PARAMION CONTRACTURE OF THE DORSAL MUSCLE OF THE LEECH DURING THE ACTION OF ANTI-CHOLINESTERASE DRUGS

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Paramion belongs to a group of compounds with an action resembling that of D-tubocurarine [1, 2, 3, 5, 6, 7]. It has the power of abolishing the effects of acetylcholine, and its action may be prevented and terminated by anticholinesterase drugs. In some objects, however, paramion produces a contractile reaction. For instance, in concentrations of $1 \cdot 10^{-4}$ and $0.2 \cdot 10^{-4}$ it produces a contracture of the isolated rectus muscle of the frog's abdomen [1, 2]. It has recently been found [4] that paramion acts on the denervated tongue of the dog to cause fibrillation of the muscles, which is intensified by prostigmine.

According to our preliminary observations, the muscle of the leech, if treated with prostigmine and eserine, also gives a contractile reaction with paramion. In the present paper we compare the effect of various anticholinesterases on the contractile reaction of the dorsal muscle of the leech during treatment with acetylcholine and paramion.

EXPERIMENTAL METHOD

Experiments were carried out by the method described by Führer [8]. A segment of muscle, consisting of 10-12 rings (1/4 of the muscle), was placed in a vessel with Ringer's solution and kept there for 2 h after isolation while oxygen was bubbled through the solution. Altogether 94 experiments with 110 tests were performed.

EXPERIMENTAL RESULTS

Acetylcholine (28 experiments). Paramion alone, in concentrations of $1 \cdot 10^{-5}$, $1 \cdot 10^{-6}$, $1 \cdot 10^{-7}$, and $1 \cdot 10^{-9}$ caused very slight relaxation of the muscle. The contracture caused by acetylcholine ($1 \cdot 10^{-4}$) was prevented by paramion only in concentrations of $1 \cdot 10^{-5}$ or higher. Lower concentrations of paramion had no significant effect on the acetylcholine contracture (the difference in the magnitude of the contractures was not statistically significant).

Anticholinesterases (prostigmine, eserine, nivalin) are known to sensitize muscle to acetylcholine. It may be seen from Table 1 that eserine caused the most marked sensitization of the dorsal muscle of the leech to acetylcholine. Subsequently the effect of the same compounds on the action of paramion was tested.

TABLE 1. Concentration of Acetylcholinesterase Compounds Causing and Not Causing Sensitization to Acetylcholine

Acetylcholinesterase substances	Maximal concentration causing sensitization to acetylcholine ($1 \cdot 10^{-6}$)	Minimal concentration causing sensitization to acetylcholine ($1 \cdot 10^{-6}$)
Eserine	$0.5 \cdot 10^{-9}$	$0.5 \cdot 10^{-8}$
Prostigmine	$0.5 \cdot 10^{-8}$	$0.2 \cdot 10^{-7}$
Nivalin	$0.5 \cdot 10^{-8}$	$1 \cdot 10^{-8}$

Eserine (23 experiments). The muscle was treated with paramion in various concentrations, after which it was rinsed in Ringer's solution for 40-60 min, and then treated with eserine solution in concentrations of $1 \cdot 10^{-6}$ or $0.2 \cdot 10^{-5}$, causing a well defined sensitization to acetylcholine, followed by paramion in the same concentration as before treatment with eserine. Paramion, in concentrations of $2 \cdot 10^{-7}$ and $1 \cdot 10^{-7}$, after preliminary treatment of the muscle with eserine, caused a contracture in 14 or 18 experiments (Fig. 1). Higher concentrations of paramion ($1 \cdot 10^{-5}$ and $1 \cdot 10^{-6}$) in the same experimental conditions caused relaxation of the muscle (5 experiments), but

