PHARMACOLOGY

CHANGES IN THE LABILITY OF THE UPPER CERVICAL GANGLION CAUSED BY THE ACTION OF QUATERNARY AMMONIUM COMPOUNDS

D. A. Kharkevich

From the Laboratory of Special Pharmacology (Director: Acting Member Acad. Med. Sci. USSR V. V. Zakusov) Institute of Pharmacology Experimental Chemotherapy and Chemoprophylaxis (Director: Acting Member Acad. Med. Sci. USSR V. V. Zakusov) Acad. Med. Sci. USSR, Moscow.

(Received June 4, 1955. Presented by Acting Member Acad. Med. Sci. USSR V. V. Zakusov)

Quaternary ammonium compounds attract the attention of pharmacologists and clinicians because they possess a great activity when used in curare-like preparations or when they are used with the aim of depressing vegetative ganglia.

The present experiments were devoted to a study of the influence exerted by some quaternary and bis-quaternary compounds on the transmission of impulses in the upper cervical ganglion.

The experiments were performed on decerebrated cats. The preganglionic fibers were excited by right angled stimuli of a frequency from 5-10 up to 200 cycles per second. The duration of each stimulus was 0.1-0.2 m/seconds. The source of each stimulus was an electronic stimulator.

The contractions of the nictitating membrane were registered. In the beginning the stimulating rhythm was of the optimal size (usually only 10 cps). As soon as the height of the mechanical graph reached a maximum, the frequency of the stimulation was gradually increased (every 3-5 seconds, 10 cps). Up to 40-45 cps the amplitude of the contractions of the third lid increased, then it stabilized, after which the tonus of the lid fell. The cells of the ganglion were then in the least optimal state. This least optimal state usually corresponded to a frequency of 70-100 cps and sometimes up to 200 cps. To prove the least optimal nature of the developing inhibiting reactions against the background of a weakened muscle, this least optimal frequency was abruptly altered to the optimal frequency. As a result, the tonus of the lid became restored.

Some experiments were done with another modification. The preganglionic fibers were stimulated with "sub-least optimal" and "least optimal" frequencies for 2-5 minutes. Usually at the beginning of the stimulations there was the greatest rise of the curve, after which ensued a more or less continued lowering. To observe the contraction curves of the third lid after the introduction of a substance the appended graphs should be studied (Fig. 2, B, C).

As a control of the action upon the ganglion of the substances studied we used the registration of the postganglionic fiber potentials. The preganglionic fibers were stimulated by stimuli of various frequencies. The bioelectricity, after the corresponding increase, was registered by one of the rays of a cathode oscillograph. After this photographs were taken.

Of the pharmacological substances we studied tetraethylammoniumiodide (TEA), pentamine,* hexonium** diacetylcholine (ditilin)*** and diplatsin. The substances were injected intravenously.

TEA was used in doses of 0.01 to 3-5 mg/kg. The shift of the least optimal in the direction of lower frequencies occurred when the dose of TEA was 0.05-0.1 mg/kg. These changes are not great and measure 10 cps. On the introduction of 1 mg/kg the lability is markedly decreased (by 30-40 cps). However the functional state of the ganglion rapidly (in the course of 10-15 minutes) returns to the original level. These

* Synthesized in the All-Union Sci. Res. Inst. of Pharmaceutical Chemistry.

** Synthesized in the Dept. of Pharmacology of the Inst. of Exptl. Med. (Dir. Acting Member Acad. Med. Sci. USSR S. V. Anichkov).

*** Synthesized in the Laboratory of Prof. A. L. Mndzhoyana (Erevan).

findings were obtained by the stimulation of the preganglionic fibers with increasing frequencies. If only "sub-least optimal" frequencies were used after the introduction of TEA the initial rise of the curve is maintained or drops very little, although the subsequent lowering occurs much more quickly and sharply. It is characteristic, that with the decreased lability changes in the height of the curve with optimal rhythms is not observed. These changes occur only with larger doses of the substance.

In connection with the fact that the initial contraction of the muscles of the third lid, when stimulated through the preganglionic fibers by various frequencies, was not altered markedly by the action of TEA, as was also the case with other quaternary ammonium compounds that we studied, we recorded the biological currents only for a definite period of time.

