cutaneous vasodilation was found to be very similar to the area from which Magoun and co-workers elicited panting. The most sensitive region was centered between the anterior commissure and the optic chiasm, and a much less sensitive region extended caudally as far as the mamillary bodies. The highly reactive area in the dog was analogous to that of the cat. These experiments did not elucidate the caudal extent of the less sensitive field.

Continuing with similar investigations, Strom (1950a) demonstrated that unilateral heating of the anterior hypothalamic/preoptic region (AH/PO region) in anesthetized cats can produce bilaterally symmetrical cutaneous vasodilation. However, even with intense bilateral hypothalamic heating, he was not able to induce panting. When urethane, which facilitates panting, was used as the anesthetic, a marked increase in respiratory rate was observed. This phenomenon was much less pronounced when pentobarbital anesthesia was employed. The author was unable to explain why panting did not occur but suggested that either vasomotor tone was much more sensitive to heating or the thermosensitive neurons initiating panting were in a different portion of the AH/PO region than those causing vasodilation. However, as stated previously, the reactive fields found by Strom and Magoun were practically identical.

Using water perfused thermodes, Strom also cooled the anterior and posterior hypothalamus to 31-36 °C. There was no change in cutaneous
blood flow or respiratory rate except when flow had previously been recently increased by hypothalamic heating; in this case, cooling of the AH/PO produced vasoconstriction. Shivering was never observed. This apparent lack of sensitivity to cold stimulation does not prove that cold receptors are lacking in the hypothalamus. As the author points out, the volume of tissue cooled may have been too small to stimulate a sufficient number of receptors, or the anesthetic may have reduced their sensitivity. In addition, at the ambient temperature at which these experiments were performed, cats are usually maximally vasoconstricted. Thus, it is not surprising that Strom was unable to elicit a cutaneous response unless vasoconstrictor tone has been previously reduced. As will be seen, further experimentation has revealed that the hypothalamus can respond to cooling.

Freeman and Davis (1959) heated and cooled the hypothalamus of anesthetized and unanesthetized cats using bilateral conductive thermodes of smaller diameter than had previously been used. Anterior hypothalamic heating in anesthetized animals yielded vasodilation of the footpad, suppression of spontaneous shivering and, occasionally, polypnea (but not true panting). Cooling the same area produced a rise in rectal temperature in about half the subjects, no change in most of the remainder and, in a few animals, a fall. No shivering or vasoconstriction was observed in those subjects which experienced an increase in body temperature, nor did the authors speculate how the rise could have been effected. Interestingly, the same effects were produced by heating and cooling the lower midbrain and pons, but the responses were smaller and less reliable. Similar effects were occasionally obtained by thermal stimulation of the posterior hypothalamus. In the majority of cases, however, heating or
cooling of the posterior hypothalamus induced rectal temperature changes that tended to follow the temperature of the stimulating thermode rather than reverse it. The significance of these anomalous responses are not understood.

In unanesthetized cats, anterior hypothalamic heating elicited vasodilation and reduction of spontaneous shivering but did not produce panting. The animals reduced their spontaneous motor activity and assumed a languid position conducive to heat loss. Anterior hypothalamic cooling produced huddling and vasoconstriction but not shivering. Heating or cooling the posterior hypothalamus had no effect in these unanesthetized subjects. The inability to produce panting or shivering by anterior hypothalamic heating or cooling, respectively, was attributed to failure to stimulate an adequate number of thermosensitive neurons due to the small diameter of the thermodes. Thus, it appears that the anterior hypothalamus is sensitive to both heating and cooling and can initiate appropriate behavioral (huddling, sprawling) as well as physiological thermoregulatory mechanisms to reduce the error signal between the setpoint and the artificially induced hypothalamic temperature. It is also apparent that there are similar receptors as far caudal as the pons and midbrain, although their sensitivity must be much less or their number fewer.

Since 1960, numerous studies using a variety of species have confirmed and expanded upon the data garnered by the workers previously cited. In unanesthetized cats, Mestyan et al. (1960), employing diathermic heating of the AH/PO, obtained panting, reduction in oxygen consumption, suppression of shivering, languid behavior and rectal temperature decreases of up to 6°C. Adams (1963) obtained similar results, except that
he was unable to elicit panting. In addition, he found that cooling of the same area increased rectal temperature and metabolic rate and induced vasoconstriction of the ears. Sundsten (1967), in an experiment similar to Adams, but using a Peltier thermode, found AH/PO heating in unanesthetized cats yielded vasodilation, reduction of shivering, languid posture and hypothermia; cooling caused vasoconstriction, huddling and an increase in rectal temperature. However, no shivering was observed. The presence or absence of panting was not reported.

Hammel et al. (1960) demonstrated that heating of the hypothalamus produced panting and a decrease in rectal temperature in the unanesthetized dog. Cooling produced vigorous shivering, vasoconstriction and a rectal temperature increase. Switching rapidly from cooling to warming induced simultaneous shivering and panting, probably indicating that there are steep thermal gradients around the thermodes, a distal portion of the hypothalamus remaining cool for a short time after warming was begun. Unfortunately, the thermodes used were large and not suitable for localization of the exact site within the hypothalamus which responded to the heating and cooling stimuli.

Fusco et al. (1961) evaluated the effect of environmental temperature on the responses observed in the dog when the anterior hypothalamus is heated. At each ambient temperature, hypothalamic heating reduced rectal temperature by about 1.0°C. However, in a cool environment the decrease was due to reduction of shivering; in a warm environment, to panting; and in a neutral environment, to a 40 percent reduction in basal metabolic rate coupled with vasodilation. Thus, the anterior hypothalamic thermoreceptors have been found to control yet another thermolytic mechanism—reduction of resting metabolic rate.
Hellstrom and Hammel (1967) reported similar results in the dog and, in addition, saw the expected behavioral responses upon heating and cooling the hypothalamus, i.e., sprawling and huddling, respectively.

The rabbit also responds to central thermal stimulation. Barbour reported wide changes in skin temperature of the rabbit ear due to artificial heating or cooling of structures at the "base of the brain" (Barbour, 1912, as quoted by Euler, 1960). Hellon (1967) reported ear vasodilation as the only response when the hypothalamus of the unanesthetized rabbit was warmed, but Grant (1968) elicited strong thermal polypnea using a similar preparation. Hellon (1967) also cooled the hypothalamus at several sites, but without effect. However, Downey et al. (1964), using the indirect method of cooling the blood flowing through the internal carotid (the external carotid supplying the skin of the face was ligated) produced vigorous shivering and an increase in rectal temperature in unanesthetized rabbits.

The goat, of all species examined, appears to be the most responsive to artificially induced changes in hypothalamic temperature. Andersson and co-workers, in a series of papers using the unanesthetized goat (Andersson et al., 1962, 1963, 1964), showed that heating of the AH/PO area, even at an ambient temperature of 2 to 5°C, yields peripheral vasodilation, polypnea, inhibition of shivering and inhibition of urinary catecholamine excretion. Rectal temperature fell to 31°C, a severely hypothermic level. Cooling of the AH/PO region produced peripheral vasoconstriction, shivering, increased plasma protein-bound iodine (PBI), increased urinary catecholamine excretion, increased blood glucose levels and a 2°C rectal temperature increase. Ambient temperature was 15 to
21°C. In one animal, shivering was absent when the hypothalamus was cooled, yet rectal temperature did not fail to increase. This indicates that a rapidly activated endocrine cold defense mechanism was able to elevate body temperature (acute non-shivering thermogenesis). Furthermore, repeated hypothalamic cooling over a period of months induced an increase in fur thickness, a gradually increasing plasma FBI level and a decrease in the shivering response. These progressive changes indicating a gradual reduction in shivering thermogenesis in favor of non-shivering thermogenesis are very similar to those seen in the process of cold acclimatization.¹ Thus, heating or cooling of the goat hypothalamus has been shown to activate every acute and chronic thermoregulatory mechanism available to this species.

In studies employing other species, Ingram and Whittow (1962) and Ingram et al. (1963) have heated the anterior hypothalamus of the ox and observed increased respiratory rate, cutaneous vasodilation, blockade of shivering and increased cutaneous evaporative heat loss. Baldwin and Ingram (1967) demonstrated that alteration of anterior hypothalamic temperature affects behavioral thermoregulation in the pig. Heating the hypothalamus reduces the rate at which the pig will press a bar to obtain radiant heat in a cool environment, whereas hypothalamic cooling increases the rate. Similar results were obtained by Murgatroyd (1966) using the

¹ Similar activation of endocrine cold defense mechanisms was obtained by Gale and Ruch (1966) in monkeys. Cooling of the AH/PO area increased rectal temperature by 0.9°C. Although no shivering occurred, there were increases in plasma FBI, serum growth hormone and urinary catecholamines. In addition, Bruck and Wunnerberg (1968) report that heating of the guinea pig hypothalamus inhibits nonshivering thermogenesis.
rat. Satinoff (1964) reported increased bar pressing for heat when the AH/PO area of the rat was cooled unilaterally. In addition, the cooling induced strong shivering. Spector et al. (1968) found hyperthermia or hypothermia was produced when the medial preoptic area of the rat was cooled or heated respectively. The most rostral portion of the preoptic area, the lateral preoptic area and the lateral hypothalamus were found to be less sensitive.

A recent study of the Virginia opossum (Roberts et al., 1969) has shown that diathermic warming of the AH/PO region reliably elicits panting, thermoregulatory grooming (licking of the fur to increase evaporative heat loss), and sleep-like relaxation (believed by the authors to be a mechanism for prevention of heat gain, in that such relaxation reduces metabolic heat production). The grooming and relaxation could be elicited only by warming of the medial preoptic area. Panting was elicited by warming this site and also by warming of the lateral preoptic area. Thermal stimulation of other hypothalamic sites and the septal area produced no response.

Hypothalamic heating and cooling has been shown to affect behavioral thermoregulation in the blue-tongued skink, a poikilotherm. This observation may indicate that hypothalamic thermosensitivity is a phylogenetically "old" phenomenon (Hammel et al., 1967).

Thermal sensitivity of the hypothalamus has also been demonstrated by recording single unit discharges while artificially changing the temperature of the neuron under observation (Nakayama et al., 1961, 1963; Hardy et al., 1964; Cabanac et al., 1967, 1968; Cunningham et al., 1967; Eisenman, 1967; Eisenman and Jackson, 1967; Wit and Wang, 1968). Both
warm-sensitive neurons (units which increase their firing rate as their local temperature increases) and cold-sensitive neurons (units which decrease their firing rate as their local temperature increases) have been found in the brains of several species. As might be anticipated, the medial preoptic area appears to have the most dense population of thermosensitive neurons. In fact, Wit and Wang (1968), studying the cat, found thermosensitive neurons only in the preoptic and anterior hypothalamic areas. None were seen in the lateral hypothalamus, paraventricular nucleus, supraoptic nucleus, or dorsomedial and ventromedial hypothalamic nuclei. On the other hand, Cabanac et al. (1968) reported that thermosensitive units lay throughout the hypothalamus and septum of the rabbit. These differences in distribution may derive from variations in technic or may represent actual species differences.

In summary, it has been shown that in numerous species, warming of the hypothalamus activates heat loss mechanisms such as panting, cutaneous vasodilation, sweating, fur wetting, inhibition of resting metabolic rate, postural changes and behavior changes, and that heat gain mechanisms are simultaneously inhibited. Local cooling of the hypothalamus activates heat gain mechanisms such as shivering, cutaneous vasoconstriction, piloerection, thyroid activation, sympathico-adrenal activation, postural changes and behavioral changes. There is considerable species variation in responsivity. At least in the cat, unilateral heating suffices to produce bilaterally equal vasodilation (Strom, 1950a) and panting (Magoun et al., 1938); in the rat, unilateral cooling was seen to elicit shivering (Satinoff, 1964). The preoptic region between the optic chiasm and the anterior commissure, is quite sensitive to local
thermal stimulation. It also seems to be the most sensitive region, according to those few studies in which other hypothalamic areas have been investigated.

However, in spite of frequent published statements to the contrary, the posterior hypothalamus does not appear to be completely insensitive to local temperature changes. Magoun et al. (1938), Strom (1950a) and Freeman and Davis (1959) saw thermoregulatory responses when the posterior hypothalamus of anesthetized cats was heated. Hellon (1967) found both warm- and cold-sensitive neurons in the posterior hypothalamus of the rabbit. On the other hand, Strom (1950a) and Freeman and Davis (1959) reported that local cooling of the posterior hypothalamus had no effect, and Hasama (1929) found it necessary to reduce posterior hypothalamic temperature to below 25°C before any counterthermoregulation was elicited. Thus, although the AH/PO region definitely exhibits the greatest thermosensitivity, the participation of posterior hypothalamic thermosensitive elements, at least in response to increases in body temperature, cannot be ruled out.

**Hypothalamic Control of Shivering**

Shivering is the primary mechanism by which warm-acclimated adult homeotherms maintain normal body temperature in a cold environment. It consists of a skeletal muscle tremor in which flexors and extensors contract simultaneously so that there is little gross movement of the limb. By this mechanism, oxygen consumption may be increased up to five times the basal level. Since shivering musculature performs no work, oxygen consumption is efficiently converted into increased heat production. Further information concerning the shivering process may be obtained
from Hemingway’s recent review (1963).

Although there are some dissenting studies, the great majority of investigations have shown that the hypothalamus is essential for effective shivering to occur. This conclusion is partially based on the observation that, while decerebrate animals cannot shiver (Bazett and Penfield, 1922; Bard and Macht, 1958), decorticate animals can (Dusser de Barenne, 1920; Pinkston et al., 1934; Aring, 1935; Stuart et al., 1962a). Decerebration and decortication leave variable amounts of midbrain and prosencephalic tissue remaining, depending on the technic used. This raises the possibility that this remaining extrahypothalamic tissue may also be involved in the control of shivering. However, Keller and McClaskey (1964) transected the dog brain by aspiration of all thalamic and hypothalamic tissue back to the level of the mammillary bodies, leaving the entire midbrain and the junctional tissue between the hypothalamus and midbrain intact. Such preparations did not shiver. Shivering was also absent in preparations in which the entire hypothalamus had been selectively dissected out or macerated (Keller and Hare, 1932). Further, dogs and cats with brain transections just anterior to the preoptic area—thus removing all cephalic connections of the hypothalamus and thalamus—shiver vigorously when exposed to cold (Keller, 1933; Keller and McClaskey, 1964; Clark et al., 1939a). Moreover, Clark et al. (1939b) placed large lesions throughout the thalamus of the cat without severely impairing shivering. Thus, it is clear that it is the hypothalamus which is responsible for effective shivering thermogenesis.

There have been many attempts to locate the hypothalamic areas involved in the control of shivering, and attention focused early on the
posterior hypothalamus. Clark et al. (1939a) reported that, although "moderate" sized lesions of the anterior hypothalamus of the cat had little effect on shivering, similar lesions placed in the lateral posterior hypothalamus produced severe impairment. Similar results were seen in monkeys (Ranson et al., 1937). Stuart et al. (1962c), using cats maintained several weeks or months following bilateral destruction of various hypothalamic regions, found that ablation of the entire posterior hypothalamus except the dorsomedial region on one side did not abolish shivering. On the other hand, bilateral destruction of this area without damage to the rest of the posterior hypothalamus abolished shivering. The critical region is described as including...

...part of the posterior hypothalamic nucleus, the dorsomedial edges of the field of Forel, the dorsal aspect of the supramammillary commissure and the posterior periventricular tract. Additionally, it includes a narrow zone immediately ventral to the posterior reuniens nucleus of the thalamus.

Since animals with lesions at this critical site could piloerect, huddle and make appropriate behavioral responses while not shivering in the cold, the authors believed that this area was not a major integrative center for heat maintenance. Rather, the implicated it in the efferent (motor) aspect of shivering. Hemingway (1963) calls this area the "primary motor center" (PMC) for shivering.

Other data corroborate the importance of the PMC as a center excitatory to shivering. Stuart et al. (1961) stimulated electrically various hypothalamic loci in anesthetized cats. Shivering was most consistently produced by stimulation at the PMC. These results have been partially confirmed in unanesthetized cats (Stuart et al., 1962b). Birzis and Hemingway (1957a) have also elicited shivering by electrical stimulation
of the brain. Most of the sites tested were posterior to the hypothalamus, but one hypothalamic site was examined. Stimulation at this site, which lay at the anterior edge of the PMC, produced shivering.

There is some evidence, however, that the PMC may not be as essential as the experiments of Stuart and co-workers suggest (Birzis and Hemingway, 1956). Anesthetized cats were observed for shivering 4.5 to 6 hours after bilateral lesioning of the brainstem. It was found that lesions in the ventrolateral posterior hypothalamus between the mammillary bodies and cerebral peduncle abolished shivering whereas lesions in other hypothalamic areas did not. However, none of the negative lesions encroached upon the PMC. Moreover, the short postoperative period before observation casts severe doubt on the interpretation of data obtained from lesions which abolished shivering since edema and hemorrhage could have stimulated or impaired the function of nearby areas. Several considerations indicate that the suppression of shivering obtained by Birzis and Hemingway after ablation of the ventrolateral posterior hypothalamus may have been due to irritative stimulation of remaining functional tissue. Both Hemingway et al. (1954) and Stuart et al. (1961) have obtained bilateral suppression of shivering by unilateral electrical stimulation of this locus, which suggests that the ventrolateral area serves an inhibitory rather than an excitatory function. That this is indeed the case is strongly indicated by the following ingenious experiment performed by Stuart et al. (1961) in anesthetized cats:

1) Unilateral electrical stimulation of the ventrolateral posterior hypothalamus suppressed spontaneous shivering for 10 minutes.
2) After shivering had returned, it was again suppressed by electrocoagulation of the area. However, shivering could still be produced by electrical stimulation of the ipsilateral PMC. Spontaneous shivering resumed after this stimulation.

3) Shivering was again suppressed by stimulation of the contralateral ventrolateral posterior hypothalamus. It was then reproduced by PMC stimulation.

4) Finally, the contralateral ventrolateral posterior hypothalamus was electrocoagulated, which again suppressed shivering.

5) Following this, shivering could not be reproduced even by PMC stimulation.

6) However, after the cats had recovered 31 days later, they were found to shiver vigorously in the cold, increasing their oxygen consumption by 320%. Thus, ablation of the ventrolateral posterior hypothalamus does not produce a permanent deficit in shivering.

Another relatively recent study is difficult to reconcile with the PMC concept (Birzis and Hemingway, 1957b). Anesthetized cats were allowed to shiver spontaneously as they reached the lighter stages of anesthesia. Microelectrode probing of the posterior hypothalamus revealed discharges in the ventrolateral posterior hypothalamus between the mammillary body and the cerebral peduncles. It is not clear what other areas of the posterior hypothalamus were examined with negative results. The "shivering" impulses appeared and disappeared as the cats' body was cooled or warmed and coincided temporally with shivering. Thus, the possibility
that this area does mediate shivering excitation cannot be completely
discounted. However, as the authors point out, the ventrolateral poste­
rior hypothalamic area bears heavy efferent traffic, and the discharges
seen may have been related to some other thermoregulatory function such
as vasoconstriction or piloerection which might be expected to occur simul­
taneously with shivering. Alternatively, they may have been shivering
discharges in efferent pathways descending ventrolaterally from the PMC
(see below).

A few reports in the older literature are also troublesome with
respect to providing support for the PMC concept. For example, the
classic paper of Ranson et al. (1937) established that large lateral
posterior hypothalamic lesions in monkeys cause severe impairment of
temperature regulation in a cold environment. There is no doubt that in
at least some of their animals there was little or no encroachment on
the PMC. However, the authors stressed that monkeys are not particularly
efficient homeotherms, and several of their control animals experienced
marked hypothermia with lack of shivering when exposed to a cool (18°C)
environment. In addition, although lesioned animals became hypothermic,
many shivered, which indicates there may have been impairment of other
thermoregulatory mechanisms such as vasomotor tone or acute nonshivering
thermogenesis. Furthermore, there is no definitive description of the
lesions in most of the animals which did not shiver. The one non-shiver­
ing animal for which a sketch of the lesion is provided had severe damage
to the dorsomedial (PMC) region also. There is no way to ascertain
whether the PMC was damaged in the remaining non-shivering animals. A
study using cats which yielded very similar results is subject to the
same criticisms (Clark et al., 1939). It should be mentioned that Stuart et al. (1962c) have also observed heat retentive deficits in cats with dorsolateral posterior hypothalamic lesions. Shivering, however, was normal. The deficit was attributed to impaired vasomotor control.

Whether or not we accept the dorsomedial posterior hypothalamus as the primary motor center for shivering, the question of how shivering impulses (regardless of their source) exit the hypothalamus must be examined. Several papers relate to this question, but clear-cut answers are not forthcoming. In cats, neither ventrolateral, nor dorsolateral nor ventromedial posterior hypothalamic lesions abolish shivering (Stuart et al., 1962c; Clark et al., 1939a). According to Stuart et al. (1962c), all posterior hypothalamic tissue with the exception of the dorsomedial area of one side can be destroyed without affecting shivering. This suggests a strong medial descending projection. On the other hand, Clark et al. (1939b) report that a huge lesion which destroyed the entire dorsomedial area just behind the PMC and a good part of the ventromedial area as well, did not affect the ability of the cat to regulate its body temperature in a cold environment. In the dog, Keller (1932, 1935) found that bilateral ablation of the ventral two-thirds of the hypothalamus or a three-quarter transverse section of the hypothalamus at the level of the mammillary bodies did not severely impair shivering. Thus, the data at hand indicate that shivering impulses do not exit the hypothalamus by a specific pathway but descend diffusely or by multiple pathways through the posterior hypothalamus.

Although the posterior hypothalamus may be necessary and, as will be seen, perhaps even sufficient for the production of shivering, the anterior
hypothalamus is not without effect on this important thermoregulatory mechanism. That heating or cooling of the AH/PO region suppresses or activates shivering, respectively, has been mentioned. Strong inhibitory influences on shivering have been obtained by electrical stimulation of the AH/PO region and the nearly septal area. Birzis et al. (1954) electrically stimulated the brains of anesthetized cats and found shivering was suppressed by unilateral stimulation at points throughout the preoptic and anterior hypothalamic areas. A few reactive points were found in the septum. Of all the sites examined, the preoptic area was the most sensitive. Similar results were obtained by Stuart et al. (1961) also in anesthetized cats. Experiments in unanesthetized goats (Andersson et al., 1956) revealed that shivering suppression could be obtained by unilateral stimulation of an area lying in the dorsal half of the region between the anterior commissure and the optic chiasm and extending laterally to the internal capsule. Stimulation 2 millimeters anterior or posterior to this area produced no response. Continuous stimulation of the reactive area when the animal was placed in a cold environment lowered rectal temperature by as much as 10°C, with no breakthrough of shivering (Andersson and Persson, 1957).

In spite of the known ability of cooling of the AH/PO region to activate shivering, electrical stimulation of this area has not generally been found to elicit shivering. The only positive report is that of Akert and Kesselring (1951) who found that stimulation at this site yielded a fine tremor in anesthetized cats. However, they found it difficult to repeat a positive result at a given point. This does not necessarily prove that the AH/PO region does not participate in the physiological elicitation of shivering, but may only be a result of the nonspecificity
of electrical stimulation. A greater number of warm- than cold-sensitive neurons have been found in this area by single unit recording technics. Thus, the excitatory effect may be masked by stimulation of the warm-sensitive neurons, which presumably activate heat loss mechanisms and suppress heat gain mechanisms, including shivering. However, electrical stimulation of the septal area, which projects to both anterior and posterior hypothalamus, is quite able to induce shivering (Andersson, 1957; Stuart et al., 1961, 1962b; Akert and Kesselring, 1951).

Most investigators who have destroyed or isolated part or all of the AH/PO region have found that shivering was only slightly impaired. Bazett et al. (1933) separated the anterior from the posterior hypothalamus by transection and found little impairment of shivering. Keller and McClaskey (1964) found that a dog with a transection removing the preoptic area, anterior commissure and part of the anterior hypothalamus could shiver and maintain a nearly normal body temperature. In another dog, complete ablation of one side of the hypothalamus combined with a lesion of the other side which spared only the extreme caudal portion of the hypothalamus did not abolish shivering; the animal shivered effectively when placed in the cold, although body temperature fell greatly before shivering was initiated (Keller and Blair, 1946).

In cats, lesions of the dorsomedial AH/PO region just below the anterior commissure have been reported to produce a decrease in oxygen consumption and chronic variability in rectal temperature with a tendency toward hypothermia. However, exposure to cold stress evoked "...appropriate if somewhat delayed and less effective thermoregulatory responses." Lesions outside this area had no effect (Jacobson and Squires, 1963;
Squires and Jacobson, 1962, 1968). It is not known whether the defect was due to a decrement in the ability to shiver. However, there is some probability that shivering was not impaired because Stuart et al. (1962c) reported that chronic unanesthetized cats with large bilateral septal, anterior hypothalamic or preoptic lesions shiver normally. In addition, Teague and Ranson (1936) placed larger bilateral lesions in the anterior hypothalamus of cats, and several days or weeks after the operation, all of these cats shivered normally. Clark et al. (1939a) found that bilateral lesions of the medial anterior hypothalamus had no effect on shivering but that lateral lesions produced some decrease in rectal temperature when the animals were placed in a cold environment. However, 2 of the 3 animals in this group were seen to shiver. Monkeys with similar lesions (Ranson et al., 1937) were hyperthermic post-operatively, but no tests were made under cold ambient conditions. Bilateral lesions in the medial AH/PO region (Hamilton and Brobeck, 1964; Han and Brobeck, 1961) had little effect on the ability of rats to maintain rectal temperature when placed in a 0-5°C environment. Presumably, shivering was present. Rats with bilateral lesions of the lateral portion of the anterior hypothalamus with some extension into the lateral tuberal area experienced a fall in rectal temperature when placed in a 10°C environment, but oxygen consumption was normal (Massopust et al., 1954). Decreased vasomotor tone was thought to be the cause of the hypothermia.

However, there are a few reports indicating that lesions of the AH/PO region produce profound impairment of shivering. Frazier et al. (1936) described "minute" lesions of the floor of the third ventricle
which produced complete loss of shivering in cats. In addition, they reported that the same deficit was seen whether the lesions were unilateral or bilateral. To the writer's knowledge, there is no other report of a unilateral lesion anywhere in the brain producing impairment, let alone, abolition of shivering. The paper, however, is totally inadequate. All tests were acute (4 days postoperative), and edema, hemorrhage and general debilitation may have produced a temporary loss of function far greater than any permanent loss attributable to the destruction of the circumscribed lesioned region. Further, the "minute" lesions described by the authors are actually huge and extend from the floor of the brain almost to the dorsal hippocampus in the steeply angled plates that are shown. The rostro-caudal extent of the lesions is not clear, but must include a considerable portion of the hypothalamus. Bond et al. (1957) reported severe hypothermia in cats with bilateral lesions in the posterior septum, fornix and medial anterior thalamus. However, most of the animals died within 5 days. Of those that survived 12 days or longer, only 3 were hypothermic, and in one of these, the lesion was restricted to the medial anterior thalamus alone. The high mortality and severe hypothermia found in this study must remain unexplained, since several studies have shown that both septum and thalamus can be removed without serious impairment of thermoregulation (see above). Clark et al. (1939b) placed very large bilateral lesions in the anterior hypothalamus of cats. Of the 12 animals that lived 10 or more days, 6 were hypothermic at room temperature. The 3 most severely hypothermic animals were tested in an environment of 10°C. Although all experienced large falls in rectal temperature, 2 of the 3 shivered; shivering in the
third was reported to be "questionable". Large lesions in the preoptic area did not produce these effects. Andersson et al., (1965) produced slow destruction of the entire preoptic area in 2 goats by proton irradiation. The septum and anterior hypothalamus were intact. Shivering was not abolished, but at an ambient temperature of 5°C, rectal temperature fell and shivering was late in onset. At −10°C, shivering started sooner and rectal temperature fell less, probably due to greater peripheral facilitation of shivering by cutaneous cold receptors. It is interesting that these animals still responded to pyrogen with an increase in body temperature and shivering. Rapid radiofrequency lesioning of the preoptic and anterior hypothalamic areas in another animal produced immediate and violent shivering. Hyperthermia rapidly developed and was maintained for 8 days. Following this, rectal temperature returned to normal, but the goat was found to be poorly resistant to cold, having reactions very similar to the proton irradiated goats.

There is little information concerning the participation of the mid-hypothalamus in the control of shivering. Hemingway et al., (1954) found that electrical stimulation of many points in the middle hypothalamus suppressed shivering but Stuart et al., (1961) were not able to evoke shivering by stimulation of this region. Teague and Ranson (1936) destroyed the medial tuberal hypothalamus in the cat with little effect on shivering. Very large middle hypothalamic lesions in cats were found by Clark et al., (1939a) to abolish shivering completely. However, all the animals died by the eleventh postoperative day, so that general debilitation may have been a factor. Goats having massive lesions of the preoptic area, anterior hypothalamus and middle hypothalamus
experienced immediate hypothermia after the lesioning process (Andersson et al., 1965). This is in contrast to the initial hyperthermia seen when the lesions were restricted to the preoptic and anterior hypothalamic areas. Evidently, shivering was impaired, since pyrogen injection, although yielding an increase in rectal temperature, did not induce shivering. However, the authors' sketch of the lesion indicates that the lesion may have destroyed part or all of the PMC in addition to the anterior and tuberal damage. Bilateral lesions in the ventrolateral portion of the midhypothalamus of rats did not affect temperature regulation in a cold environment (Han and Brobeck, 1961; Massopust, 1954). It is presumed that shivering was intact. However, rats with dorsal lesions of the medial and lateral tuberal area experienced a rapid fall in rectal temperature at 10°C ambient and had chronically low oxygen consumption rates which increased only slightly when the rats were placed in the cold. The presence or absence of shivering was not reported, but the failure of oxygen consumption to increase in a cold environment indicates that it was impaired. Commenting on these findings, Stuart et al. (1962c) suggested that because of the small size of the rat brain, the lesions could well have destroyed or impaired the function of the nearby PMC.

In summary, it has been found that effective thermoregulatory shivering is dependent upon the integrity of the hypothalamus. Although there is some controversy, it appears that the dorsomedial portion of the posterior hypothalamus acts as a primary motor center (PMC) for shivering. Ablation of this area abolishes shivering, whereas destruction of other areas of the hypothalamus may impair, but not abolish, shivering. The
anatomy of the descending pathway from this center through the remain­
ing posterior hypothalamus to the midbrain is not known, but it must be
diffuse or consist of several discrete and widely separated tracts.

According to Hemingway (1963), the PMC can be activated independently
of the rest of the hypothalamus by afferents from skin cold receptors.
However, other hypothalamic areas probably modulate the activity of the
PMC. Electrical stimulation studies have shown that the septum, the
AH/PO region between the optic chiasm and the anterior commissure, and
the ventrolateral posterior hypothalamus exert strong inhibitory in­
fluence on shivering; shivering is facilitated by impulses from the
septum and possibly the AH/PO region.

Lesions in the AH/PO region do not abolish shivering but do seem to
impair the precision with which shivering is regulated; shivering is
less vigorous and its onset is slower when the animal is subjected to a
cold environment. With the exception of two studies, lesions in the
midhypothalamic area have had little effect on shivering. In the studies
where shivering was impaired, the lesions may have encroached upon the
PMC.

Hypothalamic Control of Cutaneous Vasomotor Tone
Cutaneous vasodilation is a method of heat dissipation utilized by
many homeotherms (Hammel, 1968). Flooding the cutaneous vascular beds
with warm arterial blood increases heat dissipation through the skin.
In heavily furred animals, only the thinly furred areas of the body such
as the feet and ears in cats and dogs and the tail in the rat participate
in thermoregulatory vasomotor changes. The changes are restricted to
the skin vasculature, and consist entirely of modulation of the tonic
sympathetic tone to these vessels (Folkow et al., 1949).

It has been mentioned previously that heating or cooling of the hypothalamus produces vasodilation and constriction, respectively. Electrical stimulation of the hypothalamus has also been found to elicit changes in cutaneous vasomotor tone. Strom has stimulated the brains of anesthetized cats, dogs and rabbits (Strom, 1950b; Eliasson and Strom, 1950). Unilateral stimulation of the frontal lobe, the lateral preoptic area, the medial and lateral anterior hypothalamus, the tuberal hypothalamus and a few sites in the posterior hypothalamus produced bilateral cutaneous vasoconstriction followed by post-stimulatory vasodilation. Responses were obtained most easily in the more rostral regions of the hypothalamus. A change in stimulation parameters (from high frequency, low voltage to low frequency, high voltage) frequently produced vasodilation as the initial effect instead of vasoconstriction. Local warming of many of the AH/PO loci shown to be sensitive to electrical stimulation resulted in cutaneous vasodilation. However, some sites responded to one type of stimulus only. It was suggested that at many of the sites tested there are both vasoconstrictor (excitatory) and vasodilator (inhibitory) neurons which modulate a common path of caudal vasoconstrictor neurons. Low frequency, high voltage stimulation and local warming may excite the cells mediating vasodilation; high frequency, low voltage stimulation may excite the vasoconstrictor neurons. The poststimulatory vasodilation could have resulted either from afterdischarges in the vasodilator neurons or poststimulatory inhibition of the vasoconstrictor neurons.

In contrast to Strom's findings, Andersson and co-workers (Andersson et al., 1956; Andersson and Persson, 1957) obtained only cutaneous
vasodilation after unilateral electrical stimulation of the hypothalamus of the unanesthetized goat. Continuous stimulation of goats kept at -6°C ambient elicited ear vasodilation lasting at least two hours, even though rectal temperature had decreased by about 6°C. Interestingly, vasodilation was maintained somewhat longer in the ear ipsilateral to the site of stimulation, an indication that there may be some laterality in the thermoregulatory vasomotor efferents. The reactive area was strictly limited to a site just below the anterior commissure and 1 to 2 millimeters rostral or caudal to it. The effects were slow in onset and persisted some minutes after stimulation was stopped. Andersson suggests that the small size of the cat brain or the anesthesia used may have been responsible for Strom's observation of vasoconstriction and vasodilation obtained at the same site; i.e., the current may have spread to adjacent areas. Alternatively, there may be a species difference in neural organization of vasomotor control at the hypothalamic level, or the difference might have arisen from the different stimulation waveforms used by the two groups.

The spread of current explanation is strengthened by another of Andersson's observations (1957). Again using the unanesthetized goat, he found that electrical stimulation of the posterior septal area causes cutaneous vasoconstriction. This area lies directly dorsomedial to the area which yielded vasodilation.

A few other studies using electrical stimulation concern themselves with the hypothalamic control of cutaneous vasomotor activity. Abrahams et al. (1960) and Uvnas (1960) have defined a tract running the length of the hypothalamus in the central gray and exiting ventrally between
the mammillary bodies and cerebral peduncles and dorsally near the aqueduct. Stimulation of this tract produces cutaneous and intestinal vasoconstriction and skeletal muscle vasodilation. The function of this pathway is not well understood, but it is apparently not concerned with temperature regulation. In Strom's study (see above) the vasoconstriction and dilation observed were limited to the cutaneous vasculature; there was no change in muscle or intestinal blood flow. Thus, the two pathways appear to be independent (Uvnas, 1960).

Cragg (1961) has found that unilateral electrical stimulation of the preoptic area and the habenular, interpeduncular and dorsal tegmental nuclei can induce cutaneous vasodilation as well as panting and muscular relaxation in the rabbit. The habenular nuclei receive afferents from the preoptic area and posterior dorsal septum and relay impulses to the dorsal tegmental nucleus via the interpeduncular nucleus. The dorsal tegmental nucleus also receives afferents from the hypothalamus via the mammillo-tegmental tract. Thus, we see here the interesting possibility of an auxiliary, epithalamic route for relay of hypothalamic vasomotor impulses to more caudal centers. That this is at most an auxiliary pathway has been shown by Grant (1963). Rabbits, when restrained, exhibit "emotional hypothermia" typified by the presence of vasodilation and panting. Bilateral habenulectomy does not affect this response. Thus, the transhypothalamic route must be of considerable importance. This is also indicated by the data of Clark et al. (1939b). Three of their cats had thalamic lesions destroying the habenular nucleus and surrounding thalamus, yet there was no deficit in temperature regulation when the animals were placed in a hot environment. One of these also had
complete destruction of all the transitional tissue between the posterior hypothalamus and midbrain, with the exception of the most caudal ventrolateral area between the cerebral peduncles and the mammillary bodies. Unfortunately, vasomotor tone was not measured, but since this animal's temperature regulation was apparently entirely normal, it seems probable that vasomotor regulation was unimpaired.

This finding suggests that the ventrolateral portion of the posterior hypothalamus contains the majority of fibers subserving thermoregulatory vasomotor tone. Such a suggestion seems reasonable, since it is this area which carries most of the efferent autonomic fibers originating in the hypothalamus (Magoun, 1940; Ingram, 1960). The classical study of Clark et al. (1939a) also buttresses this viewpoint, but again, the study is weakened by the failure of the authors to report skin temperature. "Moderate sized" lesions were placed in the posterior hypothalamus of cats. Medial lesions had no effect on thermoregulation during exposure to cold or warm environments, while lateral lesions destroying the ventrolateral area yielded severe impairment in both situations. On the other hand, Stuart et al. (1962c) found that dorsolateral posterior hypothalamic lesions in cats yielded a fall in body temperature in a cold environment, although shivering was normal. It was suggested that the regulatory deficit could have been due to impaired vasomotor control, but skin temperature was not measured. It is apparent that further study is required before this issue can be resolved.

Several investigations report that the AH/PO region influences thermoregulatory vasomotor tone. Small electrolytic lesions in the medial preoptic region produced chronic hypothermia and a reduction
in oxygen consumption in cats. Inappropriate cutaneous vasomotor control was observed, i.e., vasodilation at low rectal temperatures and vasoconstriction during the occasional periods of higher rectal temperature (Squires and Jacobson, 1966; Jacobson and Squires, 1963, 1966a, 1966b). Somewhat larger lesions in the medial preoptic area of rats produced a partial impairment of vasodilation (Han and Brobeck, 1961). When exercised, these lesioned rats vasodilated, as did control animals. However, at the end of exercise, immediate vasoconstriction appeared while rectal temperature was still elevated. Control animals did not constrict until rectal temperature had fallen somewhat. These studies indicate that lesions of the AH/PO region produce a derangement in, but not total loss of, thermoregulatory cutaneous vasomotor control. Such a derangement could derive from impairment of either integrative or thermoceptive elements.

It has been mentioned that cutaneous vasomotor dilation is due solely to inhibition of tonic vasoconstrictor activity. Keller (1960) found that midbrain cats were not chronically vasodilated and, in fact, had an enhanced cutaneous vasoconstrictor tone. Hemisection of the brainstem as low as the rostral medulla did not produce vasodilation, but caudal medullary hemisection yielded ipsilateral cutaneous vasodilation. Thus, it appears that the neurons sustaining this vasoconstrictor activity lie caudal to the midbrain, possibly in the caudal medulla, rather than in the hypothalamus. Vasodilation mediated by the hypothalamus must therefore result from direct or indirect inhibition of these neurons.
There is some evidence that the latter is the case. Strom (1950a), for example, found that midbrain decerebration of anesthetized cats produced a large increase in skin blood flow "lasting many minutes." Keller and Hare (1932) reported that massive destruction of the hypothalamus back to the middle of the mammillary bodies yields chronic vasodilation associated with panting lasting up to 2 weeks. Andersson et al. (1965) found that progressive rostrocaudal destruction of the goat hypothalamus induced chronic vasodilation when the lesions encroached upon the tuberal region. These experiments suggest that the hypothalamic vasomotor output cannot consist of direct inhibition of the bulbar vasoconstrictor neurons, unless one subscribes to the remote possibility that, in each case, the vasodilation was due to irritative effects of the lesions on sites caudal to the ablations. The simplest explanation for these data is that there are neurons somewhere in the midbrain between Strom's transection (which yielded vasodilation) and Keller's transection (which yielded vasoconstriction) whose processes make inhibitory synapses with the bulbar tonic vasoconstrictor neurons. Hypothalamic efferents would inhibit these midbrain units. Thus, transection caudal to the midbrain neurons would yield enhanced cutaneous vasoconstriction, whereas transection rostral to these units would free them from hypothalamic inhibition, resulting in chronic vasodilation. Of course, much more complex neural circuitry is conceivable.

