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SELECTIVE BLOCKADE BY GALLAMINE AND
PANCURONIUM OF MUSCARINIC INHIBITORY ACTIVITY
IN CERVICAL SYMPATHETIC GANGLIA AS DETERMINED
BY NICTITATING MEMBRANE CONTRACTIONS, SURFACE
POTENTIAL RECORDINGS AND HISTOFLUORESCENT
EXPERIMENTS.
THE OHIO STATE UNIVERSITY, PH.D., 1978

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1978
SELECTIVE BLOCKADE BY GALLAMINE AND PANCURONIUM OF MUSCARINIC INHIBITORY ACTIVITY IN CERVICAL SYMPATHETIC GANGLIA
AS DETERMINED BY NICTITATING MEMBRANE CONTRACTIONS,
SURFACE POTENTIAL RECORDINGS AND HISTOFLUORESCENT EXPERIMENTS

DISSERTATION

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

By
Estelle J. Tsevdos, B.A.

* * * * *

The Ohio State University

1978

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ACKNOWLEDGMENTS

I would like to dedicate this any any future contributions I may make to my parents whose love of knowledge inspired and continues to inspire me throughout all endeavors. Only through their understanding and faith in me was I able to overcome a very difficult period of time in my life. I also would like to thank my sister, Maria, for her patience and much-needed cheerfulness during the last four years.

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INTRODUCTION

Literature Review

Early Studies in Autonomic Pharmacology

One of the first accounts of drug actions in autonomic ganglia was given by J. N. Langley in a series of papers during the last decade of the nineteenth century (Langley, 1891, 1893, 1898). In this series, the stimulating and subsequent blocking actions of nicotine on sympathetic ganglia were first described. By applying nicotine to the surface of ganglia and measuring end-organ responses (nictitating membrane, arterial vessel size in the ear, pupil size), Langley was able to distinguish between those fibers that simply coursed through the ganglion without making synaptic contacts with those that ended within it. This technique eventually enabled investigators to outline the pattern of autonomic innervation to specific organs.

The responses recorded to nicotine were very similar to those obtained by electrical stimulation of preganglionic nerve fibers. These studies raised the suspicion that certain nervous transmission was chemical in nature and warranted investigations into the identification of a possible chemical transmitter. The nicotine-like activities of choline and several choline esters were described by Dale in 1914. He found that the pressor responses resulting from intravenous doses of
acetylcholine and other choline esters in cats treated with atropine were abolished by prior administration of nicotine. Dale also differentiated between "nicotinic" and "muscarinic" actions of the esters. These early experiments set the pattern for later investigations to test other compounds in autonomic ganglia and to speculate that acetylcholine was the chemical held responsible for physiological activity.

The experiment for neurohumoral transmission came with Loewi's (1921) study on the frog heart. Upon collection of the perfusion fluid during vagal stimulation, he discovered that this fluid contained a substance which caused cardiac slowing (vagal activity) when applied to a second frog heart. Furthermore, atropine prevented the action not only of vagal stimulation but also application of the perfusate. Thus, Loewi definitively demonstrated that vagal stimulation, acetylcholine, and the released material gathered upon vagal stimulation exhibited physiologically similar responses.

When Loewi's approach was applied to the process of neuronal transmission, an acetylcholine-like substance was isolated from sympathetic ganglia (Chang and Gaddum, 1933) by utilizing a perfusion technique for the superior cervical ganglia of cat (Kibjakow, 1933). The advancement of this technique allowed an important series of experiments to be performed in Feldberg's laboratory (Brown and Feldberg, 1936; Feldberg and Gaddum, 1934; Feldberg and Vartiainen, 1934) leading to the confirmation of acetylcholine as the primary transmitter in sympathetic ganglia.
The current mechanistic concept of acetylcholine's actions in autonomic ganglia is as follows. An action potential conducted down a myelinated preganglionic nerve results in a calcium-dependent release of acetylcholine from intraneuronal storage sites. The acetylcholine then diffuses across the synaptic space and reacts with the post-junctional cells. This resulting reaction between the acetylcholine and the cell causes a change of ionic permeability and discharge of the potential difference existing across the postjunctional membrane. The cell depolarizes, and this action is referred to as the synaptic potential. The depolarization in the postjunctional membrane must reach certain threshold values to initiate a propagated action-potential. Termination of the transmitter activity occurs by means of enzymatic hydrolysis or diffusion (Volle, 1966).

**Synaptic Events in Sympathetic Ganglia**

In recent years it has become evident that the synaptic events in autonomic sympathetic ganglia are not those of simple single relay transmission. In fact, the prediction made by Eccles in the 1930's that there would be excitatory and inhibitory events discovered in ganglionic transmission has come true (Eccles, 1937).

A technique utilized for studying changes in membrane potentials in sympathetic ganglia is the recording of potential changes from the surface. One external recording electrode is placed on the surface of the ganglion and the second on crushed postsynaptic fibers. Since the resting potential is intact at the surface position and zero at the injury site, a steady current flows between the two points, giving
rise to a unique recording of cells projecting into the particular postganglionic branch. Direct coupled (DC) amplification allows the recording of not only rapid electrical events such as the action potential, but also of slower changes associated with potential differences between the surface of the ganglion and the injured nerve. These changes are produced by changes in the transmembrane potential of ganglionic cells (Mountcastle, 1974). Studies of in vivo and isolated in vitro ganglia used the demarcation potential (surface or injury) technique and were later substantiated by more direct intracellular (microelectrode) measurements.

DC recordings from isolated superior cervical ganglion of cat indicated two pharmacologically-distinct excitatory cholinoceptive sites. The first site is classified on the basis of its sensitivity to activation and blockade by nicotine and blockade by curare or hexamethonium-like drugs. Therefore, it has been labeled nicotinic. The second site is characterized by its sensitivity to activation by muscarine-like compounds and blockade by small doses of atropine. This site has been labeled muscarinic (Eccles, 1943; Eccles, 1944). Preganglionic nerve stimulation in isolated superior cervical ganglion preparations treated with curare presented a triphasic surface-potential complex. The first portion of the complex was a negative wave (f-EPSP or N wave) followed by a positive wave (s-IPSP or P wave) and then a late negative wave (s-EPSP or LN wave) (Eccles, 1952). The f-EPSP is selectively abolished with curare, and the s-EPSP and s-IPSP potentials disappear with small doses of atropine (Eccles and Libet, 1961).
The triphasic waveform has been identified in isolated ganglia of turtle (Laporte and Lorente, 1950), rabbit (Eccles and Libet, 1961), frog (Nishi and Koketsu, 1967, 1968), and cat (Libet, 1967). In addition, intracellular recording on isolated frog and rabbit ganglia have confirmed the triphasic membrane-potential change (Libet and Tosaka, 1969; and Koketsu, 1969).

On the basis that the s-IPSP was blocked by dibenamine, it was suggested that a catecholamine mediates this potential (Eccles and Libet, 1961). Libet et al. (Libet and Tosaka, 1970; Libet, 1970) supported the hypothesis that the synaptic transmitter held responsible for the s-IPSP is dopamine. This pathway is hypothesized to contain an interneuron which when stimulated by acetylcholine releases dopamine to interact with a dopaminergic receptor located on the ganglion cell itself. Evidence for this interneuron was presented when morphological studies revealed a chromaffin-like cell in the ganglion (Norberg and Sjorqvist, 1966; Eranko and Eranko, 1971; Jacobowitz, 1970; Bjorkland, 1970). These cells are small and intensely fluorescent when viewed under ultra-violet light following treatment with formaldehyde.

The role of dopamine as a synaptic transmitter was investigated by correlating changes in formaldehyde-induced fluorescence and synaptic events (Libet and Owman, 1974). Upon exposure to bethanechol, a muscarinic agent, the fluorescence specific for dopamine in isolated ganglia was decreased, and when exogenous amounts of dopamine were applied to the prepared tissues, it was found that some of the fluorescence reappeared. In addition, s-IPSP changes corresponded
directly to alterations in histofluorescence. This added to the evidence that dopamine is the neurohumoral agent held responsible for the hyperpolarization (s-IPSP).

Greengard and coworkers investigated the role cyclic nucleotides have in ganglionic synaptic events. Their results indicated that changes in cyclic AMP were associated with stimulation of the dopaminergic receptor located in the ganglion. Furthermore, an adenylate cyclase sensitive to dopamine was found in sympathetic tissue. In addition to changes associated with the dopaminergic receptor, levels of cyclic GMP were affected when the muscarinic receptors were stimulated (Greengard and Kebabian, 1974). Based upon previous work done in the area (Volle, 1969; Volle, 1970) in addition to their own results, Greengard's group presented a modified model of sympathetic ganglionic synaptic events (Figure 1).

