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THE EFFECTS OF NEOSTIGMINE UPON GANGLION RESPONSES AFTER ADMINISTRATION OF BLOCKING DRUGS

BY

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The effects of block of autonomic ganglia by chlorisondamine and by hexamethonium, the administration of neostigmine and of atropine upon blood pressure, nervous transmission through the superior cervical ganglion, stimulation of autonomic ganglia by dimethylphenylpiperazinium, and the carotid occlusion reflex, have been studied in the dog anaesthetized with sodium pentobarbitone. The results of these studies have shown: (1) A ganglion blocking agent blocks synaptic transmission in the superior cervical ganglion at the same time as it lowers blood pressure. Neostigmine administered after ganglion-block raises the blood pressure without much change in the response to stimulation of the preganglionic cervical sympathetic nerve. (2) If the effects of preganglionic nerve stimulation are recorded as a contraction of the nictitating membrane, a ganglion-blocking agent also abolishes this response at the same time as it blocks the reflex rise in blood pressure produced by occlusion of both common carotid arteries. Neostigmine administered under these conditions does not affect the responses of the nictitating membrane, but restores the carotid occlusion reflex. This restoration of reflex activity is sensitive to atropine, as is the blood pressure rise. (3) At the same time that neostigmine restores the carotid occlusion reflex, there is no restoration of sensitivity of autonomic ganglia to chemical stimulation by dimethylphenylpiperazinium. In these animals atropine not only blocked the restored carotid occlusion reflex, but produced a further inhibition of the pressor response to dimethylphenylpiperazinium. It is concluded that neostigmine may raise blood pressure by partially restoring autonomic ganglionic transmission, but that total ganglionic function is not restored.

In their studies of the pressor response to neostigmine after ganglion-block, both Hilton (1961) and Long & Eckstein (1961) postulated that this response was mediated at autonomic ganglia. However, these investigators did not measure directly the actions of neostigmine upon ganglionic transmission after ganglion-block. For this reason it was considered important that more complete studies of the effects of neostigmine upon ganglionic function be carried out.

The most desirable method of studying the effects of neostigmine upon transmission of impulses through a ganglion would be to place electrodes upon the pre- and postganglionic nerves and to record the actions of the various drugs at each stage of the experiment. This proved impractical, so the cervical sympathetic preganglionic nerves were stimulated and the response was recorded either as postganglionic nerve impulses or indirectly as the response of the innervated nictitating membrane. The effects of exogenous chemical stimulation on autonomic ganglia were also investigated.

METHODS

Dogs were anaesthetized by intravenous injection of sodium pentobarbitone. Blood pressure was recorded with a Statham transducer from the right carotid artery (Series 2 and 3) or the left femoral artery (Series 1). Three series of animals were studied. In Series 1 (twenty dogs), nerve impulses transmitted through the superior cervical ganglion were studied. The preganglionic nerve was separated from the cervical vagosympathetic trunk by blunt dissection but was not cut. The postganglionic nerve was located along the external maxillary artery and isolated by blunt dissection. In isolating both the pre- and postganglionic nerves, care was taken to avoid disruption of the blood supply to the nerves. Each isolated nerve was suspended on a pair of plastic-shielded platinum electrodes and covered with liquid paraffin. The preganglionic nerve was stimulated by means of an American Electronics Laboratory stimulator at the rate of 12 supramaximal shocks/min. Each shock was at a strength of 5 V for 5 msec. The transmitted impulses were recorded from the postganglionic trunk through a Grass low-level DC amplifier upon a Grass polygraph. The following criteria were applied to ascertain that only the sympathetic preganglionic nerve was being stimulated and that the recorded impulses were passing through the superior cervical ganglion: (1) lack of evidence of efferent vagal stimulation from the recorded pulse contour and heart rate; (2) direct relationship between shocks and recorded impulses (the rate of stimulation was increased to determine whether or not the rate of recorded impulses increased); (3) lack of retrograde conduction (stimulation of the postganglionic nerve failed to produce impulses in the preganglionic nerve); (4) block of transmission by the selected ganglion-blocking agent.

In Series 2 (ten dogs), nerve impulse transmission through the superior cervical ganglion was also studied; however, in these experiments, contractions of the nictitating membrane were measured instead of transmitted nerve impulses. The left vagosympathetic trunk was isolated and cut, but no attempt was made to dissect out the cervical sympathetic nerve. The peripheral end of the cut vago-sympathetic trunk was stimulated by means of a Harvard inductorium and the contraction of the nictitating membrane was recorded with a strain gauge from a ligature through the middle of the nictitating membrane approximately 3 mm from its outer margin. In each animal the strength of stimulus was adjusted to the weakest which would produce maximal contraction of the membrane. The stimulation period was fixed at 10 sec.