In summary, the hypothalamus has been shown to participate in the control of thermoregulatory cutaneous vasomotor tone. Heating and cooling of the AH/PO region evokes vasodilation and vasoconstriction, respectively. In cats, unilateral electrical stimulation of many
hypothalamic areas elicits vasoconstriction or vasodilation, depending upon stimulation parameters. In goats, unilateral electrical stimulation of a localized area lying just beneath the anterior commissure elicits vasodilation. Stimulation of the septal area produces vasoconstriction. In rabbits, electrical stimulation of the preoptic area, habenular nucleus, interpeduncular nucleus or dorsal tegmental nucleus yields vasodilation. This suggests an epithalamic route for efferent hypothalamic vasomotor impulses. However, since habenulectomy does not prevent vasodilation, it appears that a transhypothalamic route also exists. There is suggestive evidence that this pathway may exit the posterior hypothalamus in its dorsolateral or ventrolateral aspect. Electrolytic lesions of the AH/PO region in cats and rats produce derangement, but not complete loss of thermoregulatory vasomotor control.

Cutaneous vasodilation is due solely to inhibition of tonic vasoconstrictor tone. The neurons subserving the latter function lie in the lower brain stem, probably in the caudal medulla. The ultimate effect of hypothalamic thermoregulatory vasomotor efferents is to inhibit these neurons, the inhibition likely being mediated by a multisynaptic hypotalamobular pathway. The neurons originating this ultimately inhibitory activity appear to lie in the tuberal and/or posterior hypothalamus. The role of the excitatory and inhibitory elements in the AH/PO region may be to modulate the activity of these more caudal hypothalamic neurons.

Hypothalamic Control of Panting

Panting in the mammal or bird is the most efficient and direct route for dissipating body heat (Hammel, 1968). Panting is a highly
integrated response. In the cat and dog, it consists of 1) elevation of respiratory rate; rates up to 350 respirations per minute have been observed 2) reduction of tidal volume so that only the air in the "dead space" of the respiratory system is moved; if this were not the case, severe hyperventilation would soon supervene 3) opening of the mouth, retraction of the lips and protrusion of the tongue, which flaps in synchrony with the ventilatory movements. Exposure to heat can also activate increased respiratory rate with decreased tidal volume, but in the absence of the behavioral aspects of panting, i.e., the mouth remains closed and the tongue retracted. The respiratory rates observed during such "thermal polypnea" are usually lower than those seen during true panting.

Evidence that local warming of the hypothalamus can elicit panting in several species has been presented previously. Although the AH/PO region was found to be most sensitive, responses could be obtained throughout the length of the hypothalamus, at least in the cat. Electrical stimulation of the hypothalamus can also cause panting. Hess and Stoll (1944) obtained panting by stimulation at sites throughout the hypothalamus and preoptic area. The pathway appears to exit at the ventrolateral region of the posterior hypothalamus, but another pathway seems to ascend from the hypothalamus to the dorsal midbrain tegmentum. Even at very low ambient temperature, Andersson et al. (1956) were able to elicit panting in the unanesthetized goat by unilateral stimulation of a circumscribed region lying just beneath the anterior commissure. Continuous stimulation of this area for 3 hours at an ambient temperature of -6°C maintained true panting for about an hour, although stimulus
strength had to be gradually increased. Polypnea, however, was main-
tained for the entire 3 hours, during which time rectal temperature had
decreased by 10°C (Andersson and Persson, 1957). Stimulation of an
area in the posterior septum just above the anterior commissure inhibited
panting which had previously been initiated by placing the animal in a
60°C environment (Andersson, 1957). Cragg (1961) has stimulated the
preoptic area, habenular nuclei, interpeduncular nuclei, dorsal teg­
mental nuclei and interconnecting tracts of the rabbit. "Panting"
sufficient to lower body temperature was induced at each of these sites.
That the panting consisted of increased respiratory rate and increased
tidal volume casts some doubt on the relation of this system to tempera­
ture regulation. However, vasodilation and inhibition of shivering were
seen to occur simultaneously with the panting. This observation strongly
suggests that the pathway described by Cragg may indeed be related to
thermoregulatory systems. Interestingly, the more caudal placements
were found to be most sensitive. Stimulation of the septal area had
little effect on respiration, but the possibility that stimulation of
this area might inhibit rather than stimulate panting (as in the goat)
was not examined. The stria medullaris is a major pathway from the
preoptic area to the habenular nuclei. However, cutting of the stria
bilaterally did not influence the panting response to either electri­
cal or thermal stimulation of the preoptic area. Nevertheless, after
cutting of the stria, electrical stimulation of either the cranial or
caudal end yielded panting. The cranial response can be attributed to
fibers which ascend in the stria to reach the preoptic area. It can
be concluded that 1) the preoptic area can elicit panting via the stria
medullaris-habenular outflow and 2) there must be an important alternative route. In fact, in confirmation of the work of Hess and Stoll, Cragg was able to elicit panting by stimulation of numerous placements in the medial forebrain bundle as far posterior as the mammillary bodies. Panting could also be obtained by stimulation dorsolateral to the interpeduncular nucleus and in the midbrain tegmentum.

The habenula and surrounding caudodorsal thalamus has also been implicated in the control of panting by Lilienthal and Otenasek (1937). By a series of transection experiments, it was shown that the spontaneous panting seen after acute decortication was abolished by transections leaving the hypothalamus intact but removing the habenular area, whereas the panting remained after sections removing the hypothalamus but retaining the habenular area. However, Clark et al. (1939b) found that chronic cats with lesions destroying the habenula and associated thalamic tissue panted well during heat stress and that, when these animals were decorticated just prior to sacrifice, typical spontaneous panting was seen. The discrepancy between these two experiments remains unresolved.

Lesion and transection experiments by the Ranson and Keller groups have provided further information. Panting was severely impaired in cats with bilateral lesions involving the hypothalamus as far back as the infundibulum. When placed in a hot environment, most of them panted, but the rectal temperature at which panting was activated (panting threshold) was elevated a degree or two. However, 9 or the 38 cats did not pant even at a rectal temperature of 41°C. Even in those that panted, there was apparently a drastic alteration in the activation mechanism. Normal cats experience a slow increase in respiratory rate as their
rectal temperature increases until, finally, true panting occurs. In
the lesioned animals, respiratory rate remained steady at normal levels
until rectal temperature rose to a level 1 or 2 degrees above the normal
panting threshold. At this temperature, panting was suddenly activated
and respiratory rate rose sharply. A few cats had unimpaired panting
responses. In these, the lesions were found to be more posterior, so
that on at least one side of the hypothalamus, the tissue around the
anterior commissure was intact (Teague and Ranson, 1936). Andersson
et al. (1965) have reported that goats with bilateral preoptic lesions
also experience impairment, but not loss, of the panting response.

Clark et al. (1939a) performed experiments similar to those of
Teague and Ranson but made somewhat more discrete lesions. Lateral
lesions of the AH/PO region were found to yield more severe impairment
than more medial lesions. None of the cats with lateral lesions panted
at rectal temperatures of 41°C; medial lesions simply raised the panting
threshold about a degree. Lesions of the medial posterior hypothalamus
did not impair panting, but lateral posterior lesions prevented panting
at rectal temperatures as high as 41°C. However, if the rectal tempera-
ture of the cats with lateral posterior hypothalamic lesions was forced
higher (43°C), panting was seen. The authors concluded that panting
impulses arise in the AH/PO region, pass laterally through the hypothala-
mus with the medial forebrain bundle and exit ventrolaterally between
the cerebral peduncles and mammillary bodies. This is in agreement with
experiments previously discussed. On the other hand, the fact that the
severely impaired cats could be forced to pant if the rectal temperature
were forced high enough may indicate that the epithalamic route described
by Cragg was operative or that sites caudal to the hypothalamus can activate panting in circumstances of severe heat stress.

Keller has demonstrated that such an infrahypothalamic panting mechanism must exist. In a long series of extirpation and transection experiments (Keller, 1930, 1933, 1935; Keller and Blair, 1946; Blair and Keller, 1946; Keller and McClaskey, 1964), it was shown that 1) complete transections of the brain anterior to the preoptic area (which also removed the septum) have little effect on panting 2) complete transections as far back as the mammillary bodies impair but do not abolish panting; the more anterior transections seemed to produce more impairment than the posterior transections 3) transections separating the cephalic midbrain from the lower centers abolish panting. From these data, Keller suggested (Keller and McClaskey, 1964) that both the AH/PO region and the cephalic midbrain have the ability to activate panting, the AH/PO region being the most sensitive. The posterior hypothalamus is said to be an inhibitory region. Some thoughtwill reveal that these concepts are not inconsistent with previously cited data.

However, one report of Keller's confuses this picture. In the cat, when the entire hypothalamus (but not the preoptic area) back to the level of the mammillary bodies was dissected out (rather than being separated from lower centers by a full dorsoventral brain transection), continuous polypnea supervened. The slightest movement of the animal would activate true panting, indicating facilitation of the panting center by non-thermal peripheral efferents (Keller and Hare, 1932). In the dog, a full brain transection cutting the hypothalamus at the same level raised the panting threshold (Keller and McClaskey, 1964).
This may be a species difference in neural organization. However, there is an alternative explanation. When the hypothalamus is dissected out, the habenular facilitory circuit from the preoptic area remains intact; a full brain transection removed it. Thus, there is a possibility that in the cat experiment, continuous polypnea appeared due to facilitation of the midbrain excitatory centers by the habenular circuit unimpeded by posterior hypothalamic inhibition.

In summary, it is clear that local heating of the AH/PO region can initiate panting. A major descending pathway passes laterally through the hypothalamus and exists ventrolaterally between the cerebral peduncles and mammillary bodies. Exit via the periventricular system is also possible. An epithalamic route via the habenular complex is supported by considerable data. The possibility that the cephalic midbrain can activate panting autonomously and that the posterior hypothalamus contains inhibitory mechanisms seems well supported, but cannot be accepted as established. The significance of the localized inhibitory center in the posterior septum of the goat is unknown. As is the case for shivering and vasomotor control, unilateral thermal or electrical stimulation is able to activate or suppress panting, but unilateral lesions have no effect.

Effects of Extrahypothalamic Thermal and Non-thermal Input

The hypothalamus is unique in that, in the absence of significant peripheral thermal input, changes in its own local temperature can activate thermoregulatory responses. In a neutral environment with normal rectal temperature, the hypothalamus should be essentially
deafferented from peripheral thermoceptive stimulation. An increase or
decrease in hypothalamic temperature of 0.2-0.3°C will now activate
panting or shivering respectively. Such high sensitivity argues strongly
for the participation of hypothalamic thermoreception in the control of
body temperature. However, the problem of spontaneous fluctuations in
hypothalamic temperature remains. Fluctuations of 0.5–1.0°C with no
overt thermoregulatory consequences have been observed in several species
(see Bligh, 1966a, for references). The problem is perhaps best illus-
trated in the cat, which has an extracranial carotid rete through which
most of the blood perfusing the brain must pass. Cranial vasomotor
changes (such as changes in ear blood flow), cerebral venous blood tem-
perature, food temperature, respiratory exchange, vasculature muscle
heat production and vascular changes in the mucosa of the oral and nasal
passages may influence the temperature of the rete, and consequently
that of the hypothalamus, without affecting deep body temperature. With
such lability in its own temperature, it is difficult to see how the
hypothalamic thermosensitive elements can act effectively to control
deep body temperature with precision.

One solution, suggested by Bligh (1966a), is that extrahypothalamic
thermal and nonthermal information is relayed to the hypothalamus,
there to be integrated with that gained from hypothalamic thermorecep-
tors. Simply put, this means that extrahypothalamic input tells the
hypothalamus which of the hypothalamic temperature changes may have
thermoregulatory consequences and should be acted upon.

Some workers hold the view that hypothalamic thermoreceptors do not
participate except during extreme thermal stress and that, in the normal
state, control is dependent entirely upon extrahypothalamic information. Others feel that some thermoregulatory mechanisms are entirely dependent upon hypothalamic thermoreception whereas other mechanisms are activated entirely by extrahypothalamic thermal information. Hammel et al. (1963) have proposed that both hypothalamic and extrahypothalamic receptors participate and envision the hypothalamus as constituting a

...proportional controller which has evolved in such a way to permit its set point to be modified by skin temperature, core temperature, state of consciousness, etc. Indeed, it is a device by which the load error for driving a thermoregulatory response is achieved not by requiring the regulated hypothalamic temperature to deviate greatly from an invariant set point, but rather by offsetting the set point according to needs of the organism. Thus, by this thesis, when the skin temperature falls in a cold environment, the steady-state and phasic firing rate of cold receptors in the skin increases and elevates the set point so that the hypothalamic temperature, without changing, would be below the set point and would drive heat conservation or increase heat production. Conversely, when the skin temperature rises in a hot environment, the steady-state firing rate from the cold receptors diminishes to zero and the steady-state and phasic firing rate from the warm receptors may increase and would, thereby, lower the set-point temperature below the hypothalamic temperature and drive increased heat loss.

Whatever the mechanism of interaction with hypothalamic thermoregulatory mechanisms, the extrahypothalamic thermoreceptors cannot be limited to skin, for in every experiment cited in previous sections (with the exception of Andersson's goats) where body temperature was altered by prolonged thermal stimulation of the hypothalamus, the rectal temperature could be driven up or down only within a limited range. Thus, core temperature detectors of extrahypothalamic location must be acting to prevent further change in deep body temperature. It has been suggested that such core thermoreceptors might lie in the vena cava (Bligh, 1961), esophagus (Rautenberg, Simon and Thauer, 1963) and vertebral canal (Simon, Rautenberg, Thauer and Iriki, 1964).
It is also possible that non-thermal input to the hypothalamus, such as the level of activity of the ascending reticular activating system (Euler and Soderberg, 1957) may influence the thermoregulatory activity of the hypothalamus.

For further information concerning hypothalamic-extrahypothalamic interactions and control systems theory, the reader is referred to the reviews of Euler (1960), Hardy (1961), Bligh (1966a) and Hammel (1968).

Unfortunately, the pathways by which thermal and non-thermal afferents reach the hypothalamus and their sites of termination within the hypothalamus are entirely unknown. In the absence of this information, it becomes very difficult to interpret properly information derived from ablation, transection and electrical stimulation experiments concerned with elucidating the mechanisms of hypothalamic control of thermoregulation. Therefore, it must be stressed that the conclusions drawn from data reviewed in previous sections are tentative and must be tempered by the recognition that afferent systems, rather than efferent, integrative or thermoreceptive elements may have been altered by the experimental procedures.

Possible Neurotransmitters Involved in the Hypothalamic Control of Body Temperature

Introduction

Prior to the early 1960's, there was little information available concerning the involvement of central neurohormones in mammalian thermoregulation despite the long and intensive study of its neuroanatomical and functional aspects. Of course, until the 1950's, little was known about the existence of central neurotransmitters, let alone their
relation to specific physiological phenomena.

Quastel et al. (1936) and Stedman and Stedman (1937) had demonstrated the ability of brain homogenates to synthesize acetylcholine (ACh). Because of its prominent role in peripheral autonomic transmission, ACh was early suspected of being a central neurotransmitter. The finding that brain ACh was concentrated in specific brain areas (MacIntosh, 1941) supported this view. U. S. Von Euler demonstrated that norepinephrine (NE) and epinephrine (E) were present in brain as early as 1946, but until 1954 it was generally assumed that these amines resided in cerebral blood vessels. Vogt's (1954) demonstration that these amines were concentrated in the brainstem, and Twarog and Page's (1953) report that 5-hydroxytryptamine (5-HT) is found in brain ushered in the "Amine Era" of neuropharmacology.

By 1960, dopamine (DA), the precursor of NE, had been found in brain (Carlsson et al., 1958); its differential distribution to areas low in NE indicated that it too might have a neurohumoral function (Bertler and Rosengren, 1959). By 1964, the cerebral sites of concentration of ACh, NE, E and DA were well established. In addition, the enzymes necessary for biosynthesis and degradation of each of these compounds had been shown to exist in brain (see Glowinski and Baldessarini, 1966, for references). Strong evidence for a central neurotransmitter function for the monoamines was provided by Carlsson's group, which developed the histochemical fluorescence technic and in 1962 reported direct visualization of dopamine, 5-HT and NE in the cell bodies and terminals of central neurons (Carlsson et al., 1962).
Catecholamines and 5-Hydroxytryptamine

Approximately a decade ago, the time was propitious for implication of candidate neurotransmitters in the central control of body temperature (as well as numerous other behavioral and neurophysiological phenomena). Brodie and Shore (1957) had considered the possibility that monoamines might be involved. Curt Von Euler (1961) speculated that NE, E and 5-HT, by virtue of their known activity on the ascending reticular activating system, might be concerned with determination of the level of the "setpoint" (see Chapter I). But it was a short paper by Feldberg and Myers, "A New Concept of Temperature Regulation by Amines in the Hypothalamus" (1963) which initiated the intense interest now being given this subject.

In this report it was shown that E or NE, when injected into the lateral ventricle of unanesthetized cats, could reduce the fever induced by intraventricular pyrogen; intraventricular 5-HT was found to induce fever in a normal cat or to augment pyrogen-induced fever. Since it had been previously demonstrated that intraventricular pyrogen probably acts on the anterior hypothalamus (Sheath and Borison, 1960) and that the hypothalamus is rich in catecholamines (CA) and 5-HT (Vogt, 1954; Amin, Crawford and Gaddum, 1954), it seemed reasonable to Feldberg and Myers that the release of E, NE and 5-HT in the anterior hypothalamus might be involved in the hypothalamic control of thermoregulation. Thus, the release of E or NE would activate heat loss mechanisms and/or suppress heat gain mechanisms, whereas 5-HT would act conversely. It should be mentioned that Feldberg and Myer's original description of the action of biogenic amines on hypothalamic control of thermoregulation was
somewhat ambiguous. Control was described as being due to a "delicate balance in the release of adrenaline, noradrenaline and 5-HT in the hypothalamus." Cooper et al. (1965) have pointed out that this could be construed to mean that release is a neurosecretory process. However, in a later paper (El Howary and Feldberg, 1966), Feldberg stressed that the amines are considered to be neurotransmitters and as such, are released at synaptic junctions. Such aminergic synapses could be located in the "setpoint" generating apparatus, thermosensitive neurons of the hypothalamus, efferent pathways for elicitation of thermoregulatory responses or afferent pathways carrying thermal or other information to the hypothalamic areas responsible for integration of thermoregulatory responses.

An extension of their original study in the following year revealed that NE and E could also lower body temperature in the non-febrile cat (Feldberg and Myers, 1964). Intraventricular injection of 50-100 micrograms of either substance lowered body temperature by 0.5-1.3°C. Cutaneous vasodilation was observed during the time rectal temperature was decreasing. Intraventricular 5-HT produced a triphasic effect—a small increase in rectal temperature, followed by a short decrease, in turn followed by a large and prolonged increase to fever level, the hyperthermia lasting as long as 20 hours. Shivering and vasoconstriction were observed during the rising phases. Sedation was frequently seen. Speculating on the relation of 5-HT to the action of pyrogens, the authors suggested that pyrogens may release hypothalamic 5-HT, mimic its action, sensitize the hypothalamus to 5-HT or prevent the release of NE.
It was found subsequently (Feldberg and Myers, 1965) that unilateral microinjection of much smaller amounts (5 micrograms or less) of NE, E or 5-HT directly into the preoptic area of unanesthetized cats produces the same effects as intraventricular injection. Microinjections made into the posterior hypothalamus or ventromedial nucleus had no effect. Therefore, the original assumption that the amines were acting at the hypothalamus was confirmed, and the AH/PO area was shown to be the most sensitive site.

Kulkarni (1967) confirmed the hypothermic effect of intraventricular NE in cats and also reported restlessness, lip licking, vocalization, vomiting, urination, defecation and increased respiratory rate (but, evidently, not true panting). Intraventricular 5-HT, however, produced immediate hypothermia lasting 2 to 3 hours which was followed by hyperthermia. Restlessness, lip licking, vocalization, increased respiratory rate, tremors, ataxia and piloerection were also observed. The hypothermic effect was dose dependent, but the hyperthermia was not. The short initial rise seen by Feldberg and Myers was not present, and Kulkarni suggested that this rise may have been due to initial excitement engendered by the injection procedure. Evidently, care was taken to avoid such excitement in his own experiments. The author suggested that 5-HT, by virtue of its vasoconstrictive properties, reduces hypothermic blood flow. Since the hypothalamus is cooled by the arterial blood, reduction of flow would increase hypothalamic temperature and activate heat loss mechanisms. Thus, the hypothermia phase of the 5-HT effect was attributed to an indirect mechanism.
Banerjee, Burks and Feldberg (1968a) attempted to resolve the confusion surrounding the action of 5-HT in the cat. The major differences between Feldberg and Myers' experiment and that of Kulkarni was the vehicle used to dissolve the 5-HT. The former workers had used saline, and the latter, distilled water. With saline as the vehicle, Banerjee et al. found 5-HT produced either the triphasic effect previously described or only a rise. At high doses, e.g., 700-1050 micrograms, the initial rise was greatly attenuated and the fall became deeper and longer. However, the final hyperthermia still occurred in every case. With the 5-HT dissolved in distilled water, the initial rise became very small and lasted only 2 to 3 minutes. The falling phase was accentuated in magnitude and duration, and the final rise was attenuated. Interestingly, distilled water alone produced immediate and prolonged hyperthermia as well as sedation, but saline had little effect. It was speculated that 1) 5-HT at low concentrations is excitatory, but at higher concentrations it "paralyzes" neural function (perhaps in a manner similar to the prolonged depolarization produced at autonomic ganglia by high concentrations of ACh) 2) distilled water may release 5-HT from the ventricular walls. Thus, in saline, 5-HT at moderate concentrations would yield initial excitation followed by depression as the concentration within the AH/PO region rises; as the 5-HT is metabolized, the concentration would fall and excitation would again supervene. This sequence of events would explain the triphasic response to intraventricular 5-HT. Higher concentrations of 5-HT would shorten the initial excitatory phase and lengthen the inhibitory phase, as was observed. Administration of a given concentration of 5-HT in distilled water would be equivalent
to administration of a higher concentration of 5-HT in saline, because distilled water itself may release 5-HT. Thus, the hypothermic (inhibitory) phase would be prominent. This could account for the observations of Kulkarni.

The effects of intraventricular or intrahypothalamic injection of E, NE and 5-HT have been examined in several species in addition to the cat. The dog reacts to these substances in a manner similar to the cat (Feldberg et al., 1966). Intraventricular E or NE in dogs anesthetized with pentobarbital produced cessation of shivering, loss of muscle tone, cutaneous vasodilation, decreased respiratory rate and a fall in rectal temperature. As in the cat, E seems to be more potent than NE. Shivering is more sensitive to the effects of these substances than is the vasomotor effect. At high doses, shivering is rapidly suppressed, but vasodilation does not occur until body temperature has already begun to fall. At lower doses, vasodilation is absent. 5-hydroxytryptamine evokes an increase in temperature, but the multiphasic effect seen in cats was not observed. In the unanesthetized dog, E or NE administered into the third ventricle produced a fall in body temperature, and low doses of 5-HT (5-20 micrograms) produced a rise. In one experiment in one animal, a higher dose of 5-HT (50 micrograms) had little effect (Feldberg, Hennon and Lotti, 1967).

The monkey responds to the biogenic amines in the same way as the dog and cat (Myers and Yaksh, 1969). Unilateral or bilateral microinjections of NE and 5-HT into the AH/PO region of unanesthetized monkey produced dose-dependent hypothermia and hyperthermia, respectively. Large doses of 5-HT often elicited a short decrease in temperature which
preceded the rise. Injection of these substances at sites other than the AH/PO region produced little effect. Epinephrine also elicited hypothermia, but seemed to be slightly less potent than NE, whereas in the cat, E was about twice as potent as NE (Feldberg and Myers, 1965). Dopamine had weak hypothermic activity when injected into the AH/PO region.

In rabbit (Cooper et al., 1965), NE injected into the cerebral ventricles or anterior hypothalamus produced either fever associated with cutaneous vasoconstriction or no effect. 5-Hydroxytryptamine given intraventricularly produced no effect except when body temperature had been previously elevated by NE or pyrogen administration. In these febrile rabbits, 5-HT produced vasodilation and a decrease in body temperature. In every instance where 5-HT or NE were ineffective, administration of pyrogen at the same site yielded fever. Injection of 5-HT into the cisterna magna produced hyperthermia (Canal and Ornesi, 1961), but this is unlikely to be due to an action on the anterior hypothalamus. Possibly, there was leakage into the peripheral circulation (peripherally administered 5-HT produces hyperthermia in rabbits) or the action was exerted at some lower brain stem site (Feldberg, 1968). A more difficult problem is posed by the study of Ruckebusch et al. (1965). These workers observed that the intraventricular injections of E lowers body temperature of rabbits during pyrogen-induced hyperthermia. It is evident that further study of the thermoregulatory effects of biogenic amines in the rabbit is necessary.

Bligh (1966b) has reported that the sheep responds to biogenic amines in a manner similar to the rabbit. Intraventricular E or NE
induced a rise in temperature accompanied by vasoconstriction of the ears and a reduction in respiratory rate. However, no shivering was observed. This suggests that acute nonshivering thermogenesis may have been activated. 5-Hydroxytryptamine most frequently caused a fall in body temperature, but occasionally no effect or a rise was observed. However, the latter effect occurs only when the animal struggles during the injection procedure.

The ox presents yet another pattern of response. Intraventricular NE in oxen kept at an ambient temperature of 30°C produced no effect. However, at -1°C, the same dose of NE or E produced a 1°C decrease in rectal temperature accompanied by sedation and cessation of shivering. There was no effect on cutaneous vasomotor tone or evaporative heat loss. 5-Hydroxytryptamine administered intraventricularly to oxen kept at -1°C produced no effect, but at 15-30°C, 5-HT elicited a fall in rectal temperature, decreased oxygen consumption, increased respiratory rate and increased evaporative heat loss (Findlay and Thompson, 1968).

These experiments in oxen raise a point that requires further comment, i.e., the importance of ambient temperature in investigations of drug-induced changes in thermoregulation. Most of the experiments concerning the relation of amines to temperature regulation have been performed at ambient temperatures of 20-25°C, primarily because this is the preferred temperature range for the clothed investigator. However, this range includes or encroaches upon the thermoneutral zone of many species. The zone of thermoneutrality is the temperature range within which body temperature is maintained at normal levels with minimal
metabolic effort, usually by changes in cutaneous vasomotor tone. Treatments which cause inhibition of thermoregulatory responses such as shivering, sweating or panting but cause no change in cutaneous vasomotor tone are unlikely to produce any detectable change in the body temperature of animals kept in thermoneutral environments. In large species, because of their great thermal inertia, even alterations in vasomotor tone are not likely to have much effect on core temperature. In this connection, if NE had been tested only at thermoneutrality in the oxen experiments just discussed, it would have been dismissed as being inactive because, although it apparently reduces shivering, at thermoneutrality, there is no shivering to reduce. To detect inhibition of a particular thermoregulatory mechanism, it is obviously necessary to perform the experiments at an ambient temperature which will elicit the thermoregulatory mechanism in question.

On the other hand, agents which stimulate heat gain or heat loss mechanisms will produce readily detectable effects, even in a thermoneutral environment. Not only will panting, shivering or sweating cause changes in core temperature, but these phenomena themselves can be observed and quantified.

Another illustration of the importance of ambient temperature is provided by the accentuated hypothermic effect of NE in cat and 5-HT in rabbit during pyrogen-induced fever. Fever is considered by most workers to be an upward adjustment of the thermoregulatory "setpoint." Thus, an ambient temperature which is thermoneutral for the normal animal may well be below the zone of vasomotor control for the fevered animal. Agents which reduce vasomotor tone will now induce accentuated
decreases in rectal temperature and agents which reduce shivering will produce at least detectable effects.

Returning now to our review of species differences in the responses to intracerebrally administered biogenic amines, Andersson et al. (1956) reported that, in a thermoneutral environment, goats respond in exactly the same manner as oxen, i.e., NE has no effect and 5-HT produces hypothermia. In view of the preceding discussion, it seems imperative to test the effect of NE in animals kept at a cold ambient temperature before concluding that it has no effect on temperature regulation in the goat.

Another species which responds somewhat similarly to the ox is the mouse. Intraventricular NE or 5-HT both cause hypothermia (Brittain and Handley, 1967). However, in contradistinction to the ox, the hypothermic effect of NE is observed at ambient temperatures of 20-22°C. This may reflect a true species difference in the mechanism of response to NE. On the other hand, the high thermoneutral temperature (30°C) and low thermal inertia of the mouse would make any deficit in temperature regulation at 20°C much more readily detectible than in the ox. Epinephrine produces a short rise which precedes a decrease similar to that elicited by NE. Since isoproterenol, a beta receptor stimulant, produces a rise in temperature only, the initial increase produced by E may be due to its considerable ability to stimulate beta receptors. Norepinephrine, which elicits no initial rise, has little beta receptor stimulating activity.

Schmidt and Fahse (1964) demonstrated that intracerebral injection of NE or 5-HT produces hypothermia in the rat. The effect of 5-HT has
been confirmed by others (Feldberg and Lotti, 1967a; Myers and Yaksh, 1968; Reid et al., 1968). However, Feldberg and Lotti (1967a) and Lomax et al. (1968) found that at lower doses of NE or E, the fall was reduced in magnitude and was followed by a rise, whereas Myers and Yaksh (1968) saw only a rise, even at high doses. The latter workers suggested that strain differences or differences in degree of restraint may account for these divergent observations.

The chicken has also been examined for its response to centrally administered CA. Alpha-methyl-NE or NE, injected into the hypothalamus, was found to reduce body temperature and oxygen consumption and to produce sedation accompanied by the electroencephalographic signs of paradoxical sleep. The same effects were produced by intravenous infusion of NE or E in young chicks, which do not possess an efficient blood-brain barrier. Older chickens, in which the barrier had developed, did not respond to the intravenous infusions (Allen and Marley, 1967; Marley and Stephenson, 1969).

Although the species differences which have been described are numerous and confusing, the data support the concept of Feldberg and Myers in its most general form, i.e., that 5-HT and CA are involved in the central regulation of body temperature; every species examined (with the possible exception of the goat) responds to centrally administered CA and 5-HT with alterations in body temperature.

However, because of the high concentrations of the monoamines that must be employed to produce an effect, it could be argued that these effects may well be only pharmacological curiosities and may have no relation to the physiological functioning of the central thermoregulatory
apparatus. Cooper et al. (1965) have pointed out that such effects might be elicited by non-specific depolarization or hyperpolarization of neurons in or fibers passing through the hypothalamus. Indeed, KCl, which causes prolonged depolarization of neurons, produces profound hyperthermia when injected into the AH/PO region of the rabbit. On the other hand, the necessity for high concentrations could well be explained by rapid removal of the drug by diffusion away from the site of injection, uptake into capillaries and neurons, and extracellular binding and metabolism by catechol-O-methyltransferase; by the possible necessity that the amine must reach a small number of widely dispersed receptor sites; and by the possibility that some form of synaptic barrier prevents ready access of the drug to postsynaptic receptors. Still, Gagnon and Mélville (1966) were able to elicit cardiovascular responses in the cat by injection of as little as 10 nanograms of NE into the cerebral ventricle. In comparison, the smallest dose reported to produce an effect on temperature regulation was 0.5 micrograms, which induced a 0.5°C decrease in the rectal temperature of pyrexic cats when injected directly into the anterior hypothalamus (Feldberg and Myers, 1965). Of course, there may be differences in the sensitivity of the various noradrenergic systems in the brain.

Because of these difficulties, other technics have been devised to demonstrate the participation of the CA and 5-HT in central thermo-regulatory control. Probably the most successful was the ingenious experiment of Myers and Sharpe (1968). Perfusate was collected from the anterior hypothalamus of a donor monkey subjected to a cold environment. When the perfusate was transfused into the anterior hypothalamus
of a recipient monkey kept at room temperature, the recipient began
to shiver almost immediately, and rectal temperature rose rapidly for
20-40 minutes. Conversely, when the donor monkey had been subjected
to a warm environment, the recipient experienced a fall in body tempera­
ture followed by an overshoot above the initial level. The perfusates
from heated or cooled donor monkeys have been analyzed in an effort to
determine what substances are responsible for the respective decrease
and increase in the body temperature of the recipient monkey (Myers,
Kawa and Beleslin, 1969). Perfusates from cooled donors contain a 2 to
15 fold increase in 5-HT over resting levels. It was not possible to
detect NE in any of the perfusates, probably due to its rapid reuptake
into presynaptic neurons. Although an increased level of nonesterified
fatty acid, possibly released by NE, was found in the perfusate from
warmed donors, it is difficult to understand how NE could have affected
the recipient if no NE were in the perfusate. Perhaps a more sensitive
assay is needed.1

It is of interest that, although the donor monkeys had been sub­
jected to a hot or cold environment, they had not yet undergone any
change in rectal temperature when the perfusates were taken for analysis.

1 Stein and Wise (1969) were able to detect NE in similar perfusates.
In their experiment, rats were injected intraventricularly with radio­
active NE. The medial forebrain bundle, which contains many ascend­
ing noradrenergic neurons, was stimulated at the caudal brainstem
level and aliquots of perfusate from the anterior hypothalamus were
assayed by both chemical and radiometric technics. There was an in­
creased release of radioactive substances during stimulation of the
MFB, but 95% of the total radioactivity collected during these periods
was accounted for by physiologically inactive metabolites of NE. In
fact, the relative proportion of metabolites to NE increased during
stimulation. The authors suggested that preferential tissue binding
of NE accounts for the small amount of NE in the perfusate.
Thus, the release of these substances was not related to any change in the donor's body temperature and probably reflected the increased neuronal activity involved in activation of heat gain or loss mechanisms. These experiments provide strong evidence that specific neurotransmitters are involved in the control of thermoregulation in the monkey and that 5-HT activates heat gain mechanisms. The evidence that NE is the substance which activates heat loss mechanisms is somewhat less substantial.

In another rather convincing experiment, Banerjee et al. (1968b) demonstrated that endogenous hypothalamic monoamines released by reserpine produce changes in body temperature. In unanesthetized rabbits, intraventricular reserpine produces an increase in body temperature which, however, did not appear when the same animal was treated with reserpine on subsequent days. Since reserpine is known to release hypothalamic NE, and intrahypothalamic injection of NE produces a rise in body temperature in the rabbit, the rise produced by the first dose of reserpine can tentatively be attributed to released NE. Failure of the effect to appear upon subsequent injections strengthens this contention, since repletion of CA after reserpine does not begin for at least 24 hours. Reserpine also releases cerebral 5-HT, but in the rabbit, 5-HT has a weak and inconsistent hypothermic effect. Thus, the CA effect could be expected to predominate. In the cat, reserpine produced an initial fall in rectal temperature followed by an increase. The falling phase disappeared after repeated injection of reserpine, but the rise was sometimes still obtained. The biphasic effect could be attributed to reserpine-induced release of both NE and 5-HT which, in the cat, produce hypothermia and hyperthermia, respectively.
Failure of the rising phase to disappear after repeated reserpine treatments may be explained by more rapid repletion of 5-HT. Another possibility is that the rising phase may have been a direct effect of reserpine and the effect of released 5-HT may have been obscured by the hypo-thermic effect of NE. In both cats and rabbits, when smaller doses of reserpine were given on several consecutive days, the thermoregulatory effects did not disappear after the first dose, probably because of incomplete depletion of amine stores. In addition, even after repeated administration of the larger doses of reserpine had produced loss of the temperature rise in rabbits and the temperature fall in cats, intraventricular injection of NE and 5-HT still elicited their typical effects on body temperature in both of these species. Thus, the effect of reserpine was not to depress the sensitivity of the hypothalamus to the biogenic amines.

Further evidence that the effects of CA and 5-HT on thermoregulation are physiological rather than pharmacological is provided by Brittain and Handley (1967). In the mouse, intraventricular injection of NE or dopamine led to a fall in body temperature. Epinephrine produced a small rise followed by a fall, and isoproterenol yielded a rise only. The effect of NE was blocked by alpha- but not beta-receptor antagonists. These results strongly suggest alpha-receptor selectivity for the CA responses produced by intraventricular injections. Such receptor selectivity would be unlikely if the CA were acting in a non-specific manner. Possible receptor selectivity has also been reported in young chicks (Allen and Marley, 1967). The hypothermic effect of alpha-methyl-NE was blocked by phenoxybenzamine, an alpha-receptor
antagonist. In addition, the hyperthermic effect of alpha-methyltryptamine (which probably acts on the same receptors as 5-HT) was blocked by the 5-HT antagonist methysergide.

If central adrenergic or serotonergic neurons are involved in the regulation of heat loss and gain, then exposure to thermal stress should increase the activity of the neurons subserving these functions. Such increased activity may or may not be reflected as changes in brain amine levels, but amine turnover rate should be altered. Corrodi et al. (1967) measured the rate of depletion of whole brain 5-HT, NE and DA after blockade of synthesis of these substances. The experiments were performed using rats exposed to environmental temperatures of 40°C or 3°C. At 40°C, the rate of depletion of 5-HT and NE increased; at 3°C, 5-HT depletion was increased, but there was no effect on NE. Dopamine depletion was not affected at either ambient temperature. The authors concluded that 5-HT and NE, but not DA, are involved in the central control of thermoregulation in the rat. Reid et al. (1968), measuring NE and 5-HT turnover rates, obtained results similar to those of Corrodi and co-workers, with the exception that they found exposure to 40°C increased NE turnover, whereas the latter group had seen no effect at this temperature. Aghajanian and Weiss (1968) confirmed the increased activity of serotonergic neurons in rats exposed to a 40°C environment. Furthermore, since most of the 5-HT in the hypothalamus derives from terminals of neurons whose cell bodies lie in the raphé nuclei, and since LSD is known to inhibit completely the firing of these neurons, they reasoned that LSD could be expected to prevent the increased 5-HT turnover induced by high environmental temperatures. This was found to
be the case. LSD, given peripherally, prevented any change in 5-HT turnover and increased the hyperthermia experienced by heat stressed rats. The latter effect, however, could have been due to a peripheral action of the drug.

Investigations such as the three cited above, in which whole-hypothalamic or even whole-brain turnover rates are measured, have been justly criticized on the grounds that these rates are not necessarily representative of turnover rates in discrete brain regions, such as the preoptic region or anterior hypothalamus (Myers, 1969). However, Simmonds (1969) recently determined the individual NE turnover rates in the preoptic region, anterior hypothalamus and posterior hypothalamus of rats exposed to moderate conditions of warming and cooling (9°C and 32°C) or to near-thermoneutral temperatures (17°C and 24°C). In rats exposed to the 9°C and 32°C environments, NE turnover rates increased in the anterior and posterior hypothalamus but not in the preoptic region. Exposure to the near-thermoneutral temperatures had no effect on NE turnover rates.

Making allowances for variations in technic, the four studies discussed above are amazingly consistent in their conclusions. Three of these investigations, as well as all microinjection data (see above) indicate that, in the rat, 5-HT activates heat loss mechanisms. NE, on the other hand, produces both hypo- and hyperthermic effects when administered centrally; the work of Reid et al. and Simmonds supports the possibility of this dual role for NE.

Experiments with centrally administered monoamine oxidase (MAO) inhibitors, used to increase hypothalamic amine concentration by
inhibiting amine degradation, have yielded results in reasonable accord with the concepts of Feldberg and Myers. In anesthetized dog (Feldberg et al., 1967) and unanesthetized cat (Feldberg and Lotti, 1967b), the MAO inhibitor, tranylcypromine produced shivering and hyperthermia when given intracerebroventricularly. Since, in these species, tranylcypromine does not greatly increase brain NE but does increase 5-HT levels, the hyperthermia was attributed to an excess of 5-HT. This is in agreement with microinjection data (see above) demonstrating that 5-HT, given centrally, is hyperthermic in cats and dogs.

Intraventricularly administered tranylcypromine had no effect on the body temperature of the rabbit (Feldberg and Lotti, 1967b). In this species, MAO inhibitors increase both 5-HT and NE, but the effect upon 5-HT is more pronounced. Since, in the rabbit, intraventricularly administered NE is strongly hyperthermic and 5-HT weakly hypothermic, a large increase in 5-HT and a smaller increase in NE might be expected to produce little or no effect on body temperature.

In rat also, MAO inhibition greatly increases 5-HT concentration and augments NE levels to a lesser extent. Intraventricular injection of tranylcypromine produced hypothermia in the rat (Feldberg and Lotti, 1967a). 5-Hydroxytryptamine and NE both produce hypothermia when administered by the same route, so that the fall in body temperature induced by tranylcypromine may have been due to increased concentration of either or both of these substances.

The ox also experiences hypothermia when treated with intraventricularly injected tranylcypromine at an ambient temperature of 18-20°C (Findley and Thompson, 1968). It will be remembered that the intraventricular administration of NE had no effect at this ambient temperature,
but 5-HT produced hypothermia. Thus, it appears that tranylcypromine may have increased brain 5-HT. Unfortunately, there are no data available concerning the effect of MAO inhibition on brain amine levels in the ox.

