THE COMPETITIVE CHARACTER OF THE ACTION OF GANGLION BLOCKING AGENTS – DERIVATIVES OF QUATERNARY AMMONIUM COMPOUNDS

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Despite the large number of experimental and clinical investigations of substances which inhibit synaptic transmission of nerve impulses, the question of the mechanism of their action has not as yet received adequate study. The majority of authors tend to explain the pharmacologic activity of these compounds by competitive antagonism because anticholinergic preparations, and ganglion blocking substances in particular (quaternary ammonium derivatives) approach in their chemical structure acetylcholine, a mediator substance [1, 3, 10, 11]. In studying the so-called methonium series of ganglion blocking agents we were also led to the conclusion that their central and peripheral and anticholinergic effects could be explained on the basis of competitive interrelations with acetylcholine [8]. It follows from this that anticholine esterase preparations, which stabilize acetylcholine, should also enter into competitive relations with ganglion blocking agents when acting on cholinergic structures.

Investigations by M. Ia. Mikhelson and collaborators [4] are significant; these authors established that the central effects of the anticholinergic substance pentaphen could be blocked by means of the anticholine esterase preparation proserine. Recently M. M. Kholodenko [9] attempted to make a similar study with respect to anticholinergic substances of the quaternary ammonium derivative type. However, the method used by that author was not without defects. The tests used in his work – the cholinergic complex and hypertension on administration of eserine as well as mortality of the animals made it impossible to evaluate the observed phenomena uniformly, since these phenomena were a consequence of numerous functions of the body; therefore the subsequent administration of ganglion blocking agent by the method adopted by the author could not demonstrate the mechanism and localization of the antagonistic action of the given substances with sufficient conviction.

EXPERIMENTAL METHODS AND RESULTS

The experimental conditions for demonstration of the interrelations in the system acetylcholine-ganglion blocking agent-anticholine esterase preparation consisted of excitation of cholinergic structures by adequate stimuli with subsequent use of the ganglion blocking agent. In the second series of experiments, whose results were compared with those of the first, stimulation of one or other cholinergic structure was combined with the use of the anticholine esterase proserine; the ganglion blocking agent was then tested against this background. The most typical representative of the quaternary ammonium bases – hexamethylene-bis-trimethyl ammonium iodide (synonyms: hexamethonium, hexonium, hexatonide) • – was used as the ganglionic blocking agent.

In order to find out how universal the principle of competitive antagonism might be, various cholinergic structures affected by the specific action of hexonium were selected as objects of investigation. Such structures included ganglionic N-cholinergic structures of the vagus nerve ganglia in cats, N-cholinergic structures of skeletal musculature (frogs), N-cholinergic structures of the central nervous system (frogs) and peripheral M-cholinergic structures (mice).

• The preparation was synthesized at the Department of General Chemistry of the Chernovits Medical Institute by Assistant Professor A. I. Lopushanskii.

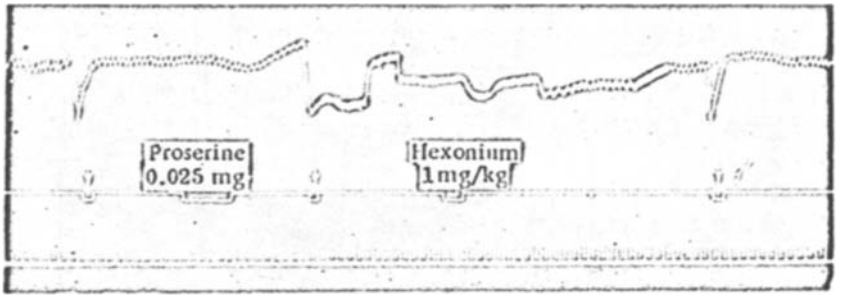

Fig. 1. Abolition of the ganglionic blocking effect of hexonium by proserine. Experiment performed on cat. Records from above down: blood pressure response to electric stimulation of preganglionic fibers of the vagus, stimulus marker, time marker (5 seconds).