In Series 3 (ten dogs), the capability of the autonomic nervous system to respond to chemical stimulation of its ganglia was tested by the use of 1,1-dimethyl-4-phenylpiperazinium iodide (25 μg/kg) injected rapidly into an exposed external jugular vein.

In each series of animals, the function to be tested was studied during each of the four experimental phases: (1) during the control period (after the completion of the surgical procedures, but before any drug other than the anaesthetic agent had been given); (2) after the administration of a ganglion-blocking agent (in experiments of all series, chlorisondamine chloride (1.0 mg/kg) was used, but in Series 1 an additional study was carried out, using hexamethonium chloride (5.0 mg/kg)); (3) after the administration of neostigmine methyl sulphate (0.1 mg/kg); and (4) after the administration of atropine sulphate (1.0 mg/kg). The drugs were always administered intravenously, and the blood pressure was allowed to stabilize after drug administration before testing the experimental procedure.

The records from these experiments were analysed in terms of mean arterial blood pressure for each stage and for each test procedure. Comparisons of changes in blood pressure were made by analysis of differences. Differences were considered statistically significant when the probability was equal to or less than 1% ($P \leqslant 0.01$).

RESULTS

The results are presented in Table 1 and Figs. 1, 2 and 3.

Mean arterial blood pressure. The effects of the different drugs upon the mean arterial blood pressure during each phase of the experiment are similar to those

previously reported by Hilton (1961). The results presented in Table 1 represent the mean responses for forty animals. Block of autonomic ganglia produced a typical fall in blood pressure; neostigmine now increased blood pressure, whereas atropine lowered it. Statistical analysis showed that the blood pressure after

TABLE 1

THE EFFECTS OF GANGLION-BLOCKADE, NEOSTIGMINE AND ATROPINE UPON VARIOUS FUNCTIONS

Values are means and standard deviations

Mean arterial blood pressure (Series 1, 2 and 3, forty dogs)

Treatment	Blood pressure (mm Hg)
Control	144·3±22·24
After chlorisondamine or hexamethonium	97·0±27·76
After neostigmine	138·3±27·95
After atropine	99·3±26·70

Impulse activity in the postganglionic nerve from the superior cervical ganglion (Series 1, ten dogs in each group). * No response in six of the ten dogs

Treatment	Chlorisondamine (μV)	Hexamethonium (μV)
Control	21±9	26±13
After chlorisondamine or hexamethonium	0·0	0·0
After neostigmine	0·0	3±4*
After atropine	0·0	3±4*
Time for complete block after chlorisondamine or hexamethonium (sec)	47·9±24·19	30·5±8·64

Blood pressure change on occlusion of both common carotid arteries, and contraction of the nictitating membrane on vagosympathetic stimulation. (Series 2, ten dogs)

Treatment	Blood pressure change on carotid arterial occlusion (mm Hg)	Nictitating membrane contraction on vagosympathetic stimulation (arbitrary units)
Control	49·6±15·9	13·9±6·7
After chlorisondamine	3·4± 3·5	4·3±2·9
After neostigmine	35·6±20·4	4·0±3·3
After atropine	3·1± 3·5	1·2±1·0

Blood pressure changes due to 25 μg/kg of dimethylphenylpiperazinium and to occlusion of both common carotid arteries. (Series 3, ten dogs)

Treatment	Dimethylphenylpiperazinium (mm Hg)	Carotid arterial occlusion (mm Hg)
Control	79·3±17·65	22·25±9·18
After chlorisondamine	19·5±13·47	6·15±5·97
After neostigmine	26·7±18·58	14·05±9·37
After atropine	7·2± 3·33	5·10±4·51

ganglion-block and the blood pressure after atropine were significantly different from the control blood pressure and the blood pressure after neostigmine, but not significantly different from each other. The control blood pressure and the blood pressure after neostigmine were not significantly different from each other.

