DICKEY, M.D., Richard Palmer, 1935—
INTERACTION OF BIOGENIC AMINES AND SEX
STEROIDS IN THE MODULATION OF GONADOTROPIN
SECRETION.

The Ohio State University, Ph.D., 1970
Pharmacology

University Microfilms, a XEROX Company, Ann Arbor, Michigan
INTERACTION OF BIOGENIC AMINES AND SEX STEROIDS IN THE MODULATION OF GONADOTROPIN SECRETION

DISSERTATION

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

by

Richard Palmer Dickey, M.D.

* * * * * * * * *

The Ohio State University
1970

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ACKNOWLEDGMENTS

I would especially like to acknowledge and thank Dr. Bernard H. Marks for his help and encouragement throughout my doctoral studies both as adviser and friend. I would also like to particularly acknowledge Dr. John C. Ullery for his encouragement, friendship and tolerance of my frequent absences, and for allowing me to continue and maintain an academic position in the Department of Obstetrics and Gynecology.

Others who have been of great help and stimulus are Drs. Harold Goldman, Philip B. Hollander, John J. O'Neill, John O. Lindower and Daniel Couri of the Department of Pharmacology and Dr. Vernon Stevens of the Department of Obstetrics and Gynecology.

I am grateful for the very able assistance of Mrs. Linda Corum in carrying out these investigations and Daria Gverna and Sandra McKinnon in preparing this manuscript.

The investigations described were supported in part by National Institute of Child Health and Human Development Contract NIH 69-2217.
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>VITA</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF ILLUSTRATIONS</td>
<td>x</td>
</tr>
<tr>
<td>DEFINITIONS AND ABBREVIATIONS</td>
<td>xii</td>
</tr>
<tr>
<td>INTRODUCTION AND STATEMENT OF PROBLEM</td>
<td>1</td>
</tr>
<tr>
<td>REVIEW OF PERTINENT LITERATURE</td>
<td>5</td>
</tr>
<tr>
<td>Effect of Autonomic Drugs on Gonadotropin</td>
<td>5</td>
</tr>
<tr>
<td>Release and Ovulation</td>
<td>5</td>
</tr>
<tr>
<td>Stimulation</td>
<td>5</td>
</tr>
<tr>
<td>Blockade</td>
<td>9</td>
</tr>
<tr>
<td>Gonadotropin, Releasing Factor, Steroid and Neurohormonal Levels During the Estrous Cycle</td>
<td>15</td>
</tr>
<tr>
<td>Effect of Endogenous and Exogenous Physiological Influences on Gonadotropin Levels</td>
<td>22</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>26</td>
</tr>
<tr>
<td>Animals</td>
<td>26</td>
</tr>
<tr>
<td>Ovariectomy</td>
<td>26</td>
</tr>
<tr>
<td>Time of Experiment</td>
<td>26</td>
</tr>
<tr>
<td>Intraventricular Injection</td>
<td>27</td>
</tr>
<tr>
<td>Sacrifice and Tissue Collection</td>
<td>32</td>
</tr>
<tr>
<td>Drugs</td>
<td>32</td>
</tr>
<tr>
<td>Radioimmunoassay for FSH and LH</td>
<td>35</td>
</tr>
<tr>
<td>Secretion Rate and Turnover Time of FSH and LH</td>
<td>42</td>
</tr>
<tr>
<td>RESULTS</td>
<td>44</td>
</tr>
<tr>
<td>Effect of Estradiol 17β on Plasma and Pituitary FSH and LH in Ovariectomized female Rats</td>
<td>44</td>
</tr>
<tr>
<td>vi</td>
<td></td>
</tr>
</tbody>
</table>
Time Response Effect of Reserpine (2.5 mg/kg) on Plasma and Pituitary FSH and LH in Ovariectomized Rats

Effect of Estrone (200 µg/kg) and Progesterone (2.0 mg/kg) on Reserpine Induced Changes in Gonadotropin Levels in Ovariectomized Rats

Effect of L-Dihydroxyphenylalanine and 1-threo-Dihydroxyphenylserine on Gonadotropin Levels in Reserpine treated Ovariectomized Rats

Effect of L-Dihydroxyphenylalanine on Gonadotropin Levels of Normal, Ovariectomized and Short Term Estrogen Treated Ovariectomized Female Rats

Effect of L-Dihydroxyphenylalanine on Gonadotropin Levels of Ovariectomized Rats Pretreated with Estradiol Valerate or Progesterone for 72 hours

Effect of Pargyline, Parachlorophenylalanine and alpha-methyltyrosine on Gonadotropin Levels in Ovariectomized Rats

Intraventricular Injection of Carbachol, Dopamine, Norepinephrine, Epinephrine or Serotonin in Normal Male Rats

Intraventricular Injection of Dopamine, Norepinephrine and Serotonin in Untreated Ovariectomized Rats

Intraventricular Injection of Dopamine, Norepinephrine and Serotonin in Reserpine Treated Ovariectomized Rats

Intraventricular Injection of Atropine and Hexamethonium in Untreated and Reserpine Treated Ovariectomized Rats
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Effect of Estradiol 17β on Plasma and Pituitary FSH and LH in Ovariectomized Rats</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td>Effect of Reserpine (2.5 mg/kg) on Plasma and Pituitary Gonadotropins of Ovariectomized Rats</td>
<td>49</td>
</tr>
<tr>
<td>3</td>
<td>Effect of Reserpine (2.5 mg/μg) on Plasma and Pituitary Gonadotropins of Ovariectomized Rats</td>
<td>52</td>
</tr>
<tr>
<td>4</td>
<td>Effect of Estrogen and Progesterone on Reserpine Induced Changes in Gonadotropin Levels in Ovariectomized Rats</td>
<td>56</td>
</tr>
<tr>
<td>5</td>
<td>Effect of L-Dihydroxyphenylalanine and Dihydroxyphenylserine on Plasma and Pituitary Gonadotropins in Reserpine Treated Ovariectomized Rats</td>
<td>61</td>
</tr>
<tr>
<td>6</td>
<td>Effect of L-Dihydroxyphenylalanine on Plasma and Pituitary Gonadotropins in the Normal Rat, Ovariectomized Rat and Ovariectomized Rat with Short Term Estrogen Pretreatment</td>
<td>65</td>
</tr>
<tr>
<td>7</td>
<td>Effect of L-Dihydroxyphenylalanine on Ovariectomized Rats Pretreated 72 Hours Before with Estradiol Valerate or 72 and 18 Hours Before with Progesterone</td>
<td>69</td>
</tr>
<tr>
<td>8</td>
<td>Effect of L-Dihydroxyphenylalanine on Ovariectomized Rats Pretreated 72 Hours before with Estradiol Valerate 72 and 18 Hours Before with Progesterone</td>
<td>70</td>
</tr>
<tr>
<td>9</td>
<td>Effect of Pargyline, Parachlorophenylalanine and α-methyltyrosine on Serum and Pituitary Gonadotropins in Ovariectomized Rats</td>
<td>74</td>
</tr>
<tr>
<td>10</td>
<td>Intraventricular Injection of Carbachol, Dopamine, Norepinephrine, Epinephrine or Serotonin in Normal Male Rats</td>
<td>78</td>
</tr>
</tbody>
</table>
Intraventricular Injection of Carbachol, Dopamine, Norepinephrine, Epinephrine or Serotonin in Normal Male Rats

Intraventricular Injection of Dopamine, Norepinephrine and Serotonin in Untreated Ovariectomized Rats

Intraventricular Injection of Dopamine, Norepinephrine and Serotonin in Reserpine Treated Ovariectomized Rats

Intraventricular Injection of Atropine and Hexamethonium in Untreated and Reserpine Treated Ovariectomized Rats
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Effect of Injection Anesthesia on Plasma and Pituitary FSH and LH</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>Effect of Intraventricular Injection of Artificial CNS Solution on Plasma and Pituitary FSH and LH</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>Radioiodinations: Calculations of Protein Label</td>
<td>38</td>
</tr>
<tr>
<td>4</td>
<td>Radioimmunoassay: Dose Response Curves for FSH and LH Standards</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>Effect of Estradiol 17 on Plasma and Pituitary FSH and LH in Ovariectomized Rats</td>
<td>46</td>
</tr>
<tr>
<td>6</td>
<td>Effect of Reserpine (2.5 mg/kg) on Plasma and Pituitary Gonadotropins of Ovariectomized Rats</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>Effect of Reserpine (5.0 mg/kg) on Plasma and Pituitary Gonadotropins of Ovariectomized Rats</td>
<td>53</td>
</tr>
<tr>
<td>8</td>
<td>Effect of Estrogen and Progesterone on Reserpine Induced Changes in Gonadotropin Levels in Ovariectomized Rats</td>
<td>57</td>
</tr>
<tr>
<td>9</td>
<td>Effect of L-Dihydroxyphenylalanine and Dihydroxyphenylserine on Plasma and Pituitary Gonadotropins in Reserpine treated Ovariectomized Rats</td>
<td>62</td>
</tr>
<tr>
<td>10</td>
<td>Effect of L-Dihydroxyphenylalanine on Plasma and Pituitary Gonadotropins in the Normal Rat, Ovariectomized Rat and Ovariectomized Rat with Short Term Estrogen Pretreatment</td>
<td>66</td>
</tr>
<tr>
<td>11</td>
<td>Effect of L-Dihydroxyphenylalanine on Ovariectomized Rats Pretreated 72 Hours Before with Estradiol Valerate or 72 and 18 Hours Before with Progesterone</td>
<td>71</td>
</tr>
<tr>
<td>Figure</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Effect of Pargyline, Parachlorophenylalanine and alpha-Methyltyrosine on Plasma and Pituitary Gonadotropins in Ovariectomized Rats</td>
<td>75</td>
</tr>
<tr>
<td>13</td>
<td>Intraventricular Injection of Carbachol, Dopamine, Norepinephrine, Epinephrine or Serotonin in Normal Male Rats</td>
<td>80</td>
</tr>
<tr>
<td>14</td>
<td>Intraventricular Injection of Dopamine, Norepinephrine and Serotonin in Untreated Ovariectomized Rats</td>
<td>84</td>
</tr>
<tr>
<td>15</td>
<td>Intraventricular Injection of Dopamine, Norepinephrine and Serotonin in Reserpine Treated Ovariectomized Rats</td>
<td>87</td>
</tr>
<tr>
<td>16</td>
<td>Intraventricular Injection of Atropine and Hexamethonium in Untreated and Reserpine Treated Ovariectomized Rats</td>
<td>91</td>
</tr>
</tbody>
</table>
### DEFINITIONS AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>catecholamine</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>DA</td>
<td>dopamine</td>
</tr>
<tr>
<td>1-DOPA</td>
<td>1-dihydroxyphenylalanine</td>
</tr>
<tr>
<td>DOPS</td>
<td>1-dihydroxyphenylserine</td>
</tr>
<tr>
<td>EPI</td>
<td>epinephrine</td>
</tr>
<tr>
<td>FSH</td>
<td>follicular stimulating hormone</td>
</tr>
<tr>
<td>FRF</td>
<td>follicular stimulating hormone releasing factor</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine (serotonin)</td>
</tr>
<tr>
<td>NE</td>
<td>norepinephrine</td>
</tr>
<tr>
<td>LH</td>
<td>lutenizing hormone</td>
</tr>
<tr>
<td>LRF</td>
<td>lutenizing hormone releasing factor</td>
</tr>
<tr>
<td>PCPA</td>
<td>para-chlorophenylalanine</td>
</tr>
</tbody>
</table>
INTRODUCTION AND STATEMENT OF PROBLEM

The first recognition of the participation of the nervous system in the regulation of gonadotrophin secretion is credited to Haighton who reported to the Royal Society of London in 1797 his observation that, in the rabbit, rupture of the Graafian follicles occurs in response to copulation. In addition to the rabbit, the cat, the ferret, and a few other mammals are reflex ovulators in response to copulation (Green, 1969). In many large mammals, which appear to ovulate spontaneously, there is strong evidence that the release of gonadotrophins is under control of nervous stimulation resulting from changes in the external environment such as length of light periods and availability of food. In only a few known animals, most notably the rat, guinea pig, primates including man, and domesticated farm animals does ovulation occur repeatedly at regular intervals unless pregnancy intervenes. Due to the frequency of these intervals and the fact that ovarian steroids are present at rising or falling levels throughout their entirety, the importance of the nervous system participation in repeat ovulators was obscured. The concept of an ovulation cycle regulated by negative feedback of ovarian steroids on the pituitary was extensively developed. The influence of external stimuli on the cycling of the rat and human even when recognized was often either ignored or considered to be mediated via an effect on steroidogenesis.

Hinsey and Markee (1933) are credited with being the first to postulate that a neural stimulus might act indirectly through a substance released into the blood and passing to the pituitary rather
than via direct innervation of the pituitary. Interest in the vascular plexus connecting the anterior pituitary and the hypothalamus was aroused by Popa and Fielding (1933), who believed blood flow was from the pituitary to the hypothalamus. It was not until 1949 that Green and Harris proved the correct direction of blood flow by observing it in the living rat. In 1952 Harris and Jacobsohn reported the results of an experiment that gave much support to the concept of neurohormonal control of the adenohypophysis. Pituitary tissue if transplanted beneath the median eminence became revascularized and rats ovulated and became pregnant. But, when pituitary tissue was transplanted under the temporal lobe, animals failed to ovulate despite good vascularization. These experiments demonstrated that control of ovulation in cyclic animals was not due to direct steroid feedback on the pituitary but involved at least one intermediary, the hypothalamic secretions. The full development of this concept led to the search for and discovery of hypothalamic pituitary stimulatory and inhibitory hormones. This subject has recently been reviewed by McCann and Porter (1969).

Most of the studies concerning the effects of pharmaceutical agents other than sex steroids and polypeptides on ovulation have been concerned with reflex ovulation or response to electrical stimulus; for a review see, Everett, 1964. Although a neural influence on ovulation was recognized for animals which ovulated in response to physical or environmental stimulus, it was assumed that the nervous system was unimportant where feedback of ovarian steroids was involved. The interposition of hypothalamic releasing factors on the pituitary-ovarian feedback type of cycle did not alter this assumption because it could be assumed that steroid feedback acted directly on hypothalamic centers responsible for synthesizing or releasing the hypothalamic releasing hormones. A few researchers, primarily
Sawyer, McCann, and their groups, however, believed that neurohormones were important for the elaboration and release of releasing factors. This view has been considerably strengthened by the discovery of McCann's group, announced during the past year, that a specific neurohormone, dopamine, could cause the release of Luteinizing Hormone Releasing Factor (LRF) (Schneider and McCann, 1969) and Follicle Stimulating Hormone Releasing Factor (FRF) (Kamberi, Schneider and McCann, 1970) from hypothalamic tissue in vitro.

We believe that evidence now indicates that the feedback influence of ovarian steroids on ovulation is mediated through adrenergic, dopaminergic, serotonergic, and cholinergic neurons and that sex steroids may profoundly modify the activities of such neurons. Pharmacologic alteration of neural activity may thus provide the means to regulate ovulation more reliably than alteration of ovarian steroids alone. At the same time, the role of sex steroids must be reevaluated as to their site and mechanism of action in modulation of gonadotropin release. It seems entirely possible that they must once again be granted an important direct action on the pituitary—an action, however, concerned with metabolism and synthesis of gonadotropins rather than with their release.

As will become apparent from the following review, the regulation of ovulation is a very complex mechanism requiring integration of steroid, polypeptide and neurohormones at several different levels. In order to investigate the relationship of a single group of drugs, the biogenic amines, to ovulation, it has been necessary to limit as many of these factors as possible. For this reason I have studied the interaction of ovarian steroids and neurohormones on FSH and LH release rather than on ovulation. In order to control
the effects of ovarian steroid feedback, I have worked primarily with the ovariectomized female rat. I have chosen as my problem, a study of the effects of neurohormones, primarily the catechol- amines and indolealkylamines, on FSH and LH release and the role of sex steroids in modifying this influence.
Effect of Autonomic Drugs on Gonadotropin Release and Ovulation.

Stimulation of Ovulation: A wide variety of pharmacological agents including metallic ions, convulsants, sympathomimetic agents and histamine have been shown to induce ovulation when administered by various routes to the rabbit (Sawyer, 1969).

Local administration of neurohormones to the hypophysis was first attempted in 1941 when Tauberhause and Soskin applied adrenergic, cholinergic and anticholinergic drugs to the exposed hypophysis. Markee, Sawyer and Hollinshead (1948) tested several autonomic agents as possible humoral mediators of gonadotropin release in the rabbit. Acetylcholine (ACh) and epinephrine (Epi) were ineffective in stimulating ovulation when injected intravenously. Local injection of Epi 1:1000, but not ACh directly into the pituitary stimulated ovulation in 5 of 10 rabbits. Both Epi and NE caused ovulation when injected into the third ventricle. Donovan and Harris (1956) were unable to duplicate the effects of intrapituitary Epi when injection was done slowly implying that effects were due to reflux to the hypothalamus. Sawyer (1962) found that intraventricular effects of NE could be blocked with small doses of intravenous pentobarbital.

In an effort to elucidate the site of action of drugs injected in the central nervous system, Sawyer (1969) studied the changes in EEG patterns in the rabbit hypothalamus and other brain areas. He considered the appearance of prolonged 5-10 second high amplitude activity in the lateral hypothalamic area to be an ovulatory pattern.
since it duplicated the type of activity seen in this area during the
critical 2:00 - 4:00 p.m. proestrous period in the rat (Critchlow
and Sawyer, 1955).

Histamine given intraventricularly was effective in eliciting
the ovulatory pattern of EEG activity only following small doses of
pentobarbital. Sympathetic phenomena appeared, the heart
accelerated, and high amplitude 30 cps EEG patterns appeared in
the lateral preoptic areas. Removal of the olfactory bulbs eliminated
the characteristic EEG pattern and blocked pituitary depletion of
gonadotrophin. Atropine, or larger doses of pentobarbital also
blocked this response.

Norepinephrine gave a response similar to histamine
(Radford and Sawyer, 1960) which did not disappear when the
olfactory bulbs were removed. During pseudopregnancy when intra-
ventricular NE did not induce ovulation the typical EEG pattern was
absent. Convulsant drugs such as picrotoxin and Metrazol in ovu-
latory doses induced EEG seizures throughout the brain. Both seiz-
ures and ovulation could be blocked by pentobarbital (Sawyer and
Markee, 1950). Many of the ovulation inhibiting drugs including
atropine, morphine and pentobarbital, at blocking doses, evoked
high amplitude slow waves suggestion of inhibition of the reticular
activating system (Sawyer et al., 1955). Sawyer (1969) concluded,
on the basis of this evidence, that the effect of the intraventricular
administration of adrenergic agents and other neurohormonal agents
such as vasopressin, oxytocin, and cholinergic substances on ovu-
lation must be considered due to pharmacological stimulation of the
central nervous system rather than to an action as humoral mediators
of hypothalamic influence on pituitary function.

In 1969 Schneider and McCann reported that dopamine (DA)
caused LRF release when added to hypothalamic fragments in vitro.
Of four biogenic amines tested, dopamine, norepinephrine, epinephrine and serotonin, only dopamine at doses of 0.5 - 5.0 μg induced release of LRF. Norepinephrine, epinephrine and serotonin creatinine were without effect. The effect of DA was blocked by phentolamine 20 μg/ml (α-adrenergic blocker) but not by pronethalol (β-adrenergic blocker). Only epinephrine 5.0 μg/ml induced LH release when added alone to pituitary halves. Dopamine 2.5 - 5.0 μg/ml also appeared to induce release of FSH-RF from hypothalamic tissue in vitro (Kamberi, Schneider and McCann, 1970). Epinephrine 1 - 5 μg/ml, norepinephrine 1 - 5 μg/ml and serotonin 1 - 5 μg/ml failed to increase FSH release when administered alone or in the presence of hypothalamic fragments. Dopamine failed to affect FSH release when incubated with pituitary in the absence of hypothalamic tissue.