It developed that the biological potentials are capable of maintaining their heights for lengthy periods of time when stimulated at low frequencies (5-10 cps). At 25-50 cps the biologic electric potentials diminish in time but not very rapidly. With 75-100 cps frequencies the potentials disappear in 3-6 seconds from the beginning of the time of stimulation, although the muscle continues to be in a tonic state. In individual experiments, in which we observe the action of pharmacological substances, the electrical potentials were recorded on 3-4 different levels of frequency. The potentials were recorded every 3-5 seconds (with a single light ray). In the majority of the experiments we were satisfied with only a partial stimulation of the preganglionic stem. Most convenient were found rhythms of 25 to 50 cps. At these levels the decrease in the biologic electric potentials proceeded slowly. In the case, however, when the substance being tested reduces the lability of the ganglion, the given frequency stops its action in a shorter time than before the introduction of the pharmacological material.

TEA in these experiments was used in relatively large doses. As an illustration see Figure 1. With the introduction of 3 mg/kg of TEA the electric potentials in the first few moments differed but little from the potentials seen before the introduction of this material. However, after 3 seconds the oscillations had already practically disappeared, whereas before the introduction of TEA the bioelectric potentials maintained themselves for a relatively long time. Analogous results were obtained in the other experiments.

These results confirm the supposition that TEA reduces the liability of the cells of the upper cervical ganglion.

Pentamine was used in doses from 0.001 to 1 mg/kg. Experiments with a mechanical recording of the contractions of the muscles of the third lid showed that changes in lability begin with the introduction of pentamine in doses of 0.005-0.01 mg/kg. A marked shift of the "least optimal" to the side of lower frequencies occurs with the use of 0.05-0.1 mg/kg. On Figure 2, A, the "least optimal" frequency before the introduction of pentamine in the dose of 0.2 mg/kg corresponded to 100 cps. After the introduction of this substance (within a minute) the "least optimal" braking action appeared at a frequency of 70 cps, this being accompanied by a sharp weakening of the lid muscle. This, apparently, fully corresponds to the concept of the totally "least optimal" situation, characterized by the absence of any fluctuations whatever — mechanical or electrical. Characteristic is the height of the curve which does not change with stimuli of 10 cps. All changes commence only at higher frequencies.

In the experiments in which the preganglionic fibers were stimulated with only one frequency, it was shown that the most distinct effect appears with the higher frequencies of stimulation. Most convenient appear to be the "sub-least optimal" frequencies. Figure 2, B and C, confirms these suppositions. The changes appearing after pentamine introduction with stimulation of the preganglionic fibers at a frequency of 50 cps are more pronounced than those with 35 cps, although the difference between the given frequencies is not great.

The introduction of pentamine in doses 0.5-1 mg/kg is frequently accompanied by apnea and convulsive muscular movements of the animal. Analogous action is exhibited by TEA although to a lesser degree.

Oscillographic recording of the electric potentials exhibited by the postganglionic fibers shows that pentamine depresses the lability of the upper cervical ganglion. With this the electric biologic potentials disappear at a time when before the introduction of this substance there had been observed definite potentials, synchronous with the stimulating rhythm.

Pentamine differs from TEA in the length of its activity. Thus as can be seen from Figure 2, 1, with 0.2 mg/kg dose of pentamine the lability of the ganglion decreases over 40-50 minutes. Taking into consideration the wide therapeutic range of this substance (Sharapov, 1955; Bein and Meier, 1951; Heim and Wagner, 1953, and others), we can consider pentamine as being a rather effective substance for the depression of vegetative ganglia.

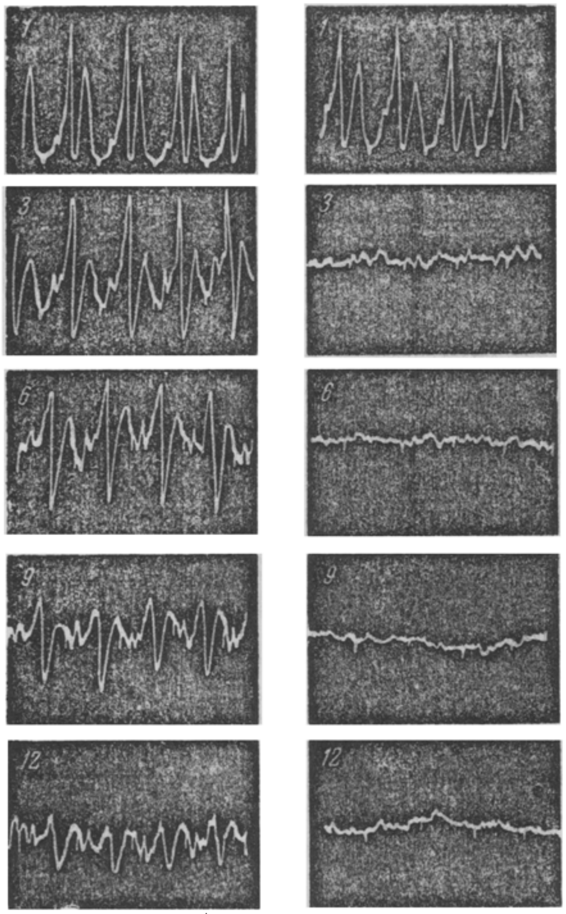

Fig. 1. Influence of tetraethylammonium (TEA) on the biopotentials of postganglionic fibers.
A) Before introduction, B) after introducing TEA (3 mg/kg).
Frequency of preganglionic fiber stimulation −20 cps (1, 3, 6, 9, 12 – time in seconds from beginning of stimulation).

