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MODIFICATION OF SELECTIVE ADSORPTION OF MUSCARINIC ANTAGONISTS ON BRAIN MEMBRANES DURING CHLOROPHOS* POISONING

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The traditional method of treatment of poisoning by organophosphorus pesticides, chlorophos* in particular, is by the combined use of cholinolytics and cholinesterase reactivators, in order to protect the acetylcholine (ACh) receptor against the action of an excess of ACh and to restore activity of inhibited cholinesterase. The pharmacologic effects of cholinolytics, due to ACh receptor blockade, have now been confirmed at the level of specific binding with membranes of the brain and peripheral organs [4-7]. It has also been shown that certain cholinesterase reactivators bind selectively with ACh receptors [2, 3, 9]. Accordingly, the phenomenon of potentiation of therapeutic effects observed during combined treatment with cholinolytics and reactivators [1] could be the result of their interaction at the ACh receptor level.

The aim of this investigation was to study selective adsorption of muscarinic antagonists on rat brain membranes during chlorophos poisoning and the effect of the cholinesterase reactivator dipyroxime (trimedoxime bromide) on this process.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 130-160 g. To study binding of radioligands the brain of the decapitated animals, without the cerebellum, was homogenized in a glass homogenizer with Teflon pestle (11,000 rpm, 10 passages) in an ice-cold solution containing 10 mM Tris-HCl buffer (pH 7.4) and 1 mM EDTA. The homogenate (0.1%) was centrifuged for 20 min at 50,000 g. The residue was resuspended by rehomogenization in the same volume of solution containing 20 mM Tris-HCl buffer (pH 7.4) and 0.14 M NaCl, and centrifuged again under the above conditions. The residue was resuspended in twice the volume of the same buffer and used in experiments to study radioligand binding. Protein was determined by Lowry's method [8]. Aliquots of homogenate were incubated with increasing concentrations (0.02-6.0 nM) of ^{3}H-quinuclidinyl benzoate (^{3}H-QB; 1.11 TBq/mmole, from "Amersham," England), or with ^{14}C-cyclosyl (1.67 TBq/mole, State Institute of Applied Chemistry, Leningrad), in concentrations of between 0.5 and 60 mM. Binding of the radioligand inhibited by 10^{-5} M unlabeled atropine was regarded as specific. Nonspecific binding was 10% in the experiments with ^{3}H-QB and about 40% in those with cyclosyl. The samples were incubated for 30 min at 0°C and the reaction was stopped by

*O,O-dimethyl-1-hydroxy-2,2,2-trichloroethylphosphonate.

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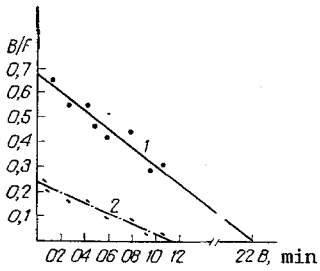

Fig. 1

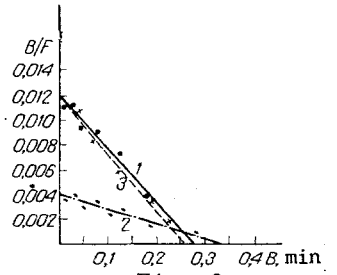

Fig. 2

Fig. 1. Kinetics of binding of ^{3}H-QB with brain membranes of intact rats (1) and rats poisoned with chlorophos (2), on Scatchard plot.

Fig. 2. Kinetics of binding of ^{14}C-cyclosyl with rat brain membranes on Scatchard plot. 1) Membrane preparation from brain of intact rats, 2) of rats poisoned with chlorofos, 3) of rats poisoned with chlorophos with the addition of dipyroxime to incubation medium.

addition of ice-cold buffer containing 10^{-6} M atropine. The bound and free radioligand was separated by filtration under a vacuum through glass fiber GF/B filters ("Whatman," England). The filters were washed twice in 5 ml buffer and placed in flasks containing dioxan scintillator. Radioactivity was measured on a Mark III counter.

