PHARMACOLOGY

THE INFLUENCE OF ANTICHOLINERGIC SUBSTANCES ON THE FUNCTIONAL STATE OF THE SUPERIOR CERVICAL GANGLION

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The present communication presents a comparative evaluation of the activity of a number of anticholinergic substances with respect to their influence on the functional state of the superior cervical ganglion.

EXPERIMENTAL METHOD

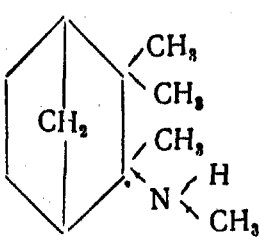
Experiments were performed on decerebrate and anesthetized (urethane) cats. Contractions of the nictitating membrane and bioelectric currents of the postganglionic fibers were recorded with various frequencies and definite duration of preganglionic trunk stimulation. The level of lability before and after administration of pharmacologically active substances was established (D.A. Kharkevich [3,4,5,6]). In control experiments the pessimal reaction usually occurred at frequencies of 60-100 cps, although sometimes inhibition was not marked even at frequencies of 150-200 cps. This is connected not only with individual variations between animals but also with the technique of the operation itself. Thus, for example, when transection of the preganglionic sympathetic trunk precedes decerebration the pessimum occurs at higher frequencies than when the sequence is reversed, and so on.

In some experiments, in addition to electric stimulation of preganglionic fibers, chemical stimulation of the ganglion was also used. This was achieved by acetylcholine which was introduced in amounts of 40-80 γ in a retrograde manner into the lingual artery. The external carotid artery was tied in these experiments.

The following anticholinergic substances were investigated: mecamine* (mecamylamine), atropine, scopolamine, d-tubocurarine and decamethonium. The substances were injected into the femoral vein.

EXPERIMENTAL RESULTS

The first of the substances investigated – mecamine – is a new long-acting ganglion – blocking agent (C. A. Stone, M. L. Torchiana, A. Navarro and K. H. Beyer [12]; A. E. Doyle, E. A. Morphy, G. H. Neilson [8]). Chemically, the preparation is 3-methylaminoisocamphane, i.e. a secondary amine (see formula):



* Mecamine was synthesized in the Chemistry Department of the Institute of Pharmacology and Chemotherapy, AMN SSSR.

