Brit. J. Pharmacol. (1948), 3, 219.

THE ACTION OF THE VENOM OF A MEXICAN SCORPION (*CENTRUROIDES NOXIUS*, HOFFMANN) ON CHOLINESTERASES

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(Received December 4, 1947)

Some of the effects of scorpion venom resemble those of eserine and other cholinesterase inhibiting substances. For instance the venom augments the tension response of skeletal muscle to maximal motor nerve volleys and may produce spontaneous contractions (del Pozo and Anguiano, 1947). This potentiation was shown to be due to repetitive response of the muscle fibres similar to that produced by eserine (Burns and del Pozo, 1947). If an anticholinesterase property of the venom were the cause of this effect, the venom should also produce eserine-like actions on other organs. For instance, substances which act by inhibition of cholinesterase produce in the isolated intestine a characteristic contraction which develops slowly and disappears only gradually when the drug is washed out. The effect contrasts with the immediate and quick contractions produced by drugs acting directly on the smooth muscle. The effect of venom was therefore examined on the isolated intestine of the guinea-pig and rabbit. In addition, *in vitro* experiments were performed to find out if the activity of true- as well as pseudo-cholinesterase could be affected by the venom.

METHODS

Pieces of the guinea-pig's and rabbit's small intestine were suspended in 16 c.c. Mg-free Tyrode solution. The contractions of the fibres of the longitudinal muscle layer were recorded by a Lovatt Evans frontal writing lever. The contractions of the circular muscle layer were observed with the naked eye through the glass wall of the tank, and were seen to cause a lengthening of the preparation. Thus relaxation of the longitudinal muscle layer and contractions of the circular layer both affected the record in the same direction. The bath was emptied by overflow and the substances were added in 0.2–0.5 c.c. saline, with a syringe. Air was bubbled continuously through the Tyrode solution, the temperature of which was kept between 32 and 36° C.

Human serum was used as a source of pseudo-cholinesterase and minced tissue of the caudate nucleus of rabbits as a source of true-cholinesterase. Since human serum contains practically no true- and the tissue of the caudate nucleus practically no pseudo-cholinesterase, their effects on acetylcholine hydrolysis are due in each case to one of the two enzymes only. The effect of venom was compared with that of eserine, which inhibits both cholinesterases (Hawkins and Mendel, 1947).

The procedure adopted was as follows: In preliminary experiments the amounts of cholinesterase preparation necessary to hydrolyse in 10 min. about 90 per cent of 200 or 400 μg. of acetylcholine at 37° C. were determined. Acetylcholine was estimated with the eserinized frog rectus muscle. It was found that 0.2 to 0.25 c.c. human serum or the equivalent of about 10 mg. tissue of the caudate nucleus made up in a final volume of 3 to 3.5 c.c. were required for this purpose. Several samples were then set up simultaneously. Each contained the same amount of the cholinesterase preparation, to which was added either venom or eserine in varying concentrations; the total volume was then made up to 2 or 2.5 c.c. with saline. Ten minutes later the acetylcholine (in 1 c.c.) was added to the samples; these were kept for another 10 min. before the enzyme action was stopped by acidification and boiling. The samples set up with true cholinesterase (a suspension of finely ground brain tissue) were shaken during the whole 20 min. The acetylcholine content of the samples after neutralization was assayed on the eserinized frog rectus muscle. The venom present in some of these samples was found not to interfere with the assay.

The scorpion venom was obtained from ground telsons of a Mexican scorpion (*Centruroides noxius*, Hoffmann) by extraction with saline and precipitation with acetone (Anguiano, 1947). A yellowish greenish powder is obtained which is stable and soluble in saline. The certainly lethal dose of the powder on intravenous injections into 20 g. mice was 1.1 mg.; this dose killed the mice within 15 to 30 min.

RESULTS

1. *Isolated intestine preparations*

Guinea-pig's intestine.—The addition of 2 to 10 mg. of venom extract to the 16 c.c. bath caused,

after a latent period of 30 to 60 sec., a slowly developing contraction of the longitudinal muscle layer. After washing out the venom, relaxation proceeded gradually (Fig. 1b). The effect resembled that of eserine by its long latency, its gradual development and disappearance; it differed, however, from that of eserine in the following respects. After replacing the bath with fresh Tyrode solution the effect of eserine subsided even more gradually that that of the venom extract (see Fig. 1a and b). In addition eserine has a more powerful action on the circular muscle. The preparation, first shortened by eserine owing to the contraction of the longitudinal muscle, frequently shows short periods of lengthening as a result of strong contractions of the circular muscle layer. This is seen in Fig 1a during the first four minutes of the eserine action. With the scorpion venom, contractions of the circular muscle may be absent or occur only once or twice. In the record of Fig. 1b one such period of lengthening owing to contraction of the circular muscle is seen after the administration of the venom.