Brain amine concentrations can also be augmented by supplying increased amounts of precursor. Low concentrations of 5-hydroxytryptophan (5-HTP), the immediate precursor of 5-HT, have been perfused through the third ventricle of anesthetized cats (El Howary and Feldberg, 1966), usually resulting in increased 5-HT content of the effluent, shivering, tachypnea, and increased body temperature. On some occasions the response was a small rise followed by a fall of varying magnitude, followed, in turn, by a pronounced rise. A similar triphasic effect was seen when larger quantities of 5-HTP were injected into the lateral ventricle. It was suggested that the falling phase, which decreases in magnitude as the dose decreases, was produced by a direct depressant effect of 5-HTP, while the rising phases were thought to be due to the action of 5-HT. However, such triphasic effects are often seen when 5-HT alone is injected into the lateral ventricle of cats. The supposition that 5-HTP depression participates in this effect is therefore not necessary. Selective perfusion of the anterior or inferior horn of the lateral ventricle yielded a smaller increase of 5-HT concentration in the effluent then was obtained when the third ventricle was perfused, and there was no effect on rectal temperature.

Intraventricular injection of 5-HTP in a monkey anesthetized with pentobarbital produced only a rise in temperature, the same effect that 5-HT produced when administered by this route (Feldberg et al., 1967).
Not all investigators agree that the biogenic amines participate in hypothalamic control of thermoregulation. Cranston and Rosendorff (1962), for example, reasoned that inhibition of MAO in the rabbit should affect the animal's response to thermal loads even though there might be no change in resting body temperature. It was felt that if such a load increases the activity of serotonergic and noradrenergic neurons, then MAO inhibition should increase the amount of amine release, producing an alteration in response to this load in comparison to an untreated animal. Since they found no difference in the responses of normal and tranylcyromine treated animals, it was concluded that the amines may not play a role in temperature regulation in the rabbit. However, if reuptake into the presynaptic neuron is not blocked, MAO inhibition would not necessarily increase the amount of amine released at the synapse as a function of the firing rate of the presynaptic neuron. Both noradrenergic and serotonergic neurons are capable of storing large quantities of amines after MAO inhibition as long as uptake is not impeded. Any increased stimulation of postsynaptic receptors will be dependent upon 1) the rate of leakage from the cell body and axon and the amount of this which diffuses to the synaptic area and 2) the rate of leakage at the synapse itself. Neither of these factors will necessarily be dependent upon the activity of the presynaptic neuron. Thus, these data cannot be taken as a valid argument against the participation of NE and 5-HT in the central control of temperature regulation in the rabbit.

Other contradictory data are provided by Sheard and Aghajanian (1967). These workers found that electrical stimulation of the raphé nuclei of rats produces release of 5-HT in the forebrain and an increase in body
temperature. Not only do these data disagree with the proposed hypo-
thermic action of 5-HT in rats but they also contradict another experi-
ment performed by these same authors in which LSD-induced reduction of
firing of raphé units impeded heat loss. The contradiction remains un-
resolved, but it should be noted that peripherally administered LSD un-
doubtedly acts at many sites other than the raphé nuclei and that
electrical stimulation of these nuclei probably activates many extra-
hypothalamic as well as hypothalamic serotonergic terminals.

The single unit recording technic has furnished criticism of another
sort (Cunningham et al., 1967). Discharges from single neurons in the
preoptic and septal areas of the dog were recorded before and after
intraventricular injection of 5-HT and E. 5-Hydroxytryptamine depressed
firing rates of both temperature sensitive and temperature insensitive
units. Epinephrine usually depressed temperature sensitive units whereas
the effect on temperature insensitive units was variable. It was con-
cluded that the data do not support the antagonistic role proposed for
5-HT and the CA in the maintenance of body temperature in the dog. How-
ever, whether or not 5-HT and the CA have physiological functions in the
control of body temperature, they patently do have antagonistic effects,
at least on the pharmacologic level. The failure of single unit studies
to substantiate data derived from stimulation of a large population of
neurons can only reflect insufficient or inappropriate sampling of in-
dividual units.

A final comment concerning the physiological role of 5-HT and NE
in the control of temperature regulation, assuming such a role exists,
is necessary. Although the hypothalamus contains high concentrations of
monoamines, the histochemical fluorescence technic has demonstrated that very few NE-containing and no 5-HT-containing cell bodies are found in the hypothalamus (Dahlström and Fuxe, 1964). The few NE-containing cells found there are not associated with the AH/PO region. It appears that hypothalamic monoamines derive from nerve terminals which arise from cell bodies located in the lower brain stem. Thus, physiologically, 5-HT and NE release are associated with ascending influences. This immediately suggests that the thermosensitive cells of the AH/PO region do not release either of these substances as their neurotransmitter and that none of the interneurons in the hypothalamic thermoregulatory system are aminergic.

What, then, is the function of the biogenic amines in temperature regulation? One possibility is that these ascending aminergic systems provide the hypothalamic thermoregulatory system with thermal information from the periphery. The previously cited experiment of Myers and Yaksh, in which hypothalamic perfusate from a heated or cooled donor monkey that had not yet undergone a change in body temperature induced appropriate counterthermoregulation in a recipient monkey kept in a neutral environment supports this possibility. Recently, however, many of the brainstem amine-containing cells have been implicated in the control of sleep and wakefulness (see review of Jouvet, 1969). Since temperature regulation is profoundly affected by such alterations in attentional state, it seems possible that NE and 5-HT may mediate these changes when released from the terminals of the ascending aminergic systems.
In summary, 5-HT and the catecholamines, NE and E, have been shown to alter body temperature in a number of species when administered directly into the AH/PO region or into the cerebral ventricles. However, species differences are great, and even in a given species, there is disagreement concerning the effects of these biogenic amines. In chicken and mouse, receptor selectivity has been demonstrated, indicating that in these species the amine effects are probably not due to non-specific hyperpolarization or depolarization. Alteration of hypothalamic amine concentrations by treatment with precursors, MAO inhibitors or depleting agents also influences temperature regulation. Exposure to high or low environmental temperatures alters hypothalamic amine turnover rate. Perfusate from the hypothalamus of cooled or warmed donor monkeys contains substances which activate thermoregulatory mechanisms in recipient monkeys kept in a neutral environment. There is little information concerning the mechanisms by which 5-HT and NE influence hypothalamic control of temperature regulation; the absence of amine-containing cell bodies in the hypothalamus suggests that these substances mediate some ascending influence on the hypothalamus. In general, the totality of the evidence strongly implicates 5-HT and NE in the physiological regulation of body temperature by the hypothalamus. However, in this writer's opinion, such a role has not been unequivocally established.

Acetylcholine

Although NE and 5-HT may be involved in hypothalamic control of thermoregulation, there is evidence that cholinergic synapses also participate in this function. The first indication of this was provided in 1931 by the neurosurgeon Harvey Cushing. Cushing injected the
cholinergic stimulant, pilocarpine, into the lateral ventricle of man and observed sweating, decreased metabolic rate and a fall in body temperature (Cushing, 1931a). These effects could be antagonized by pre-treatment with atropine (Cushing, 1931b). A few years later, Henderson and Wilson (1936) observed that acetylcholine (ACh) or the anticholinesterase, physostigmine (eserine), also produced hypothermia when administered intraventricularly. Since physostigmine has no direct cholinergic action, there was good evidence, even in 1936, that accumulation of endogenously occurring cerebral ACh can markedly affect thermoregulation.

Recently, central cholinergic stimulation has been shown to produce similar effects in rats. Injection of oxotremorine or carbamylcholine (CCh) (both potent muscarinic stimulants) into the AH/PO region induced profound hypothermia. This effect could be prevented or reversed by intrahypothalamic or intraperitoneal injection of muscarinic blockers having a tertiary amine structure. Antagonists having a quaternary structure, which cannot cross the blood-brain barrier, blocked the hypothermia only when given by intrahypothalamic injection. Intrahypothalamic injection of the muscarinic antagonists alone produced hyperthermia. It was thus speculated that ACh may participate in the activation of heat loss mechanisms; blockade of these synapses could tip the balance in favor of heat gain (Lomax and Jenden, 1966; Kirkpatrick et al., 1967a, 1967b). Acetylcholine also produces hypothermia in rats when applied to the AH/PO region either by a new gel iontophoresis technic (Lomax, 1969) or by microinjection (Beckman and Carlisle, 1969). The latter authors also demonstrated that intrahypothalamic ACh
depresses the rate that rats kept in a cold environment will press a lever to obtain bursts of infrared heat. Thus, both physiological and behavioral thermoregulation may be affected by cholinergic stimulation within the AH/PO region of rats.

Intraventricular injection of ACh and/or eserine has yielded less consistent results (Myers and Yaksh, 1968). Low doses of ACh or eserine produced either no effect or moderate hypothermia. Eserine-ACh mixtures or higher doses of ACh or eserine alone caused hyperthermia, sometimes preceded by a short decrease in temperature. This study is not strictly comparable to those of Lomax and co-workers or that of Beckman and Carlisle. The latter workers maintained their animals in an environment of about -5°C and the rats were completely shorn of fur; under such conditions, any hyperthermic effect is unlikely to appear. Myers and Yaksh' rats were unrestrained and kept at room temperature, whereas in the experiments of Lomax and co-workers, although the animals were also kept at room temperature, they were lightly restrained. Further, the Lomax group administered the ACh by iontophoresis rather than by micro-injection.

In view of these differences in technic, it is difficult to decide whether these variables or the route of injection (intrahypothalamic versus intraventricular) is responsible for the disparate results observed. However, in the absence of further information, it seems reasonable to conclude tentatively that the effect of ACh upon the AH/PO region of the rat is to initiate a fall in body temperature. This conclusion stems from three considerations: 1) the iontophoresic delivery used by Lomax assures a steady, slow application of ACh to discrete areas within
the AH/PO region 2) Lomax and co-workers have never observed a secondary hyperthermia when oxotremorine or CCh were microinjected into the AH/PO region 3) Myers and Yaksh frequently saw a fall in body temperature which preceded the rising phase. The most logical explanation of the secondary rise seen by Myers and Yaksh is that it is due to action at some other paraventricular site, perhaps in the lower brain stem. However, it is also possible that the higher doses of ACh or eserine produced, after an initial period of stimulation, persistent depolarization of the synapses mediating heat loss.

Although cholinergic agents have not been injected directly into the brains of mice, tremorine and pilocarpine, muscarinic agonists which can cross the blood brain barrier, elicit hypothermia in this species. Muscarinic antagonists capable of entering the central nervous system prevent this effect, whereas antagonists incapable of crossing the blood-brain barrier do not (Spencer, 1965; Zetler, 1968; Friedman and Jaffe, 1969). Thus, it appears that there may be cholinergic mechanisms involved in the central regulation of body temperature in the mouse as well as in the rat. However, because of the peripheral route of administration of the cholinergic agents, one cannot be sure what area of the central nervous system was affected.

Injection of ACh or ACh in combination with eserine into the AH/PO region of the rabbit had no thermoregulatory consequences (Cooper et al., 1965). However, Borison and Clark (1967) cite older literature (no reference given) as demonstrating that intracisternal administration of pilocarpine caused shivering and hyperthermia in the rabbit.
The ox also seems to be insensitive to central cholinergic stimulation; 2 milligrams of ACh perfused through the cerebral ventricles did not alter body temperature (Findley and Thompson, 1968).

Peripherally administered tremorine produces an increase in body temperature in the cat (Everett, 1964), but intraventricular injection of ACh, eserine or CCh have no effect (Feldberg and Sherwood, 1954; Connor et al., 1966a). However, Connor et al. (1966a, 1966b, 1967) found that injection of ACh, CCh and other choline esters into the caudate nucleus produced a hyperthermia of 1.2-1.4°C which was temporally correlated with the occurrence of "tremor." Sites other than the caudate (red nucleus, anterior hypothalamus, midbrain reticular formation, hippocampus) were unresponsive. The tremor could be blocked by intraperitoneal injection of low doses of atropine or pentobarbital and by intracaudate injection of scopolamine, atropine, benztropine and procaine, but not by hexamethonium, tetraethylammonium, decamethonium, erythroidine or tubocurarine. It is also blocked by ipsilateral or contralateral intracaudate injection of beta-receptor adrenergic agonists such as E or isoproterenol. Thus, the tremorogenic response is muscarinic and is inhibited by a crossed and uncrossed adrenergic system of the beta-receptor type. Other workers have found, however, that the caudate is not the only site at which cholinergic stimulation induces tremor. Tremor of various forms, intensities and durations have been elicited by microinjection of oxotremorine or CCh into the brainstem tegmentum, preoptic area, anterior hypothalamus, diagonal band of Broca, septal area, anterior thalamus, posterior thalamus, mesencephalic reticular formation and pontine reticular formation (George et al., 1964, 1966;
The relationship between this cholinergic "tremor" and temperature regulation is not clear. The above workers have considered the tremor to be an extrapyramidal reaction akin to parkinsoniac tremor, whereas Myers and Yaksh (see below), who observed a similar phenomenon in the monkey, considered it to be shivering. At least in the cat, there is some evidence that cholinergic muscarinic synapses may not be involved in the control of shivering. Atropine sulfate, 5 mg/kg intraperitoneally, does not reduce physiological shivering in the cat (Stuart et al., 1961). On the other hand, the tremor evoked by injection of cholinergic agents at the several cerebral loci previously mentioned is readily blocked by intracerebral injection of atropine. In addition, the tremor which follows cholinergic stimulation of the caudate is blocked by small doses of atropine given intraperitoneally (George et al., 1964, 1966; Yng-She et al., 1968). Although these data indicate that ACh may not be involved in the control of physiological shivering, they do not necessarily prove that cholinergic tremor is not shivering; it is quite possible that the muscarinic stimulants used could be acting upon neurons which are physiologically excited or inhibited during shivering by another neurotransmitter or transmitters. Sensitivity of neurons to more than one neurohumor has been observed frequently. Bloom et al. (1963), for example, found that many neurons in the cat hypothalamus respond to both ACh and NE or 5-HT. The tremor-shivering problem might be resolved by a detailed electromyographic analysis of normal shivering and the tremor induced by central cholinergic stimulation.
The effect of hypothalamic cholinergic stimulation in the monkey has recently been examined by Myers and Yaksh (1969). Microinjection of ACh, ACh-esterine mixtures, or CCh into the hypothalamus of unanesthetized, restrained rhesus monkeys usually resulted in vigorous shivering and an immediate and precipitous rise in body temperature. Points reacting in this manner were scattered throughout the hypothalamus; the posterior hypothalamus being most sensitive. The latency of the response was as short as 30 seconds, making it very unlikely that the drug could have diffused into the ventricle and then to a more distal site of action. In view of the tremorogenic action of hypothalamic cholinergic stimulation in the cat, there is some question whether the "shivering" seen was equivalent to thermoregulatory shivering. Although this question remains to be investigated, the observation that intense vasoconstriction occurred simultaneously with the shivering favors the probability that the response was indeed a generalized stimulation of heat gain and heat retention mechanisms.

Although hyperthermia was the most frequent response, in one circumscribed area lying adjacent to the mammillary body and ventral to the mammillary fasciculus princeps at the midbrain-hypothalamic junction, cholinergic substances induced hypothermia. The authors speculated that both heat gain and heat loss pathways had cholinergic synapses, the heat gain pathway beginning in the AH/PO region and descending through the hypothalamus in multisynaptic fashion, and the heat loss pathway beginning in the posterior hypothalamus.

In summary, although there is a paucity of data concerning the cholinergic involvement in hypothalamic regulation of body temperature,
it appears that cholinergic mechanisms may play a role in mouse, rat, cat and monkey, but not in dog, rabbit and ox. With the exception of rat and monkey, the data are weak and further investigation is required. In monkey, cholinergic stimulation of the hypothalamus can induce both hyperthermia and hypothermia, depending on the site of stimulation. There are indications that the same may be true in rat. There is a need to determine whether the rhythmic movements induced in cat and monkey by central cholinergic stimulation are identical to physiological shivering or represent a tremor unrelated to temperature regulation.

**The Effect of Chlorpromazine on Thermoregulation**

The thermoregulation-disrupting properties of the potent tranquilizing agent, chlorpromazine (CPZ), have been of particular interest to the writer. The hypothermic effect of CPZ was noted by Courvoisier et al. (1953) in their comprehensive report of the pharmacology of this drug and has since been found to occur in numerous species including man, horse, dog, rabbit, hamster, guinea pig, ground squirrel, rat, mouse and pigeon (Hoffman and Zarrow, 1958; Jacobsen, 1960).

CPZ-induced hypothermia has been attributed to both increased heat loss and decreased heat production. The ability of CPZ and related phenothiazine derivatives to augment heat loss by inducing peripheral vasodilation is well documented (Courvoisier et al., 1953--rat; Chevillard and Geono, 1956--guinea pig; Dobkin et al., 1956--dog; LeBlanc, 1958b--rat; Richter, 1964--mouse; Kollias and Bullard, 1968--rat). Although LeBlanc (1958b) observed moderate vasodilation in the hindpaw of the CPZ-treated rat, he concluded that this could not entirely explain the hypothermic effect. He reported that the treated animals
no longer huddled in a cool environment and suggested that this effect, rather than vasodilation, was responsible for the hypothermia. However, LeBlanc failed to measure tail skin temperature, and Kollias and Bullard (1968) have shown that CPZ greatly increases heat loss in the rat by increasing tail blood flow. They also demonstrated that lack of huddling is of little thermoregulatory consequence at the mildly cool ambient temperatures in which LeBlanc's experiments were conducted.

Somewhat more controversial is the ability of CPZ to decrease heat production. Several investigators have reported that CPZ prevents or reduces cold-induced shivering (Dundee et al., 1954—dog; Moyer et al., 1957—dog; Mueller and McDonald, 1966—rat). Johnson et al. (1963) and Lettau et al. (1964), measuring oxygen consumption in rats, found that CPZ, given peripherally, greatly reduced the increase in metabolic rate experienced by warm-acclimated rats placed in a cold environment. This indicates that CPZ had markedly reduced shivering, since warm-acclimated rats depend primarily upon shivering rather than non-shivering thermogenesis when exposed to cold. In addition, both investigations revealed that CPZ reduces basal metabolic rate by about 20 percent, an observation earlier reported by Courvoisier et al. (1953). On the other hand, Jackson et al. (1959) reported that a "maximal" shivering response was observed in unanesthetized dogs treated with as much as 70 mg/kg of CPZ. Chatonnet and Tanche (1955) found that 5 mg/kg of CPZ administered intravenously had little effect on oxygen consumption in conscious dogs subjected to severe cold stress. Giaja and Markovic-Giaja (1954) stated that CPZ had no effect on the basal metabolic rate of rats and that the reduction in oxygen consumption seen in cold-exposed CPZ-treated rats
was a consequence rather than a cause of the concomitant hypothermia. Kollias and Bullard (1968) found no change in basal metabolic rate in CPZ-treated rats, and Dandiya et al. (1960) saw only a slight reduction. Popovic (1954) found that rats treated with a combination of dinitrophenol (which increases metabolic rate by uncoupling oxidative phosphorylation) and CPZ experienced hypothermia at an ambient temperature of 20°C even though metabolic rate was greatly increased. It was concluded that a reduction in heat production cannot be responsible for CPZ-induced hypothermia.

In general, the evidence seems to indicate that, in rats, CPZ produces a slight reduction in metabolic rate at neutral ambient temperatures ("basal" metabolic rate). On the other hand, it is not entirely clear whether CPZ reduces cold-induced shivering in this species. To be sure, several studies have shown that the expected increase in oxygen consumption in cold-exposed rats is severely reduced after CPZ treatment. But, as has been pointed out, this could be a consequence rather than a cause of a decreased body temperature brought about by some other action of CPZ (such as vasodilation). However, there are two considerations that make this unlikely: 1) rats experiencing a fall in body temperature due to some mechanism other than blockade of shivering have been observed to shiver vigorously until their body temperature reached lethal levels (Leduc, 1961) 2) Mueller and McDonald (1966) observed visually that shivering was depressed after CPZ treatment. Thus, the evidence, meager as it is, indicates that CPZ does reduce shivering in cold-exposed rats. In dogs, the two experiments suggesting that CPZ reduced shivering were performed on anesthetized animals, whereas the
two reporting negative results were performed on conscious animals. Possibly, anesthesia facilitates the anti-shivering effect of CPZ in this species. Unfortunately, the effect of CPZ on heat production in other species has not been examined.

The possibility that CPZ may impair nonshivering thermogenesis (NST) as well as shivering thermogenesis has not been entertained, but cannot be dismissed out of hand. Nonshivering thermogenesis is actually an inappropriate term for the phenomenon with which it is currently associated. In current usage, any cold-induced metabolic heat production above the basal level which cannot be attributed to tonic or phasic contraction of skeletal muscle is considered to be NST. However, another efficacious source of heat production is "thermoregulatory muscle tonus", which consists of very low amplitude, high frequency fasciculations of individual muscle fibers (Ivanov, 1963). This tonic activity is certainly "nonshivering" but is not classed as an NST mechanism.

NST is not a unitary phenomenon confined to a single organ. Nor is it necessarily the same phenomenon in young versus old, and warm-acclimated versus cold-acclimated animals. There are also species differences in the importance and, possibly, the mechanism by which nonshivering heat is generated. There is fair agreement that NE, derived from sympathetic nerve terminals, E, from the adrenal medulla, the adrenal cortical hormones and thyroid hormone are involved in NST, these substances acting in concert to modulate the metabolic activity of many bodily tissues. Exogenous catecholamines increase oxygen consumption in many species, particularly in the new-born and in cold-acclimated animals. The latter maintain body temperature during cold exposure almost entirely by NST and have large amounts of brown adipose tissue.
Thus, a connection has been drawn between NST, brown adipose tissue and catecholamine calorigenesis. Although it has not been conclusively proven, there is good evidence that a major portion of nonshivering heat production is derived from increased lipid oxidation in brown fat, the process being activated by NE released from the dense sympathetic innervation of this tissue. [See Masoro (1966) and Hemingway (1963), for references and excellent discussions of NST.]

Within the present context, the import of the nonshivering thermogenic process lies in the fact that it may be of some importance in adult warm-acclimated animals as well as in neonates and cold-acclimated animals. Further, at least in the rat, activation and termination of NST in warm-acclimated adults has been reported to be just as rapid as shivering thermogenesis. Thus, although thermoregulatory muscle tonus and shivering are the primary methods by which normal rats (and possibly other species) resist hypothermia upon cold exposure, NST, rapidly activated and under direct central nervous control via the sympathetic outflow, may also play a role. (For references see Masoro, 1966; Davis, 1963; Chattonet, 1963; Davis and Mayer, 1955; Leduo, 1961.) A dissenting opinion is offered by Hemingway (1963) who has concluded that NST is of little importance in warm-acclimated animals.

Even if we accept the existance of a nonshivering thermogenic mechanism in warm-acclimated animals, is there any indication that CPZ may interfere with this process? A review of the literature reveals data of an indirect and inconclusive nature which both support and oppose such an action.
On the negative side, LeBlanc (1958a) and Johnson et al. (1963) found that cold-acclimated rats, which depend almost entirely on NST, experience less hypothermia when given CPZ than do warm-acclimated rats, which depend primarily upon shivering thermogenesis. Johnson (1964) demonstrated that CPZ did not decrease the release of NE and E in cold- and warm-acclimated rats kept at 2°C and 20°C respectively, and Popovic (1954) reported that CPZ does not antagonize the calorogenic effect of E in warm-acclimated rats. Weak supportive evidence is provided by the observation that warm-acclimated rats subjected to an ambient temperature of 2°C and treated with phenoxybenzamine, a potent alpha-receptor blocker (as is CPZ), all died rapidly due to hypothermia although they shivered vigorously (Leduc, 1961). An injection of E prevented death and almost abolished the fall in body temperature. Leduc concluded that phenoxybenzamine had interfered with an important nonshivering mechanism for heat production. There is, however, considerable doubt whether the effect of phenoxybenzamine was due to blockade of alpha-receptors (Ellis, 1967; Nickerson and Hollenberg, 1967) and therefore, whether CPZ would also produce this phenomenon.

It is obvious that one cannot come to any conclusion concerning the ability of CPZ to interfere with NST from the data available. Such a decision must await further investigation. However, as previously pointed out, the possibility of such an action cannot be completely ruled out.

Although CPZ consistently produces hypothermia at ambient temperatures lower than thermoneutrality, there are many reports that it and related phenothiazines have little effect on body temperature at
ambient temperatures equal to or slightly above the thermoneutral range (Dandiya et al., 1960—rat; Johnson et al., 1963—rat; Higgins et al., 1964—dog; Bartlett, 1965—mouse; Kollias and Bullard, 1968—rat).

This would indicate that CPZ does not actively lower body temperature, as would be the case if it were lowering the setpoint, but allows a passive fall to occur in a cool environment.

At even higher ambient temperatures, CPZ has been observed to produce hyperthermia. Kollias and Bullard (1968) found that CPZ decreases survival time of rats maintained in a 34°C environment. Wetting of the fur with saliva, an important heat loss mechanism in the rat, was prevented. Resistance hygrometry confirmed that evaporative heat loss was inhibited. At 43°C, CPZ prevented the maximal tail vasodilation observed in control rats. Dandiya et al. (1960) observed that CPZ decreases heat tolerance in rats and mice in a 36°C environment. Chlorpromazine induced hyperthermia in rats performing a conditioned avoidance-escape task at 31°C, whereas at 24°C, hypothermia was evoked (Rudy and Wolf, 1967). On the other hand, Shemano and Nickerson (1958) did not see CPZ-induced hyperthermia unless ambient temperature was greater than 36°C and Bagdon and Mann (1965) claim that CPZ induces hypothermia in mice even at 38°C ambient.

Kollias and Bullard (1968) and Shemano and Nickerson (1958), among others, have concluded that the ability of CPZ to inhibit both heat loss and heat gain mechanisms is indicative of a central action of the drug, possibly at the hypothalamus where thermoregulation is coordinated. However, this speculation is predicated almost entirely upon data obtained from mice and rats. The often ignored data of Decourt et al.
(1953) must be taken into account. These workers found that rabbits, guinea pigs and dogs die in hyperthermia when exposed to temperatures ranging from 39 to 43°C. If CPZ (20 mg/kg) is injected just before rectal temperature reaches lethal levels, body temperature is lowered and death is prevented, at least temporarily. Unfortunately, no attempt to confirm these results has been made.

However, it is not necessary to ignore these data or to limit discussion to rats and mice in order to entertain the idea that some or all of the thermoregulatory disrupting effects of CPZ may be attributable to effects upon the central nervous system. There is evidence other than the fact that it may produce a quasi-poikilothermic state in rats and mice which suggests that the drug has a central action on thermoregulation.

It has been previously pointed out that CPZ reduces shivering in the rat and possibly in the dog. Since shivering is not controlled by the autonomic nervous system, it is difficult to explain this action by the peripheral alpha receptor blocking action of the drug. CPZ directly depresses skeletal muscle fibers, has a curare-like action at the neuromuscular junction, blocks release of ACh at the neuromuscular junction and has local anesthetic activity capable of blocking conduction in gamma efferent and alpha motoneuron fibers. However, these activities are unlikely to be obtained except when the drug is administered by close intra-arterial injection (Su and Lee, 1960). On the other hand, Hudson has discussed the abundant evidence that CPZ exerts a motor depressant effect by acting within the central nervous system (Hudson, 1966, 1968; Hudson and Domino, 1963). Although CPZ does act at the spinal level to modulate descending influences from the brainstem, its
primary effect appears to be exerted at the brainstem reticular formation, where it significantly depresses the mesencephalic facilitory area, and to a lesser extent, the medullary inhibitory area, resulting in a relatively selective depression gamma motor outflow. Euler (1961) believes the effect of CPZ on shivering to be related to this reduction in gamma efferent activity. In addition, this could account for the splayed posture observed in CPZ-treated rats. However, these spinal and bulbar actions do not obviate the possibility that CPZ may also act within the hypothalamus to disrupt shivering and huddling.

The remaining effects of CPZ on thermoregulation—production of vasodilation, possible interference with NST, prevention of piloerection, prevention of salivation and fur wetting—could also be centrally mediated if CPZ has the ability to interfere with central sympathetic outflow. There is evidence supporting this conjecture. Specter et al. (1957) reported that intracisternal injection of CPZ, in amounts too small to be effective peripherally, produced relaxation of the nictitating membrane, miosis and bradycardia, all effects that would appear if sympathetic influence were reduced. Dasgupta and Werner (1954) and Dasgupta et al. (1954) found that intracisternal administration of CPZ in anesthetized monkeys lowered blood pressure and abolished the baroreceptor reflex, whereas the pressor response to intravenously injected E was unaffected. Further, pressor responses elicited by electrical stimulation of hypothalamic or medullary pressor areas, and the sciatic pressor reflex, are specifically abolished in decorticate cats by intravenous doses of CPZ as low as 50 μg/kg. In the normal cat, however, such effects could not be elicited consistently even at a dose of 1 mg/kg. As little as
250 µg/kg given intravenously to decorticate or diencephalic cats completely abolished both the somatic and autonomic signs of sham rage and produced complete loss of muscular tone. Again, however, the same dose had no effect in intact cats.

These experiments demonstrate that CPZ has definite effects on central sympathetic outflow when given intracisternally in normal animals or intravenously to decorticate or diencephalic animals. Unfortunately, they do not answer the question whether these effects are seen when CPZ is administered by a peripheral route in the normal animal. There is, however, direct evidence that CPZ can block sympathetic discharge in the intact animal. Elliott (1967), in etherized cats, recorded and quantitated the rate of discharge in fibers teased out of the preganglionic trunk of the superior cervical ganglion. Intravenous injection of 0.5-1.0 mg/kg of CPZ reduced the average discharge rate by 56 and 74 percent, respectively. The higher dose maintained this suppression for at least 2 hours. Thus, it seems probable that CPZ would severely reduce sympathetic outflow at the relatively high doses necessary to disrupt thermoregulation.

Even though CPZ evidently has the potential to impair thermoregulation by central actions, such impairment could also be attributed partially to peripheral effects of the drug. Vasodilation is almost certainly due in part to the well-known alpha-receptor blocking action of CPZ. In addition, CPZ possesses anticholinergic activity. Salivation is mediated by both the sympathetic and parasympathetic systems. Thus, the alpha adrenergic and cholinergic blocking properties of CPZ could be brought to bear here, resulting in inhibition of thermoregulatory
fur wetting. Further, CPZ has a direct inhibitory effect at the salivary glands (Bradley, 1963) which could intensify the anti-salivation effect. If NST is reduced by CPZ (and this is questionable), this effect could possibly be due to the peripheral alpha adrenergic blocking action of the drug.

As has been previously mentioned, the depressant properties of CPZ exerted at the neuromuscular junction, skeletal muscle fibers or associated nerves cannot be responsible for its ability to suppress shivering. However, there may be another peripheral mechanism involved. Malckel et al. (1967) found that adrenal demedullated, "chemically sympathectomized" rats died rapidly when exposed to low ambient temperatures. These animals did not increase plasma free fatty acids (FFA) or blood glucose levels, nor was shivering or vasoconstriction seen. Pretreatment with E in oil prolonged survival time; FFA and glucose levels were increased and shivering and vasoconstriction were evident. It was concluded that an intact sympathetic nervous system is essential for shivering to appear. Although there is no direct sympathetic innervation of skeletal muscle, the failure to mobilize glucose and FFA could deprive skeletal muscle of the energy substrates necessary for the intense activity involved in shivering thermogenesis. The agents used to produce "chemical sympathectomy" were syrosingopine, which depletes peripheral catecholamines; a bretylium-like agent, which prevents release of NE at sympathetic nerve endings; and chlorisondamine, a ganglionic blocking agent. It is possible that each of these agents produced the observed effects by an action within the central nervous system. However, if this were the case, it is difficult to understand how
peripherally administered E, which does not cross the blood-brain barrier, could reverse their effect on shivering. Further, the experiments of Maickel et al. are supported by investigations in the goat (Andersson et al., 1964) which demonstrated that the increases in urinary catecholamine excretion, the increased blood glucose levels, the shivering and the vasoconstriction produced by cooling of the anterior hypothalamus are all antagonized by chlorisondamine. The blockade could be reversed by an intravenous epinephrine infusion.

However, there are contrary data indicating that ganglionic blockade does not reduce shivering. Mecamylamine did not reduce shivering in rats exposed to cold, although they died in hypothermia. The author described the shivering in dying rats as "tremendous" (Leduc, 1961). Similarly, Moyer et al. (1957) reported that mecamylamine augmented shivering in the dog, and Dundee et al. (1954) found that hexamethonium or pentolinium did not affect shivering in this species.

Even if we accept the possibility that ganglionic blockade reduces shivering and that it does so by decreasing sympathetic activity, does this mean that CPZ, which blocks that portion of the sympathetic activity mediated by alpha receptors, will exert a similar effect? There are indications that it would not. For example, in the goat experiments discussed above, administration of the potent alpha-receptor antagonist, phenoxybenzamine, did not reduce the effects of central cooling and actually increased the intensity of shivering. Several other experiments have demonstrated that alpha adrenergic blockade per se does not reduce shivering (Leduc, 1961—phenoxybenzamine in rat; Moyer et al., 1957—hydergine and phenoxybenzamine in dog; Dundee et al., 1954—phentolamine, piperoxane, tolazoline in dog; Alexander and Williams,
1968-phenolamine and phenoxybenzamine in lamb). On the other hand, Maickel et al. (1967) cite a personal communication from E. Costa showing that the shivering blockade produced by chlorisondamine is also seen in cats and that this effect can also be produced by the beta-receptor blocking agent, N-isopropyl-methoxamine. Furthermore, Alexander and Williams (1968) have demonstrated a clear reduction in shivering in young lambs treated with the beta-receptor blocking agent, propranolol. Thus, if sympathetic activity is necessary for shivering, it appears that the component mediated by beta-receptors is the critical one. Since CPZ has little or no beta-receptor blocking activity, it seems unlikely that it could inhibit shivering by the peripheral mechanism described by Maickel et al.\footnote{Also, since all of the alpha-receptor blocking agents which were ineffective in blocking shivering enter the central nervous system to some extent (Nickerson and Hollenberg, 1967), it seems likely that central alpha-receptor blockade does not reduce shivering.} Therefore, although most of the thermoregulatory disrupting effects of CPZ may be effected at either central or peripheral sites (or both), blockade of shivering is in all probability a central effect.

Few data are available concerning the thermoregulatory effects of discrete intracerebral microinjection of CPZ, although this route of administration would seem helpful in determining which, if any, of the thermoregulatory effects of CPZ are centrally mediated. That intracisternal injection of CPZ reduces sympathetic outflow in cat and monkey has been mentioned, but the autonomic parameters measured were not directly related to temperature regulation. However, Reigle and Wolf (1969) reported that CPZ produced dose-dependent hypothermia when injected into the AH/PO region of hamsters maintained at 5°C ambient.
Shivering and cutaneous vasomotor tone were apparently not affected, and it was speculated that nonshivering thermogenesis may have been impaired. Rewerski and Jori (1968), on the other hand, found that intrahypothalamic injection of CPZ induced hyperthermia in rats kept at 20°C. The effect was seen only when CPZ was injected into the anterior hypothalamus or the third ventricle; posterior hypothalamic, thalamic or temporal lobe injections were ineffective. The hyperthermic effect was reasonably dose-dependent and was only partially reduced by pre-treatment with a large dose of reserpine. Unfortunately, no attempt was made to determine the mechanism by which this hyperthermia was effected. It is unlikely that vasoconstriction was affected, since rats normally exhibit maximal vasoconstrictor tone at 20°C. Since hyperthermia also occurred in reserpinized rats, which are essentially sympathectomized except for adrenomedullary function, the rise was likely due to increased shivering or the calorogenesis produced by E released from the adrenal medulla.

The hamster experiments support the contention that, at least in this species, the hypothermia produced by peripherally injected CPZ is mediated by the drug's action within the AH/PO region. As has been mentioned, CPZ given peripherally in the hamster produces hypothermia without affecting vasomotor tone or shivering. Since similar results were observed after intrahypothalamic injection, the thermoregulatory effects produced by the two routes correlate well. The speculation that nonshivering thermogenesis was impaired remains to be proven. The rat data, however, do not argue well for the concept that CPZ produces hypothermia by a hypothalamic action. In fact, since injections
into the third ventricle are likely to reach the fourth ventricle as well, it would appear that CPZ does not activate heat loss or impair heat gain mechanisms by an action at any site in the brainstem which lies near the ventricular walls. However, the possibility remains that peripherally administered CPZ may act at deeper structures to produce a hypothermic effect which would outweigh the AH/PO-mediated hyperthermic action. Obviously, further microinjection studies in rat and other species are needed before the central effects of CPZ on thermoregulation can be understood.

In summary, at ambient temperatures lower than thermoneutrality, CPZ has been found to produce hypothermia in several species. This effect is mediated primarily by cutaneous vasodilation and blockade of shivering. However, huddling and piloerection are also reduced, and these factors may augment the hypothermia. Reduction of nonshivering thermogenesis possibly occurs, but the supportive evidence for this is meager. At thermoneutral ambient temperatures, CPZ has little effect on body temperature. At elevated environmental temperatures, hyperthermia is produced in rat and mouse, but in dog, guinea pig and rabbit, CPZ reportedly protects against hyperthermia.

Because CPZ produces a pseudo-poikilothermic state in rat and mouse, it has been suggested that it may be acting within the hypothalamus to disrupt thermoregulation. Considerable evidence indicates that CPZ has the capability of blocking most thermoregulatory mechanisms by a central (but not necessarily hypothalamic) action. However, it also could impair these mechanisms by virtue of its peripheral activity, one exception being reduction of shivering, which is almost certainly a consequence of an action within the central nervous system.
Intrahypothalamic microinjection of CPZ in hamsters lowers rectal temperature, possibly by reducing nonshivering thermogenesis, since no vasodilation was seen and shivering was not impaired. Peripheral injection of CPZ in hamsters produces similar effects. However, similar injections in rats were seen to produce hyperthermia at 20°C ambient; at this environmental temperature peripheral CPZ yields hypothermia in rats. This result does not speak well for the concept that the hypothermic effects of CPZ are mediated by an action within the hypothalamus. It is suggested that further microinjection studies in several species are required before firm conclusions can be drawn.

Statement of the Problem

It is evident from the preceding introduction that 1) currently there is considerable interest in the participation of the candidate central neurotransmitters, epinephrine and norepinephrine, in the hypothalamic control of body temperature, and that 2) intracerebroventricular and/or intrahypothalamic injection of these substances does affect body temperature in several species. However, the pharmacology of these centrally elicited alterations in thermoregulation has not been studied to any great extent. Those reports that have appeared have dealt with the effects of intracerebroventricularly administered amines; the pharmacological aspects of catecholamines injected directly into cerebral tissue (microinjection) have been almost totally neglected. In particular, the problem of the receptor specificity of the thermo-regulatory effects of microinjected catecholamines has not been examined.

An important criticism of the concept that the catecholamines have a physiological function in the hypothalamic control of body temperature
has been that the high concentrations of putative neurohumors that
must be microinjected to produce an effect on thermoregulation may lead
to non-specific depolarization or hyperpolarization of neurons in or
fibers passing through the AH/PO region. It was felt that if receptor
specificity for effects of catecholamines injected into the hypothalamus
could be demonstrated, the probability that the catecholamines actually
participate in the hypothalamic regulation of body temperature would be
enhanced. Moreover, demonstration of specificity would provide much-
needed information concerning the properties of central adrenergic re-
ceptors. Furthermore, the experimental procedures developed to establish
the existence or nonexistence of receptor specificity could be used to
obtain data relevant to the effects of these amines on the parameters
responsible for changes in body temperature, i.e., shivering, peripheral
vasomotor tone and respiratory heat exchange, information not yet avail-
able in the literature.

Therefore, the primary objective of the investigations to be des-
cribed was to determine the receptor specificity of the thermoregulatory
changes induced by intrahypothalamic injection of catecholamines and
to gather as much information as possible pertaining to the mechanisms
through which these changes are effected. In addition to the experi-
ments necessary to achieve this primary objective, three additional
series of experiments were performed:

1. Although it had been frequently stated that the catecholamines
had an effect on thermoregulation only when injected into the AH/PO
region, the evidence for this was meager. It was therefore felt valu-
able to examine the effect of catecholamines injected into a variety of
hypothalamic sites.

