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EFFECT OF PREGANGLIONIC NERVE STIMULATION ON SENSITIVITY OF THE SUPERIOR CERVICAL GANGLION TO NICOTINIC BLOCKING AGENTS

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1 Periodic stimulation (every 10 min) of the cervical sympathetic nerve increased the ganglionic block by low concentrations of chlorisondamine (CHL) in the superior cervical ganglion of the cat when compared to the contralateral unstimulated side.

2 Periodic stimulation of the postganglionic nerve was ineffective in increasing the block.

3 Ganglionic block by low concentrations of mecamylamine had the same stimulus dependency, but ganglionic block by any dose of hexamethonium was not influenced by nerve stimulation.

4 Physostigmine infused together with CHL increased the rate of onset of block produced by CHL. Atropine had no apparent effect on the development of ganglionic block by CHL.

5 Repeated intra-arterial injections of 1,1-dimethyl-4-phenylpiperazinium into the circulation of the superior cervical ganglion increased the magnitude of block produced by CHL. Similar injections of methacholine had no effect on ganglionic block produced by CHL.

6 The results are interpreted to indicate that activation of ganglionic nicotinic receptors increased the affinity of receptors for CHL and mecamylamine.

Introduction

Repetitive stimulation of the preganglionic nerve may alter the sensitivity of autonomic ganglion cells to drugs as well as to subsequent nerve stimulation. Iorio & McIsaac (1966) showed that the responses of the nictitating membrane to stimulation of the superior cervical ganglion by non-nicotinic stimulants were augmented by short periods of conditioning preganglionic nerve stimulation. In addition, repetitive preganglionic nerve stimulation may, under certain conditions, unmask an atropine-sensitive response to exogenously administered acetylcholine (Takeshige & Volle, 1962). Ganglionic block by hexamethonium or tubocurarine is well known to be dependent on the frequency of preganglionic nerve stimulation (Paton & Zaimis, 1951; Winters & Volle, 1968).

Traber, Carter & Gardier (1967) reported that after a single intravenous injection of chlorisondamine (CHL) or hexamethonium, stimulation of the cervical sympathetic nerve initially elicited action potentials and a contraction of the nictitating membrane which disappeared in 3-20 seconds. Subsequent stimulations caused no response. This delay in onset of block was independent of time between drug administration and beginning of stimulation.

Our investigation was conducted to study an initial observation in which the magnitude of ganglionic block by CHL appeared to depend on the amount of preganglionic nerve stimulation applied during the infusion of CHL. It was found that periodic preganglionic nerve stimulation increased the apparent sensitivity of ganglion cells to the blocking effect of CHL and mecamylamine. Physostigmine enhanced the effect of nerve stimulation and repeated injections of 1,1-dimethyl-4-phenylpiperazinium (DMPP) mimicked the effect of nerve stimulation.

Methods

Cats weighing between 1.8 and 2.5 kg (mean of 2.0 kg) were anaesthetized with dial-urethane. Both left and right nictitating membranes were prepared for recording the isometric tension in response to supramaximal stimulation of the pre- or postganglionic nerves, or to direct stimulation of the superior cervical ganglion by intra-arterially

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injected drugs. Responses of the nictitating membrane were recorded with a Grass FT-03 force-displacement transducer connected to a Grass Model 5 polygraph. A resting tension of 5 g was applied to the nictitating membranes in each experiment. The preganglionic cervical sympathetic nerves were separated from the vagi, tied and crushed at the level of the clavicle. The nerves were stimulated by square pulses of 0.5 ms duration at 40 Hz and twice the voltage required for maximum responses of the nictitating membrane.

After obtaining control responses of the nictitating membrane to preganglionic nerve stimulation, infusion of the ganglion blocking drug was started and continued for the duration of the experiment. At 0.5 h after the start of drug infusion, the preganglionic nerve of one side was stimulated every 10 min alternately for 10 s (test volley) and 40 s (conditioning volley). The magnitude and rate of block was determined from responses to the 10 s test volley. The contralateral side in the same animal was first tested at periods ranging from 1.5 to 3.5 h after start of infusion of the ganglion blocking drug.

