EFFECT OF DI-ISOPROPYLFLUOROPHOSPHATE, PARAOXON, AND DICHLOROVOS ON BINDING OF [^{3}H]QUINUCLIDINYL BENZYLATE WITH SYNAPTIC MEMBRANES OF THE RAT STRIATUM

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Exposure to anticholinesterase poisons is accompanied by disturbances of the physiological state of neuronal receptors, especially of the muscarine-sensitive kind [1, 2]. This is manifested as a change in the characteristics of binding of a specific ligand for muscarinic (M) acetylcholine receptors, namely [^{3}H]quinuclidinyl benzylate ([^{3}H]QNB). The mechanisms of these changes are unknown. In this investigation the state of M acetylcholine receptors of the striatum was assessed in rats exposed to the convulsive action of di-isopropylfluorophosphate (DFP) and also the effect of DFP, paraoxon, and dichlorovos (DDVP) on binding of [^{3}H]QNB with striatal membranes of intact animals.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 170-200 g. DFP (from "Serva," Germany) was dissolved in physiological saline and injected intraperitoneally in a dose of 6 mg/kg (1-5 LD_{50}). The control animals received an equivalent volume of the solvent. The rats were decapitated 10 min after the beginning of convulsions. The P_2 fraction isolated from the striatum was frozen in 100 volumes of water for 18 h at −20°C. After thawing, the membranes were washed three times in 100 volumes of 50 mM K-phosphate buffer, pH 7.5. [^{3}H]QNB (from "NEN," Germany, 45.7 Ci/mmole, concentration 32-500 pM) was used for radioligand analysis. The volume of the incubation medium was 1 ml. The protein content of the membranes in the sample did not exceed 130-170 μg. After incubation for 2 h at 20°C on GF/C filters ("Whatman," England) the unbound label was separated. Nonspecific binding was determined in the presence of 1 μM atropine. The effect of DFP, DDVP ("Serva," Germany), and paraoxon on binding of [^{3}H]QNB (0.5 nM) with striatal membranes of intact rats also was assessed. The organophosphorus compounds (OPC) were used in concentrations of 10^{-11}-10^{-5} M. Incubation began after simultaneous addition of the anticholinesterase agent and the radioligand. AChE activity was estimated by Ellman's method [3]. Binding characteristics of [^{3}H]QNB were calculated from the results of four separate experiments. Protein was determined by Lowry's method [5].

EXPERIMENTAL RESULTS

The convulsant action of DFP was accompanied by significant changes in binding of [^{3}H]QNB with striatal membranes of the poisoned rats (Table 1).

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TABLE 1. Binding of [^{3}H]Quinuclidinyl Benzylate with Rat Striatal Membranes after Seizures Induced by DFP

Substance	K_d, nM	B_{max}, fmoles/mg protein
DFP	0,34+0,03**	413,0+37,2*
Physiological saline	0,53+0,05	619,5+62,5

Legend: *p < 0.05, **p < 0.02.

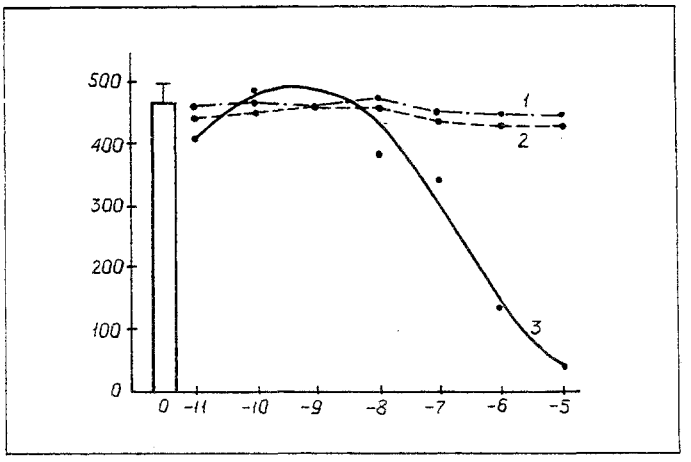

Fig. 1. Effect of DFP, DDVP, and paraoxon on binding of [^{3}H]quinuclidinyl benzylate (0.5 nM) with striatal membranes of intact rats. Abscissa, concentration of OPC (log); ordinate, binding of [^{3}H]QNB (in fmoles/mg protein). O) Binding of ligand in control, 1) DFP, 2) DDVP, 3) paraoxon. Binding of ligand in control was 463 ± 31 fmoles/mg protein.