Drug-evoked Alterations of Synaptic Activity in Sympathetic Ganglia

Previous to the present research there had been no specific proof that the two muscarinic receptors are pharmacologically distinct. Originally, Koppanyi (1932) demonstrated that pilocarpine injected into the circulation of cat's superior cervical ganglion causes contraction of the nictitating membrane. This first suggested the existence of atropine-sensitive ganglionic responses. Extending this work, other investigators were able to demonstrate that introduction of muscarinic agents into the circulation of sympathetic ganglia elicited changes in synaptic events as indicated by surface potential recordings. One such drug tested was acetyl-beta-methylcholine (methacholine) which caused
FIGURE 1

The relationship between the various neuronal elements, the various transmitters, the sensitivity of the synaptic receptors to different classes of antagonists, the electrical signs that accompany activation of the post-ganglionic stimulation, and the postulated involvement of cyclic nucleotides in the production of electrophysiological changes (Greengard and Kebabian, 1974).
FIGURE 1

Muscarinic Cholinergic

- blocked by hexamethonium

Cyclic GMP

PREGANGLIONIC CHOLINERGIC FIBERS

ACH - blocked by hexamethonium

POSTGANGLIONIC NEURON

Nicotinic Cholinergic

- blocked by α-adrenergic antagonists

Initial EPSP

DOPAMINE

Cyclic AMP

POSTGANGLIONIC SYNAPTIC POTENTIALS

IPSP

INTERNEURON

ACH - blocked by atropine

Late EPSP

DOPAMINE
ganglionic stimulation in the form of electrically-recorded hyperpolarization and slow depolarization, both of which are susceptible to atropine (Pappano and Volle, 1962; Takeshige et al., 1963). Takeshige and Volle (1964) extended their work by injecting pilocarpine into the animal prior to the intraarterial injections of methacholine. In the presence of pilocarpine only hyperpolarization developed to injections of acetylcholine and methacholine, rather than the usual hyperpolarization followed by the slow depolarization. These data resulted in speculation that pilocarpine is a "selective agonist for muscarinic excitatory (slow depolarization) receptors" (Haefely, 1972).

The Effect of Neuromuscular Junction Blocking Agents on Ganglionic Transmission

Extending a study of neuromuscular junction blocking agents' effect on sympathetic ganglionic transmission, the neuromuscular drug, gallamine, was tested in the pharmacological nictitating membrane experiment (Figure 2). The results indicated that gallamine enhanced the muscarinic component of the nictitating membrane contraction in response to preganglionic nerve stimulation (Gardier et al., 1974). It was of interest to see if pancuronium, a new neuromuscular junction blocker, behaved as gallamine since it resembles gallamine clinically. In contrast to d-tubocurarine, both drugs are devoid of a hypotensive response either by a blocking action at ganglionic sites or by a release of histamine (Buckett et al., 1968). Next, both gallamine and pancuronium may exhibit a marked increase in mean arterial pressure, tachycardia, and increased cardiac output (Brown and Crout, 1970; McIntyre and Gain, 1971; Stoelting, 1972).
FIGURE 2

Enhancement of the ganglionic muscarinic response of the nictitating membrane by gallamine in the presence of incomplete nicotinic blockade. The top row reflects the inability of gallamine to affect normal ganglionic transmission. Stimulation was with 2 V, 20 Hz. A, preganglionic; between A and B, gallamine, 2 mg/kg, i.v.; B, preganglionic; between B and C, atropine, 0.1 mg/kg i.v.; C, preganglionic. The middle row shows the enhancement by gallamine of ganglionic muscarinic activity in the absence of a residual nicotinic effect. Conditions of stimulation were as above. D, preganglionic; between D and E, chlorisondamine, 4 mg/kg, i.v.; E, preganglionic; between E and F, gallamine, 4 mg/kg, i.v.; F, preganglionic. The bottom row demonstrates that ganglionic muscarinic response is not modified by d-tubocurarine. Stimulation was with 5 V, 20 Hz. G, preganglionic; between G and H, chlorisondamine, 2 mg/kg, i.v.; H, preganglionic; between H and I, d-tubocurarine, 0.4 mg/kg, i.v.; I, preganglionic.
FIGURE 2
Preliminary nictitating membrane experiments in which pancuronium was tested on the muscarinic component of the contraction elicited upon preganglionic nerve stimulation showed that it also enhanced muscarinic events (Jackson et al., 1975).

**Hypothesis**

The preliminary investigations with the nictitating membrane experiment of the cat disclosing enhanced muscarinic contraction to electrical stimulation of the preganglionic nerve prompted more direct measurements of ganglionic events. It was suspected that the muscarinic slow depolarization (s-EPSP) was exaggerated by gallamine. However, in situ surface potentials in response to brief tetanic electrical stimuli indicated that both gallamine and pancuronium, a new neuromuscular drug, selectively reduced the hyperpolarization (s-IPSP).

The possibility of dopamine blockade of ganglionic dopaminergic receptors by pancuronium or gallamine was investigated further by comparing the effects of a known dopamine antagonist with little alpha-adrenergic activity, haloperidol (Byck, 1975) with the neuromuscular drugs (Carlsson and Lindqvist, 1963; Carlsson, 1974).

The results of these experiments indicate that gallamine and pancuronium are selectively occupying the interneuronal muscarinic receptor labeled $M_1$. 
METHODS AND MATERIALS

Animal and General Preparation

Adult cats of either sex weighing between 1.9 and 4.0 kg were pre-medicated with 12.5 mg of pentothal sodium i.v. as an induction agent. They were then carried on 37.5 mg/kg of chloralose and 250 mg/kg of urethane. Anesthesia was maintained thereafter with chloralose in urethane supplements at one-half the original anesthetic dose. With the animal loosely restrained in a supine position, drug injections and fluid replacement (isotonic saline) were made via a polyethylene catheter placed in the right femoral vein. A second catheter filled with heparinized isotonic saline was placed into the right femoral artery and connected to a Statham P23AA transducer for blood pressure recordings. A ventral cervical midline incision from the mandibular arch to the upper border of the sternum exposed the surgical field, and a tracheal cannula was inserted through a tracheostomy incision at the latter level. Ventilation was controlled by a Palmer pump with room air in a semi-open system at delivered volumes ranging between 60 and 100 cc during the period of neuromuscular blockade. Rectal temperature was maintained at 36 ± 1°C with a warming pad placed beneath the animal. All responses were visualized on an Electronics for Medicine V-6 Simultrace recorder.
In order for muscarinic transmission to be studied, the normal nicotinic pathways in the ganglion must be abolished. This was accomplished by the use of a classical ganglionic blocking agent. Chlorisondamine (Ecolid®) (2 mg/kg) was used for this purpose throughout the study.

**Nictitating Membrane Experiments**

The right cervical sympathetic trunk was exposed and separated from the vagus and aortic nerves. The longus muscles were freed from their insertions on the lateral cervical processes in the area of the tympanic bulla. Sufficient muscle was resected in the ganglionic area to provide maximal exposure of the superior cervical ganglion and the internal carotid postganglionic nerve fibers. Supramaximal stimulation, consisting of rectangular electrical pulses of 2 to 6 V and 0.1 msec duration, was delivered to the preganglionic nerve via bipolar platinum electrodes from Grass models S4C stimulator and S1U-4B stimulus isolation unit for 15 second periods at frequencies varying between 2 and 40 Hz. The nerves and electrodes were covered with warm liquid paraffin, and isometric contractions of the right nictitating membrane were detected through a Grass FT03B force displacement transducer. The membrane was always placed under 5 g of resting tension.

In order to rule out a postganglionic mechanism, postganglionic stimulation with recordings of the nictitating membrane contraction were accomplished in an identical manner to that described for preganglionic nerve excitation recordings.
Ganglionic Surface Potential Experiments

These experiments were separate from those on the nictitating membrane but followed the above description with these sequential additions. The larynx, trachea and esophagus were dissected free of their attachments and their cranial stumps everted through the mouth. The head was immobilized with the cut skin edges retracted and tied to a ringstand to form a cavity. Under a dissecting microscope, the superior cervical ganglion was carefully released from its attachment to the nodose ganglion and its ventral surface desheathed, using caution to keep the blood supply intact. Connective tissue was removed from the postganglionic trunk which then was crushed and suspended on a unipolar platinum electrode. A second platinum electrode was placed on the exposed desheathed ventral surface of the ganglion, and both electrode leads were fed into first, a Tektronix 26A2 D.C. differential amplifier with $10^6$ ohm input resistance and then, into a D.C. V20 amplifier of the Simultrace V-6 recorder. A third indifferent electrode was inserted into the body wall close to the ganglion and used as ground.

Preganglionic nerve stimulation parameters differed from the nictitating membrane experiments in that 6 V, 0.1 msec pulse duration at frequencies of either 40 Hz for 0.5 sec or 32 Hz for 0.25 sec were used. These trains of stimuli were controlled by a Tektronix 26G2 ramp generator. Two minute intervals separated the periods of stimulation since previous experiments on the nictitating membrane indicated that a tachyphylaxis resulted to muscarinic enhancement when a shorter time interval was used.
(Gardier et al., 1974). The cervical cavity was filled with warm liquid paraffin sufficient to cover both stimulating and recording electrodes. Ganglionic depolarization was indicated by an upward deflection of the recording beam.

Frequency and Voltage Response Experiments

Experiments in which the frequencies and voltages varied were performed in order to observe any preferential development of the s-IPSP or s-EPSP and to establish optimum stimulation parameters for further work. The experimental procedure utilized was similar to that described above with the duration set at 0.1 msec for 0.5 sec. Once threshold values for voltage were obtained (2-4 V), that setting was tested throughout a series of frequencies beginning with 2 Hz. The frequency was then doubled progressively until an upper limit of 128 Hz was reached. Then, the entire procedure was repeated at settings of 4, 6 and 8 V (supramaximal).

Sectioned Preganglionic Cervical Sympathetic Nerve Experiments

These experiments were carried out in the same manner as those for frequency-voltage responses, but once baseline recordings of the surface potential were obtained, the cervical sympathetic trunk was surgically divided using microdissecting techniques. Stimulating electrodes were left stationary during the surgical dissection of preganglionic fibers in order to avoid any change in recording position. At the end of each procedure, the ganglion and connected cervical sympathetic nerve were dissected free from the preparation and preserved in 10% formalin. Histological examination of the
tissue revealed the extent of surgical sectioning, and the resulting tissue was then photographed.