Impulse transmission through the superior cervical ganglion. Fig. 1 shows the results of a typical experiment in which the preganglionic nerve was stimulated and

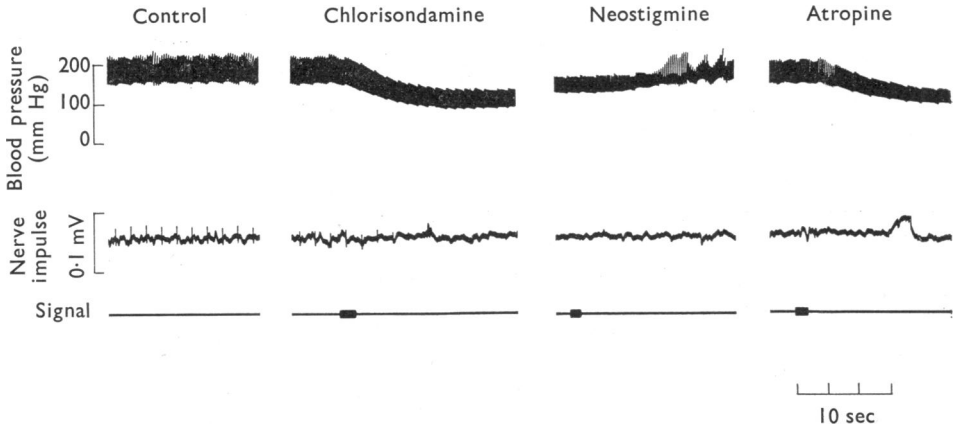

Fig. 1. Dog, 14.5 kg, female. Record of a typical experiment in which nervous activity from the postganglionic nerve from the superior cervical ganglion was recorded during preganglionic nerve stimulation. Uppermost trace: femoral arterial blood pressure; middle trace: impulse activity in postganglionic nerve; lowest trace: injection marker. The dose of chlorisondamine chloride was 1.0 mg/kg; of neostigmine methyl sulphate, 0.1 mg/kg; and of atropine sulphate, 1 mg/kg. The record cut for illustration purposes.

action potentials were recorded from the postganglionic trunk. The mean results for ten animals receiving chlorisondamine and for ten animals receiving hexamethonium are presented in Table 1. Chlorisondamine blocked all transmission for the remainder of the experiment despite the fact that neostigmine raised the blood pressure and atropine abolished the rise of blood pressure due to neostigmine. Hexamethonium also totally blocked ganglionic transmission: this block persisted throughout the experiment in six of the ten animals tested. In the other four animals, there was a slight return of transmission on the administration of neostigmine, which amounted to between 10 and 20% of the original action potential activity. In these four animals atropine did not affect transmission despite the fall in the blood pressure.

Carotid arterial occlusion and nictitating membrane responses. Since it had been previously shown (Hilton, 1961) that the blood pressure responses to electrical stimulation of the peripheral end of the cut vagus nerve and to carotid arterial occlusion were re-established by the administration of neostigmine after ganglion-block, it seemed of interest to determine whether or not another physiological structure would show return of ganglionic transmission after the administration of neostigmine. The function tested was the contraction of the nictitating membrane in response to preganglionic nerve stimulation, using the entire vagosympathetic trunk. The results of these experiments are shown in Table 1 and Fig. 2. Ganglion-block markedly reduced both the contraction of the nictitating membrane and the pressor response to occlusion of both common carotid arteries. Neostigmine partially restored the response to carotid occlusion, but did not alter the block of the nictitating membrane contraction. Atropine blocked the restored carotid occlusion