The density of M acetylcholine receptors was lowered (by 33%) and their affinity for the ligand increased (by 36%). It can be tentatively suggested that the changes observed may be attributed both to dysfunction of cholinergic structures and the membrane-toxic effects of OPC [4, 8]. On the other hand, evidence has been obtained of interaction of anticholinesterase poisons directly with the active zone of the M acetylcholine receptors [9]. Is such a mechanism probable in this case? To answer this question, we studied the effect of DFP, paraoxon, and DDVP (10^{-11}-10^{-5} M) on binding of [^{3}H]QNB with striatal membranes of intact rats. The results show that neither DFP nor DDVP have any effect on binding of the ligand (Fig. 1). Changes in the binding characteristics of [^{3}H]QNB in the striatum during the convulsive action of DFP are unconnected with any direct effect of the poison on M acetylcholine receptors. This conclusion is in agreement with results obtained by other workers, who found that DFP does not affect binding of [^{3}H]QNB with brain membranes of intact animals [7]. Meanwhile, paraoxon effectively inhibited binding of the ligand (Fig. 1). IC_{50} was $5.5 \cdot 10^{-7}$ M. Paraoxon inhibited acetylcholinesterase of striatal membranes in vitro more actively than DFP and DDVP. IC_{50} was $6.8 \cdot 10^{-9}$, $6.4 \cdot 10^{-7}$, and $5.1 \cdot 10^{-6}$ M respectively. It can be tentatively suggested that active zones of AChE and M acetylcholine receptors of striatal synaptic membranes have definite structural similarity. This is in agreement with views regarding the marked homology in the amino acid sequence of these zones [6]. Paraoxor, thanks to its special chemical structure, can probably interact with the active center of the M acetylcholine receptor. Aliphatic OPC DFP and dichlorovos do not possess this property.

The convulsive action of DFP was thus accompanied by changes in binding of [^{3}H]QNB with membranes of the rat striatum: the affinity of the receptors was increased but their density reduced. In experiments in vitro DFP did not affect binding of the ligand with membranes from intact rats, whereas paraoxone effectively inhibited this process. Direct action of DFP on M acetylcholine receptors of the striatum is evidently not the cause of the changes in bind-

ing of [^{3}H]QNB during seizures induced by the anticholinesterase poison. It can be tentatively suggested that the differences thus revealed may be due both to dysfunction of cholinergic structures and to the membranotropic effects of DFP.

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ULTRASTRUCTURE OF NORMAL HUMAN BLOOD LYMPHOCYTES INCUBATED WITH DEATH-HEAD (*Amanita phalloides*) TOXIN

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Functional characteristics of lymphocytes, which are responsible for the main types of immunologic reaction, including antibody production and accumulation of sensitized lymphocytes, capable of recognizing and eliminating foreign substances [4], have now been well studied [5, 9]. Investigations have shown that lymphocytes constitute a quite heterogeneous population.

There is much evidence in the literature of the effect of immunocompetent cells on tumor growth [1, 8], and on diseases of the hepatobiliary system [2, 3, 6]. An important group of diseases is formed by those produced by exogenous poisons, including poisoning by the most deadly of the Hymenomycetes, the death-head (*Amanita phalloides*) [7]. The problem of the effect of death-head toxin (DHT) on immunocompetent human blood cells has not been discussed in the literature.

The aim of this investigation was to study the action of DHT on human blood lymphocytes in relation to dose and duration of exposure.

EXPERIMENTAL METHOD

Heparinized (12 IU/ml) blood was obtained from healthy donors and incubated at 37°C with DHT in a dose of 0.05 and 0.5 LD_{100} for 1 and 3 h. The lymphocytes were sedimented by centrifugation, fixed with a 1% solution of glutaraldehyde in 0.1 M phosphate buffer, postfixed in a 1% solution of osmium, dehydrated in alcohols, and embed-

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