2. The experiments of Lomax and Jenden (1966) had shown that cholinergic stimulation of the AH/PO region in the rat produces hypothermia. Early exploratory experiments of our own, using high doses of carbamylcholine, suggested that the same response was produced in the cat. However, in 1969, Myers and Yaksh reported that cholinergic stimulation of the monkey hypothalamus usually yielded hyperthermia. In view of the fact that monkey and cat respond to catecholamines in an identical manner, it was felt necessary to pursue the matter of cholinergic stimulation in the cat more thoroughly to determine whether this similarity of response also might hold for cholinergic as well as adrenergic stimulation. Accordingly, the effects of several dose levels of two cholinergic agents were examined by injection into the AH/PO and other regions of the cat hypothalamus.

3. Although the psychotropic agent, chlorpromazine, had been shown to produce hypothermia in several species, the effect of CPZ in the cat had apparently not been reported. Furthermore, although it was widely reported that CPZ-induced hypothermia was due, in part, to reduction of shivering, this effect had been convincingly demonstrated only in the rat. Finally, although it had been hypothesized that the thermoregulatory disrupting effects of CPZ may be due to an action within the AH/PO region, this suggestion had never been subjected to experimental test. It was therefore thought important to determine whether CPZ disrupts thermoregulation in the cat, and by what means and at what locus such disruption might be produced.
CHAPTER II
GENERAL PROCEDURES

**Animals**

Experiments were performed using 17 male and 2 female adult mongrel cats. The animals were obtained from several local animal suppliers and quarantined in our animal facilities for 2 weeks, during which time they were freed from intestinal parasites and treated with feline dis-
temper and feline pneumonitis vaccines. The cats were maintained in individual stainless steel cages at an ambient temperature of $21 ^\circ \text{C} \pm 2 ^\circ \text{C}$. Except on the day they were to be used in an experiment, the cats were fed daily at 3 P.M. Water was available at all times except during experimental sessions.

**Drugs**

The following drugs were employed: $1$-epinephrine d-bitartrate (Sterling-Winthrop), $1$-norepinephrine d-bitartrate monohydrate (Sterling-Winthrop), $1$-isoproterenol d-bitartrate dihydrate (Sterling-Winthrop), $1$-phenylephrine HCl (Gane's), phentolamine methane sulphonate (Ciba), propranolol HCl (Ayerst), chlorpromazine HCl (Smith, Kline and French), carbamylcholine Cl (Aldrich), acetylcholine Cl (Aldrich) and physostigmine $\text{SO}_4$ (Merck and Co.).

All drug solutions were prepared the day of the experiment in which they were to be used. Phentolamine and propranolol were dissolved
in sterile water for injection (pyrogen-free, no bacteriostat). Acetylcholine, physostigmine, carbamylcholine and chlorpromazine were dissolved in sterile water for injection and the resulting solution was brought to isotonicity by the addition of sodium chloride. The sympathomimetic amines were dissolved in sterile water for injection containing 1 mg/ml (0.1%) sodium metabisulfite as an antioxidant, and the final solution was brought to isotonicity by the addition of sodium chloride.

Every effort was made to insure that all drug solutions were sterile and pyrogen-free. However, since the raw drugs were not known to be pyrogen-free and none could be subjected to the high temperatures necessary to destroy pyrogens, there is no certainty that any of the drug solutions were completely free of pyrogen contamination.

All utensils used in preparing the solutions were baked at 190°C for at least 2 hours. The completed solutions were sterilized by forcing them through a seitz filter using a Swinny filter adapter (Becton, Dickinson and Co., Rutherford, N.J.). The filter and adapter had been autoclaved at 121°C and 15 pounds pressure for 30 minutes. The sterilized solutions were received directly into a disposable, sterile, pyrogen-free syringe.

Construction of Injection Cannulae

The construction of the injection guides, injection cannulae and stilettes is illustrated in Figure 1. The hypodermic tubing was obtained from Popper and Sons, Inc., 300 Park Avenue South, New York, N.Y. The epoxy resin can be obtained at almost any hardware or department store. Curing of the resin can be hastened by heating at 100°C for 10 to 15 minutes.
Figure 1. Construction of microinjection cannulae.

The guide cannula (B) is constructed from a 35 mm length of 22 G hypodermic tubing. A blob of epoxy resin is centered approximately 3 mm from the upper end of the cannula. This provided gripping surface for the dental acrylic used to affix the cannula to the skull.

The injection cannula (C) is constructed from a 41 mm length of 28 G hypodermic tubing. The collar is a 3 mm length of 22 G tubing glued to the injection cannula with epoxy cement. The distance between the lower end of the collar and the end of the injection cannula is 36 mm. Thus, when inserted into the guide, the injection cannula protrudes 1 mm beyond the guide cannula tip. The lateral orifice is ground 0.5 mm from the injection cannula tip as described in the text.

The stilette (A) is constructed from a 38 mm length of the reaming wire usually provided in boxes of 22 G non-disposable syringe needles. A bend is made 2 mm from the upper end so that when the stilette is inserted into the guide, the stilette tip protrudes slightly (0.1-0.2 mm) from the end of the guide cannula.
The lateral orifice in the injection cannulae was found to be an absolute necessity. The experimental protocol required leaving the loaded cannula in place for up to 20 minutes after its insertion into the injection guide. Very frequently, an injection cannula with only a ventral orifice became plugged with blood or tissue debris so that the injection could not be made. Cannulae with both lateral and ventral orifices very rarely became occluded during this time period.

The orifice was ground using a high-speed hand grinder clamped solidly to a table and an aluminum oxide solid abrasive cylinder (#5 Foredom abrasive cylinder, Techni-Tool, Inc., 1216 Arch Street, Philadelphia, Pennsylvania). The tubing is placed at a 45° angle to the edge of the cylinder and ground about half-way through. The thin film of metal occluding the opening can then be punctured and reamed out with a 27 or 28 gauge hypodermic stilette wire.

If resin rather than solder is used to make the ball at the dorsal end of the guide cannula, it is necessary to grind several shallow pits in the guide cannula at the site where the ball will be formed. The cannula should also be thoroughly cleaned with alcohol and dried before applying the resin. If these procedures are not followed, the resin will not grip the cannula solidly, and the guide may eventually slip down further into the brain.

Since guide cannulae of 35 mm length could be used for investigation of any site within the cat hypothalamus, it was necessary to make only one injection cannula (see Figure 1).
Surgical Procedures

Animals brought to surgery weighed 2.5-3.5 kg and had been deprived of food and water for 24 hours previous to the operation. Under pentobarbital or methoxyflurane anesthesia and using aseptic technic, the animal's head was placed in a stereotaxic head holder and the dorsal surface of the skull was exposed. Guide cannulae were inserted through holes drilled in the skull and lowered to the appropriate stereotaxic locus. Since the injection cannulae were 1 mm longer than the guide cannulae, the tips of the guides were positioned 1 mm above the intended site of injection. Four small stainless-steel machine screws were screwed into holes previously tapped into the bone at sites surrounding the array of guide cannulae. The entire array of guide cannulae and screws was then covered with several layers of dental acrylic. After the acrylic had hardened and Neosporin antibiotic ointment (Burroughs Wellcome & Co.) had been applied to the wound, the stilettes were inserted into the guides and the skin and muscle sutured loosely around the base of the mound of acrylic. Detailed surgical protocol may be found in the Appendix.

With the exception of 2 animals which had intraventricular guide cannulae, each cat had one bilaterally symmetrical set of injection guides with their tips lying within the AH/PO region. Most animals were also implanted with a second set of injection guides located at various hypothalamic sites posterior to the AH/PO region. In a few cats, no second set was implanted or the second set was not bilaterally symmetrical. Some of the animals also had an 18 guage guide cannula positioned in one frontal cortex from which brain temperature could be
measured by inserting a small thermistor into the guide.

Recording of Physiological Parameters

Rectal Temperature

Rectal temperature was recorded in all experimental runs. A flexible thermistor probe (#401, Yellow Springs Instrument Co., Yellow Springs, Ohio) was coated liberally with a local anesthetic ointment (Nupercainal ointment), inserted 15 cm into the colon and taped firmly to the base of the tail. Most cats tolerated the probe well for many hours. The probe was connected to a Yellow Springs Model 43 or 47 Telethermometer. The output from this instrument was recorded continuously on either a VOM 7 (Bausch and Lomb) or a Model FMWSE6C Multiriter recorder (Texas Instruments). Temperature records could be read accurately to 0.01°C. The rectal probes were calibrated periodically against a standard thermometer in a water bath.

Brain Temperature

Brain temperature was recorded in a few experimental sessions utilizing the same technic and equipment used to measure rectal temperature, except that a Yellow Springs #520 18 gauge thermistor probe was employed. The probe, which had previously been sterilized in 1:500 Zephrin chloride solution and rinsed in sterile water for injection, was inserted down the guide cannula into the frontal cortex. These probes are quite fragile, and the probe leads broke frequently. In addition, brain temperature was found to be quite unstable, varying widely with the attentional state and head position of the animal. Thus, the more stable rectal temperature was taken as the sole measure of core temperature in the majority of experimental sessions.
Ear Skin Temperature

The temperature of the dorsal surface of the ear pinna was taken as an index of cutaneous vasomotor tone. A Yellow Springs #409 thermistor probe was attached to the tip of the ear pinna using Davol surgical appliance cement. Output from a Yellow Springs Model 43 Telethermometer was fed to a Yellow Springs Model 80 recorder. Readings were accurate to 0.1°C.

The cement used to attach the ear probe is quite irritating until it has dried thoroughly. Therefore, it was necessary to apply a thin layer of the cement to the ear the day prior to the experimental session. Just before the experiment, a thin layer was applied to the thermistor and allowed to dry to tackiness. The thermistor was then applied to the ear. If the cement was applied to the ear the same day as the experiment, irritative vasodilation frequently developed. Ear temperature was measured from the ear ipsilateral to the site of drug injection except where otherwise noted.

Respiratory Rate

Respiratory rate was obtained using impedance pneumography. An area approximately 5 x 10 cm on both sides of the thorax was shaved and depilated (Nair brand depilatory) prior to placement of the recording electrodes. The size and position of the depilated areas were kept as constant as possible throughout the entire series of experiments. A gold-plated cup electrode (E5G, Grass Instruments, Quincy, Mass.) filled with Grass EC2 electrode cream was affixed to each side of the thorax using disposable adhesive disks (Beckman Instrument Co., Spinco Division, Palo Alto, California). The electrode leads were connected to
an impedance pneumograph (E & M Instrument Co., Inc., Houston, Texas) driven by an E & M driver amplifier. The pneumograph output was fed to a Grass 7P3A Wide Band A.C. Preamplifier and D.C. Driver Amplifier for the production of pen records on a Grass pen recorder. The low frequency one-half amplitude attenuation on the 7P3A was set at 0.3 Hz; high frequency one-half amplitude attenuation on the D.C. Driver was set at 3 Hz for respiratory rates less than 100/min. and at 15 Hz for rates greater than this. These parameters minimized thoracic electromyographic interference.

Respirations were automatically counted and totaled during each 10-minute period of the run by banks of relay-activated counters. A length of surgical suture was tied to the end of the recording pen and the other end attached to a microswitch. Each excursion of the pen activated the microswitch which, in turn, activated the counting mechanism.

Electromyogram

The electromyogram from the right lateral thigh muscles (principally biceps femoris) was taken as an index of shivering activity. The thigh area was shaved and depilated, and electrodes were placed about 5 cm apart in the center of the thigh. The type of electrodes and method of affixation were the same as for respiratory rate recording. An attempt was made to place the electrodes in the same position for each run, but the depilation process removed any markings that might be made on the skin. Small variations in electrode position are therefore probable. A grounding electrode was affixed to any available depilated area next to one of the pneumograph electrodes.
Electrode leads were connected to a Grass 7P3A Wide Band AC Amplifier and Integrator, which drove a Grass DC Driver Amplifier for recording of the raw electromyogram on a Grass penwriter. The control settings on the 7P3A were: low frequency one-half amplitude attenuation, 3 Hz; threshold, 6.0; rectification, full wave; sensitivity, maximum. High frequency one-half amplitude attenuation on the DC Driver was set at 0.5 Hz. The sensitivity was usually set so that 75 µV produced 1 cm of pen movement. However, at maximum sensitivity, the system required only 10 µV input per cm of pen movement. Thus, electromyographic activity at the 1 µV level was detectible.

Output from the 7P3A was also lead to a Grass 7P10A Polygraph Integrator. This integrator has an extremely long decay time and low drift. The rectified and integrated EMG activity was written out as a slow upward movement of the pen, the pen height at any given time representing accumulated electromyographic activity (voltage) to this time. Pen reset to baseline was automatic as maximum excursion was reached. The sensitivity setting of the 7P10A was 1.5. Bandwidth of the 7P3A-7P10A combination was 0.15 Hz to 10 KHz. Since the integrator was incapable of any meaningful calibration, integrated activity was recorded as mm of pen rise per unit time.

The background noise in the integrated electromyographic records was obtained at the end of the run by increasing the ambient temperature in the environmental room in which the cat was restrained. Shivering was suppressed by this maneuver, and background noise due to 60 Hz interference, the electrocardiogram, and electromyographic activity from the respiratory muscles could be determined and subtracted from the
experimental values for the electromyographic integration of shivering activity.

Microinjection Procedure

The construction of the injection cannulae has been described. During experimental runs, the cannula was connected to a 10 μl microsyringe (Hamilton Co., Inc., Whittier, Calif.) by 1 meter of size 10 polyethylene tubing. The microsyringe was locked into a Multi-Speed injection pump (Harvard Apparatus Co., Dover, Mass.) located at the same level as the cat’s head. Before the experimental run, the syringe plunger was removed and the system was back-filled and flushed with 1:500 Zephrin Chloride disinfectant. This solution was allowed to remain in the system at least 1 hour before an injection was made.

Just before the time of injection, the system was back-flushed thoroughly with sterile water for injection followed by drug solution. The system was back-filled with drug solution and an air bubble 1 to 2 mm long was placed in the injection tubing so that, when the system was ready for injection, the bubble lay in the polyethylene tubing just past the tip of the injection syringe. The bubble served to confirm that the drug was actually being ejected when the pump was started. For a 1 μl injection, the distance the bubble moved down the tubing was constant from run to run. Any deviation from this distance or compression of the bubble was taken as an indication that the injection cannula was occluded, and the experiment was terminated. The syringe plunger was then inserted and run forward to a standard mark on the syringe, thus ejecting some of the drug solution. The excess solution adhering to the tip of the injection cannula was carefully removed with sterile gauze. The syringe
pump was then run backward in order to place a 0.2 μl air bubble in the injection cannula tip. This procedure prevented any seepage into the brain between the times of insertion of the injection cannula and injection of the drug.

At the time of injection, the pump driving the microsyringe was run forward rapidly to eject the 0.2 μl air bubble and then was allowed immediately to a rate which ejected 1 μl of drug solution in exactly 2 minutes.

Experimental Protocol

Two days preceding the experimental run, the subject was fed only one-third of his normal ration and the day preceding the run was completely deprived of food. On the experimental day, he was allowed to eat to satiation a few hours prior to the beginning of the experiment. This procedure was found to be necessary, since many of the drug treatments elicited defecation. The period of food deprivation ensured an empty or near-empty colon, thus preventing expulsion of the rectal probe or possible rupture of the bowel.

Just before the experiment, the areas previously described were depilated and the cat was thoroughly dried. The rectal probe was then inserted and the cat placed in a restraining device.

The type of restraint used is illustrated in Plate I. The carpet was provided not only for the subjects' comfort but also to prevent rapid conductive heat loss to the cool table top. The leg ties were omitted for some cats, but, in those animals which tended to twist to the side, the ties were necessary to prevent biting of the injection tubing or ear temperature probe wiring. Training of the animals to tolerate restraint usually required two or three 5-hour sessions in the
Plate I. Cat in restraining device during experimental session.
restrainer. Once they were trained, most cats would lie quietly or sit in the typical feline dozing posture. However, struggling was occasionally seen when the cat was first placed into the restrainer at the beginning of the run and after certain drug treatments.

The cat and restraining device were kept in a walk-in environmental chamber (Forma Scientific) which, unless otherwise noted, was maintained at 20°C ± 0.5°C. During experimental sessions, the thick walls of the chamber and the noise produced by blowers inside the chamber produced considerable isolation from external sounds.

After the cat had been placed in restraint, the respiratory rate, electromyogram and grounding electrodes and ear temperature thermistor were affixed. The stilette of the injection guide to be used was removed, cleaned of any debris with 1:500 Zephrin Chloride solution, rinsed in sterile water for injection and replaced. The door to the environmental chamber was then closed and the session begun. During experimental sessions the animals were observed either through a one-way window or by closed-circuit television.

At least one hour was allowed for rectal temperature and the other parameters to stabilize. The chamber door was then opened, the stilette of the appropriate injection guide was removed and a loaded injection cannula inserted into the guide and the door was closed. Before the injection was begun, enough time (usually 5-10 minutes) was permitted to elapse to be certain that the insertion process was not going to have thermoregulatory consequences. Usually, inserting the cannula (or simply opening the chamber door) produced a temporary interruption of shivering and induced a small and short-lasting vasodilation of the
ear. After all parameters had restabilized, the drug was injected. The injection cannula was permitted to remain in place until the end of the session or until rectal temperature had returned to baseline after a drug effect.

Histology

Following completion of the experiments, the cats were anesthetized with pentobarbital and the thorax was opened. The aorta was clamped at its origin and a perfusion cannula inserted in the descending aorta, which was then clamped just caudal to the cannula. The superior vena cava was then cut and the brain perfused with isotonic saline followed by 10% formalin solution. The head was removed and the acrylic mound securing the injection guide array to the skull was cut free using a small circular saw driven by a high speed hand grinder. This permitted the injection guide array to be removed from the skull.

The brain was then exposed as much as possible without destroying the auditory meatus, the infraorbital ridge or the roof of the mouth. With the brain thus exposed, the head was placed in a stereotaxic head holder and two 15 gauge needles lying in the same stereotaxic vertical plane were lowered into the brain a few millimeters posterior to the area where the injection guide array had been located. The needles were used to guide a razor blade cut through the brain. The same procedure was repeated a few millimeters anterior to the location of the injection guide array. The tissue block thus created was removed and placed in 10% formalin for 24 hours. Since the anterior and posterior faces of the tissue block containing the injection sites were cut in a true stereotaxic vertical plane, the block could be readily cut in
a microtome so that the entire injection guide tract could be visualized in a single frontal section. The block was then embedded in celloidin and sectioned at 25 microns. The sections were stained by a modification of the method of Kluver and Barrera and examined microscopically.

1 Embedding, sectioning and staining were accomplished using the methods of Lauber (1970). The celloidin embedding process was the standard length Cat Brain Pyridine Celloidin Embedding procedure described by this author. Sections were cut at 30 micra according to her method A. It should be stressed that, although embedded brains may be stored in 70% alcohol for indefinite periods, severe shrinkage (1/3 to 1/2) is seen after 30 days. It is strongly suggested that the brains be cut within a few days after completion of the embedding process.

Cut sections were stained prior to mounting on slides using Lauber's modification of the Kluver and Barrera procedure, except that: (1) the Cresyl Violet Acetate was prepared in 0.025% concentration rather than the suggested 0.1% concentration (2) 10-20 seconds of staining in this solution, rather than the suggested 3 minutes, were found to be adequate (3) the Cresyl Violet staining was accomplished at room temperature rather than the suggested 57°C (4) the Luxol Fast Blue was prepared as a 0.1% solution rather than 1.0% as suggested; the latter concentration is a typographical error (5) it was found that 6 hours of staining in Luxol Fast Blue at 37°C was adequate; staining for 16-24 hours at 57°C as suggested resulted in grossly overstained sections.
CHAPTER III
THE THERMOREGULATORY EFFECTS OF INTRACEREBRAL
INJECTION OF SYMPATHOMIMETIC AMINES

Introduction

Investigation of the receptor selectivity for the hypothermic response induced by microinjection of sympathomimetic amines into the AH/PO region was the primary goal of the experiments to be described. In addition, it was desired to obtain quantitative information concerning the mechanisms (e.g., vasodilation, cessation of shivering, panting) by which these changes in body temperature are produced. Finally, the effect of sympathomimetic amines injected at sites other than the AH/PO region was examined.

Pharmacologic receptors in the adrenergic portion of the peripheral autonomic nervous system have been classified operationally into the alpha and beta types. These receptor types have been differentiated in various organs by utilizing one or both of the following technics: 1) dose-response curves for a series of sympathomimetic amines acting on a given effector system can be constructed and the relative potency of these amines compared 2) the ability of certain substances to antagonize specifically the response to one or more agonists can be determined. Alpha-receptors, for example, are readily stimulated by epinephrine but are relatively insensitive to isoproterenol; other sympathomimetic amines, such as norepinephrine and phenylephrine, fall between these two agonists on the potency
continuum. Thus, the typical alpha-receptor potency series is:
epinephrine > norepinephrine > phenylephrine > isoproterenol. Beta-
receptors, on the other hand, are most sensitive to isoproterenol and
least sensitive to phenylephrine, yielding the potency series:
isoproterenol > epinephrine > norepinephrine > phenylephrine. Alpha-
receptor stimulation can be blocked relatively specifically by such
substances as phentolamine and phenoxybenzamine, whereas beta-receptor
activation is antagonized selectively by substances such as propranolol,
and dichloroisoproterenol.

It was felt that, by combining these two technics, that is, by
looking at the relative potency of several agonists as well as the
blocking activity of specific antagonists, a stronger statement con­
cerning the receptor selectivity of the hypothermic response could be
made.

In most previous pharmacological studies of thermoregulation, deep
core (rectal) temperature has been taken as the chief indicator of
thermoregulatory status. Thus, in the present study, the maximum fall
in rectal temperature produced by a intrahypothalamic microinjection
was of great interest. However, this parameter is not necessarily the
best choice when one is interested in comparing the potency of several
compounds. Clearly, the maximum fall in body temperature observed will
be a function of the time that the drug remains at the site of injec-
tion. A weak, but slowly removed agonist could conceivably produce as
great a decrease in rectal temperature as a strong agonist which is
rapidly removed.
A measure of potency which is relatively independent of the sojourn of the drug at the injection site is the initial rate of fall of body temperature, that is, the rate of fall produced during the first few minutes after the microinjection, when drug concentration has been little influenced by the various mechanisms of disposition. As will be discussed later, another measure, which under certain circumstances yields the same information as initial rate of fall is the average rate of fall of rectal temperature (maximum fall in rectal temperature divided by the time required to attain this degree of hypothermia).

Another approach to the problem of obtaining relative drug potencies uninfluenced by the rate of drug disposition would be to record thermoregulatory parameters which are capable of being measured soon after the drug injection. In the cat kept in a 20°C environment (the ambient temperature used in these experiments), passive fall in body temperature is dependent upon vasodilation and cessation of shivering. Conceivably, the rate of fall could be actively augmented by panting. Thus, by monitoring peripheral vasomotor tone, level of shivering and respiratory rate, it is possible to gain information concerning the mechanisms underlying the catecholamine-induced hypothermic effect as well as three independent measures of drug potency.

**Methods**

**Construction of Dose-Response Curves**

The experimental subjects were 12 male cats. Ten were surgically implanted with a bilaterally symmetrical set of injection guides with their tips lying in the AH/PO region. Two were implanted with
unsymmetrical guides, the guide on one side being 1 mm anterior to the other. The standard site of aim for the injection guide tip was A (14.0), L (1.5), H (-2.5), according to the atlas of Snider and Niemer (1961). The tips of the injection cannulae, when they were inserted into the guides, would lie 1 mm lower, hopefully in the ventral half of the region between the optic chiasm and the anterior commissure. Each of the subjects also had a second set of injection guides located at various sites more posterior in the hypothalamus. These posterior cannulae were to be used in the exploratory phase of the study.

In the present dose-response experiments, all microinjections were unilateral and were made into the anterior site thought to be located at A (14.0), L (1.5), H (-3.5). In the 10 cats with bilaterally symmetrical anterior cannulae, the side shown to be most sensitive in preliminary experiments was used throughout the remainder of the study.

The drugs and concentrations used are given in Table 1. Although this table provides several expressions of the drug concentrations in order to facilitate comparisons with the literature, unless otherwise specified, all drug concentrations cited in the text or figures refer to the molar concentration of the solution injected. Since the volume of fluid injected was always 1 μl, the molar concentration is numerically equal to the number of micromoles of drug injected. The pH of all sympathomimetic amine solutions ranged between 3.0 and 3.5.

Two control solutions were used. As a control for the bitartrate ion in the epinephrine, norepinephrine and isoproterenol salts, a solution containing a bitartrate ion concentration close to that contained in the highest drug concentration used (0.10 molar) was prepared.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Molar concentration</th>
<th>Millimolar concentration</th>
<th>Total number of µmoles injected</th>
<th>µg injected (as the salt)</th>
<th>µg injected (as the base)</th>
</tr>
</thead>
<tbody>
<tr>
<td>l-epinephrine</td>
<td>0.003</td>
<td>3.0</td>
<td>0.003</td>
<td>1.00</td>
<td>0.55</td>
</tr>
<tr>
<td>d-bitartrate</td>
<td>0.01</td>
<td>10.0</td>
<td>0.01</td>
<td>3.33</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>30.0</td>
<td>0.03</td>
<td>10.00</td>
<td>5.50</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>100.0</td>
<td>0.10</td>
<td>33.33</td>
<td>18.32</td>
</tr>
<tr>
<td>l-norepinephrine</td>
<td>0.01</td>
<td>10.0</td>
<td>0.01</td>
<td>3.37</td>
<td>1.69</td>
</tr>
<tr>
<td>d-bitartrate</td>
<td>0.03</td>
<td>30.0</td>
<td>0.03</td>
<td>10.11</td>
<td>5.08</td>
</tr>
<tr>
<td>monohydrate</td>
<td>0.10</td>
<td>100.0</td>
<td>0.10</td>
<td>33.73</td>
<td>16.92</td>
</tr>
<tr>
<td>l-phenylephrine</td>
<td>0.01</td>
<td>10.0</td>
<td>0.01</td>
<td>2.04</td>
<td>1.67</td>
</tr>
<tr>
<td>hydrochloride</td>
<td>0.03</td>
<td>30.0</td>
<td>0.03</td>
<td>6.11</td>
<td>5.02</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>100.0</td>
<td>0.10</td>
<td>20.37</td>
<td>16.72</td>
</tr>
<tr>
<td>l-isoproterenol</td>
<td>0.01</td>
<td>10.0</td>
<td>0.01</td>
<td>3.97</td>
<td>2.11</td>
</tr>
<tr>
<td>d-bitartrate</td>
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<td>30.0</td>
<td>0.03</td>
<td>11.92</td>
<td>6.34</td>
</tr>
<tr>
<td>dihydrate</td>
<td>0.10</td>
<td>100.0</td>
<td>0.10</td>
<td>39.73</td>
<td>21.12</td>
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</tbody>
</table>
Its exact composition was: 17 mg/cc d-tartaric acid, 1 mg/cc sodium metabisulfite, 3.0 mg/cc sodium chloride, the final solution being brought to pH 3.45 (the average pH of the epinephrine, norepinephrine and isoproterenol solutions) with 4 N sodium hydroxide. The control solution for the chloride ion in phenylephrine HCl contained 1.0 mg/cc sodium metabisulfite and 8.34 mg/cc sodium chloride, the final solution being brought to pH 3.40 (the average pH of the phenylephrine solutions) with 0.05 N HCl. All control solutions were prepared and sterilized in the same manner as the drug solutions.

In each drug or control session, rectal temperature, ear skin temperature, respiratory rate and electromyographic activity were obtained as discussed in Chapter II. From these measured parameters, a number of computed parameters were derived as described below:

$\Delta T_{r}$ (in °C). - The maximum decrease in rectal temperature following a microinjection. The decrease is measured relative to the subject's rectal temperature just prior to the onset of the fall.

$\Delta T_{e}$ (in °C). - The maximum increase in ear skin temperature following a microinjection. The increase is measured relative to the subject's ear skin temperature just prior to the onset of the increase.

$\Delta EMG$ (in %). - The percentage reduction in EMG activity produced by a microinjection. The method for quantitation of EMG activity has been described (Chapter II). The average mm/min of pen rise for the 30-minute period preceding the injection was taken as a baseline. Following the injection, the 8-minute period during which the least amount of EMG activity occurred was found by inspection, and the percentage reduction from baseline was computed after correcting for background
noise.

The 30 minute baseline period was long enough to average out short-term variations in shivering but short enough to exclude the variable shivering activity which often occurred during the first 10-20 minutes of the 1-hour preinjection acclimatization period. The 8-minute post-injection measurement interval was selected for similar reasons; it was long enough to average out the many very short variations in shivering rate but not so long as to "smear" the actual drug effect, which occasionally lasted only 10 minutes.

50% $\Delta T_r / \Delta T$ (in °C/hr). - The desirability of obtaining a measure of drug effect on rectal temperature which is relatively independent of rate of drug disposition has been mentioned. Although the true initial rate of rectal temperature fall ($dT_r / dT$) would best serve this purpose, it was found that this could not be obtained in many cases. The "dead zone" of the rectal temperature recorder and small, but sudden, shifts in rectal temperature due to changes in probe position inside the colon or passage of feces down the colon frequently made measurement of true initial rate of fall unreliable. However, it was observed that the time required for rectal temperature to fall to 50 percent of $\Delta T_r$ was long enough to reduce the influence of small, sudden changes in rectal temperature and that, taken as a whole, the rate of fall during this period was reasonably linear. Usually, rate of fall did not level off appreciably until 60 to 70 percent of $\Delta T_r$ had been reached. Therefore, $50% \Delta T_r / \Delta T$, i.e., 50 percent of $\Delta T_r$ divided by the time (in hours) required to reach this level was taken as an approximation of the initial rate of rectal temperature fall.
90% $\Delta T_r / \Delta T$ (in °C/hr). - Another method for compensating for the
varying rates of disposition of microinjected drugs is to compute the
average rate of fall of rectal temperature, i.e., $\Delta T_r$ divided by the
time of fall. If the degree of physiological counterthermoregulation
against a drug-induced fall in rectal temperature is independent of the
magnitude of the decrease, average rate of fall will provide the same
information with respect to relative potency as will true initial
rate of rectal temperature decrease. However, as has been previously
discussed, it is highly probable that there is a proportional component
in thermoregulatory control, and it is therefore likely that the greater
the hypothermia, the greater will be the physiological resistance.
Thus, average rate of body temperature fall shares with $\Delta T_r$ itself the
possibility that the true activity of strongly hypothermic agents will
be underestimated. Further, it is frequently impossible to determine
the exact time when minimum body temperature is reached, since this
level is usually approached asymptotically. However, the time when
rectal temperature had reached 90 percent of $\Delta T_r$ was almost always
easily determinable. Therefore 90% $\Delta T_r / \Delta T$ was taken as an approxi-
mation of true average rate of body temperature decrease. In spite
of the possible disadvantages discussed, it was felt that this parameter
would prove valuable for comparison with data derived from other thermo-
regulatory parameters.

$T_r$ Latency (in minutes). - The time between the beginning of a
microinjection and the beginning of the hypothermic effect. It should
be remembered that the duration of the microinjection was 2 minutes.
Thus, a $T_r$ latency of less than 2 minutes means that rectal temperature
began to fall before the entire 1 µl droplet had been ejected.

\( T_e \) _Latency_ \(_1\) (in minutes). - The time from the beginning of a microinjection to the first sign of an increase in ear temperature.

\( T_e \) _Latency_ \(_2\) (in minutes). - The time from the beginning of a microinjection to the beginning of the large and rapid increase in ear skin temperature. Changes in \( T_e \) were usually sudden and of large magnitude. However, on occasion, a slow upward drift preceded the rapid increase. Thus, the need for two measures of latency for this parameter.

\( EMG \) _Latency_ (in minutes). - The time between the beginning of a microinjection and the first sign of a persistent reduction in electromyographic activity.

\( T_e \) _Duration_ (in minutes). - The period between the onset of hypothermia and the onset of a persistent tendency for rectal temperature to return toward the preinjection level.

\( T_e \) _Duration_ (in minutes). - The period between the onset of the major increase in ear temperature and the onset of its return to baseline.

\( EMG \) _Duration_ \(_1\) (in minutes). - The period between the onset of EMG suppression and the first sign of return of EMG activity toward preinjection levels.

\( EMG \) _Duration_ \(_2\) (in minutes). - The period between the onset of EMG suppression and the return of EMG activity to the preinjection baseline level. Two measures of EMG duration were necessary because EMG recovery was usually a gradual process, and it was not clear which of the two "durations" was of the greatest significance.
The protocol for a typical experimental session is the same as that described in Chapter II.

The experimental design, as it was initially envisioned, would have consisted of administration of all drug and control treatments to each of six cats in a completely random fashion. Unfortunately, it was not possible to complete the study in accordance with this design, primarily because several cats became insensitive to the injected sympathomimetic amines, probably because of necrosis or gliosis at the injection site. In a dose-response study of this nature, such decrements in sensitivity are intolerable. Thus, although randomized treatment was desirable from the standpoint of statistical treatment of the data, the design was altered in the following ways to minimize the chance of sensitivity changes influencing the results:

1) Six new cats were introduced into the study. In these animals the treatment sequence was begun with either 0.03 molar epinephrine or 0.03 molar norepinephrine, a dose which always produced a large hypothermic effect. In most subjects (and in all subjects included in the analysis of variance tables in the results section), this initial drug-dose combination was repeated at least once in the treatment sequence as a check on site deterioration. In many animals, other treatments were also replicated as a further check on site sensitivity. If a sensitivity loss was detected, the animal was withdrawn from the experiment and any experimental runs thought to be influenced by the sensitivity decrement were discarded.

2) In order to reduce the number of microinjections in the treatment sequence, the lowest dose (0.003 molar) was at first limited to
epinephrine and later deleted entirely.

3) The 0.03 molar concentrations of isoproterenol and phenylephrine were also moved forward in the treatment sequence to maximize the probability that at least one common dose of each amine would be administered to each animal before site deterioration occurred.

Pretreatment with Alpha- and Beta-Receptor Antagonists

Five male and one female cat were used in this series of experiments. Four of these animals had bilateral sets of anterior and posterior cannulae as in the dose-response subjects. The other two subjects had no posterior cannulae. The intended site of injection was the same as in the dose-response experiments, i.e., A (14.0), L (1.5), H (-3.5), according to the atlas of Snider and Niemer (1961). In those subjects having bilateral anterior sets of injection guides, the side demonstrating the greatest sensitivity was used.

The drugs and the concentrations administered were as follows:

1) l-epinephrine d-bitartrate, 0.02 molar (6.7 μg/μl as the salt)
2) phentolamine methanesulphonate, 0.27 molar (100 μg/μl as the salt)
3) propranolol HCl, 0.27 molar (78.4 μg/μl as the salt).

The measured and computed parameters were the same as in the dose-response experiments. However, the session protocol was slightly different than that described in Chapter II. One hour or more after the beginning of the session, the chamber door was opened and the stilette of the injection cannula was removed and a loaded injection cannula was inserted into the guide. After the recorded parameters had restabilized, 1 μl of 0.9% saline, phentolamine or propranolol was microinjected. Five minutes later, the injection cannula was removed and the
stilette reinserted. After 8 more minutes, the stilette was removed and the injection cannula reinserted. At exactly 15 minutes after the beginning of the first microinjection, the same brain site was microinjected with a standard dose of epinephrine (0.02 molar). Controls consisted of injection of saline, phentolamine or propranolol alone. At least 5 days intervened between treatments.

The treatment sequence is given below:

1) Saline + epinephrine
2) Phentolamine + epinephrine
3) Saline + epinephrine
4) Propranolol + epinephrine
5) Saline + epinephrine
6) Phentolamine alone
7) Propranolol alone
8) Saline alone

The multiple saline controls were inserted to insure that any apparent reduction in the response to epinephrine by pretreatment with phentolamine or propranolol was not due to a decrease in the sensitivity of the injection site.

"Mapping" of the Hypothalamus

After completion of the dose-response and antagonist studies, each subject received one or more microinjections of 0.02-0.03 molar epinephrine (a dose which produced strong hypothermia when injected into the AH/PO region) into each of its remaining implantation sites. The parameters measured and the session protocol were identical to those used in the dose-response experiments. After the subjects had been sacrificed and the injection sites verified histologically, an attempt was made to correlate the sites of stimulation and the responses observed.
Results

Construction of Dose-Response Curves

Figure 2 illustrates the thermoregulatory consequences of micro-injecting 0.1 µmole of epinephrine, norepinephrine, phenylephrine or isoproterenol into the AH/PO region of a single cat. Epinephrine (a) produced, after a short latency, vasodilation of the ear skin and suppression of shivering, and rectal temperature decreased by more than 2.5°C. Rectal temperature did not begin to rise until shivering had returned and vasoconstriction had occurred. (The profundity of the inhibition of shivering produced in this experimental session is illustrated in Figure 3). Norepinephrine (b) evoked similar but less intense effects, and the duration of action was shorter. Phenylephrine (c) failed to elicit vasodilation and evoked only slight inhibition of shivering. Isoproterenol (d) had no effect on any parameter and, consequently, failed to produce hypothermia.

Figure 2 is presented primarily to provide the reader with a visual impression of the kinds of data that were gathered. The data obtained with this particular cat are not presented as "typical" in the sense that the magnitude of the effects on the various parameters is representative of the remaining subjects. As will be seen, there was considerable variability in this respect.

Of the 12 animals which began the dose-response experiments, only 4 completed the entire treatment sequence. Of the 8 remaining animals, 1 ripped the entire array of 4 injection guides from his skull, 2 destroyed the utility of the critical injection guide by bending it, 1 died from cystitis and the other 4 experienced a partial loss of
Figure 2. Effect of intrahypothalamic microinjection of 0.1 molar epinephrine (a), norepinephrine (b), phenylephrine (c) and isoproterenol (d) on rectal temperature, ear skin temperature and electromyographic activity in a single cat.

E = epinephrine bitartrate  
NE = norepinephrine bitartrate monohydrate  
PE = phenylephrine HCl  
ISO = isoproterenol bitartrate dihydrate

\[ T_r = \text{rectal temperature} \quad T_e = \text{ear skin temperature} \quad \text{EMG} = \text{electromyographic activity of the lateral thigh} \]

The sequence in which the four sympathomimetic amines were injected was E, NE, ISO, PE. At least 5 days intervened between injections. EMG is presented histographically as the mean rate of pen rise for each 10-minute period of the experimental session (see text).
Figure 3. Reduction in EMG produced by intracerebral injection of 0.1 molar epinephrine bitartrate.

Upper panel: direct pen writeout of electromyographic activity.
Lower panel: integrated EMG activity. See text (Chapter II) for details.

Epinephrine was injected at the arrows labelled "E".
sensitivity to the injected amines. Unfortunately, the data from those animals which did not complete the study cannot be included in any legitimate statistical comparison of the relative potencies of the various sympathomimetic amines. Nevertheless, data derived from all 12 subjects will be presented for visual comparison with that derived from only the 4 animals which completed the sequence. For convenience, these latter animals will henceforth be referred to as "complete" cats.

The relative ability of microinjected epinephrine, norepinephrine, phenylephrine and isoproterenol to lower rectal temperature in the 4 "complete" cats is presented in Figure 4. Similar data for all 12 subjects may be found in Figure 5. In each figure, the dose of sympathomimetic amine in micromoles is plotted against the mean maximum decrease in rectal temperature produced. The vertical bracketed lines represent 1 standard error of the mean. The data presented in Figure 3 were subjected to an analysis of variance, and a highly significant effect due to drugs was found (see Table 2). The relative ability of microinjected epinephrine, norepinephrine, phenylephrine and isoproterenol to lower rectal temperature in the 4 "complete" cats is presented in Figure 4. Similar data for all 12 subjects may be found in Figure 5. In each figure, the dose of sympathomimetic amine in micromoles is plotted against the mean maximum decrease in rectal temperature produced. 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Multiple Range Test was used to make individual comparisons between the amines at the 3 dose levels. These data are given in Table 3.

Examination of these tables and figures indicates that the ability of the sympathomimetic amines to lower body temperature is dose dependent and that the 4 amines differ in potency with respect to this parameter. At all dose levels, epinephrine was shown to produce significantly greater hypothermia than did isoproterenol. Further, at the middle and high dose levels, norepinephrine and phenylephrine also lowered rectal temperature to a significantly greater extent than did isoproterenol, and, at these dose levels, epinephrine was significantly more active than norepinephrine. However, irrespective of the dose, norepinephrine and phenylephrine could not be shown to differ in their hypothermia-producing ability. Thus, at the middle and high dose levels, the potency series with respect to $\Delta T_r$ was epinephrine $>$ norepinephrine $=$ phenylephrine $>$ isoproterenol.