Drug Injection Experiments

The experimental procedure for these studies was carried out in a manner similar to that of Volle (1962), and Takeshige and Volle (1964). Cats were anesthetized with the same chloralose and urethane anesthetic concentrations mentioned in the Animal Preparation section and surgically treated as in the surface potential experiments. In addition, a 27-gauge needle connected through polyethylene tubing to a tuberculin syringe was inserted into the thyroid branch of the common carotid artery.

Once the needle was secure, acetyl-beta-methylcholine (10 μg) was injected as a 0.9% NaCl solution in a volume of 0.05 ml. Responses were considered to be "control" when they were reproduced at least three times. Pancuronium, gallamine and atropine were given, as usual, into the femoral vein with all results recorded on the Electronics for Medicine V-6 Simultrace recorder.

Utilizing the methacholine procedure documented above, intra-arterial injections of pilocarpine HCl (10 μM) in a 0.9% NaCl solution at 0.1 ml volume were made. However, in addition to the recorded alterations, preganglionic electrical stimuli of 4-6 V at 0.1 msec pulse duration and frequency of 16 or 32 Hz for 0.5 sec were delivered to the preparation (Haefely, 1974). Therefore, during the pilocarpine-induced muscarinic slow depolarization (s-EPSP) which was observable upon blockade by chlorisondamine (2 mg/kg), stimulation of the
preganglionic cervical sympathetic nerve allowed the development of purely "s-IPSP" potentials.

**Histofluorescence**

Rats of either sex weighing between 250 and 350 g were anesthetized with pentobarbital, 100 mg/kg i.p. The animal was then restrained in a supine position and a midline incision from the edge of the mandible to the upper border of the sternum exposed the surgical area. A tracheal cannula was inserted and further dissection exposed the cervical sympathetic trunk. This was followed cranially to reveal the left superior cervical ganglion which is located near the bifurcation of the common carotid artery. The ganglion with 2-4 mm of attached postganglionic nerve and 8-10 mm of preganglionic cervical sympathetic nerve was dissected free of its connections. This entire preparation was then placed in cold oxygenated (95% O₂/5% CO₂) Kreb's Ringer solution. Using a dissection microscope, the ganglionic connective tissue capsule was carefully removed from the ganglion and attached neuronal fibers.

The preparation was placed in a 37°C oxygenated Kreb's Ringer bath for thirty minutes. The right superior cervical ganglion subsequently was treated in an identical manner with the exception that it was placed in a Kreb's Ringer bath containing 0.5 mM bethanechol (Libet and Owman, 1974).

This procedure was repeated in the presence of pancuronium and gallamine. Pretreatment of pancuronium (2 mg/kg) was made in vivo,
while gallamine was given *in vivo* and *in vitro* (concomitant with betanechol). Table 1 provides the protocol of the histofluorescent studies.

After the thirty minutes of incubation, the tissue was placed in isopentane submerged in liquid nitrogen. Following this, each ganglion was dehydrated in a lyophillizer for 6-7 days. These fixed and freeze-dried tissues were exposed to formaldehyde vapors for one hour (Falck *et al.*, 1962). Next, the prepared tissues were embedded in paraplast and sectioned at 14 microns. Upon sectioning, each slide which contained 4-6 sections was observed under a Leitz Wetzlar #573576 microscope utilizing ultra-violet light from a HB0200 W/2 mercury lamp and an oil immersion lens (Leitz Wetzlar #B26469). Figure 3 (Cooper *et al.*, 1974) presents the resultant chemical reaction which takes place when biogenic amines are treated with formaldehyde vapors. The dihydroisoquinoline formed fluoresces upon exposure to ultra-violet light.

SIF cells per section were visually counted and compared among sections. Photographs were taken of various sections (usually, between 5 and 8 sections were examined and quantitated per experiment) to insure lack of bias on the part of the observer.

**Drugs and Solutions**

The ganglionic blocking agent used to unmask muscarinic pathways in the superior cervical ganglion was chlorisondamine (Eclid®) at a dose of 2 mg/kg dissolved in doubly distilled water. Dr. Charles A. Brownley, Jr. of CIBA Chemical Corporation supplied the drug for these experiments.
### TABLE 1

**PROTOCOL FOR HISTOFLUORESCENCE**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Left Superior Cervical Ganglion</th>
<th>Incubation Time</th>
<th>Right Superior Cervical Ganglion</th>
<th>Incubation Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Kreb's Control</td>
<td>3</td>
<td>bethanechol (0.5 mM)</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>bethanechol (0.5 mM)</td>
<td>3</td>
<td>gallamine (2 mg/kg, i.v.)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>bethanechol (0.5 mM)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>or</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>pancuronium (0.1 mg/kg, i.v.)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>bethanechol (0.5 mM)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>gallamine (2 mg/kg, i.v.)</td>
<td>3</td>
<td>gallamine (2 mg/kg, i.v.)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>bethanechol (0.5 mM)</td>
<td></td>
</tr>
</tbody>
</table>
FIGURE 3

The reaction by which dopamine forms a fluorescent isoquinoline derivative upon exposure to formaldehyde and viewed under ultra-violet light.
FIGURE 3

DOPAMINE + HCHO → 3,4-DIHYDROISOQUINOLINE

HO

\[
\begin{array}{c}
\text{HO} \\
\text{H} \\
\text{C} \\
\text{H} \\
\text{CH} \\
\text{NH}_2 \\
\text{HO}
\end{array}
\]

\[
\begin{array}{c}
\text{HO} \\
\text{H} \\
\text{C} \\
\text{H} \\
\text{CH} \\
\text{CH} \\
\text{C} \\
\text{N}
\end{array}
\]

\[
\begin{array}{c}
\text{HO} \\
\text{H} \\
\text{C} \\
\text{H} \\
\text{CH} \\
\text{CH} \\
\text{C} \\
\text{N}
\end{array}
\]

\[
\begin{array}{c}
\text{HO} \\
\text{H} \\
\text{C} \\
\text{H} \\
\text{CH} \\
\text{CH} \\
\text{C} \\
\text{N}
\end{array}
\]
Both gallamine triethiodide (Flaxedil™) and pancuronium bromide (Pavulon™) were obtained from The Ohio State University Hospital Pharmacy. The structures of these agents are presented in Figure 4. Gallamine was always administered in the optimum neuromuscular blocking dose of 2 mg/kg, i.v. (Gardier et al., 1974). Doses of pancuronium bromide varied between 0.04 and 0.2 mg/kg, i.v. with the optimum dose level being set at 0.1 mg/kg (Figure 5).

Haloperidol was given at a 0.5% concentration in a solution composed of 1.5% tartaric acid and 0.1% methylparaben in a 40% aqueous solution of propylene glycol. Injection of the vehicle alone did not have any pharmacological effect on the preparation. Phentolamine was used as an alpha-adrenergic blocker with which haloperidol was compared. The dosage given intravenously for nictitating membrane experiments was 2 mg/kg, and the molar concentrations of haloperidol and phentolamine were 1.33 μM/kg and 1.78 μM/kg, respectively. The dopamine agonist, apomorphine HCl, was given at a dose of 0.1 mg/kg, i.v. Aside from those experiments involving apomorphine, atropine sulfate was given at the end of each experiment in a dose of 0.1 mg/kg to confirm the muscarinic nature of the responses studied.

Acetyl-beta-methylcholine (methacholine) was prepared according to Takeshige and Volle (1964). Ten μg of drug was dissolved in 0.9% NaCl and administered intraarterially in a volume of 0.05 ml. Pilocarpine (10 μM) was also dissolved in a 0.9% NaCl solution and given at doses of 0.1 ml (Haefely, 1972).
FIGURE 4

Chemical structures of the neuromuscular blocking agents, gallamine and pancuronium.
GALLAMINE (FLAXEDIL<sup>R</sup>)

PANCURONIUM (PAVULON<sup>R</sup>)

FIGURE 4
FIGURE 5

Pancuronium-induced reduction of the s-IPSP with no change in s-EPSP. Optimal effect on the s-IPSP was observed at 0.1 mg/kg since addition of 0.16 mg/kg caused no additional inhibition of the s-IPSP.
The composite of Kreb's Ringer solution used for histofluorescent studies is presented in Table 2. Bethanechol was given in vitro at 0.5 mM concentration.

**Statistical Analysis of Data**

A typical response for control readings and maximal drug effect responses were selected in each of three experiments. Response amplitudes for nictitating membrane experiments were measured at 5 sec intervals, averaged and plotted as an individual trace. For surface potential responses amplitudes were measured at 0.25 sec intervals, averaged and plotted as an individual trace. Standard error of the mean values were computed for each point of the averaged graph, and statistical significance was determined using the paired Student t test (Dixon and Massey, 1969). Significance level a $p$ less than 0.05 was used. A $p$ less than 0.05 was considered significant.
<table>
<thead>
<tr>
<th></th>
<th>G/2L</th>
<th>mM</th>
</tr>
</thead>
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<td>117.0</td>
</tr>
<tr>
<td>KCl</td>
<td>0.70</td>
<td>4.7</td>
</tr>
<tr>
<td>MgCl</td>
<td>0.48</td>
<td>1.2</td>
</tr>
<tr>
<td>NaH₂PO₄</td>
<td>0.32</td>
<td>1.2</td>
</tr>
<tr>
<td>glucose</td>
<td>4.14</td>
<td>11.2</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>4.20</td>
<td>25.0</td>
</tr>
<tr>
<td>oxygenate for 5 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.55</td>
<td>2.5</td>
</tr>
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</table>
RESULTS

Normally, nicotinic transmission is the prevalent pathway in sympathetic ganglia. With the administration of a nicotinic blocker, chlorisondamine (2 mg/kg), alternative pathways are unmasked as registered by DC recordings of the s-EPSP and s-IPSP in ganglionic surface potential experiments. Atropine sulfate (0.1 mg/kg) abolishes these potentials, thereby confirming their muscarinic nature. All data, except where specifically noted, were collected in the presence of nicotinic blockade. Atropine injections were given at the end of each experiment to reaffirm recording of muscarinic events.