Drugs were usually administered by constant infusion into the cannulated right femoral vein. CHL was usually infused at a rate of 25 $\mu g\ kg^{-1}\ min^{-1}$. The concentration was adjusted to contain that dose in 0.2 ml/minute. DMPP and acetyl-β-methylcholine were injected retrogradely into the blood supply of the superior cervical ganglion via the lingual artery during occlusion of the external carotid artery (Trendelenburg, 1959). The volume of solution injected intra-arterially was 0.1 ml followed by 0.2 ml wash with heparinized physiological salt solution. The time required for the injection and wash was 10 seconds. The physiological salt solution had the following composition (mM): NaCl, 135.9; KCl, 5.6; $CaCl_2$, 2.2; $MgCl_2$, 1.2; $NaHCO_3$, 16.0; NaH_2PO_4, 1.2; and glucose, 11.

The following drugs were used: chlorisondamine chloride (CHL), mecamylamine hydrochloride, hexamethonium chloride, atropine sulphate, physostigmine sulphate, 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) and acetyl-β-methylcholine chloride (methacholine). Doses are expressed as weight of the salt.

Results

Ganglionic block by chlorisondamine

Alkadhi & McIsaac (1973) have shown that during continuous infusion of a ganglion blocking drug (CHL, mecamylamine or hexamethonium) a 10 s volley applied to the preganglionic nerve evoked a biphasic increase in tension of the nictitating membrane. The first component was designated a nicotinic response and it reached a peak tension during stimulation. The amplitude of this response decreased with subsequent tests and continued infusion of the blocking drug. The second component of the response reached a maximum 2-3 s after cessation of stimulation and was sensitive to block by small doses of atropine. It was suggested that the latter response was due to activation of ganglionic muscarinic receptors.

Initial observations indicated that the magnitude of block of the nicotinic response in CHL-treated cats was increased when the preganglionic nerve was frequently stimulated. In order to test the effect of repeated stimulation of the preganglionic nerve on the rate of onset of block of the nicotinic response, a series of experiments were done as outlined in the methods section comparing the onset of block in ganglia in which the preganglionic nerve was stimulated with the onset of block in the contralateral unstimulated ganglia. Figure 1 shows records from a representative experiment in which the rested side was first tested at 3.5 h after start of infusion of CHL. At 3.5 h, the nicotinic response of the side that had been periodically stimulated was almost abolished and only the atropine-sensitive component was seen (lower trace, at 3.5 hours). However, the nicotinic response of the rested side was still about 30% of the pre-CHL control (upper trace at 3.5 h) on the first test. With subsequent periodic stimulation the nicotinic response of the rested side was diminished (upper trace at 5.5 hours).

The time course of decrease of the nicotinic responses of both the stimulated and rested sides in five experiments is summarized in Figure 2. The nicotinic response of the stimulated side decreased at an exponential rate, and 3.5 h after start of infusion it had decreased to about 2% of control. However, at 3.5 h, the response of the contralateral, rested side was still about 30% of control with the first test. The response of the rested side decreased rapidly with subsequent tests which were alternated every 10 min with 40 s conditioning volleys.

The rate of development of block of the nicotinic response by CHL in the absence of periodic stimulation was studied in different groups of cats in which the first test of the rested ganglia was made at 0.5, 1.5, 2.5 or 3.5 h after start of infusion of CHL (Figure 3). With the first test at 0.5 h, the response of the rested ganglia was about 50% of control. After that, however, the mean responses changed very little and there was only about 20% reduction in response in cats