Hexonium (hexamethonium) was tried in doses from 0.001 to 1 mg/kg. Threshold doses are of the order 0.005-0.01 mg/kg. Introduction of a dose of 0.25- 1 mg/kg was usually accompanied by apnea, markedly disturbed respiratory rhythm, and muscular fibrillation in the decerebrated cats. It should be observed that in experiments when we utilized urethane narcosis these respiratory disturbances and muscular fibrillatory twitchings were not observed when TEA, pentamine and hexonium was used in the indicated doses.

Hexonium markedly depresses the functional capacity of the ganglion. The "least optimal" responses move sharply over to the region of lower frequencies. With stimulations just short of the worst responsive rhythms the introduction of hexonium decreases markedly the tone of the muscle of the nictitating membrane (Fig. 3, A). Analogous results were obtained when the potentials of the postganglionic fibers were recorded. Potentials fail to reproduce themselves much more rapidly than before the introduction of the material.

We can form an idea of the length of the action by Figure 3, A. From it can be seen that hexonium in a dose of 0.1 mg/kg depresses the functional response of the ganglion for more than 40 minutes.

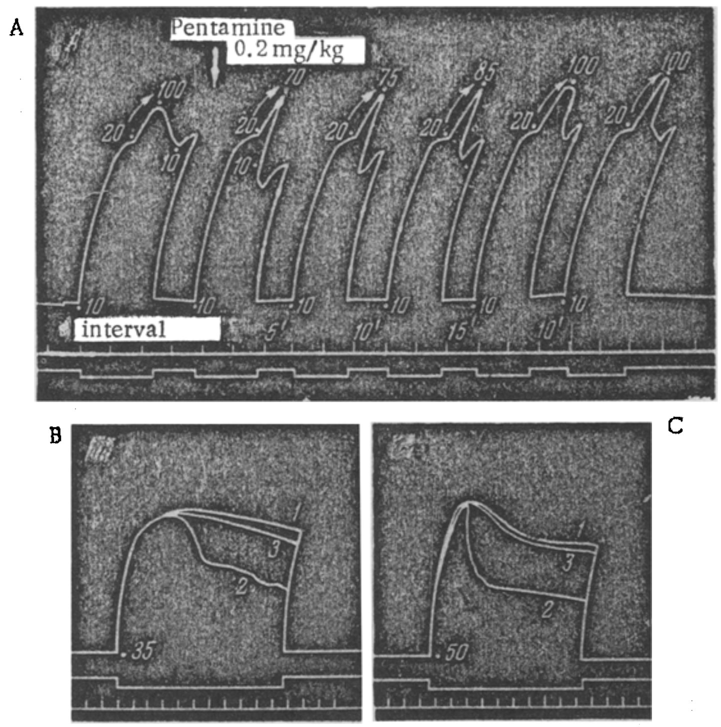

Fig. 2. Influence of pentamine (↓) upon the functional state of the upper cervical ganglion.
The upper curve (in each picture) – is a mechanical graph of contractions of the third lid. The numbers indicate preganglionic fiber stimulation in cps. Arrows correspond with the gradual change from one frequency to the other. The point of stimulation, the time marker for A) – 30 seconds, for B and C) – 15 seconds.
B and C: 1) before introducing pentamine 2) – one minute after internal administration of pentamine (0.1 mg/kg), 3) – after 15 minutes.

Diacetylcholine (ditilin) was used in much larger doses (50-100 fold), than those used for immobilization. The experiments were done with the use of artificial respiration. The introduction of ditilin in doses up to 3-5 mg/kg caused no changes in the functional capacity of the upper cervical ganglion. No changes were seen when a preliminary injection of eserin (0.015 mg/kg) was given, this drug, as is well known, being a strong reinforcer of ditilin activity.

Diplatsin was used in doses from 0.01 to 5 mg/kg. It developed that the threshold doses, changing the lability of the nerve-muscle synapses (experiments conducted in our laboratory by A. V. Valdman), coincide with those for the upper cervical ganglion. In this way diplatsin materially differs from diacetylcholine. The decrease in the lability of the ganglion (just as with the nerve-muscle synapses) occurs when diplatsin is introduced in the dosage of 0.1-0.2 mg/kg.