Electrical Stimulation of Cervical Sympathetic Nerve

Frequency-Voltage Response Experiments

Experiments to determine optimal stimulation parameters were accomplished with the intention of observing preferential development of any component of the triphasic surface potential. Threshold values for voltage ranged from 0.8 V to 2 V. Once threshold was obtained, it was held constant through a series of test frequencies beginning with 8 Hz and progressively doubling the value until supramaximal results (128 Hz) were attained. The voltage systematically was then increased by two, and the entire sequence of frequencies was again tested. Supramaximal voltage was 8 V.
Figure 6 presents the dose-effect curves for the development of the s-IPSP in incremental geometrical progression from threshold to supramaximal values (Table 3). Following the recording of control responses, pancuronium was administered to maximal effect. At that point, haloperidol was administered to a maximum dose of 0.5 mg/kg. Only pancuronium produced a significant change, this being an inhibition of the s-IPSP (Figure 7). The s-EPSP was unaffected by any drug treatment (Table 4). The procedure was repeated with the exception that haloperidol preceded pancuronium. The curves followed the same pattern in that only the s-IPSP was appreciably depressed by pancuronium without an effect on the s-EPSP (Figures 8 and 9; Tables 5 and 6).

**Nictitating Membrane Experiments**

**Effect of Pancuronium on the Nictitating Membrane**

The muscarinic contraction resulting from preganglionic nerve stimulation was enhanced significantly by pancuronium. The n for pancuronium consisted of four nictitating membrane experiments in which the drug was used alone and three experiments wherein haloperidol was administered subsequent to the full pancuronium effect. Haloperidol administered after the pancuronium was not able to significantly overcome the enhanced muscarinic contraction but a change of direction was observable (Figure 10, Table 7).

**Effect of Haloperidol on the Nictitating Membrane**

When haloperidol preceded pancuronium, its fully effective dose was 0.2 mg/kg. As can be seen in Figure 11, the muscarinic contraction
Frequency-voltage response curves plotted in three separate experiments and indicated that maximum response occurred at 16–32 Hz. At this setting, the curves plateaued at all voltages tested. Optimal voltage actually was observed at 4.0 V, but due to the variability between experiments was not visualized in this set of data. 0.8 V was threshold for only two experiments. This curve is the control set for the curve following in which drug responses were tested.
FIGURE 6
FIGURE 7
Pancuronium alone preceding haloperidol significantly inhibited the s-IPSP in comparison to control values in Figure 6 without altering the pattern of development for the surface potential. Again, optimal parameter settings were observed at 4 V and 16-32 Hz.
Amplitude (µV) "p" wave

PANCURONIUM (0.1 - 0.2 mg/kg) BEFORE HALOPERIDOL

8.0 Volts
6.0 Volts
4.0 Volts
0.8 Volt
2.0 Volts

Frequency (Hz.)

FIGURE 7
<table>
<thead>
<tr>
<th>Voltage (V)</th>
<th>Frequency (Hz)</th>
<th>Control (mean ± SE (μV))</th>
<th>Pancuronium (0.1-0.2 mg/kg) (mean ± SE (μV))</th>
<th>Haloperidol (0.5 mg/kg) (mean ± SE (μV))</th>
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</thead>
<tbody>
<tr>
<td>2.0</td>
<td>8</td>
<td>42.5 ± 22.6</td>
<td>17.5 ± 7.5</td>
<td>20.0 ± 10.0</td>
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<td>16</td>
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<td></td>
<td>32</td>
<td>75.0 ± 41.0</td>
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<td>48.3 ± 26.8</td>
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<td>64</td>
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<td>28.3 ± 13.0</td>
<td>33.3 ± 6.7</td>
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<td>73.3 ± 24.1</td>
<td>81.7 ± 20.3</td>
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<td></td>
<td>64</td>
<td>148.0 ± 24.6 *</td>
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<td>195.0 ± 45.4 *</td>
<td>83.3 ± 32.2</td>
<td>128.3 ± 18.6</td>
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</tbody>
</table>

*p < 0.05
TABLE 4

SLOW DEPOLARIZATION
(S-EPSP)

<table>
<thead>
<tr>
<th>Voltage (V)</th>
<th>Frequency (Hz)</th>
<th>Control (mean ± SE (µV))</th>
<th>Pancuronium (0.1-0.2 mg/kg) (mean ± SE (µV))</th>
<th>Haloperidol (0.5 mg/kg) (mean ± SE (µV))</th>
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<tr>
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<td>128</td>
<td>-43.4 ± 6.7</td>
<td>-45.0 ± 2.9</td>
<td>-48.3 ± 8.8</td>
</tr>
</tbody>
</table>

| 4.0        |               |                          |                                             |                                           |
| n=3        | 8             | -43.3 ± 13.3             | -43.3 ± 8.8                                 | -36.7 ± 1.7                               |
|            | 16            | -53.3 ± 7.3              | -55.0 ± 10.4                                | -53.3 ± 6.0                               |
|            | 32            | -63.3 ± 10.9             | -75.0 ± 22.9                                | -71.7 ± 19.7                              |
|            | 64            | -71.7 ± 19.2             | -83.3 ± 28.5                                | -73.3 ± 18.6                              |
|            | 128           | -81.7 ± 17.6             | -81.7 ± 26.8                                | -83.3 ± 24.1                              |

| 6.0        |               |                          |                                             |                                           |
| n=3        | 8             | -31.7 ± 7.3              | -50.0 ± 13.2                                | -46.7 ± 16.9                              |
|            | 16            | -63.3 ± 8.8              | -58.3 ± 15.9                                | -61.7 ± 17.7                              |
|            | 32            | -73.3 ± 13.6             | -81.7 ± 26.8                                | *                                         |
|            | 64            | -83.3 ± 17.4             | -88.3 ± 30.9                                | -91.7 ± 32.2                              |
|            | 128           | -83.3 ± 18.6             | -91.7 ± 34.2                                | -90.0 ± 28.5                              |

| 8.0        |               |                          |                                             |                                           |
| n=3        | 8             | -35.0 ± 10.4             | -63.3 ± 28.9                                | -43.3 ± 18.4                              |
|            | 16            | -60.0 ± 10.4             | -68.3 ± 26.2                                | -63.3 ± 15.9                              |
|            | 32            | -75.0 ± 13.2             | -85.0 ± 33.3                                | -71.6 ± 21.7                              |
|            | 64            | -90.0 ± 25.2             | -95.0 ± 37.8                                | -91.7 ± 34.2                              |
|            | 128           | -93.3 ± 24.1             | -95.0 ± 30.6                                | -91.7 ± 29.2                              |

*p < 0.05
FIGURE 8

The compiled results of three separate experiments in which the frequency-voltage varied in the same manner as in Figure 6. The plateauing effect at 16-32 Hz is more obvious in this set of results. Optimal voltage registered at 4 V with 8 V always being supramaximal.
CHLORISONDAMINE (2-6 mg/kg)

Amplitude (μV) "p" wave

- 8.0 Volts
- 6.0 Volts
- 4.0 Volts
- 2.0 Volts
- 0.8 Volt

Frequency (Hz.)

n=3

FIGURE 8
FIGURE 9

Drug order administration for these experiments was reversed in that haloperidol (0.5 mg/kg) preceded pancuronium (0.1 mg/kg). The results herein were taken after both drugs were administered to the preparation. Haloperidol was unable to significantly alter the s-IPSP, but pancuronium was able to overcome any haloperidol effect and inhibit the s-IPSP in comparison to control (Figure 8).
Amplitude (pV) "p" wave

PANCURONIUM (0.1-0.3 mg/kg)
AFTER
HALOPERIDOL (0.5 mg/kg)

Frequency (Hz.)