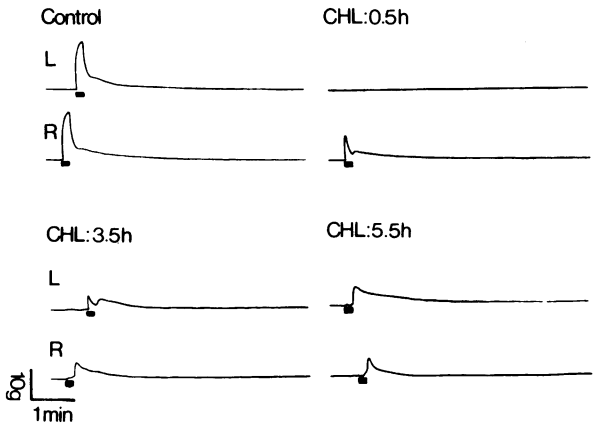

Fig. 1 Typical biphasic responses of the nictitating membrane to preganglionic nerve stimulation in a cat treated with chlorisondamine (CHL). In this experiment, the left nerve (L) was not stimulated until 3.5 h after start of infusion of chlorisondamine after control tests were complete but the right nerve (R) was stimulated every 10 min as described in the methods section. At 3.5 h, the left side was stimulated for the first time and between 3.5 and 5.5 h both sides were stimulated every 10 minutes. Bars under the traces indicate the period of stimulation.

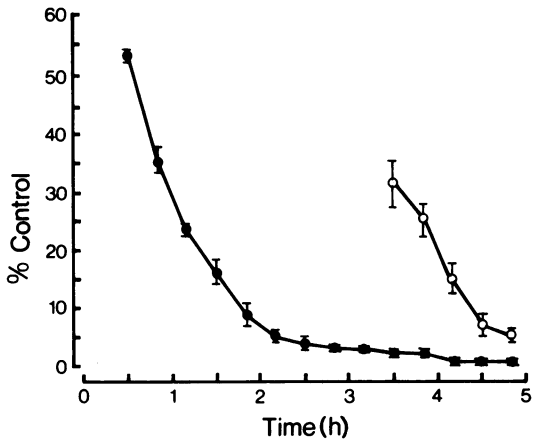

Fig. 2 The time course of decrease of the nicotinic response of the nictitating membrane with preganglionic nerve stimulation during infusion of chlorisondamine (CHL). One nerve was stimulated every 10 min alternately for 10 and 40 s, beginning 0.5 h after start of infusion, only responses to 10 s volleys are shown (•). The contralateral, rested side was first tested at 3.5 h after start of infusion of CHL (○). Amplitude is expressed as % pre-chlorisondamine response (ordinates), abscissa scale is time after start of infusion of CHL. Vertical bars are s.e. mean of five experiments.

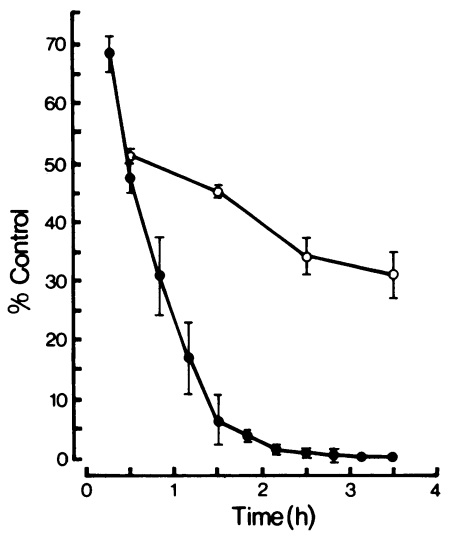

Fig. 3 Comparison of the time course of decrease of nicotinic response of the nictitating membrane during continuous infusion of chlorisondamine in rested (○) and stimulated (•) ganglia. Each value for the rested series is from a separate group of cats (3-11). Each point in the stimulated series is from a single group of three cats. The abscissa scale is time after start of infusion, vertical bars are s.e. mean.

tested after 3 h of continuous infusion. For comparison, Fig. 3 also shows the rate of development of block of the nicotinic response in a group of three cats in which the preganglionic nerve was periodically stimulated beginning at 15 min after start of infusion of CHL.

To test if periodic preganglionic nerve stimulation in the absence of CHL caused a decrease of the response of the nictitating membrane, salt solution was infused instead of CHL (two cats). The responses of the stimulated side and rested contralateral side (tested at 2.5 h

after start of salt solution infusion) were similar in amplitude (range: 79-87% stimulated and 74-76% rested side) and were well maintained for as long as 5 h of infusion of salt solution.