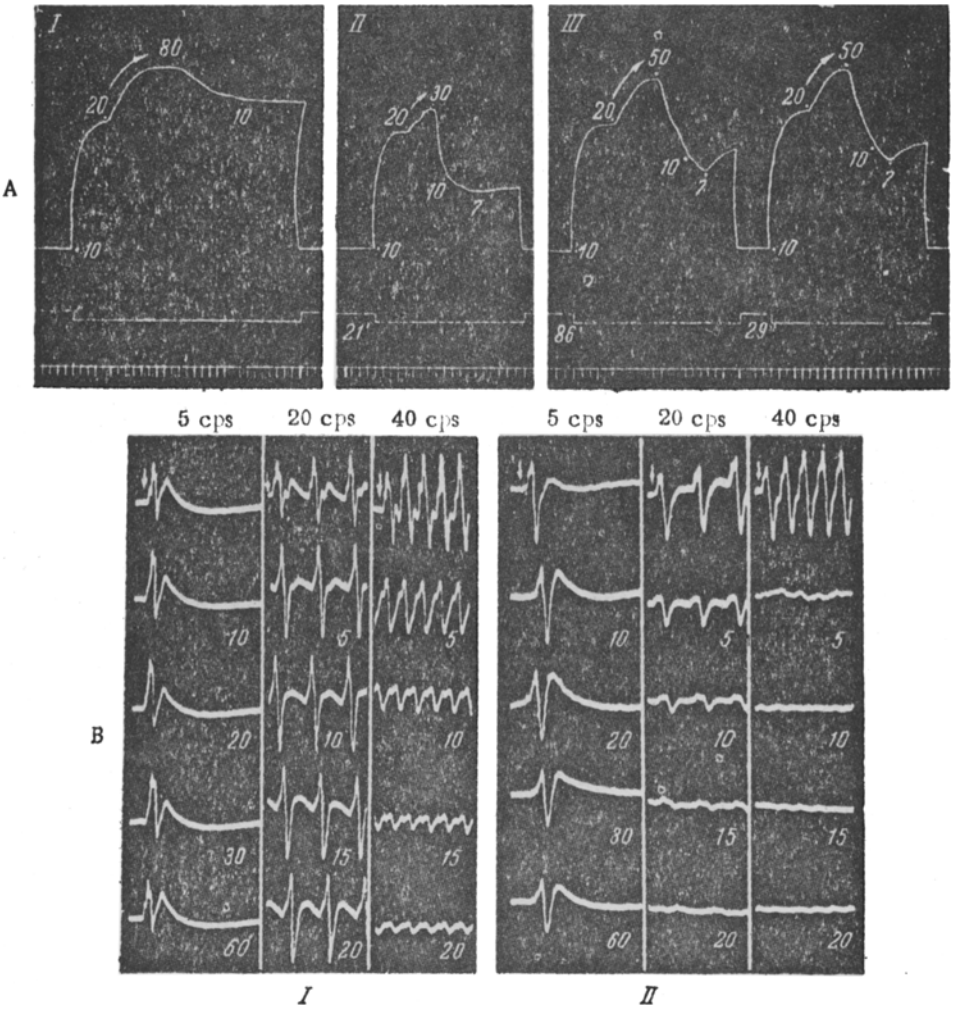


Fig. 1. The effect of mecamine on lability of the superior cervical ganglion. A) Mechanograms of nictitating membrane contractions. Figures designate frequency of stimulation of preganglionic trunk (in cps). Arrows correspond to gradual transition from one frequency to the next (I) before administration of mecamine; II) 21 minutes after injection of 0.5 mg/kg mecamine; III) 107 minutes after injection. B) Biocurrents of postganglionic fibers. Frequency of preganglionic trunk stimulation is marked over each column of oscillograms. Vertical arrow (↓) marks beginning of stimulation. Figures over each oscillogram designate time from beginning of stimulation in seconds (read from above down) (I) before mecamine injection; II) 9 minutes after injection 0.5 mg/kg mecamine). Experiments A) and B) were performed on decerebrate cats with maximal amplitude of stimulation and duration of each stimulus of 0.5 millisecond (A) and 0.1 millisecond (B).

As shown experimentally, mecamine has a profoundly depressing effect on the functional state of the superior cervical ganglion. The threshold dose for this substance is 5-10 γ/kg. In doses of 0.25-0.5 mg/kg mecamine shifts the pessimum markedly towards the lower frequencies, the duration of this effect being several hours. Thus, Fig. 1, A) demonstrates that 21 minutes after administration of 0.5 mg/kg mecamine the pessimal reaction occurred at the frequency of 30 cps, where as it was barely perceptible at 80 cps prior to injection

of the substance. It is characteristic that the change from the pessimal frequency of stimulation (30 cps) to the optimal (10 cps) was not accompanied by restoration of the level of the curve. At the frequency of 7 cps relaxation of the nictitating membrane ceased and its tonus began to increase slowly. After 2-2½ hours following injection of mecamine the pessimum occurred at 50 cps despite the fact that the initial amplitude of nictitating membrane contraction at 10 cps as well as the total height of the curves practically reached the original level. In this case too the pessimal reaction was not abolished by an abrupt change of stimulation frequency from 50 to 10 cps. However, when the frequency of stimulation was decreased to 7 cps transmission of excitation in the ganglion was restored. The nictitating membrane tonus began to increase perceptibly. The duration of the mecamine effect can also be judged from Fig. 1, A) which shows that after administration of 0.5 mg/kg mecamine the reduction of ganglionic lability continues for well over 2½ hours. According to data obtained by Yu. V. Uranov therapeutic doses (1-2.5 mg/kg) of mecamine depresses autonomic ganglia for periods of 6-18 hours.