The discovery that DA had an effect in vitro on the release of LRF from stalk median eminence (SME) encouraged new studies on the effects of DA in vivo. Using cannulas chronically implanted in the third ventricle or pituitary of rats Kamberi, Mical, and Porter (1970a) infused solutions of biogenic amines and collected blood from the jugular vein. Within 10 minutes after DA 1.25 μg was injected into the third ventricle, serum LH increased fourfold, at 20 minutes serum LH increased nine-fold, at 30 and 60 minutes after the injection there was a moderate decline followed by a steady rise. By 2 hours, the serum LH had risen 20-fold. However, when quantities of DA greater than 1.25 μg were injected, the serum levels were inversely related to dose. Intraventricular injection of 100 μg but not of 2.5 or 5 μg of Epi or NE caused a 5 to 8-fold increase in LH. Intraventricular injections of serotonin (2.5 and 5 μg) or melatonin (1, 5, and 50 μg) caused statistically significant decreases in the concentrations of plasma LH during the first hour.
after injection. Lutenizing hormone release was not affected by injection of any of the catecholamines or indolalkylamines directly into the pituitary. Schneider and McCann (1970d) reported similar results in a less elaborate experiment. Determination of LRF activity in the pituitary stalk blood of hypophysectomized rats following intraventricular infusion of DA and NE proved that LRF was released by DA (Kamberi, Mical and Porter, 1969). This finding strongly suggested to these authors that the observed elevation in plasma LH levels observed following intraventricular infusion of DA was the result of LRF release.

Reports of RSH concentration following intraventricular injection by these authors are available only in preliminary form (Kamberi, Mical and Porter, 1970b), but it appears that the patterns of serum FSH release are similar to those seen for LH and that FSH release is also stimulated by DA.

Schneider and McCann (1970a) injected biogenic amines in doses of 4 μg into the third ventricle at noon on each day of the estrous cycle in normal female rats and castrate males and females and determined serum LH levels 15 minutes later. Dopamine raised serum LH levels 8 to 10-fold to 10-20 μg/ml when injected on proestrus and 2 to 4-fold to 6-14 μg/ml on the day preceding proestrus (Diestrous 2). Injection on the day of estrous resulted in equally great increases in comparison to resting value, but the final levels were no greater than 5 μg/ml. Injection on the first day of diestrous had little or no effect (maximum 1 x increase) in 5 or 7 animals tested. Phenoxybenzamine (α-blocker) blocked the increase of LH due to DA during proestrus or diestrous 2 while pro-nethalol (β-blocker) was ineffective. Norepinephrine injection was without effect on the day of proestrus or diestrous day 1 or 2 but caused a 2 to 3-fold increase in LH to 5-12 μg/ml on the day of
estrous. 5-Hydroxytryptamine was without consistent effect either positive or negative on all days studied. Injection of 5-HT into castrate females however, caused significant (averate 50%) decrease in serum LH levels. Dopamine and NE produced variable results in female castrate animals. Treatment of castrate females with estradiol valerate and progesterone 25 mg lowered serum LH levels by 50-70%. Intraventricular administration of DA in these animals resulted in a 10-fold increase (from 5-10 μg/ml to 50-60 μg/ml) in LH. Administration of NE to steroid blocked castrates was without effect.

Schneider and McCann (1970b) found that the release of LRF initiated by intraventricular injection of DA was blocked by 0.1 μg estradiol injected two hours prior to DA. They have also showed a similar effect of estrone on DA stimulation of hypothalamus fragments in vitro (1970c).

**Blockade of Gonadotropin Release and Ovulation:** Drugs found effective in blocking the release of pituitary gonadotropins include: anti-adrenergic, anticholinergic, anesthetic, analgesic and tranquilizing agents (Sawyer, 1969).

Blockage of ovulation in the pregnant mare serum (PMS) treated immature rats has proven to be a convenient means of studying the effect of CNS acting drugs on gonadotropin secretion. Systemic administration of methyl dopa (300 mg/kg), Syrosin gopine (10 mg/kg), or tetrabenzyline (15 mg/kg) 53 hours after administration of PMS completely suppress ovulation in the immature rat (Coppola et al., 1966). Similar treatment with tyramine 14 mg/kg, guanethidine 30 mg/kg, p-chloro-n-methylamphetamine 25 mg/kg or α-aminocaproic acid 300 mg/kg is without effect. The ovulatory blockade produced by reserpine (0.4 mg/kg) is reversed by
iproniazid (50 mg/kg), pheniprazine (10 mg/kg) or human chorionic gonadotropin (HCG) 1-4 IU. Since syrosingopine and tetrabenzylidine are specific depleters of brain catecholamine and not of serotonin (Brodie et al., 1959) it appears that either dopamine, norepinephrine or epinephrine is essential for synthesis or release of LH under these conditions.

It was found by Lippman et al., 1967 that the doses of reserpine and alpha-methyl-para-tyrosine required to block ovulation also lowered hypothalamic CA in rats. Iproniazid pretreatment prevented the fall in hypothalamic CA and the blockade of ovulation following reserpine and α-MT. Pargyline failed to prevent either of these effects following reserpine or α-MT. Neither iproniazid nor pargyline alone affect ovulation, although both increased hypothalamic CA levels.

Treatment with reserpine, chlorpromazine or pentobarbital protects neonatal female rats against the sterilizing effect of testosterone propionate (Arai and Gorski, 1968). The importance of CA in neonatal sterilization is uncertain since reserpine treatment during the first 14 days of life is without effect on CA content in the hypothalamus of the mature rat (Bjorklund et al., 1969).

Ovulation in PMS treated immature rats can also be blocked with phenobarbital 3.0 - 7.5 mg, atropine 40 mg, chlorpromazine 0.3 mg, or butazolidine 12 mg per rat (average body weight 50 gm). Blockage in these cases was overcome by progesterone 0.5 mg and desoxycorticosterone 1.0 mg, estradiol 17β and estrone (Ying and Meyer, 1969). Significantly the amount of estrogen was critical. Estrone 10μg was optimal for overcoming blockade by phenobarbital. Estrone 4 μg and estradiol 5 μg were only 40% effective, estradiol 1 μg was 20% effective. Estrone 30 μg was completely ineffective in overcoming blockade.
A difference in the time of blockade was seen for different active drugs by Quinn and Zarrow (1964). Atropine (40 mg) was most effective in blocking ovulation 49 hours post-PMS, nembutal (3 mg) was active at two times: 50 hours and 54-55 hours. Chlorpromazine (2 mg) blocked all ovulation up to 55 hours. Hypophysectomy blocked all ovulation at 42 hours, about 50% at 56 hours and at 60 hours was equal to control nontreated animals. LH release thus appears to occur between 55 and 56 hours after PMS. Cholinergic blockade is effective 52 hours following PMS primarily during a 30 minute critical period while adrenergic blockage is effective up to 55-56 hours which is the latest time that hypophysectomy is effective.

A specific requirement for dopamine rather than norepinephrine in ovulation was found by Kordon and Glowinski (1969). Alphamethyl-p-tyrosine blocked ovulation when administered between 3:00 and 4:00 p.m. of proestrus. L-dihydroxyphenylalanine (DOPA) a precursor of NE and DA restored ovulation while 1-threodihydroxyphenylserine (DOPS) which restores NE synthesis only was ineffective. The blockade of superovulation which occurs following administration of monoamine oxidase inhibitors appeared to be mediated by increased brain serotonin levels rather than by increased brain NE, since blockade of ovulation did not occur when animals were pretreated with PCPA, but did occur after pretreatment with DOPS (Kordon et al., 1968). Kordon and Glowinski (1969) speculate that LH release from the pituitary involves a double aminergic control system with a positive DA component and an inhibitory serotonergic one.

Kordon (1969) attempted to localize the sites of aminergic action in the central nervous system. Monoamine oxidase inhibitors were effective in blocking ovulation only when injected into the hypothalamus and not when injected in the pituitary. Prior inhibition of
5-HT synthesis with PCPA prevented this blockade while prior inhibition of CA synthesis was without effect.

Inhibition of cyclic ovulation has been observed with many of the drugs effective in the PMS stimulated immature animal. Reserpine (5 mg/kg) blocks ovulation in mature cycling rats if given prior to 2:00 p.m. of proestrus (Meyerson and Sawyer, 1968). Pargyline (25 mg/kg) or nialamide (200 mg/kg) prevented the blocking effects of reserpine but were without effect when given alone. Pargyline (50 mg/kg) blocked ovulation if given prior to 2:00 p.m. of proestrus. However, neither of these drugs or L-Dopa could advance the time of LH release. Chlorpromazine also blocks ovulation in mature rats (Schally et al., 1967). Copulation overcomes chlorpromazine blockade, presumably as a result of LH release.

Everett, Sawyer and Markee (1949) found that anticholinergic and specific anti-adrenergic agents were effective in blocking cyclic ovulation only if injected before 2:00 p.m. on the day of proestrus. Merely anesthetizing rats with ether or barbiturates during the critical period (2:00 - 4:00 p.m.) the day of proestrus blocked ovulation for 24 hours (Everett and Sawyer, 1950). Other agents effective if given just prior to the 2:00 - 4:00 p.m. critical period include the post-ganglionic (e.g., muscarinic) anticholinergic agents banthine and Pathilon (Gitsch and Everett, 1958), a ganglionic (e.g., nicotinic) anticholinergic agent (Everett and Sawyer, unpublished, cited in Sawyer, 1969) an alpha adrenergic blocking agent, dibenzylamine (Moore, 1961) an anticonvulsant agent, diphenylhydantoin (Quinn, 1965), morphine (Barraclough and Sawyer, 1955) and the MAO inhibitor tranylcypromine (Alleva et al., 1965) and alcohol (Barraclough, unpublished, cited in Sawyer, 1969).
Sawyer, Markee and Everett (1950) found that rapid post-coital injection of beta chlorehylamine adrenergic blocking agents inhibit release of ovulation hormone in the rabbit if given within 1 minute of copulation. The ovulation blocking capacities of such agents as dibenamine, SKF 501, and dibenzylaminoethylol were found to be proportional to their adrenergic blocking capacities. Rapid post-coital injection of the anticholinergic agents atropine (Sawyer, Markee and Townsend, 1949) and banthine (Sawyer, Markee and Everett, 1951) also blocked copulation induced ovulation in the rabbit. Sawyer suggested that the post-coital ovulation in the rabbit might employ a cholinergic-adrenergic sequence such as is known to exist for the sympathetic autonomic nervous system (Sawyer, Markee and Townsend, 1949).

Intravenous administration of amines also blocks copulation-stimulated ovulation in the rabbit (Currie, et al., 1969). When administered within one minute of ovulation epinephrine (100 μg - 300 μg/kg) was also highly effective in blocking ovulation while NE was ineffective. Isoproterenol was also effective at 100 μg/kg. Serotonin was highly effective at 1.0 mg/kg while amphetamine to 7 mg/kg was ineffective.

Many of the studies concerning the effects of drugs on ovulation and the gonadotropins involve electrical stimulation. In the pro-estrous cyclic rat, spontaneous ovulation can be blocked with appropriately timed injections of pentobarbital (Everett and Sawyer, 1953). Electrical stimulation of the tuberal region just dorsal to the median eminence in such a preparation was shown to induce ovulation (Critchlow, 1958). Stimulation of this area was also effective in appropriately primed rats previously rendered anovulatory by treatment with testosterone in infancy (Barraclough, 1961).
Saul and Sawyer (1957) investigated the critical sites of the blocking action of CNS drugs by applying ovulation inducing electrical stimulation to areas of the rabbit brain. Atropine sulfate, morphine sulfate and sodium pentothal blocked ovulation following stimulations in the basal tuberal regions, while reserpine and SKF 501 were ineffective. However, ovulation in response to electrical stimulation of the medial preoptic region is not blocked by atropine or pentobarbital (Everett, 1961), morphine, chlorpromazine (Everett et al., 1964), or diphenylhydantoin (Quinn, 1965).

There is a small body of evidence that some of the CNS active agents can prevent induction of ovulation by steroids. In the hen, phenobarbital blocked progesterone advancement of ovulation (Fraps and Case, 1963). Also, in the hen, ovulation advanced by progesterone as well as spontaneous ovulation was blocked by dibenamine (Tienhoven et al., 1954), SKF 501 or atropine (Zarrow and Bastain, 1953). Lutenizing hormone release induced by estrogen during pseudopregnancy or the progravid phase of pregnancy is subject to blockade by dibenamine, atropine or the barbiturates (Presl, 1961; Sawyer, Everett and Markee, 1949). In the cow, atropine sulfate blocked both spontaneous ovulation (Hansel and Trimberger, 1951) and ovulation produced by progesterone (Hough et al., 1955).

The effect of reserpine on pituitary FSH and LH levels has been studied by Labhsetwar (1967). Reserpine (50 μg/day) for 9-10 days lowered total LH content and blocked the post-castration rise in pituitary LH. Pituitary FSH content was increased despite reduced pituitary size but no inhibition of post-castration rise in pituitary FSH was seen. Estrogen priming of castrate rats (5 μg/day) for three days plus 2.5 μg for 1 day before reserpine treatment reversed this effect. Estrogen alone in these bilaterally ovariectomized animals caused a decrease in pituitary LH but an increased pituitary FSH content as compared to ovariectomized controls. After estrogen priming
reserpine resulted in an increase in LH content and LH per mg and a slight decrease in FSH content but no change in FSH per mg compared to controls. This data suggested to Labhsetwar that decreased LH output in reserpinized rats is secondary to decreased synthesis whereas, decreased FSH results primarily from impairment of its release rather than its synthesis. In another study, Negro-Vilar and Meites (1968) found that reserpine (25 μg/rat/day for 30 days) resulted in significant reduction of both plasma and hypothalamic FSHRF. By contrast, testosterone propionate reduced plasma but not hypothalamic FSHRF.

Gonadotropin-Releasing Factor, Steroid and Neurohormonal Levels During the Estrous Cycle.

Changes in pituitary (McClintock and Schwartz, 1968; Goldman and Mahesto, 1968; Caligaris et al., 1967) and plasma (McClintock and Schwartz, 1968) FSH, and pituitary (Chowers and McCann, 1965; Monroe et al., 1969) and plasma (Monroe et al., 1969; Lawton and Smith, 1970) LH during the estrous cycle have been measured. Caligaris et al. (1967) found pituitary FSH highest on the morning of proestrus (15.7 ± 1.1 μg/mg wet weight) with a marked fall in the morning of proestrus to 5.3 ± 0.3 μg/mg and a return to 80% of peak levels by the morning of estrus. Ovariectomy at 9:00 a.m. the morning of proestrus abolished the fall in pituitary FSH content. Administration of progesterone 2 mg/rat, but not estriadiol benzoate 2 mg/rat at 11:00 a.m. on proestrus to the rats ovariectomized at 8:30 a.m. resulted in a fall in FSH content almost as great as that seen in the intact animal. Goldman and Mahesto (1968) noted similar changes in 4 day and in 5 day cycling rats. In 5 day cycling rats, however, pituitary FSH levels remained low throughout the day of estrous. McClintock and Schwartz (1968) observed that plasma FSH rose from 2.45 μg/ml the morning of
proestrus to 3.28 the afternoon of proestrus. They also noted a fall in pituitary FSH content on the afternoon of proestrus to 17.45 μg/mg from 25.12 μg/mg the morning of proestrus.

Employing radioimmunoassay, Monroe et al. (1969) noted peak levels of 200 to 450 ng/ml LH occurring between 4:00 and 6:00 p.m. on proestrus. When serial plasma levels were determined on the same animals at 5 minute intervals, a single LH peak was observed usually at 4:00 p.m., but in some cases as late as 6:00 p.m. Pituitary LH content measured by radioimmunoassay fell by about 50% on the afternoon of proestrus and continued low the morning of proestrus. In the study of Monroe et al. (1969) no prolonged release of plasma LH was observed as reported by Lawton and Smith (1970) who pooled plasma from groups of normal rats sacrificed at different times on the afternoon of proestrus. By contrast with their findings in normal females, Lawton and Smith found a single LH peak at 2:00 p.m. in groups of castrate female animals, indicating that LH release always occurred at the same time in the ovariectomized animal but at variable times on the afternoon of proestrus in the intact animals. It is possible that the ovarian steroids are responsible for the variation in time of LH release. In humans given oral estrogen from the fifth to tenth day of the cycle, the time between the last dose and the increase in Basal Body Temperature ovulation was longer with high than with low doses of estrogen (Boutselis and Dickey, in press).

Levels of releasing factors during the estrous cycle have been reported for LRF (Chowers and McCann, 1965; Ramirez and Sawyer, 1965) and FRF (Negro-Villar and Meites, 1970). Chowers and McCann (1965) found LRF activity of stalk median eminence (SME) sections as judged by Ovarian Ascorbic Acid Depletion (OAAD) assay reached its highest levels at 4:00 - 8:00 p.m. the evening preceding
proestrous and then fell significantly (P<.01) by noon of proestrous. Levels at 4:00 - 8:00 p.m. on the day of proestrous were similar to those found for estrous, diestrous and the morning of diestrous.

In contrast to the findings of Chowers and McCann, Ramirez and Sawyer (1965) found LRF highest during the afternoon of proestrous with a rapid fall to 20% of peak levels during the first two and a half hours of darkness the evening of proestrous. Low levels continued the morning of estrous and rose the afternoon of estrous.

Negro-Villar and Meites (1970) measured FRF content of the SME by in vitro incubation. These workers found a sharp fall in FRF activity between 11:00 a.m. and 4:30 p.m. the day of proestrous. In this study pituitary FSH content paralleled SME FRF activity.

Estrogen levels have been determined in the rat ovarian venous plasm during the estrous cycle by intravaginal bioassay (Yoshinaka, et al., 1969). The estrogen secretion rate rose in the afternoon and evening of the day preceding proestrous. Estrogen concentration was highest from 10:00 a.m. and 3:00 p.m. or proestrous when it plateaued at 4.5 ng estradiol/ovary/hour. It then declined by about 20% at 8:00 p.m. and then fell precipitously to 15% of peak levels at midnight and was undetectable at 5:00 a.m. of estrous. Estrogen concentration rose to detectable levels between 10:00 and 3:00 p.m. both on the day of estrous and again on the first day of diestrous. The apparent daily increase in estrogen secretions between 10:00 a.m. and 3:00 p.m. thus is a consistent pattern. The marked increase in estrogen production on proestrous may be seen as a part of this pattern.

The secretory rates of progesterone (P) and 20α-OH progesterone (20α-OHP) have been followed throughout the estrous cycle and differ significantly from that seen for estrogen. 20α-OH progesterone is the major produce in the rat (Hashimoto et al., 1968).
Maximum levels of P plus 20 α-OHP were found at 7:00 p.m. the evening of proestrus 4.4 μg/hour/ovary P, 17.6 μg/hour/ovary, 20 α-OHP. Values fell by 50% during estrous. A second rise to less than peak values for P and to peak values for 20 α-OHP occurred at noon the following day, the first day of diestrous.

Catecholamine content of the hypothalamus during the estrous cycle has also been determined. The presence of serotonin (5-HT), dopamine (DA) and Norepinephrine (NE) containing neurons in the hypothalamus was demonstrated by fluorescence microscopy (Hillarp Fuxe and Dhalstrom, 1966). Dopamine containing neurons originate in the vicinity of the arcuate nucleus and end near the primary plexus of the hypophyseal portal vessels in the median eminence (Fuxe and Hokfelt, 1967).