Diplatsin powerfully moves the "least optimal" response to the region of lower frequencies, with which the height of the curves at optimal rhythms is not altered even with a large dose of the substance (Figure 3, B). The electric potentials after the injection of diplatsin drop rapidly and disappear, whereas, before its introduction, the biopotentials decrease in amplitude quite gradually.

In this fashion all the studied substances, with the exception of diacetylcholine, alter the lability of the upper cervical ganglia facilitating the development of "least optimal" inhibition. However, each of these

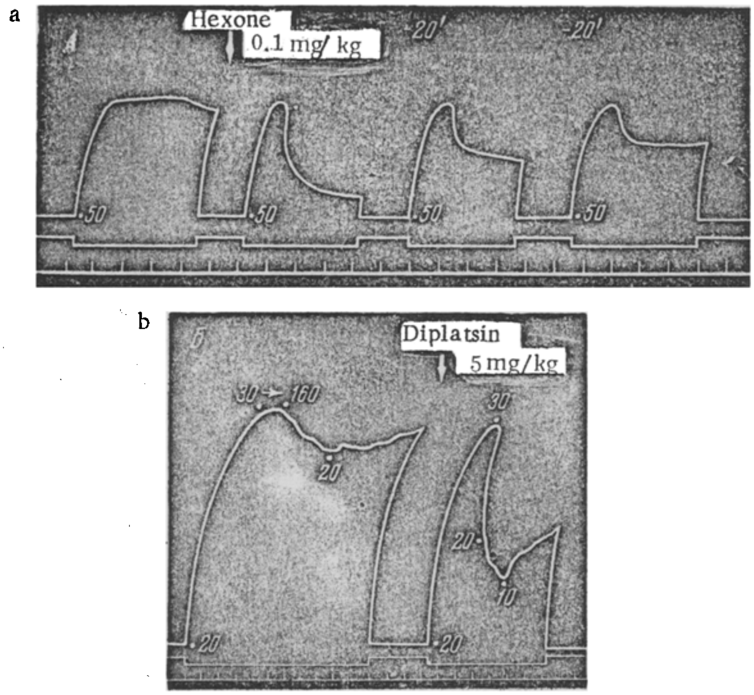

Fig. 3. Influence of hexonium (A) and diplatsin (B) upon the lability of the upper cervical ganglion.
Significance of curves (from above down): mechanogram of contractions of the third lid, point of stimulation, time marker (for A 30 seconds, for B – 15 seconds). ↓ introduction of preparation, figures indicate stimulation frequencies in cps.

substances possesses its own peculiarities. For example, tetraethylammonium lowers quite actively the lability of the ganglia, while its influence is only fleeting. Much more prolonged is the activity possessed by pentamine and hexonium. Intermediate between them is diplatsin.

The degree of lability is an excellent criterion for estimating the degree of activity possessed by ganglionic poisons. Characteristically, with the decrease in lability under the influence of quaternary ammonium compounds, the excitability of the ganglionic cells may not be changed. This last was shown by us when we determined the threshold of excitability with various rhythms of stimulating the preganglionic stem, besides which this finding is further confirmed by the fact that the amplitude of the mechanogram is not infrequently maintained even with a marked decrease in the lability of the ganglia. A special interest is seen in the capacity of the ganglion cells to respond to various rhythms at specified intervals of time. It became evident that the higher the frequencies at a given level of stimulation, the more rapidly do the biopotentials disappear in the postganglionic fibers. After the introduction of quaternary ammonium compounds the length of time during which the biopotentials are preserved is markedly shortened. The potentials, registered in the first few seconds and especially at the very beginning moment of stimulation, disappear or are markedly diminished very late or only with the use of relatively large doses of these substances. Therefore it seems rational to us that during the investigation of ganglion-blocking agents the registration of biological currents (and contractions of the muscle of the nictitating membrane) at various frequencies and for a definite period of stimulation time is an excellent index of the condition of the preganglionic stem. In this fashion, the study of ganglionic poisons has as one of its basic parameters the determination of the lability and functional condition of the cells of the ganglion which is done with the additional consideration of the time factor.

LITERATURE CITED

[1] Sharapov, I. M. Farmakol. Toksikol. 18, 1, 8-13 (1955).

[2] Bein, H. J. and Meier, R. Schw. Med. Wschr. No. 19, 446-452 (1951).

[3] Heim, F. and Wagner, H. Arch.Exptl. Pathol. Pharmakol. Bd. 218, 343-348 (1953).