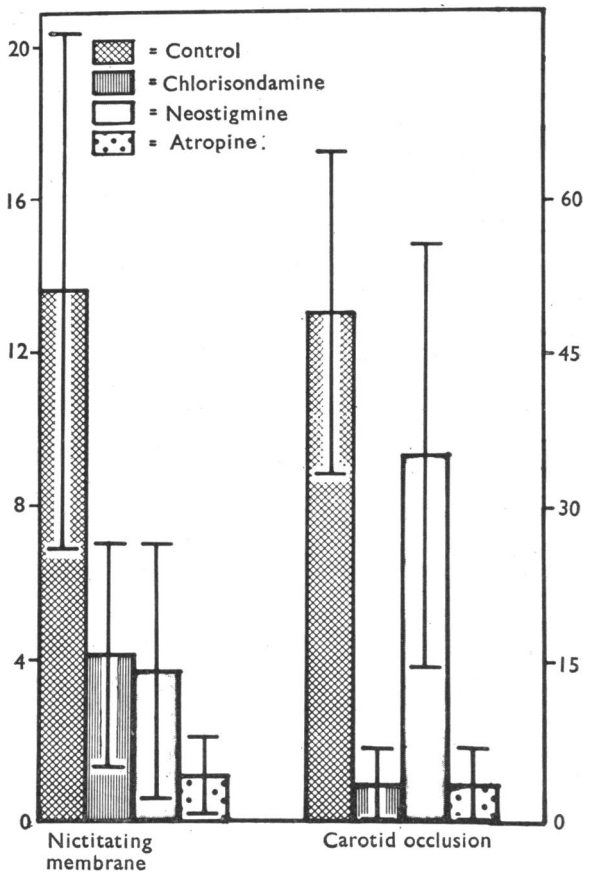

Fig. 2. Response of the nictitating membrane to stimulation of the cervical vagosympathetic trunk, and of femoral arterial blood pressure to occlusion of both common carotid arteries. Each value represents the mean of ten animals. Standard deviations are shown by the central vertical lines. Nictitating membrane responses are given in terms of arbitrary units of contraction, blood pressure changes are increases in mm Hg.

response and further reduced the response of the nictitating membrane to electrical stimulation of the preganglionic nerve.

Response to dimethylphenylpiperazinium and carotid arterial occlusion. The results of administration of dimethylphenylpiperazinium and of occlusion of the common carotid arteries are presented in Table 1 and Fig. 3. During the control period, dimethylphenylpiperazinium greatly increased the blood pressure, by approximately 80 mm Hg. The ganglion-blocking agent, chlorisondamine, reduced this rise to approximately one-quarter of the control increase and reduced the response to occlusion of the carotid arteries. Neostigmine partially restored the carotid occlusion response, but did not greatly alter the ganglion-stimulating effect of dimethylphenylpiperazinium. Atropine blocked the response to dimethylphenylpiperazinium more completely than did chlorisondamine alone and blocked the partially restored carotid occlusion response.

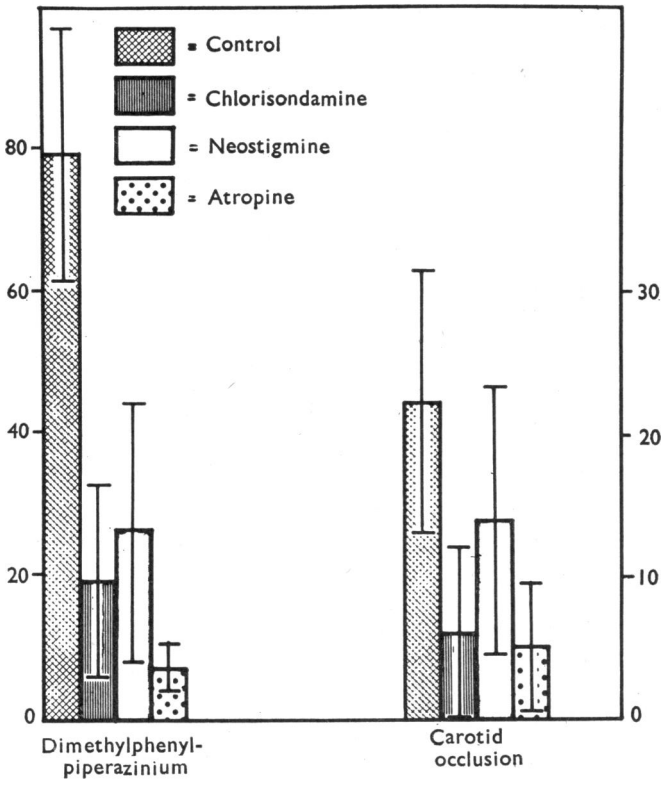

Fig. 3. Changes in femoral arterial blood pressure (in mm Hg) elicited by intravenous injection of dimethylphenylpiperazinium (25 μg/kg), and by occlusion of both common carotid arteries. Each value represents the mean of ten experiments. Standard deviations are shown by the central vertical lines.

DISCUSSION

Our results show that after a ganglion-blocking drug neostigmine has a mixed effect upon ganglionic transmission. Some functions are restored while others remain blocked. Thus, the cardiac response to electrical stimulation of the peripheral end of the cut vagus nerve is restored (Hilton, 1961), as is the blood pressure to, or nearly to, the levels before ganglion-block. The reflex rise in blood pressure due to occlusion of the common carotid arteries is also restored. However, transmission of preganglionic electrical stimulation through the superior cervical ganglion remains blocked, as indicated by the failure to re-establish either the action potentials in the postganglionic nerve, the contraction of the nictitating membrane or the response elicited by chemical stimulation with dimethylphenylpiperazinium.