The effect of antidromic activation of ganglionic cells on the magnitude of block of the nicotinic response was tested in three cats. In this group, the postganglionic nerve was stimulated for 40 s every 10 min beginning at 0.5 h after start of infusion of CHL. At 1.5 h after start of infusion, the preganglionic nerve was tested for the first time. The evoked nicotinic response was 32% of control. Although this response is significantly ($P < 0.05$) higher than the nicotinic response (16%) evoked at the same time after periodic preganglionic nerve stimulation, it is lower in amplitude than that of the previous series of rested ganglia tested at 1.5 h after start of infusion (45%) (Figures 2 and 3). This small decrease with periodic postganglionic stimulation may be due to spread of stimulus to the ganglion due to its close proximity to the stimulating electrodes.

In all of the preceding series CHL was infused at 25 $\mu g\ kg^{-1}\ min^{-1}$. At a higher rate of infusion, 75 $\mu g\ kg^{-1}\ min^{-1}$, the nicotinic response of the stimulated side completely disappeared within 50 min of infusion of CHL. When the contralateral, rested side was tested at 1.5 h after start of infusion, the nicotinic response was also completely blocked.

Block by mecamylamine and hexamethonium

To determine whether or not the phenomenon observed with CHL is unique for this agent, the effect of preganglionic nerve stimulation on ganglionic block by mecamylamine and hexamethonium was also investigated as in CHL experiments.

Mecamylamine. When mecamylamine was infused at 25 $\mu g\ kg^{-1}\ min^{-1}$, the nicotinic response of the stimulated side decreased gradually with repeated testing as with CHL, and was about 7% of control at 2 h after start of infusion. The response of the contralateral, rested side averaged about 40% of control when first tested at 2 h after start of infusion.

Infusion of mecamylamine at a higher rate, 75 $\mu g\ kg^{-1}\ min^{-1}$, resulted in a rapid onset of block of the nicotinic response of the stimulated side, and the response of the contralateral, rested side was completely blocked when tested at 2 h after start of infusion of mecamylamine.

Hexamethonium. Infusion of hexamethonium at 500 $\mu g\ kg^{-1}\ min^{-1}$ caused a rapid decrease of the response of the stimulated side. At 2 h after start of infusion of hexamethonium, the nicotinic response of both the rested and stimulated sides were completely blocked. When hexamethonium was infused at a rate of 100-150 $\mu g\ kg^{-1}\ min^{-1}$ (one and two cats respectively), the nicotinic response decreased to 17-35% of control 0.5 h after start of infusion. Stimulation of the preganglionic nerve, thereafter, every 10 min alternately for 10 and 40 s did not cause a further decrease of the response for as long as 3 h after start of infusion. The response of the rested contralateral side, when tested, at 2 h after start of infusion, was similar in amplitude to that of the stimulated side.

Effect of atropine on rate of block with chlorisondamine

In order to determine whether the late atropine-sensitive response influenced in any way the block of the nicotinic response, a mixture of atropine, 1 or 3 $\mu g\ kg^{-1}\ min^{-1}$, and CHL, 25 $\mu g\ kg^{-1}\ min^{-1}$, was infused and periodic testing began 0.5 h after start of infusion. The rates and half-times for decrease of the nicotinic response were calculated and compared with those obtained with infusion of CHL alone. Although the infusion of atropine with CHL caused marked reduction or complete block of the atropine-sensitive component, the rate of decrease of the nicotinic response with preganglionic nerve stimulation in either group was not significantly ($P > 0.05$) different from the rate with infusion of CHL alone (Table 1). Infusion of atropine alone under the conditions of these experiments has been previously shown to have no significant effect on the responses of the nictitating membrane to periodic preganglionic nerve stimulation (Alkadhi & McIsaac, 1973).