Amplitude (μV) "p" wave

8.0 Volts
6.0 Volts
4.0 Volts
2.0 Volts
0.8 Volt

FIGURE 9
TABLE 5

HYPERPOLARIZATION
(s-IPSP)

<table>
<thead>
<tr>
<th>Voltage (V)</th>
<th>Frequency (Hz)</th>
<th>Control (mean ± SE (μV))</th>
<th>Haloperidol (0.5 mg/kg) (mean ± SE (μV))</th>
<th>Pancuronium (0.1-0.3 mg/kg) (mean ± SE (μV))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>8</td>
<td>48.3 ± 10.9</td>
<td>36.7 ± 3.3</td>
<td>15.0 ± 2.9</td>
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<tr>
<td></td>
<td>16</td>
<td>73.3 ± 13.6</td>
<td>78.3 ± 28.3</td>
<td>38.3 ± 7.3</td>
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<tr>
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<td>83.3 ± 20.9</td>
<td>88.3 ± 20.9</td>
<td>43.3 ± 7.3</td>
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<tr>
<td></td>
<td>64</td>
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<td>35.0 ± 5.8</td>
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<td>4.0</td>
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<td>53.3 ± 6.7</td>
<td>57.7 ± 6.1</td>
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<tr>
<td></td>
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<td>78.3 ± 18.6</td>
<td>88.3 ± 18.3</td>
<td>46.7 ± 1.7</td>
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<td>100.0 ± 22.9</td>
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<td>110.0 ± 17.6</td>
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<td>111.7 ± 16.7</td>
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<td>45.0 ± 10.4</td>
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<td>60.0 ± 5.0</td>
<td>*</td>
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<td>48.3 ± 19.2</td>
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<td>128</td>
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<td>128.0 ± 19.2</td>
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</table>

*p <0.05
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<thead>
<tr>
<th>Voltage (V)</th>
<th>Frequency (Hz)</th>
<th>Control (mean ± SE (μV))</th>
<th>Haloperidol (0.05 mg/kg) (mean ± SE (μV))</th>
<th>Pancuronium (0.1-0.3 mg/kg) (mean ± SE (μV))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>8</td>
<td>-31.7 ± 4.9</td>
<td>-30.0 ± 5.0</td>
<td>-33.3 ± 7.3</td>
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<td>-48.3 ± 8.8</td>
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<td>-56.7 ± 10.2</td>
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<td>-40.0 ± 5.8</td>
<td>-35.0 ± 5.0</td>
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<tr>
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<td>-58.3 ± 10.1</td>
<td>-63.3 ± 9.3</td>
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<td>-51.7 ± 14.8</td>
<td>-66.7 ± 13.7</td>
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<tr>
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<td>128</td>
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<td>-70.0 ± 12.6</td>
<td>-70.0 ± 17.6</td>
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<tr>
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<td>8</td>
<td>-40.0 ± 7.6</td>
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<td>-41.7 ± 8.8</td>
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<td>-41.7 ± 6.0</td>
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<td></td>
<td>128</td>
<td>-73.3 ± 12.0</td>
<td>-71.1 ± 10.9</td>
<td>-68.3 ± 16.9</td>
</tr>
</tbody>
</table>

*p < 0.05
of the nictitating membrane was not significantly altered (p > 0.05) although a tendency to be reduced was observable. Pancuronium given after the haloperidol tended to enhance the contraction, but again, statistical significance was not demonstrated (Table 7).

Additional experiments were performed to determine whether alpha-adrenergic blocking effects were responsible for the nictitating membrane results observed with haloperidol at the highest dose of 0.5 mg/kg. This dose was used to insure that sufficient haloperidol was applied in attempting to balance or antagonize the effect produced by pancuronium. In order to substantiate that the haloperidol was indeed working at ganglionic rather than at alpha-adrenergic post-ganglionic neuroeffector sites, a comparison was made with phentolamine, a known alpha-adrenergic antagonist. Figure 12 presents the results of one of those experiments. Haloperidol at the highest dose of 0.5 mg/kg (administered in stepwise progression from 0.1 to 0.2, 0.4, and 0.5 mg/kg) decreased nictitating membrane contractions approximately 20%. On the other hand, phentolamine administered in a like manner to 0.5 mg/kg was able to decrease the nictitating membrane contraction by approximately 60%. Thus, on a molar basis, haloperidol has about 25% of the alpha-adrenergic blocking activity of phentolamine.

Effect of Apomorphine on the Nictitating Membrane

Unlike gallamine and pancuronium, but like atropine, apomorphine abolished the membrane contraction even after its enhancement by gallamine (3 experiments) or pancuronium (2 experiments) (Figure 13). The nictitating membrane contraction resulting from supramaximal post-ganglionic stimulation was not affected by this dose of apomorphine.
### TABLE 7

**MAXIMUM RESPONSE DEVELOPED TO PREGANGLIONIC STIMULATION**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dose</th>
<th>n</th>
<th>Nictitating Membrane (mean ± SE (g))</th>
<th>n</th>
<th>Hyperpolarization s-IPSP (mean ± SE (μV))</th>
<th>n</th>
<th>Slow Depolarization s-EPSP (mean ± SE (μV))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallamine</td>
<td>(2.0 mg/kg)</td>
<td>5</td>
<td>51 ± 17.6</td>
<td>9</td>
<td>5.0</td>
<td>2.93*</td>
<td>-38.0 ± 5.8</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>7</td>
<td>1.8 ± 0.42</td>
<td>4.7 ± 0.72</td>
<td>3.99*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancuronium (0.1 mg/kg)</td>
<td>3</td>
<td>1.9 ± 1.07</td>
<td>3.45*</td>
<td>4.5 ± 1.24</td>
<td>2.53</td>
<td>40 ± 20.8</td>
<td>2.82</td>
</tr>
<tr>
<td>Haloperidol (0.5 mg/kg)</td>
<td>2.4 ± 0.44</td>
<td>2.53</td>
<td>67 ± 19.2</td>
<td>2.53</td>
<td></td>
<td>60.0 ± 15.3</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>3</td>
<td>3.0 ± 1.10</td>
<td>2.25</td>
<td>3.85*</td>
<td>3.85*</td>
<td>1.67</td>
</tr>
<tr>
<td>Haloperidol (0.5 mg/kg)</td>
<td>1.7 ± 0.52</td>
<td>2.43</td>
<td>30 ± 11.5</td>
<td>28.40*</td>
<td>-58.3 ± 10.2</td>
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</tr>
<tr>
<td>Pancuronium (0.1 mg/kg)</td>
<td>3.8 ± 1.34</td>
<td>2.43</td>
<td></td>
<td>1.10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ All data collected following nicotinic blockade with chlorisondamine

* p < 0.05
FIGURE 10

Muscarinic contraction of the nictitating membrane to supramaximal electrical stimulation of the cervical sympathetic trunk. A typical response, after maximal drug effect, was selected in each of three experiments. Response amplitudes were measured at 5-sec intervals, averaged and plotted as an individual trace. The sequence of drug administration was control (chlorisondamine, 2 mg/kg), pancuronium, 0.1 mg/kg, and haloperidol, 0.5 mg/kg. I = S.E.M.
FIGURE 10

TENSION (gms) vs TIME (Sec)

- CONTROL
- PANCURONIUM
- HALOPERIDOL
Muscarinic contractions of the nictitating membrane to supramaximal electrical stimulation of the cervical sympathetic trunk. Traces were derived in the same manner as in Figure 10. The sequence of drug administration was control (chlorisondamine, 2 mg/kg), haloperidol, 0.5 mg/kg, and pancuronium, 0.1 mg/kg.

I = S.E.M.
TENSION (gms)

CONTROL

HALOPERIDOL

PANCURONIUM

TIME (Sec)
FIGURE 12

Nictitating membrane contractions to supramaximal electrical stimulation of the postganglionic cervical sympathetic nerve. A1 and B1 are respective control responses. A2 through A5 is the response after haloperidol at cumulative milligram per kilogram doses of 0.1, 0.2, 0.4 and 0.5. B2 through B5 displays the response after cumulative milligram per kilogram doses of phentolamine equal to its counterpart in the A series.
FIGURE 12

HALOPERIDOL

PHENTOLAMINE
FIGURE 13

Muscarinic contraction of the nictitating membrane was handled and plotted as indicated in Figure 6. The sequence of drug administration was control (chlorisondamine, 2 mg/kg), gallamine (2 mg/kg) or pancuronium (0.1 mg/kg), and apomorphine at 0.1 mg/kg.
FIGURE 13
Surface Potential Experiments

Effect of Gallamine on Ganglionic Surface Potentials

Gallamine enhanced the muscarinic contraction of the nictitating membrane resulting from preganglionic nerve stimulation (Gardier et al., 1974). More direct measurements of ganglionic function were indicated in order to explain the mechanism for the alterations in ganglionic muscarinic-mediated nictitating membrane contractions. D.C. recording of the ganglionic surface potential was elected as the most promising approach to a resolution of this problem. Gallamine given at the optimal neuromuscular blocking dose of 2 mg/kg reduced the s-IPSP by more than 80% but had no significant effect on the s-EPSP (Table 7).

Effect of Pancuronium on Ganglionic Surface Potentials

Like gallamine, pancuronium reduced the s-IPSP elicited through muscarinic ganglionic transmission (Figure 14, Table 7). Three experiments separate from those on the nictitating membrane were completed and indicated that the residual s-IPSP following treatment with either gallamine or pancuronium was susceptible to atropine (Figure 15).