Dose-response curves for the 50% $\Delta T_r / \Delta T$ parameter are presented in Figures 6 and 7. The analysis of variance table and comparisons of individual means are given in Tables 4 and 5, respectively. It will be seen that this estimate of initial rate of rectal temperature decrease is dose dependent. There is also a significant effect due to drugs, but few significant differences between individual means were found. However, at the intermediate and high doses, norepinephrine and epinephrine produced significantly greater rates of rectal temperature fall than did isoproterenol, and, at the middle dose, epinephrine was significantly more active than phenylephrine. Moreover, in spite of the lack of statistical significance, Figures 6 and 7 depict a
Figure 4. Mean maximum decrease in rectal temperature ($\Delta T_r$) produced by microinjection of various doses of sympathomimetic amines into the anterior hypothalamic/preoptic region.

Since the injected volume was always 1 $\mu l$, the number of micromoles injected is numerically equivalent to the molar concentration of the drug solution.

E = epinephrine bitartrate  NE = norepinephrine bitartrate monohydrate
PE = phenylephrine HCl  ISO = isoproterenol bitartrate dihydrate
C = control solution

Data are derived from only the four cats which completed the entire treatment series. Vertical bracketed lines represent standard errors of the mean.
\[ \Delta T_r \] (°C)

NUMBER OF MICROMOLES INJECTED\(^a\)
(LOG SCALE)
Figure 5. Mean maximum decrease in rectal temperature (ΔT<sub>r</sub>) produced by microinjection of various doses of sympathomimetic amines into the anterior hypothalamic/preoptic region.

Since the injected volume was always 1 μl, the number of micromoles injected is numerically equivalent to the molar concentration of the drug solution.

E = epinephrine bitartrate  NE = norepinephrine bitartrate monohydrate
PE = phenylephrine HCl  ISO = isoproterenol bitartrate dihydrate
C = control solution

Data are derived from all subjects which received at least one microinjection. Figures in parentheses indicate the number of subjects included in each data point. Vertical bracketed lines represent standard errors of the mean.


\[ \Delta T_r \quad (°C) \]

\[ \text{NUMBER OF MICROMOLES INJECTED}^a \quad (\text{LOG SCALE}) \]

\[ 0.003 \quad 0.01 \quad 0.03 \quad 0.10 \]

- E
- NE
- PE
- ISO
- C

\[ (6) \quad (7) \quad (11) \quad (6) \quad (8) \quad (8) \]
TABLE 2
ANALYSIS OF VARIANCE TABLE FOR AT
DATA PRESENTED IN FIGURE 4

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F&lt;sub&gt;.01&lt;/sub&gt;</th>
<th>F&lt;sub&gt;.05&lt;/sub&gt;</th>
<th>F&lt;sup&gt;e&lt;/sup&gt;</th>
<th>F&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drugs</td>
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<td>3.28</td>
<td>4.49</td>
<td>2.91</td>
<td>31.23</td>
<td>.01</td>
</tr>
<tr>
<td>Doses</td>
<td>20.45</td>
<td>3</td>
<td>6.82</td>
<td>4.49</td>
<td>2.91</td>
<td>64.23</td>
<td>.01</td>
</tr>
<tr>
<td>Subjects</td>
<td>2.18</td>
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<td>0.73</td>
<td>4.49</td>
<td>2.91</td>
<td>6.95</td>
<td>.01</td>
</tr>
<tr>
<td>Interaction</td>
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<td>9</td>
<td>0.61</td>
<td>3.05</td>
<td>2.20</td>
<td>5.81</td>
<td>.01</td>
</tr>
<tr>
<td>Error</td>
<td>3.26</td>
<td>31</td>
<td>0.11</td>
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<td></td>
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<td>Total</td>
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<td>49</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a SS = sum of squares.
b DF = degrees of freedom.
c MS = mean square.
d F<sub>.01</sub> and F<sub>.05</sub> = F values which must be exceeded for the source effect to attain significance at the .01 or .05 levels, respectively.
e F = calculated F statistic.
f P = the level at which the source effect is significant. P values > .05 are entered as NS, indicating that the source effect is not significant.
<table>
<thead>
<tr>
<th>Comparison</th>
<th>0.01</th>
<th>0.03</th>
<th>0.10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoproterenol vs Epinephrine</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Isoproterenol vs Norepinephrine</td>
<td>NS</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Isoproterenol vs Phenylephrine</td>
<td>NS</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Phenylephrine vs Norepinephrine</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Phenylephrine vs Epinephrine</td>
<td>NS</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Epinephrine vs Norepinephrine</td>
<td>NS</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

a Duncan's Multiple Range Test.
b Each comparison was made at the three dose levels indicated.
c Micromoles of drug injected.
d * = compared means differ significantly at the 0.05 level.
e NS = compared means are not significantly different at the 0.05 level.
Figure 6. Mean "initial" rate of rectal temperature decrease (50% $\Delta T_p/\Delta T$) produced by microinjection of various doses of sympathomimetic amines into the anterior hypothalamic/preoptic region.

$^{a}50\% \Delta T_p/\Delta T$. The rate of rectal temperature decrease during the time required (AT) for rectal temperature to attain 50 percent of its maximum decrease (50% $\Delta T_p$). This rate serves as an approximation of the true initial rate of decrease. See text for details.

$^{b}$Since the injected volume was always 1 $\mu l$, the number of micromoles injected is numerically equivalent to the molar concentration of the drug solution.

E = epinephrine bitartrate       NE = norepinephrine bitartrate monohydrate
PE = phenylephrine HCl          ISO = isoproterenol bitartrate dihydrate
C = control solution

Data are derived from only the four cats which completed the entire treatment series. Vertical bracketed lines represent standard errors of the mean.
Figure 7. Mean "initial" rate of rectal temperature decrease (50% $\Delta T_r/\Delta T$) produced by microinjection of various doses of sympathomimetic amines into the anterior hypothalamic/preoptic region.

$a^{50\%} \Delta T_r/\Delta T$. The rate of rectal temperature decrease during the time required ($\Delta T$) for rectal temperature to attain 50 percent of its maximum decrease ($50\% \Delta T_r$). This rate serves as an approximation of the true initial rate of decrease. See text for details.

$b$Since the injected volume was always 1 $\mu l$, the number of micromoles injected is numerically equivalent to the molar concentration of the drug solution.

$E =$ epinephrine bitartrate $\quad NE =$ norepinephrine bitartrate monohydrate
$PE =$ phenylephrine HCl $\quad ISO =$ isoproterenol bitartrate dihydrate
$C =$ control solution

Data are derived from all subjects which received at least one microinjection. Figures in parentheses indicate the number of subjects included in each data point. Vertical bracketed lines represent standard errors of the mean.
50% ΔTf/Δt° (°C/HOUR)

NUMBER OF MICROMOLES INJECTED
(LOG SCALE)

- E
- NE
- PE
- ISO
- C

(3)
(6)
(7)
(10)
(6)
(10)
(7)
(8)
(5)
(5)
(6)
(8)
### TABLE 4

ANALYSIS OF VARIANCE TABLE FOR 50% ΔT /ΔT
DATA PRESENTED IN FIGURE 6

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F .01</th>
<th>F .05</th>
<th>F^e</th>
<th>P^f</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drugs</td>
<td>58.72</td>
<td>3</td>
<td>19.57</td>
<td>4.51</td>
<td>2.92</td>
<td>10.31</td>
<td>.01</td>
</tr>
<tr>
<td>Doses</td>
<td>104.23</td>
<td>3</td>
<td>34.74</td>
<td>4.51</td>
<td>2.92</td>
<td>18.30</td>
<td>.01</td>
</tr>
<tr>
<td>Subjects</td>
<td>27.87</td>
<td>3</td>
<td>9.29</td>
<td>4.51</td>
<td>2.92</td>
<td>4.89</td>
<td>.01</td>
</tr>
<tr>
<td>Interaction</td>
<td>28.73</td>
<td>9</td>
<td>3.19</td>
<td>3.07</td>
<td>2.21</td>
<td>1.68</td>
<td>NS</td>
</tr>
<tr>
<td>Error</td>
<td>56.93</td>
<td>30</td>
<td>1.90</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>276.48</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a SS = sum of squares.
b DF = degrees of freedom.
c MS = mean square.
d F .01 and F .05 = F values which must be exceeded for the source effect to attain significance at the .01 or .05 levels, respectively.
e F = calculated F statistic.
f P = the level at which the source effect is significant. P values > .05 are entered as NS, indicating that the effect is not significant.
<table>
<thead>
<tr>
<th>Comparison</th>
<th>0.01</th>
<th>0.03</th>
<th>0.10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoproterenol vs Epinephrine</td>
<td>NS(^d)</td>
<td>*(^e)</td>
<td>*</td>
</tr>
<tr>
<td>Isoproterenol vs Norepinephrine</td>
<td>NS</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Isoproterenol vs Phenylephrine</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Phenylephrine vs Norepinephrine</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Phenylephrine vs Epinephrine</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Epinephrine vs Norepinephrine</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^a\) Duncan's Multiple Range Test.

\(^b\) Each comparison was made at the three dose levels indicated.

\(^c\) Micromoles of drug injected.

\(^d\) NS = compared means are not significantly different at the 0.05 level.

\(^e\) * = compared means differ significantly at the 0.05 level.
clear trend for phenylephrine to be more potent than isoproterenol. It also appears that norepinephrine and epinephrine are approximately equipotent. Thus, the potency series for this parameter appears to be: epinephrine = norepinephrine > phenylephrine > isoproterenol.

Very similar trends for the estimate of average rate of rectal temperature fall (90% \( \Delta T_r/\Delta T \)) can be seen in Figures 8 and 9 and Tables 6 and 7. Again the effect is clearly dose dependent. At all doses tested, epinephrine and norepinephrine produced significantly greater average rates of fall than did isoproterenol. At the low and high doses, these two agents are also more active than phenylephrine. However, there is no significant difference between epinephrine and norepinephrine at any dose, nor is there any suggestion of one in the figures. On the other hand, although phenylephrine and isoproterenol are also not significantly different, both Figures 8 and 9 indicate a trend for phenylephrine to be more potent. Therefore, again we see the relative potency series: epinephrine = norepinephrine > phenylephrine > isoproterenol.

Examination of the EMG data in Figures 10 and 11 and Tables 8 and 9 reveals that this parameter is also dose dependent. At all dose levels, epinephrine and norepinephrine reduce electromyographic activity to a significantly greater degree than isoproterenol. At the intermediate dose, epinephrine and norepinephrine are significantly more suppressive than phenylephrine. However, there are no significant differences between epinephrine and norepinephrine or phenylephrine and isoproterenol, nor are any trends apparent in the dose-response plots. Thus, the best that can be said with regard to the relative
Figure 8. Mean "average" rate of rectal temperature decrease (90% $\Delta T_r/\Delta t$) produced by microinjection of various doses of sympathomimetic amines into the anterior hypothalamic/preoptic region.

$^a$90% $\Delta T_r/\Delta t$. The rate of rectal temperature decrease during the time required ($\Delta t$) for rectal temperature to attain 90 percent of its maximal decrease (90% $\Delta T_r$). See text for details.

$^b$Since the injected volume was always 1 ul, the number of micromoles injected is numerically equivalent to the molar concentration of the drug solution.

E = epinephrine bitartrate   NE = norepinephrine bitartrate monohydrate
PE = phenylephrine HCl      ISO = isoproterenol bitartrate dihydrate
C = control solution

Data are derived from only the four cats which completed the entire treatment series. Vertical bracketed lines represent standard errors of the mean.
Figure 9. Mean "average" rate of rectal temperature decrease (90% $\Delta T_r/\Delta T$) produced by microinjection of various doses of sympathomimetic amines into the anterior hypothalamic/preoptic region.

$^{a}90\% \Delta T_r/\Delta T$. The rate of rectal temperature decrease during the time required ($\Delta T$) for rectal temperature to attain 90 percent of its maximum decrease (90% $\Delta T_r$). See text for details.

$^{b}$Since the injected volume was always 1 $\mu l$, the number of micromoles injected is numerically equivalent to the molar concentration of the drug solution.

E = epinephrine bitartrate  ME = norepinephrine bitartrate monohydrate
PE = phenylephrine HCl  ISO = isoproterenol bitartrate dihydrate
C = control solution

Data are derived from all subjects which received at least one microinjection. Figures in parentheses indicate the number of subjects included in each data point. Vertical bracketed lines represent standard errors of the mean.
### TABLE 6

**ANALYSIS OF VARIANCE TABLE FOR 90% ΔΤ/ΔΤ**

*DATA PRESENTED IN FIGURE 8*

<table>
<thead>
<tr>
<th>Source</th>
<th>SS^a</th>
<th>DF^b</th>
<th>MS^c</th>
<th>F_01</th>
<th>F_05</th>
<th>Fe</th>
<th>Pf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drugs</td>
<td>26.09</td>
<td>3</td>
<td>8.70</td>
<td>4.51</td>
<td>2.92</td>
<td>14.46</td>
<td>.01</td>
</tr>
<tr>
<td>Doses</td>
<td>37.55</td>
<td>3</td>
<td>12.52</td>
<td>4.51</td>
<td>2.92</td>
<td>20.81</td>
<td>.01</td>
</tr>
<tr>
<td>Subjects</td>
<td>12.47</td>
<td>3</td>
<td>4.16</td>
<td>4.51</td>
<td>2.92</td>
<td>6.91</td>
<td>.01</td>
</tr>
<tr>
<td>Interaction</td>
<td>9.77</td>
<td>9</td>
<td>1.09</td>
<td>3.07</td>
<td>2.21</td>
<td>1.81</td>
<td>NS</td>
</tr>
<tr>
<td>Error</td>
<td>18.05</td>
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<td>0.60</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>103.93</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a SS = sum of squares.  
^b DF = degrees of freedom.  
^c MS = mean square.  
^d F_01 and F_05 = F values which must be exceeded for the source effect to attain significance at the .01 or .05 levels, respectively.  
^e F = calculated F statistic.  
^f P = the level at which the source effect is significant. P values > .05 are entered as NS, indicating that the source effect is not significant.
TABLE 7

TESTS FOR SIGNIFICANCE OF DIFFERENCES BETWEEN INDIVIDUAL MEANS FOR 90% ΔT_x/ΔT DATA PRESENTED IN FIGURE 8a

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Dose</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.01</td>
<td>0.03</td>
<td>0.10</td>
</tr>
<tr>
<td>Isoproterenol vs Epinephrine</td>
<td>*d</td>
<td>*</td>
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</tr>
<tr>
<td>Isoproterenol vs Norepinephrine</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Isoproterenol vs Phenylephrine</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Phenylephrine vs Norepinephrine</td>
<td>*</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>Phenylephrine vs Epinephrine</td>
<td>*</td>
<td>NS</td>
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<tr>
<td>Epinephrine vs Norepinephrine</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

a Duncan's Multiple Range Test.
b Each comparison was made at the three dose levels indicated.
c Micromoles of drug injected.
d * = compared means differ significantly at the 0.05 level.
e NS = compared means are not significantly different at the 0.05 level.
Figure 10. Mean percentage decrease in electromyographic activity of the lateral thigh (ΔEMG) produced by microinjection of various doses of sympathomimetic amines into the anterior hypothalamic/preoptic region.

ΔEMG. Percentage reduction in electromyographic activity from preinjection baseline levels. See text for details.

bSince the injected volume was always 1 μl, the number of micromoles injected in numerically equivalent to the molar concentration of the drug solution.

E = epinephrine bitartrate  NE = norepinephrine bitartrate monohydrate
PE = phenylephrine HCl  ISO = isoproterenol bitartrate dihydrate
C = control solution

Data are derived from only the four cats which completed the entire treatment series. Vertical bracketed lines represent standard errors of the mean.
AEMG (%)

NUMBER OF MICROMOLES INJECTED (LOG SCALE)

ΔEMG (°)

(%)
Figure 11. Mean percentage decrease in electromyographic activity of the lateral thigh ($\Delta$EMG) produced by microinjection of various doses of sympathomimetic amines into the anterior hypothalamic/preoptic region.

$^a$AEMG. Percentage reduction in electromyographic activity from preinjection baseline levels. See text for details.

$^b$Since the injected volume was always 1 $\mu$l, the number of micromoles injected is numerically equivalent to the molar concentration of the drug solution.

$E = $ epinephrine bitartrate  $NE = $ norepinephrine bitartrate monohydrate
$PE = $ phenylephrine HCl  $ISO = $ isoproterenol bitartrate dihydrate
$C = $ control solution

Data are derived from all subjects which received at least one microinjection. Figures in parentheses indicate the number of subjects included in each data point. Vertical bracketed lines represent standard errors of the mean.
NUMBER OF MICROMOLES INJECTED *
(LOG SCALE)

∆EMG (%)
TABLE 8
ANALYSIS OF VARIANCE TABLE FOR $\Delta$EMG
DATA PRESENTED IN FIGURE 10

<table>
<thead>
<tr>
<th>Source</th>
<th>SS^a</th>
<th>DF^b</th>
<th>MS^c</th>
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<th>F .05</th>
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</thead>
<tbody>
<tr>
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<td>2.94</td>
<td>25.81</td>
<td>0.01</td>
</tr>
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<td>Interaction</td>
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<td>656</td>
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<td>46</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

a SS = sum of squares.
b DF = degrees of freedom.
c MS = mean square.
d $F_{.01}$ and $F_{.05}$ = F values which must be exceeded for the source effect to attain significance at the .01 or .05 levels, respectively.
e $F$ = calculated F statistic.
f $P$ = the level at which the source effect is significant. $P$ values > .05 are entered as NS, indicating that the source effect is not significant.
**TABLE 9**

TESTS FOR SIGNIFICANCE OF DIFFERENCES BETWEEN INDIVIDUAL MEANS FOR ΔEMG DATA PRESENTED IN FIGURE 10a.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Dose 0.01</th>
<th>Dose 0.03</th>
<th>Dose 0.10</th>
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</thead>
<tbody>
<tr>
<td>Isoproterenol vs Epinephrine</td>
<td>#d</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Isoproterenol vs Norepinephrine</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Isoproterenol vs Phenylephrine</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Phenylephrine vs Norepinephrine</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Phenylephrine vs Epinephrine</td>
<td>*</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Epinephrine vs Norepinephrine</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*a_ Duncan's Multiple Range Test.*
*b_ Each comparison was made at the three dose levels indicated.*
*c_ Micromoles of drug injected.*
*d_ # = compared means differ significantly at the 0.05 level.*
*e_ NS = compared means are not significantly different at the 0.05 level.*
ability of the sympathomimetic amines to reduce shivering is that epinephrine = norepinephrine > phenylephrine > isoproterenol.

In contrast to rectal temperature and electromyographic activity, which change in a graded fashion following microinjection of sympathomimetic amines, changes in ear skin temperature were quantal. The ear skin in all cats prior to injection was always vasoconstricted, and ear skin temperature was 1-2°C above the ambient temperature of 20°C. Microinjection of a monoamine produced one of two responses: there was either a slight (< 2°C) or no change in ear skin temperature, or the ear vasodilated maximally, yielding an increase in ear temperature of more than 8°C. Occasionally, the two types of response were combined so that T_e drifted slowly upward by 1 or 2°C and then suddenly rose the remaining 7 or more degrees in 1 or 2 minutes. Vasodilation intermediate between 2 and 8°C was never observed.

Because of the all-or-none nature of the vasodilatory response, the data concerning this parameter have been handled in a somewhat different manner. Figure 12 portrays the percentage and proportion of subjects vasodilating maximally after microinjection of monoamines into the AH/PO region (all 12 cats are included in the figure). Figure 13 portrays the same data but includes only the "complete" cats. It is clear from these figures that isoproterenol is decidedly inferior to the other three amines with respect to producing vasodilation. Unfortunately, because of the non-normal distribution of the data population and the fact that the data presented in Figure 11 are partly dependent and partly independent (i.e., some cats were common to each treatment group and some were not), it was difficult to
Figure 12. Percentage of animals vasodilating in response to microinjection of various doses of sympathomimetic amines into the anterior hypothalamic/preoptic region.

aVasodilation was defined as an increase in ear skin temperature of 8°C or more. See text.

bSince the injected volume was always 1 μl, the number of micromoles injected is numerically equivalent to the molar concentration of the drug solution.

E = epinephrine bitartrate  NE = norepinephrine bitartrate monohydrate
PE = phenylephrine HCl  ISO = isoproterenol bitartrate dihydrate
C = control solution

Data are derived from all subjects which received at least one microinjection. Figures inside or above the vertical bars indicate the number of animals vasodilating over the number tested.
Figure 13. Percentage of animals vasodilating in response to microinjection of various doses of sympathomimetic amines into the anterior hypothalamic/preoptic region.

\textsuperscript{a}Vasodilation was defined as an increase in ear skin temperature of $\geq 0^\circ C$ or more. See text.

\textsuperscript{b}Since the injected volume was always 1 $\mu l$, the number of micromoles injected is numerically equivalent to the molar concentration of the drug solution.

$E$ = epinephrine bitartrate \hspace{1cm} $NE$ = norepinephrine bitartrate monohydrate
$PE$ = phenylephrine $HCl$ \hspace{1cm} $ISO$ = isoproterenol bitartrate dihydrate
$C$ = control solution

Data are derived from only the four cats which completed the entire treatment series.
test this intuitive conclusion. However, the nonparametric "binomial test" (Siegel, 1956) indicates that, at the 95% level of confidence, there is a significant difference between the number of animals vasodilating after microinjection of epinephrine and isoproterenol at the 0.03 molar concentrations.¹ No differences could be demonstrated at the low and high dose. No other comparisons were attempted.

The effect of microinjected sympathomimetic amines on the rate and depth of respiration were not marked. Usually, respiratory rate was unchanged or slightly decreased. Frequently, the rhythm of respiration became more regular; this effect was often associated with sedation and EMG suppression. Thus, the effect may not have been a direct one but may have been engendered by reduced disruption of the respiratory rhythm by voluntary movements, shifts in attentional state and bouts of shivering invading the thoracic musculature. One subject (SA-4), however, responded with increased respiratory rate after microinjections of 0.03 or 0.10 molar epinephrine or 0.10 molar norepinephrine and phenylephrine. Interestingly, isoproterenol, even at the 0.10 molar concentration, did not increase respiratory rate in this animal. In each instance, the maximum rate obtained was 100-110 respirations/minute, but there was never any sign of open-mouthed

¹ The binomial test is valid only for related samples. In the present context, this means that only data from animals common to both the epinephrine and isoproterenol treatment groups shown in Figure 11 could be used. At the 0.01 molar dose level, 3 animals were common; at the 0.03 level, 6 animals; and at the 0.10 level, 6 animals. The proportion of these subjects which vasodilated after the various isoproterenol and epinephrine treatments was: E (0.01) 2/3, ISO (0.01) 0/3; E (0.03) 5/6, ISO (0.03) 0/6; E (0.10) 6/6, ISO (0.10) 2/6.
panting. The onset of this tachypnea was gradual, the maximum rate occurring at 20 minutes after the injection of 0.10 molar epinephrine or norepinephrine and at 60 and 90 minutes after injection of 0.03 molar epinephrine and 0.10 molar phenylephrine, respectively.

In addition to the various parameters previously discussed, the latency from the time of drug injection to the onset of these responses was measured (see Methods). The mean, standard error and range of these latencies are summarized in Table 10. No statistical treatment was attempted.

Although no conclusions can be drawn from this table, examination of the data for each individual experimental session revealed that, with few exceptions, the first parameter to be affected by a microinjection was shivering. In 32 of the 81 experimental sessions listed (39.5%), the order in which the parameters were affected was EMG, $T_e$, $T_r$. In another 7 sessions (8.6%), EMG suppression and vasodilation occurred simultaneously, and rectal temperature began to fall shortly thereafter. In 18 instances (22.2%), shivering was reduced and rectal temperature began to fall before vasodilation occurred, and in 13 experiments, (16.0%), no vasodilation was seen, even though shivering was reduced. In 1 session (1.2%), shivering was impaired but no vasodilation or hypothermia ensued. Thus, in 71 of 81 experiments (87.6%), the electromyogram was affected prior to or simultaneously with ear temperature, or no change in ear temperature occurred.

In 4 of the remaining 10 experiments, although EMG suppression preceded vasodilation, rectal temperature began to fall before any detectable change in EMG activity had occurred. Vasodilation caused
<table>
<thead>
<tr>
<th>Parametera</th>
<th>Drug</th>
<th>Dose (pmoles)</th>
<th>Nᵇ</th>
<th>Mean latency (minutes)</th>
<th>SEᶜ</th>
<th>Range (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectal</td>
<td>Eᵈ</td>
<td>0.003</td>
<td>3</td>
<td>3.83</td>
<td>1.09</td>
<td>2.5-6.0</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>6</td>
<td>4.18</td>
<td>0.81</td>
<td>2.5-8.0</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>4.02</td>
<td>0.82</td>
<td>1.0-12.0</td>
</tr>
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<td></td>
<td></td>
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<td>3.99</td>
<td>0.82</td>
<td>1.7-7.0</td>
</tr>
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<td>-----</td>
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<td>4.0-14.0</td>
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<td>9.0-11.5</td>
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<td>2.00</td>
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<td>-----</td>
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</table>

**TABLE 10**

LATENCIES TO ONSET OF DRUG-INDUCED CHANGES IN RECTAL TEMPERATURE, EAR SKIN TEMPERATURE AND ELECTROMYOGRAPHIC ACTIVITY
**TABLE 10 (CONTINUED)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Drug</th>
<th>Dose (µmoles)</th>
<th>N</th>
<th>Mean latency (minutes)</th>
<th>SE  (minutes)</th>
<th>Range (minutes)</th>
</tr>
</thead>
<tbody>
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<td>Electromyographic activity</td>
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<td>2.47</td>
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<td>6</td>
<td>3.02</td>
<td>0.63</td>
<td>1.3-5.2</td>
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<td>12</td>
<td>2.47</td>
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<td>0.4-6.0</td>
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<td>0.72</td>
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</tr>
<tr>
<td>ISO</td>
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<td>0</td>
<td>----</td>
<td>----</td>
<td>6.4-8.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>2</td>
<td>7.20</td>
<td>0.80</td>
<td>6.4-8.0</td>
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</tr>
<tr>
<td></td>
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<td>6</td>
<td>2.23</td>
<td>0.85</td>
<td>0.8-6.4</td>
<td></td>
</tr>
</tbody>
</table>

a See text for definition of "latency" for each parameter.
b N = number of animals included in the mean.
c SE = standard error of the mean.
d E = epinephrine bitartrate   NE = norepinephrine bitartrate monohydrate   PE = phenylephrine HCl   ISO = isoproterenol bitartrate dihydrate.
a fall in rectal temperature in the absence of detectable changes in shivering in 2 sessions, and, in another 2 experiments, vasodilation preceded EMG suppression. To complete the list of permutations, in one experiment, although there was considerable EMG suppression followed by vasodilation, rectal temperature failed to decrease; in another, there was a decrease in rectal temperature, but no detectable change in shivering or ear temperature.

The durations of the effect of microinjected sympathomimetic amines on $T_r$, $T_e$ and EMG were also computed and are presented in Table 11. Because of the great variability between cats, only the mean and range are given. However, after examination of the various effects within each experimental session, the relationship becomes clearer. Seventy-two sessions provided reliable data for the duration of at least 2 of the 3 parameters ($T_r$, $T_e$ and EMG). In 37 experiments (52.8%), EMG began to recover most rapidly from the effects of the microinjection. In another 20 sessions (27.8%), there was no vasodilation, but EMG began to recover before rectal temperature began to return toward baseline. EMG suppression and vasodilation had identical durations in 19 experiments (12.6%), and, in 1 experiment (1.4%), all 3 parameters had the same duration. In 3 sessions (4.2%), vasodilation occurred, but no EMG reduction was observed; in these instances, the beginning of the return of rectal temperature toward baseline was coincident with vasoconstriction. Finally, there were 4 clear-cut examples (5.6%) of vasodilation having a shorter duration than EMG suppression.
TABLE 11

DURATION OF DRUG-INDUCED CHANGES IN RECTAL TEMPERATURE, EAR SKIN TEMPERATURE AND ELECTROMYOGRAPHIC ACTIVITY

<table>
<thead>
<tr>
<th>Parametera</th>
<th>Drug</th>
<th>Dose (μmoles)</th>
<th>Nb</th>
<th>Mean duration (minutes)</th>
<th>Range (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectal temperature</td>
<td>E</td>
<td>0.003</td>
<td>3</td>
<td>36.3</td>
<td>14.5-60.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01</td>
<td>6</td>
<td>45.8</td>
<td>30.0-96.0</td>
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<tr>
<td></td>
<td></td>
<td>0.03</td>
<td>11</td>
<td>71.5</td>
<td>28.0-105.0</td>
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<td>6</td>
<td>96.3</td>
<td>65.0-143.0</td>
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<tr>
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<td>NE</td>
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<td>6</td>
<td>41.1</td>
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<td>6</td>
<td>73.5</td>
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<tr>
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<td>PE</td>
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<td>5</td>
<td>72.5</td>
<td>60.0-95.5</td>
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<td>Mean duration (minutes)</td>
<td>Range (minutes)</td>
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a See text for definition of "duration" for each parameter.
b N = number of animals included in the mean.
c E = epinephrine bitartrate  NE = norepinephrine bitartrate monohydrate  PE = phenylephrine HCl  ISO = isoproterenol bitartrate dihydrate.
Two phenomena of non-thermoregulatory nature, sedation and defecation, were frequently observed after microinjections of sympathomimetic amines. In many sessions, sedation could not be assessed because the subjects were resting or sleeping prior to the microinjection. However, if the subjects were vocalizing or attending to the environment, the sedative effect could be readily observed. In several instances, the effect resembled the onset of anesthesia after an intravenously administered barbiturate. Before the end of the 2-minute microinjection period, the subjects' eyes closed and the head dropped slowly to the ground, the body becoming limp. In other cases, the animal simply assumed a resting posture and remained motionless for many minutes with eyes closed or half-closed. Sedation typically persisted until shortly before the time of maximum hypothermia. At this time, the animals usually arose and became very restless for several minutes. This period of activity was usually followed by mild sedation during which the cat shivered vigorously to return body temperature to normal levels.

The early phase of intense sedation was temporally correlated with inhibition of shivering. In addition, the depth of sedation appeared to be related to the magnitude of shivering inhibition. Careful observation revealed that the first sign of return of EMG activity was usually very close to the first indication of behavioral "awakening." It should be pointed out that there was no paralysis during the period of sedation and EMG suppression, nor were these phenomena related to the loss of muscle tone reportedly found during paradoxical sleep. Even during the period when the electromyogram
was flat down to 1 µV and the cat very unresponsive to stimulation, occasional spontaneous head movements, opening of the eyes or weak meowing were seen. On several occasions the sedation-EMG suppression phenomenon was interrupted by shifts of position during which the cat alerted slightly and EMG activity was recorded. The EMG activity, however, was obviously related to the voluntary movements; there was no sign of shivering activity.

Because no attempt was made to quantitate sedation, no definitive statement can be made concerning the relative sedative activity of the 4 monoamines. However, it is the author's impression that these activities are similar to the potencies of the amines as hypothermic agents.

In spite of the food deprivation schedule (see Methods), defecation was seen commonly after microinjection of the 0.03 and 0.10 molar concentrations of epinephrine and norepinephrine; phenylephrine and isoproterenol did not elicit defecation in these deprived animals. Usually the feces were liquid or semi-liquid, indicating intense peristaltic activity. The most common time of occurrence was near the time of maximum hypothermia, but defecation was also observed shortly after the microinjection or as long as several hours later. If defecation occurred during the period of sedation previously discussed, the cat usually gave no behavioral indication of his awareness of this. However, defecation after sedation had subsided usually caused great excitement and intense EMG activity as the animal assumed the typical posture. It seems possible that the restlessness commonly observed near the time maximum hypothermia in non-defecating
cats may have been due to intense peristaltic activity, even though there was no actual evacuation.

Histologic examination of the brains of the animals participating in the dose-response studies revealed that all injection sites lay within the tissue volume delineated by the stereotaxic coordinates A (12.3-15.0), L (1.0-3.0), H (-3.0)-(5.0), according to the atlas of Snider and Niemer (1961). (A complete listing of these sites may be found in Table 13). Thus, all the sites lay within the anterior hypothalamic/preoptic region.

Tissue destruction at the site of injection varied widely between animals. A few of the injection sites are illustrated in Plate II. The damage seen in "A" of Plate II was the most severe encountered in the entire series of animals. The edema and demyelinization are due to the formation of a septic abscess at the injection site. The most smaller lesions depicted in "B" and "C" are more typical of the damage found in most of the subjects. Minimal destruction of the tissue at the injection site is portrayed in "D."

Pretreatment with Alpha- and Beta-Receptor Antagonists

Six animals began the antagonist experiments, but 3 developed irreversible decreases in site sensitivity after injection of phentolamine. However, in the remaining 3 subjects, phentolamine produced a substantial and reversible blockade of epinephrine-induced hypothermia. Figure 14 illustrates this effect. Note the reproducibility of the control injections, and that in all 3 subjects, good sensitivity to epinephrine was demonstrable the week following the injection of phentolamine.
Plate II. Photographs of frontal sections containing the microinjection sites of cats SA-18 (A), SA-5 (B), SA-8 (C) and SA-6 (D).
Figure 14. Change in rectal temperature elicited by intrahypothalamic microinjection of epinephrine after pretreatment with phentolamine.

Data derived from 3 cats (SA-10, SA-9, SA-1) are presented. Normal saline (in experiments labeled C₁ and C₂) or 100 µg phentolamine methanesulphonate (in experiments labeled PTA) was microinjected into the AH/PO region at the first arrow and 0.02 µmole of epinephrine bitartrate given 15 minutes later at the second arrow. At least 5 days intervened between consecutive treatments in a given subject. The treatment sequence was C₁, PTA, C₂. Cat SA-1 did not receive the first control treatment. See text for details.
CHANGE IN RECTAL TEMPERATURE (°C)
The effect of phentolamine pretreatment on epinephrine-induced changes in the other thermoregulatory parameters is given in Table 12. In each of the 3 cats, phentolamine prevented vasodilation. However, the effect on shivering was equivocal; EMG suppression was completely blocked in one cat, partially blocked in another and little affected in the third. Both measures of rate of fall were clearly reduced.

Figure 15 and Table 12 illustrate the effect of the beta-receptor antagonist, propranolol, on epinephrine-induced hypothermia in cats SA-9 and SA-1 (cat SA-10 displaced his injection guide before the effect of propranolol could be tested). Again, the reproducability of the control injections given the weeks before and after injection of propranolol should be noted. Although propranolol appeared to be less effective than phentolamine in blocking the hypothermic response to epinephrine, the drug did produce partial reduction in $\Delta T_r$ in cat SA-1. In both subjects, the indicators of rate of fall were reduced by propranolol pretreatment, but to a lesser extent than by phentolamine. The effects on vasodilation and EMG suppression were inconclusive; EMG suppression was partially blocked in one subject but vasodilation was unaffected, whereas in the other subject, EMG suppression was unaffected, but vasodilation was prevented.

The effect of microinjection of phentolamine, propranolol and saline alone are illustrated in Figure 16. Each of these agents appears to produce a slow increase in rectal temperature. There was no detectable effect on shivering or ear skin temperature.
Figure 15. Change in rectal temperature elicited by intrahypothalamic microinjection of epinephrine after pretreatment with propranolol.

Data derived from 2 cats (SA-9 and SA-1) are presented. Normal saline (in experiments labeled C1 and C2) or 78.4 μg propranolol HCl (in experiments labeled PP) was microinjected into the AH/PO region at the first arrow and 0.02 μmole of epinephrine bitartrate given 15 minutes later at the second arrow. At least 5 days intervened between consecutive treatments in a given subject. The treatment sequence was C1, PP, C2. See text for details.
CHANGE IN RECTAL TEMPERATURE (°C)
Figure 16. Change in rectal temperature elicited by intrahypothalamic microinjection of saline, phentolamine, or propranolol.

Data derived from 2 cats (SA-9 and SA-1) are presented. At the arrow, normal saline (C), 100 μg phentolamine methanesulphonate (PTA) or 78.4 μg propranolol HCl (PP) was microinjected into the AH/PO region. At least 5 days intervened between injections. The treatment sequence was C, PTA, PP. See text for details.
### TABLE 12
THE EFFECT OF PHENTOLAMINE OR PROPRANOLOL PRETREATMENT ON EPINEPHRINE-INDUCED ALTERATIONS OF THERMOREGULATION

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<thead>
<tr>
<th>Cat</th>
<th>Treatment</th>
<th>$\Delta T_r$</th>
<th>$50% \Delta T_r$</th>
<th>$90% \Delta T_r$</th>
<th>$\Delta T_e$</th>
<th>$\Delta EMG$</th>
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<td>SA-1</td>
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<td>3.38</td>
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a Normal saline, 100 µg phentolamine methanesulphonate, or 78.6 µg propranolol HCl was microinjected into the AH/PO region 15 minutes prior to microinjection of 0.02 µmoles epinephrine bitartrate at the same site. Treatments were administered in the order listed.
b $\Delta T_r$ = maximum decrease in rectal temperature in °C.
c $50\% \Delta T_r/\Delta T$ = rate of rectal temperature decrease during the time required ($\Delta T$) for rectal temperature to attain 50 percent of its maximum decrease ($50\% \Delta T_r$). See text.
d $90\% \Delta T_r/\Delta T$ = rate of rectal temperature decrease during the time required ($\Delta T$) for rectal temperature to attain 90 percent of its maximum decrease ($90\% \Delta T_r$). See text.
e $\Delta T_e$ = maximum increase in ear skin temperature in °C.
f $\Delta EMG$ = percentage reduction in electromyographic activity of the lateral thigh. See text.
"Mapping" of the hypothalamus

The effects of intracerebral microinjection of 0.02-0.03 µmole of epinephrine bitartrate into various diencephalic loci are presented in Table 13 and Figures 17, 18 and 19. With few exceptions, these injections produced one of three types of response: 1) vasodilation of the ear skin, suppression of shivering and hypothermia 2) suppression of shivering and hypothermia in the absence of vasodilation 3) no effect on ear skin temperature, shivering or rectal temperature.

Invariably, injection into the tissue bounded by the coordinates A (13.5)-(15.5), L (1.0)-(3.0), H (-2.0)-(5.5) (10 sites) produced vasodilation, EMG suppression and hypothermia. These coordinates encompass most of the lateral and medial preoptic region.

All injections into the tissue bounded by the coordinates A (12.3)-(13.4), L (1.2)-(2.5), H (-3.0)-(-4.0) (12 sites), i.e., the anterior hypothalamic region, elicited EMG suppression and hypothermia. However, vasodilation was not always seen.

Microinjections of epinephrine into the lateral tuberal or lateral posterior hypothalamic regions bounded by the coordinates A (7.5)-(10.5), L (2.0)-(4.5), H (-2.0)-(-3.5) (6 sites) had no effect on the parameters measured. Single injections into the hypothalamic-midbrain junctional region between the habenulo-interpeduncular tract and the red nucleus [ A (6.0), L (2.0), H (-3.0)], or just lateral to the central grey [ A (6.2), L (2.0), H (0.0)], were also ineffective, as was an injection into the lateral habenular nucleus [ A (7.5), L (2.0), H (+4.0)].

Thus, most of the microinjection data indicate that there exists
within the hypothalamus a sensitivity gradient with respect to
catecholamine-induced hypothermia, injection into sites within the
AH/PO region producing a maximum response and injections into the
posterior region producing no effect. However, not all the experiments
supported such a clear-cut concept. For example, EMG suppression and
hypothermia were produced by injection of epinephrine into the anterior
thalamus [A (13.0), L (1.5), H (-3.0)], the lateral tuberal area [A
(11.0), L (2.0), H (-4.5)] and the posterior hypothalamus [A (8.0),
L (1.0), H (-4.5)]. At one posterior hypothalamic site, [A (9.0),
L (1.5), H (-2.0)], the response produced was in every respect similar
to that elicited by injection into the AH/PO region.

Finally, a few examples of responses not fitting any of the
three previously mentioned categories were seen. Injection into the
tuberal hypothalamus [A (11.5), L (2.0), H (-4.5)] produced an immedi­
ate and profound, but short-lasting decrease in EMG activity and a
small fall in rectal temperature. This was followed by massive shiver­
ing and hyperthermia of more than 1.0 degree. Injections into the ex­
treme lateral posterior hypothalamus [A (7.5), L (3.5-4.0), H (-4.5)]
produced, after a 13 to 20 minute delay, behavior strongly indicative
of paradoxical sleep, i.e., assumption of sleep posture, relaxation
of the body, rapid and complete loss of electromyographic activity, and
a sudden return of this activity 8 minutes later simultaneously with
behavioral signs of arousal (see footnotes h and i of Table 13 for a
complete description of this effect).
Figures 17 and 18. Serial frontal sections through the cat brain illustrating the approximate locations of sites stimulated with epinephrine and the thermoregulatory responses produced.