In the dose of 1 mg/kg, mecamine causes considerable reduction in ganglionic excitability. The pessimum is shifted even more sharply towards the lower frequencies and occurs at rhythms which had been optimal prior to administration of this substance. The events are shown to be occurring in the ganglion itself by changes in the biopotentials of the postganglionic fibers. Results of one such experiment are presented in Fig. 1, B). The oscillograms show that following administration of 0.5 mg/kg mecamine the most definite changes are observed at the higher frequencies of stimulation. Thus, following administration of mecamine the biopotentials, at 40 cps, almost completely disappeared by the 5th second after beginning of stimulation. At the 15th-20th second barely perceptible oscillations were observed at 20 cps. Prior to administration of mecamine definite biocurrents were recorded at these frequencies and with analogous intervals of time; these biocurrents were synchronous with the rhythm of preganglionic trunk stimulation. It must be mentioned that at 5 cps the biocurrents were somewhat increased under the influence of mecamine.

Fig. 2 and 3 show that following administration of 1 mg/kg mecamine, the biopotentials of the postganglionic fibers at frequencies of 20-60 cps only appeared in response to the first few stimuli after which they disappeared. Considerable changes were also observed at 5 cps, i.e. at such a frequency at which, under ordinary conditions, the potentials were of high voltage and maintained their amplitude for prolonged periods of time.

Comparison of the results obtained with data published earlier concerning quaternary ammonium bases (D. A. Kharkevich [4,5,6]) leads to the conclusion that mecamine lowers the lability of the superior cervical ganglion for a substantially longer period of time than hexonium and pentamine. Mecamine approaches hexonium* in its activity.

Besides mecamine, compounds with predominant activity in the region of cholinereactive structures were investigated. The first of these – atropin – was used in doses from 0.5 to 4 mg/kg. It was found that this substance in doses of 1-3 mg/kg slightly diminished the functional lability of the ganglion (by 10-20 cps). These changes are only observed in those cases in which the excitability of the ganglion is appreciably decreased. Transmission of excitation in the superior cervical ganglion when atropine was used in the dose of 1 mg/kg diminished by an average of 15-25%. More pronounced lowering of the amplitude of nictitating membrane contractions (of the order of 50%) was noted in isolated experiments, even when the dose of the substance was increased to 2-4 mg/kg.

When the postganglionic fiber biocurrents were recorded it was found that atropine diminished the amplitude of the potentials slightly (Fig. 2).

The second preparation in the atropine group – scopolamine – proved to be an even less active compound. Maximal changes in ganglionic transmission which were expressed in diminution of the nictitating membrane reaction did not exceed 5-10%, and were, moreover, inconstant. No changes in the functional state of the superior or cervical ganglion could be detected. The doses of scopolamine used in these experiments were from 0.3 to 5 mg/kg.

* Transliterated from Russian – probably hexamethonium.

The third group of anticholinergic substances investigated included two muscle relaxants with different mechanism of action – d-tubocurarine and decamethonium.

D-tubocurarine was used in doses 0.15-0.3 mg/kg. This preparation caused definite decrease in ganglionic lability. The experiments showed that following administration of this substance the pessimum was shifted towards the lower frequencies and relaxation of the nictitating membrane occurred more sharply than in the control experiment. The excitability of the ganglion changed only slightly (usually not more than by