Effect of physostigmine

The effect of physostigmine on the rate of block by CHL was studied to determine the role of acetylcholine released from preganglionic nerve terminals on the stimulation-enhancement of ganglionic block. Physostigmine, 5 $\mu g\ kg^{-1}\ min^{-1}$, was infused with CHL. The rate of decrease of the nicotinic response with periodic preganglionic nerve stimulation in the presence of physostigmine was significantly greater ($P < 0.01$) than in experiments in which CHL alone was infused (Table 1). It has been shown previously that infusion of physostigmine alone at the rates used did not significantly influence the responses of the nictitating membrane to periodic preganglionic nerve stimulation (Alkadhi & McIsaac, 1973).

Table 1 The effect of physostigmine and atropine on the rate of ganglionic block during chlorisondamine infusion

Treatment	*Rate constant** *(proportion decrease/min)*	$T_{½}$** *(min)*
Chlorisondamine	0.0214 ± 0.003 (3)†	32.4
Chlorisondamine + physostigmine, 5 μg kg^{-1} min^{-1}	0.0541 ± 0.007 (3)	12.8
Chlorisondamine	0.0253 ± 0.003 (3)	27.4
Chlorisondamine + atropine, 1 μg kg^{-1} min^{-1}	0.0256 ± 0.012 (3)	27.1
Chlorisondamine + atropine, 3 μg kg^{-1} min^{-1}	0.0272 ± 0.004 (4)	25.5

The response of the nictitating membrane was tested every 20 min beginning 0.5 h after start of CHL infusion, 25 μg kg^{-1} min^{-1}. A 40 s conditioning volley was given 10 min after each test.
* Mean with s.e. of the rate constant (k) in: $Y_t = Y_o e^{-kt}$.
** The time required for the response to decrease to one-half of its original value: $T_{½} = 0.693/k$.
† Number of experiments.

Conditioning stimulation prior to infusion of chlorisondamine

The effect of repetitive stimulation prior to the infusion of CHL on the rate of block of the nicotinic response was examined, because ganglionic block has been reported to be more intense in ganglia subjected to repetitive preganglionic nerve stimulation just prior to administration of the ganglion blocking agent (Paton & Zaimis, 1951; Winters & Volle, 1968). Three control responses of the nictitating membrane to 10 s preganglionic volleys were obtained for each side, and 1 h was allowed to elapse to minimize the effect of these tests. Then, the preganglionic nerve of one side was continuously stimulated for 5 min and CHL infusion was started 5 min after the end of the stimulation period. Both the conditioned and the contralateral (unconditioned) sides were stimulated every 10 min alternately for 10 and 40 s beginning at 15 min after start of infusion of CHL. Although the amplitude of the response of the conditioned side at 15 min after start of infusion (mean of 67% of control) was significantly higher ($P < 0.05$) than that of the contralateral unconditioned side (57%), there was no significant ($P > 0.05$) difference between the rates of decrease of the nicotinic response of the two sides with periodic stimulation, 0.0391 ± 0.008 and 0.0368 ± 0.009 min^{-1}, respectively.

Stimulation of ganglion nicotinic and muscarinic receptors

Direct stimulation of the ganglion by cholinomimetic drugs was done to investigate whether activation of ganglionic cholinoceptors would increase the magnitude of ganglionic block. Retrograde intra-arterial injections of DMPP or methacholine were delivered into the blood supply of the superior cervical ganglion via the lingual artery every 10 min beginning 0.5 h after start of infusion of CHL. Although the response of the nictitating membrane to DMPP gradually decreased and was completely abolished about 1 h after start of infusion of CHL, periodic injections of DMPP were continued. In each experiment, intra-arterial injections of physiological salt solution were delivered into the lingual artery of the contralateral side. At 2.5 h after start of infusion, intra-arterial injections were stopped and the preganglionic nerves of both sides were tested for the first time to evaluate the magnitude of block.