Each site was tested with one or more microinjections of 0.02 or 0.03 molar epinephrine bitartrate. Consult Table 13 for exact coordinates of injection sites.

Solid circles represent sites at which epinephrine produced reduction in electromyographic activity, peripheral vasodilation and a fall in rectal temperature of 0.25°C or more.

Open circles represent sites at which epinephrine produced reduction in electromyographic activity and a fall in rectal temperature of 0.25°C or more, but not vasodilation.

Triangles represent sites at which epinephrine produced no change in electromyographic activity, peripheral vasomotor tone or rectal temperature.

Neuroanatomical abbreviations:

- ac—Anterior commissure
- cau—Caudate nucleus
- cg—Central grey of midbrain
- cm—Central medial thalamic nuc.
- cp—Cerebral peduncle
- db—Diagonal band of Broca
- en—Endopuduncular nucleus
- f—Fornix
- ff—Field of Forel
- gp—Globus pallidus
- hi—Habenulo-Interpeduncular tract
- ic—Internal capsule
- in—Interstitial nucleus of Cajal
- lh—Lateral habenular nucleus
- lgn—Lateral geniculate nucleus
- m—Mammillothalamic tract
- md—Medial dorsal thalamic nuc.
- mgn—Medial geniculate nucleus
- mm—Medial mammillary nucleus
- nr—Red nucleus
- oc—Optic chiasm
- ot—Optic tract
- pc—Posterior commissure
- ph—Posterior hypothalamic area
- pu—Putamen
- re—Reuniens nucleus
- rm—Reticular nucleus of thalamus
- s—Septal region
- sn—Substantia nigra
- va—Ventral ant. thalamic nucleus
- vm—Ventral medial hypothalamic nuc.
- zi—Zona incerta
- 3—Oculomotor nerve
Figure 19. Parasagittal projection of the cat brain illustrating the approximate locations of sites stimulated with epinephrine and the thermoregulatory responses produced.

Each site was tested with one or more microinjections of 0.02-0.03 molar epinephrine bitartrate. Site loci are projected on a parasagittal section 1.2 mm lateral to the midline.

Solid circles represent sites at which epinephrine produced reduction in electromyographic activity, peripheral vasodilation and a fall in rectal temperature of 0.25°C or more.

Open circles represent sites at which epinephrine produced reduction in electromyographic activity and a fall in rectal temperature of 0.25°C or more, but not vasodilation.

Triangles represent sites at which epinephrine produced no change in electromyographic activity, peripheral vasomotor tone or rectal temperature.

AC—anterior commissure
ACC—nucleus accumbens
AM—anteromedial nucleus
CM—central medial nucleus
DH—dorsal hypothalamic nucleus
DMH—dorsomedial hypothalamic nucleus
F—fornix
haa—anterior hypothalamic area
hda—dorsal hypothalamic area
HM—medial habenular nucleus
hpa—posterior hypothalamic area
MC—mammillary complex
MD—mediodorsal thalamic nucleus
MV—medioventral nucleus
NR—red nucleus
OC—optic chiasm
PAG—periaqueductal grey
PC—posterior commissure
ptm—medial pretectal area
SC—superior colliculus
SLN—lateral septal nucleus
SM—striate medullaris thalami
V 3—3rd ventricle
VM—ventromedial thalamic nucleus
VMH—ventromedial hypothalamic nucleus
TABLE 13
THERMOREGULATORY EFFECTS OF 0.02-0.03 MOLAR EPINEPHRINE BITARTRATE
MICROINJECTED INTO VARIOUS DIENCEPHALIC LOCI\textsuperscript{a}

<table>
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<tr>
<th>Cat</th>
<th>Injection coordinates\textsuperscript{b}</th>
<th>( \Delta EMG )</th>
<th>( \Delta T_e )</th>
<th>( \Delta T_r )</th>
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<tr>
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### TABLE 13 (CONTINUED)

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</table>

a Sites in cats SA-8 and SA-9 received 0.02 µmole in 1 µl. All other sites received 0.03 µmole in 1 µl.

b According to the coordinate system and stereotaxic atlas of Snider and Niemer (1961). All site locations were determined with respect to the Weil stained plates in this atlas. A, L and H refer to the distance in millimeters anterior to the interaural line, lateral to the midsagittal plane and above or below the horizontal zero plane, respectively.

c ΔEMG = percentage reduction in electromyographic activity of the lateral thigh as compared to a preinjection baseline. See text.

d ΔT<sub>e</sub> 0 indicates that ear skin temperature increased by less than 2.0°C. + indicates that ear skin temperature increased by 8.0°C or more.

e ΔT<sub>r</sub> = maximum observed change in rectal temperature in °C.

f Sites of injection from which data were taken for construction of agonist dose-response curves are marked with an asterisk.
g Injection of epinephrine produced an immediate and profound (98\%) but short-lasting decrease in electromyographic activity and a small decrease in rectal temperature. This was followed in 15 minutes by massive shivering and hyperthermia.

h Injection of epinephrine at this site had no immediate effect. However, 20 minutes later, the subject lowered its head between its forepaws and EMG activity trailed off to complete electrical silence which lasted 8 minutes. The subject then suddenly lifted its head and EMG activity returned at full preinjection intensity about 20 seconds later. Rectal temperature, which had fallen during the period of EMG silence, rose to preinjection levels and remained steady.

i The effect produced at this site is similar to that described in the previous footnote. However, in this case, latency was only 13 minutes and a second hypothermic episode followed the first immediately after rectal temperature had returned to the preinjection level.

j Parentheses indicate that there is doubt whether the observed effect was a consequence of the drug injection.

k The effect produced by microinjection of epinephrine into this posterior hypothalamic site was in every respect similar to that produced by injection into the AH/PO region.
Discussion

The results show that, at 0.03 and 0.10 molar concentrations, both epinephrine and norepinephrine are significantly more effective in lowering body temperature as reflected by $\Delta T_r$, $50\% \Delta T_r / \Delta T$, $90\% \Delta T_r / \Delta T$ and $\Delta EMG$, than is isoproterenol (Tables 3, 5, 7, and 9). In addition, epinephrine (0.03 molar) was found to produce vasodilation in a significantly greater number of cats than an equimolar concentration of isoproterenol.

These results, when combined with other statistically significant differences and certain suggestive but non-significant trends, indicate that the relative-potency series presented in Table 14 may exist.

Several aspects of these data require comment. For example, as has been previously discussed, it is possible that the relatively low potency of isoproterenol with respect to alteration of thermoregulation may be due to the possibility that this amine is removed from the vicinity of the injection site more rapidly than are the other sympathomimetic amines which were tested. However, the observation that isoproterenol produces a very slow rate of body temperature decrease indicates that this is probably not the case, since the rate measures employed are unlikely to be influenced by duration of drug action (see Introduction). Also, ear skin temperature and electromyographic activity, two other parameters unlikely to be influenced by rate of disposition of the injected substance, are more sensitive to epinephrine and norepinephrine than isoproterenol. Furthermore, since isoproterenol is a poor substrate for monoamine oxidase and is less readily taken up into the presynaptic neuron than epinephrine or norepinephrine
TABLE 14

RELATIVE POTENCIES OF FOUR SYMPATHOMIMETIC AMINES WITH RESPECT TO FIVE THERMOREGULATORY PARAMETERS AFTER MICROINJECTION INTO THE AH/PO REGION

<table>
<thead>
<tr>
<th>Parametera</th>
<th>Relative Potencies</th>
</tr>
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<tr>
<td>$\Delta T_r$</td>
<td>$E &gt; NE \geq PE \leq ISO$</td>
</tr>
<tr>
<td>$50% \Delta T_r / \Delta T$</td>
<td>$NE = E &gt; PE &gt; ISO$</td>
</tr>
<tr>
<td>$90% \Delta T_r / \Delta T$</td>
<td>$E = NE &gt; PE &gt; ISO$</td>
</tr>
<tr>
<td>$\Delta EMG$</td>
<td>$E = NE &gt; PE = ISO$</td>
</tr>
<tr>
<td>$\Delta T_e$</td>
<td>$NE = PE = E &gt; ISO$</td>
</tr>
</tbody>
</table>

a See Chapter II for a full description of the various parameters.

b An asterisk above a sign of inequality signifies that the indicated inequality is significant at the 0.05 level.

c An equality sign indicates the lack of any significant difference or trend toward such a difference between the conjoined members.

d A sign of inequality in the absence of an asterisk indicates a suggestive but non-significant trend in the direction indicated.
(Furchgott, 1967), it seems unlikely that the sojourn of isoproterenol at the injection site would be less than that of these latter two substances.

On the other hand, the greater ability of epinephrine than norepinephrine to lower body temperature and perhaps the equipotency of norepinephrine and phenylephrine in this connection may indeed be due to differences in the rate of disposition of these amines. This state of affairs could arise if norepinephrine were removed from the site of injection more rapidly than the other amines. This view is reinforced by the observation that, with respect to several parameters less likely to be affected by duration of drug action (50% $\Delta T_r/\Delta T$, 90% $\Delta T_r/\Delta T$, $\Delta EMG$), epinephrine and norepinephrine are nearly equipotent, and the potency of phenylephrine is nearer that of isoproterenol than norepinephrine. Thus, parameters which are affected by the rate of removal of the drug from the injection site, e.g., $\Delta T_r$, would assign a lower relative potency to norepinephrine than parameters which are independent of rate of drug disposition.¹ Thus, in agreement with the original report of Feldberg and Myers (1965), epinephrine was found to

¹ One exception to this line of reasoning is the equipotency of norepinephrine and phenylephrine with respect to their ability to modify peripheral vasomotor tone ($\Delta T_e$). $\Delta T_e$ was measured soon after the microinjection, when the concentrations of the injected amines were presumably not yet affected by varying rates of removal from the site. However, this apparently real similarity in potency of norepinephrine and phenylephrine with respect to this parameter cannot be responsible for their equipotency with respect to alteration of rectal temperature because the parameters reflecting rate of body temperature decrease (50% and 90% $\Delta T_r/\Delta T$), although necessarily influenced by the equal ability of norepinephrine and phenylephrine to produce vasodilation, nevertheless indicate that norepinephrine induces a more rapid fall in rectal temperature than does phenylephrine.
be more "potent" than norepinephrine with respect to hypothermia- producing ability. But with respect to activation (or inhibition) of the central thermoregulatory mechanisms which mediate the fall in rectal temperature, the two substances are approximately equipotent.

In general, the potency series reported in the present experiments are quite similar to the series produced when the actions of sympathomimetic amines at alpha-receptors in the (peripheral) autonomic nervous system are analyzed (Furchgott, 1967). This finding supports the hypothesis that the various sympathomimetic amines induce hypothermia by acting at central receptors similar to the alpha-receptors found in the periphery.

Experiments with relatively specific adrenergic receptor antagonists further support this viewpoint. It was found that local pretreatment with the alpha-receptor antagonist, phentolamine, partially or completely prevented epinephrine-induced hypothermia, whereas propranolol, a beta-receptor antagonist, was much less effective (Figures 13 and 15, Table 12). The ability of propranolol to partially block the epinephrine-induced hypothermia might be attributed to the alpha-receptor blocking activity of the drug, which appears at high concentrations (Krnjevic and Lincir, 1967), or perhaps to its considerable local anesthetic activity (Levy, 1968).

It could be argued, of course, that the blockade of the epinephrine response by phentolamine was not due to antagonism at alpha-receptors, but to some non-specific, high concentration effect not possessed by propranolol. In this connection, the irreversible loss of sensitivity of the injection site in 3 of the 6 test animals after
treatment with phentolamine suggests that there may even be neural
damage produced by the concentration used. On the other hand, the
other 3 animals suffered little or no loss of sensitivity after these
injections. Furthermore, Connor et al. (1967), studying catecholamine
antagonism of carbachol-induced tremor, an effect apparently mediated
by central beta-receptors, found that the effect could be antagonized
by microinjections of propranolol (25 μg) but not by up to 200 μg of
phentolamine. These animals apparently suffered no ill effects from
the phentolamine injections. These considerations suggest that the
phentolamine antagonism observed in the present experiments was
specific.¹

The conclusion drawn from the present experiments, that the hypo­
thermia induced by intrahypothalamic microinjection of sympathomimetic
amines is mediated by central receptors similar to alpha-receptors
found in the periphery, is also supported by the findings of others.
Domer and Feldberg (1960) demonstrated that pentobarbital-induced
tremor (possibly shivering) could be suppressed by perfusion of
catecholamines from the lateral ventricles to the aqueduct, the rela­
tive potency of these amines being: E > NE > ISO. Banerjee et al.
(1968c) reported that ergotamine, an alpha-receptor blocking agent,
when administered into the lateral ventricles, could prevent the
hypothermic effects of intraventricularly administered norepinephrine

¹ To obtain reasonable assurance of this, one should obtain dose­
response curves for at least one of the agonists in the presence
and in the absence of a given concentration of phentolamine. If
the antagonism is competitive and specific as is suggested, the
dose-response curve obtained in the presence of the antagonist
will be parallel to the curve obtained in its absence and shifted
to the right along the dose axis (Furchgott, 1964).
or epinephrine. Recently, Burks (1970) reported that the hypothermia provoked in cats by intraventricular administration of 100 μg of norepinephrine could be blocked by prior intraventricular injection of 100 μg of phentolamine but not by up to 200 μg of MJ-1999 (Sotalol). Neither of the blocking agents produced any consistent behavioral or thermoregulatory effects when administered alone. These data of Burks', in particular, reinforce the present author's conclusions concerning the specificity of the hypothermia-antagonizing effects of phentolamine.

Although the primary goal of these experiments was to demonstrate the receptor selectivity just discussed, a second objective was to compile data concerning the mechanisms through which intracerebrally injected sympathomimetic amines cause hypothermia in the cat. The results demonstrate that, at 20°C ambient, the 4 amines tested induce hypothermia by suppression of shivering and reduction of cutaneous vasomotor tone. Panting was not elicited.

Shivering was usually the first parameter to be affected, although on several occasions vasodilation occurred simultaneously with inhibition of shivering. Moreover, partial return of EMG activity was usually the first sign of recovery from the microinjected sympathomimetic amine. In most experiments, changes in rectal temperature were clearly linked with the level of shivering, rectal temperature falling after EMG activity had been suppressed and returning to baseline only after EMG activity had at least partially recovered.

But not all the observations supported this relationship between rectal temperature and level of EMG activity. In a few experiments,
rectal temperature fell following the microinjection, but before EMG activity had begun to return and while the ears were still dilated, the rate of rectal temperature decrease gradually slowed until body temperature had ceased to decline. The sensitivity of the EMG recorder was normally adjusted so that EMG activity at the 10 μV level was observable, but only barely so. On several occasions, the sensitivity was increased during the period of apparent EMG silence just described, and EMG activity in the 1-10 μV range was found. Possibly, this very low-level activity, perhaps equivalent to the "thermoregulatory muscular tonus" of Ivanov (1963), is capable of preventing further decrease in body temperature. Another possible explanation is that true shivering had begun in muscles other than those which lay beneath the EMG recording electrode. Evidence that the distribution of shivering musculature is of great importance has been cited (Chapter I). A third possibility is that some non-shivering thermogenic mechanism had been activated either by the microinjection of the fall in body temperature. In both goat and monkey, cooling of the anterior hypothalamus rapidly activated endocrine cold defense mechanisms, resulting in increased body temperature in the absence of shivering (Andersson et al., 1962; Gale and Ruch, 1966). One or more of these 3 possibilities might also explain the one experiment in which EMG activity was suppressed and vasodilation occurred, but rectal temperature failed to decrease.

In general, ear skin temperature seemed to be a reasonably dependable and easily obtainable measure of peripheral vasomotor tone. Vasodilation, as reflected by this parameter, usually did not occur until shivering had begun to subside and frequently not until rectal
temperature had begun to decline. These effects are similar to those seen in anesthetized dog after intraventricular injection of norepinephrine or epinephrine (Feldberg et al., 1966). In contrast to the dog, however, vasoconstriction did not always occur before rectal temperature began to return toward baseline. In fact, in many cases vasodilation persisted even after rectal temperature had reached its initial level. However, in 10 of 72 experiments, vasodilation and inhibition of shivering had similar durations and both shivering and vasoconstriction had returned before rectal temperature began to rise.

The temperature of the ear contralateral to the site of injection was not recorded. However, whenever it was necessary to enter the environmental chamber for some other reason, the temperature of the contralateral ear was estimated by palpation. When this was done during the period of rapid rectal temperature decrease immediately following a microinjection, the vasodilation was always found to be bilateral. However, vasodilation was not always found to be bilateral after rectal temperature had reached its lowest level. Eight examples of vasodilation limited to the ipsilateral ear were found during this period.

Since bilateral ear temperatures were not uniformly measured, it is fruitless to speculate about the relative frequency of occurrence of unilateral vasodilation or its effectiveness as a heat loss mechanism in comparison to bilateral vasodilation. However, in view of the substantial electrophysiological and neuroanatomical evidence that autonomic outflow is bilaterally balanced (Harrison et al., 1939; Pitts and Bronk, 1942), it is interesting that the unilateral effect
was observed. To the author's knowledge, unilateral ear skin dilation, as a normal physiological response or as a response to central chemical, electrical or thermal stimulation, has not been previously reported. However, Andersson et al. (1956) observed that electrical stimulation of the goat hypothalamus elicited ear skin vasodilation which persisted longer on the ipsilateral than the contralateral side, and Pinkston et al. (1934) reported that hemidecortication in the cat led to small contralateral increases in the temperature of the skin of the trunk. It is evident that there may be more laterality to sympathetic outflow than has been suspected.

Another interesting phenomenon is the all-or-none vasodilation observed after microinjection of sympathomimetic amines. There is some question as to whether this is the normal thermoregulatory response of the cat, or whether the dose-response curve for amine-induced vasodilation is extremely steep. That is, would the correct dose of sympathomimetic amine produce an intermediate degree of vasodilation, or is vasodilation in the cat intrinsically a quantal response?

Forster and Ferguson (1952) reported that cats maintained at 25-27°C ambient experienced cyclical changes in ear skin temperature. The changes were rapid and maximal; intermediate values were not observed. However, Adams (1963) found that anterior hypothalamic heating in cats kept at 23°C ambient caused vasodilation of varying intensity. The regression line of increase in ear skin temperature on increase in hypothalamic temperature was quite steep, maximal vasodilation being produced by a 1.0 to 1.5°C increase in hypothalamic temperature. Thus, it seems that by the correct choice of stimulus level, intermediate degrees of ear skin dilation can be elicited. It is probable then,
that within a narrow dose range, microinjected sympathomimetic amines would also elicit a graded response.

In contrast to shivering and vasomotor tone, respiratory rate was only rarely affected by the microinjections, even though panting is a highly effective heat loss mechanism in the cat. Intraventricular injections of large quantities of epinephrine (up to 2 mg) reportedly increased respiratory rate to over 200/minute (Rothballer, 1959). Although the author refers to this as panting, he gives no indication of whether true behavioral panting was present. Feldberg and Sherwood (1954), using the same preparation as Rothballer, did not see polypnea. Nor did up to 1 mg of norepinephrine, injected intraventricularly in the anesthetized dog cause polypnea (Feldberg et al., 1966). The single animal in the present study which exhibited polypnea after sympathomimetic amine microinjections did not pant, and respiratory rate did not exceed 110/minute.

Clearly, the centrally administered sympathomimetic amines which produce marked vasodilation and inhibition of shivering do not induce panting, except perhaps at very high dose levels. There are several possible explanations for this. There may be, for example, sites at which the amines could elicit panting but they were not tested in the present experiments. Or, the amine-sensitive receptors eliciting panting may be located on neurons so diffusely distributed that a single microinjection could not activate a sufficient number to indicate the response. A further possibility is that the sympathomimetic amines did facilitate the panting mechanism but that a certain degree of additional facilitation by central warm receptors may be
necessary to elicit the response (see Bligh, 1966a, for references).

The final objective of this series of experiments was to test the suggestion that the sympathomimetic amines elicit thermoregulatory effects only when injected into the AH/PO region (Feldberg and Myers, 1965). In general, the data support this concept. Suppression of shivering, vasodilation and hypothermia were most consistently elicited by monoamines injected into the AH/PO region. Injections of these substances into loci outside this region usually, but not always, failed to induce thermoregulatory responses. Microinjections into a site just dorsal to the mammillary complex, into a locus bordering on the ventromedial hypothalamic nucleus and into the paraventricular nucleus of the thalamus elicited suppression of shivering and hypothermia. Injections into one dorsal posterior hypothalamic site produced vasodilation, suppression of electromyographic activity and hypothermia, the total effect closely resembling that produced after adrenergic stimulation of the AH/PO region.

Thus, although it cannot be flatly stated that thermoregulatory responsiveness to microinjected monoamines is limited to the AH/PO region, this region is clearly more responsive than many other hypothalamic loci. A considerably greater number of microinjections at various hypothalamic loci would be necessary to determine whether the responsive sites lying outside this region represent a gradient of responsive sites with a focus within the AH/PO region or whether they represent discrete areas of thermoregulatory function independent of the AH/PO region.
This brings us to a discussion of the possible central mechanisms through which the sympathomimetic amines could be eliciting the thermoregulatory responses which have been described. If one assumes that the amines are acting primarily on the hypothalamic thermoregulatory apparatus, there are relatively few mechanisms through which these substances might act:

a) They may imitate peripheral or central thermal stimulation, thus initiating counterregulation which would lower body temperature.

b) They may interfere with hypothalamic integrative mechanisms, perhaps resulting in a downward readjustment of the "setpoint" or in disorganized and/or inappropriate activation of peripheral thermoregulatory effectors.

c) They may directly activate (or inhibit) hypothalamic efferent outflow destined to control the thermoregulatory effectors in the periphery.

The experiments at hand, when considered alone, offer insufficient data to distinguish between these possibilities. However, when considered in light of other available information, it will be seen that only one retains any plausability.

The dose-response and antagonist studies already discussed indicate that the thermoregulatory responses to intrahypothalamically injected monoamines are due to the action of these substances at central receptors similar in nature to the alpha-receptors found in the periphery. Thus, these effects are not due to a non-specific change in membrane permeability of all neurons at the site of injection.
or to an action at other pharmacological receptors, such as those reacting with acetylcholine, histamine or 5-HT. It also seems reasonable to assume that the presence of adrenergic receptors in the AH/PO area indicates the release of norepinephrine as a neurotransmitter at these synapses. However, as has been pointed out in Chapter I, almost all hypothalamic catecholamines reside in nerve terminals belonging to monoaminergic cells located in the lower brainstem which project their axons rostrally. Very few monoamine-containing cell bodies lie in the hypothalamus and the few cell groups that are there are not located in the AH/PO region. Thus, it seems highly probable that intrahypothalamically injected norepinephrine activates the cells which are in synaptic contact with the ascending adrenergic fiber systems.

If this is true, then the only reasonable explanation of the action of the monoamines is that some of the ascending amineergic fibers provide information from peripheral (or at least subhypothalamic) thermal receptors and that the microinjected amines introduce false information about the thermal status at the periphery. The other suggested mechanisms require the presence of adrenergic neurons within the AH/PO region, neurons which apparently are not there.

Since it is well known that peripheral warm stimulation can inhibit shivering and induce peripheral vasodilation in the cat (Euler, 1960), this proposed mechanism of action for the monoamines explains the activation of these heat loss mechanisms. It does not as readily explain the lack of monoamine-induced panting. Peripheral warming has been shown to produce panting in some cats, even in the absence of an increase in hypothalamic temperature (Forster and Ferguson, 1952).
However, other workers have shown that central warming facilitates the effect of peripheral warming on panting and that central cooling inhibits peripherally-induced panting (Bligh, 1966). Thus, perhaps the lack of central warming and/or the central cooling engendered by suppression of shivering and vasodilation were sufficient to prevent activation of panting.

Up to this point, the implicit assumption has been made that the actions of the sympathomimetic amines must be explained by a mechanism which lends to them an intimate role in the control of body temperature, a role for which much supporting evidence has been adduced (see Chapter I). However, one cannot ignore the fact that microinjected monoamines were seen to cause effects which are probably not at all related to thermoregulation, e.g., defecation and profound sedation. It could be argued that these represent the effects of the injected amines on other ascending aminergic systems, unrelated to temperature regulation. On the other hand, it could be just as reasonably argued that the so-called thermoregulatory effects of these substances are no more than actions upon these other systems. After all, the peripheral effectors which bring about changes in body temperature are controlled by several systems and participate in activities other than thermoregulation. Defecation and vasodilation, for example, may simply be consequences of a generalized inhibition of sympathetic outflow. Or perhaps these phenomena represent abstracted portions of the generalized emotional state so frequently elicited by electrical stimulation of the hypothalamus. Possibly, the behavioral depression and inhibition of shivering which were reported are related to paradoxical sleep, a state
during which skeletal muscle atonia is usually present (Jouvet, 1969). This possibility is particularly interesting since centrally administered catecholamines have been found to induce sleep or drowsiness in several species (Baas, 1914—dog; Brittain and Handley, 1967—mouse; Marley and Stephenson, 1969—chick; Rothballer, 1959—cat; Sherwood, 1955—man). Suppression of skeletal muscle tone was seen in conjunction with this behavioral sleep in mouse (Brittain and Handley, 1967), chick (Marley and Stephenson, 1969), cat (Rothballer, 1959) and man (Sherwood, 1955). In chick and cat, the electroencephalogram during amine-induced sleep and muscular relaxation was measured and was found to be characterized by low amplitude, high frequency activity indicative of paradoxical sleep (Allen and Marley, 1967; Rothballer, 1959). The potency series for EMG suppression and elicitation of the electroencephalogram typical of paradoxical sleep in the chick was E > NE >> ISO = PE, a series similar to that found for sympathomimetic amine-induced EMG suppression in the present study. Moreover, again in the chick, phenoxybenzamine, an alpha-receptor antagonist, prevented both EMG suppression and the sleep-like state (Allen and Marley, 1967; Marley and Stephenson, 1969).

The possibility that the norepinephrine may be involved in the control of attentional state is also strengthened by data reviewed by Jouvet (1969) which demonstrate that 1) destruction of brainstem nuclei within which are located norepinephrine-containing cells results in selective suppression of paradoxical sleep and a reduction in forebrain norepinephrine content 2) reduction of forebrain norepinephrine content by various drug treatments also suppresses
paradoxical sleep and 3) there is an increased turnover of cerebral norepinephrine in rats during the rebound of paradoxical sleep following its selective deprivation.

Although the present experiments indicate that the thermoregulatory effects of the sympathomimetic amines probably result from activation of neural systems which are physiologically activated by norepinephrine released at the synapse, it remains for further research to determine whether these systems are part of the central thermoregulatory apparatus per se or whether they have some other function, perhaps related to the control of state of consciousness.
CHAPTER IV

THE THERMOREGULATORY EFFECTS OF INTRACEREBRAL INJECTION OF CHOLINOMIMETIC AGENTS

Introduction

The available information concerning the possible involvement of cholinergic synapses in the central control of body temperature has been reviewed (Chapter I). Here it was noted that muscarinic stimulation of the rat anterior hypothalamus leads to severe hypothermia (Lomax and Jenden, 1966). Similar effects have been observed after intrahypothalamic injection of carbamylcholine (carbachol, CCh) in the cat (Hull et al., 1967).

Early exploratory experiments of the present author also demonstrated that large (about 25 μg) doses of CCh induced hypothermia when injected into the AH/PO region of the cat. Since these observations agreed with those of Hull (in cats) and Lomax and Jenden (in rats), investigation of the effects of cholinergic stimulation was not pursued at this time. However, in 1969, Myers and Yaksh reported that cholinergic stimulation of sites throughout the hypothalamus of the rhesus monkey produced strong shivering, peripheral vasoconstriction, piloerection, huddling and hyperthermia. Only after stimulation of one circumscribed posterior hypothalamic site was hypothermia obtained.

In view of the similar responses of cat and rhesus monkey to intrahypothalamic injection of 5-HT and catecholamines (see Chapter I), which suggests that there may be a close similarity in the hypothalamic
neural organization for the control of body temperature in these two species, it seemed of interest to explore more thoroughly the thermo-regulatory responses of the cat to cholinergic stimulation of the hypothalamus.

Methods

The experimental subjects were 13 male and 2 female cats. Of these 15 animals, 11 had participated in the sympathomimetic amine studies. These latter animals had injection guide cannulae implanted at various hypothalamic loci, including the AH/PO region, as previously described (Chapter III). The remaining 4 subjects were implanted with bilaterally symmetrical sets of guide cannulae, one set aimed at the AH/PO region, the other at more caudal sites in the hypothalamus.

The drugs and concentrations used are listed in Table 15. Because of its resistance to the cholinesterases, carbachol was chosen as the primary cholinergic stimulant. However, to insure that the effects observed were not peculiar to carbachol, acetylcholine (ACh) in combination with an equal weight of the cholinesterase inhibitor, physostigmine sulfate (eserine), was also administered at 6 of the 31 sites tested. This 1:1 ACh-eserine mixture was also used by Myers and Yaksh (1969) for cholinergic stimulation of the monkey hypothalamus. In addition, the .01 and .03 µmole doses of CCh and ACh used in the present study are quite similar to the quantities of these substances administered by Myers and Yaksh. The ACh-eserine mixtures were prepared by dissolving the ACh and eserine in sterile, pyrogen-free water in the same vessel, and bringing the resulting solution to isotonicity
<table>
<thead>
<tr>
<th>Drug</th>
<th>Molar concentration</th>
<th>Millimolar concentration</th>
<th>Total number of μmoles injected</th>
<th>μg injected (as the salt)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>3.0</td>
<td>0.003</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>10.0</td>
<td>0.01</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>30.0</td>
<td>0.03</td>
<td>5.48</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>100.0</td>
<td>0.10</td>
<td>18.27</td>
</tr>
<tr>
<td>Acetylcholine chloride</td>
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<td>30.0</td>
<td>0.03</td>
<td>5.45</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>100.0</td>
<td>0.10</td>
<td>18.16</td>
</tr>
</tbody>
</table>
by the addition of sodium chloride. Sterile, pyrogen-free 0.9% saline served as the control solution.

The protocol for a typical experimental session and the methods for recording rectal temperature, ear skin temperature, respiratory rate and the electromyographic activity of the lateral thigh, the 4 parameters measured in most sessions, have been described in Chapter II.

Ten sites were treated with a single dose of CCh, 8 with .01 micromole, 1 with .03 micromole, and 1 with .10 micromole. Twenty-one sites were treated with more than one dose of CCh. Eleven of these loci received two dose levels, .01 and .03 micromole, administered in that order. The remaining 10 sites were tested with three dose levels, 5 sites receiving the dose sequence, .01, .03, .10 micromole, and the other 5 sites receiving the dose sequence, .01, .03, .003 micromole. At least 5 days intervened between treatments in a given cat.

Results

The thermoregulatory responses to intrahypothalamic microinjection of CCh were greatly dependent upon the dose of CCh and the hypothalamic site stimulated. The response most commonly observed was a biphasic change in rectal temperature consisting of initial hypothermia followed by an increase in temperature above the preinjection level. The magnitude of the temperature increase was usually many times greater than that of the temperature decrease, although biphasic curves manifesting predominant hypothermia or nearly equal hypothermic and hyperthermic phases were also seen. Examples of several types of biphasic response
can be found in Figure 20 (SA-15, .01; SA-18, .003; SA-13, .01), Figure 21 (SA-15, .01; SA-10, .01 and .03), and Figure 23 (SA-14, all dose levels).

This type of response was produced by at least one dose-level of CCh at 24 of the 31 sites investigated. Cholinergic stimulation of 5 of the remaining 7 sites yielded hyperthermia only. However, 4 of the 5 received only the .01 μmole dose-level and the fifth received only the .01 and .03 μmole levels. As will be seen, it is possible that higher doses at these sites would have elicited the typical biphasic response. Carbachol stimulation at the other 2 sites elicited immediate hypothermia without a secondary overshoot above the baseline level.

Although 60% of all responses to central CCh stimulation were biphasic, it was found that at some loci, administration of doses higher or lower than those which elicited the biphasic response altered the shape of the time-response curve for rectal temperature so that either the hypothermic or hyperthermic phase was accentuated. This is illustrated by cat SA-15 (Figure 20), in which microinjection of .003 μmole yielded hyperthermia only, .03 μmole produced hypothermia only and .01 μmole elicited the biphasic response. Similar accentuation of the hypothermic phase with increasing dosage is seen in cats SA-18 and SA-13 (Figure 20).

Figure 21 illustrates somewhat similar responses in 2 other cats. Here, however, hypothermia appeared only after the highest dose had been administered. On the other hand, in one subject, CCh stimulation elicited hypothermia even at the lowest dose level (Figure 22). In all, 13 of the 21 sites tested with more than one dose yielded increasing
Figure 20. Change in rectal temperature elicited by intrahypothalamic microinjection of carbamylcholine at varying dose levels.

Data from 3 cats are presented (SA-15, SA-18, SA-13). Each panel represents rectal temperature changes elicited by 3 doses of carbamylcholine chloride (CCh) microinjected into the same diencephalic site. The time of drug injection is indicated by the arrow. The dose of CCh in micromoles is given at the end of each time-response curve.

At least 5 days intervened between successive injections in a given cat. The absolute rectal temperature at the time of injection was between 38.5 and 39.5°C.

Sites of injection for each cat according to the atlas of Snider and Niemer (1961):

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>SA-15</td>
<td>A</td>
<td>12.5</td>
<td>L</td>
<td>1.5</td>
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<td>SA-18</td>
<td>A</td>
<td>12.5</td>
<td>L</td>
<td>1.8</td>
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<tr>
<td>SA-13</td>
<td>A</td>
<td>13.3</td>
<td>L</td>
<td>1.5</td>
</tr>
</tbody>
</table>
Figure 21. Change in rectal temperature elicited by intrahypothalamic microinjection of carbamylcholine at varying dose levels.

Data from 2 cats are presented (SA-15, SA-10). Each panel represents rectal temperature changes elicited by 3 doses of carbamylcholine chloride (CCh) microinjected into the same diencephalic site. The time of drug injection is indicated by the arrow. The dose of CCh in micromoles is given at the end of each time-response curve.

At least 5 days intervened between successive injections in a given cat. The absolute rectal temperature at the time of injection was between 38.5 and 39.5°C.

Sites of injection for each cat according to the atlas of Snider and Niemer (1961):

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>L</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA-15</td>
<td>13.0</td>
<td>2.5</td>
<td>-3.5</td>
</tr>
<tr>
<td>SA-10</td>
<td>8.0</td>
<td>3.0</td>
<td>-4.5</td>
</tr>
</tbody>
</table>
CHANGE IN COLONIC TEMPERATURE (°C)

TIME (HOURS)

SA-10

SA-15

CCh
Figure 22. Change in rectal temperature elicited by intrahypothalamic microinjection of carbamylcholine at varying dose levels.

Rectal temperature changes elicited by 3 doses of carbamylcholine chloride (CCh) microinjected into the same diencephalic site in cat SA-17 are presented. The time of drug injection is indicated by the arrow. The dose of CCh in micromoles is given at the end of each time-response curve.

At least 5 days intervened between successive injections. The absolute rectal temperature at the time of injection was between 38.5 and 39.5°C.

Site of injection according to the atlas of Snider and Niemer (1961):

SA-17  A 12.3  L 2.5  H -3.0
CHANGE IN COLONIC TEMPERATURE (°C)

TIME (HOURS)

SA-17
hypothermia with increasing dosage of CCh.

In contrast to those sites at which stimulation with increasing dosage of CCh augmented the hypothermic phase, stimulation of 2 loci elicited hyperthermia as the predominant response, and even the highest dose failed to induce predominant hypothermia (Figure 23). It should be noted, however, that increasing the CCh dosage at one of these sites slightly increased the hypothermic phase of the biphasic response, and increasing the dose at the other site reduced the rate of rectal temperature increase. Injection of various concentrations of CCh into 6 other loci revealed no tendency toward increased rectal temperature fall with increased CCh dosage. However, these sites did not receive the highest dose of CCh.

CCh-engendered hypothermia, regardless of the size of the rectal temperature decrease, was almost always accompanied by a reduction in EMG activity. Complete inhibition of EMG activity, however, was never observed. The rising phase of the time-response curve for rectal temperature, whether there was an increase above baseline or only a return to baseline from hypothermic levels, was frequently characterized by intense electromyographic activity. On the few occasions where this was not the case, visual inspection of the cat revealed that shivering activity which had previously been limited to the hindquarters had now become generalized to most of the body. This whole-body tremor could usually not be distinguished visually from intense physiological shivering. Further, although no frequency analysis was made, gross inspection of the electromyographic records did not reveal any marked differences in the patterns produced during shivering and
Figure 23. Change in rectal temperature elicited by intraphyothalamic microinjection of carbamylcholine at varying dose levels.

Data from 2 cats are presented (SA-4, SA-8). Each panel represents rectal temperature changes elicited by 3 doses of carbamylcholine chloride (CCh) microinjected into the same diencephalic site. The time of drug injection is indicated by the arrow. The dose of CCh in micromoles is given at the end of each time-response curve.

At least 5 days intervened between successive injections in a given cat. The absolute rectal temperature at the time of injection was between 38.5 and 39.5°C.

Site of injection for each cat according to the atlas of Snider and Niemer (1961):

- SA-4: A 13.5 L 1.5 H -4.0
- SA-8: A 13.5 L 2.5 H -3.5
during CCh-induced tremor.

Changes in cutaneous vasomotor tone during CCh-induced hypothermia, as reflected by alterations in ear skin temperature, were related to the magnitude of the rectal temperature decrease. Ear temperature data are available for 29 experiments during CCh-induced hypothermic episodes of less than 0.5°C. Each of these episodes constituted the initial phase of the typical biphasic response. In only 8 instances was vasodilation seen. In 4 instances the vasodilation was well coordinated with 
EMG and rectal temperature, i.e., vasodilation occurred during the hypothermic phase and was replaced by tonic vasoconstriction during the hyperthermic phase. However, in the other 4 instances, vasodilation was maintained throughout the entire biphasic effect.

Ear temperature data are available for 10 of the 19 instances of CCh-induced hypothermia greater than 0.5°C. Vasodilation occurred in 7 of these experiments. In 6 cases it was coordinated with the other thermoregulatory parameters throughout the session, but in one experiment, the ear remained vasodilated even while the subject shivered vigorously during the rising phase of rectal temperature.

Although observation of the behavioral responses to intracerebral injection of CCh was not a major goal of the present experiments, the animals were closely watched by closed circuit television during experimental sessions, and written protocols were kept. The environmental chamber within which the subjects were restrained was kept closed, and air blowers within the chamber provided sufficient background noise to mask most external sounds. The behavior observed was therefore elicited in response to internal stimuli or stimuli within the environmental
chamber. The only novel stimulus regularly presented was a standard "click" delivered next to a small porthole through the chamber wall.

In non-treated cats, the click stimulus elicited no response or a slight twitch or rotation of the ears. In CCh-treated cats, ear rotation, turning of the head toward the source of the sound and sometimes intense visual inspection of the environment were produced. The arbitrary endpoint for this "attending" response was a clear turning of the head immediately following presentation of the stimulus. All other responses to CCh injection were "spontaneous" in the sense that they could not be reliably related to any stimulus external to the environmental chamber. These behavioral and autonomic responses included ear twitching; rotation of the ears similar to that seen in the attentional response but not related to presentation of the click stimulus; visual "searching" of the environment in a hallucinatory manner; "searching" followed by a fear response such as a startle, withdrawal, or struggle; struggling not related to fear behavior; increased alertness, manifested by a change in demeanor from apparent nonattending to the environment to apparent awareness of the environment; sedation, manifested by a reduction in vocalization or movement and/or staring fixedly straight ahead; hissing; growling; lip licking; piloerection; changes in respiratory rate; tremor, broadly defined as a visually observable movement of any of the skeletal musculature in a rhythmic manner (no attempt is made to differentiate shivering from nonshivering tremor); small shifts in postural attitude ("position shifts"); probable paradoxical sleep (PDS), characterized by sudden and complete cessation of EMG activity, slumping of the body and head, twitching of the ears or legs and sudden reappearance of EMG activity and behavioral alertness;
meowing; small, apparently involuntary, movements of the head in a "tic-like" manner (IHM).

The behavioral signs observed in each experiment are listed in Table 16 in their order of appearance. Because of mediocre resolution in the television monitoring system, failure to observe tremor or piloerection did not reliably indicate that these phenomena were not present.

The most frequently observed behavioral signs of central cholinergic stimulation were increased alertness, "attending", ear twitch and "searching." Panting and sprawling, behavioral patterns of possible thermoregulatory significance, were never observed. Huddling, another thermoregulatory behavior, was such a common phenomenon both before and after microinjections that its occurrence could only rarely be attributed to the drug treatment.

