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THE ANTICURARE ACTIVITY OF ESERINE ON THE SUPERIOR CERVICAL GANGLION OF THE CAT

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Eserine and prostigmine augment the contractions of skeletal muscle produced by stimulation of the motor nerve. This observation is one which supports the view that acetylcholine plays a part in neuromuscular transmission as both substances are inhibitors of cholinesterase. They are, in addition, powerful antagonists to curare on skeletal muscle.

The transmission in sympathetic ganglia is also believed to be effected by acetylcholine, but in the perfused cervical ganglion it is difficult to observe any potentiation of the effects of preganglionic stimulation by eserine or prostigmine. In concentrations from 10^{-5} to 10^{-4} eserine causes a depression of ganglionic transmission, though Feldberg and Vartiainen (1934) found that a weak eserine solution (10^{-6}) could be shown to potentiate the response to submaximal and infrequent stimulation as well as that to injection of small doses of acetylcholine.

We have now used another method to examine the action of eserine on the ganglion, which is to see if the depression of ganglionic transmission by tubocurarine is relieved by eserine.

METHOD

Cats were anaesthetized with pentobarbitone and the superior cervical ganglion was prepared by Kibjakow's method (1933) as modified by Feldberg and Gaddum (1934). Warm, oxygenated Locke solution was perfused through a cannula in the carotid artery at a pressure around 120 mm. of mercury and the venous outflow from the ganglion was collected. The preganglionic fibres were stimulated with maximal stimuli at a rate of 8 per second for periods of 15 sec. at 3 min. intervals. In some experiments the stimulation was continuous so that the contraction of the nictitating membrane was recorded as an uninterrupted plateau. All contractions of the nictitating membrane were recorded with an isotonic lever. Curare was given as *d*-tubocurarine chloride. Eserine and prostigmine were given as sulphate and methylsulphate respectively.

RESULTS

The effect of eserine was observed in five experiments. After recording at least three maximal contractions of the nictitating membrane, tubocurarine was perfused at a concentration of 1–4 μg./c.c. Gradually the contractions declined and if no antagonist was injected the response became progressively smaller. When the contraction was diminished by 30-40 per cent the antagonist was injected. It was found that eserine in doses of 0.1–0.4 μg. caused an increase of contractions, while doses of 1–5 μg. had no effect or possibly caused a further depression. An illustration of the action of eserine on the curarized ganglion is given in Fig. 1. Two contractions of the nictitating membrane in response to maximal stimulation are shown in (a). After 21 min. perfusion with 4 μg. tubocurarine chloride per c.c., the contractions diminished to about half the size (b). Now 0.2 μg. eserine was injected 1 min. before each stimulation and (c) shows the contractions after the 9th and 10th dose of eserine. The effect of tubocurarine was not entirely abolished but the contractions recovered to 86 per cent of their original size. When the perfusion with tubocurarine was continued without adding any further eserine, the contractions declined once more to 57 per cent of their original height (d). Finally, when Locke solution containing no tubocurarine was perfused, the contractions recovered once more to 86 per cent of their initial size (e). From this experiment it can be seen that the eserine effect is only maintained by repeated injections of 0.2 μg. This was confirmed in other experiments. The eserine was injected regularly 1 min. before stimulating the preganglionic nerve.

It has not been possible to demonstrate any anticurarine action of prostigmine on the perfused ganglion. Repeated doses of 0.05 μg., 0.4 μg., 2.0 μg., and 20 μg. were tried in four experiments, but the contractions of the nictitating membrane declined progressively in spite of the addition of prostigmine.

The effect of tubocurarine given by single injections of 5 μg. to 10 μg. or by perfusion of a

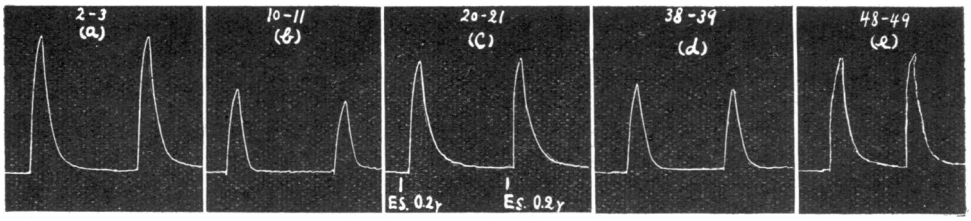

FIG. 1.—Ganglion perfusion. The numbers above each section are the serial numbers of the contractions of the nictitating membrane.