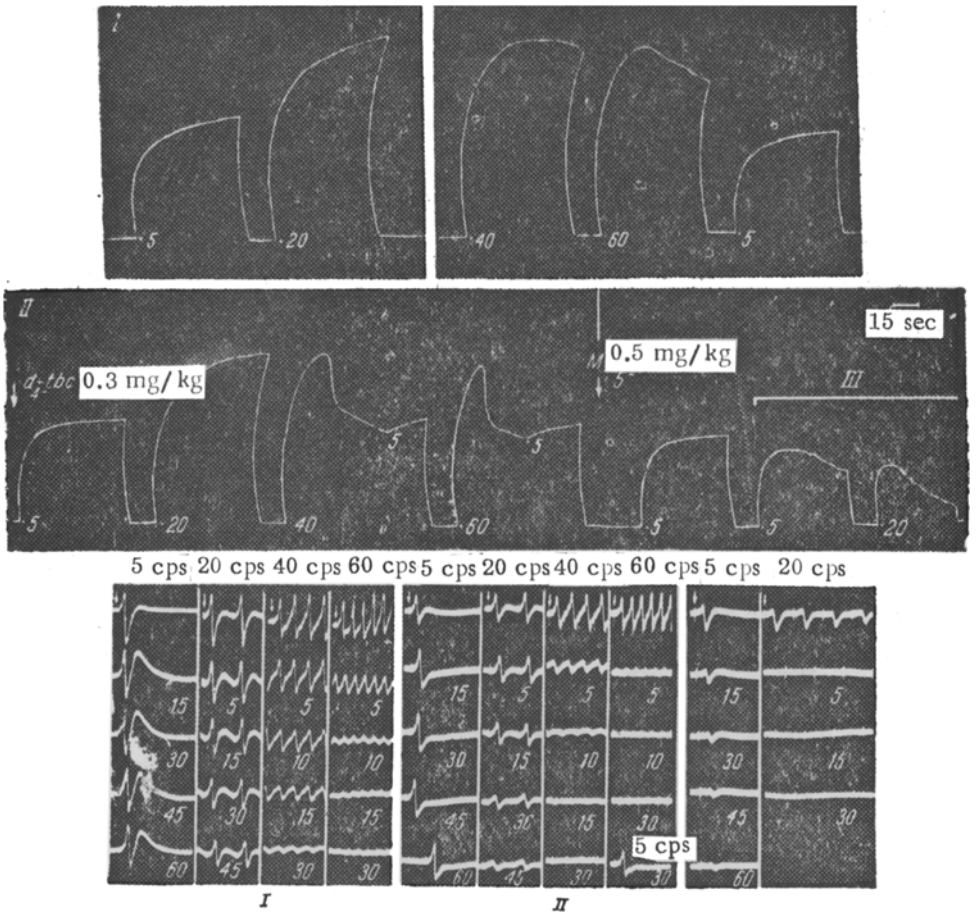


Fig. 2. Postganglionic fiber biocurrents. Vertical arrow (↓) beginning of stimulation. Figures underneath each oscillogram – time from the beginning of stimulation in seconds (read from above down). Frequency of preganglionic trunk stimulation (in cps) marked under each column of oscillograms.
I) Before administration of the substance, II) 4 minutes after injection of atropine 1 mg/kg; III) 8 minutes after injection of mecamine 1 mg/kg. Experiment performed on decerebrate cat. Duration of each stimulus 0.1 millisecond. Amplitude of stimulus – supramaximal.

10-20% at 10 cps). Duration of d-tubocurarine action in such experiments was on the average 1-1½ hours.

The inhibitory action of d-tubocurarine on the functional lability of the superior cervical ganglion was also confirmed oscillographically. The postganglionic fiber biocurrents were somewhat diminished following administration of the substance and ceased to be reproduced after a shorter interval of time than prior to its use. Some experiments were staged with simultaneous recording of biopotentials and of nictitating membrane contractions (Fig. 3). These experiments demonstrated particularly convincingly that d-tubocurarine enhances

pessimal inhibition in the ganglion. If at the moment of pessimal reaction, accompanied by absence of biopotentials and relaxation of the nictitating membrane muscle, the pessimal frequency was replaced by a known optimal (e.g. 5 cps) frequency restoration of the membrane tonus and appearance of biopotential were observed (Fig. 3, II).