In a typical experiment in which a biphasic effect consisting of a small fall in rectal temperature followed by a rise was produced, the first behavioral signs, usually occurring before or during the temperature decrease, were increased alertness, liplicking, increased respiratory rate or position shifts. As the rectal temperature approached its minimum value or leveled off, ear twitching, "searching", "alerting" and tremor appeared and usually remained until rectal began to fall after the hyperthermic phase. Manic excitement, rage behavior such as hissing and growling, or voluntary movements were usually not observed. In fact, during the hyperthermic phase especially, the subjects often stared straight ahead fixedly or dozed between "searches."
Spontaneous rage behavior was observed in 3 instances following microinjection of CCh. In 2 other experiments, the experimenter's opening of the environmental chamber door elicited rage in cats which, in the absence of external stimuli, had appeared to be normal or even slightly sedated. Each of these examples of rage occurred during CCh-induced hypothermia in sessions where hypothermia was the predominant effect produced. On the other hand, 9 other examples of predominant hypothermia were not accompanied by rage.

Probable paradoxical sleep was observed in 4 animals following intrahypothalamic microinjection of CCh. In 2 of these animals, the effect, consisting of sudden and complete disappearance of EMG activity, relaxation of the body and head and twitching of the ears, tail and legs, appeared 1.5-2.0 hours postinjection as body temperature was returning toward baseline following the hyperthermic phase of the biphasic effect. Signs of central cholinergic stimulation were minimal at this time. Although this PDS-like phenomenon was never seen in either of these 2 animals at any other time, 3 other cats were observed to undergo similar PDS episodes in the absence of drug treatment. In each of these 5 animals, PDS episodes were accompanied by a fall in rectal temperature of 0.1 to 0.5°C. Body temperature returned to baseline without overshoot immediately following the period of atonia. During this recovery period, EMG activity was greatly intensified.

In contrast to the first 2 animals, in which the postinjection interval before appearance of the PDS-like episodes was long, 2 other subjects experienced episodes of apparent paradoxical sleep soon after the microinjection of CCh. In the first of these animals, CCh, 0.01
umole, induced "searching," hissing and tremor within 5 minutes. At 18 minutes postinjection, the animal experienced a PDS-like episode of short duration. In the following 17 minutes, 9 such episodes, ranging in duration from 15 to 100 seconds, were observed; the rapid alternation between hyperexcitation and apparent unconsciousness was striking.

The other animal in which CCh produced a PDS-like state of relatively rapid onset reacted uniquely in several other respects. Rectal temperature tracings for this animal after microinjection of 3 doses of CCh at A 11.0, L 2.0, H -4.5 are presented in Figure 2a. The lowest dose of CCh rapidly elicited increased shivering or tremor as well as increased alertness and lip licking. During the short plateau phase, "searching" became very intense, and piloerection, increased respiratory rate (70/minute), and sniffing and licking of the restraining device were conspicuous. There was, however, no vasodilation or reduction in EMG activity. The second portion of the rising phase was characterized by continued high respiratory rate and gradually diminishing behavioral excitation.

The .03 umole dose elicited increasing EMG activity within one minute after completion of the microinjection. Increased alertness, "searching" and lip licking followed 4 minutes later. Dozing between successive "searches" and Biot's breathing (in which every fourth or fifth inspiration is held for several seconds) appeared in the plateau phase, but again, there was no vasodilation or EMG reduction to account for the sudden halt in rectal temperature increase. During the second portion of the rising phase, cholinergic behavioral signs increased
Figure 24. Change in rectal temperature elicited by intrahypothalamic microinjection of carbamylcholine at varying dose levels.

Rectal temperature changes elicited by 3 doses of carbamylcholine chloride (CCh) microinjected into the same diencephalic site in cat SA-3 are presented. CCh was injected at the first arrow. The dose of CCh in micromoles is given at the end of each time-response curve. At the .03 micromole dose, the arrow labeled A represents intrahypothalamic microinjection of 40 μg of atropine sulfate at the same site. At the .10 micromole dose level, the arrows labeled A represent two intraperitoneal injections of atropine sulfate, 2 mg/kg.

At least 5 days intervened between successive CCh injections. The absolute rectal temperature at the time of injection was between 38.5 and 39.5°C.

Site of injection according to the atlas of Snider and Niemer (1961):

SA-3   A 11.0   L 2.0   H -4.5
CHANGE IN COLONIC TEMPERATURE (°C)

TIME (HOURS)

SA-3
markedly in intensity, but alertness still appeared to alternate with brief periods of drowsiness. Hissing and strong tremor were also observed during this period. Atropine sulfate, 40 μg in 1 μl, injected into the same hypothalamic site while EMG activity and rectal temperature were still elevated, brought about an immediate reduction in these two parameters. EMG activity returned when rectal temperature reached the preinjection baseline.

The immediate response to .10 μmole of CCh was quite similar to that produced by .03 μmole. The intensity of the behavioral signs increased more rapidly, however, and at 10 minutes postinjection, jerking of the hindquarters and constant hissing and growling were observed. Two minutes later, rectal temperature began to fall, although EMG activity was still intense and the ears remained vasoconstricted. At 15 minutes after the microinjection, the subject suddenly collapsed into unconsciousness and the electromyogram became totally flat. This state persisted for 43 minutes, interrupted only by a 30-second period during which the cat suddenly looked up, hissed and growled strongly and then collapsed again. At the end of this PDS-like state, strong tremor, constant hissing, intense licking of the restraining device and small involuntary movements of the head suddenly appeared. Two intraperitoneal doses of atropine sulfate (2 mg/kg) given 1.5 and 2.0 hours later, reduced the tremor and behavioral manifestations, and rectal temperature gradually returned to the preinjection level.

The thermoregulatory effects of ACh-esterine mixtures injected at 6 hypothalamic sites, compared to the most similar effect produced by injection of CCh at the same sites are illustrated in Figures 25 and 26.
Figure 25. Change in rectal temperature elicited by intrahypothalamic microinjection of carbamylcholine and acetylcholine–eserine mixtures.

Data from 3 cats are presented (SA-13, SA-1, SA-10). Each panel represents rectal temperature changes elicited by carbamylcholine chloride (CCh) and acetylcholine chloride–eserine sulfate mixtures (ACh) microinjected into the same diencephalic site. The drugs were injected at the arrow. Figures in parentheses represent doses of CCh and ACh in micromoles. ACh–eserine mixtures were 1:1 by weight.

At least 5 days intervened between the two injections in each cat. The absolute rectal temperature at the time of injection was between 38.5 and 39.5°C.

Sites of injection for each cat according to the atlas of Snider and Niemer (1961):

<table>
<thead>
<tr>
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<td>-4.0</td>
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<tr>
<td>SA-10</td>
<td>8.0</td>
<td>1.0</td>
<td>-4.5</td>
</tr>
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</table>
CHANGE IN COLONIC TEMPERATURE (°C)

SA-13

SA-1

SA-10

TIME (HOURS)

CCh (.003)

ACh (.03)

ACh (.03)

CCh (.01)

ACh (.03)

CCh (.01)

ACh (.10)

SA-13

SA-1

SA-10
Figure 26. Change in rectal temperature elicited by intrahypothalamic microinjection of carbamylcholine and acetylcholine-esorine mixtures.

Data from 3 cats are presented (SA-8, SA-18, SA-9). Each panel represents rectal temperature changes elicited by carbamylcholine chloride (CCh) and acetylcholine chloride-esorine sulfate mixtures (ACh) microinjected at the arrow. Figures in parentheses represent doses of CCh and ACh in micromoles. ACh-esorine mixtures were 1:1 by weight.

At least 5 days intervened between the two injections in each cat. The absolute rectal temperature at the time of injection was between 38.5 and 39.5°C.

Sites of injection for each cat according to the atlas of Snider and Niemer (1961):

<table>
<thead>
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<th>A</th>
<th>L</th>
<th>H</th>
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</thead>
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<td>SA-9</td>
<td>15.0</td>
<td>2.5</td>
<td>-3.5</td>
</tr>
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</table>
Of particular interest is an injection in cat SA-9 (Figure 26) which demonstrates that ACh, like CCh, can lower as well as increase body temperature. In this example, vasodilation occurred simultaneously and in perfect coordination with the respective rectal temperature decrease produced by CCh and ACh-eserine.

Although increased alertness, "searching" and tremor were observed after microinjection of ACh-eserine mixtures, in general, the behavioral effects were less prominent than those produced by CCh. Moreover, sedation and PDS-like episodes did not occur.

The anatomical distribution of the diencephalic loci stimulated and the various thermoregulatory and behavioral responses produced are listed in detail in Table 16. In Figures 27 and 28, each site has been classified with respect to the major response produced at each dose level and plotted on stylized frontal sections of the cat brain. Unfortunately, these sections do not correspond exactly with those of Snider and Niemer (1961), so that the site locations presented in Figures 27 and 28 are only close approximations. Nevertheless, it is clear that at the intermediate CCh dose levels (.01 and .03 μmole), predominant hypothermia was elicited by injection only into sites located in the AH/PO region, whereas the biphasic effect and/or predominant hyperthermia could be elicited by stimulation of every rostro-caudal hypothalamic level tested. There were no obvious dorso-ventral or medio-lateral segregation of the various types of responsive sites.

Discussion

Since it was known that the thermoregulatory responses of the cat to intrahypothalamic adrenergic and serotonergic stimulation are similar
Figures 27 and 28. Serial frontal sections through the cat brain illustrating the approximate locations of sites stimulated with carbamylcholine and the thermoregulatory responses produced.

Each injection site lies at the center of a complete or partial circle. The response to 1 to 3 dose levels of carbamylcholine (CCh) injected at this site is indicated by the following scheme:

Upper semicircle—predominant change in rectal temperature at the 0.01 micromole dose level.
Lower semicircle—predominant change in rectal temperature at the 0.03 micromole dose level.
Arrow—predominant change in rectal temperature at the 0.10 micromole dose level.

Filled symbols—temperature increase.
Open symbols—temperature decrease.
Stippled symbols—temperature decrease followed by temperature increase of approximately equal magnitude.
Arrow pointing up—temperature increase.
Arrow pointing down—temperature decrease.

Semicircle or arrow missing indicates site not tested at these doses. Biphasic effects with unequal falling and rising phases have been classified according to the predominant effect. See text for details.

Neuroanatomical abbreviations:

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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>cau</td>
<td>Caudate nucleus</td>
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<tr>
<td>cg</td>
<td>Central grey of midbrain</td>
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<tr>
<td>cm</td>
<td>Central medial thalamic nuc.</td>
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<tr>
<td>cp</td>
<td>Cerebral peduncle</td>
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<tr>
<td>db</td>
<td>Diagonal band of Broca</td>
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<td>en</td>
<td>Endopuduncular nucleus</td>
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<tr>
<td>f</td>
<td>Fornix</td>
</tr>
<tr>
<td>ff</td>
<td>Field of Forel</td>
</tr>
<tr>
<td>gp</td>
<td>Globus pallidus</td>
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<td>hi</td>
<td>Habenulointerpeduncular tract</td>
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<tr>
<td>ic</td>
<td>Internal capsule</td>
</tr>
<tr>
<td>in</td>
<td>Interstitial nucleus of Cajal</td>
</tr>
<tr>
<td>lh</td>
<td>Lateral habenular nucleus</td>
</tr>
<tr>
<td>lgn</td>
<td>Lateral geniculate nucleus</td>
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<tr>
<td>m</td>
<td>Mamillothalamic tract</td>
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<tr>
<td>md</td>
<td>Medial dorsal thalamic nuc.</td>
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<tr>
<td>mgn</td>
<td>Medial geniculate nucleus</td>
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<td>mm</td>
<td>Medial mammillary nucleus</td>
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<td>nr</td>
<td>Red nucleus</td>
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<td>ot</td>
<td>Optic tract</td>
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<tr>
<td>pc</td>
<td>Posterior commissure</td>
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<td>Posterior hypothalamic area</td>
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<td>Putamen</td>
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<td>Reuniens nucleus</td>
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<tr>
<td>rm</td>
<td>Reticular nucleus of thalamus</td>
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<td>s</td>
<td>Septal region</td>
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<td>sn</td>
<td>Substantia nigra</td>
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<tr>
<td>va</td>
<td>Ventral ant. thalamic nucleus</td>
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<td>Ventromedial hypothalamic nuc.</td>
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<td>zi</td>
<td>Zona incerta</td>
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<td>3</td>
<td>Oculomotor nerve</td>
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### TABLE 16

**THERMOREGULATORY AND BEHAVIORAL RESPONSES TO INTRAHYPOTHALAMIC MICROINJECTION OF CARBAMYLCHOLINE AND ACETYLCHOLINE-ESERINE MIXTURES**

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<th>Drug</th>
<th>Dose</th>
<th>( T_r ) response</th>
<th>( T_r ) response latencies</th>
<th>( T_e ) response</th>
<th>EMG during:</th>
<th>Behavioral responses</th>
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<td>Rise</td>
<td>Fall</td>
<td>Rise</td>
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*a coordinates:

*b Drug:

*C Dose:

*d \( T_r \) response:

*e \( T_r \) response latencies:

*f \( T_e \) response:

*g EMG during:

*h Behavioral responses:
<table>
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<tr>
<th>Cat</th>
<th>Site coordinates</th>
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<th>T&lt;sub&gt;e&lt;/sub&gt; response</th>
<th>EMG during: T&lt;sup&gt;r&lt;/sup&gt; + T&lt;sup&gt;r&lt;/sup&gt;</th>
<th>Behavioral responses</th>
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TABLE 16 (CONTINUED)
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<th>Dose</th>
<th>Drug Site</th>
<th>Cept Coordinates</th>
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TABLE 16 (CONTINUED)
TABLE 16 (CONTINUED)

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a According to the coordinate system and stereotaxic atlas of Snider and Niemer (1961). All site locations were determined with respect to the Weil-stained plates in this atlas. A, L and H refer to the distance in millimeters anterior to the interaural line, lateral to the midsagittal plane and above or below the horizontal zero plane, respectively.
b CCh-carbamylcholine chloride  ACh-acetylcholine chloride (mixed with an equal weight of physostigmine sulfate).
c Doses are given in micromoles of CCh or ACh.
d T_{r} response: the maximum fall and/or rise in rectal temperature in °C, measured with respect to preinjection baseline temperature.
e Latency for the falling phase of rectal temperature is the time in minutes between drug microinjection and the first downward trend in rectal temperature. Latency for the rising phase is the time in minutes between drug microinjection and the first sustained upward trend in rectal temperature.
f T_{e} responses: A-Maximal or partial ear skin vasodilation during the period of rectal temperature fall, followed by vasoconstriction during the rising phase of rectal temperature. B-Similar to A except that the ear remains vasodilated during the rising phase of rectal temperature. C-Maximal or partial vasodilation at or near the peak of the hyperthermic phase, apparently as a counterthermoregulatory action. D-No vasodilation.
g The level of electromyographic activity during the falling and rising phases of rectal temperature, relative to the preinjection activity level. - = decrease in activity, + = increase in activity, 0 = no change, ? = effect not clear.
**TABLE 16 (CONTINUED)**


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i Dashes indicate data are missing or not applicable.

j In this animal, at the .01 and .03 micromole doses of CCh, the rectal temperature increase was interrupted by a short plateau. At .10 micromoles, the initial rise of 0.20°C (not indicated in the table) is followed by a large fall and then a large rise. See text and Figure 24 for full explanation of thermal and behavioral effects seen after stimulation of this site.

k Head torsion consisted of strong involuntary tonic twisting of the head in a direction ipsilateral to the side of the microinjection.

l-m As long as the animals remained undisturbed in the closed environmental chamber, they appeared to be sedated. However, hissing was elicited when the chamber door was opened.
to those of the rhesus monkey, it seemed possible that cholinergic stimulation of the hypothalamus might also elicit identical thermoregulatory effects in these two species. Indeed, in agreement with the experiments of Myers and Yaksh (1969) in the rhesus monkey, it was found that in the cat also, .01 μmole of CCh elicits shivering (or shivering-like activity) and hyperthermia when injected into many hypothalamic loci. In this respect, then, the reactions of cat and monkey are comparable. However, there are several differences. In cat, at the .01 μmole dose, the hyperthermia is usually preceded by a small decrease in rectal temperature; in monkey, the rise in temperature is immediate. In cat, increasing the carbachol dose frequently augments the initial hypothermia; in monkey, increasing the dose merely accentuates the hyperthermia. In cat, at some anterior hypothalamic and preoptic sites, .01 μmole of carbachol induces considerable hypothermia as the predominant effect; this phenomenon has not been observed in monkey. However, Myers and Yaksh report that cholinergic stimulation of one circumscribed posterior hypothalamic locus between the mammillary bodies and the peduncle does elicit a fall in body temperature in the monkey. Injection of the same dose of carbachol into analogous sites in the cat induced only an increase in body temperature.

These differences could wholly or partially arise from factors other than gross dissimilarities in the hypothalamic thermoregulatory apparatus of the two species. For example, the inability of Myers and Yaksh to produce hypothermia with CCh injections in the AH/PO region of the monkey does not necessarily mean that such a response cannot be produced but possibly only that the correct loci were not stimulated. In view of
the larger size of the monkey brain and the fact that Myers and Yaksh
tested only 12 sites in the AH/PO region, there is a reasonable possi­
bility that the reactive sites were missed. Similarly, the posterior
hypothalamic sites yielding hypothermia in the monkey may have been
missed in the cat.

Further, although Myers and Yaksh reported that, in monkey, in­
creasing the dose of CCh increased the magnitude of the hyperthermic
response, they were not specific about the sites from which dose-response
data were obtained and the doses used. It seems possible that the dif­
ference between cat and monkey may be primarily one of sensitivity of
the hypothalamus to cholinergic stimulation. If sufficiently high dos­
age had been used, perhaps hypothermia would have been elicited by CCh
stimulation of sites which yielded only hyperthermia at lower doses,
as was found to be the case in the cat.

More difficult to explain is the lack of biphasic rectal tempera­
ture responses after stimulation of any of the 62 hypothalamic sites
tested in the monkey. In comparison, such responses were observed after
stimulation of 24 of 32 sites tested in the cat. It was seen at 17
different sites at the .01 μmole dose level, the same dose used by Myers
and Yaksh. Differences in sensitivity between the two species could
again be called upon to resolve this problem, but the almost universal
delay of the hyperthermic response in cats, even at sites or at dosages
which produced hyperthermia as the only effect, compared to the almost
instantaneous hyperthermic response of the monkey indicates that
sensitivity difference is not the only factor involved.
Of course, whether any of the speculations offered here actually account for the observed differences in the responses of cat and monkey to cholinergic stimulation of the hypothalamus remains to be investigated. Clearly, further exploration of the hypothalamus with cholinergic stimulation is necessary, particularly in the AH/PO region of the monkey and the posterior ventrolateral area in cat. Until such experiments are performed, it would be unrealistic to conclude that the differences in response of cat and monkey to cholinergic stimulation represent significant differences in hypothalamic organization for the regulation of body temperature in these species.

However, irrespective of whether these differences should prove to be substantial or insubstantial, it might be legitimately asked whether ACh is physiologically involved in the central control of body temperature in either cat or monkey and thus whether species differences in response to cholinergic stimulation actually have relevance to thermoregulatory organization.

A review of data pertinent to this question has been presented in Chapter I. As was discussed there, one of the important problems is whether the tremor induced by microinjection of cholinergic agents into cat or monkey hypothalamus is physiological shivering or a form of tremor not related to shivering. At least in monkey, cholinergic stimulation causes a coordinated thermoregulatory effort to increase the body temperature, consisting of shivering-like tremor, vasoconstriction, piloerection and behavioral huddling (Myers and Yaksh, 1969). This strongly implies that the tremor was a part of this effort and was therefore partly or wholly shivering. Because of the problems involved in observing drug-induced piloerection and huddling in the
present study, it was not possible to determine if these phenomena were involved in drug-induced thermoregulatory changes. Changes in ear skin temperature, however, were usually well coordinated with EMG activity. Large decreases in rectal temperature were usually associated with vasodilation which reverted to vasoconstriction as tremor and the upward swing in rectal temperature began. Since the cats were normally tonically vasoconstricted at 20°C ambient, in those experiments in which no initial vasodilation occurred, it was impossible to determine whether the cholinergic stimulation tended to increase peripheral vasomotor tone during the rectal temperature rise. However, the coordinated vasomotor activity that was observed reinforces the notion that cholinergic stimulation of the hypothalamus of the cat induces coordinated thermoregulatory changes.

Moreover, in most instances visual inspection could not differentiate CCh-induced tremor from physiological shivering. This is not to deny that there was considerable stereotyped motor activity such as ear twitching and involuntary head movements which are usually not seen during normal shivering. These movements, however, were usually limited to the head and when they subsided, the underlying tremor was seen to resemble cold-induced shivering.

The electromyogram after CCh treatment also resembled that of intense shivering. The EMG frequency of each type of tremor ranged between 26 and 33 Hz. CCh usually increased the amplitude of the EMG activity and the frequency of occurrence of spindle-bursts as compared to predrug control activity, while apparently leaving the basic shivering rhythm unchanged. Furthermore, in 2 cats which
developed high amplitude spindle-bursting after CCh treatment, the bursts were closely synchronous with inspirations, a phenomenon characteristic of shivering. Finally, in 2 other experiments, increasing the ambient temperature during CCh-induced tremor strongly suppressed CCh-induced tremor. As previously mentioned, warming of the environment rapidly suppresses shivering (Euler, 1960); it is unlikely that a form of tremor not related to temperature regulation would be so affected.

Taken together, the circumstantial evidence cited above indicates that CCh-induced tremor is shivering and strengthens the idea that cholinergic synapses play a role in central regulation of body temperature. The ability of environmental heating to inhibit cholinergic tremor is particularly convincing. However, these experiments require repetition in a larger number of animals.

On the other hand, the experiments of Stuart et al. (1961), which demonstrated that up to 5 mg/kg of atropine sulfate given intraperitoneally does not reduce cold-induced shivering in the cat, argue strongly that cholinergic synapses are not involved in the control of shivering. This argument seems especially strong since the present study as well as that of George et al. (1966) have shown that centrally or peripherally administered atropine can inhibit the tremor induced by the intrahypothalamic injection of CCh. As mentioned in Chapter I, it is possible that microinjected cholinergic stimulants act upon neurons which are physiologically excited or inhibited by another neurotransmitter during shivering. In this respect, sensitivity of hypothalamic neurons to more than one transmitter has been reported
Thus, the possibility arises that CCh-induced tremor may be shivering but that the synapses controlling physiological shivering are not cholinergic. It is also possible, however, that central cholinergic synapses participate in the control of shivering thermogenesis but that, for unknown reasons, the normal activity of these synapses is resistant to atropine blockade whereas the activity induced by the injection of exogenous muscarinic stimulants is readily antagonized by atropine. Muscarinic sites having these characteristics have been reported to exist in the peripheral autonomic nervous system (Koelle, 1965). Thus, in view of the contradictory evidence, it is not presently possible to define what role, if any, cholinergic synapses play in the hypothalamic control of shivering.

One of the most frequent effects produced by cholinergic intervention in hypothalamic thermoregulatory control was a biphasic change in rectal temperature (hypothermia followed by hyperthermia). Several speculative explanations for this phenomenon come to mind. Perhaps the simplest possibility is that microinjection of CCh at a given hypothalamic locus induces hypothermia by an action at this site and induces subsequent hyperthermia by diffusion or transport via the blood or cerebrospinal fluid to some more distant site. Such an explanation has been proposed to explain the delayed appearance of rage after forebrain implantation of CCh crystals in contrast to the immediate rage produced after brainstem implantation (Baxter, 1968).

However, in the present context such an explanation does not seem tenable. For example, biphasic effects could be elicited from loci as far lateral as 4 mm as well as from periventricular loci. Moreover,
biphasic responses obtained from sites widely spread throughout the hypothalamus were, with one exception, characterized by initial hypothermia followed by hyperthermia. It would seem that if the hyperthermia-producing site were within the hypothalamus, then hyperthermia should precede hypothermia at some sites, or, at the least, there should be a gradient of hyperthermia response latencies centered around this site. In only one instance did an increase in temperature precede the decrease, and there was no clear gradient of response latencies centered about this point. Thus, if one adheres to the diffusional hypothesis, the hyperthermia-inducing site must be extrahypothalamic. However, if this is true, one should expect to see a polarization of short hyperthermia response latencies toward this area. But short latencies were not limited to any one hypothalamic area. In addition, at 2 sites, the latency of the hyperthermic phase seen at low doses was similar to the latency of the hypothermic phase seen at higher doses, which suggests that the hyperthermia-inducing locus was at least as near as the site producing hypothermia.

A second possible explanation of the biphasic effect is suggested by the finding that carbachol produces surface potentials in the superior cervical ganglion of the cat consisting of initial hyperpolarization and blockade of transmission followed by depolarization and ganglionic firing (Brown, 1966). Although this series of events fits nicely with the initial fall in rectal temperature and the ensuing rise seen in the present experiments, the time course of these ganglionic potentials is only a few seconds, far too short to account for the biphasic temperature changes.
Large doses of ACh initially stimulate and then paralyze transmission at autonomic ganglia (Koelle, 1965). It is possible that these excitatory and inhibitory phases correspond with the hypothermic and hyperthermic phases of the biphasic effect seen when CCh is microinjected into the hypothalamus of the cat. Although there is again a problem with the time-course of the ganglionic effect versus the proposed central effect, another objection may also be raised. Invocation of this mechanism to explain the biphasic effect implies that increasing doses should reduce the initial excitatory (hypothermic) stage and augment the inhibitory (hyperthermic) stage. At most hypothalamic sites tested, just the opposite was found to occur.

A variant of this hypothesis is somewhat more acceptable. Here it is assumed that for an undetermined length of time following a microinjection, the highly concentrated solution remains localized near the tip of the microinjection cannula. Because of the high solute concentration and relatively rapid rate of injection, initial excitation is very short or not detectible and persistent depolarization occurs rapidly, producing initial hypothermia. Then, as the drug spreads to fill a 1 to 2 mm diameter sphere of tissue (Myers, 1966) and the concentration becomes less at the periphery, excitation begins. Depending on the rate of diffusion and/or metabolism of the injected drug, excitation and hyperthermia would become the dominant effect as more and more tissue was stimulated by the lower concentration. At first glance, this model of diffusion within a homogeneous neuronal pool would appear to explain many of the observed effects of micro-
injected cholinomimetics. However, as discussed previously (Chapter I), it has been demonstrated empirically many times that unilateral hypothalamic lesions have no detectable effects on thermoregulation. Since local persistent depolarization represents a functional lesion, it is unlikely that the initial hypothermia can be explained by this mechanism.

A final (and more speculative) suggestion assumes that there exist within the cat hypothalamus two diffuse and partially overlapping multisynaptic neural systems which project, respectively, excitatory and inhibitory influences on lower brainstem neurons which control or influence skeletal muscle tone and coordination. Cholinergic excitation of the inhibitory system would produce crossed inhibition, consequent atonia and/or reduction of shivering and hypothermia. Conversely, stimulation of the excitatory system would initiate shivering (or tremor) and hyperthermia. Where overlap of the two system is least, only hypothermia or hyperthermia would be produced, depending on which of the systems was stimulated. At points of overlap, cholinergic stimulation would yield biphasic responses. If it is assumed that the inhibitory neurons are less sensitive to cholinergic stimulation but have a proportionately greater effect on the lower brainstem neurons controlling skeletal muscle tone and phasic activity, then 1) with adequate dosage, hypothermia would usually be the first effect seen and 2) low doses would produce hyperthermia only, and increasing the dose would augment the hypothermic phase. By invoking the possibility of a few areas where there are sharp variations in the cell density of the two systems within the sphere of influence of the microinjected
droplet, it is even possible to explain the clockwork precision of the triphasic effect seen in cat SA-3 (Figure 24).

That a diffuse hypothalamic system modulating skeletal muscle tone and phasic activity exists is indicated by the finding that, at every site tested, microinjection of CCh produced thermoregulatory responses effected by changes in skeletal muscle activity. However, there is no direct evidence indicating the existence of the hypothetical excitatory and inhibitory components of this system or their pharmacologic reactivity. Nevertheless, it is interesting that the older electrical stimulation and lesion data pertaining to hypothalamic control of shivering (Chapter I) suggest that such antagonistic systems may exist and that the primary inhibitory center lies in the AH/PO region, the region where, in fact, it was found that microinjection of CCh most readily induced predominant hypothermia.
CHAPTER V

THE THERMOREGULATORY EFFECTS OF INTRAPERITONEAL, INTRACEREBROVENTRICULAR AND INTRACEREBRAL INJECTION OF CHLORPROMAZINE

Introduction

The tranquilizing agent, chlorpromazine (CPZ), has been shown to produce hypothermia in several species (Hoffman and Zarrow, 1958; Jacobsen, 1960). As has been discussed in considerable detail in Chapter I, there is some controversy concerning the mechanism through which this is accomplished. It is well known that CPZ enhances heat loss by producing cutaneous vasodilation, this effect being mediated, at least in part, by blockade of peripheral alpha adrenergic receptors. However, it is not well established that CPZ reduces shivering thermogenesis in any species except the rat. The finding of Kollias and Bullard (1968), that CPZ blocks all mechanisms of thermoregulation in the rat, led these investigators to suggest that the drug may act within the hypothalamus to disrupt central thermoregulatory control. Indeed, the ability of CPZ to suppress shivering in this species strongly indicates that this is the case, since there is no known mechanism by which CPZ might inhibit shivering by a peripheral mechanism. If CPZ could be shown to block shivering thermogenesis in animals other than the rat, then a central component of chlorpromazine's action would be indicated in these species as well. Therefore, as a first step, it was decided to examine the ability of intraperitoneally
injected CPZ to induce hypothermia in the cat, a species apparently not yet tested, and to determine whether impairment of shivering is involved in any fall in body temperature which might be found. Further, a preliminary examination of the ability of CPZ to disrupt thermo-regulation by an action within the central nervous system was made utilizing direct intracerebroventricular injections of the drug in order to prevent its access to peripheral receptors while simultaneously providing a high drug concentration at central pharmacologic receptors.

Since the results of these two experiments suggested that at least some of chlorpromazine's thermoregulatory disrupting effects were exerted at a central site, microinjections of a fixed dose of CPZ were made into several areas of the hypothalamus to test the possibility that these effects are mediated within this structure.

**Methods**

**Intraperitoneal Injections**

The experimental subjects were 6 male cats. Five of these had microinjection cannulae implanted in various diencephalic loci. One of these 5 had, in addition, a cannula positioned so that injections could be made into a lateral ventricle. The sixth animal had a ventricular injection cannula only.

The general treatment of these animals and the experimental protocol are as described in Chapter II, with the exception that after the control period the drug was injected intraperitoneally rather than intracerebrally. Ear skin temperature, respiratory rate, EMG activity and rectal temperature were monitored until rectal temperature had
ceased to decline or until it had fallen to a level which endangered the life of the subject (about 30°C). In the latter case, the environmental temperature was increased and the animal was released from restraint.

Chlorpromazine hydrochloride was dissolved in distilled water at a concentration of 50 mg/cc, the resulting solution being brought to isotonicity by the addition of NaCl. Five cats received 10 mg/kg of CPZ HCl intraperitoneally at an environmental temperature of 20°C. One cat received 20 mg/kg intraperitoneally at an environmental temperature of 5°C. One of the first 5 cats also received 2.5, 5, and 20 mg/kg intraperitoneal injections of CPZ HCl, again at 20°C ambient, in order to establish a dose response relationship. In this animal, at least 5 days intervened between the successive doses.

Intracerebroventricular Injections

Two animals received intracerebroventricular injections of CPZ HCl. One subject was injected with 0.5, 1.0 and 3.0 mg of the drug in that order and at an environmental temperature of 20°C. The treatments were separated by at least 5 days. The other subject received 1.0 mg of CPZ HCl only; the ambient temperature was 5°C. Again, the experimental protocol was the same as previously described (Chapter II) except that the drug was injected into the lateral ventricle rather than into the cerebral tissue. Drug solutions were sterile, pyrogen-free and isotonic, and contained the amount of CPZ HCl to be injected in 100 μl. The loaded injection cannula was placed into its guide 10 or more minutes prior to the time of injection. At the designated time, 100 μl of solution was injected at an even rate over a 2-minute period. The injection cannula was left in place until the session was terminated.
Intracerebral Injections

A total of 13 cats, 12 male and 1 female, were included in the intracerebral microinjection experiments. Each of these animals had participated in the studies described in Chapters III and IV. The handling of the animals, experimental protocol and preparation of the drug solutions have been described (Chapter II). Each cat had 2 to 4 guide cannulae implanted so as to permit microinjections into various diencephalic loci.

Each site was treated with a standard dose of 100 μg of CPZ HCl contained in 1 μl of solution. In several instances, bilateral as well as unilateral injections were made. A few sites which proved to be very active were treated with 10 μg of CPZ HCl as well as the standard dose. Most sites were also treated with 1 μl of sterile, pyrogen-free isotonic saline as a control for volume and tonicity effects.

Results

Intraperitoneal Injections

The change in rectal temperature caused by 10 mg/kg of CPZ HCl injected intraperitoneally into 5 cats kept at 20°C ambient is shown in Figure 29 (lower panel). In one of these cats (SA-13), the injection may inadvertently have been given intravenously rather than intraperitoneally, for, in this animal, strong drug effects were apparent only 1.5 minutes after the injection.

The mean maximum fall in rectal temperature produced by the intraperitoneal CPZ was 2.9°C (excluding SA-13). The individual data were: SA-1, 3.5°; SA-12, 2.6°; SA-7, 2.3°; SA-16, 3.2°; SA-13, > 6.9°C.
Figure 29. Rectal temperature changes produced by intraperitoneal injection of chlorpromazine.

Upper panel—rectal temperature change in cat SA-12 after intraperitoneal injection of 2.5, 5, 10 and 20 mg/kg of chlorpromazine HCl (CPZ). Injections were made at the arrow. At least 5 days intervened between doses. Ambient temperature: 20°C.

Lower panel—rectal temperature changes in cats SA-1, SA-7, SA-12, SA-13 and SA-16 after intraperitoneal injection of 10 mg/kg of chlorpromazine HCl (CPZ). Injections were made at the arrow. Ambient temperature: 20°C.
CHANGE IN RECTAL TEMPERATURE (°C)
Intraperitoneal injections of normal saline had little or no effect on body temperature.

Vocalization, excitation and struggling were frequent occurrences after the CPZ injections, but were particularly prominent in the early postinjection period when rectal temperature was falling most rapidly. However, when the environmental chamber was opened and the animals were stimulated by the experimenter they were seen to be quite unresponsive to mildly nociceptive stimuli even during the periods of spontaneous excitement. Nictitating membrane relaxation and ptosis were observed in each animal treated with the 10 mg/kg dose, but the magnitude of these effects seemed to vary considerably between subjects.

The duration of the various thermoregulatory effects induced by 10 mg/kg of CPZ was not measured. However, except for SA-16 and SA-13, the subjects were still vasodilated at the end of the recording period indicated in Figure 29 (lower panel). SA-16 experienced a very short ear skin vasodilation, the effect lasting only 15 minutes. SA-13, which probably received the dose intravenously rather than intraperitoneally, vasoconstricted as rectal temperature reached 34°C, 2.5 hours after the injection. In all cats except SA-13, electromyographic activity indicative of shivering had begun to return by 3 hours after the CPZ injection. In cat SA-13, the EMG had not recovered by 4 hours postinjection. The ambient temperature in the environmental chamber was increased to 26°C as this animal's body temperature approached 32°C, and the cat was released from restraint. At 22 hours after the drug injection, rectal temperature was still only 32.5°C. The animal treated with 20 mg/kg at 5°C ambient was also released into
a warm environment as rectal temperature reached 30°C. However, in
spite of the rapid fall in rectal temperature, this animal was shiver­
ing strongly when released. After being released into the warm envi­
ronment, shivering became violent.

One animal received several different doses of CPZ in addition
to the standard 10 mg/kg dose. The resulting changes in rectal tem­
perature are illustrated in Figure 29 (upper panel). The CPZ-induced
hypothermia is clearly dose-dependent.

As might be expected, the subject which was treated with 20 mg/kg
of CPZ HCl at an ambient temperature of 5°C experienced extreme hypo­
thermia. By 3.5 hours after the injection, rectal temperature had
fallen to 30°C and the experiment was terminated. After an intra­
peritoneal injection of saline, the same animal maintained his rectal
temperature at 38°C during 5 hours of exposure to the 5°C environment.

The fall in rectal temperature elicited by intraperitoneally
injected CPZ was mediated, at least in part, by peripheral vasodilation
and suppression of shivering. The changes in vasomotor tone and shiver­
ing produced by intraperitoneal injection of 5 mg/kg of CPZ HCl in one
animal are portrayed in Figure 30 (ambient temperature = 20°C). Simi­
lar effects were seen in animals treated with 10 mg/kg of the drug,
although in one case the vasodilation lasted only 15 minutes. Ear
skin temperature was not measured in the cat treated in the 5°C envi­
ronment, but suppression of EMG activity after injection of CPZ was pro­
found.

Irrespective of the dose employed, the first effect produced by
the drug was suppression of EMG activity. This was followed within a
Figure 30. Effect of intraperitoneal injection of 5 mg/kg of chlorpromazine on rectal temperature ($T_r$), ear skin temperature ($T_e$) and electromyographic activity (EMG) in a single cat (SA-12).

Chlorpromazine HCl (CPZ) was injected at the arrow. EMG is presented histographically as the mean rate of pen rise for each 10-minute period of the experimental session (see Chapter II).
few minutes by bilateral vasodilation of the ear skin. Rectal tempera-
ture began to decrease as soon as shivering was suppressed. At the
same time, the subjects assumed a splayed posture and usually remained
in this abnormal configuration for the remainder of the session or
until strong shivering had returned.

Intracerebroventricular Injections

The rectal temperature changes induced in the 2 cats receiving
intraventricular injections of CPZ HCl are shown in Figure 31. In
cat SA-16, 0.5 or 1.0 mg of CPZ HCl produced immediate sedation and,
later, ptosis. Nictitating membrane relaxation was observed at the
1.0 mg dose. Each of these 2 doses produced a small reduction in EMG
activity soon after the injection, and rectal temperature fell slightly
(0.4°C in each case). There was no vasodilation.

The highest dose, 3.0 mg, produced an effect resembling that
elicited by 10 mg/kg of CPZ injected intraperitoneally. There was
immediate sedation followed by excitation and struggling. The animal
then stretched out in a splayed position, and the EMG was almost
abolished, except for occasional episodes of struggling. Simultane-
ously with EMG suppression, vasodilation appeared, and rectal tempera-
ture fell precipitously. Vasoconstriction and sporadic shivering re-
appeared 60 minutes after the injection. During the rising phase of
rectal temperature, shivering was extremely intense, but the huddled
posture typically assumed during intense shivering was not resumed.
Nictitating membrane relaxation and ptosis were observed at about 1
hour postinjection, but their times of appearance were not ascertained.
After the session, when the animal was removed from restraint,
Figure 31. Rectal temperature changes produced by intracerebroven-
tricular injection of chlorpromazine.

Chlorpromazine HCl (CPZ) was injected at the arrow. The total amount injected in indicated at the end of each curve. The injection volume in each case was 100 μl.

The 3 upper curves represent data obtained from cat SA-16. At least 5 days intervened between doses. Ambient temperature: 20°C.

The lower curve represents data obtained from cat C-2. Ambient temperature: 5°C.
nictitating membrane relaxation, ptosis, moderate catatonia, akinesia and sluggish righting reflexes were seen. The following morning, the cat appeared to be normal in every respect.

Intraventricular injection of 100 μl of 0.9% saline produced no observable effect in SA-16. Nor did 3.0 mg of CPZ HCl given intraperitoneally have any discernable effect except slight relaxation of the nictitating membrane.

With the exception that ear skin temperature was not recorded, cat C-2, which received 1.0 mg CPZ HCl at an ambient temperature of 5°C, experienced effects very similar to those seen in SA-16 at the 3.0 mg dose.

**Intracerebral Injections**

The response produced by micrc'jection of the standard dose of 100 μg of CPZ HCl into the diencephalon depended upon the area injected and sometimes on whether the injections were made bilaterally or unilaterally. Simultaneous bilateral injections into loci in the diagonal band (A 15.5, L 2.5, H -5.0; A 15.0, L 2.0, H -5.0) in cat SA-3 elicited a startle response, urination, defecation and struggling but no change in rectal temperature except the slight increase attributable to the struggling. A unilateral injection into the medial pre-optic nucleus (A 15.0, L 1.0, H -3.0) elicited in cat SA-9 an increase in EMG activity and a slow increase in rectal temperature, the maximum rise of 0.7°C being reached at 1.5 hours postinjection.