Cyclic variation of the catecholamine content of the hypothalamus has been investigated with the idea of correlating changes of CA content with steroid levels. Fuxe, Hokfelt and Nilsson (1967) noted only slight increase in the fluorescence of DA nerve cell bodies in the tuberoinfundibular area during diestrous. Ovariectomy had no effect nor did estrogen or progesterone treatment. Marked increase in fluorescence occurred in pregnancy and pseudopregnancy. Lippmann et al. (1967) measured hypothalamic CA levels and LH release following various treatments and found that the rate of synthesis, uptake and release of CA appeared to be more critical than absolute concentrations of CA. Both reserpine and αMT lowered hypothalamic CA content and induced pseudopregnancy in rats. Iproniazid blocked both affects of reserpine. Pargyline was ineffective in blocking either CA depletion of the hypothalamus or pseudopregnancy following α-MT. Stefano and Donoso (1967) found that the total CA content, measured fluorometrically, of the anterior and middle hypothalamus
was minimal at estrous, rose significantly during diestrous and was at a peak on both the morning (9:00 a.m.) and evening (6:00 p.m.) of proestrous. No changes were observed in the CA content of the posterior hypothalamus. Sandler (1968) failed to find any change in hypothalamic CA content during the estrous cycle. Sandler used more specific methods of determination than did Stefano and Donoso--methods designed to assay only NE and exclude other biogenic amines. Lichtensteiger (1969) using microfluorometry of nerve cells as a determinant, also distinguished increasing CA content during the cycle from diestrous to estrous. He was unable to distinguish between NE and DA but believed the change was due to increased NE since no such change occurred in the DA rich fibers of the substantia nigra. The potential error in this conclusion is that no steroid sensitive receptors capable of responding to estrogen may be present in the latter tissue.

Anton-Tay, Pelham and Wurtman (1969) have reported that castration resulted in an increased rate of turnover of $^3$H-norepinephrine in the hypothalamus and midbrain following intraventricular injection of NE. This could be duplicated by administration of FSH but not LH, thus suggesting a means whereby FSH could modulate hypothalamic levels of a catecholamine which may effect FRF. Zigmond and Wurtman (1970) reported that the rate at which brain CA is synthesized from circulatory $H^3$-tyrosine is significantly higher in the middle of the light period than in the middle of the dark period, indicating a diurnal rhythm.

Kamberi and Kobayashi (1970) found that monoamine oxidase (MAO) activity is highest in the hypothalamus at 10:00 a.m. or proestrous, and becomes elevated again by 10:00 a.m. estrous. The possibility of diurnal variation with low points at 6:00 a.m. was not
excluded by this study since assays were done only at 10:00 a.m. and 3:00 p.m. except for the day of proestrous. Highest activity was found in the median eminence area. Monoamine oxidase activity in the pituitary rose on the morning of proestrous and revealed its highest level on the afternoon of proestrous.

Green and Miller (1966) measured plasma concentrations of norepinephrine (NE) and epinephrine (E) in the rat during the estrous cycle and pregnancy. The concentrations of NE in plasma from estrous and pregnant animals was significantly lower than from first or second day diestrous animals. Epinephrine levels were highest in estrous and pregnant animals. However, plasma levels were not measured on the day of proestrous.

A very pertinent recent observation regarding the interrelation­ship between sex steroids and CA is the finding that estrogen and progesterone effect the uptake of $^3$H-NE into synaptosomes (Janowsky and Davis, 1970). Estradiol $10^{-5}$M and progesterone $10^{-4}$M decreased the amount of $^3$H-NE taken up by synaptosomes isolated from whole rat brain. $^3$H-norepinephrine efflux was also effected when synaptosomes had been incubated with $^3$H-NE for 10 minutes prior to adding steroids. Hydrocortisone and desoxycorticosterone were without effect.

Changes in indolamine synthetis and metabolism in the pineal have been correlated with the estrous cycle in the rat. Activity of hydroxyindole-o-methyl-transferase (HIOMT) in the rat pineal gland varies two-fold during the estrous cycle (Wurtman, Axelrod, Sawyer, and Chu, 1965). HIOMT, measured at 9:00 a.m. was highest the second day of diestrous and lowest at 9:00 a.m. on both proestrous and estrous. Estradiol (10 $\mu g$/day, s. c.) the day before killing caused a slight decrease in HIOMT activity. Castration was without effect.
Weiss and Crayton (1970) studied the ability of norepinephrine (NE) to stimulate adenylcyclase activity of the pineal gland. Unstimulated adenylcyclase activity was lowest during proestrous. Norepinephrine stimulated adenylcyclase activity of rat pineal gland in all phases of the estrous cycle except proestrous. Administration of estradiol, 0.05 mg/kg s.c. for three days increased non-stimulated adenylcyclase activity slightly and inhibited norepinephrine stimulation of activity significantly. These authors suggested that estrogen levels might cause a decrease in the biosynthesis of melatonin by competing with norepinephrine. A possible role for melatonin in the regulation of LRF is suggested by the studies of Fraschini, Mess and Martini (1968). They found that implantation of melatonin on pineal fragments into the median eminence caused a depletion of both pituitary LH stores and plasma LH levels. Implantation into the pituitary was without effect. By contrast, pinealectomy of adult male rats caused increased pituitary LH stores and testicular hypertrophy.

Light decreases sympathetic impulses to the pineal gland of the rat (Taylor and Wilson, 1969). Norepinephrine activates adenylcyclase activity of the pineal gland (Weiss and Costa, 1967). Summarizing the above studies it can be suggested that, during periods of light, NE activation of adenylcyclase activity is reduced, less melatonin formation occurs and, because less melatonin is available to inhibit release or synthesis of hypothalamic releasing factors, the pituitary and plasma LH levels increase. During periods of darkness the reverse process occurs with the difference that estrogen in sufficiently high concentrations is able to block NE stimulation of adenylcyclase activity and subsequent melatonin synthesis.
Endogenous and Exogenous Influence on Gonadotropin Levels.

Effect of age on plasma and pituitary gonadotropin levels:
Pituitary stores of FSH and LH have been determined in female rats of ages from 12-14 days to 280 days (Labhsetwar, 1969a). Pituitary FSH concentration was high at 12-14 days (16 µg/mg) but by 33 days had fallen considerably (5 µg/mg). However, total pituitary FSH content does not change remarkably over the period of puberty and had increased slightly by the 33rd day. In very old animals, over 280 days of age, both pituitary FSH content and FSH µg/mg are increased. Similar changes occur in LH content and a progressive rise from age 76 days on. Aged rats failed to have compensatory increase in ovary weight after unilateral ovariectomy. Labhsetwar considers that with aging the rat develops an inability to release, but not to synthesize gonadotropin in response to hemi-ovariectomy.

Effect of castration on plasma and pituitary gonadotropin levels:
Gay and Midgley (1969), using radioimmunoassay, did a definitive study of plasma LH levels of males and female rats following castration. Within eight hours of gonadectomy, but not a five hours, plasma LH levels in the males were significantly elevated and by 24 hours they were 50 times above control levels. Over the next 60 days they increased only two-fold. Following ovariectomy of females, LH levels increased gradually and took two weeks to reach levels seen in the male after one day. Final female levels were about 60% of male levels. These authors found that LH levels were not stable in castrated animals, but varied by as much as 80% over a one hour interval.

Parlow (1964) commented on the difference between FSH and LH ratios in pituitary and plasma of ovariectomized rats. In the
anterior pituitary of rats castrated 50 days previously the mean concentration of FSH was 161 µg/mg and of LH was 23.2 µg/ml, for a FSH to LH ratio of 7/1. Plasma values of FSH were 9.2 µg/ml and of LH were 0.08 µg/ml for a FSH to LH ratio of 115/1. A similar differential may exist in the intact animals.

Lawton and Smith (1970) reported that a daily cycle of plasma LH levels persisted in castrate females that was identical to that seen on the day of proestrous in intact females. A similar cycle of LH release was not observed in males castrated after the 10th day of life. Yamamoto, Diebel and Bogdanove (1970) failed to find a diurnal variation of plasma LH in ovariectomized females but may have missed the critical time periods.

The effect of short term steroid treatment of male and female castrates was studied by Gay and Bogdanove (1969). Testosterone propionate (5 mg/kg) and estradiol benzoate (.05 mg/rat) and progesterone (25 mg/rat) caused decrease in plasma LH in 24 hrs but no change in pituitary LH. Following the same doses of testosterone propionate, pituitary FSH levels increased but plasma FSH was unchanged. Estradiol benzoate plus progesterone consistently lowered plasma FSH levels without altering pituitary levels in the male.

Several studies have been made on the effect of estrogens or progesterone on pituitary and plasma FSH and LH (Chowers & McCann, 1965; Kobayashi, Hara and Miyaka, 1969; Hagino and Goldzielher, 1970). For an older review see McCann and Ramirez (1964). In general, all of these have confirmed the negative feedback effect of steroids on FSH and LH synthesis and release.

There is some evidence that low doses of estrogen may stimulate LH release. Callentine et al. (1966) have reported that
estradiol (0.6 μg/kg, s.c.) for seven days caused a significant elevation in plasma LH and a fall in pituitary LH concentration. This dose is about 4 times the production rate of estrogen per day determined by Yoshinaga, Hawkins and Stocker (1969).

McCann and Ramirez (1964) have calculated that 1.2 μg/kg/day of estradiol benzoate was required in order to lower the plasma LH of a mature ovariectomized female rat. Pituitary LH was suppressed in the castrate animal at 1.0 μg/kg/day E₂K but pituitary FSH was slightly increased at this dose and not suppressed until 4.0 μg/kg/day was given.

Corbin and Corbin (1966) reported that LH implanted in the median eminence inhibited LH secretion by the pituitary and suggested the existence of a short loop (e.g., pituitary to hypothalamus) inhibition of FRF by pituitary FSH. This led to rethinking of certain concepts regarding control of ovulation. Although originally this finding was criticized as being due to nonspecific factors, their work has gained increasing acceptance. Median eminence (Corbin and Corbin, 1966; David et al., 1966; Corbin, 1966 implants of LH or FSH (Corbin and Story, 1967) are capable of lowering pituitary content of these respective gonadotropins in intact and castrate females. More recently, however, Ojeda and Ramirez (1969, 1970) have suggested that FSH has a positive feedback on FRF, and that FSH may compete with estrogen for the same hypothalamic receptor since FSH blocked $^3$H-E₂ uptake by the hypothalamus. Corbin et al. (1970) have challenged this finding. They found only inhibitory effects of endogenous FSH on SME FRF content in ovariectomized rats which were subsequently hypophysectomized. Ovariectomy alone had no effect on SME FRF content or plasma FRF levels leading them to postulate that FSH synthesis and release is entirely dependent on the short loop systems.
Effect of steroids on pituitary gonadotropin content in vitro:
Piacsek and Meites (1966) found that estradiol (0.025μg/ml) increased LH release from pituitary halves in vitro while higher concentrations were less effective. Schneider and McCann (1970c) found that estradiol alone incubated with pituitary at doses of 0.5 and 1.0 μg/ml stimulated LH release from pituitary while 5.0 μg/ml inhibited LH release. Puromycin and cycloheximide blocked the effect of E₂ on LH synthesis. Estradiol 0.2 - 12.5 μg/ml slightly inhibited the response of pituitary to LRF in vitro and completely blocked the increase in LH release seen when dopamine 2.5 μg/ml was incubated with SME plus pituitary. Puromycin and cycloheximide also blocked this effect of estradiol to inhibit the LRF release due to DA. Testosterone 0.08 - 2.0 mg/ml had no effect on C¹⁴-leucine incorporation into LH. FSH release in vitro was not found to be increased by either estradiol (0.25 μg/ml) or testosterone (0.5 - 0.1 μg/ml) (Mittler and Meites, 1966).

The implications of these studies require further investigation. It would appear that estrogen has two sites of action on LH secretion. At one site in the pituitary stimulates LH synthesis and/or release, on the second estrogen blocks LRF release perhaps by competing with DA. Both actions of estrogen are dependent on RNA and protein synthesis as evidenced by the blocking action of puromycin and cycloheximide, respectively.
MATERIALS AND METHODS

Animals:

Female and male Sprague Dawley rats 180 - 200 grams, obtained from Laboratory Supply, Indianapolis, Indiana were used for all experiments.

Ovariectomized females were used for the majority of experiments both to eliminate feedback of ovarian steroids as a variable and because plasma gonadotropin levels in normal females are at the limits of detectability in our radioimmunoassay.

Animals were housed 5 to a cage in an airconditioned room with a light cycle of 14 hours on and 10 hours off, 5:00 a.m. - 7:00 p.m. Water and Purina Rat chow were provided ad lib.

Ovariectomy:

Ovariectomy in females was carried out on 180 - 200 gram rats through bilateral flank incisions under ether anesthesia. All animals of a group of 30 were operated within 3 days of each other. Ovariectomized animals were kept with other animals for 21 to 30 days prior to use in experiments.

Time of experiment:

Experiments were timed so that animals could be sacrificed between 10:00 a.m. and noon. Female rats following ovariectomy may continue to have daily plasma peaks in LH between 2 and 4 p.m. (Lawton and Smith, 1970). Rats were returned to the animal housing overnight during experiments.
Intraventricular injection:

Preliminary experiments proved that both pentobarbital and chlorolose anesthesia blocked FSH and LH release and perhaps synthesis (Figure 1). Thereafter intraventricular injections were done without anesthesia. Animals were gently but firmly restrained with one hand while with the other, the scalp was swiftly incised with a single stroke of the scalpel. The site for injection 0.5 cc caudal and 1.5 mm lateral to the bregma was identified. A hole was rapidly bored through the skull at this site by rotating a 22 gauge needle between the fingers. If profuse bleeding occurred at this point, the animals were discarded. If no bleeding occurred intraventricular injection was performed immediately. Injection was done with a 50 μl Hamilton syringe and a 27 gauge needle. Correct depth was achieved by using a sheath which allowed only 4 mm of the needle tip to protrude. Initially 30 μl and later 15 μl of Merle's solution (NaCl 8.98, KCl 0.25, CaCl₂ 0.14, MgCl₂ 0.11, NaH₂PO₄ 0.07, Urea 0.13, Glucose 0.16, gram/liter) an artificial cerebrospinal fluid (Glowinski and Axelrod, 1965) was introduced with no pressure other than the weight of the plunger. Spontaneous depression of the plunger under gravity was taken as evidence that the needle was properly placed in the lateral ventricle. Animals demonstrated little or no pain when this procedure was rapidly and efficiently performed. Preliminary experiments (Figure 2) demonstrated that changes in plasma and pituitary gonadotropin did occur in response to intraventricular injection of Merle's solution alone which had returned to near control levels 60 minutes later. However, following the publication of studies by Kamberi, Mical and Porter (1969, 1970a, 1970b) 15 minutes was most often accepted as the time used for sacrifice after intraventricular studies.
Figure 1

Effect of Injection Anesthesia on Plasma and Pituitary FSH and LH in Normal Males

Groups of three normal (150 - 180 g) Sprague Dawley males were given intraperitoneal injection of 0.6 cc normal saline, nembutal (35 mg/kg) or choloralose (55 mg/kg) dissolved in saline and sacrificed by decapitation 90 minutes later.
LH mg/ml PLASMA

FSH mg/ml PLASMA

PITUITARY

PLASMA

CONTROL  SALINE  NEMBUTAL  CHOLORALOSE
0.6 ml  35 mg/Kg  35 mg/Kg

CONTROL  SALINE  NEMBUTAL  CHOLORALOSE
0.6 ml  35 mg/Kg  55 mg/Kg
Normal male Sprague Dawley rats (450 g) were anesthetized with nembutal (35 mg/kg, i.p.). Intraventricular injection of 15 μl Merles solution, pH 7.5 was perfused 30 minutes later and animals were sacrificed by decapitation at the indicated times.
The figure shows the changes in FSH and LH levels in plasma and pituitary over time. The control group shows baseline levels, while the 30 and 60 MIN. groups show an increase in levels. The bars indicate a significant difference (p < 0.05) in FSH levels at 30 MIN. compared to the control group. LH levels also increase significantly (p < 0.01) at 30 MIN. compared to control, but not at 60 MIN.
Sacrifice and Tissue Collection:

Animals were sacrificed by decapitation between 10:00 a.m. and noon. Blood from the aorta was collected in heparinized 10 ml tubes packed in ice. The cranium was removed after cutting along suture lines and the brain lifted out. The dura and the posterior pituitary were separated from the anterior pituitary. The anterior pituitary was then removed, weighed on a glassene paper and placed in a homogenizing tube, packed in ice which contained 1 ml 0.005 M of phosphate buffer solution, pH 7.6 containing 1% bovine serum albumin. These samples were homogenized at the end of the experiment and immediately centrifuged to separate solid material from buffer solution containing FSH and LH polypeptides. Pituitary extract and separated plasma were frozen (-20°C) until assay. Gonadotropins are stable for at least six months at -20°C temperature.

Experimental Conditions:

**Drugs:** Drugs were used in this study as their salts unless otherwise specified.

a. Reserpine (Serpasil, Ciba) 5 mg 2 cc ampules. Dose was 2.5 mg/kg or 5.0 mg/kg given by intraperitoneal injection. Controls received the same volume of saline.

b. Pargyline hydrochloride (Entonyl, Abbott Laboratories) Dose was 50 mg/kg intraperitoneal given in 1 ml/kg saline. Animals were sacrificed at 24 hours. Pargyline is a potent monoamine oxidase inhibitor. After treatment with this drug, increased content of catecholamines and serotonin has been found in the brain (Crout, 1961).

c. dl, Parachlorophenylalanine methyl ester HCl (PCPA, Calbiochem.). Dose was 500 mg/kg intraperitoneal. PCPA is
almost insoluble in water. Solubilization was achieved by adding 4 N NaOH to a heated saline suspension until complete solubility was achieved. pH was then adjusted to 10.4 -10.6 with 4 N HCl. Animals were sacrificed at 48 hours. This compound selectively inhibits tryptophan hydroxylase in the biosynthetic pathway for 5-hydroxytryptamine (Koe and Weissman, 1966).

d. 1, alpha-methyl-tyrosine ( -MT). Dose 300 mg/kg intraperitoneal in pH adjusted saline 1.0 ml/kg. This solution was prepared by adding 4 N HCl to a heated saline suspension until complete solubilization occurred. pH was then adjusted to 2.5 by addition of 4 N NaOH. Animals were sacrificed at six hours. Alpha methyl tyrosine inhibits tyrosine hydroxylase in the biosynthetic pathway of catecholamine synthesis (Spector et al., 1965).

e. 1-norepinephrine bitartrate (Levophen, Winthrop) 2 mg norepinephrine/1 ml ampule. A final dilution of 5.0 µg or 0.5 µg in 15 µl Merles solutions were used for intraventricular injection.

f. epinephrine HCl (Adrenaline, Parke Davis) M. W. 183.2 1 mg/ml ampule. A final dilution of 5.0 µg or 0.5 µg in 15 µl Merles solution was used for injection.

g. serotonin creatinine sulfate (Nutritional Biochemical Corp.) M. W. 405.4, a dilution of 10.0 µg, 5.0 µg, 1.0 µg or 0.5 µg in 15 ml Merles solution was used for injection.

h. 1, 3,4 dihydroxyphenylalanine HCl (L-DOPA Nutritional Biochemical Corp.) M. W. 197.19. Dose 400 mg/kg intraperitoneal This solution was prepared by adding 4 N HCl to heated saline suspension until complete solubilization occurred. pH was then adjusted to 2.5 by addition of 5 N NaOH. L-Dopa 400 mg/kg intraperitoneal in mice caused a ten-fold increase in brain DA with no increase in brain NE and a 63 % decrease in brain 5-HT, 30 minutes after injections. The fall in brain 5-HT was accompanied by increase in
urine indolacetic acid indicating increased release and metabolism
of 5-HT (Everett and Borcherding, 1970).

i. 1-threo-3,4 dihydroxyphenylserine (Phenyl Serine, Pfaltz
and Bauer, Inc.). Dose 200 mg/kg i.p. in normal saline. Phenyl
Serine restores NE synthesis in the -MT blocked animal (Creve-
ling et al., 1968).

j. dihydroxyphenylethylamine (Dopamine-DA, Nutritional Bio-
chemical Corp.) M.W. 188.53. Dose 5.0 or 0.5 µg in 15 ml Merles
solution, i.v. Freely soluble in aqueous solution.

k. carbamylcholine chloride U.S.P. (Carbachol) (City Chem-
icical Corporation, New York). Dose 50 µg or 25 µg in 15 µl Merles
solution. Stable in aqueous solution. Nicotinic and muscarinic
activity resistant to both AChE and nonspecific cholinesterase
(Koelle, 1970).

l. Methacholine Chloride U.S.P. (Mecholy1, Merck Sharp and
Dohme). Dose 10 µg in 15 µl Merles solution. Predominantly mus-
carinic action. Slowly hydrolyzed by ACH esterase (Koelle, 1970).

m. atropine sulfate U.S.P. Dose 100 µg in 10 µl Merles solution.
antimuscarinic.

n. hexamethonium HCl (Burroughs Wellcome and Co.) Dose
100 µg in 10 µl Merles solution. Antinicotinic.

o. Estradiol valerate U.S.P. (Delestrogen, E. R. Squibb and
Sons, Inc.) 20 mg/ml in castor oil and 20 % benzyl benzoate. Slowly
absorbed estrogen preparation.

p. estradiol 17β (Elite Chemical). Dissolved in EtOH and dil-
uted to 0.5 % with normal saline.

q. estrone (Elite Chemical) Dissolved in EtOH and diluted to
0.5 % with normal saline.
r. progesterone (Upjohn) 25 mg/ml aqueous suspension.
Crystalline suspension used for injection.