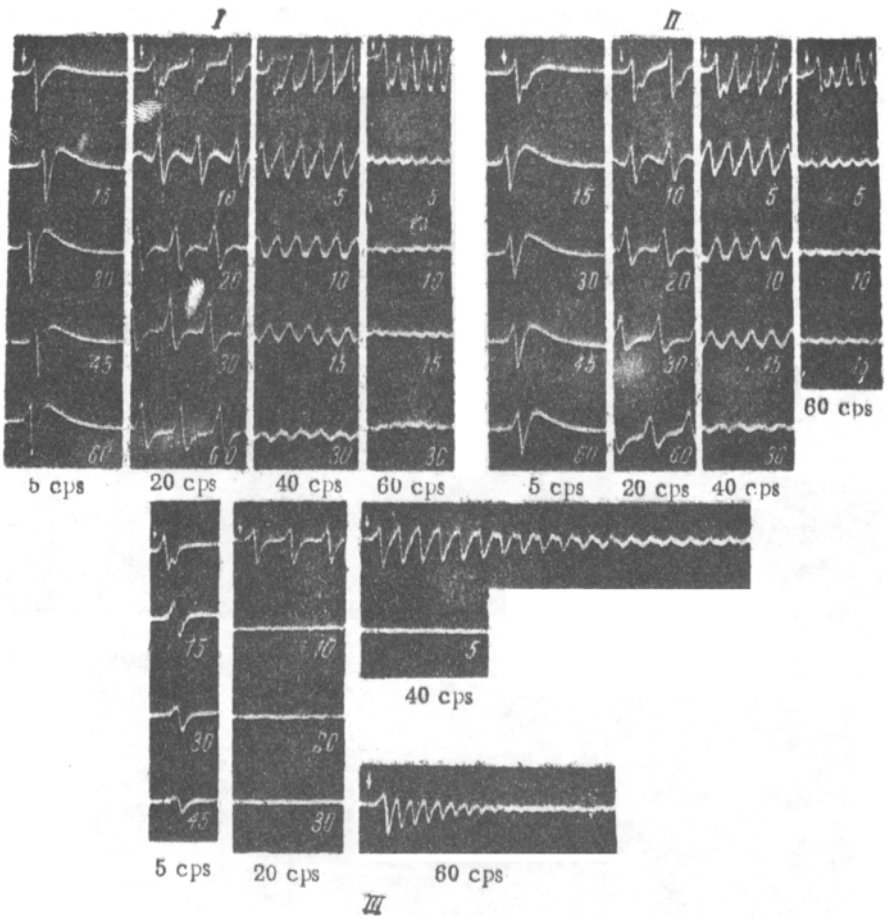


Fig. 3. Simultaneous recording of nictitating membrane contractions (A) and postganglionic fiber biopotentials (B).*
Corresponding biopotentials and mechanograms are designated by the same figures (I, II, III). Figures at the beginning of each row of mechanograms – frequency of stimulation of the preganglionic trunk (in cps). Figures underneath each oscillogram – time from the beginning of stimulation (in seconds, see from above down). Vertical arrow (|) beginning of stimulation. I) Before administration of the substances; II) 4 minutes after injection of d-tubocurarine 0.3 mg/kg; III) 9 minutes after injection of mecamine 1 mg/kg. The experiment was performed on decerebrate cat with artificial respiration. Duration of each stimulus 0.1 millisecond. Amplitude of stimulation – maximal.

D-tubocurarine thus possesses the ability to diminish the functional lability of the superior cervical ganglion. This effect is sufficiently clearcut but considerably less pronounced than that of mecamine, hexonium or pentamine.

* A and B not identified in figure – Publisher.

Unlike d-tubocurarine, decamethonium proved to be little active with respect to the superior cervical ganglion. Changes in the height of the mechanograms following the use of decamethonium in doses from 30 to 100 γ/kg. were negligible. The ganglion lability also remained unchanged. In order to make sure that the substance reached the ganglion, the animal was given tetraethylammonium at the end of the experiment. This was accompanied by consistent lowering of the amplitude of nictitating membrane contractions, together with a shift of the pessimum towards the lower frequencies. Decamethonium actively inhibited neuromuscular transmission in doses considerably smaller than those which were used in experiments on the superior cervical ganglion.

Decamethonium can thus be taken to be without effect on the functional state of the superior cervical ganglion cells.

SUMMARY

The investigations showed that the substances tested exert an inhibitory influence on the functional state of the ganglion. The most active compound was mecamine. It lowered ganglionic lability very markedly and for a prolonged period of time. D-tubocurarine in doses which inhibit neuromuscular transmission possesses analogous but considerably less pronounced action. Decamethonium is practically without effect on the functional lability of the cervical ganglion. In this respect it resembles ditilin* which is also practically devoid of ganglioplegic activity. It can thus be taken that curare-like substances with a depolarizing type of action do not exert a negative effect on transmission of impulses in the superior cervical ganglion. In this they differ substantially from muscle relaxants with a competitive type of action (d-tubocurarine, diplacin**).