Effect of Haloperidol on Ganglionic Surface Potentials

When surface potential experiments were carried out with haloperidol, the drug was able to enhance the s-IPSP. However, pancuronium was able to overcome the haloperidol effect, but not the reverse (Figure 16, Table 7).
FIGURE 14

Superior cervical ganglionic surface potential changes after brief tetanic stimuli to the cervical sympathetic trunk. A typical response, after maximal drug effect, was selected in each of three experiments. Response amplitudes were measured at 0.25 sec intervals, averaged and plotted as an individual trace. The sequence of drug order was control (chlorisondamine, 2 mg/kg), pancuronium, 0.1 mg/kg, and haloperidol, 0.5 mg/kg. I = S.E.M.
FIGURE 14

- CONTROL
- PANCURONIUM
- HALOPERIDOL
FIGURE 15

Superior cervical ganglionic surface potential's susceptibility to 0.1 mg/kg atropine sulfate in two typical experiments. Experiment I had stimulation parameters of 6 V, 40 Hz, 0.1 msec pulse for 0.5 sec. The previous sequence of drug administration was 2 mg/kg chlorisondamine followed in order by 0.5 mg/kg haloperidol, 2 mg/kg gallamine, 0.5 mg/kg of haloperidol and 2 mg/kg of gallamine. Experiment II had stimulation parameters of 6 V, 32 Hz, 0.1 msec pulse for 0.25 second. The previous sequence of drug administration was 2 mg/kg of chlorisondamine followed in order by pancuronium totalling 0.2 mg/kg and haloperidol totalling 0.1 mg/kg.
FIGURE 16

Superior cervical ganglionic surface potential changes after brief tetanic stimuli to the cervical sympathetic trunk. Traces were derived in the same manner as in Figure 14. The sequence of drug administration was control (chlorisondamine, 2 mg/kg), haloperidol, 0.5 mg/kg, and pancuronium, 0.1 mg/kg. I = S.E.M.
**Figure 16**

- **CONTROL**
- **HALOPERIDOL**
- **PANCURONIUM**

**Graph Details**
- **Y-Axis:** Amplitude (µV)
- **X-Axis:** Time (Sec)
- Time range: 0-6 seconds
- Amplitude range: -60 to 60 µV
Effect of Apomorphine on Ganglionic Surface Potentials

The above mentioned observations on the dopamine antagonist, haloperidol, were complemented by similar studies with the dopamine receptor agonist, apomorphine. Figure 17 demonstrated that apomorphine behaved like gallamine and pancuronium by significantly reducing the s-IPSP resulting from a brief tetanic stimulus. Moreover, like gallamine and pancuronium, apomorphine had no significant effect on the subsequent development of the s-EPSP.

Pre-versus Post-junctional Site of Drug Action

Partial Section of Preganglionic Cervical Sympathetic Nerve

A series of experiments was performed in which the preganglionic nerve was microscopically sectioned once control ganglionic surface potentials were obtained. In this way, the amount of transmitter released from brief tetanic stimulation to the cervical sympathetic trunk could be controlled, and some indication as to the competition between acetylcholine and pancuronium for interneuronal muscarinic receptors could be determined. This technique was devised in order to determine the possibility of presynaptic action on the part of pancuronium (Riker and Okamoto, 1969).

The percentage s-IPSP decrease change between sectioned nerve and whole nerve experiments was significantly different in that the percent decrease was greater in the sectioned nerve preparations. This is indicative of a competitive action at the postjunctional site (Table 8). The s-EPSP, on the other hand, was not similarly affected. In fact, after administration of pancuronium the percentage change in sectioned nerve experiments increased in most instances (Table 9).
FIGURE 17

Superior cervical ganglionic surface potentials before and after 0.1 mg/kg of apomorphine. The traces were derived as in other surface potential experiments and response amplitudes were measured about every 0.2 sec during the first sec, and every 0.5 and 1-sec intervals thereafter. Control refers to the presence of chlorisondamine, 2 mg/kg.
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<th>Frequency (Hz)</th>
<th>Control Nerve (µV)</th>
<th>t</th>
<th>Split Nerve (µV)</th>
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<td>4 V</td>
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<td>55.2%</td>
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<td>82.3%</td>
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<td>10.37**</td>
<td>78.6% + 1.16***</td>
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*p=3
*p<0.05
***S.E.M.
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<thead>
<tr>
<th>Voltage (V)</th>
<th>Frequency (Hz)</th>
<th>Control Nerve (µV)</th>
<th>t</th>
<th>Split Nerve (µV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 V</td>
<td>16</td>
<td>+3.2%</td>
<td></td>
<td>+6.4%</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>+18.5%</td>
<td></td>
<td>+7.3%</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>+16.2%</td>
<td></td>
<td>+38.2%</td>
</tr>
<tr>
<td>6 V</td>
<td>16</td>
<td>+7.9%</td>
<td></td>
<td>+30.4%</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>+14.6%</td>
<td></td>
<td>+13.2%</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>+6.0%</td>
<td></td>
<td>+12.4%</td>
</tr>
<tr>
<td>8 V</td>
<td>16</td>
<td>+13.8%</td>
<td></td>
<td>+54.6%</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>+13.3%</td>
<td></td>
<td>+18.7%</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>+5.6%</td>
<td></td>
<td>+23.7%</td>
</tr>
</tbody>
</table>

| mean      | 11.01% ± 1.80** | 2.26  | 21.34% ± 5.54** |

* n=3
** ± S.E.M.
Once the experiments were completed, the ganglion with attached preganglionic nerve (8-10 mm) was dissected free of its connections and then carefully desheathed of its connective tissue capsule. It was placed in 10% formalin for preservation until histological examination to determine the extent of the nerve sectioning could be made. Plates I and II present two separate experiments in which photographs of the cut were taken.

Direct Drug Injections - Pilocarpine

When pilocarpine was administered intraarterially to the superior cervical ganglion, it caused a long-acting slow depolarization as described by Haefely (1974). Stimulation of the preganglionic nerve during this event resulted in an exaggerated s-IPSP and significantly reduced s-EPSP (statistically equivalent to zero). The s-IPSP was found to be susceptible to inhibition by gallamine (2 mg/kg) (Figure 18, Table 10).

Direct Drug Injections - Methacholine

Close intraarterial injections of methacholine were used to confirm the postsynaptic activity of the muscle relaxants. In Figure 19 are plotted the mean responses to methacholine given before and after gallamine in five experiments (in the absence of nicotinic blockade). As in the experiments in which the surface potential was developed by preganglionic stimulation, gallamine significantly reduced the s-IPSP without altering the s-EPSP (Table 11).
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PLATE I

Photograph of the sectioned preganglionic nerve indicating the extent of damage. The length of the preganglionic nerve ranged from 8 to 10 mm with the cut located near the ganglion body. The damage in this particular experiment shows that a relatively small section (approximately 25% of the diameter) decreased the response by roughly 50%. G indicates location nearest the ganglion.
PLATE II

Second photograph of a separate experiment showing a slight section of the preganglionic cervical sympathetic nerve caused a decrease in surface potential response of approximately 50%. G indicates ganglionic side of the sectioned nerve.
FIGURE 18

Superior cervical ganglionic surface potentials elicited from brief tetanic stimuli preganglionically (control response with nicotinic blockade). Following this, pilocarpine (10 μM) injection i.a. caused a long-acting slow depolarization and preganglionic stimulation during this event produced an enhanced hyperpolarization (s-IPSP) and residual slow depolarization (s-EPSP) presented herein. Gallamine (2 mg/kg) effect was then recorded. The data plotted as in Figure 14 were collected from three experiments. Measurements were made at 0.2 sec intervals during the first sec and at approximately 0.5 sec intervals thereafter.
Superior cervical ganglionic surface potential developed in 10 μg of i.a. methacholine, in the absence of nicotinic blockade, and as affected by 2 mg/kg of gallamine. The data plotted as for Figure 14 were collected from five experiments. Measurements were made at 0.2 sec intervals during the first sec, at 0.5 sec intervals during the next three sec and at 1 sec intervals thereafter.
FIGURE 19

METHACHOLINE CONTROL
METHACHOLINE AFTER GALLAMINE

AMPLITUDE (µV)

TIME (SEC.)

-60
-50
-40
-30
-20
-10
0
10
20
30
40
50
60

--- METHACHOLINE CONTROL
--- METHACHOLINE AFTER GALLAMINE

75
TABLE 10
MAXIMUM GANGLIONIC RESPONSE DEVELOPED TO TETANIC NERVE STIMULATION AS AFFECTED BY INTRAARTERIAL PILOCARPINE AND FURTHER MODIFIED BY GALLAMINE

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dose</th>
<th>n</th>
<th>Hyperpolarization (s-IPSP) (mean ± SE (μV))</th>
<th>t</th>
<th>Slow Depolarization (s-EPSP) (mean ± SE (μV))</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control @</td>
<td></td>
<td>3</td>
<td>65.0 ± 20.80</td>
<td>1.75</td>
<td>-36.7 ± 10.90</td>
<td></td>
</tr>
<tr>
<td>Pilocarpine</td>
<td>10 μM*</td>
<td></td>
<td>88.3 ± 27.30</td>
<td>3.07**</td>
<td>-18.3 ± 10.00</td>
<td>3.05**</td>
</tr>
<tr>
<td>Gallamine +</td>
<td>2 mg/kg</td>
<td></td>
<td>16.7 ± 12.00</td>
<td>3.07**</td>
<td>-11.7 ± 7.30</td>
<td>0.71</td>
</tr>
<tr>
<td>Pilocarpine</td>
<td>10 μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Following nicotinic blockade with 2 mg/kg chlorisondamine
*Volume injection of 0.1 ml
**p < 0.05
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dose</th>
<th>n</th>
<th>Hyperpolarization (s-IPSP) (mean ± SE (µV))</th>
<th>t</th>
<th>Slow Depolarization (s-EPSP) (mean ± SE (µV))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methacholine</td>
<td>10 µg*</td>
<td>5</td>
<td>30.4 ± 4.95</td>
<td>7.83**</td>
<td>-55.6 ± 9.53</td>
</tr>
<tr>
<td>Gallamine +</td>
<td>2 mg/kg</td>
<td></td>
<td>9.4 ± 3.26</td>
<td></td>
<td>-55.2 ± 14.13</td>
</tr>
<tr>
<td>Methacholine</td>
<td>10 µg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*volume injection of 0.05 ml

**p <0.05
Histofluorescent Studies

The selective postsynaptic blockade suggested by the electropharmacological studies was evaluated further by the histochemical fluorescent technique of Falck et al. (1962). *In vitro* exposure of rat superior cervical ganglion to bethanechol, a muscarinic stimulant, reduced fluorescence in both ganglionic neurons and small intensely fluorescent (SIF) interneuronal cells (Libet and Owman, 1974). Plate III presents the result of a ganglion exposed to a control Kreb's solution for thirty minutes. Plate IV illustrates that the fluorescence was greatly reduced with exposure to bethanechol for a comparable incubation period.