Radioimmunoassay for FSH and LH:

Materials for the radioimmunoassay were obtained from the National Institute of Arthritis and Metabolis Disease, NIH. Rat Pituitary Distribution Program as follows:

1. Purified rat follicle stimulating hormone for radiiodination NIAMD-Rat FSH-1-1. Biological potency approximately 100 x NIH FSH-S1 (HCG augmentation assay). LH contamination less than 0.002 x NIH-LH-S1 (OAAD Assay). Provided as 100 µg in 1.0 ml phosphate buffer solution (PBS) (0.01 M Phosphate, 0.15 M NaCl, 0.01% merthiolate, pH 7.6).

2. Purified rat luteinizing hormone for radiiodination NIAMD-Rat LH-1-1. Biological potency approximately 1.0 x NIH-LH-S1 (OAAD assay), FSH contamination less than 0.04 x NIH-FSH-SL (GCG augmentation assay). TSH contamination approximately 0.4 U.S.P. units/mg (McKenzie Assay) Provided as 100 µg in 1.0 ml PBS.

3. Antiserum to rat FSH prepared in rabbits: NIAMO-Anti-Rat FSH Serum-1. provided undiluted.

4. Antiserum to rat LH prepared in rabbits. NIAMO-Anti-Rat LH Serum-2. provided undiluted.

5. Rat Follicle Stimulating Hormone Reference Preparation for Radioimmunoassay. NIAMO-Rat FSH RP-1. Biological Potency 2.1 x NIH FSH-S1 (HCG Augmentation Assay) LH contamination - 0.02 x NIH-LH-S1 (OAAD Assay) TSH contamination - 0.3 U.S.P. units/mg McKenzie Assay provided as lyophylized powder.
6. Rat Luteinizing Hormone Reference Preparation for Radioimmunoassay: NIAM-Rat-LH-RP-1. Biological potency 0.03 x NIH-LH-S1 (OAAD assay). FSH contamination 0.54 x NIH-FSH-S1 (HCG augmentation assay). TSH contamination 0.22 U.S.P. (Bovine) TSH units/mg McKenzie assay.

Other materials used were:

1. Sheep anti-rabbit serum (2nd antibody) obtained from Mr. John Powell, Department of Obstetrics and Gynecology, Ohio State University. Provided undiluted: Diluted 20:1 with phosphate buffer pH 7.6 immediately prior to use.

2. $^{131}$Iodine: Sodium$^{131}$ Iodide in NaOH. Approximate concentration 500 mCi/ml, 0.002 ml containing 1.30 10% mCi at time of shipping (Cambridge Nuclear Corporation).

3. Chloramine-T (Lilly Pharmaceutical Co.) 25 mg/10 ml of 0.01M PO$_4$, 0.15 M NaCl pH 7.6 buffer, prepared immediately prior to use.

4. Sodium metabisulfite (J.T. Baker Chemical Co.) 25 mg/ml of 0.01M PO$_4$, 0.15 M NaCl, pH 7.6 buffer, Prepared immediately prior to use.

5. Bio-Gel P-60 (BioRad Laboratories)

Radioiodination Procedure:

Radioiodination of either purified rat LH or FSH was carried out on alternate weeks according to the method described by Monroe, Parlow and Midgeley (1968). This procedure uses chloramine-T to oxidize iodide to iodine, which reacts with available tyrosine residues in LH or FSH. Excess iodine is then reduced to iodide by sodium
metabisulfite. To 1 mCi of carrier and the sulfate free sodium\textsuperscript{131}I in NaOH dispensed at the bottom of a 1 ml plastic vial was added 25 μl of 0.5 M sodium phosphate, pH 7.6 and 2.0 μg purified rat FSH (NIAMD-Rat-FSH-1-1 in 20 μl of H\textsubscript{2}O. A stopper was inserted and the contents mixed by tapping. Chloramine T, 20 μg in 10 μl was next added and the reaction mixture was agitated for one and one half minutes. Sodium metabisulfite, 50 μg in 10 ul of buffer was added and the reaction mixture agitated again.

This reaction mixture was then layered beneath the buffer on the surface of a 1 x 15 cm disposable column of Bio Gel P 60--prepared 18 hours earlier and equilibrated with 0.5 M phosphate. Before use, 1 ml of a 5% solution of bovine serum albumin (BSA) was passed through the column to reduce nonspecific binding of rat FSH \textsuperscript{131}I or LH \textsuperscript{131}I. Blue dextran was added to identify the solvent front.

One ml aliquots were collected in 13 to 15 tubes containing 1 ml 0.05 M phosphate buffer with 5% BSA. Specific activity of the radiolabeled rat FSH \textsuperscript{131}I or LH \textsuperscript{131}I was determined by counting the collection tubes in a lead container set on top of the well of an automatic gamma scintillation spectrometer, Packard Model 3001.

The maximum activity occurred in two peaks. The first, usually in tubes 3 or 4, contained \textsuperscript{131}I labeled FSH or LH. The second usually in tubes 7-10 contained free \textsuperscript{131}I. Calculation of the appropriate dilution to yield .0025 μg of labeled protein/ml FSH or LH was then carried out. Radiolabeled FSH \textsuperscript{131}I was usually at 4°C for 5 days and LH \textsuperscript{131}I for 10 days. Average results of this procedure were 64% ± 15% utilization of \textsuperscript{131}I, 29% ± 15% recovery of rat LH. A typical run showing the calculations used is seen in Figure 3.
**Figure 3**

**Iodination No. 18**

<table>
<thead>
<tr>
<th>Date</th>
<th>Antigen Source</th>
<th>NIH Hormone</th>
<th>FSH</th>
<th>File No.</th>
<th>Concentration</th>
<th>Antigen</th>
<th>1 μg</th>
<th>10 μl</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-29-70</td>
<td>NIH</td>
<td>FSH</td>
<td></td>
<td></td>
<td>100 μg/ml</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Chloramine T. (2 mg/ml)** 10 μl  
**Metabisulfite (1 mg/ml)** 50 μl  
**Pot. Iodides (10 mg/ml)** 100 μl  
**Buffer** 50 μl  

<table>
<thead>
<tr>
<th>Column Size</th>
<th>Gel Type</th>
<th>P-60 Biogel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 x 15 cm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Calculation of Protein Label**

<table>
<thead>
<tr>
<th>Vial Count</th>
<th>Free Iodine</th>
<th>Protein Count</th>
<th>Concentration (desired)</th>
</tr>
</thead>
<tbody>
<tr>
<td>65119</td>
<td>43913</td>
<td>16172</td>
<td>0.025 μg/ml</td>
</tr>
</tbody>
</table>

\[
\text{Vial Count} - \text{free iodine} = 21206 \quad A
\]

\[
\text{Protein Count} + A = 76.2\%
\]

\[
\text{Protein B} \quad \text{recovered} = \frac{\text{Antigen (μg)}}{B} \times 76.2\%
\]

\[
\text{Protein C} \quad \text{recovered} = 76.2\% \quad \text{TP Count in Tube} = 0.76 \quad \mu g/\text{protein/ml}
\]

**Labelled Antigen**

<table>
<thead>
<tr>
<th>No. of tubes used</th>
<th>CPM (0.1 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>20,000</td>
</tr>
</tbody>
</table>

\[
\text{Protein C} \quad \% \quad \text{TP Count in Tube} = 0.76 \quad \mu g/\text{protein/ml}
\]

\[
\text{DX/ml} = 305/1 \quad \text{dilution} \quad 0.0025
\]

\[
\text{Picogram of label/tube} = 250
\]
Radioimmunoassay:

Radioimmunoassay was also conducted according to the method of Monroe, Parlow and Midgley (1968). To reduce the possibility of contamination disposable culture tubes (10 x 75 mm) were used. Either 100 μl or 200 μl of sample and 100 μl of standard were added to each tube. Sufficient phosphate buffer, pH 7.6, containing 1% BSA was added so that each tube contained 500 μl. Two hundred microliters of anti rat FSH serum diluted 1:635 or anti rat LH serum diluted 1:25,000 were added to each tube. At these dilutions anti rat FSH serum should bind 25% of labeled rat FSH and anti rat LH serum should bind 40% of labeled rat LH. One hundred microliters of the labeled FSH 131I or LH 131I was added and the tubes agitated on a vortex mixer. Incubation was then carried out at 4°C for 5 days. At the end of 5 days, 200 μl of sheep anti-rabbit serum diluted 20:1 was added to each tube. At this dilution sheep anti-rabbit serum maximally precipitated the antibody bound labeled rat FSH or LH. Tubes were incubated at 4°C for 1 more day. At the end of this period all tubes were centrifuged at 1,000 x g for 30 minutes at 4°C. The supernate was discarded and the precipitate counted in an automatic well type gamma counter for 30 seconds. All serum and tissue samples were run in duplicate. Initially duplicate samples were run at two dose levels, however, no difference was seen for pituitary extract at two different doses. Serum consistently showed less activity at 200 μl than at 100 μg, but there was no difference in activity between 100 μl and 50 μl. Therefore, all samples were run at 100 μl in the later experiments. Dose response curves were constructed from data obtained over the range 4 ng to 2000 ng with each new label. Satisfactory dose-response curves were usually obtained from 16 μg to 2000 μg (90°-20% bound).

Typical dose-response curves for FSH and LH are shown in Figure 4.
Figure 4

Radioimmunoassay: Dose Response Curve for FSH and LH Standards

Abscissa represents cpm minus background expressed as percent of the cpm in tubes where FSH - $^{131}$I or LH - $^{131}$I was precipitated in the absence of unlabeled hormone (tubes containing PBS-1 % BSA instead of sample). Ordinate represents nanograms of standard FSH or LH Reference Preparation; NIAMD-Rat-FSH-RP-1 or NIAMD-Rat-LH-RP-1.
Adenohypophysial secretory kinetics for FSH and LH have been examined by Bogdanove and Gay (1969) using long term castrate, acutely hypophysectomized male and female rats. These authors were able to accurately define the half life of endogenous LH (Gay and Bogdanove, 1968) and FSH (Bogdanove and Gay, 1969) in the plasma. Endogenous LH activity disappears from the plasma four times as rapidly as endogenous FSH activity. The decay constant \( K = 0.693/t^{1/2} \) in minutes) for LH was 0.0219, and for FSH was 0.0046. The disappearance rate \( dQ_{LH}/dt \) which is equal to the metabolic clearance rate times the LH concentration in plasma equals the pituitary release rate during a steady state condition.

Using their average estimates for \( K_{FSH} \) and \( K_{LH} \), and using \( V_{FSH} \) and \( V_{LH} \), the distribution volume of gonadotropin in the rat of 3% they were able to calculate the secretion rate of FSH and LH in an average long term castrate male rat (body weight 350 g) as follows:

\[
\frac{dQ_{LH}}{dt} = -K_{LH} \times (LH)_0 \times V_{LH} \\
= 0.0219 \times 0.78 \times (0.03 \times 350) \\
= 0.0179 \mu g \text{ LH-S1 equivalents/minute} \\
= 1.08 \mu g \text{ equivalents/hour} \\
= 25.8 \mu g \text{ equivalents/350 g castrate rat/day}
\]

\[
\frac{dQ_{FSH}}{dt} = -K_{FSH} \times (FSH)_0 \times V_{FSH} \\
= -0.0046 \times 11.8 \times (0.03 \times 350) \\
= -562 \mu g \text{ FSH-S3 equivalents/minute} \\
= 33.7 \mu g \text{ equivalents/hour} \\
= 809 \mu g \text{ equivalents/350 g castrated rat/day}
\]

Numerically the secretion and disappearance rate of FSH (809 \mu g equivalent/rat/day) is about 25 times the corresponding rate for LH (26 \mu g equivalent/rat/day). According to Wilhelme, cited as a personal communication (Bogdanove and Gay, 1969) the NIH-FSH
used in this study is not more than 1% pure FSH while NIH-LH is about 25% pure LH. In terms of active components of these reference materials, the average castrated male rat secretes each day the equivalent of 6.5 μg ovine LH and less than 8.1 μg ovine FSH.

By referring to the total pituitary content of FSH and LH Bogdanove and Gay (1969) also calculated a pituitary turnover time for each hormone. Pituitary turnover time (TTp) is calculated by dividing the secretory rate for each hormone (expressed as units of activity/unit time) into the total quantity of intrahypophysial stores of each hormone. Using average pituitary content of 112 μg equivalents LH and 280 μg equivalents FSH for a castrate 350 g rat, they calculated that TTp LH required over 4 days (103.61 hours) and TTp FSH required about 8 hours (8.31 hours).

Based on these estimates, it is apparent that changes in pituitary content of LH will occur too slowly (0.96% an hour) to be apparent even after abrupt cessation of LH release. Following abrupt cessation of FSH release pituitary contents can be expected to double by 8.3 hours, or an approximate rate of 12% an hour.

These estimates of hypophysial kinetics will be used in attempting to interpret the data in this study. Exact applications are not possible however, because (1) total suppression of either synthesis or release probably does not occur in short term investigations and, (2) LH at least is probably not released tonically but rather comes out in a series of spurts found to occur about every 60 minutes in the monkey (Dierschke et al., 1970).
RESULTS

Effect of Estradiol 17β on Plasma and pituitary FSH and LH in ovarioctomized females:

The effect of estradiol 17β on plasma and pituitary FSH and LH is shown in Table 1, Figure 5. Estradiol 17β dissolved in 0.5% alcohol solution was injected (i.p.) into 250mg female Sprague-Dawley rats ovariectomized three weeks before. Animals were sacrificed by decapitation two hours later. This is the time at which brain levels of estrogen are highest after i.p. administration of estradiol-H³ (McEwen, 1970).

There is an apparent 30-fold difference in the amount of estrogen effective in suppressing FSH and LH release. The ED₅₀ for suppression of plasma LH was approximately 0.3 μg/kg, the ED₅₀ for suppression of plasma FSH was 10 μg/kg.

Plasma LH was maximally decreased to 23% of control levels and pituitary LH maximally elevated (3x) at a dose of 10 μg/kg estradiol. The lowest dose tested 0.3 μg/kg still caused a marked inhibition of the release of plasma LH. At doses greater than 10 μg/kg plasma LH rose and pituitary LH fell but did not reach control levels.

Plasma FSH was only slightly decreased at 0.3 μg/kg and was maximally suppressed to 43% of control levels at 30.0 μg/kg. Pituitary FSH was maximally increased (two and one half times) at 3.0 μg/kg. Higher doses of estrogen caused a return of plasma FSH and pituitary levels to near control values.
Table 1

Effect of Estradiol 17β on Plasma and Pituitary FSH and LH in Ovariectomized Rats

<table>
<thead>
<tr>
<th>Dose (µg/kg)</th>
<th>Plasma µg/ml</th>
<th>FSH Pituitary mg/gland</th>
<th>Plasma µg/ml</th>
<th>LH Pituitary mg/gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.11±.14</td>
<td>.68±.18</td>
<td>1.12±.26</td>
<td>2.50±.80</td>
</tr>
<tr>
<td>0.3</td>
<td>1.07±.05</td>
<td>.84±.18</td>
<td>0.66±.12</td>
<td>4.56±.31</td>
</tr>
<tr>
<td>1.0</td>
<td>1.03±.16</td>
<td>.52±.40</td>
<td>0.46±.17</td>
<td>4.43±.38</td>
</tr>
<tr>
<td>3.0</td>
<td>0.84±.13</td>
<td>1.68±.05</td>
<td>0.53±.20</td>
<td>4.95±.65</td>
</tr>
<tr>
<td>10.0</td>
<td>0.52±.06</td>
<td>.42±.03</td>
<td>0.26±.06</td>
<td>7.45±.84</td>
</tr>
<tr>
<td>30.0</td>
<td>0.47±.09</td>
<td>.62±.07</td>
<td>0.41±.07</td>
<td>6.60±.60</td>
</tr>
<tr>
<td>100.0</td>
<td>1.06±.01</td>
<td>.96±.02</td>
<td>0.62±.18</td>
<td>5.70±.95</td>
</tr>
</tbody>
</table>

Female rats ovariectomized 3 weeks previously were injected (i.p.) with estradiol 17β dissolved in 0.5% EtOH in the indicated doses and sacrificed by decapitation 2 hours later. Values represent mean radio-immunoassayable N1AND-FSH-Rat-RP-1 or N1AMD + Rat LH-RP-1, ± standard error of the mean. Numbers in parenthesis represent number of animals used.
Female rats ovariectomized 3 weeks previously were injected (i. p.) with estradiol 17β dissolved in 0.5% EtOH in the indicated doses and sacrificed by decapitation 2 hours later.
Stimulation of FSH release or LH release was not seen at the lowest doses of Estradiol 17β tested (0.3 μg/kg). This dose exceeds by 12 times the endogenous production of estrogen by both rat ovaries which has been determined as a maximum of 22 ng/hour (Yoshimaka et al., 1969). The apparent release of FSH and LH, seen as increased plasma levels and decreased pituitary levels of both gonadotropins, which occurred at the highest levels of estrogen tested must be considered pharmacological rather than physiological effects since these doses far exceeded endogenous production. The use of the intraperitoneal route of injection may have considerable influence on the response to estrogen since endogenous estrogen is released into the inferior vena cava via the ovarian and renal veins, thus largely bypassing the liver while intraperitoneal estrogen would be absorbed largely by mesenteric vessels and pass through the portal system and liver.

The 30-fold difference in the effective dose of estrogen for acute suppression of FSH and LH release suggest that real differences exist in the mechanisms responsible for release of the two gonadotropins.

Effect of reserpine (2.5 mg/kg) and reserpine (5.0 mg/kg) on plasma and pituitary FSH and LH in ovarietomized rats:

The effect of reserpine administered intraperitoneally to ovarietomized female rats which were sacrificed 1-48 hours later is seen in Table 2 and 3, Figures 6 and 7. Administration was timed so that all rats would be sacrificed between 10:00 a.m. and noon.

