NATURE OF INTERACTION OF ANTICHOLINESTERASE DRUGS WITH ALLOSTERIC AND ISOSTERIC PARTS OF CHOLINERGIC RECEPTORS

M. L. Tarakhovskii and Ya. G. Timoshenko

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In the presence of nonanticholinesterase concentrations of neostigmine and myotol the bologram of logarithm of concentration versus effect of acetylcholine was significantly shifted to the left along the concentration scale, parallel to the original line. Cadmium chloride (1×10^{-6} M) abolished this shift. With an increase in the cadmium chloride concentration to 1×10^{-4} M, the curve of logarithm of concentration versus effect of acetylcholine was shifted to the right, parallel to the bologram obtained in the absence of cadmium chloride; the contractile response of the intestine to myotol was absent.

Acetylcholinesterase drugs have been shown to have a direct action on cholinergic receptors [1, 5, 10, 16, 23, 24, 26, 28]. In particular, potentiation of acetylcholine effects by the action of nonanticholinesterase concentrations of neostigmine and armine* have been shown to be due to the direct effect of these drugs on cholinergic structures [14, 15, 29,30]. This is a further argument in support of the hypothesis that anticholinesterase drugs interact with allosteric areas of cholinergic receptors [4, 7].

In the investigation described below an attempt was made to obtain additional evidence regarding the nature of interaction between anticholinesterase drugs and the allosteric and isosteric areas of cholinergic receptor structures.

EXPERIMENTAL METHOD

The effect of neostigmine (a reversible cholinesterase inhibitor) and of myotol (an irreversible cholinesterase inhibitor [2, 19]) was investigated in 130 experiments on isolated duodenal segments of sexually mature noninbred albino rats. The segments of intestine were placed in a jar filled with oxygenated Kravkov's solution at 37°C.

The relationship between concentration and effect of acetylcholine in the presence of nonanticholinesterase concentrations of neostigmine and myotol was studied by the cumulative curve method [31]. The action of acetylcholine was investigated after exposure of the segment of intestine for 30 min to a solution containing these drugs. To determine the degree of action of the drugs on acetylcholinesterase activity in sections through the rat duodenal wall, a histochemical method was used to determine its activity [27]. In the experiments of series II the effect of neostigmine and myotol in nonanticholinesterase concentrations was studied on the acetylcholine effects in the presence and in the absence of cadmium chloride (1×10^{-6} M to 1×10^{-4} M).

The cumulative curves were subjected to regression analysis [22].

*The ethyl-p-nitrophenyl ester of ethylphosphinic acid.

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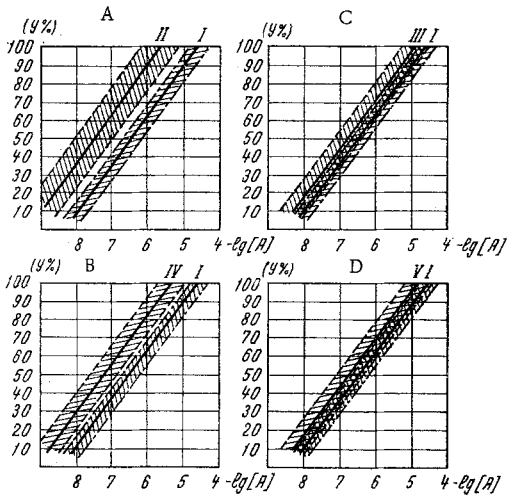

Fig. 1. Choline-sensitizing action of nonanticholinesterase concentrations of meostigmine (A) and myotol (B) and its abolition by cadmium chloride (C and D). I) Regression line for bologram of logarithm of concentration versus effect of acetylcholine; II) for bologram of logarithm of concentration versus effect of acetylcholine in the presence of neostigmine (3×10^{-8} M); IV) for the same bologram in the presence of myotol (1×10^{-16} M); III) for bologram of logarithm of concentration versus effect of acetylcholine against the background of action of cadmium chloride (1×10^{-6} M) in the presence of neostigmine; V) for same bologram in the presence of myotol. Confidence limits of corresponding curves are shaded.

EXPERIMENTAL RESULTS

The investigation showed that in the presence of nonanticholinesterase concentrations of neostigmine (3×10^{-8} M) and myotol (1×10^{-16} M), the bologram of the logarithm of concentration versus effect of acetylcholine was significantly shifted to the left (between the limits of 20 and 80% of the maximum effect) along the concentration scale parallel to the original curve (Fig. 1A, B).

In the next series of experiments, the concentration of cadmium chloride (1×10^{-6} M) which, with an exposure of 50 min, did not change the character of the cumulative curves for acetylcholine and produced no significant effect on the tone and contractile properties of the smooth muscles of the rat duodenal segment was determined.