1,1-Dimethyl-4-phenylpiperazinium (DMPP). The effect of DMPP was studied in three cats (5 μg, two cats, and 10 μg, one cat). Records from the single experiment in which multiple 10 μg DMPP injections were used are shown in Fig. 4a, and the result from two additional experiments are summarized in Figure 5. The nicotinic response of the DMPP-treated side was either completely abolished (10 μg, Fig. 4a) or reduced to about 8% (range 7-9%) of control (5 μg, Fig. 5), whereas that of the contralateral, control side was still about 39% (range 25-52%) of control. The possibility that the effect of DMPP in increasing block of the nicotinic response is due to desensitization of the ganglion was tested in two experiments. In these experiments, physiological salt solution was infused instead of CHL, and one side received DMPP (10 μg) injections, whereas the contralateral side received salt solution injections retrogradely as in CHL-treated cats. The responses of the nictitating membranes of both sides to

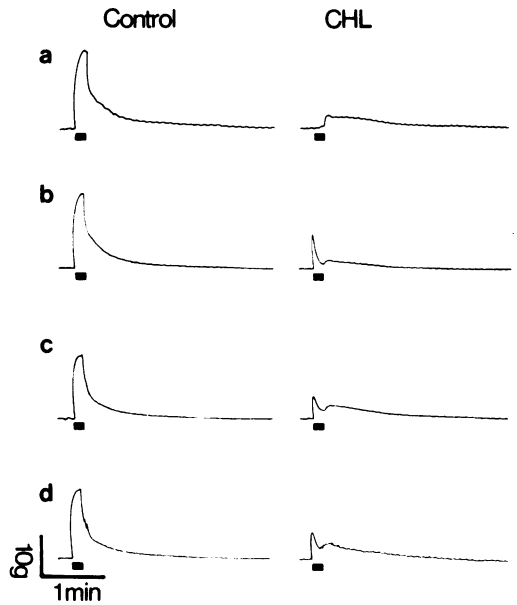

Fig. 4 Responses of the nictitating membrane to preganglionic nerve stimulation evoked before (control) and after 2.5 h infusion of chlorisondamine (CHL). In experiments a and b, close arterial injections of 1,1-dimethyl-4-phenylpiperazinium (DMPP; 10 μg) into the left ganglion (a) and physiological salt solution (saline) to the right ganglion (b) were given every 10 min beginning 30 min after start of infusion of CHL. At 2.5 h after start of infusion, the response of the nictitating membrane of each side was tested for the first time. Experiments c and d were done similarly except methacholine (25 μg) injections were given to the left side (c) instead of DMPP, and saline to the right. Bars under traces indicate time period of stimulation. Calibrations apply to all traces.

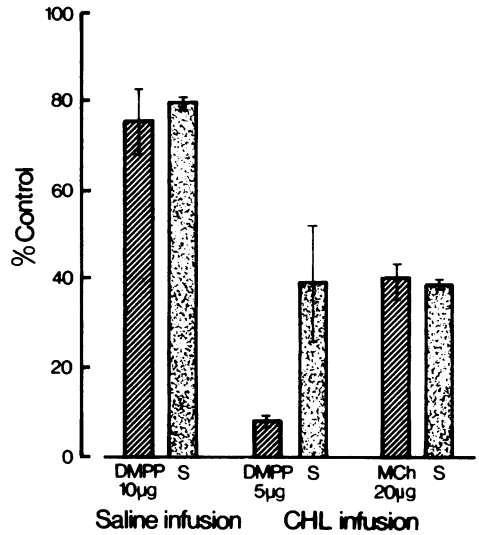

Fig. 5 Effect of multiple injections of 1,1-dimethyl-4-phenylpiperazinium (DMPP) and methacholine (MCh) on response of the nictitating membrane. In each of the three groups of experiments, one side received multiple intra-arterial injections of DMPP or MCh, the contralateral side received injections of physiological salt solution (S) (see Fig. 4 for details). At 2.5 h after start of infusion, responses of the nictitating membrane to 10 s preganglionic nerve volleys were obtained for both drug-injected (hatched bars) and salt solution (saline)-injected (stippled bars) sides. Each bar is the mean of two experiments, vertical lines are ranges.

preganglionic test stimulation at 2.5 h after start of infusion of physiological salt solution were not significantly ($P > 0.05$) different (Figure 5).