Following injection of 2.5 mg/kg reserpine, plasma LH fell to 85% of control level at 1 hour and reached its lowest point 37% of control at 2 hours (significant at P<.05). By 6 hours, plasma LH had returned to control levels. Plasma FSH rose by 25% at 1 hour
Table 2

Effect of Reserpine 2.5 mg/kg on Plasma and Pituitary Gonadotropins of Ovariectomized Rats

<table>
<thead>
<tr>
<th>Time</th>
<th>Plasma µg/ml</th>
<th>FSH Pituitary mg/gland</th>
<th>LH Plasma µg/ml</th>
<th>LH Pituitary mg/gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (5)</td>
<td>1.03±.26</td>
<td>.82±.20</td>
<td>1.16±.30</td>
<td>1.76±.49</td>
</tr>
<tr>
<td>1 hr. (4)</td>
<td>1.24±.42</td>
<td>.57±.07</td>
<td>.99±.32</td>
<td>2.16±.47</td>
</tr>
<tr>
<td>2 hr. (5)</td>
<td>1.55±.31</td>
<td>.74±.17</td>
<td>.43±.18a</td>
<td>2.38±.78</td>
</tr>
<tr>
<td>6 hr. (4)</td>
<td>1.84±.33a</td>
<td>.24±.09a</td>
<td>1.11±.33</td>
<td>3.69±.39a</td>
</tr>
<tr>
<td>12 hr. (4)</td>
<td>1.04±.37</td>
<td>.21±.04a</td>
<td>1.20±.35</td>
<td>2.01±.09</td>
</tr>
<tr>
<td>24 hr. (4)</td>
<td>1.31±.32</td>
<td>.40±.10</td>
<td>1.14±.28</td>
<td>2.15±.55</td>
</tr>
<tr>
<td>48 hr. (4)</td>
<td>1.64±.31</td>
<td>.22±.03a</td>
<td>1.26±.29</td>
<td>2.94±.40</td>
</tr>
</tbody>
</table>

a different from control (Student t test) P < .05

Female rats ovariectomized 3 weeks previously were injected with reserpine 2.5 mg/kg (i.p.) and sacrificed at the indicated times. Injections were timed so that all animals were sacrificed between 10 a.m. and 12 noon. Values represent radioimmunoassayable FSH and LH expressed as NIAMD-Rat FSH-RP-1 or NIAMDO-Rat LH-RP-1, ± standard error of the mean. Numbers in parenthesis represent number of animals used.
Figure 6

Effect of Reserpine (2.5 mg/kg) on Plasma and Pituitary Gonadotropins of Ovariectomized Rats

Female rats ovariectomized 3 weeks previously were injected with reserpine 2.5 mg/kg (i.p.) and sacrificed at the indicated times. Injections were timed so that all animals were sacrificed between 10:00 a.m. and 12 noon. $P =$ difference from control.
Table 3

Effect of Reserpine 5.0 mg/kg on Plasma and Pituitary Gonadotropins of Ovariectomized Rats

<table>
<thead>
<tr>
<th>Time</th>
<th>Plasma µg/ml</th>
<th>FSH Pituitary mg/gland</th>
<th>LH Plasma µg/ml</th>
<th>LH Pituitary mg/gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>.89±.16</td>
<td>.73±.10</td>
<td>2.06±.46</td>
<td>1.52±.14</td>
</tr>
<tr>
<td>1 hr.</td>
<td>.56±.04</td>
<td>.91±.19</td>
<td>1.39±.42</td>
<td>1.14±.15</td>
</tr>
<tr>
<td>2 hr.</td>
<td>.43±.08</td>
<td>1.33±.25</td>
<td>1.76±.29</td>
<td>1.78±.14</td>
</tr>
<tr>
<td>12 hr.</td>
<td>.29±.12</td>
<td>.89±.16</td>
<td>1.32±.28</td>
<td>1.27±.27</td>
</tr>
<tr>
<td>24 hr.</td>
<td>.86±.14</td>
<td>.98±.18</td>
<td>1.23±.29</td>
<td>1.71±.33</td>
</tr>
<tr>
<td>48 hr.</td>
<td>.65±.10</td>
<td>.74±.12</td>
<td>1.46±.30</td>
<td>1.58±.33</td>
</tr>
</tbody>
</table>

a different from control (Student t test) P < .05
b different from control (Student t test) P < .01

Female rats ovariectomized 2 weeks previously were injected with reserpine 5.0 mg/kg (i.p.) and sacrificed at the times indicated after injection. Injections were timed so that all animals were sacrificed between 10 a.m. and 12 noon. Values represent radioimmunoassasble FSH and LH expressed as NIAMD-Rat FSH-RP-1 or NIAMD-Rat LH-RP-1, ± standard error of the mean. Numbers in parenthesis represent number of animals used.
Figure 7

Effect of Reserpine (5.0 mg/kg) on Plasma and Pituitary Gonadotropins of Ovariectomized Rats

Female rats ovariectomized 2 weeks previously were injected with reserpine (5.0 mg/kg, i.p.) and sacrificed at the times indicated after injection. Injections were times so that all animals were sacrificed between 10:00 a.m. and 12 noon. P = difference from control.
and reached its maximal level 179% of control at 6 hours (significant at P<.05). By 12 hours plasma FSH had returned to control levels but rose again at 24 and 48 hours. Pituitary FSH was significantly decreased (P<.05) and pituitary LH was significantly increased (P<.05) corresponding to periods of maximal serum changes.

Following injection of 5.0 mg/kg reserpine, plasma LH reached its lowest level (60% of control levels) at 24 hours and had not returned to control levels at 48 hours. Plasma FSH fell to 56% of control levels at 1 hour and reached its lowest level 33% of control at 12 hours (significant at P<.01). By 24 hours plasma FSH had returned to control levels. Pituitary FSH was elevated significantly at 2 hours (P<.08) and did not return to normal until 48 hours.

Even though only 2 doses of reserpine were studied, the difference in effect on FSH and LH is striking. At the lower dose tested, 2.5 mg/kg of reserpine stimulated FSH release (rise in plasma levels and decrease in pituitary content) while it blocked LH release (fall in plasma levels and rise in pituitary content). Both plasma effects had returned to control levels by 12 hours but pituitary effects had not returned by 48 hours.

At the higher dose (5.0 mg/kg) plasma FSH fell reaching its maximum decrease at 12 hours and returned to control levels at 24 hours. Plasma LH had not returned to control levels at 48 hours. It thus appears that LH release is blocked at a lower dose and for a longer time than is FSH release.

Effect of estrone (200 μg/kg) and progesterone (2.0 mg/kg) on reserpine-induced changes in gonadotropin levels in ovariectomized rats:

The effect of reserpine (2.0 mg/kg, i.p.) on rats ovariectomized 4 weeks previously and simultaneously treated with estrone 200
Table 4
Effect of Estrogen and Progesterone on Reserpine Induced Changes in Gonadotropin Levels in Ovariectomized Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FSH Plasma µg/ml</th>
<th>FSH Pituitary mg/gland</th>
<th>LH Plasma µg/ml</th>
<th>LH Pituitary mg/gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (4)</td>
<td>1.80±.24</td>
<td>.93±.10</td>
<td>2.42±.16</td>
<td>2.49±.48</td>
</tr>
<tr>
<td>Reserpine (4)</td>
<td>1.70±.38</td>
<td>.56±.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.93±.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.17±.11</td>
</tr>
<tr>
<td>Estrone (4)</td>
<td>2.04±.33</td>
<td>.58±.19</td>
<td>1.83±.47</td>
<td>3.17±.22</td>
</tr>
<tr>
<td>Progesterone (4)</td>
<td>1.95±.70</td>
<td>.38±.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.14±.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.62±.36</td>
</tr>
<tr>
<td>Reserpine + Estrone (4)</td>
<td>1.21±.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.34±.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.52±.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.45±.50</td>
</tr>
<tr>
<td>Reserpine + Progesterone (4)</td>
<td>3.95±1.05&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>.47±.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.30±.07&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.92±.25&lt;sup&gt;bd&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> different from control (Student t test) P < .05  
<sup>b</sup> different from control (Student t test) P < .01  
<sup>c</sup> different from Reserpine alone (Student t test) P < .05  
<sup>d</sup> different from Reserpine alone (Student t test) P < .01

Female rats ovariectomized 4 wks previously were injected with reserpine 2.0 mg/kg (i.p.), Estrone in peanut oil 200 µg/kg (s.c.) or progesterone in peanut oil 2.0 mg/kg (s.c.) 20 hrs. before sacrifice, control animals received 0.6 ml peanut oil 20 hrs. before sacrifice.
Figure 8

Effect of Estrogen and Progesterone on Reserpine Induced Changes in Gonadotropin Levels in Ovariectomized Rats

Female rats ovariectomized 4 weeks previously were injected with reserpine (2.0 mg/kg, i.p.), Estrone in peanut oil 200 µg/kg (s.c.) or progesterone in peanut oil 2.0 mg/kg (s.c.) 20 hours before sacrifice, control animals received 0.6 ml peanut oil 20 hours before sacrifice. $P =$ difference from control.
PLASMA and PITUITARY levels of FSH and LH after treatment with Reserpine, Estrone, Progesterone, and Control.

- FSH (μg/mL): 
  - Control: <0.01 μg/mL
  - Reserpine: 1.0 ± 0.5 μg/mL
  - Estrone: 1.5 ± 0.1 μg/mL
  - Progesterone: 2.0 ± 0.2 μg/mL
  - Reserpine: 3.5 ± 1.0 μg/mL

- LH (μg/mL): 
  - Control: <0.01 μg/mL
  - Reserpine: 1.0 ± 0.5 μg/mL
  - Estrone: 1.5 ± 0.1 μg/mL
  - Progesterone: 2.0 ± 0.2 μg/mL
  - Reserpine: 3.5 ± 1.0 μg/mL
\( \mu g/kg \) or progesterone 2.0 \( \mu g/kg \) is shown in Table 4 and also in Figure 8. Animals were sacrificed by decapitation 20 hours after injection.

Reserpine treatment alone resulted in a significant \( (P < .01) \) fall in plasma LH and no change in plasma FSH but a fall in pituitary FSH \( (P < .05) \) at 20 hours.

Estrogen alone had only a slight effect on decreasing pituitary FSH and increasing pituitary LH content. Estrogen potentiated the effect of reserpine in decreasing plasma and pituitary FSH and in blocking LH release \( \text{decreased plasma LH, increased pituitary LH} \text{).} \)

Progesterone alone significantly decreased plasma LH \( (P < .05) \) and decreased pituitary FSH \( (P < .01) \). Progesterone significantly potentiated the reserpine induced blockade of LH release \( (P < .01) \) and the reserpine induced release of FSH \( (P < .05) \).

Both sex steroids thus acted similarly in potentiating the reserpine induced blockade of LH release but differently in their effect on FSH release. It appears that estrogen potentiates reserpine blockade of FSH synthesis \( \text{plasma and pituitary levels decreased} \) while progesterone may have reversed this blockade and in addition caused FSH release.

The elevated levels of plasma FSH over reserpine alone \( (P < .05) \) while pituitary content was not changed from reserpine alone, and the maintenance of elevated FSH levels 20 hours after treatment suggests that both synthesis and release are increased. However, an equally satisfactory explanation would be that FSH clearance from plasma was decreased in the presence of progesterone. Bogdanove and Gay \( (1969) \) found that estrogen and testosterone do not effect FSH or LH clearance but did not study the effect of progesterone on gonadotropin clearance.
Effect of 1-Dihydroxyphenylalanine and 1-threo-dihydroxyphenylserine on plasma and pituitary gonadotropins in reserpine treated ovariectomized rats:

The effect of the simultaneous administration of 1-dihydroxyphenylalanine (L-Dopa) 300 mg/kg, i.p. or 1-threo-dihydroxyphenylserine (DOPS) 200 mg/kg, i.p. on the reserpine 2.5 mg/kg, i.p. induced changes in plasma and pituitary gonadotropins at 2 and 24 hours are shown in Table 5, Figure 9. As noted previously (Table 2, Figure 6) reserpine (2.5 mg) appeared to increase FSH release and block LH release. Neither L-Dopa or DOPS administration altered the increase in plasma FSH at 2 or 24 hours following reserpine, L-Dopa significantly reduced (P .05) FSH synthesis at 2 hours compared to animals treated with reserpine alone. This effect is similar to that noted with estrogen (Table 4, Figure 8). Dihydroxyphenylserine, but not L-Dopa reversed the reserpine blockade of LH release as shown by significantly (P< .01) increased plasma LH levels at 2 hours. L-Dopa resulted in increased mean plasma LH at 24 hours which was not significant due to the wide range of values and in significantly increased pituitary LH levels at 2 hours (P< .01).

The observation that blockade of LH release by reserpine is reversed by DOPS and not by L-Dopa at 2 hours strongly suggests that norepinephrine and not dopamine is necessary for the release of LH, however, dopamine may play a role in LH synthesis as shown by the increased pituitary LH content following L-Dopa. No conclusions about the role of specific neurohormones in FSH release are possible from this study.

Effect of L-Dihydroxyphenylalanine on gonadotropin levels of normal, ovariectomized and short term estrogen treated ovariectomized female rats:

The effect of L-dihydroxyphenylalanine (L-Dopa) 300 mg/kg i.p. on gonadotropin levels in normal females, females ovariectomized
Table 5

Effect of L-Dihydroxyphenylalanine and Dihydroxyphenylserine on Plasma and Pituitary Gonadotropins in Reserpine Treated Ovariectomized Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma µg/ml</th>
<th>FSH Pituitary mg/gland</th>
<th>Plasma µg/ml</th>
<th>LH Pituitary mg/gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.06±.52</td>
<td>0.82±.20</td>
<td>1.16±.30</td>
<td>1.76±.49</td>
</tr>
<tr>
<td>2 Hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reserpine (5)</td>
<td>3.10±.61</td>
<td>0.74±.17</td>
<td>.43±.18</td>
<td>2.38±.78</td>
</tr>
<tr>
<td>Reserpine + L-Dopa (5)</td>
<td>3.04±.55</td>
<td>0.34±.06a</td>
<td>.62±.05</td>
<td>6.10±.55b</td>
</tr>
<tr>
<td>Reserpine + DOPS (5)</td>
<td>3.74±.94</td>
<td>0.68±.13</td>
<td>.92±.06b</td>
<td>3.58±.78</td>
</tr>
<tr>
<td>24 Hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reserpine (5)</td>
<td>2.64±.68</td>
<td>0.49±.10</td>
<td>.77±.15</td>
<td>3.48±.95</td>
</tr>
<tr>
<td>Reserpine + L-Dopa (5)</td>
<td>2.42±.57</td>
<td>0.31±.08</td>
<td>1.40±.48</td>
<td>4.42±.30</td>
</tr>
</tbody>
</table>

\[a\] different from reserpine treatment alone (Student t test) \(P < .05\)
\[b\] different from reserpine treatment alone (Student t test) \(P < .01\)

Females ovariectomized 3 weeks previously were injected with reserpine 2.5 mg/kg (i.p.). Either L-dihydroxyphenylalanine (L-Dopa) 300 mg/kg or 1-threo-dihydroxyphenylserine (DOPS)200 mg/kg (i.p.) or saline \(0.3 \text{ ml}\) were injected immediately following reserpine. Animals were sacrificed 2 or 24 hrs. later.
Figure 9

Effect of L-Dihydroxyphenylalanine and Dihydroxyphenylserine on Plasma and Pituitary Gonadotropins in Reserpine Treated Ovariectomized Rats

Females ovariectomized 3 weeks previously were injected with reserpine (2.5 mg/kg i.p.). Either L-dihydroxyphenylalanine (L-Dopa) 300 mg/kg or 1-threo-dihydroxyphenylserine (DOPS) 200 mg/kg, i.p. or saline 0.3 ml were injected immediately following reserpine. Animals were sacrificed 2 or 24 hours later. $P =$ difference from reserpine treatment alone.
3 weeks previously, and ovariectomized females treated with estradiol 17β (500 μg/kg, i.p.) one hour previously is shown in Table 6, Figure 10. Ovariectomized rats were sacrificed at 30, 60 minutes and 20 hours after L-Dopa. Normal rats were sacrificed at 60 minutes. Estrogen treated animals were sacrificed 30 minutes after L-Dopa, 90 minutes after estrogen injection.

The effects of L-Dopa on FSH were markedly different in the groups studied. L-Dopa treatment resulted in an increase in plasma FSH in normal females at 1 hour (P<.01) and a marked decrease in plasma FSH in ovariectomized females at 30 minutes, 60 minutes and 20 hours (P<.05). Changes in pituitary FSH content indicate that FSH release was blocked by L-Dopa in the ovariectomized animals.

The large increase (4 x) in plasma FSH at 1 hour in normal animals probably represents synthesis plus release. This finding in the normal animal is similar to that seen previously for the reserpine plus progesterone treated ovariectomized animal. L-Dopa administration 30 minutes before sacrifice and the estrogen pretreated animals resulted in changes similar to those seen in normal animals.

L-Dopa had only a minimal effect on LH release resulting in a slight but nonsignificant increase (maximal 61 %) in plasma LH in all 3 groups and a slight decrease in pituitary LH content, significant only in the ovariectomized group at 60 minutes (P<.05). These findings may be contrasted to those following L-Dopa treatment of reserpinized ovariectomized rats (Table 5, Figure 9). In the later group, L-Dopa caused pituitary LH content to increase but did not overcome reserpine blockade of LH release.

Effect of L-Dihydroxyphenylalanine on ovariectomized rats pre-treated with estradiol valerate or progesterone:

The effect of L-Dihydroxyphenylalanine (L-Dopa) 300 mg/kg
Table 6

Effect of L-Dihydroxyphenylalanine on Plasma and Pituitary Gonadotropins in the Normal Rat, Ovariectomized Rat and Ovariectomized Rat with Short Term Estrogen Pretreatment

<table>
<thead>
<tr>
<th>Treatment/Time</th>
<th>Plasma µg/ml</th>
<th>FSH Pituitary mg/gland</th>
<th>LH Plasma µg/ml</th>
<th>LH Pituitary mg/gland</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.85±.34</td>
<td>.34±.06</td>
<td>.42±.07</td>
<td>.89±.12</td>
</tr>
<tr>
<td>L-Dopa 60'</td>
<td>4.00±1.13a</td>
<td>.27±.10</td>
<td>.46±.02</td>
<td>.50±.10</td>
</tr>
<tr>
<td><strong>Ovariectomized Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.13±.41</td>
<td>.69±.17</td>
<td>.87±.07</td>
<td>2.88±.18</td>
</tr>
<tr>
<td>L-Dopa 30'</td>
<td>1.95±.31</td>
<td>1.09±.31</td>
<td>.63±.12</td>
<td>2.67±.28</td>
</tr>
<tr>
<td>L-Dopa 60'</td>
<td>1.06±.17a</td>
<td>.94±.20</td>
<td>1.22±.27</td>
<td>2.22±.21a</td>
</tr>
<tr>
<td>L-Dopa 20 hr.</td>
<td>.89±.26a</td>
<td>.88±.12</td>
<td>1.40±.29</td>
<td>2.40±.19</td>
</tr>
<tr>
<td><strong>Estrogen Treated Ovariectomized Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrogen 90 min.</td>
<td>1.22±.43</td>
<td>.84±.14</td>
<td>.56±.06</td>
<td>2.40±.14</td>
</tr>
<tr>
<td>Estrogen 90 min. + L-Dopa 30'</td>
<td>.85±.14</td>
<td>.56±.06</td>
<td>2.40±.14</td>
<td>2.40±.14</td>
</tr>
<tr>
<td>L-Dopa 30'</td>
<td>1.57±.20</td>
<td>.52±.14</td>
<td>.63±.08</td>
<td>2.55±.71</td>
</tr>
</tbody>
</table>

\*Different from control (Student t test) P < .05

Normal females or females ovariectomized 3 wks previously were injected with L-dihydroxyphenylalanine (L-Dopa)300 mg/kg (i.p.) at the indicated times prior to sacrifice. Controls were injected with saline, pH adjusted to 2.4 with HC1. Two groups were also injected with Estradiol 17β, 50 µg/kg in 0.5% EtOH (i.p.) Values represent radioimmunoassayable FSH or LH expressed as N1AMD-Rat FSH-RP-1 or N1AMD-Rat LH-RP-1, ± standard error of the mean.
Normal females or females ovariectomized 3 weeks previously were injected with L-dihydroxyphenylalanine (L-Dopa) 300 mg/kg, i.p. at the times indicated prior to sacrifice. Controls were injected with saline, pH adjusted to 2.4 with HCl. Two groups were also injected with Estradiol 17β, 50 µg/kg in 0.5% EtOH (i.p.).
i.p. 1 hour before sacrifice on animals ovariectomized 8 weeks previously and pretreated with estradiol valerate, s.c. 72 hours before or progesterone suspension 72 and 18 hours before L-Dopa is shown in Tables 7, 8 and Figure 11. L-Dopa caused significant increases in FSH synthesis (P<.01) (increased FSH content with no change or increase in plasma FSH) in control animals and progesterone or progesterone and estradiol valerate treated animals but not in animals pretreated with 5 or 50 µg of estrogen alone. Plasma FSH was significantly elevated in animals pretreated with progesterone (P<.01) or progesterone plus estradiol valerate (P<.05).