Against the background of cadmium chloride in this concentration, and in the presence of neostigmine (3×10^{-8} M) and myotol (1×10^{-16} M) the shift of the bolograms of logarithm of concentration versus effect of acetylcholine was abolished (Fig. 1C, D). Meanwhile, during exposure for 5 min to cadmium chloride in a concentration of 1×10^{-4} M, the smooth muscle of the duodenal segment was relaxed and its tone lowered. Against this background the bologram of logarithm of concentration versus effect of acetylcholine was significantly shifted to the right, parallel to the original line, along the concentration scale (within the limits of 20 and 80% of the maximal effect), while the contractile response to myotol, which was clearly visible before administration of cadmium chloride, was absent (Fig. 2).

With an increase in the duration of action of cadmium chloride (1×10^{-4} M) to 20 min, the contractile response to acetylcholine also disappeared. Nicohexonium (2×10^{-4} M) had no effect on the character of the cumulative curves for acetylcholine and myotol.

The experiments thus showed that in nonanticholinesterase concentrations, neostigmine and myotol had no substantial effect on the contractile properties and tone of isolated segments of the rat duodenum, but had a choline-sensitizing action. This was manifested as a significant parallel shift to the left of the bologram of logarithm of concentration versus effect of acetylcholine along the concentration scale. The choline-sensitizing action of neostigmine and myotol in these concentrations was evidently the result of their effect on allosteric areas of the cholinergic receptors; in their interaction with these areas, these compounds noncompetitively potentiated the effects of acetylcholine.

A similar action of the anticholinesterase drugs was also seen relative to the allosteric areas of muscarine-like cholinergic receptors, for in the presence of the gangliolytic agent nicohexonium (2×10^{-4} M), both neostigmine and myotol, in nonanticholinesterase concentrations, continued to exert their choline-sensitizing action.

These results give experimental confirmation that anticholinesterase drugs can act as allosteric effectors, noncompetitively increasing the effectiveness of interaction between acetylcholine and isosteric areas of cholinergic receptors [25, 29].

These experiments also indicate the role of SH-groups in the mechanism of interaction of neostigmine and myotol with allosteric and isosteric areas of muscarine-like cholinergic structures. In fact, against the background of cadmium chloride (1×10^{-6} M) which does not significantly alter the character of the cumulative curves for acetylcholine, the shift of the bolograms of the logarithm of concentration versus effect of acetylcholine produced by neostigmine and myotol was abolished.

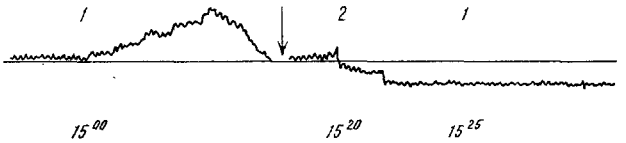

Fig. 2. Inhibition of contractile response of duodenum to myotol against the background of blocking of thiol groups by cadmium chloride (isolated segment of the rat duodenum): 1) cumulative curve for myotol starting with a concentration of 3×10^{-7} M; 2) cadmium chloride (1×10^{-4} M) for 5 min. Arrow indicates time of rinsing with Kravkov's solution.

This fact confirms the presence of thiol groups in allosteric areas of the muscarine-like cholinergic receptors and their role in the mechanism of the choline-sensitizing action of nonanticholinesterase concentrations of neostigmine and myotol.

The results suggest that the sulfhydryl groups of allosteric areas of muscarine-like cholinergic receptors are evidently more sensitive to the blocking effect of calcium chloride than the thiol groups of the isosteric areas of the cholinergic receptor structures.

The role of SH-groups of isosteric areas of muscarine-like cholinergic receptors was demonstrated in another series of experiments in which, against the background of higher concentrations of cadmium chloride (1×10^{-4} M) with a 5-min exposure, the contractile response to myotol was abolished and the bolo-gram of logarithm of concentration versus effect of acetylcholine was significantly shifted parallel to the original to the right along the concentration scale. With an increase in the exposure to 20 min, the response to acetylcholine likewise was abolished.

The results of these experiments are in agreement with the writers' earlier data for the role of SH-groups of nicotine-like cholinergic receptors in the mechanism of action of neostigmine [17].

The results showing the role of thiol groups of allosteric and isosteric areas of cholinergic receptors in the mechanism of action of anticholinesterase drugs correlate well with data in the literature on the importance of these structures during interaction of a number of choline-positive and choline-negative drugs with cholinergic receptor structures [6, 9, 11, 18, 20, 21].

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