The mechanism involved in the partial restoration of ganglionic function by neostigmine after ganglion-block is still not completely clear. One explanation for this partial restoration lies in the possible existence of two different ganglion receptor sites, postulated by Levy & Ahlquist (1962) and by Jones & Trendelenburg (1963). Levy & Ahlquist classified these as category I and category II sites, while

Jones & Trendelenburg referred to nicotinic and muscarinic ganglion sites. The evidence for the two sites is the existence of both cardiovascular and nictitating membrane reactions after ganglion-block by established agents (hexamethonium and chlorisondamine) and by the inhibition of these reactions by agents not usually considered as ganglion-blocking drugs (atropine).

Volle (1962) has also shown partial restoration of ganglionic function by anti-cholinesterase drugs. In the presence of a ganglion-blocking agent, dyflos does not restore synaptic transmission through the superior cervical ganglia, but does produce an asynchronous postganglionic nerve discharge. Because of limitations of equipment, no attempt was made to study this asynchronous discharge; therefore, the experiments presented here were able to confirm only the failure of transmission in response to stimulation of the preganglionic nerve. This asynchronous discharge has been reported by Takeshige & Volle (1963) to occur after administration of neostigmine and could explain the restoration of blood pressure and of the carotid occlusion reflex which was seen in our experiments. This asynchronous discharge is transient when the preganglionic nerve is not stimulated but is reinforced and has a prolonged duration when the preganglionic nerve is stimulated. Since the preganglionic fibres to blood vessels are intact in these experiments, the preganglionic nerve potentials should reinforce the asynchronous discharge and could produce the long duration of cardiovascular effects in the experiments reported here.

The failure of neostigmine to restore transmission through the superior cervical ganglion, and thereby allow contraction of the nictitating membrane, partially disagrees with previous results of Mason (1962a). He found that neostigmine produced a direct contraction of the nictitating membrane even in the presence of small doses of the reversible ganglion-blocking agent hexamethonium. There may be two differences between our experiments and those of Mason: first, we used a dose of ganglion-blocking agent considerably larger than that of Mason and, second, Rossum & Ariens (1959) and Trendelenburg (1961) have reported that chlorisondamine is, as a blocking agent, noncompetitive compared to hexamethonium which is considered to be competitive. Our results, then, would agree more closely with Mason's (1962b) later results which showed that ganglion-block did interfere with direct depolarization of the superior cervical ganglia by neostigmine.

Levy & Ahlquist (1962) have demonstrated that dimethylphenylpiperazinium fails to produce a response after ganglion-blockade, while neostigmine continues to do so. Our results extend their observation and demonstrate that during the restoration of blood pressure by neostigmine, the pressor response to dimethylphenylpiperazinium is still blocked. These results would still fit into their concept of two distinct receptors. There are two differences between our results and those of Levy & Ahlquist. The first is our observation that neostigmine seems to return the blood pressure to, or nearly to, the level before ganglion-block. Although duration of response was not measured in the present study, previous work indicates that this response lasts longer than would be expected from simple stimulation. The second difference is the observation that during the pressor response to neostigmine there was a return of the blood pressure responses to carotid arterial occlusion and to stimulation of the peripheral end of the cut vagus nerve. The return of these

reflex responses seems to indicate that neostigmine acts in some manner other than by simple stimulation of a ganglion receptor.

The results of the experiments reported here, together with the work of other authors, have led us to postulate that the pressor response to neostigmine is due to the partial restoration of ganglionic transmission. This is probably due to the preservation of acetylcholine by inhibition of cholinesterase, since Hilton (1961) has shown that this response also occurs after physostigmine. This restoration of function seems to require intact preganglionic fibres and consists of at least two different phases. The first of these is the increase in blood pressure above control level with the fall to, or nearly to, the level before ganglion-block. This phase probably corresponds to the direct ganglion stimulating effects postulated by Levy & Ahlquist (1962). The second phase is the relatively prolonged maintenance of blood pressure at the level which was seen before ganglion-block. This phase seems to correspond to the prolonged asynchronous discharge reported by Volle (1962) to occur on preganglionic stimulation after neostigmine. The fact that there is not full restoration of all ganglionic function is of possible interest, since this may indicate that the nerve fibres controlling the cardiovascular system (the efferent arc of the baroreceptor reflexes and the efferent vagal fibres to the heart) respond differently to the fibres controlling the nictitating membrane and to those involved in the cardiovascular responses to dimethylphenylpiperazinium.

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