PHARMACOLOGY

THE SIGNIFICANCE OF SULFHYDRYL GROUPS IN THE ACTION OF DERIVATIVES OF QUATERNARY AMMONIUM BASES (RE: THE ACTION MECHANISM OF THE GANGLIOBLOCKING DRUGS)

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Substances blocking the synaptic transmission of impulses in the autonomic ganglia have become widely used of recent years since the most different branches of practical medicine have found them of value. However, the all-purpose use of these drugs is hampered because of the insufficient information available on the nature of the processes which occur when synaptic transmission is blocked.

It has now been recognized that acetylcholine plays a leading role in stimulation transmission through the autonomic ganglia, and this has made it possible to divide all ganglioblocking substances into two groups: depolarizing substances (which act like excess concentrations of acetylcholine) and competitive substances (which prevent the action of acetylcholine on the ganglionic cholinergic structures) [17]. Because of their chemical proximity to acetylcholine, one group of ganglioblocking agents – derivatives of quaternary ammonium bases – are regarded by a majority of authors [1, 10, 18 et al.] as possessing the competitive type of action. There has been experimental verification of this proposal, and we ourselves recently obtained results corroborating the fact that the action mechanism of this group of gangliolytics is competitive [12].

Although there has been some progress in research investigating the intimate action mechanism of ganglioblocking agents, the literature still contains hardly any information concerning the biochemical structures which participate in the processes effecting the conduction of impulses through the ganglia. The works of M. L. Belen'kii [3, 4 et al.] have demonstrated the role played by disturbances in the carbohydrate metabolism and by the associated accumulations of high-energy phosphorus compounds in the action of substances influencing the cholinergic structures of the carotid sinus. N. B. Vysotskaya [5] succeeded recently in showing that ganglionic blockade by means of the depolarizing type of substance, particularly nicotine, is attended by changes in the content of high-energy phosphorus compounds, while the content of ATP in the ganglionic tissue is not materially affected by substances with the competitive type of action.

The question of the possible participation of ganglioblocking substances as in the metabolic processes, and particularly in the protein metabolism, is of special interest. In their many works, Kh. S. Koshtoyants and his co-workers [6, 7, 10, 11, 13 et al.] have assembled a considerable number of factors, which suggest that cholinergic stimulation, as well as the action of artificially introduced acetylcholine, is a process immediately connected with nerve cell metabolism and which occurs with the active participation of acetylcholine in the biochemical transformations of the innervated tissues. In these investigations, Kh. S. Koshtoyants lays particular emphasis on the role of the sulfhydryl groups as the most reactive and labile of the groups contained in the protein molecule.

Considering the role of sulfhydryl groups in the action mechanism of acetylcholine and taking into account the competitive effects caused by gangliolytics which are derivatives of quaternary ammonium groups, we decided to demonstrate the effect of the latter under conditions in which the content of sulfhydryl groups was changed.

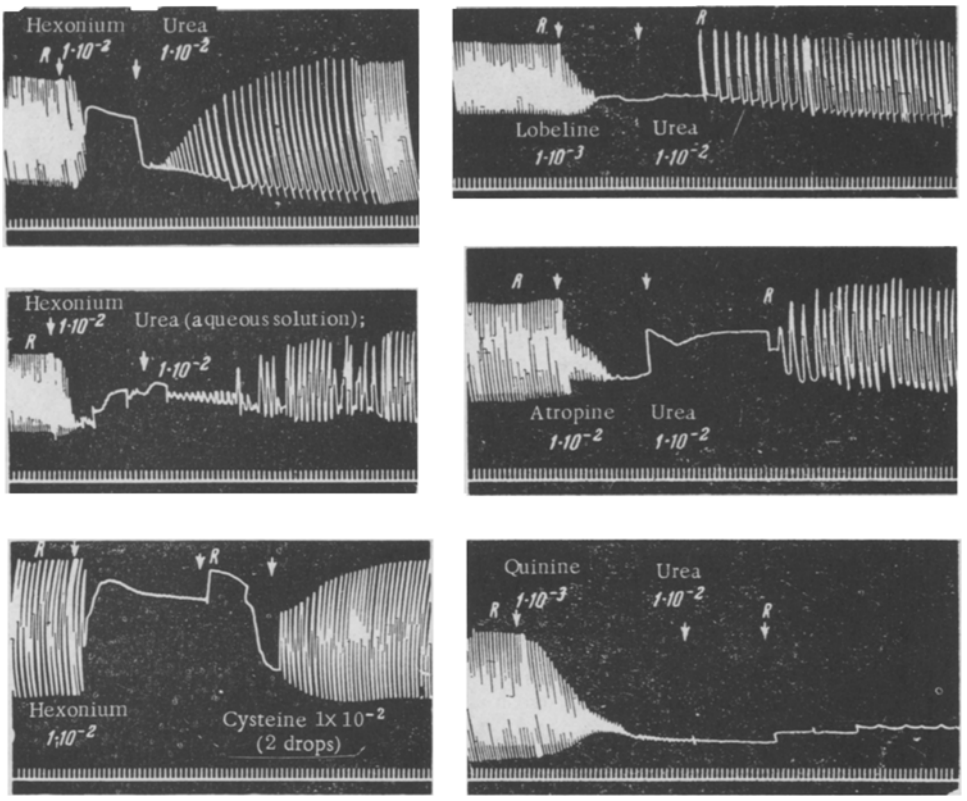

Fig. 1. Restoration of the operation of a frog's heart (isolated according to Straub) arrested with Hexonium, with the help of urea and cysteine. Time indicated in 5-second marks. R – introduction of Ringer's solution.