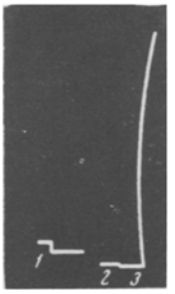

Fig. 1

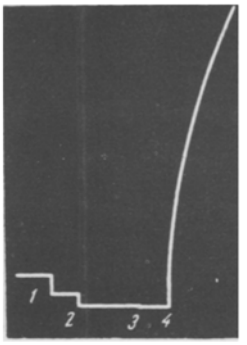

Fig. 2

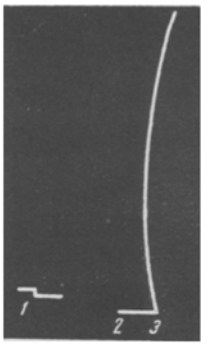

Fig. 3

Fig. 1. Paramion contracture of the dorsal muscle of the leech after preliminary treatment of the muscle with eserine. 1) paramion $2 \cdot 10^{-7}$ (15 min); 2) eserine $1 \cdot 10^{-6}$ (5 min); 3) paramion $2 \cdot 10^{-7}$ (15 min).

Fig. 2. Paramion contracture after preliminary treatment of the muscle with eserine and acetylcholine. 1) acetylcholine $1 \cdot 10^{-6}$ (1 min); 2) eserine $0.5 \cdot 10^{-9}$ (30 min); 3) acetylcholine $1 \cdot 10^{-6}$ (1 min); 4) paramion $2 \cdot 10^{-7}$ (10 min).

Fig. 3. Paramion contracture after preliminary treatment of the muscle with prostigmine. 1) paramion $2 \cdot 10^{-7}$ (30 min); 2) prostigmine $2 \cdot 10^{-6}$ (2 min); 3) paramion $2 \cdot 10^{-7}$ (5 min).

did not prevent the development of the acetylcholine contracture. Particular attention was drawn to the fact that after addition of acetylcholine in a concentration of $1 \cdot 10^{-6}$, not causing a contracture of the eserinized muscle, paramion ($1 \cdot 10^{-7}$) produced a contracture (Fig. 2).

Prostigmine (28 experiments). The conduct of the e xperiment was the same as in the preceding series. Paramion in concentrations of $0.2 \cdot 10^{-6}$ and $1 \cdot 10^{-7}$, not causing a contracture of the muscle, did so in 8 of 12 experiments after preliminary treatment of the muscle with prostigmine (Fig. 3). In the remaining 4 experiments the subsequent action of acetylcholine led to a clear contracture. In contrast to eserine, when prostigmine was used in concentrations not causing sensitization to acetylcholine sensitization to paramion was likewise not observed. However, even if only very slight sensitization to acetylcholine was present the addition of paramion ($0.2 \cdot 10^{-6}$) caused a further increase in the contracture (Fig. 4). Hence eserine, in lower concentrations than prostigmine, facilitates the development of the paramion contracture.

Nivalin – a Bulgarian preparation identical with the Soviet drug galanthamine – (15 experiments). After treatment of the muscle with nivalin, even in concentrations of $0.5 \cdot 10^{-4}$, $1 \cdot 10^{-6}$, and $0.2 \cdot 10^{-6}$, producing obvious sensitization to acetylcholine, the administration of paramion in concentrations of $1 \cdot 10^{-6}$, $1 \cdot 10^{-7}$, and $0.5 \cdot 10^{-6}$ caused no contracture in any one of 14 experiments. However, with a concentration of nivalin causing very slight sensitization to acetylcholine, the contracture caused by acetylcholine was sharply intensified by the addition of paramion (Fig. 5). Paramion concentrations of $1 \cdot 10^{-5}$ and higher, as in the experiments with prostigmine and eserine, depressed the reaction to acetylcholine stimulated by nivalin.

Table 2 shows that of the anticholinesterase drugs tested, only eserine in concentrations not causing sensitization to acetylcholine caused sensitization of the muscle to paramion in the presence of acetylcholine.

The experimental results demonstrated that the curare-like drug paramion may induce not only a cholinolytic effect in the dorsal muscle of the leech, but also a contracture. This latter effect was observed after preliminary treatment of the muscle with eserine and prostigmine (but not with nivalin). The efficacy of the anticholinesterase drugs tested (prostigmine, eserine, nivalin) varied considerably, and the results are capable of differentiation. After treatment with eserine, paramion produced a contracture even though sensitization to added acetylcholine was absent (in the presence of acetylcholine). It is assumed that eserine, by inhibiting the enzymic hydrolysis of acetylcholine, thereby leads to synergism between acetylcholine and paramion.