Approximately 5 mg. of venom were found to have an action equal to or somewhat smaller than that of 10 μg. of eserine; i.e., eserine was about 500 times more active than the venom extract. On repeated administration the venom did not lose its power to cause contraction of the smooth muscle in the intestine; thus no desensitization to the venom took place, unlike the characteristic desensitization which occurs with snake venoms.

Atropine had an antagonistic effect on the action of the venom extract, but the antagonism was less pronounced than with eserine. In the experiment of Fig. 1, atropine 1 in 1.5 million had abolished the action of 5 and even of 10 mg. of venom extract, but 20 mg. of venom extract still produced a pronounced contraction; on the other hand the preparation had become practically insensitive to 150 μg. of eserine (at c). In some experiments one part of atropine in a million reduced but did not abolish the effect of even 5 mg. of venom extract.

Rabbit's intestine.—The effect of the venom extract on the rabbit's intestine resembled that on the guinea-pig's intestine. The addition to the 16 c.c. bath of 1 mg. extract and, in some preparations, even of 0.5 mg. produced small contractions. Stronger effects were obtained with the addition of 5 to 10 mg. of venom extracts. When these amounts were kept in the bath for a few minutes a gradually developing contraction of the longitudinal muscle layer with usually slight contractions only of the circular muscle occurred after a latent period of 30–90 sec. Relaxation of the muscle after washing out the extract proceeded as gradually as after eserine; the venom also produced a contraction of the circular muscle layer shortly after being washed out, but to a less extent than eserine. In nearly all experiments the effect

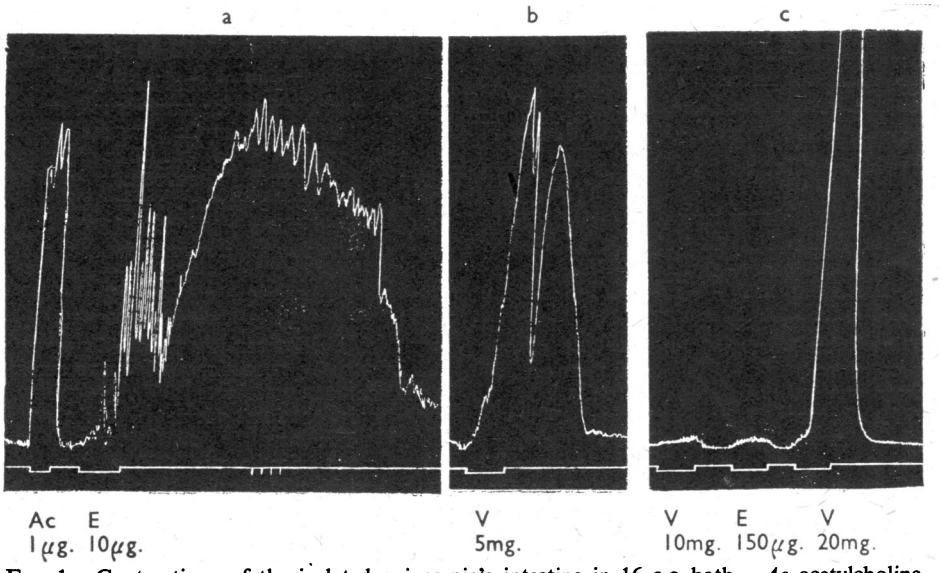

FIG. 1.—Contractions of the isolated guinea-pig's intestine in 16 c.c. bath. *Ac*, acetylcholine washed out after 1 min. *V* and *E*, scorpion venom extract and eserine sulphate respectively washed out after 2 min. *a* and *b* before, *c* during atropine sulphate, 1 in 1.5 million. Time in 30 sec. indicated in *a*. For details see text.)

on the rabbit's intestine of 5 mg. venom extract was slightly weaker, and that of 10 mg. much stronger, than that of 10 μg. of eserine.