Atropine slightly lowers the functional activity of the ganglion and only with decrease in its excitability which is also slight. Scopolamine affects ganglionic transmission of impulses to an even lesser extent. Practically, these changes in ganglionic transmission can be considered as negligible. The lability of the ganglion is unaffected by scopolamine.

The present investigation and investigations published earlier (D. A. Kharkevich [3,5,6], A. V. Valdman, Z. N. Ivanova and D. A. Kharkevich [1]) have been concerned with the study of the effect of many anticholinergic substances on the functional state of the superior cervical ganglion. These have included ganglioplegic agents (mecamine, hexonium, pentamine tetraethylammonium, nicotine, pachycarpine,* sparteine), muscle relaxants (d-tubocurarine, diplacin, decamethonium, ditilin) as well as substance which are mainly active in the region of cholinereactive structures (atropine, scopolamine, spasmolytin*). Comparison of the data obtained leads to the conclusion that all the substances which inhibit transmission of impulses in the ganglion lower its functional activity. Restoration of these two parameters occurs in the reverse order. These relationships are the rule in the case of ganglioplegic substances. For some substances the influence on lability can be considered as the main manifestation of their action on the ganglion (e.g. for diplacin).

The mechanism of pessimal inhibition and its shift, under the influence of ganglion blocking agents, towards the lower frequencies still remains uncertain. Many authors (A. G. Ginetsinsky and I. M. Shamarina [2]; G. L. Brown and W. Feldberg [7] and others) consider that acetylcholine plays the chief role in the development of the pessimum. However, in recent years H. Kewitz [9,10,11] has published work in which he denies altogether participation of acetylcholine in the transmission of nervous excitation in the ganglion. This is based on the results of experiments on the perfused superior cervical ganglion of cat. The author established that atropine, scopolamine, novocain and trasentine abolished the excitatory action of acetylcholine on the ganglion without affecting the transmission of excitation associated with electric stimulation of preganglionic sympathetic fibers.

In our investigation analogous experiments were performed but on the intact animal with preservation of blood supply to the superior cervical ganglion. Acetylcholine was given by retrograde injection into the

* Transliteration of Russian – Publisher's note.

** Russian trade name.

lingual artery. It was found that atropine and novocain reduced the ganglionic reaction to acetylcholine and at the same time inhibited synaptic transmission in the ganglion associated with electric stimulation of preganglionic fibers. Scopolamine was without effect in either case.

Thus in our view there is yet no sufficient basis for denying the participation of acetylcholine both in the transmission of nervous excitation and the development of pessimal inhibition.

LITERATURE CITED

[1] A. V. Valdman, Z. N. Ivanova and D. A. Kharkevich, Farmakol. i Toksikol, 2,3-11 (1955).

[2] A. G. Ginetsinsky and I. M. Shamarina, Trudy Fiziolog. Inst. im. I. P. Pavolov , Leningrad, 4, 139-148 (1949).

[3] D. A. Kharkevich. Thesis, The Effect of Ganglion Blocking and Narcotic Substances on the Transmission of Excitation in the Superior Cervical Ganglion at Various Frequencies of Preganglionic Trunk Stimulation,* Leningrad (1953).

[4] D. A. Kharkevich, Farmakol. i Toksikol, 1, 3-9 (1956).**

[5] D. A. Kharkevich, Farmakol. i Toksikol, 3, 3-9 (1956).**

[6] D. A. Kharkevich, Byull Eksptl. Biol. i Med. 10, 34-38 (1956).**

[7] G. L. Brown and W. Feldberg, J. Physiol. , 86, 290-305 (1936).

[8] A. E. Doyle, E. A. Morphy, G. H. Neilson, Brit. Med. J., 24, 1209-1211 (1956).

[9] H. Kewitz, Arch Exp. Path. u Pharmakol., 222, 1-2 235-237 (1954).

[10] H. Kewitz, Arch Exp. Path. u Pharmakol., 222, 3, 323-329 (1954).

[11] H. Kewitz, Arch Exp. Path. u Pharmakol., 225, 1-2, 111-112 (1955).

[12] C. A. Stone, M. L. Torchiana, A. Navarro and K. H. Beyer, J. Pharmacol. a exp. Therap., 117, 2, 169-183. (1956).

* In Russian.

** Original Russian pagination. See C.B. Translation.