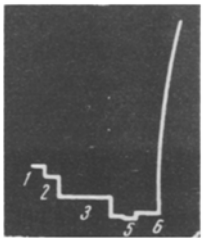

Fig. 4

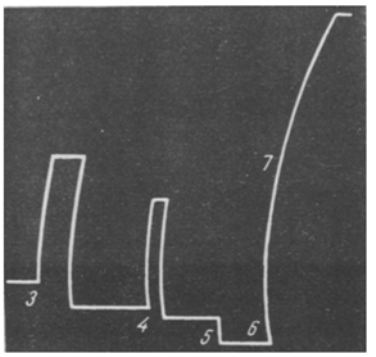

Fig. 5

Fig. 4. Paramion contracture after preliminary treatment of the muscle with prostigmine and acetylcholine. 1) acetylcholine $1 \cdot 10^{-6}$ (1 min); 2) paramion $2 \cdot 10^{-7}$ (10 min); 3) prostigmine $1 \cdot 10^{-8}$ (30 min); 5) acetylcholine $1 \cdot 10^{-6}$ (1 min); 6) paramion $2 \cdot 10^{-7}$ (10 min).

Fig. 5. Potentiation of acetylcholine contracture by paramion after preliminary treatment of the muscle with nivalin. 3) acetylcholine $1 \cdot 10^{-6}$ (5 min); 4) acetylcholine $1 \cdot 10^{-6}$ (30 sec); paramion $2 \cdot 10^{-7}$ (5 min); 5) nivalin $1 \cdot 10^{-8}$ (20 min); 6) acetylcholine $1 \cdot 10^{-6}$ (30 sec) 7) paramion $2 \cdot 10^{-7}$ (5 min).

TABLE 2. Action of Anticholinesterases on the Effects of Acetylcholine and Paramion

Anticholinesterases	Concentration	Effect of sensitization to acetylcholine (in the presence of added drug)	Contracture from paramion ($1 \cdot 10^{-7}$, $2 \cdot 10^{-7}$)	
			present	absent
			No. of experiments	
Eserine	$1 \cdot 10^{-6}$	+	14	4
	$0{,}5 \cdot 10^{-9}$	—	5	1
Prostigmine	$1 \cdot 10^{-6}$	+	8	4
	$0{,}5 \cdot 10^{-8}$	—	1	3
Nivalin	$1 \cdot 10^{-6}$	+	0	10
	$0{,}5 \cdot 10^{-8}$	—	0	4

A similar explanation may be applied to prostigmine, although this drug was effective only in concentrations causing sensitization of the muscle to acetylcholine added from an external source.

After treatment of the muscle with nivalin, even in concentrations causing marked sensitization to acetylcholine, paramion did not produce a contracture. When an acetylcholine contracture was produced, paramion potentiated it. Qualitative differences thus exist between the three anticholinesterases. Paramion, in the presence of anticholinesterases, caused a contracture only if given in comparatively low concentrations; in higher concentrations it suppressed the acetylcholine contracture. The fact that eserine and prostigmine facilitated the development of the paramion contracture, like that of the acetylcholine contracture, suggests that depending on its concentration paramion may act both in the manner of D-tubocurarine (blocking cholinergic structures) and of decamethonium (causing persistent depolarization). Consequently, in small doses paramion potentiates the action of acetylcholine, and in large doses depresses it.

SUMMARY

Experiments were staged on the dorsal muscle of the leech (110 tests). High concentrations of paramion relax

the acetylcholine contracture of the muscle. Low doses may cause contracture after preliminary eserine or prostigimine (but not nivalin) treatment of the muscle. Eserine concentrations which fail to produce cholinosensitization promote the development of paramion contracture in the presence of acetylcholine. Low concentrations of paramion, an agent usually included in the D-tubocurarine group, may cause an effect similar to that of acetylcholine and intensify the action of the latter, which is characteristic of decamethonium and compounds of the ditilin type.

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