Fig. 2. Effect of cysteine and urea on Hexonium and Hexonate blockade of transmission in the superior cervical sympathetic ganglion. Experiment made October 10, 1957. Male cat weighing 2.8 kg. Anesthesia: urethan, 1 g/kg; cysteine, 4 mg/kg intravenously; urea, 10 mg/kg intravenously; Hexonium, 3 mg/kg intravenously; Hexonate, 10 mg/kg intravenously.

The experiments were performed on isolated biological subjects (isolated heart of a frog, rectus abdominis muscle of a frog) and on intact animals (cats and white mice). Cysteine was used to provide surplus sulfhydryl groups; urea was also used, as in the opinion of many authors, it possesses the ability to break up the protein molecule and liberate the reactive sulfhydryl groups [2, 8, 14 et al.]. We used a thiol poison, cadmium chloride, to bind the tissue sulfhydryl groups. The ganglioblocking substances used were different salts of hexamethylene-1, 6-bis-trimethylammonium: the iodide (Hexonium), the pyridine-3-carbonate (Hexonate), and the benzoate. All the substances were dissolved in a Ringer's solution for use in the experiments on the isolated organs, while aqueous solutions were used in the experiments on the animals. A total of 45 experiments were performed on a frog's heart isolated according to Straub; 20 experiments were performed with the frog's rectus abdominis muscle, and 12 acute experiments were performed on cats. We used 50 mice in the experiments determining the toxicity of the gangliolytics.

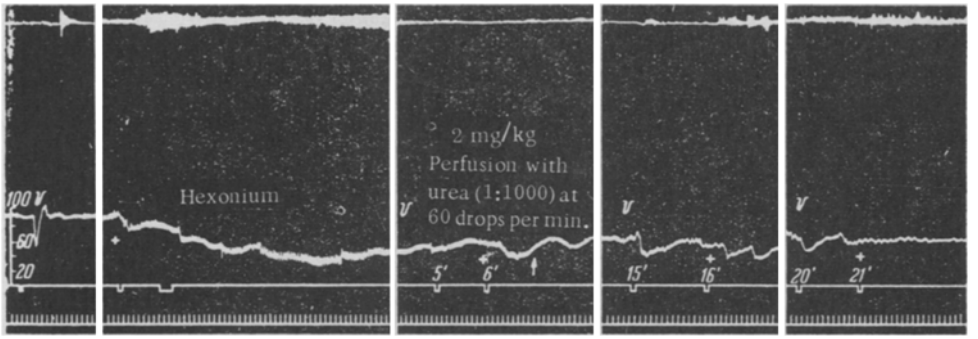

Fig. 3. Effect of urea on ganglionic action of Hexonium. Curves (from top to bottom) show: respiration, blood pressure, indication of stimulation and injection of preparations; time indicated in 5-second marks; + intravenous injection of lobeline; V — stimulation of preganglionic trunk of vagus nerve; — start of urea perfusion.

EXPERIMENTAL RESULTS

In the first series of experiments, the effect of cysteine (1×10^{-2}) and of urea (1×10^{-2}) on the operation of an isolated frog's heart, which had been preliminarily stopped with Hexonium, was studied. In the control experiments, Hexonium in a concentration of 1×10^{-2}) consistently caused a negative inotropic effect, rapidly succeeded by cardiac arrest. The replacement of the Hexonium solution with the urea solution led in all the experiments to the restoration of the original amplitude of the contractions; rinsing the heart with an aqueous solution of urea, as was done in control experiments, had a similar effect. The same effect also resulted from the addition of tiny quantities of cysteine (2-3 drops of a 1% solution) to the solution nourishing the heart. In order to examine the specificity of the symptoms we observed, we studied the effect of urea on the cardiac depression induced by a stimulator of N-cholinergic structures (lobeline, 1×10^{-3}), and M-cholinolytic (atropine, 1×10^{-2}) and a protoplasmic poison (quinine, 1×10^{-3}). In these concentrations, all these substances caused a negative inotropic effect followed by cardiac arrest.

In contrast to the experiments with Hexonium, urea did not eliminate the effect of these substances; the subsequent rinsing of the heart with a Ringer's solution did, however, lead to the restoration of the amplitude of the heart contractions (Fig. 1), except in the experiments with quinine.