Peripheral N-cholinergic structures. In the first series of experiments the relation between hexonium and proserine in their effect on N-cholinergic structures of the cardiac ganglia of the vagus was studied. Experiments (10) were performed on cats under urethane anesthesia or on decerebrate preparations. Hexonium was injected into the femoral vein in doses of 0.1-5 mg/kg. The ganglion blocking action of the compound was judged by the hypotensive reaction when the preganglionic fibers of the vagus were stimulated by induction current of suprathreshold strength. Control experiments established that hexonium in doses of 1-5 mg/kg produced constantly complete and prolonged (up to 30 minutes and longer) ganglionic blocking effect.

Preliminary intravenous injection of proserine in doses 0.01-0.025 mg abolished the ganglion blocking effect of hexonium (Fig. 1).

The curare-like action of hexonium was used for the study of competitive interrelations on N-cholinergic structures of skeletal musculature. Frogs were used in these experiments. The results of the experiments were recorded on kymograms.

The first series of investigations was carried out on the hind limbs of frogs prepared by Pisemskii's method (7 experiments). Perfusion with a hexonium solution of 1×10^{-4} concentration evoked progressively increasing curare-like effect which was manifested in a sharp decrease of reaction to stimulation of the peripheral end of the sciatic nerve by single shocks of induction current of suprathreshold strength at the rate of one shock per minute. Subsequent perfusion with proserine in 1×10^{-4} concentration was accompanied by restoration of the amplitude of contraction in the frog's leg.

In the second series of experiments (9 experiments) the isolated frog rectus abdominis was used. Hexonium in concentration of $3 \times 10^{-3} - 3 \times 10^{-6}$ caused relaxation of acetylcholine-induced contraction (acetylcholine in concentration of 3×10^{-7}). Administration of proserine in concentration of 1.5×10^{-6} protected the muscle from relaxation (Fig. 3). It is characteristic that higher concentrations of hexonium again exerted a curare-like effect.

Peripheral M-cholinergic structures. We could find no literature references to the effect of ganglion blocking substances of the quaternary ammonium derivative type on peripheral M-cholinergic structures. Nonetheless it appeared interesting to attempt to obtain a blocking effect on these structures since here too the competitive interrelations under consideration could, presumably, take place.

Experiments were carried out on 28 white mice weighing 18-30 g. In control experiments subcutaneous injection of 5-6 mg acetylcholine was accompanied by increased salivation, micturition and defecation. All these manifestations were easily abolished by preliminary or subsequent subcutaneous injection of small doses of atropine (0.02-0.04 mg) which proved the peripheral character of the observed phenomena.

Like atropine, hexonium given subcutaneously in doses of 0.2-0.4 mg 10 minutes prior to acetylcholine caused marked diminution, and in most cases complete disappearance, of the manifestations mentioned above. Preliminary administration of 0.05-0.5γ proserine with subsequent administration of hexonium and acetylcholine in appropriate doses restored the original picture of acetylcholine intoxication in mice.

Central N-cholinergic structures. Manifestations of competitive antagonism occurring in central N-cholinergic structures were studied in two series of experiments. The first series made use of the property of this group of ganglion blocking preparations of preventing the specific reaction of frogs to nicotine (this property was established earlier by us [7]), the central character of this reaction having been proved by many authors [2, 5, 6].

Experiments were performed on 24 Rana esculenta frogs weighing 40-50 g. Preliminary experiments established that hexonium in doses of 8-10 mg blocked the characteristic nicotine reaction (1 mg nicotine) completely or partially. Preliminary (5-10 minutes earlier) administration of proserine in doses of 0.5-0.75 mg with subsequent administration of hexonium and nicotine in the doses indicated above was not accompanied by any substantial changes in the reaction of the frogs to nicotine.

In the second series consisting of 12 experiments, the blocking effect of hexonium on central N-cholingergic structures was studied on spinal forgs with determination of the latent period of the Turk flexor reflex. The experimental results were recorded on kymograms.

It was found that introduction of 2-4 mg hexonium into the dorsal lymph sac of the frog caused a considerable (2-4 fold) increase of the latent period for the reflex. Preliminary administration of proserine in the dose of 0.5 mg abolished this effect of hexonium.