In experiments to study the action of organophosphorus compounds (OPC) on specific binding of the radioligand, chlorophos (2 LD_{50}) was injected intramuscularly into the rats. The rats were decapitated 15 min after the beginning of convulsions and the brain used in experiments in vitro by the method described above. Distilled water was injected into animals of the control group. To study the effect of the cholinesterase reactivator on the kinetics of radioligand binding aliquots of homogenate were incubated with increasing concentrations of ^{14}C-cyclosyl and dipyroxime, used in the ratio of 1:25. The statistical significance of differences was determined by Student's test.

EXPERIMENTAL RESULTS

In the experiments of series I specific binding of ^{3}H-QB was studied with a membrane preparation from the brain of intact rats and of rats poisoned with chlorophos.

It will be clear from Fig. 1 that a marked reduction in maximal binding (B_{max}) was observed in animals poisoned with chlorophos. A tendency was noted for binding of the radioligand with the membranes, recorded as an increase in values of the dissociation constant K_d, to weaken (Table 1).

The next step was to verify the existence of a similar relationship for other cholinolytics, with less affinity for the ACh receptor than the muscarinic antagonist QB. In the experiments of series II specific binding of cyclosyl with rat brain membranes was investigated under similar conditions. The results of a study of specific binding are shown in Fig. 2 on a Scatchard plot. During poisoning no significant change was found in the values of maximal binding, but fourfold weakening of the bond was discovered (Table 1). Addition of the cholinesterase reactivator dipyroxime to the reaction medium neutralized the effect of poisoning (Fig. 2; Table 1).

In accordance with accepted views, specific binding of cholinolytics can be identified with their highly selective binding with muscarinic ACh receptors. The results of the present experiments point to an inhibitory action of chlorophos on the kinetics of selective binding of cholinolytics in the rat brain as shown by the maximal binding test for ^{3}H-QB and/or the value of K_d for ^{14}C-cyclosyl. The particular features of the action of poisoning on the kinetics of binding of ^{3}H-QB and ^{14}C-cyclosyl can be explained by the considerable differences in affinity of these radioligands for the muscarinic ACh receptor. Interaction of a certain hypothetical factor, induced by the poison (or of the poison itself) with the ACh receptor and/or its membrane environment leads to reduction of the binding sites without any significant change in affinity for the powerful muscarinic antagonist ^{3}H-QB. The significant decrease in the values of K_d for cyclosyl suggests the presence of heterogeneity of the specific binding sites under the conditions of poisoning, mainly

TABLE 1. Kinetic Parameters of Specific Binding of ^{3}H-QB and ^{14}C-Cyclosyl with Brain Membranes of Intact Rats and Rats Poisoned with Chlorophos

Group of animals	Radioligand	K_d, nM	B_{max}, fmoles/mg protein
Intact	^{3}H-QB	3,3±0,3	5866±472
	^{14}C-cyclosyl	23,2±2,0	746±54
Poisoned with chlorophos (2 LD_{50}; intramuscularly)	^{3}H-QB	4,5±0,42	2933±212*
	^{14}C-cyclosyl	83,3±4,5*	880±67
	^{14}C-cyclosyl in presence of dipyroxime	21,7±2,1	693±52

Legend. Asterisk indicates significant differences from values in intact animals ($p < 0.01$).

regions of lower affinity, which may include presynaptic muscarinic ACh receptors [11]. This line of argument suggests that the positive action of dipyroxime, leading to an increase in K_d for cyclosyl, can be explained by its ability to compete with the "poisoning factor" at the level of muscarinic ACh receptors, including presynaptic receptors also. Actually in recent years certain bis-pyridine oximes have been shown to be able not only to block postsynaptic, but also to activate presynaptic muscarinic ACh receptors [10]. It can be concluded from these results that interaction of cholinolytic and cholinesterase reactivator at the level of muscarinic ACh receptors during poisoning may be one mechanism of potentiation of their therapeutic effects.

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