We used the rectus abdominis muscle of a frog to determine how the curare-like effects of Hexonium were affected by blockade of the sulfhydryl groups. The muscle was placed in a small glass containing a Ringer's solution, through which oxygen was passed continuously. The introduction of acetylcholine in a concentration of 3×10^{-5} caused a stable contracture of the muscle. In the control experiments, the addition of Hexonium in a concentration of 3×10^{-3} for 30 minutes caused the muscle to relax 40-60%. Cadmium chloride added in a concentration of 3×10^{-5} at the height of the contraction considerably weakened the curare-like effect of Hexone (by 10-25% in relation to the original height of the contraction).

The experimental subjects used in the experiments on cats under ether-urethan anesthesia were the cholinergic structures of the superior cervical sympathetic ganglion and the cardiac ganglia of the vagus nerve. The gangliolytics were injected as 1% solutions in doses of 2-10 mg/kg. Cysteine was used in doses of 2-4 mg/kg and urea in doses of 10-20 mg/kg; for perfusion purposes, both these substances were introduced intravenously in a dilution of 1 : 1000 at a rate of 50-60 drops per minute.

In the control experiments, all the experimental gangliolytics caused, in these doses, complete blockade of the cardiac ganglia of the vagus nerve, lasting not less than 1½ hours from the moment of injection. The reaction of the nictitating membrane was retained, although it became considerably diminished, and the original reaction was restored no less than an hour after the injection.

The reaction to lobeline was also completely blocked for a long time, especially after the injection of Hexonium benzoate; for this reason, it was primarily the benzoate which was used in the experiments studying the reactivity of the carotid sinus receptor zones.

On a background of ganglionic blockade, the administration of cysteine and urea diminished, and in many cases eliminated, the lytic effect of the ganglioblocking agents. This effect was especially clearly manifested

by urea perfusion, which caused the liberation of the sulfhydryl groups in the organism (Figs. 2 and 3).

We used mice to study the effect of urea on the toxic effect of Hexonium and Hexonate. We first established the lethal doses of these two gangliolytics; with intraperitoneal administration, the LD50 and LD100 were 122 and 150 mg/kg respectively for Hexonium and 148 and 160 mg/kg for Hexonate.

In the first series of experiments, mice were given intraperitoneally simultaneous injections (with two syringes) of Hexonium, in the LD100 dose, and urea, in a dose of 300 mg/kg. Ten of the twenty mice who received these injections survived. Eight mice were injected with 300-500 mg/kg of urea 10 minutes before they were injected with the LD100 of Hexonium. The death rate was not lowered in these experiments, but death did occur considerably later in a majority of cases (after 1-24 hours) than in the control experiments, in which the animals died after 3-6 minutes. In both these experimental series, the convulsive effect of the lethal dose of Hexonium was considerably diminished. Respiratory insufficiency and its associated symptoms (cyanosis, exophthalmos) were also less pronounced. Urea also showed a marked "defensive" effect with the injection of lethal doses of Hexonate. The intraperitoneal injection into 16 mice of 200 mg/kg of Hexonate (1.25 LD100) and 300 mg/kg of urea led to the death of 6 of the mice (37.5%).

Additional experiments were performed to determine the effect of this group of gangliolytics on the content of free SH groups in the tissues. The SH groups present in extracts of the brain, muscle and liver tissue of mice and in extracts of the femoral muscles of a rabbit were determined by A. Mirskii's ferricyanide method [16], as modified by A. S. Tsyperovich and A. L. Loseva [15]. From the data obtained, we drew the preliminary conclusions that the content of sulfhydryl groups in the liver and muscles of mice and in the muscles of rabbits is considerably decreased under the influence of Hexonium. There was no significant change in the content of SH groups in the brain tissue of the mice.

The experimental data which we have cited indicate that the sulfhydryl groups are of definite significance in the processes connected with ganglionic blockade by means of cholinolytics – derivatives of quaternary ammonium bases.

Recent data, especially those which T. M. Turpaev [13] obtained with the help of labeled atoms, have confirmed the existence of competitive interrelations between acetylcholine and thiol poisons during their action on the protein structures of the tissues. Since the experimental group of gangliolytics are also competitive in respect to acetylcholine, one can propose them to possess the property of inhibiting the sulfhydryl groups of the protein molecule.

At the same time, it must be emphasized that the ideas which we have expressed in regard to the type of action exerted by the gangliolytics of this class are by no means an attempt to simplify the nature of the problem by implying that the action mechanism of these suhstances consists only in blockade of the sulfhydryl groups. In all probability, the effect of these substances is caused not so much by direct structural changes in the protein molecule as by a considerable change in the reactivity of the molecule, with the change affecting primarily the reactivity of the sulfhydryl groups, as the most labile components of the molecule. Further investigation is required before we can approach an understanding of the nature of the biochemical changes which are caused by ganglioblocking substances.

SUMMARY

The author studied the effect of cysteine and urea and thiol poison (cadmium chloride) on the effect of ganglioblocking substances, i.e. the derivatives of the quarternary ammonium bases. Experiments demonstrated that the action of different salts of hexamethylene bis-trimethylammonium – ganglioblocking, curare-like and depressing the work of the isolated heart – may be inhibited by the substances which change the concentration of the free SH-groups in the innervated tissues. These facts allow one to conclude that the reactivity of the sulfhydryl groups change when impulse conductivity through the ganglionic synapses is blocked.

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