(a) Two contractions in response to maximal stimulation.

(b) After 21 min. perfusion with 4 μg. tubocurarine chloride per c.c. the contractions diminished to about 50 per cent.

(c) Shows the contractions after the 9th and 10th dose of 0.2 μg. eserine injected 1 min. before each stimulation; the effect of tubocurarine was not entirely abolished but the contractions recovered to 86 per cent of their original size.

(d) The perfusion with tubocurarine was continued without adding any further eserine and the contractions declined once more to 57 per cent of their original height.

(e) When Locke solution containing no tubocurarine was perfused the contractions recovered once more to 86 per cent of their initial size.

solution containing 4 μg. per c.c. was also observed during a sustained contraction of the nictitating membrane produced by continuous preganglionic stimulation. When the tubocurarine had reduced the height of the plateau to about 50 per cent of its original height, eserine or prostigmine was injected; but neither eserine nor prostigmine had any antagonistic action under these conditions.

The effect of eserine methiodide was observed in one experiment. After recording three maximal contractions of the nictitating membrane, tubocurarine was perfused at a concentration of 2 μg. per c.c. Gradually the contractions declined and the responses became progressively smaller. When the contraction was diminished to 32 per cent, eserine methiodide was injected 1 min. before each stimulation. It was found that doses of 0.2 μg. and 1.0 μg. had no effect, while doses of 5.0 μg. caused an increase of contractions which recovered to 51 per cent of their original size after the fourth dose of 5.0 μg. of eserine methiodide. When the perfusion with tubocurarine was continued without adding any further eserine methiodide the contractions declined once more to 27 per cent of their original height; after this injections of 25 μg. of eserine methiodide 1 min. before stimulation had no effect. Finally, when Locke solution containing no curarine was perfused, the contractions recovered once more to 67 per cent of their initial size. From this experiment it can be seen that the anticurare effect of eserine methiodide is only maintained by repeated injections of 5.0 μg.

DISCUSSION

According to Schweitzer, Stedman, and Wright (1939) the difference between the action of eserine and prostigmine on the spinal reflexes is due to the difference in the chemical structure of the basic nitrogen radicle rather than to their difference in anticholinesterase activity. They found that eserine caused an excitatory action of the spinal cord whereas prostigmine caused an inhibition. Eserine methiodide, a quaternary derivative of eserine, also produced an inhibition. They explained the difference between the effect of these substances by their different relative solubility in water and in lipoid. It is well known that a quaternary ammonium salt such as prostigmine produces a cation which is soluble only in water. On the other hand, the salt of a tertiary ammonium base, such as eserine, on hydrolytic dissociation produces a free base in addition to the cation, and this free base will be soluble in lipoid. We found that eserine, if administered in small doses and by repeated injections, had anticurarine activity, while prostigmine had no such action. By changing the tertiary ammonium salt, eserine, into the quaternary salt, eserine methiodide, its anticurarine activity became 25 times less than that of eserine itself. Since the spinal reflex involves a synaptic transmission not very different from that occurring in the ganglionic synapse, it is possible to explain by this hypothesis the difference between eserine, eserine methiodide, and prostigmine which has been found in our experiments. The fact that eserine when given in

large doses or when given during continuous electrical stimulation, no longer has anticurare action, does not contradict the foregoing assumption. Under such conditions there is usually an excessive accumulation of acetylcholine, which, being a quaternary ammonium compound itself, is quite sufficient to cause depression of the ganglion cells.

The picture may, however, not be so simple. Bülbring and Burn (1941) have found that eserine and prostigmine were equally depressant to the knee jerk, and both increased the flexor reflex. They believe that there is only a quantitative difference between the action of these two drugs on the reflexes, eserine being more potent than prostigmine.

Summary

Eserine antagonizes the action of tubocurarine on transmission in the perfused superior cervical ganglion.

Eserine methiodide has an anticurare action on transmission in the perfused superior cervical ganglion; its relative potency to that of eserine is about 25 times weaker.

No such antagonistic effect could be observed with prostigmine.

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