By contrast, L-Dopa resulted in significant (P<.01) increases in LH release only in animals pretreated with estrogen 5 µg or 50 µg. Only slight (15-25%) increase in plasma LH occurred in control animals and estrogen plus progesterone treated animals. No increase in plasma LH occurred in progesterone treated animals.

The effects of increasing brain catecholamine levels, by administration of the dopamine precursor, L-Dopa, on gonadotropin release thus appears to be modulated by the sex steroids. A differential effect is seen for the two sex steroids in the modulation. Prior treatment with estrogen 72 hours before results in increased LH release in response to L-Dopa administration which is not due to increased pituitary stores of LH. This can be contrasted to the failure of estrogen pretreatment 1 hour before to cause ovariectomized females to respond to L-Dopa with increased levels of plasma LH (Table 6, Figure 10). Prior treatment with progesterone resulted in increase in both synthesis and release of FSH. Either progesterone or estrogen alone resulted in increase of both pituitary and plasma FSH but not LH. The increase in FSH synthesis and release following L-Dopa was in addition to that occurring following administration of either sex steroid alone.
Table 7

Effect of L-Dihydroxyphenylalanine on Ovariectomized Rats
Pretreated 72 Hours Before with Estradiol Valerate or 72 and 18 Hours Before with Progesterone

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>Plasma µg/ml</th>
<th>FSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.60±.60</td>
<td>1.67±.17</td>
</tr>
<tr>
<td>Control + L-Dopa</td>
<td>1.53±.94</td>
<td>3.00±.27b</td>
</tr>
<tr>
<td>Estradiol Valerate 50 µg + Progesterone 25 mg</td>
<td>.97±.63</td>
<td>1.50±.12</td>
</tr>
<tr>
<td>Estradiol Valerate 50 µg + L-Dopa + Progesterone 25 mg</td>
<td>2.43±.18b</td>
<td>3.25±.32b</td>
</tr>
<tr>
<td>Estradiol Valerate 50 µg + L-Dopa</td>
<td>2.70±.30</td>
<td>2.47±.52</td>
</tr>
<tr>
<td>Estradiol Valerate 50 µg + Progesterone 25 mg</td>
<td>1.85±.35</td>
<td>2.25±.30</td>
</tr>
<tr>
<td>Estradiol Valerate 5 g + L-Dopa</td>
<td>1.28±.72</td>
<td>2.13±.86</td>
</tr>
<tr>
<td>Estradiol Valerate 5 g + Progesterone 25 mg</td>
<td>1.08±.22</td>
<td>1.86±.41</td>
</tr>
<tr>
<td>Progesterone 25 mg + L-Dopa</td>
<td>4.00±.04b</td>
<td>3.10±.37b</td>
</tr>
</tbody>
</table>

a Three animals used per treatment
b Different from treated group without L-Dopa (Student t Test) P < .01
Animals ovariectomized 8 weeks previously were pretreated with either estradiol valerate (s.c.) 72 hrs before or aqueous suspension of progestrone (s.c.) 72 and 15 hours before sacrifice. One hour before sacrifice 1-dihydroxyphenylalanine 300 mg/kg or saline 0.3 ml was injected (i.p.). Values represent mean radiolimnoassable FSH or LH expressed as NIAMD-Rat FSH-RP-1 or NIAMD-Rat LH-RP-1, ± standard deviation of the mean.
### Table 8

**Effect of L-Dihydroxyphenylalanine on Ovariectomized Rats**

Pretreated 72 Hours before with Estradiol Valerate or 72 and 18 Hours before with Progesterone

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma μg/ml</th>
<th>LH Pituitary mg/gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(3) 4.72±.25</td>
<td>2.10±.15</td>
</tr>
<tr>
<td>Control + L-Dopa</td>
<td>(3) 5.43±.67</td>
<td>1.85±.07</td>
</tr>
<tr>
<td>Estradiol Valerate 50μg + Progesterone 25 mg</td>
<td>(3) 3.45±.75</td>
<td>1.65±.06</td>
</tr>
<tr>
<td>Estradiol Valerate 50μg + L-Dopa</td>
<td>(3) 4.31±.09</td>
<td>1.81±.09</td>
</tr>
<tr>
<td>Estradiol Valerate 50μg</td>
<td>(3) 4.50±.20</td>
<td>1.80±.05</td>
</tr>
<tr>
<td>Estradiol Valerate 50μg + L-Dopa</td>
<td>(3) 8.20±.60b</td>
<td>1.78±.12</td>
</tr>
<tr>
<td>Estradiol Valerate 5 μg</td>
<td>(3) 4.55±.45</td>
<td>1.80±.15</td>
</tr>
<tr>
<td>Estradiol Valerate 5 μg + L-Dopa</td>
<td>(3) 8.40±.60b</td>
<td>1.72±.08</td>
</tr>
<tr>
<td>Progesterone 25 mg</td>
<td>(3) 5.15±.35</td>
<td>1.84±.19</td>
</tr>
<tr>
<td>Progesterone 25 mg + L-Dopa</td>
<td>(3) 5.47±.62</td>
<td>1.69±.14</td>
</tr>
</tbody>
</table>

*a* different from treated group without L-Dopa (Student t test)  P < .05

*b* different from treated group without L-Dopa (Student t test)  P < .01

Animals ovariectomized 8 weeks previously were pretreated with either estradiol valerate (s.c.) 72 hrs before or aqueous suspension of progesterone (s.c.) 72 and 15 hrs before sacrifice. One hour before sacrifice l-dihydroxyphenylalanine 300 mg/kg or saline 0.3 ml was injected (i.p.). Values represent mean radioimmunoassable FSH or LH expressed as NIAMD-Rat FSH-RP-1 or NIAMD-Rat-LH-RP-1, ± standard deviation of the mean.
Figure 11

Effect of L-Dihydroxyphenylalanine on Ovariectomized Rats
Pretreated 72 Hours Before with Estradiol Valerate or
72 and 18 Hours Before with Progesterone

Animals ovariectomised 8 weeks previously were pretreated
with either estradiol valerate (s. c.) 72 hours before or aqueous
suspension of progesterone (s. c.) 72 and 15 hours before sacrifice.
One hour before sacrifice L-dihydroxyphenylalanine 300 mg/kg or
saline 0.3 ml was injected (i. p.). $P =$ difference from treated
group without L-Dopa.
Effect of pargyline, parachlorophenylalanine and alpha-methyltyrosine on serum and pituitary gonadotropins in ovariectomized rats:

Female rats ovariectomized 4 weeks previously were injected either with MAO inhibitor pargyline 50 mg/kg, i.p. 24 hours before sacrifice with an inhibitor of serotonin synthesis, parachlorophenylalanine (PCPA) 500 mg/kg, i.p. 48 hours before sacrifice with an inhibitor of catecholamine synthesis, alphamethyltyrosine (α-MT) 300 mg/kg, i.p. 6 hours before sacrifice or with a combination of drugs. These results are reported in Table 9, Figure 12.

Pargyline alone caused an increase in FSH synthesis (significant increase in plasma (P<.05) and pituitary (P<.01) FSH, but no increase in plasma or pituitary LH.

Blockade of serotonin synthesis with PCPA had no significant effect on FSH or LH. Alpha methyltyrosine alone caused a significant increase in FSH synthesis (P<.05) but no release. Alphamethyltyrosine also produced a significant (P<.01) reduction of LH release.

Following PCPA administration, pargyline failed to cause an increase in plasma FSH but pituitary FSH content increased slightly indicating stimulation of FSH synthesis but not release in the presence of increased catecholamines. LH release was blocked by PCPA plus pargyline.

When α-MT was given to block CA synthesis following pargyline administration both FSH release and synthesis were significantly increased (P<.01). LH release was partially but not significantly blocked.

These studies taken alone indicate that both catecholamine and indolamines are involved in gonadotropin synthesis and release. It is
Table 9

Effect of Pargyline, Parachlorophenylalanine and alpha-Methyltyrosine on Plasma and Pituitary Gonadotropins in Ovariectomized Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma µg/ml</th>
<th>FSH Pituitary mg/gland</th>
<th>Plasma µg/ml</th>
<th>LH Pituitary mg/gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>(4) 5.72± .94</td>
<td>2.25± .27</td>
<td>1.99± .46</td>
<td>2.19± .29</td>
</tr>
<tr>
<td>Pargyline</td>
<td>(4) 10.60±2.02\textsuperscript{a}</td>
<td>5.77±1.02\textsuperscript{b}</td>
<td>2.09± .46</td>
<td>2.58±.56</td>
</tr>
<tr>
<td>PCPA</td>
<td>(4) 7.20± .72</td>
<td>2.20± .44</td>
<td>1.18± .26</td>
<td>2.32±.29</td>
</tr>
<tr>
<td>KT</td>
<td>(4) 6.15±1.13</td>
<td>4.61±1.10\textsuperscript{a}</td>
<td>0.75± .05\textsuperscript{b}</td>
<td>2.32±.19</td>
</tr>
<tr>
<td>Pargyline + PCPA</td>
<td>(4) 6.26±0.41</td>
<td>6.45± .94\textsuperscript{b}</td>
<td>0.75± .22\textsuperscript{a}</td>
<td>2.84±.40</td>
</tr>
<tr>
<td>Pargyline + KT</td>
<td>(4) 12.92±2.35\textsuperscript{b}</td>
<td>4.73± .53\textsuperscript{b}</td>
<td>1.02± .47</td>
<td>2.28±.20</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Different from control group (Student t test) P < .05
\textsuperscript{b} Different from control group (Student t test) P < .01

Females ovariectomized 6 wks previously were injected with Pargyline 50 mg/kg (i.p.) 24 hrs before sacrifice. Parachlorophenylalanine (PCPA) 500 mg/kg (i.p.) 48 hrs before sacrifice. Alpha-methyltyrosine (aMT) 300 mg/kg (i.p.) 6 hrs before sacrifice or a combination of drugs. Animals not injected with pargyline received saline 0.3 ml (i.p.) 24 hrs before sacrifice. Values represent radioimmunoassayable FSH or LH NIAMD-Rat-FSH-RP-1, NIAMD-Rat-LH-RP-1, ± standard error of the mean.
Figure 12

Effect of Pargyline, Parachlorophenylalanine and alpha-Methyltyrosine on Plasma and Pituitary Gonadotropins in Ovariectomized Rats

Females ovariectomized 6 weeks previously were injected with pargyline (50 mg/kg, i.p.) 24 hours before sacrifice. Alpha-methyltyrosine (300 mg/kg, i.p.) 6 hours before sacrifice. Parachlorophenylalanine (500 mg/kg, i.p.) 48 hours before sacrifice or a combination of drugs. Animals not injected with pargyline received saline 0.3 ml (i.p.) 24 hours before sacrifice. $P = \text{difference from controls.}$
difficult to determine the exact role of specific neurohormones since it is unlikely that the drugs given totally inhibit action of their respective enzymes. Further studies are necessary to understand the implication of these findings.

Intraventricular injection of carbachol, dopamine, epinephrine, and norepinephrine in unanesthetized normal males:

Normal 180-220 gm Sprague Dawley males received intraventricular injection of carbachol 50 µg, dopamine, epinephrine or norepinephrine either 5 µg or 0.5 µg in 30 µl Merles solution. Animals were sacrificed 60 minutes later (Table 10 and 11, Figure 13). Controls received 30 µl Merles solution.

Of the drugs injected into the CNS only dopamine increased FSH release as seen by a seven and one half times increase in plasma FSH at 5 µg and a 3 x increase at 0.5 µg both significant at $P < .01$. Both doses of dopamine decreased pituitary FSH content. Carbachol caused increased pituitary FSH content ($P < .05$) and increased plasma FSH. None of the drugs significantly elevated plasma LH levels.

Serotonin at the higher dose decreased LH synthesis ($P < .01$) and caused increased pituitary FSH content ($P < .01$) possibly due to inhibition of release.

An unexpected finding was that norepinephrine at both the 5 µg and 0.5 µg dose inhibited FSH synthesis as seen by a decrease in plasma FSH ($P < .01$ both doses) and a decrease in pituitary FSH content ($P < .05$ both doses). The failure of carbachol to evoke significant gonadotropin changes was also unexpected in view of the fact that intraventricular carbachol activates ACTH release (Hall and Marks, 1970).
Table 10

Intraventricular Injection of Carbachol, Dopamine, Norepinephrine, Epinephrine or Serotonin in Normal Male Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma µg/ml</th>
<th>FSH Pituitary mg/gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (3)</td>
<td>.46± .05</td>
<td>.13± .05</td>
</tr>
<tr>
<td>Carbachol 50 µg (3)</td>
<td>.58± .10</td>
<td>.29± .08</td>
</tr>
<tr>
<td>Dopamine 5 µg (3)</td>
<td>3.90±1.30b</td>
<td>.04± .01b</td>
</tr>
<tr>
<td>Dopamine 0.5 µg (3)</td>
<td>1.97±1.07b</td>
<td>.09± .05</td>
</tr>
<tr>
<td>Epinephrine 5 µg (3)</td>
<td>.52± .10</td>
<td>.07± .04</td>
</tr>
<tr>
<td>Epinephrine 0.5 µg (3)</td>
<td>.42± .04</td>
<td>.12± .02</td>
</tr>
<tr>
<td>Norepinephrine 5 µg (3)</td>
<td>.14± .02b</td>
<td>.04± .01b</td>
</tr>
<tr>
<td>Norepinephrine 0.5 µg(3)</td>
<td>.18± .01b</td>
<td>.08± .01b</td>
</tr>
<tr>
<td>Serotonin 5 µg (3)</td>
<td>.34± .15</td>
<td>.20± .03b</td>
</tr>
<tr>
<td>Serotonin 0.5 µg (3)</td>
<td>.51± .24</td>
<td>.10± .01</td>
</tr>
</tbody>
</table>

Different from control group (Student t test) P < .05
Different from control group (Student t test) P < .01

Unanesthetized normal males were injected in the lateral ventricle with Carbachol, Dopamine HC1, Epinephrine HC1 or Serotonin creatinine in the doses indicated, dissolved in 20 µl Marles solution. Control animals were injected with 20 µl Marles solution. Animals were sacrificed by decapitation 1 hour after injection. Values represent mean radioimmunoassayable FSH or LH expressed as NIAMD-Rat-FSH-RP-1 or NIAMD-Rat-LH-RP-1, ± standard error of the mean.
### Table 11

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma μg/ml (3)</th>
<th>LH Pituitary mg/gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>.58±.06</td>
<td>3.00±.54</td>
</tr>
<tr>
<td>Carbachol 50 μg</td>
<td>.59±.01</td>
<td>3.45±.40</td>
</tr>
<tr>
<td>Dopamine 5 μg</td>
<td>.44±.05</td>
<td>3.90±.15</td>
</tr>
<tr>
<td>Dopamine 0.5 μg</td>
<td>.52±.09</td>
<td>2.18±.37</td>
</tr>
<tr>
<td>Epinephrine 5 μg</td>
<td>.76±.13</td>
<td>2.56±.74</td>
</tr>
<tr>
<td>Epinephrine 0.5 μg</td>
<td>.57±.03</td>
<td>4.05±.40</td>
</tr>
<tr>
<td>Norepinephrine 5 μg</td>
<td>.43±.07</td>
<td>2.18±1.08</td>
</tr>
<tr>
<td>Norepinephrine 0.5 μg</td>
<td>.54±.02</td>
<td>2.70±.57</td>
</tr>
<tr>
<td>Serotonin 5 μg</td>
<td>.57±.13</td>
<td>1.38±.21</td>
</tr>
<tr>
<td>Serotonin 0.5 μg</td>
<td>.46±.06</td>
<td>2.45±.54</td>
</tr>
</tbody>
</table>

*different from control group (Student t test) P < .05

Unanesthetized normal males were injected in the lateral ventricle with Carbachol, Dopamine HCl, Epinephrine HCl, Norepinephrine HCl or Serotonin creatine in the doses indicated, dissolved in 20 μl Merles solution. Animals were sacrificed by decapitation 1 hr after injection. Values represent mean radioimmunoassable FSH or LH expressed as NIAMD-Rat RSH-RP-1 or NIAMD-Rat-LH-RP-1, ± standard error of the mean.
Figure 13

Intraventricular Injection of Carbachol, Dopamine, Norepinephrine, Epinephrine or Serotonin in Normal Male Rats

Unanesthetized normal males were injected in the lateral ventricle with carbachol, dopamine HCl, epinephrine HCl or serotonin creatinine in the doses indicated, dissolved in 20 µl Merles solution. Control animals were injected with 20 µl Merles solution. Animals were sacrificed by decapitation 1 hour after injection. \( P \) = difference from control.
Intraventricular injection of dopamine, norepinephrine and serotonin in untreated ovariectomized rats:

Ovariectomized female rats received (i. v.) injection of dopamine or norepinephrine (5 µg or 0.5 µg) or serotonin (10 µg or 1.0 µg) and were sacrificed 15 minutes later (Table 12, Figure 14).

Only Dopamine evoked FSH release. This effect was accompanied by increased pituitary FSH content indicating the FSH synthesis had occurred. Differences however, were not significant. Norepinephrine and serotonin both blocked FSH release at either dose, as shown by decreased plasma and increased pituitary FSH content. No significant changes in plasma or pituitary LH occurred with any drug.

This study can be contrasted with the previous one in which drugs were injected into the lateral ventricle of normal males (Table 7, Figure 13). In the normal male, dopamine stimulates release but not synthesis of FSH while norepinephrine blocks synthesis. In the present study of ovariectomized females, dopamine stimulated synthesis and release of FSH while norepinephrine blocked release but not synthesis. LH synthesis and release were not effected in either normal males or ovariectomized females.

Effect of intraventricular injection of dopamine, norepinephrine or serotonin on unanesthetized ovariectomized females pretreated with reserpine (5 mg/kg):

Female rats ovariectomized 21 days previously and were given reserpine (5 mg/kg , i. p.) 2 hours prior to intraventricular injection. Animals were sacrificed by decapitation 15 minutes after injection (Table 13, Figure 15).