Exposure of ganglia to gallamine or pancuronium alone did not have any observable effect on the fluorescence either in ganglionic neurons or SIF cells indicating no significant difference from control values (p < 0.05) (Plate V). However, pretreatment of the superior cervical ganglion with gallamine or pancuronium prevented the decrease in SIF cells fluorescence (p < 0.05) but not the ganglionic fluorescence resulting from *in vitro* exposure to bethanechol (Plate VI, Figure 20).
PLATE III

Photograph of one experiment in which excised rat superior cervical ganglion was exposed to Kreb's Ringer solution. There are two types of fluorescence studied, the general ganglionic neuronal and the interneuronal (SIF) cell type. The arrow is pointing to a cluster of three SIF cells. \( G \) indicates a ganglionic cell.

Filter = K550 barrier filter  
Magnification = 240x
PLATE IV

In vitro exposure to bethanechol (0.5 mM). The fluorescence both in ganglionic neurons and interneuronal (SIF) cells was significantly depressed. Paired ganglia were used as a comparison between control tissues and bethanechol-treated ones. G indicates a ganglionic cell.

Filter = K550 barrier filter  Magnification = 240X
PLATE IV
PLATE V

Exposure of superior cervical ganglia of rats to gallamine (2 mg/kg) or pancuronium (0.1 mg/kg) alone.
No change from the fluorescent pattern observed in control ganglia. G marks ganglionic cells.
Filter = K550 barrier filter      Magnification = 240X
Pretreatment in vivo (2 mg/kg gallamine or 0.1 mg/kg pancuronium) and in vitro (concomitant with bethanechol) of rat superior cervical ganglia. The treatment prevented the decrease in SIF cell fluorescence, but not the ganglionic resulting from in vitro exposure to bethanechol. This data was compared with either paired ganglia exposed to bethanechol alone or ganglia subjected to treatment with the neuromuscular drugs. The arrow is pointing to a SIF cell, and G is marking a ganglionic cell.

Filter = K550 barrier filter  Magnification = 240X
FIGURE 20

Graph presenting the results of the histofluorescent studies. Paired data were used throughout the experiments and later evaluated for significance. If none was indicated, the results of two groups were pooled. Therefore, for the bethanechol treatment, there are six experiments, three of which were paired with controls and three paired with bethanechol + drug. The same procedure was followed for the bethanechol + drug series in that of the total six experiments, three were paired with bethanechol alone and three with gallamine. Drug indicates either pancuronium (0.1 mg/kg) or gallamine (2.0 mg/kg).

I = S.E.M.
DISCUSSION

Frequency-Voltage Response

Classical nictitating membrane and surface potential experiments presented the tools necessary in observing ganglionic function in response to doses of the neuromuscular junction blocking agents, gallamine and pancuronium. Frequency-voltage response experiments performed on the surface potentials indicated that there is no preferential development of any one component of the triphasic waveform. In addition, the development both of the s-IPSP and s-EPSP followed a similar pattern i.e., there was a sharp observable rise in amplitude at all voltages with increasing frequency until a plateau was reached at approximately 32 Hz. Once this plateau was attained, increasing the frequency had virtually no effect on the ensuing amplitudes. Voltages found to be maximal varied between 4-6 V with 6 V usually and 8 V always being supramaximal.

Nictitating Membrane and Surface Potential Results

Cholinergic Antagonists – Gallamine and Pancuronium

Although the possible existence of distinctive agonists and/or antagonists for the two muscarinic sites in sympathetic ganglia has been proposed (Volle, 1968; Haefely, 1972), the studies herein are the first to demonstrate a separation of muscarinic ganglionic hyperpolarization (s-IPSP) and slow depolarization (s-EPSP) by means of drug-induced selective inhibition. This hypothesis is based on the fact that
gallamine (Gardier et al., 1974) and later, pancuronium enhanced the muscarinic-mediated nictitating membrane contraction, while reducing ganglionic hyperpolarization without altering slow depolarization (Gardier et al., 1978).

Pancuronium significantly reduced the s-IPSP resulting from preganglionic tetanic stimulation regardless of experimental conditions. However, the enhancement of the nictitating membrane by pancuronium was dependent on the order of drug administration in that it was not significant when preceded by haloperidol.

**Dopaminergic Antagonist - Haloperidol**

Haloperidol significantly enhanced the s-IPSP when given alone but was unable to statistically alter the muscarinic component of the nictitating membrane-response, although the direction of change is observable. In order to rule out any possible post-ganglionic effect on the part of haloperidol, comparative experiments of the influence by haloperidol and phentolamine, a known alpha-adrenergic antagonist, were conducted. The resultant data indicate that on a molar basis, haloperidol has about one-fourth the alpha-adrenergic blocking activity of phentolamine. This potency is calculated to be of insufficient magnitude to account for the reduction in nictitating membrane response. Direct ganglionic action was not questioned because an alpha-adrenergic blockade at that site would have resulted in decreased rather than increased hyperpolarization.

The inhibition of ganglionic hyperpolarization (s-IPSP) by the neuromuscular drugs was originally construed as involving some blockade of postjunctional dopamine receptors and prompted the investigation of
a dopamine antagonist. On the basis of the haloperidol effects, pancuronium cannot be considered anti-dopaminergic. Furthermore, although haloperidol did not behave as expected i.e., abolish hyperpolarization, it conforms to earlier studies of Carlsson and Lindqvist (1963) as well as more recent experiments of Bunney et al. (1973) (Figure 21). Haloperidol intensifies the s-IPSP presumably by a pre-eminent blocking action on prejunctional receptors, thereby preferentially antagonizing the autoinhibitory activity of released dopamine. Phillipson and Horn (1976) demonstrated that greater inhibitory activity on dopamine-sensitive adenylate cyclase in substantia nigra vs. striatum exists for haloperidol and conforms to a suggested preference for pre vs. post-junctional receptor blockade observed by these experiments. Thus, in the presence of haloperidol, stimulation of the interneuron produces an exaggerated firing and enhanced s-IPSP.

**Dopaminergic Agonist - Apomorphine**

When apomorphine, a dopamine agonist, was tested on the ganglionic surface potential in response to preganglionic nerve stimulation an effect opposite to that of haloperidol was observed. A likely explanation is that apomorphine is stimulating the autoinhibitory presynaptic dopamine receptor, and consequently, it is lowering the firing rate of the speculated dopaminergic interneuron making it less responsive. The possibility was considered that apomorphine was hyperpolarizing the ganglionic noradrenergic cell and thus decreasing its excitatory response to preganglionic stimulation. No alteration in the magnitude of the s-EPSP obviates that argument. Apomorphine reduces the muscarinic-mediated nictitating membrane contraction rather
(A) Effect of chlorpromazine on the activity of a dopaminergic cell in the zona compacta of a rat anesthetized with chloral hydrate. (CPZ) Chlorpromazine administered intravenously (i.v.) in divided doses of 0.5, 0.5, and 1.0 mg/kg caused a marked acceleration of the firing of this dopaminergic neuron.

(B) Effect of haloperidol on the activity of a dopaminergic cell in a non-anesthetized rat. An intravenous (i.v.) injection of haloperidol (HAL) 0.04 mg/kg increased basal activity about 100 percent.
than an expected enhancement. The possibility of postganglionic involvement was ruled out when no change could be demonstrated to supramaximal electrical stimulation of postganglionic fibers.

A possible explanation for the nictitating membrane response following apomorphine is that the drug may be hampering norepinephrine release from the noradrenergic nerve terminal which is not evident during supramaximal postganglionic nerve stimulation. Long et al. (1976) have shown that apomorphine inhibits the end-organ response to low levels of stimulation of postganglionic sympathetic nerves. Since the normal nicotinic pathways are blocked in these experiments, the residual weaker muscarinic ganglionic transmission is theoretically equivalent to submaximal stimulation of postganglionic fibers.