Atropine had only a slight antagonistic action on the effect of venom. It was even less effective in antagonizing the action of the venom extract than in the corresponding experiments on the guinea-pig's intestine. The effect of 5 mg. venom extract was only slightly if at all reduced by a concentration of atropine (1 : 10^6) which abolished the effect of 10 μg. eserine sulphate.

2. *Cholinesterase preparations*

Venom extract had only a slight inhibiting action on pseudo- as well as on true-cholinesterase. In the experiments of Table I a 0.1 per cent solution of the venom had no effect on the enzyme activity in the samples and 1 to 2 per cent solutions caused partial inhibition. When this effect was compared with the inhibition produced by eserine sulphate it was found that, at room temperature, eserine 1 in 80 millions had a much weaker and eserine 1 in 20 millions a slightly stronger action than 1 to 2 per cent venom extract ; in the one experiment with true-cholinesterase carried out at 37° C. the difference in potency between the two substances was even greater.

Discussion

The extract of venom of the Mexican scorpion used in the present experiments exerted an anti-cholinesterase activity only if tested in very high concentrations, and there was no difference between its effects on pseudo- or true-cholinesterase preparations. When the inhibitory action of the venom extract on these cholinesterases was compared with that of eserine in *in vitro* experiments it was found that, weight for weight, the venom extract was 200,000 to 800,000 times less potent. The necessity of using high concentrations of venom extract for demonstrating its cholinesterase inhibitory action raises the question of how far the effect is due to the venom proper or to impurities present in the extract or, on the other hand, how far such impurities may have reduced any inhibitory action of the venom proper. This question can be answered only if purified preparations of the venom become available.

It is difficult to see how the feeble cholinesterase inhibiting action of the venom extract can contribute more than to a slight extent to the smooth muscle contracting effects on the isolated intestine. The effect has a superficial resemblance to that of eserine, but 0.5 mg. of the venom extract were found to be about as potent as 1 μg. of eserine sulphate, whereas in the *in vitro* experiments on cholinesterase preparations 1 μg. of eserine was as potent as 200 to 800 mg. of venom extract. In addition the smooth muscle contracting effect of the venom was more resistant to atropine than that of eserine. The action of the venom extract on the intestinal preparations appears therefore to be independent of its feeble cholinesterase inhibiting property. Similarly the spontaneous contractions of skeletal muscle, and the potentiating effect which the venom extract has previously been found to exert on the twitch response to single motor nerve volleys in cats, cannot be attributed, or can be attributed to a slight extent only, to inhibition of cholinesterase. These " eserine like " effects of the venom extract can more reasonably be explained as an action either directly on the motor nerve endings or on the motor end plates or on both structures.

TABLE I

COMPARISON OF THE INHIBITORY ACTION OF SCORPION VENOM EXTRACT AND ESERINE SULPHATE ON CHOLINESTERASE ACTIVITY

Cholinesterase source	Temp.(°C.) at which samples were kept	μg. acetylcholine found after 10 minutes action of cholinesterase.						
		Concentration of venom extract				Concentration of eserine sulphate		
		0	0.1%	0.33–0.5%	1–2%	1.25×10^{-8}	5×10^{-8}	$1–2 \times 10^{-7}$
(1) Human serum ..	14.5	42	50	75	90	—	150	200
(2) Human serum ..	15.5	21	28	52	110	60	160	200
(3) Caudate nucleus	16.5	9	9	12	45	12	40	150
(4) Caudate nucleus	37.0	80	90	95	110	275	325	325

In experiments (1) to (3) 200 μg. acetylcholine were used as substrate; in (4) 400 μg.

SUMMARY

The effects of extracts of the venom of a Mexican scorpion (*Centruroides noxius*, Hoffmann) were examined on cholinesterase preparations with the view of finding out if the "eserine-like" effects this venom exerts on isolated tissues and in animals on intravenous injections could be explained by a cholinesterase inhibiting property. The venom extract was only found to inhibit pseudo- as well as true-cholinesterase when present in very high concentrations. It is concluded that the symptomatology of the venom poisoning is independent of, or dependent to a very slight degree only on, inhibition of cholinesterase.

I should like to make grateful acknowledgment to Dr. W. Feldberg for his help and interest in this work, to Prof. E. D. Adrian for the hospitality afforded in his Institute, and to the British Council for their invitation to come to England.

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