Plasma LH increased with all the drugs given unlike normal male or ovariectomized female. Serotonin caused depression of
Table 12

Intraventricular Injection of Dopamine, Norepinephrine and Serotonin in Untreated Ovariectomized Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma µg/ml</th>
<th>FSH</th>
<th>Pituitary mg/gland</th>
<th>Plasma µg/ml</th>
<th>LH</th>
<th>Pituitary mg/gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (5)</td>
<td>0.56±0.12</td>
<td>0.44±0.13</td>
<td>0.72±0.15</td>
<td>0.64±0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine 5 µg (4)</td>
<td>0.91±0.30</td>
<td>0.88±0.16</td>
<td>0.67±0.05</td>
<td>1.08±0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine 0.5 µg (4)</td>
<td>0.62±0.06</td>
<td>1.00±0.20^a</td>
<td>0.86±0.11</td>
<td>0.50±0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine 5 µg(4)</td>
<td>0.27±0.04</td>
<td>3.84±1.44^b</td>
<td>0.83±0.09</td>
<td>0.65±0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine 0.5 µg (4)</td>
<td>*</td>
<td>1.60±0.14^b</td>
<td>0.62±0.12</td>
<td>0.29±0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serotonin 10 µg (4)</td>
<td>0.13±0.03^b</td>
<td>0.88±0.08^a</td>
<td>0.99±0.12</td>
<td>1.02±0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serotonin 1.0 µg (4)</td>
<td>0.17±0.04^b</td>
<td>1.12±0.07^b</td>
<td>0.62±0.06</td>
<td>1.17±0.37</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a different from control (Student t test) P < .05
b different from control (Student t test) P < .01
* below detectable limits of assay

Female rats ovariectomized 14 days previously received i.v. injections of the indicated drugs dissolved in 15 µl Merles solution. Control animals received 15 µl Merles solution only. Animals were sacrificed by decapitation 15 minutes later.
Figure 14

Intraventricular Injection of Dopamine, Norepinephrine and Serotonin in Untreated Ovariectomized Rats

Female rats ovariectomized 14 days previously received i.v. injections of the indicated drugs dissolved in 15 µl Merles solution. Control animals received 15 µl Merles solution only. Animals were sacrificed by decapitation 15 minutes later. P = difference from control.
FSH µg/ml PLASMA

- Plasma
- Pituitary

<table>
<thead>
<tr>
<th>Condition</th>
<th>5 µg</th>
<th>0.5 µg</th>
<th>5 µg</th>
<th>0.5 µg</th>
<th>10 µg</th>
<th>1 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serotonin</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LH µg/ml PLASMA

- Plasma
- Pituitary

<table>
<thead>
<tr>
<th>Condition</th>
<th>5 µg</th>
<th>0.5 µg</th>
<th>5 µg</th>
<th>0.5 µg</th>
<th>10 µg</th>
<th>1 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serotonin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 13

Intraventricular Injection of Dopamine, Norepinephrine and Serotonin in Reserpine Treated Ovariectomized Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma µg/ml</th>
<th>FSH Pituitary mg/gland</th>
<th>Plasma µg/ml</th>
<th>LH Pituitary mg/gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(4) 1.92±.47</td>
<td>0.51±.09</td>
<td>.18±.02</td>
<td>3.10±.53</td>
</tr>
<tr>
<td>Dopamine 5 µg</td>
<td>(4) 10.67±5.40</td>
<td>1.59±.28b</td>
<td>.34±.15</td>
<td>2.04±.22b</td>
</tr>
<tr>
<td>Dopamine 0.5 µg</td>
<td>(4) 1.23±.54</td>
<td>1.23±.27a</td>
<td>.31±.07</td>
<td>1.65±.21b</td>
</tr>
<tr>
<td>Norepinephrine 5 µg</td>
<td>(3) 0.40±.02b</td>
<td>1.03±.31a</td>
<td>.31±.08</td>
<td>2.94±.34</td>
</tr>
<tr>
<td>Norepinephrine 0.5 µg</td>
<td>(3) 0.34±.09b</td>
<td>0.77±.43</td>
<td>.20±.02</td>
<td>1.94±.23a</td>
</tr>
<tr>
<td>Serotonin 5 µg</td>
<td>(3) 4.37±.92b</td>
<td>0.68±.12</td>
<td>.33±.06a</td>
<td>1.53±.24a</td>
</tr>
<tr>
<td>Serotonin 0.5 µg</td>
<td>(4) 3.47±1.45</td>
<td>3.49±.73</td>
<td>.33±.13</td>
<td>2.21±1.20</td>
</tr>
</tbody>
</table>

*different from control group (Student t test) P < .05
*b different from control group (Student t test) P < .01

Female rats ovariectomized 3 wks previously were injected with reserpine 5 mg/kg (i.p.) 2 hrs before sacrifice. Dopamine HCl, norepinephrine HCl or serotonin creatine is dissolved in 15 ul Merles solution in the doses were injected into the right lateral ventricle of the unanesthetized animal 15 min before sacrifice by decapitation.
Figure 15

Intraventricular Injection of Dopamine, Norepinephrine and Serotonin in Reserpine Treated Ovariectomized Rats

Female rats ovariectomized 3 weeks previously were injected with reserpine (5 mg/kg, i.p.) 2 hours before sacrifice. Dopamine HCl, norepinephrine HCl or serotonin creatine is dissolved in 15 μl Merles solution in the doses indicated, injected into the right lateral ventricle of the unanesthetized animal 15 minutes before sacrifice by decapitation. P = difference from control.
pituitary LH coincidently with increased plasma LH. Pituitary LH increased most with norepinephrine (5 µg).

Dopamine again stimulated FSH synthesis and release at the higher dose. Norepinephrine blocked FSH release (P<.01). Serotonin appeared to stimulate FSH synthesis (increased plasma levels with unchanged or increased pituitary content). Except for serotonin these effects are similar to those seen for ovariectomized females (Table 12, Figure 14) rather than to those seen for normal males (Tables 10, 11 and Figure 13).

Unlike normal males and ovariectomized females, where these drugs had no effect on LH, dopamine at both doses and serotonin at the higher dose appeared to stimulate LH released as seen by significant (P<.01) decrease in pituitary content and an increase in plasma LH which was not significant. Despite the observation in an earlier study that DOPS reversed reserpine blockade of LH release in ovariectomized rats (Table 5, Figure 9), no significant increase in plasma LH levels occurred following intraventricular injection of NE. This may have been due to the small number of animals used in this experiment.

Effect of intraventricular injection of atropine and hexamethonium in untreated and reserpinized ovariectomized females:

Atropine sulfate (100 µg) or hexamethonium (100 µg) dissolved in 10 µl Merles solution was injected into the lateral ventricle of unanesthetized females, ovariectomized 11 days previously. Animals were sacrificed 15 minutes later by decapitation. Control animals received 10 µl Merles solution only. Half of these animals were pretreated with reserpine (5 mg/kg, i.p.) 1 hour before intraventricular injection (Table 14, Figure 16).
### Table 14

**Intraventricular Injection of Atropine and Hexamethonium in Untreated and Reserpine Treated Ovariectomized Rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FSH Plasma µg/ml</th>
<th>FSH Pituitary mg/gland</th>
<th>LH Plasma µg/ml</th>
<th>LH Pituitary mg/gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>.68±.14</td>
<td>.38±.04</td>
<td>.69±.10</td>
<td>.90±.08</td>
</tr>
<tr>
<td>Atropine</td>
<td>.11±.07</td>
<td>1.42±.75</td>
<td>.26±.14</td>
<td>.89±.04</td>
</tr>
<tr>
<td>Hexamethonium</td>
<td>.95±.09</td>
<td>.15±.07</td>
<td>.48±.19</td>
<td>.98±.03</td>
</tr>
<tr>
<td>Reserpine</td>
<td>.22±.05</td>
<td>.20±.14</td>
<td>1.95±.08</td>
<td>.84±.04</td>
</tr>
<tr>
<td>Reserpine + Atropine</td>
<td>.06±.01</td>
<td>.83±.52</td>
<td>.60±.15</td>
<td>.73±.06</td>
</tr>
<tr>
<td>Reserpine + Hexamethonium</td>
<td>2.53±.36</td>
<td>.23±.18</td>
<td>.33±.10</td>
<td>.41±.07</td>
</tr>
</tbody>
</table>

*different from control (Student t test) P < .05
b*different from control (Student t test) P < .01
c*different from reserpine alone (Student t test) P < .05
d*different from reserpine alone (Student t test) P < .01

Female rats ovariectomized 11 days previously were injected with reserpine 5 mg/kg (i.p.) or saline 30 ml 1 hr later. Unanesthetized animals were injected in the lateral ventricle with atropine 100 µg or hexamethonium 100 µg in 10 µl Merles solution. Controls received 10 µl Merles solution. Animals were sacrificed 15 min after intraventricular injection by decapitation. Values represent mean radioimmunoassayable FSH or LH expressed as NIAMD-Rat FSH-RP-1 or NIAMD-Rat LH-RP-1, ± standard error of the mean.
Figure 16

Intraventricular Injection of Atropine and Hexamethonium in Untreated and Reserpine Treated Ovariectomized Rats

Female rats ovariectomized 11 days previously were injected with reserpine (5 mg/kg, i.p.) or saline 30 ml one hour later. Unanesthetized animals were injected in the lateral ventricle with atropine 100 μg or hexamethonium 100 μg in 10 μl Merles solution. Controls received 10 μl Merles solution. Animals were sacrificed 15 minutes after intraventricular injection by decapitation. P = difference from control.
This study was performed to determine if the effects of reserpine on FSH and LH release might be due to stimulation of a cholinergic pathway. Unlike previous studies reserpine stimulated rather than blocked LH release.

Atropine alone caused significant decrease of plasma FSH ($P < .01$) and LH ($P < .05$). Atropine appeared to block FSH release as seen by a rise in mean pituitary FSH content. There was no increase in pituitary LH content following atropine.

In reserpine pretreated animals atropine injection resulted in a further decrease in plasma FSH levels which was significantly different ($P < .01$) than the effect of reserpine alone. It would appear from this study that reserpine blocked FSH synthesis while atropine blocked FSH release. The increase of plasma LH in reserpine treated animals was completely reversed by atropine ($P < .05$).

Hexamethionium alone elevated plasma FSH and significantly ($P < .05$) decreased pituitary FSH content. Hexamethionium caused a slight but significant block of LH release.

In reserpine pretreated animals hexamethionium injection resulted in a marked increase in plasma FSH, significantly ($P < .01$) different from either control or reserpine treated animals. Hexamethionium markedly inhibited LH synthesis in reserpine treated animals seen as significant ($P < .01$) decreased plasma LH and pituitary LH content compared to either control or reserpine treated animals.

This study clearly shows the importance of the cholinergic system in modulation of gonadotropin secretions. Atropine blockade of PMS induced ovulation (Quinn and Zarrow, 1964) and cyclic ovulation (Sawyer, 1969) is well known. As the present study shows, atropine acts at least in part by blocking release rather than synthesis of FSH and LH.
An effect of ganglionic blocking agents has not previously been demonstrated due to the difficulty of introducing ganglionic blocking agents into the CNS. In the present study, hexamethonium injection resulted in FSH release. The most impressive effects of hexamethonium were seen in the reserpine treated animals where injection was followed by a 3 x increase in FSH release and a 50% reduction of LH synthesis. The dual cholinergic mechanism in modulation of gonadotropin secretion demonstrated here further illustrate the complex interaction of the neuroendocrine system with ovulation mechanisms.

This dual cholinergic effect perhaps also explains the lack of activity of carbachol introduced intraventricularly (Table 10, 11 and Figure 13). Carbachol activates both muscarinic and nicotinic receptors. Since these receptor mechanisms produce different (in some cases opposing) responses, the administration of carbachol was not effective. This suggests that carbachol administration should be performed in atropine blocked and hexamethonium blocked rats in order to demonstrate its cholinergic effects.
DISCUSSION

Role of specific biogenic amines in modulation of gonadotropin secretion:

Evidence for a modulating effect of the biogenic amines on gonadotropin release or ovulation can come from two sources: (1) experiments demonstrating the release of a specific tropic hormone following administration of a biogenic amine in vitro or in vivo and (2) experiments showing an effect on gonadotropin release or ovulation following depletion or blockade of specific biogenic amines. Although some of the evidence reviewed and presented here is strongly suggestive of such an action, neither line of evidence provides absolute proof of a role for a specific neurotransmitter in gonadotropin release or ovulation control. The effect of direct administration of a proposed neurotransmitter in vivo or in vitro are open to the criticism that they may cause a generalized CNS effect (Sawyer, 1969), or that they escape into the systemic circulation and cause peripheral effects. Similarly, following depletion or blockade of transmitters, the experiments are subject to the question of nonspecific effects.

The question whether there is a unique neurotransmitter reserved for gonadotropin release will be answered only when all other functions are excluded for the neurotransmitter in question. The small number of known neurotransmitters and the large number of neuroendocrine functions makes such a unique function for one of them unlikely. More likely it will be found that the relatively greater participation of one neurotransmitter in modulation of gonadotropin release is due to a predominance of that compound in the neural
pathways in which integration of the factors controlling gonadotropin release takes place. Unique neurohormones do occur; these are the hypothalamic releasing factors whose precise numbers, composition and mechanism of action is still much in doubt. The role of biogenic amines in modulation of gonadotropin release must then be that of neurotransmitters, in a still but vaguely defined series of synapses, the last of which may directly stimulate the secretion of hypothalamic substances (LRF and FRF) into the portal system supplying the anterior pituitary. Such a system may not only involve stimulatory but also inhibitory neuronal input.

In the present investigation evidence was found linking each of the neurotransmitters studied to gonadotropin secretion in some manner. Dopamine was wound by Kamberi, Mical and Porter (1970a, 1970b) and Schneider and McCann (1970a) to release FSH and LH when injected into the third ventricle or added to SME slices plus pituitary in vitro, was effective primarily in releasing FSH in the present study. The failure of dopamine to release LH in males or castrate females may be comparable to the failure of dopamine to release LH on diestrous day 1, observed by Schneider and McCann (1970a). The experiments performed by Kamberi, Mical and Porter (1970a, 1970b) in which LH was consistently released were done on steroid primed ovariectomised rats. LH release in response to dopamine was shown (Schneider, McCann, 1970a) to be blocked by phenoxybenzamine (alpha-adrenergic blocker) but not by pronethalol (beta-adrenergic blocker).

Norepinephrine which was found by the above authors to be much less effect than dopamine in releasing LH appeared to exert a greater effect on LH release then dopamine in some of our studies. In marked contrast, norepinephrine inhibited FSH synthesis or release. Thus following reserpine treatment of ovariectomised
females systemic administration of phenylserine, a precursor of norepinephrine, restored plasma and pituitary LH to control levels while L-Dopa, a precursor of both dopamine and norepinephrine, caused increased synthesis but not release of LH. In each of the three experiments with intraventricular injection of biogenic amines, norepinephrine blocked either synthesis or release of FSH, but caused LH release only in reserpine pretreated animals and not in normal males or ovariectomized females.

Serotonin has been suggested as a specific blocker of gonadotropin release by Kordon et al. (1969) who found that blockade of ovulation in the PMS stimulated rat with a MAO inhibitor did not occur if animals were pretreated with PCPA, an inhibitor of serotonin synthesis, but did occur if catecholamine synthesis was inhibited. In the present investigation (Kordon et al., 1969) when the experiment was repeated in ovariectomized adult animals results indicated that under some conditions, serotonin stimulated rather than inhibited FSH release. Administration of the monoamine oxidase inhibitor, pargyline, caused a 2-fold increase in serum and pituitary FSH content without affect LH levels. Pretreatment with PCPA to inhibit serotonin synthesis completely blocked the increase in plasma FSH due to MAO inhibition and further increased FSH content of the pituitary. Pretreatment with α-MT to block catecholamine synthesis had no effect on the increase in plasma FSH due to MAO inhibition. Thus serotonin appears to facilitate rather than to block FSH release.

Previous reports of the intraventricular injections of serotonin showed that it was either without effect (Kamberi, Mical and Porter, 1970a, 1970b) or inhibited LH release (Schneider and McCann, 1970a) in the estrogen primed castrate female. In the present studies, intraventricular injections of serotonin were without effect on FSH or LH in normal males or castrate females but stimulated both FSH
and LH release in the reserpine pretreated ovariectomized female confirming the results found following systemic administration of MAO inhibitor.

The differences between our findings and those of Kordon et al. (1969) may be explained by the presence of sex steroid stimulation in his animals after PMS injection. As was the case with the catecholamines, the action of serotonin on the CNS on gonadotropin secretion depends to a large extent on the neuroendocrine state at the time of administration as determined by the levels of sex steroids and possibly other neurohormones.

To recapitulate, dopamine causes release of LH only in the estrogen primed rat (Schneider and McCann, 1970a), but caused FSH release and synthesis in both normal, castrate and reserpinized animals (present study). Norepinephrine caused LH release only in reserpine pretreated ovariectomized animals (present study) and not in normally cycling (Schneider and McCann, 1970a) or estrogen plus progesterone primed ovariectomized females (Kamberi, Mical and Porter, 1970a). Norepinephrine blocked FSH synthesis or release in normal males, or reserpine treated ovariectomized females but not in ovariectomized females (present study). Serotonin blocked ovulation in PMS stimulated immature rats (Kordon et al., 1969) ovariectomized females and normal males but stimulated FSH release in reserpine treated ovariectomized females (present study). This may or may not imply that the availability of receptor sites for a specific neurotransmitter is dependent on sex steroids or other neurohormones.

The second type of evidence for a role of biogenic amines in regulation of ovulation is that seen with reserpine blockade of FSH and LH release. The effect of reserpine on blocking ovulation has been widely reported as noted in the present review. As little
reserpine as 0.4 mg/kg blocks ovulation in the PMS primed immature rat (Coppola et al., 1966). This blockade is overcome by MAO inhibitors indicating that its effect is mediated through biogenic amines, or indolalkylamines, and by human chorionic gonadotropin 1-4 iu indicating that LH release is blocked. Specific depleters of brain catecholamines such as syrosingopine and tetrabenazine also block ovulation in these animals while PCPA, which blocks serotonin synthesis, is without effect. Reserpine (5 mg/kg) also blocks ovulation in mature cycling rats when given prior to 2:00 p.m. the day of proestrus (Meyerson and Sawyer, 1968). In the human, a number of phenothiazide agents are known to not only block ovulation but also to cause lactation (Hooper et al., 1961).

In the present investigation when two doses of reserpine (2.5 mg/kg and 5.0 mg/kg) were administered to groups of ovariectomized rats a differential effect on FSH and LH release was observed. At the lower dose FSH release was stimulated while LH release was blocked. At the higher dose, both FSH and LH release were blocked equally but plasma and pituitary FSH content had returned to control levels at 12 hours while LH levels were still affected at 48 hours.

In a third study, reserpine (2 mg/kg) was injected simultaneously with estrogen or progesterone and animals sacrificed 20 hours later. In these animals reserpine alone blocked LH release and caused reduction in FSH synthesis. Estrogen potentiated both these effects of reserpine slightly, suggesting that estrogen blockade and catecholamine depletion act at the same site. Progesterone significantly potentiated reserpine blockade of LH release but reversed blockade of FSH synthesis and release. This suggests again a specific effect of progesterone on FSH and further emphasises the difference in the sex steroid mechanisms for modulation of FSH and LH secretion.
These effects of reserpine on gonadotropin release in the rat can be contrasted with its effect on ACTH release. Bhattacharya and Marks (1969) have reported that reserpine as well as chlorpromazine cause ACTH release in the rat and potentiate the release caused by intraventricular carbachol administration (Hall and Marks, 1970). Intraventricular injection of biogenic amines blocks ACTH release in the rat (Hall and Marks, 1970).

Studies of reserpine effects in the CNS are complicated by the fact that more than one neurotransmitter is being affected. Thus, depletion of neuronal stores of DA, NE and 5-HT all may occur following reserpine administration. It is apparent that drugs with more specific activity need to be studied. Particularly interesting drugs to study would be alpha methyl dopa alpha methyl norepinephrine and alpha adrenergic blocking agents.

The role of a nicotinic receptor in modulation of gonadotropin release has not been previously reported to the best of our knowledge. The ability of atropine and other antimuscarinic agents to block ovulation is well known and led Saywer, Markee and Townsend (1949a) to postulate a cholinergic-adrenergic sequence in post-coital ovulation in the rabbit.

Atropine blocks ovulation following electrical stimulation of basal tuberal region of the rabbit brain (Saul and Saywer, 1957) but not following electrical stimulation of the medial preoptic region (Everett, 1961). Atropine blocks spontaneous ovulation in the cow (Hausel and Trimberger, 1951) and ovulation produced by progesterone (Hough et al., 1955). In the rat estrogen induced LH release is blocked by atropine (Presl, 1961). Quinn and Zarrow (1961) showed that following PMS stimulation of immature rats cholinergic blockade with atropine was most effective over a 30 minute period.
8 hours prior to ovulation while adrenergic blockade was effective up to 4 hours preceding ovulation.

In the present study atropine blocked both FSH and LH release in the ovariectomized female. In the reserpine treated ovariectomized female only FSH release was blocked. Since reserpine alone blocked LH release the latter finding suggests that atropine and biogenic amine depletions act on similar mechanisms in the pathway to LH release.

The previously noted initial period of action of atropine 8 hours preceeding ovulation and the finding in the present study that atropine acts mainly to block FSH release may be explained if in fact FSH release is necessary for LH release to occur. One mechanism whereby FSH may act to facilitate LH release is through stimulation of steroidogenesis in the ovary. Increased production of both estrogen 8-24 hours prior to LH release (Miyake, 1969; Miyake et al., 1969) and progesterone or 20 OH progesterone immediately preceeding or coincidental with LH release have been suggested as being a necessary prerequisite for LH release (Hillard et al., 1967).