**Revision of Ganglionic Model**

Based upon the results of these experiments, some revisions to the ganglionic model first suggested by Eccles and Libet (1961) have been made (Figure 22). Normal transmission is by way of the N or nicotinic receptors which have been blocked in these studies with chlorisondamine (2 mg/kg). The alternative routes of transmission involve a monosynaptic excitatory muscarinic pathway and a multisynaptic inhibitory pathway (Greengard and Kebabian, 1974). The latter contains an interneuron possessing a muscarinic receptor identified in the figure as M4 at the primary synapse. Upon stimulation of this receptor, an inhibitory neurohumor, presumably dopamine, is released and reacts with pre- and postjunctional receptors at a second synapse. The net ganglionic effect of this is a registered surface hyperpolarization (s-IPSP).
Proposed modification of the sympathetic ganglion model to distinguish the muscarinic receptor on the inhibitory interneuron as $M_i$, which is the suggested site of blockade by pancuronium and gallamine. The muscarinic receptor responsible for the slow depolarization and not affected by the neuromuscular junction drugs is identified as $M_e$. D, the site on the interneuron, indicates the autoinhibitory (prejunctional) dopamine receptor considered to be preferentially blocked by haloperidol. DA distinguish the interneuron as dopaminergic and D on the postganglionic neuron acknowledges the postjunctional dopamine receptor.
FIGURE 22

POSTGANGLIONIC NEURON

INTERNEURON

ACh

N

D

M_e

DA

M_i

ACh

D
The data indicate that pancuronium (0.1 mg/kg) and gallamine (2 mg/kg) block the chinoceptive muscarinic site at the primary synapse \( \text{M}_1 \) thereby preventing stimulation of the interneuron. The decreased release of dopamine reduces the activity at the ganglionic secondary synapse and is consequently recorded as a reduced s-IPSP.

As explained above, the evidence indicates a preference by haloperidol for presynaptic dopaminergic receptors not allowing dopamine to "feedback", resulting in an exaggerated firing rate and enhanced s-IPSP. Irrespective of the site of the hypothesized dopaminergic receptors, cholinergic stimulation at the primary synapse is necessary for demonstrating a haloperidol effect at subsequent synapses. Therefore, when pancuronium and haloperidol were combined in the same experiment, pancuronium overcame the haloperidol effect, but not the reverse.

**Pre- vs. Postjunctional Effect**

Another possible explanation for the effects of gallamine and pancuronium could be that these drugs act prejunctionally to decrease the release of acetylcholine (Riker, 1969; Gergis, 1972). If the muscle relaxants were functioning prejunctionally to decrease acetylcholine release, there would be a decrease in the s-EPSP as well. Since this was not evident in any experiment, another explanation would have to be based on a reduction in acetylcholine release from a select population of preganglionic fibers or alternatively, that the ganglion geometry favors diffusion to the postganglionic neuron. Neither of these is more plausible than the proposed hypothesis of two distinctive muscarinic receptors.
Another possibility is the ability of pancuronium blocking dopamine receptors or inhibiting release of dopamine by an action at the nerve terminal. Inhibition of transmitter release has been associated with prejunctional cholinergic receptors at noradrenergic nerve terminals (Lindmar et al., 1968) and this action was reversed by atropine blockade of the prejunctional cholinergic receptors. If such an action existed at the interneuronal terminal, then the predicted result would be an increased dopamine release by pancuronium, and thus, an opposite action to the one obtained. The following studies were completed to provide additional evidence pro or con that the drug effects are prejunctival in nature.

**Sub-total nerve section**

In order to control the amount of transmitter released and to get some idea as to the competition between the neuromuscular blocking drugs and acetylcholine, surface potentials were recorded wherein the preganglionic sympathetic trunk was partially sectioned. Theoretically, if the pancuronium were indeed acting postjunctionally, it would be logical to assume that the percentage decrease from paired control in s-IPSP should be greater after partial sectioning of the nerve in comparison to the intact nerve. This reasoning is based on the available titer of acetylcholine and the presumed competitive nature of the actions. The percentage decrease in partially sectioned nerve for the s-IPSP indeed significantly exceeded that recorded in whole nerve. The s-EPSP, on the other hand, was not affected in a similar manner. In fact, in the majority of cases there was an increase in s-EPSP amplitude for divided nerve following pancuronium.
This is interpreted as signifying that under conditions of limited availability of acetylcholine, a greater amount of acetylcholine present reacts with the ganglionic muscarinic receptors subserving the s-EPSP since pancuronium is blocking the alternative receptors usually occupied by the neurohumoral agent.

**Drug Injections - Pilocarpine**

Previous reports (Takeshige and Volle, 1968; Haefely, 1972) indicated that pilocarpine, a cholinergic stimulant with muscarinic activity, injected into the ganglionic circulation exhibits somewhat selective activity in producing a long-acting slow depolarization (s-EPSP). Upon stimulation of the cervical sympathetic nerve during this depolarization, Haefely (1974) produced "pure" s-IPSP potentials. This experimental procedure was used in order to observe gallamine's effect on those "pure" s-IPSP's.

When injected intraarterially into the circulation of the superior cervical ganglion in the presence of nicotinic blockade, pilocarpine caused slow depolarization. Subsequent stimulation of the preganglionic nerve exhibited an exaggerated s-IPSP and significantly decreased s-EPSP. In fact, statistical evaluation of the residual s-EPSP correlates it with zero. This is appropriate if pilocarpine occupies the $M_e$ receptors allowing acetylcholine to react with $M_i$ receptors. Interaction with the $M_i$ receptor subsequently releases dopamine and causes an exaggerated s-IPSP during the pilocarpine-induced depolarization. With gallamine (2 mg/kg, i.v.), the s-IPSP is reduced without altering the s-EPSP. This conforms to the proposed mode of action by gallamine and/or pancuronium on $M_i$ receptors.
Drug Injections - Methacholine

Methacholine injections into the arterial circulation of the superior cervical ganglion result in postjunctional stimulation of muscarinic sites. This is recorded as purely ganglionic hyperpolarization and slow depolarization corresponding to the s-IPSP and s-EPSP generated by electrical stimulation of the cervical sympathetic trunk. Gallamine given i.v. inhibits only the hyperpolarization without altering the other muscarinic potential. This presents additional evidence for a purely postjunctional site of action due to the inability of gallamine to alter the magnitude or duration of the slow depolarization potential.

Histofluorescence

Exposure of rabbit superior cervical ganglion to oxygenated bethanechol (0.5 mM) at 37°C depleted catecholamines from both interneuronal (SIF) and ganglionic neuronal fibers when prepared for histochemical examination (Libet and Owman, 1972; Libet, 1977). The same observation is substantiated in rat superior cervical ganglia treated with formaldehyde vapors. That is, the total number of observable SIF cells decreased when exposed to bethanechol in comparison to paired controls. When gallamine or pancuronium was given in vivo and supplements in vitro to the control bath, the SIF cell population was comparable to control values. However, experiments in which ganglia were exposed to the neuromuscular drug and then treated with bethanechol revealed that ganglionic fluorescence resembles that of tissues treated with bethanechol alone, but the number of SIF cells increased in these experiments when compared to bethanechol treatment alone. These results substantiate the assumption that pancuronium
and gallamine are occupying $M_1$ receptors; consequently, depletion by the muscarinic agonist, bethanechol, is prevented.
SUMMARY AND CONCLUSIONS

Optimal stimulation parameters for surface potential experiments obtained were 16 Hz and 4 V, and in all cases, the recordings were made at optimal or supramaximal settings. The development of the triphasic surface potential was singular. That is, there was no preferential development of any one component of the recorded potential.

Gallamine (2 mg/kg) enhanced contraction of the cat's nictitating membrane resulting from ganglionic muscarinic transmission. When more direct observations of ganglionic function were made by recording ganglionic surface potentials, it was found that gallamine reduced the atropine-sensitive s-IPSP without affecting the atropine-sensitive s-EPSP. Pancuronium (0.1 mg/kg), a new neuromuscular junction blocking agent with clinical characteristics similar to gallamine, behaved in a manner identical to gallamine. The inhibited s-IPSP is held responsible for the enhancement of the end-organ (nictitating membrane) response by both gallamine and pancuronium.

Since the development of the s-IPSP theoretically results from stimulation of dopamine receptors on the ganglion cell, the effects of a dopamine antagonist, haloperidol, on the s-IPSP were determined. These were opposite to the muscle relaxants in that haloperidol reduced the muscarinic contraction and enhanced the s-IPSP without altering the s-EPSP.

Apomorphine, a dopamine receptor agonist, inhibited the s-IPSP but depressed the muscarinic contraction of the nictitating membrane. The data from the studies on apomorphine and haloperidol are suggestive.
of a prejunctional dopaminergic site of action for both drugs.

Attempts to record purely postsynaptic events were carried out in order to rule out any prejunctional activity for the neuromuscular blocking drugs. One approach was partial sectioning of the cervical sympathetic nerve. When pancuronium was given in intact nerve surface potential experiments, there was a 50% decrease in the s-IPSP. In comparison, pancuronium in the same dose given in partially-bisected nerve experiments, decreased the s-IPSP by 75%. Theoretically, if the action of pancuronium were prejunctional, its percent occupation of prejunctional sites would be the same, and the same percentage reduction would result in both intact and partially-sectioned nerve. The fact that the inhibition was greater in the partially-sectioned preparation indicated a lower acetylcholine titer competing with pancuronium for postjunctional sites.

A second approach was the direct injection of muscarinic agents such as methacholine into the superior cervical ganglion's circulation which resulted in the production of s-IPSP's and s-EPSP's. Gallamine reduced the s-IPSP without affecting the other muscarinic potential.

Injection of pilocarpine close to the ganglion during nicotinic blockade produced a long-lasting slow depolarization (s-EPSP). Brief tetanic stimuli during the pilocarpine-induced slow depolarization resulted in an enhanced s-IPSP and significantly reduced s-EPSP. Again, gallamine successfully abolished the s-IPSP without altering the s-EPSP.

These data are discussed in terms of unequivocal evidence favoring the proposal of two pharmacologically distinct muscarinic receptors in sympathetic ganglia. These sites have been labeled \( M_1 \) on the dopaminergic interneuron and \( M_4 \) on the ganglion cell itself.
BIBLIOGRAPHY