The remainder of the microinjection data are summarized in
Figures 32-38. Illustrated in these figures are the sites\(^1\) of CPZ injections, both unilateral and bilateral, and the rectal temperature responses that were elicited.

Responses to CPZ injected into the caudal portion of the preoptic region are seen in Figure 32. In cat SA-8 (panel A), unilateral injection of CPZ yielded a very slight fall in rectal temperature due to delayed vasodilation (30 minutes postinjection) followed by vasoconstriction and an increase in rectal temperature. In cat SA-15 (panel B), unilateral injection of CPZ caused, after a 15 minute latency, struggling and a short but considerable decrease in shivering, resulting in a small rectal temperature decrease; there was no vasodilation. Unilateral injection on either side or bilateral injection of CPZ in cat SA-13 (panels C, D and E) caused a considerable rectal temperature fall but no change in ear skin temperature or detectible reduction of shivering. Interestingly, the effect of bilateral injection was less than that produced by the unilateral injection illustrated in panel E. Unilateral injection of 10 \(\mu g\) of CPZ HCl into this site had no effect (not illustrated).

Several injections were made into the anterior hypothalamic and lateral hypothalamic regions just posterior to the optic chiasm (Figure 33). Electromyographic activity was slightly reduced during the small decrease in rectal temperature produced by unilateral injection of CPZ in cat SA-1 (panel A), but no EMG increase could be detected during the rectal temperature rise which followed. The

\(^1\) It should be noted that the illustrated placements are approximations only. The exact site coordinates according to the atlas of Snider and Niemer (1961) can be found in the individual figure legends.
Figure 32. Rectal temperature changes produced by intracerebral injection of chlorpromazine.

The approximate sites of injection (filled circles) are plotted on a stylized frontal section of the cat brain. AC = anterior commissure  CAU = caudate nucleus  F = fornix GP = globus pallidus  IC = internal capsule  OC = optic chiasm  PU = putamen  S = septal region

The graphs depict rectal temperature changes in °C (ordinate) over time in hours (abscissa) after injection of 100 μg of chlorpromazine HCl at the arrow.

Solid arrows extending from injection sites point to graphs portraying responses produced after unilateral injection of chlorpromazine into these sites.

Dashed arrows point to graphs portraying responses produced after simultaneous bilateral injection of chlorpromazine at the sites indicated.

Actual sites of injection according to the atlas of Snider and Niemer (1961):

Panel A..........A 13.5  L 2.5  H -3.5
Panel B..........A 13.3  L 2.5  H -3.5
Panel C..........A 13.3  L 1.5  H -4.0
Panel E..........A 13.3  L 2.5  H -4.0

SA-8, etc. are cat identification codes.
Figure 33. Rectal temperature changes produced by intracerebral injection of chlorpromazine.

The approximate sites of injection (filled circles) are plotted on a stylized frontal section of the cat brain. CAU = caudate nucleus EN = endopeduncular nucleus F = fornix GP = globus pallidus IC = internal capsule OT = optic tract PU = putamen RN = reticular nucleus of the thalamus

The graphs depict rectal temperature changes in °C (ordinate) over time in hours (abscissa) after injection of 100 μg of chlorpromazine HCl at the arrow.

Solid arrows extending from injection sites point to graphs portraying responses produced after unilateral injection of chlorpromazine into these sites.

Dashed arrows point to graphs portraying responses produced after simultaneous bilateral injection of chlorpromazine at the sites indicated.

Actual sites of injection according to the atlas of Snider and Niemer (1961):

Panel A..........A 13.0 L 1.2 H -3.5
Panel B..........A 12.3 L 2.5 H -3.0 (left site)
Panel C..........A 12.5 L 1.5 H -3.5
Panel D..........A 13.0 L 1.0 H -3.0
Panel E..........A 12.5 L 1.8 H -4.0
Panel G..........A 12.8 L 1.8 H -4.0
Panel H..........A 12.3 L 1.5 H -4.0

SA-1, etc. are cat identification codes.
bilateral and unilateral injections illustrated in panels B and D, respectively, had little effect on any thermoregulatory parameter. There was likewise little rectal temperature change in cat SA-15 following a unilateral microinjection (panel C). However, the injection did elicit an immediate increase in EMG activity, increased alertness and, 45 minutes later, defecation. Unilaterally injected CPZ caused behavioral restlessness and a slow rectal temperature increase in cat SA-12 (panel H). Unilateral injection on either the right or left side in cat SA-18 (panels E and G) precipitated partial vasodilation and a slight reduction in EMG activity. Bilateral injections at these sites (panel F) produced greater vasodilation and EMG reduction as well as sedation during the rectal temperature decline.

Figure 34 illustrates the effects of simultaneous bilateral injections of CPZ in 2 cats whose injection sites were found to lie in different rostrocaudal planes. The 2 sites for each animal have been plotted on 2 half-brain diagrams cut at the preoptic and anterior hypothalamic levels, respectively. With the exception of panel F, the effects of the unilateral injections have been discussed. Bilateral injections in cat SA-15 caused EMG suppression during the falling phase and EMG activation during the rising phase of the rectal temperature plot. At no time was vasodilation or any change in the cat's demeanor observed. The effect in cat SA-1 was almost identical.

When injected just lateral to the ventromedial nucleus, CPZ produced profound changes in thermoregulation (Figure 35, panels A, B and C). Unilateral injection into either side produced no change in vasomotor tone but slightly suppressed shivering, resulting in a
Figure 34. Rectal temperature changes produced by intracerebral injection of chlorpromazine.

The approximate sites of injection (filled circles) are plotted on two stylized partial frontal sections of the cat brain (see text). AC = anterior commissure CAU = caudate nucleus EN = endopeduncular nucleus F = fornix GP = globus pallidus IC = internal capsule OC = optic chiasm OT = optic tract RN = reticular nucleus of the thalamus S = septal region

The graphs depict rectal temperature changes in °C (ordinate) over time in hours (abscissa) after injection of 100 μg of chlorpromazine HCl at the arrow.

Solid arrows extending from injection sites point to graphs portraying responses produced after unilateral injection of chlorpromazine into these sites.

Dashed arrows point to graphs portraying responses produced after simultaneous bilateral injection of chlorpromazine at the sites indicated.

Actual sites of injection according to the atlas of Snider and Niemer (1961):

Panel A...........A 13.3 L 2.5 H -3.5
Panel C...........A 12.5 L 1.5 H -3.5
Panel D...........A 13.0 L 1.2 H -3.5
Panel F...........A 13.5 L 1.0 H -3.5

SA-15, etc. are cat identification codes.
Figure 35. Rectal temperature changes produced by intracerebral injection of chlorpromazine.

The approximate sites of injection (filled circles) are plotted on a stylized frontal section of the cat brain. CAU = caudate nucleus EN = endopeduncular nucleus F = fornix GP = globus pallidus IC = internal capsule NR = reticular nucleus of the thalamus OT = optic tract PU = putamen RE = reuniens nucleus VA = ventromedial hypothalamic nucleus

The graphs depict rectal temperature changes in °C (ordinate) over time in hours (abscissa) after injection of 100 μg of chlorpromazine HCl at the arrow.

Solid arrows extending from injection sites point to graphs portraying responses produced after unilateral injection of chlorpromazine into these sites.

Dashed arrows point to graphs portraying responses produced after simultaneous bilateral injection of chlorpromazine at the sites indicated.

Actual sites of injection according to the atlas of Snider and Niemer (1961):

Panel A........A 11.5 L 2.0 H -4.5
Panel B........A 11.0 L 2.0 H -4.5

SA-3 is a cat identification code.
correspondingly small rectal temperature decrease. However, simultaneous injections into the 2 nearly bilaterally symmetrical sites caused immediate cessation of EMG activity, partial vasodilation of the ear skin and a rectal temperature fall of more than 2°C. Interestingly, the cat remained alert and seemed completely unaware that these events were transpiring. Close inspection of the animal at the end of the temperature fall revealed no ptosis, nictitating membrane relaxation or behavioral sedation. A bilateral injection of 10 µg of CPZ at the same sites had no effect on temperature regulation.

Microinjections of CPZ into the dorsal hypothalamic area (Figure 36, panels A and B) had little effect on temperature regulation. However, the injections did cause restlessness and excitement. The small perturbations visible in the temperature tracings are due to this excitation.

Unilateral or bilateral injections of CPZ into the posterolateral hypothalamic area, illustrated in Figure 36, yielded a slight decrease in rectal temperature (panels C, D and E) but no vasodilation or EMG suppression could be detected. In each case the cat, which had been vocalizing frequently prior to drug injection, ceased to do so soon after the microinjection. Paradoxical sleep (PDS) or a PDS-like state was observed following the bilateral injection (visible as a slight dip in the rectal temperature curve at time 5 hours in panel D) and after one of the unilateral injections (visible as 2 dips at 3.5 and 4.5 hours in panel E). In each instance of this PDS-like state there was a sudden and unheralded cessation of EMG activity and complete loss of postural tone for about 90 seconds. Postural tone and EMG activity
Figure 36. Rectal temperature changes produced by intracerebral injection of chlorpromazine.

The approximate sites of injection (filled circles) are plotted on a stylized frontal section of the cat brain. EN = endopeduncular nucleus  F = fornix IC = internal capsule  M = mammillothalamic tract OT = optic tract  PH = posterior hypothalamic area RE = reuniens nucleus  RN = reticular nucleus of the thalamus  VA = ventral anterior thalamic nucleus

The graphs depict rectal temperature changes in °C (ordinate) over time in hours (abscissa) after injection of 100 µg of chlorpromazine HCl at the arrow.

Solid arrows extending from injection sites point to graphs portraying responses produced after unilateral injection of chlorpromazine into these sites.

Dashed arrows point to graphs portraying responses produced after simultaneous bilateral injection of chlorpromazine at the sites indicated.

Actual sites of injection according to the atlas of Snider and Niemer (1961):

Panel A.................A 9.0 L 1.5 H -2.0
Panel B.................A 9.0 L 2.0 H -2.0
Panel C.................A 9.0 L 2.0 H -3.5
Panel E.................A 9.0 L 2.0 H -3.5

SA-17, etc. are cat identification codes.
returned just as quickly at the end of this period.

Injections of CPZ into the most caudal portion of the posterior hypothalamus seemed to elicit two effects, a slow decrease in rectal temperature and repetitive PDS-like episodes (Figure 37). Two such episodes can be seen in panel A. The sharp decreases in rectal temperature were associated with loss of postural tone and complete block of EMG activity. Note that the episodes are superimposed on a slow decrease/increase in rectal temperature which begins about 20 minutes after the injection.

A somewhat similar pattern was produced in cat SA-10 (panel D) after injection of CPZ into the supramammillary region. However, although the EMG spindled-off repetitively for short periods, there was never complete cessation of activity; nor was a change in posture or behavior observed.

In the effect depicted in panel B, the initial drop in rectal temperature was mediated by a trailing-off of EMG activity similar to that observed after injection of catecholamines into the AH/PO region. The fall was interrupted by a bout of struggling but then continued steeply due to complete blockade of shivering and concomitant vasodilation. During this period, the animal abandoned his usually huddled position and lay stretched out on his stomach. The vasodilation subsided at the next plateau, which was caused by a prolonged burst of shivering. The remaining dips and rises in the record were produced by periodic bursts of shivering followed by periods of EMG suppression. It should be noted that in panels B and D, the tops of the temperature rises can be connected by an imaginary line to form a smooth curve of
Figure 37. Rectal temperature changes produced by intracerebral injection of chlorpromazine.

The approximate sites of injection (filled circles) are plotted on a stylized frontal section of the cat brain. CM = central medial thalamic nucleus
CP = cerebral peduncle  FF = field of Forel  LGN = lateral geniculate nucleus  MM = medial mammillary nucleus
NR = reticular nucleus of the thalamus  OT = optic tract  SN = substantia nigra  ZI = zona incerta

The graphs depict rectal temperature changes in °C (ordinate) over time in hours (abscissa) after injection of 100 µg of chlorpromazine HCl at the arrow.

Solid arrows extending from injection sites point to graphs portraying responses produced after unilateral injection of chlorpromazine into these sites.

Dashed arrows point to graphs portraying responses produced after simultaneous bilateral injection of chlorpromazine at the sites indicated.

Actual sites of injection according to the atlas of Snider and Niemer (1961):

Panel A............A 7.5  L 2.0  H -3.0
Panel B............A 8.0  L 3.0  H -4.5
Panel C............A 7.5  L 3.5  H -4.5 (left site)
Panel D............A 8.0  L 1.0  H -4.5 (right site)

SA-16, etc. are cat identification codes.
rectal temperature decrease.

Panel C of Figure 37 illustrates the effect of injecting CPZ into the vicinity of the substantia nigra and subthalamic nucleus in cat SA-11. Here, as in panel A, the sharp drops in body temperature were apparently due to repeated episodes of PDS. The second injection, made into the contralateral site, which impinged slightly upon the peduncle, did not augment the effect of the initial injection and may have antagonized it. Note the smooth curve formed by a line connecting the tops of the temperature rises and that this line is almost horizontal during the latter portion of the session.

In spite of the frequent occurrence of the PDS-like episodes after microinjections of CPZ into the areas portrayed in Figure 37, when the cats were awake, there did not appear to be any sedation, ptosis or nictitating membrane relaxation.

The most caudal sites tested lay at the hypothalamo-midbrain junction at the level of exit of the third nerves, as illustrated in Figure 38. Sequential injections into these nearly bilaterally symmetrical loci (upper panel) caused a small reduction in EMG activity and a slight rectal temperature fall after each injection. However, there was no clear PDS or PDS-like state produced. On the other hand, simultaneous bilateral injections elicited an immediate PDS-like state followed by 2 more such episodes. The flatness of the imaginary line connecting the peaks in the temperature record should again be noted.

With two exceptions, each of the unilateral or bilateral injections illustrated in Figures 32-38 was repeated at least once using isotonic
Figure 38. Rectal temperature changes produced by intracerebral injection of chlorpromazine.

The approximate sites of injection (filled circles) are plotted on a stylized frontal section of the cat brain. cg = central gray matter of the midbrain cp = cerebral peduncle lgn = lateral geniculate nucleus mgn = medial geniculate nucleus nr = red nucleus pc = posterior commissure an = substantia nigra

The graphs depict rectal temperature changes in °C (ordinate) over time in hours (abscissa) after injection of 100 μg of chlorpromazine at the arrow(s).

The upper panel illustrates the effects of sequential injection of chlorpromazine at the two nearly symmetrical sites.

The lower panel illustrates the effects of simultaneous bilateral injection of chlorpromazine at these sites.

Actual sites of injection according to the atlas of Snider and Niemer (1961):

A 6.0 L 2.0 H -3.0 (left site)
A 6.0 L 2.5 H -3.5 (right site)

SA-15 identifies the experimental subject.
saline or a lower concentration of CPZ (10 µg in 1 µl) as a control for volume and tonicity effects. No control injection was made for the effect shown in Figure 38 (lower panel). The effect illustrated in Figure 37, panel C was not controlled for by injections of saline or low CPZ concentrations, but epinephrine (0.03 molar) elicited a PDS-like episode after sequential injection into these 2 sites. The latency in each case was 20 minutes.

The control injections usually produced little or no change in behavior or thermoregulation. However, in 2 instances, the saline injections elicited effects similar to those produced by the standard dose of CPZ. The rise in temperature shown in Figure 34, panels D, E and F, was reproduced by the saline controls. However, the initial temperature fall seen in D and E was not reproduced. The temperature rise portrayed in Figure 33, panel H was also elicited by a microinjection of saline.

**Discussion**

Chlorpromazine HCl, administered intraperitoneally in doses of 2.5 to 20 mg/kg, produced hypothermia in cats maintained at an ambient temperature of 20°C. This not unexpected finding is in agreement with numerous reports of CPZ-induced hypothermia in other species (see Chapter I).

The fall in body temperature was mediated by cutaneous vasodilation and inhibition of shivering. Thus, both heat-loss and heat-gain mechanisms were affected. The ability of CPZ to elicit peripheral vasodilation, at least in part by blockade of peripheral alpha-receptors
is well documented (see Chapter I). However, CPZ-induced suppression of physiological shivering has not been convincingly demonstrated in any species except the rat. In the present experiments, shivering, muscle tone, and postural reflexes were markedly impaired. Since shivering suppression was always the first effect to appear after drug administration and body temperature fell in synchrony with this phenomenon before the appearance of cutaneous vasodilation, it is certain that shivering inhibition was an important factor in the hypothermic effect. Further, since there is no acceptable mechanism which can explain shivering inhibition on a peripheral basis (Borison and Clark, 1967), this phenomenon and therefore at least a portion of the hypothermic effect can be attributed to an action of CPZ within the central nervous system.

Intracerebroventricular injection of 3 mg of CPZ HCl in one cat kept in an ambient temperature of 20°C and 1 mg in another cat kept in an environmental temperature of 5°C gave further evidence that CPZ-induced hypothermia is of central origin. Administered by this route, CPZ induced cutaneous vasodilation (in one cat; ear skin temperature was not recorded in the other), inhibition of shivering, loss of postural reflexes and profound hypothermia. Furthermore, the behavioral effects produced were quite similar to those observed after high doses of CPZ are given intraperitoneally. Although there is some possibility of leakage of the drug from the ventricular spaces into the systemic circulation (Rech, 1968), intraperitoneal injection of 3 mg of CPZ had no effect on thermoregulation in the same cat which experienced hypothermia after intraventricular administration of an
identical dose.

It might be argued that 1000 to 3000 μg of CPZ is an "unpharmacological" amount in comparison to the quantities found in brain after peripheral administration. However, it should be remembered that the intraventricularly administered drug is rapidly diluted by the cerebrospinal fluid (CSF) (Rech, 1968) so that only a small portion of the drug comes in contact with the ventricular walls. Further, the drug is likely to be rapidly cleared by bulk caudalward flow of CSF long before more than a small fraction of the total amount of drug injected can be absorbed into the structures surrounding the ventricles. Thus, it is not likely that the drug concentration in the structures forming the walls of the ventricles was unduly excessive.

The results of the intraventricular injection experiments, although requiring verification in a larger number of animals, strongly suggest that not only the thermoregulatory effects, but also some of the autonomic effects of CPZ, e.g., ptosis and relaxation of the nictitating membrane, may be mediated partially by central mechanisms.

The rapid onset of thermoregulatory disruption produced by intraventricular injection of the drug indicates that the responses were probably mediated by neural structures lying in or at a short distance from the ventricular walls (Feldberg, 1963; Rech, 1968). However, since no attempt was made to limit the diffusion of the injected solutions to a specific area of the ventricles, the exact locus of action could not be determined from these experiments. The report of Kollias and Bullard (1968), demonstrating that CPZ blocks all mechanisms of thermoregulation in the rat suggested that the hypothalamus, well
known to be intimately involved in the control of body temperature and forming the ventral half of the walls of the third ventricle, could be the structure mediating CPZ-induced hypothermia.

Bilateral and unilateral microinjections of CPZ into several areas of the hypothalamus in an effort to replicate the effects produced by intraperitoneal and intraventricular injection yielded encouraging results. Fifteen sites lying in the AH/PO region were subjected to CPZ microinjections. Clearcut thermoregulatory effects were seen after unilateral injections into 5 of these loci. The largest fall in body temperature occurred in the absence of vasodilation or any detectible change in shivering (Figure 32, panels C, D and E). It seems likely that the rectal temperature fall was due to a subtle but generalized inhibition of shivering that was not detected by the recording techniques used. Another possibility is that vasodilation occurred at areas other than the ears, such as the footpads (Strom, 1950a). However, the possibility that the drug inhibited some form of nonshivering thermogenesis cannot be discounted (see Chapter I). On the other hand, suppression of shivering evidently was responsible for the slight hypothermic effect seen after injection of CPZ into another AH/PO site (Figure 33, panel A), and vasodilation, EMG suppression and hypothermia were produced by injections into 2 other loci within the AH/PO region (Figure 33, panels G, E and F).

However, the most profound hypothermic effects were seen after CPZ injections into the region between the fornix column and the ventromedial hypothalamic nucleus and into the posterolateral hypothalamus. Bilateral injections into the first locus or unilateral injections into
the second produced almost complete blockade of EMG activity, partial or complete vasodilation and a rapid rectal temperature decrease. In addition, injections into the posterior hypothalamic site produced the "spread-eagle" posture typically elicited by peripherally administered CPZ.

These results are encouraging in that they demonstrate that direct application of CPZ to various regions of the hypothalamus can induce thermoregulatory changes similar to those seen after intraventricular or peripheral administration of the drug. Thus, they support the hypothesis that CPZ may act within the hypothalamus to alter the homeostatic regulation of body temperature.

However, the high drug concentration used casts some doubt on the interpretation of these data. Chlorpromazine is a potent local anesthetic (Bradley, 1963), and the 10% solution used for the microinjections cannot have failed to produce local anesthesia at the tip of the injection cannula. That the responses produced may have depended upon this high drug concentration is indicated by the failure of 10 µg to produce any effect when injected into the sites most responsive to the 100 µg dose. But, as explained in Chapter I, it is commonly found that high concentrations are necessary for microinjected drugs to elicit an effect, and there are several reasonable explanations for this necessity. Furthermore, there is experimental support for the contention that these high drug concentrations do not obviate the possibility that the drug is acting by the same specific pharmacological mechanisms by which it acts when presented to the brain via the general circulation. For example, Lotti et al. (1965) demonstrated that the hypothermic
effect of morphine injected in 50 μg quantities into the AH/PO region
of the rat (yielding a probable local concentration within the radius
of spread of the drug solution of 12,500 μg/gm of tissue) is selectively
blocked by centrally or peripherally administered nalorphine. Thus,
the effect was receptor specific. That such receptor specificity
can be seen at high local concentrations is also supported by the ex-
periments described in Chapter III of this thesis.

The repetitive PDS or PDS-like episodes induced by CPZ when it
is injected into the most caudal portion of the hypothalamus and into
the hypothalamo-midbrain junctional region, although of intrinsic
interest, also have implications with respect to the thermoregulatory
effects of CPZ. So little is known about the neural mechanisms in-
volved in the control of paradoxical sleep that it seems fruitless to
conjecture how locally injected CPZ could elicit this state. However,
the fact that these episodes were superimposed upon a general fall in
rectal temperature is of potential significance.

As previously discussed (Chapter I), the prime criterion for
demonstration of an altered thermoregulatory "setpoint" is that body
temperature indeed be regulated about this point. This has been shown
to be the case during pyrogen-induced fever. Inspection of the rectal
temperature curves in Figure 37 reveals that the strong shivering after
each hypothermic episode brings rectal temperature back to a level
delineated by a smooth line drawn along the peaks of the record. In
other words, body temperature seems to be regulated around a set-
point which slowly decreases and returns toward normal. If this is
indeed the case, it is an important finding since, as Borison and
Clark (1967) have pointed out, there is no known example of the set-point being artificially lowered. Moreover, it is of interest that these effects were activated by the posterior hypothalamus; the anterior hypothalamus has usually been considered as the source of the thermoregulatory setpoint (Kammel, 1968). For these reasons, the effect of CPZ injected into the posterior sites described would bear further investigation.

The experiments just discussed were not designed to examine the pharmacological mechanisms through which CPZ exerts its effects on the central control of body temperature. Indeed, their purpose was to look at the thermoregulatory effects of peripherally injected CPZ in the cat and to determine whether these effects could arise from an action of the drug within the central nervous system. This has been accomplished, and several sites within the hypothalamus have been shown to be sensitive to locally injected CPZ. Further research concerned with mechanisms of action can concentrate upon these areas. Nevertheless, the author feels moved to speculate a bit about possible pharmacological mechanisms. Since these thoughts are related to results reported in Chapters III and IV of this dissertation, they are presented in the General Discussion which follows.
CHAPTER VI
GENERAL DISCUSSION, SUMMARY AND CONCLUSIONS

The experimental results, interpretations and conclusions described in detail in Chapter III, IV and V of this dissertation are summarized below:

**Experiments with sympathomimetic amines (Chapter III)**

It was found that the sympathomimetic amines, epinephrine, norepinephrine, phenylephrine and isoproterenol caused dose-dependent hypothermia when injected into the hypothalamus of the cat. The relative potency of these agents with respect to several thermoregulatory parameters was similar to that described for the actions of sympathomimetic amines at peripheral alpha receptors, isoproterenol being much less active than epinephrine or norepinephrine and phenylephrine having a potency lying between these extremes. The hypothermia induced by epinephrine could be blocked by pretreatment of the hypothalamic site with the alpha-receptor blocker, phentolamine, but not by propranolol, a beta-receptor antagonist. Hypothermia was most reliably elicited by injection of the sympathomimetic amines into the anterior hypothalamic/preoptic (AH/PO) region, but a few loci outside this region were also sensitive. The fall in body temperature produced by these agents was effected by peripheral vasodilation and inhibition of shivering. These effects were often accompanied by profound sedation. It was concluded that the
sympathomimetic amines, after microinjection into the AH/PO region, induce hypothermia by an action at receptors within the central nervous system which have characteristics similar to peripheral alpha-receptors. The possibility that they act by some non-specific mechanism is thus discounted.

Although these data support the hypothesis that endogenous hypothalamic norepinephrine may participate in the central control of body temperature, the possibility must be considered that the thermoregulatory changes observed may represent the actions of endogenous norepinephrine within hypothalamic neural systems not directly concerned with temperature regulation, but activating (or inhibiting) common peripheral effectors.

Experiments with cholinomimetic agents (Chapter IV)

It was found that microinjection of carbamylcholine (CCh) into the hypothalamus produced thermoregulatory changes that were dependent upon the dose of drug and the site of administration. The most common effect was a rectal temperature fall followed by a larger rise. This biphasic response was seen after microinjections of CCh at sites scattered throughout the hypothalamus, usually after application of intermediate CCh concentrations (0.01 or 0.03 molar). Stimulation of many sites with doses of CCh which exceeded those eliciting the biphasic effect augmented the hypothermic phase, and the fall in rectal temperature became the major effect. After similar stimulation of other loci, hyperthermia remained the predominant effect, even at the highest doses. At the two intermediate doses, CCh elicited predominant hypothermia only when injected into the anterior hypothalamic/preoptic region. In
a limited number of tests, acetylcholine-eserine mixtures produced responses similar to CCh.

Hypothermia was usually brought about by inhibition of shivering, but occasional vasodilation was also seen. Hyperthermia was usually mediated by rhythmic skeletal muscle activity that appeared to be shivering. However, the nature of this tremor was not examined experimentally.

Microinjections of CCh usually elicited behavioral excitation and hyperalertness, but sedation and paradoxical sleep (or a paradoxical sleep-like state) were also observed.

The thermoregulatory consequences of intrahypothalamic injections of CCh or ACh in the cat resembled those elicited in the rhesus monkey after similar intrahypothalamic injections (Myers and Yaksh, 1969), in that both species often responded with shivering and/or tremor and hyperthermia. However, there are also significant differences in the responses of the two species to these cholinomimetic agents. The initial hypothermia preceding the temperature rise, the augmentation of this hypothermic phase by increased dosage, and the predominant hypothermia occasionally seen after cholinergic stimulation of the AH/PO region have not been observed in the monkey but do occur in the cat. Conversely, the hypothermia produced by cholinergic stimulation of certain areas of the posterior hypothalamus of the monkey was not seen after stimulation of analogous sites in the cat. The comparability of the hypothalamic mechanisms for the control of body temperature in cat and rhesus monkey therefore remains in doubt.
Experiments with chlorpromazine (Chapter V)

It was found that intraperitoneally administered chlorpromazine HC1 (CPZ) produces hypothermia in cats at an ambient temperature of 20°C. This effect could be attributed to peripheral vasodilation and inhibition of shivering. Intracerebroventricular injection of the drug elicited similar responses. Injections directly into various hypothalamic loci (anterior hypothalamic/preoptic region, region of the ventromedial nucleus, perimammillary region) also caused hypothermia. These data support the contention that CPZ may disrupt temperature regulation by a central action, possibly within the hypothalamus.

Although the three groups of experiments summarized above were conceived, performed and interpreted relatively independently, each was concerned with the thermoregulatory effects of substances injected directly into the feline hypothalamus. Thus, perhaps some unitary explanation for the data derived from these experiments could be found. In particular, the possibility of adrenergic-cholinergic interaction in the hypothalamic control of body temperature and the perturbations induced by the administration of chlorpromazine (which may affect both these systems) must be briefly considered.

Lomax et al. (1969) have demonstrated that muscarinic stimulants depress body temperature in the rat when injected into the anterior-hypothalamic/preoptic region. This effect could be blocked by previous injection of catecholamines into the same hypothalamic site. The blockade, in turn, could be prevented by local pretreatment with an alpha-receptor antagonist. These results and a previous report (DeGroat and Volle, 1966) indicating that norepinephrine blocks cholinergic
transmission within the superior cervical ganglion but not after pre-treatment of the ganglion with alpha-adrenergic antagonists led Lomax and co-workers to speculate that endogenous catecholamines may modulate (inhibit) the activity of cholinoceptive neurons involved in thermoregulation within the AH/PO region.

Myers and Yaksh (1969) have suggested a similar inhibitory role for norepinephrine in the anterior hypothalamic/preoptic region of the monkey. However, in their scheme the neurons inhibited are normally activated by 5-hydroxytryptamine and, in turn, release ACh at their terminals to activate a multisynaptic cholinergic heat gain pathway which passes caudally through the hypothalamus.

The results of the present studies using cats are not incompatible with these models of central thermoregulatory control suggested for the rat and monkey. In this connection, it is suggested that noradrenergic neurons having their perikarya in the brainstem send axons rostrally to synapse at cholinoceptive neurons lying throughout the hypothalamus but primarily within the anterior hypothalamic/preoptic region. Release of norepinephrine at these terminals would inhibit firing in these cells which are normally activated by cholinergic synapses. It is further supposed that, similar to the construct of Myers and Yaksh, the cholinoceptive cells release ACh at their terminals to activate a diffuse polysynaptic cholinergic descending pathway which ultimately brings about increased heat gain (see Figure 39).

Although quite simple, such a system would explain most of the data obtained in the present experiments with microinjected sympathomimetic and cholinomimetic agents. Sympathomimetic amines, injected into
Figure 39. Schematic diagram of a hypothetical neural mechanism explaining the thermoregulatory effects of intrahypothalami cally microinjected catecholamines, cholinomimetics and chlorpromazine.

ACh—acetylcholine acting as an excitatory transmitter at the indicated synapses.
NE—norepinephrine acting as an inhibitory transmitter at the indicated synapses.
CCh—carbamylcholine, applied by microinjection and acting at the indicated sites.
CPZ—chlorpromazine, applied by microinjection and acting at the indicated site.
H.G.—efferent heat gain pathway.

A multisynaptic cholinergic efferent heat gain pathway (B-E), originating in the AH/PO region, is normally activated by synapses arising from cholinergic neurons involved in anterior hypothalamic thermal detection or thermoregulatory integration (A). A noradrenergic pathway ascends from the brainstem (F) and synapses throughout the hypothalamus but primarily in the AH/PO region, where it inhibits the activity of the cholinergic neurons forming the heat gain pathway.

Norepinephrine or other catecholamines, when injected into the AH/PO region, act at the noradrenergic synapses to inhibit heat gain. Heat loss may also be augmented by reciprocal innervation (not illustrated). Hypothermia is less likely to be elicited by injections at more posterior sites where inhibitory synapses are sparse.

Chlorpromazine may produce hypothermia when injected into the AH/PO region by acting at inhibitory synapses to mimic the action of norepinephrine.

Carbamylcholine produces hypothermia followed by hyperthermia by releasing norepinephrine from inhibitory terminals and then directly stimulating the efferent heat gain pathway. The magnitude and duration of the hypothermic phase depends on the density of inhibitory innervation at the injection site.
the AH/PO region, would mimic the inhibitory function of the endogenous transmitter and suppress heat gain or activate heat loss mechanisms. Injections elsewhere in the hypothalamus would be less effective because of the lower density of the inhibitory synapses in these areas. Injections of cholinomimetic agents anywhere in the multisynaptic descending cholinergic chain would activate heat gain mechanisms (e.g., shivering). The fall in body temperature that frequently preceded the hyperthermia induced by microinjections of carbamylcholine and the ability of this substance to induce hypothermia as the primary effect when injected into the AH/PO region could be explained by the presence of an overlapping cholinergic inhibitory system as suggested in Chapter IV. However, the recent demonstration that ACh augments the release of norepinephrine from the feline hypothalamus may provide another explanation (Philippu et al., 1970). Whether or not this release is a physiological process, it seems possible that in the present experiments, carbamylcholine, mimicking the action of ACh, caused an initial release of norepinephrine from the terminals of the inhibitory fibers synapsing on the cholinceptive cells just discussed. Thus, the initial effect of carbachol would be to release norepinephrine and thereby inhibit the cholinergic heat gain system. Following this, the drug would stimulate heat production by direct excitation of the cholinceptive cell. The duration and depth of the initial hypothermia should depend upon the density of the inhibitory noradrenergic innervation. Thus, carbachol would produce more profound and longer-lasting hypothermia when injected into the AH/PO region, which is postulated to receive the greatest portion of such innervation.
It will also be seen that at least some of the thermoregulatory effects of microinjected chlorpromazine can be explained within this speculative framework. Chlorpromazine (CPZ) is a potent antagonist at alpha-receptors within the peripheral autonomic nervous system (Gokhale et al., 1964), and crosses the blood-brain barrier with ease (Jacobs et al., 1962). Thus, there has been a tendency to attribute the central effects of CPZ to blockade of the effects of norepinephrine at central alpha-receptors. Indeed, iontophoretic application of CPZ to neurons of the cat hypothalamus revealed that it specifically inhibited the effect of norepinephrine on units which were excited by this catecholamine. However, the action of CPZ at neurons inhibited by norepinephrine was to mimic this inhibition (Bradley et al., 1966).

It appears that the ability of CPZ to mimic the depressive effect of norepinephrine may be of importance. This action, for example, has recently been invoked to explain the paradoxical eating induced by CPZ when it is injected into the "eating centers" of the rat hypothalamus (Leibowitz and Miller, 1969). Since norepinephrine usually elicits eating when it is injected into these centers, it was expected that CPZ would antagonize this action due to its alpha-receptor blocking activity. The authors explained the unexpected CPZ-induced eating by suggesting that norepinephrine elicits eating by local neuronal inhibition and that CPZ mimics this effect. The finding that CPZ elicited hyperthermia in rats when injected into the AH/PO region, a region where, local injection of norepinephrine also elicited hyperthermia (Rewerski and Jori, 1968), may also be explained by the assumption that CPZ mimics the action of norepinephrine at some central receptors.
Such an explanation might also account for the hypothermia seen in the present studies after microinjection of CPZ into the AH/PO region of the cat. Since injection of norepinephrine within this region causes hypothermia, CPZ-induced receptor blockade would be expected to produce hyperthermia. However, if the drug mimics the depressive effects of norepinephrine—at the cholinceptive cells in the model discussed above—then inhibition of heat gain mechanisms and hypothermia would be the expected result. This general explanation may also account for the profound hypothermia induced by injections of CPZ into the vicinity of the ventromedial hypothalamic nucleus, particularly since epinephrine-induced hypothermia when injected into the same site. However, the requirement that the CPZ injections be bilateral to be effective casts some doubt on this possibility. On the other hand, the delayed rise in body temperature occasionally seen after CPZ microinjections into the AH/PO region might be due to its anticholinesterase activity (Maickel, 1968) which would increase the concentration of endogenous ACh at the cholinceptive cells of the heat gain system. The association of paradoxical sleep or a similar state with the hypothermia elicited by injection of CPZ into the region surrounding the mammillary bodies and the ineffectiveness of norepinephrine injections at these sites suggests that CPZ may act at such loci by a different mechanism than that proposed for its actions in the rostral hypothalamus.

With the abovementioned exception, the theoretical model presented in Figure 39 appears to account reasonably well for the alterations in thermoregulation elicited by microinjections of sympathomimetic amines, cholinomimetics and chlorpromazine which have been described in this
dissertation. Whether the system, in fact, functions as has been suggested can be established only by further experimentation. Regardless of the implications for this particular theoretical construct, the information derived from such additional experiments cannot help but increase our inadequate knowledge of the pharmacology and physiology of central thermoregulatory control.
APPENDIX

SURGICAL PROCEDURES

Animals brought to surgery weighed 2.5 to 3.5 kg and were deprived of food and water for 24 hours prior to operation. Regardless of the anesthetic used, the animals were pretreated with 0.04 to 0.08 mg/kg atropine sulfate given intraperitoneally 1/2 hour before initiation of the anesthetic procedures.

Two anesthetic regimens were successfully employed: intravenous pentobarbital, 30 mg/kg, and methoxyflurane administered by inhalation. Pentobarbital, 60 mg/cc, injected into the cephalic vein, provided convenient and rapid induction. However, recovery was sometimes slow, particularly if supplementary injections were required during the operation. Methoxyflurane anesthesia required greater preparation but had the advantages of very rapid recovery and complete control of the depth of anesthesia during the operative procedures. The surgical protocol found below includes a description of the methoxyflurane anesthetic technique.

1) One-half hour after the administration of atropine sulfate, 13-22 mg/kg of Surital (Parke, Davis & Co.) was given intravenously as a 2.5% solution. The solution was injected slowly over a 30 second period in order to avoid the induction of apnea. The needle was left in the vein in case further injections were needed.
2) During the 5 to 10 minutes that the cat remained anesthetized by this ultrashort-acting barbiturate, the animal was placed in the headholder of the stereotaxic instrument and the trachea was intubated, the endotracheal tube being tied securely to the lower jaw with string. Intubation was facilitated by swabbing the pharynx with 4% lidocaine (Xylocaine, Astra) prior to inserting the catheter.

3) The endotracheal tube was connected to the gas machine and the vaporizer opened full until surgical anesthesia was achieved. This usually required 1/2 hour. The vaporizer could then be set approximately half open for the remainder of the operation.

4) During the time required for induction of anesthesia, the cat's head was depilated and scrubbed with PhisoHex and Zephirin Cl solution (1:590), followed by 70% alcohol. The surgeon then scrubbed thoroughly and donned sterile gloves.

5) The surgical packs were opened, and the head was draped. Only dry goods were autoclaved; all instruments were kept in Zephirin Cl (1:590) during the operation.

6) A midline incision was made, and the muscle and integument were reflected and clamped to narrow strips of sterile muslin using 8 small hemostats.

7) The calvarium was scraped free of fascia; blood was removed with sterile saline and gauze pads. The field was thoroughly air-dried and the 2 anterior electrode carriers of the stereotaxic instrument (C. H. Stoelting Co., Chicago, Illinois) were placed on the machine.

8) The electrode carriers, each holding one microinjection guide cannula, were moved to the predetermined anterior-posterior and lateral
coordinates, and the sites on the skull directly beneath the cannulae were marked with a sterile lead pencil. Lateral zero was determined from the position of the sagittal suture.

9) The electrode carriers were moved aside temporarily, and holes for the 2 cannulae were drilled in the skull using a dental drill. Two holes for 0-80 machine screws were tapped into the bone at the same time. These usually were placed slightly anterior and lateral to the cannula holes.

10) The electrode carriers were replaced in their correct positions and, after puncturing the dura with a 22 G needle, the guide cannulae were lowered into positions 1 mm above the intended injection site.

11) The cannulae were affixed to the skull by applying several layers of dental acrylic (L. D. Caulk Co., Milford, Delaware) around the cannulae and screws. Care was taken not to cover the intended placement sites for the posterior set of cannulae. After the acrylic had hardened (about 10 minutes), the cannulae were freed from the electrode carriers and the latter were removed from the stereotaxic apparatus.

12) The same procedure (8-11) was repeated for implantation of the posterior set of guides.

13) After the posterior set of electrode carriers had been removed, the stiletts were placed in their respective guide cannulae.

14) The mound of dental cement surrounding the array of guides and screws was then built up in successive layers until only about 1 mm of the guide tip protruded. This prevented the guides from being bent once the cat was replaced in his cage.
15) At this time, the methoxyflurane vaporizer was turned to the "off" position.

16) After the final application of dental cement had hardened completely, the wound was infiltrated with Neosporin antibiotic ointment (Burroughs Wellcome & Co.). The wound edges were then secured loosely around the base of the mound of cement by the placement of 1 or 2 sutures at the anterior and posterior ends of the incision. Frequently, no sutures were necessary.

17) Usually, the cat had regained corneal, cough and withdrawal reflexes at the time of suturing. The tracheal tube was then removed and the cat was released from the ear bars and placed in a padded recovery box under a heat lamp.
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