The role of nicotinic receptor mechanisms in modulation of ovulation was most apparent in the reserpine-blocked ovariectomized animals. Here blockade with hexamethonium resulted in a three and one half-fold increase in FSH release. Although the exact nature of the cholinergic modulation of gonadotropin release is still unknown it is apparent that both muscarinic and nicotinic receptors are involved. It could perhaps be further stated that the muscarinic receptor serves primarily to promote FSH rather than LH release and that the nicotinic receptor serves primarily to inhibit FSH release but also possibly to stimulate LH synthesis. Many further experiments will be required to define these cholinergic mechanisms.
In one preliminary study which was performed, nicotine (5 mg/kg, i.p.) failed to effect LH release and caused a nonsignificant decrease in plasma FSH and a significant \( P < .01 \) increase in pituitary FSH. Systemic effects of nicotine which were not monitored could have been responsible for this effect rather than CNS action.

Interaction of biogenic amines and sex steroids in modulation of gonadotropin secretion:

Despite a great deal of effort the site and mechanism of feedback of sex steroids in the regulation of gonadotropin secretion is not clearly defined. To be sure anatomical sites of estrogen localization have been identified in the hypothalamus and pituitary (Eisenfeld and Axelrod, 1967). Whether estrogen is acting on cells responsible for synthesizing gonadotropins in the pituitary, releasing factors in the hypothalamus or even melatonin in the pineal is not known. Likewise, whether estrogen is directly involved with the synthesis, release, or receptor activation of neurotransmitter at stimulatory and inhibitory synapses is unknown. The general action of estrogen to stimulate protein synthesis in sensitive cells (Segal and Scher, 1967; Hamilton, 1964) and the fact that several known actions of estrogen on gonadotropin (Schneider and McCann, 1970b, 1970c) or LRF release can be blocked by prior treatment with inhibitors of protein synthesis suggest that estrogen action in the CNS may not be drastically different from its action in the uterine muscle or other sex steroid sensitive tissues. Where and what then is the site of interaction between neurotransmitters and sex steroids in the modulation of gonadotropin release? Several possibilities can be proposed: (1) Sex steroids may stimulate synthesis of gonadotropins in the pituitary (Piascek and Meites, 1966) just as estrogen apparently stimulates synthesis of myosin and actinomyosin in the
myometrium (Csapo, 1950), (2) Sex steroids may effect ionic transport across the cell membrane thereby sensitizing or blocking cells which release gonadotropin or hypothalamic releasing factors. This occurs in the case of uterine muscle contractility, where progesterone hyperpolarized and estrogen depolarized the myometrial cell membrane (Kumar, 1967), (3) Sex steroids may compete with catecholamines and indolalkylamines at cell receptors or in the re-uptake from the synaptic cleft (Janowsky and Davis, 1970), (4) Sex steroids may act on some intracellular function directly or indirectly activated by neurotransmitters.

Presumptive evidence for an interaction of sex steroids and biogenic amines can be found in the relationship between steroid levels and biogenic amine content of the CNS or between biogenic amine content of the CNS and gonadotropin secretion. To summarize these lines of evidence: the catecholamine content of the hypothalamus as determined by fluorescence changes only slightly during the ovulatory cycle of the rat and is increased in pseudopregnancy or pregnancy but not in response to ovariectomy, estrogen or progesterone treatment (Fuxe et al., 1967). Total catecholamine content of the anterior and middle hypothalamus measured fluorometrically was found to be minimal at estrous, rising during diestrous, and highest on both the morning and evening of proestrus (Stefano and Donoso, 1967). However, other studies in which norepinephrine was measured specifically have failed to detect changes in hypothalamic content during the estrous cycle (Sandler, 1968). MAO activity in both the hypothalamus and pituitary have been found to be higher on the morning of proestrus than during the evening (Kamberi and Kobayashi, 1970). Plasma concentrations of norepinephrine have been found to be high during diestrous and low during estrous while plasma epinephrine shows the reverse pattern (Green
and Miller, 1966). FSH but not LH has been found to increase the rate of \(^3\)H-norepinephrine turnover in the midbrain (Anton-Tay et al., 1969). The most direct evidence to date of a role for biogenic amines in gonadotropin secretion is that of intraventricular injection mentioned previously. Significantly the action of dopamine and LH release is dependent on the stage of the cycle, being highest during proestrus, much less during estrus and insignificant on diestrus 1 (Schneider and McCann, 1970b).

In the pineal hydroxyindol-o-methyl transferase measured at 9:00 a.m. each day varies with the cycle being highest on diestrus and lowest on proestrus and estrus (Wurtman et al., 1965). More direct evidence for the role of pineal neurohormone is the finding that implantation of melatonin or pineal fragments into the median eminence caused a depletion of both pituitary and plasma LH (Fraschini et al., 1968).

In order to avoid the criticism of nonspecific CNS effect (Sawyer, 1969), and with the end in view of eventual clinical application of our findings we chose to study more closely the effects of a systemically administered catecholamine precursor 1-dihydroxyphenylalanine (L-Dopa) rather than perform further intraventricular injections. Our results must be tempered by the report published after these studies were completed that, in the mouse, intraperitoneally administered L-Dopa at high doses increased the rate of serotonin turnover and depletes brain serotonin levels (Everett and Borcherding, 1970). In this same study it was found that maximal brain dopamine levels occurred 30 minutes after i.p. injection of L-Dopa and that brain norepinephrine content was not significantly increased at this time with any of the doses tested.

In the first experiment L-Dopa and DOPS were injected simultaneously with reserpine (2.5 mg) in ovariectomized rats and all
animals were sacrificed 2 hours later. Only DOPS released LH in reserpine treated animals. In the next experiment L-Dopa (300 mg/kg) was injected in normal, ovariectomized and ovariectomized females treated with estrone (500 mg/kg) 1 hour before. In normal diestrous females L-Dopa released only FSH. In untreated ovariectomized animals L-Dopa significantly blocked FSH release at 60 minutes and 20 hours. This effect was blocked by prior treatment with estrone. LH release rose slightly but not significantly in ovariectomized animals following L-Dopa.

In the third experiment L-Dopa (300 mg/kg) was injected in animals which had been pretreated with either sustained release estrogen 72 hours before or with progesterone suspension 72 and 18 hours before. A marked difference in the effect of L-Dopa was noted in the estrogen as compared with progesterone pretreated groups. In estrogen pretreated animals L-Dopa caused a significant (P< .01) two-fold increase in serum LH following doses of both 5 μg and 50 μg estradiol valerate/rat. No increase in serum FSH occurred and a notable but not significant decrease in plasma FSH was seen following L-Dopa with the larger dose of estrogen.

By contrast, significant (P< .01) increases in both plasma and pituitary FSH occurred following L-Dopa injection in animals pretreated with progesterone or progesterone plus estradiol. Since the ratio of progesterone to estrogen was 500/1 the later group can be considered to be essentially progesterone treated.

A final piece of evidence for the interaction of estrogen and catecholamines in modulating gonadotropin secretion is the observation by Schneider and McCan (1970b) that estrogen blocked DA induced release of LH following intraventricular injection and following addition of DA to hypothalamic slices in vitro (1970c).
The site of interaction between sex steroids and biogenic amines has not been defined. Perhaps several sites exist. In addition to a direct interaction in hypothalamic tissue suggested above (Schneider and McCann, 1970b, 1970c) estrogen has been found to inhibit norepinephrine stimulation of adenylcyclase activity in the pineal (Weiss and Crayton, 1970).

The mechanisms whereby sex steroids and biogenic amines interact is likewise unknown. One possibility recently reported by Janowsky and Davis (1970) is that estradiol and progesterone inhibit uptake of norepinephrine into synaptosomes. The effective dose estradiol $10^{-5}$ M and progesterone $10^{-4}$ M were so large however as to preclude this as a physiological effect. A more likely action is one already suggested by several studies. The receptor concerned with DA stimulation of gonadotropin release appears to be alpha, blocked by phenoxybenzamine (Schneider and McCann, 1970). The action of biogenic amine on gonadotropin release is therefore not a direct one, but is mediated through a secondary messenger, perhaps adenylcyclase. Estrogen blockade of DA activation of gonadotropin release is also not direct for it can be inhibited in turn by puromycin and cycloheximide (Schneider and McCann, 1970c). It is possible therefore that direct competitive inhibition between sex steroids and biogenic amines does not occur but rather that sex steroids alter some link in a chain reaction activated by biogenic amines. Such an action is in keeping with the known actions of estrogen as an intracellular stimulator of RNS and protein synthesis (Hamilton, 1964; Segal and Scher, 1967). The previously mentioned action on the cell membrane or on calcium and electrolyte transport across the membrane cannot be discounted. Progesterone seems to act by such a mechanism in the myometrium (Kumar, 1967) in addition to possessing a relatively nonspecific antiestrogen action on the described anabolic actions of estrogen.
Further studies on the site and mechanism of interaction between sex steroids and biogenic amines are indicated. A fruitful approach may be to investigate possible interaction in the pineal. The pineal may play an important role in the biological clock so essential to ovulation in the rat (Schwartz, 1969). Estrogen interference with synthesis of an inhibitory indolalkylamine in the pineal could explain the continual stimulus to further estrogen production during the afternoon and evening of proestrus. A second site of interaction may involve blockade of biogenic amine stimulation of LRF or FRF release during times of peak estrogen levels in the CNS.

Although the primary emphasis has been placed in this discussion on the role of estrogen as a stimulus to intracellular protein synthesis, the possible role for a catechol estrogen in direct receptor or competition with a catecholamine remains. Catechol estrogens 2 OH estrone and 2 OH estradiol form a small but significant part of total estrogen in the human and have been found to compete with catecholamines for catechol-o-methyl transferase (Axelrod, 1964). The principal drawback to such a role for catechol estrogens is that, to date, all the known effects of catecholamines on gonadotropin release appear to be mediated through alpha adrenergic receptors. The catechol estrogens would be expected to compete with catecholamines primarily at beta receptors since this is the site specific for the catechol moiety.

The relationship between biogenic amines and the sex steroids has not been resolved by the present study. It is hoped, however, that further directions for study have been discovered which pursued to their conclusion will furnish useful information about the control of gonadotropin secretion and ovulation.
Scheme for regulation of ovulation in the rat:

Schemes designed to explain the cyclicity of the human menstrual cycle have relied primarily on the concept of negative feedback since Moore and Price (1932) recognized that hormones from the pituitary stimulate ovarian secretion while ovarian hormones inhibit pituitary secretions. In the rat, this cycle is profoundly influenced by the light and dark rhythm (Sawyer, 1969). A number of schemes have been proposed in recent years to explain the rat cycle (Bogdanove, 1964; Barraclough, 1966; Lisk, 1966). In the main these have been intentionally limited in scope. The most ambitious attempt to date at bringing together all the endocrine environmental and neurological factors into one plan is that of Schwartz (1969). She brought information together from a number of sources to draw a time map of the essential variables during the estrous cycle. From this she has constructed a formal systems analysis model of the rat cycle. A description of the events of the 4 day cycle as visualized by Schwartz is as follows: On day 1 (estrous) follicular growth begins possibly under the influence of FSH released along with LH on proestrus. Estrogen secretion is minimal at this time. Follicular growth continues during metestrus. On diestrus, the day proceeding proestrus, between 11:00 a.m. and 3:00 p.m. there is increased secretions of LH which stimulates the ovarian production of estrogen necessary for all following events including ovulation. At 2:00 p.m. of proestrous estrogen has reached the threshold which stimulates the ovulation of LH and FSH. The cycle then repeats itself. This plan is elaborated to explain vaginal cornification, uterine destention and mating behaviour. Taken by itself this very simple plan has no major flaws yet it fails to explain how the biological clock operates. A basic assumption of such a hypothesis is the dualistic action of estrogen first to inhibit LH release during proestrus and then, when it has reached threshold to stimulate LH release after 2:00 p.m. on proestrus.
If we first examine the proposition that stimulation of LH release is due to estrogen reaching a threshold we find that recent evidence using radioimmunoassay for LH shows that in the sheep (Knobil, 1970) and in the human (Burger et al., 1969) LH release actually occurs a few hours after peak serum estrogen levels. The very fine study of estrogen secretions from the rat ovary by Yoshimaka et al. (1969) which was not available to Schwartz when she proposed here scheme, shows a plateau in estrogen secretions between 10:00 a.m. and 3:00 p.m. of proestrous rather than a continuing rise to threshold. Furthermore, Monroe et al. (1969) found that the LH peak in their rats might occur anywhere between 2:00 p.m. and 6:00 p.m. Finally Boutselis and Dickey (in press) have reported that when low doses of estrogen were given from the 5th to 10th day of the cycle an attempt to regulate the day of ovulation higher doses delayed ovulation for 2 days. Progesterone has the dual effect in either delaying the day of ovulation if given on diestrous (Everett, 1948) or advancing the time of ovulation if given within 6 hours of the expected LH release (Nallar et al., 1966). This suggests that the latter effect of progesterone might be to inhibit the action of estrogen to block LH release and that in fact it is the fall in estrogen or failure to rise rather than a continued rise that overcomes blockade of LH release at proestrous. The essential question in regard to estrogen then is where and how does it manifest this dual action of blocking LH release but increasing the amount of LH release when it does occur. An initially attractive hypothesis is that estrogen may stimulate LH synthesis in the pituitary as demonstrated by Piacsek and Meites (1966) in vitro at the same time it is blocking FRF release in the hypothalamus. Analysis of our own data and others however fails to show increased pituitary LH content more than a few hours following estrogen administration. More important the sensitivity to a constant
dose of LRF does not change during the estrous cycle. LRF is essentially similar in effectiveness in increasing plasma LH regardless of cycle stage (Autunes, Rodrigues et al., 1966). LRF however does rise during the cycle reaching a peak in early afternoon of proestrous and falls late afternoon and early day of proestrous (Ramsey and Sawyer, 1965). Chowers and McCann (1965) found similar results except that LRF content fell by noon of proestrous. FRF also falls between 11:00 a.m. and 4:30 p.m. of proestrous (Negro-Villar and Meites, 1970). It thus may be that estrogen blocks release but stimulates synthesis of LRF rather than LH. Progesterone by contrast may stimulate synthesis of pituitary FSH or hypothalamic FRF (Tables 7, 8 and Figure 11).

The mechanism of estrogen blockade of LRF secretion and possibly stimulation of LRF synthesis requires analysis in terms of the findings in these investigations and recent reports by Schneider and McCann (1970a, 1970b, 1970c) and Kamberi, Mical and Porter (1970a, 1970b). In particular, Schneider and McCann (1970a) found that intraventricular injection of dopamine was most effective in releasing LH of the cycling rat during proestrous and was completely ineffective during diestrous. These authors also found (1970c) that estrogen blocked the effect of dopamine on releasing LRF from hypothalamic fragments in vitro, an effect that could be overcome by prior inhibitors of protein synthesis. The implications then are that estrogen antagonizes, perhaps indirectly, some effect of catecholamine on release of LRF.

The facilitation of ovulation in the rat ovary during a very brief period each afternoon requires interpretation. It is likely that this is mediated through neurons containing biogenic amines. One way in which this may occur is that during periods of light NE activation of melatonin formation is reduced, therefore blockade of LRF
synthesis or release by melatonin is revoked and LH release occurs
(Taylor and Wilson, 1969; Weiss and Costa, 1967). The fact that
estrogen also blocks the NE activation of adenylcyclase and there-
fore possibly of melatonin formation may explain how estrogen acts
to stimulate LH synthesis. However, more acutely acting mechani-
sms must be postulated to explain critical periods for LH release on
the afternoon of proestrous rather than morning. Here a vital role
for FSH is suggested by the fact that the effect of atropine and hexa-
methonium administration is directed toward FSH more than LH
and the fact that FSH release was more affected by intraventricular
injection of biogenic amines than was LH release. As shown in this
study, muscarinic receptors seem to stimulate and nicotinic receptors
seem to block FSH release. FSH release was evoked by dopamine
and blocked by norepinephrine following intraventricular injection of
doses which had no effect on LH. This apparently richer nature of
the innervation of the FSH releasing mechanism suggest that environ-
mental stimuli or internal stimuli influencing the finely timed release
of trophic hormones may act through FSH. The possibility can be ad-
vanced that FSH release precedes LH release and is responsible for
LH release via direct-short loop competition with hypothalamic
estrogen binding sites (Ojeda and Ramirez, 1970b) or indirect -
secondary to increased ovarian progesterone inhibition of the block
to LRF release. These and many more questions raised by the
present investigation may provide a fruitful area of study for the
future.
SUMMARY AND CONCLUSIONS

The effects of systemic and CNS administration of biogenic amines and cholinergic blocking agents in ovariectomized, ovariectomized-reserpinized and ovariectomized rats treated with sex steroids on the plasma and pituitary levels of FSH and LH was investigated in an attempt to discern a mechanism of interaction between autonomic agents and sex steroids in the regulation of gonadotropin secretion.

Notable observations were as follows:

1. There is an apparent 30-fold difference in the amount of estradiol effective in suppressing release of FSH and LH at 2 hours, the time of maximal estrogen content in the brain (McEwen, 1970). An effect of estrogen on stimulation of FSH and LH release was not seen until doses 10 (FSH) to 100 (LH) times the ED$_{50}$ for suppression were given. This suggests that different mechanisms are involved in release of FSH and LH from the pituitary and may be presumptive evidence for two separate hypothalamic releasing factors.

2. Reserpine administration also demonstrated a differential effect on FSH and LH release. Reserpine 2.5 mg/kg caused a blockade of LH release (maximal at 6 hours) and stimulated FSH release (maximal at 6 hours). Both effects had returned to normal by 12 hours. Reserpine (5 mg/kg) caused a blockade of both FSH and LH release, with the FSH effect disappearing by 24 hours and the LH effect still present at 48 hours. These results suggest that either reserpine acts differently at sites of action for FRF and LRF or that the action is the same but that at the lower dose of reserpine a biogenic amine is depleted which acts differently on FRF and LRF blocking the former and stimulating the latter. At higher doses of reserpine
other events occur which result in blockade of both FRF and LRF. Such differential events might involve depletion of NE at lower doses and of both NE and DA at higher doses. Alternatively, low doses of reserpine might augment stimulatory cholinergic pathways and high doses block stimulatory biogenic amine pathways.

This differential effect was also seen in the fact that following intraventricular administration, DA effected and NE blocked FSH but not LH release in normal males and reserpine treated ovariectomized females but had little effect in untreated ovariectomized females. Finally, blockade of biogenic amine degradation with a MAO inhibitor significantly increased FSH release without affecting LH release. Simultaneous inhibition of CA synthesis and block of serotonin degradation resulted in significant increase of FSH release.

3. The effect of biogenic amines on release of gonadotropins is dependent on sex steroid levels. This was illustrated in an experiment in which administration of L-Dopa to ovariectomized rats pretreated with estrogen 72 hours earlier resulted in increase of plasma LH but not plasma FSH whereas administration of L-Dopa to progesterone primed animals resulted in release and synthesis of FSH but not LH (Tables 7, 8). Pretreatment with estrogen one and one-half hours before was without effect (Table 6). This finding is corroborated by the report of Schneider and McCann (1970) that intraventricular administration of DA evoked significant LH release only during proestrus of the estrous cycle.

4. A significant role for nicotinic as well as muscarinic receptors in modulating gonadotropin secretion was found in experiments in which hexamethonium and atropine were injected into the lateral ventricle of ovariectomized and reserpine-treated ovariectomized rats. Atropine alone blocked both FSH and LH release. Pretreatment with
reserpine potentiated blockade of FSH release and prevented atropine blockade of LH release. Hexamethonium alone slightly stimulated release of FSH and in the presence of reserpine markedly stimulated FSH release while inhibiting LH synthesis and release. These results demonstrate the dual nature of the cholinergic mechanism acting on FSH secretion, with muscarinic receptors stimulating and nicotinic receptors inhibiting release of FSH. Such a dual mechanism explains the failure of carbachol injection to effect FSH release. The relatively greater effect of anticholinergic agents and biogenic amines on FSH than LH release suggest that in the ovariectomized animal FSH release is more closely modulated by the autonomic nervous system.

Conclusions:

The conclusions to be drawn from the present study are that (1) biogenic amines and cholinomimetic agents have different actions on FSH and LH release, (2) the effects of biogenic amines on gonadotropin release is dependent on the hormonal milieu provided by the sex steroids, and (3) interactions of biogenic amines and sex steroids must be given important consideration in any attempt to explain the functioning of the estrous cycle.
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