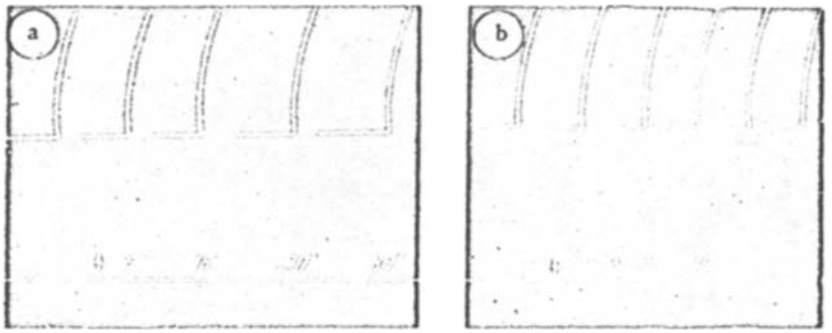

Fig. 2. Effect of hexonium on the length of the latent period of the flexor reflex according to Turk (a) and abolition of this effect by proserine (b). In both traces the stimulus marker shows the latent period for the reflex.
↑ – hexonium, 4 mg; ↑↑– proserine, 0.5 mg.

Since prolongation of the latent period of the reflex could have been a consequence of changed functional state of the efferent part of the reflex arc, the same frogs were subjected to stimulation of the peripheral end of the sciatic nerve by single shocks of induction current of suprathershold strength and the contraction of the hind limb muscle in response to this stimulation was determined. Administration of hexonium did not alter the observed reaction appreciably. In other words, hexonium given in the doses indicated and by the route employed did not exert a curare-like effect and its influence on the duration of the latent period of the reflex was apparently determined by the blocking of the central portions of the reflex arc.

The experimental material obtained confirms the postulated hypothesis concerning the competitive character of phenomena occurring at cholinergic structures under the influence of the ganglionic blocking substances of the group under consideration. If the extensive functions of acetylcholine in the transmission of impulses in the interneuronal synapses of the central and peripheral components of the reflex arcs are taken into account,

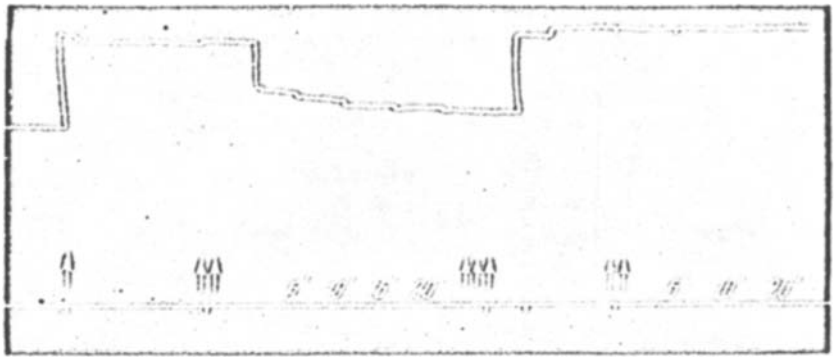

Fig. 3. Abolition of the curare-like effect of hexonium by proserine. Experiment on frog rectus abdominis preparation. ↑ – acetylcholine, 3×10^{-7}; ↑ ↑ – hexonium, 6×10^{-5}; ↑↑↑ – proserine, 1.5×10^{-6}.

it becomes clear why the stabilizing influence of the anticholine esterase preparation proserine inhibits various manifestations of the anticholinergic action of hexonium which approaches acetylcholine in its structure.

It appears to us that further investigations directed towards the study of the intimate mechanisms of action of anticholinergic preparations of this series can be of use in searching for therapeutic substances capable of normalizing the nervous regulation of various functions.

SUMMARY

An attempt was made to prove experimentally the competitive character of the ganglion blocking preparations – derivatives of the quaternary ammonium compounds. The stabilizing effect of proserine on the mediator function of acetylcholine was used for that purpose. It was established by these experiments that proserine inhibits the blocking effect of hexonium (which is very similar in structure to acetycholine) on the various peripheral and central N and M cholinergic structures.

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