Acetyl-β-methylcholine (Methacholine). Methacholine (20 μg, two cats and 25 μg, one cat) injections to one side and physiological salt solution injections to the other side were administered retrogradely into the ganglion circulation during infusion of CHL exactly as with DMPP experiments. Records for one experiment are shown in Figure 4b. The nicotinic response of the methacholine-treated side was similar in amplitude to that of the salt solution-treated side. The response was about 39% (36-44) of control in both sides (Figure 5).

Discussion

Stimulation of the preganglionic nerve increased the ganglionic block by moderate doses of both CHL and mecamylamine, but not by hexamethonium. Since the drugs were infused continually, it was assumed that the plasma concentrations were approaching equilibrium after some hours of infusion and that both the stimulated and contralateral unstimulated ganglia were exposed to equal concentrations of blocking drugs. Traber *et al.* (1967) speculated that the complete blockade which appeared in their series only after stimulation of the preganglionic nerve was due to an increased circulation to the ganglia as a result of increased metabolic activity. However, results reported here do not support this hypothesis. No increase in blockade was observed with repeated nerve stimulation during hexamethonium infusion. Furthermore, stimulation of the ganglion by methacholine, a vasodilator, did not increase the magnitude of CHL block. Lastly, antidromic stimulation of the ganglia had no

influence on ganglionic block. Assuming that the antidromic action potential invaded the soma of the cell (Erulkar & Woodward, 1968), antidromic stimulation would be expected to increase the metabolic activity of the cell.

Our results extend the initial observations by Traber *et al.* (1967) but are not in agreement with their report that hexamethonium block also depended on preganglionic nerve activity. We observed a rapid disappearance of response of the nictitating membrane on the stimulated side when the test was made 15 min after start of infusion of large doses of CHL or hexamethonium. However, the plasma concentrations of drug are probably still rising at this time. When the contralateral nictitating membrane of the unstimulated side was tested after an hour or more of infusion, complete block was attained with the first test. Such a control was not done by Traber *et al.* (1967).

The reason for lack of effect of preganglionic nerve stimulation on the magnitude of block by low doses of hexamethonium is unknown. The explanation may be related to a difference in the mechanism of action between hexamethonium, CHL and mecamylamine. Hexamethonium is believed to be a competitive blocking agent, whereas mecamylamine has a dual mechanism, both competitive and non-competitive, and CHL is a non-competitive blocking agent (Van Rossum, 1962).

The stimulus-dependence of blockade to CHL and mecamylamine was observed only with moderate (submaximal) doses and is different from the well known stimulus frequency dependence of ganglion block (Paton & Zaimis, 1951; Winters & Volle, 1968). The fact that the phenomenon described here was seen only with moderate doses of CHL or mecamylamine does not exclude its occurrence in the first few minutes after administration of high doses. However, large doses probably saturate all the available receptors rapidly and any change in sensitivity of the receptor population may be obscured.

The results described here may be explained by a hypothesis that the increase in magnitude of ganglionic block following preganglionic nerve stimulation is a consequence of interaction of acetylcholine released from preganglionic nerves with nicotinic receptors. The changes initiated by this interaction are unknown, but they may result in a conformational change in the nicotinic receptor and/or surrounding membrane that increases the affinity of the receptor for blocking drugs. This hypothesis is supported by the observations that the effect of nerve stimulation was enhanced by physostigmine, and that intra-arterial injections of DMPP had an effect similar to nerve stimulation. On the other hand, antidromic activation of ganglion cells had no effect and intra-arterial injections of methacholine did not cause enhancement of ganglionic block. The lack of effect of preganglionic nerve stimulation prior to infusion of CHL on the rate of block may indicate that these changes are transient unless CHL is concurrently present. This finding is at variance with those of Winters & Volle (1968) who showed that stimulation prior to injection increased the subsequent ganglionic block. However, an important complicating factor in their experimental procedure is the possibility of transmitter depletion with continuous stimulation.

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