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ACETYLCHOLINE METABOLISM OF NORMAL AND AXOTOMIZED GANGLIA

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Transmission of impulses through a sympathetic ganglion is abolished by section of its postganglionic axons (axotomy) (Brown, McLennan & Pascoe, 1952; Brown & Pascoe, 1954). The loss of function is attributable to a loss by the ganglion cells of their excitability to acetylcholine. The output of acetylcholine by the perfused ganglion in response to stimulation of the preganglionic trunk falls within normal limits, but the axotomized ganglion cells respond neither to this nor to acetylcholine reaching them from the circulation. The crude test of perfusion gives little information about this metabolism of acetylcholine in the ganglion, particularly about the time course of its destruction. One possible cause of the transmission block is the accumulation in the ganglion of amounts of acetylcholine which could cause depolarization of the cells and a loss of excitability. For this reason it appeared desirable to study the quantitative changes of the enzymes responsible both for the destruction and the synthesis of acetylcholine.

METHODS

The superior cervical ganglia of rats have been used. The animals were anaesthetized with ether, and the postganglionic axons from the ganglion on one side were sectioned with aseptic precautions, the nerves being cut close to the ganglion. The contralateral ganglion served as a control. A period of 3 weeks was allowed for complete degeneration.

For the determination of cholinesterase, both ganglia were removed from a pair of rats. Immediately after excision the ganglia were placed in a dish of saline medium, and after the removal of the capsule with scissors, they were blotted and weighed on a torsion balance. The ganglia were placed in a glass homogenizer of the Potter-Elvehjem type, together with 2 ml. of saline medium, and ground to give a uniform tissue suspension. 1·8 ml. of this suspension was placed in the main compartment of a Warburg vessel, and 0·2 ml. of acetylcholine chloride solution of a strength sufficient to give a final concentration in the vessel of 4 m.mole/l. was placed in the side bulb. The estimation was carried out at 37° C. The saline medium contained, in m.moles/l.: Na 148, K 3, Ca 1·5, Mg 1, Cl 129, HCO_3 25, SO_4 1. In the cholinesterase determination this was equilibrated with a gas phase consisting of 95 % N_2–5 % CO_2, which at 37° gives a pH of 7·4.

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For the measurement of the amount of acetylcholine synthesized, the ganglia from two to six rats were excised, decapsulated, and weighed as before. The ganglia were incubated, either whole or divided in two with scissors, in 2 ml. of saline medium. The medium was of composition similar to the above, but contained in addition 10 m.mole/l. glucose and 0·4 m.mole/l. eserine sulphate, and was equilibrated with 95% O_2–5% CO_2 at 37°. The high potassium medium which was used in some of the experiments contained 27 m.moles/l. K, and the Na was reduced to 124 m.moles/l. to preserve isotonicity.

At the end of the incubation period, the vessels were taken from the bath, the tissue was removed, and the amount of acetylcholine formed was determined directly in the suspending fluid. The assay was performed on the isolated eserinized dorsal muscle of the leech.

RESULTS

A consistent observation in the course of this work has been that the weight of an axotomized ganglion is greater than that of the control from the same animal. This is due to the development of a neuroma of variable size at the point of section of the axons. Comparisons between normal and axotomized tissue on a weight basis are therefore impossible, and the results to be presented below are expressed per ganglion, rather than per gram of tissue. This does provide a fair basis for comparison, as the weights of two normal ganglia from the same animal agree to within 3% of each other, and it is probable that the amount of actual ganglionic tissue remains unaltered after axotomy.

TABLE 1. Cholinesterase content of normal and axotomized ganglia

Values are expressed as μl.CO_2 evolved/ganglion in an incubation period of 30 min.

Normal	Axotomized
66	15
27	15
25	19
46	27
41	18

The cholinesterase content of axotomized ganglia has been found to be reduced. The values obtained are shown in Table 1. The loss of activity is variable, the axotomized ganglia containing between 23 and 76% of the enzyme content of the controls. This drop represents a loss of 'true' cholinesterase, since 'pseudo' cholinesterase, as judged from the complete lack of hydrolysis of either triacetin or benzoylcholine, is not present in this tissue.

Table 2 shows the effects of axotomy on the synthesis of acetylcholine. If the tissue is incubated in a medium containing the normal low concentration of potassium, then the amount of acetylcholine synthesized is approximately the same in the axotomized and in the control ganglia. Raising the potassium concentration to 27 m.moles/l. causes a considerable increase in the synthesis of acetylcholine in normal ganglia, as is also observed in brain tissue (McLennan & Elliott, 1950), but this increase is not obtained with the axotomized ganglia, the amount of acetylcholine synthesized being the same in both media.

TABLE 2. Acetylcholine synthesis by normal and axotomized ganglia

Values are expressed as μg/acetylcholine chloride synthesized per ganglion during the time of incubation.

Normal		Axotomized	
3 m.moles K^+	27 m.moles K^+	3 m.moles K^+	27 m.moles K^+
1 hr incubation			
0·005	—	0·005	—
0·013	—	0·011	—
—	0·035	—	0·010
2 hr incubation			
—	0·150	—	0·020
—	0·090	—	0·010
0·006	0·034	0·007	0·006

DISCUSSION

The finding that about one-half of the cholinesterase of sympathetic ganglia is lost after axotomy is difficult to understand. The amount of cholinesterase present in normal tissue could hydrolyse about 0·2μg of acetylcholine per second (i.e. 10,000 times the observed maximum rate of synthesis), and the loss of even three-quarters of this activity should leave enough to cope with the amounts of acetylcholine likely to be encountered in the tissue. In the superior cervical ganglion of the cat, 'true' cholinesterase has been demonstrated to be present in some, but not all, ganglion cells, but most is found in the preganglionic fibres and their ramifications (Koelle, 1951). None is present in the postganglionic axons. After preganglionic section the enzyme disappears from the fibres, and remains only in the cells and their prolongations (Koelle, 1951). Cholinesterase in ganglia determined by chemical rather than by histological means is reduced to about one-half by preganglionic section (von Brücke, 1937). Axotomy should have no effect on the enzyme of the preganglionic fibres, but might perhaps cause a reduction of the cellular cholinesterase such that a local excess of acetylcholine could develop. Histochemical localization of the enzyme in axotomized ganglia might provide valuable information in this connexion.

The significance of the results obtained for the synthesis of acetylcholine is obscure. The fact that synthesis in the normal low potassium medium is unaffected by axotomy, coupled with the finding that the output of acetylcholine from the ganglion on preganglionic stimulation is also unchanged, combine to suggest that the supply of transmitter is adequate under ordinary conditions. The mechanism by which potassium ions enhance the synthesis of acetylcholine in normal tissues is not clear: that they have a direct effect on the synthesizing enzyme is certain, but it is also probable that in surviving tissue preparations, such as that used here, there are other effects as well. The failure of potassium to stimulate the synthesis in axotomized ganglia might indicate

that the tissue store of acetylcholine is more easily exhausted, or that it is more slowly restored; but it is difficult to see how this phenomenon can be invoked as an explanation for the observed transmission block. Neither does the finding indicate a defective movement of potassium ions, for the rate and extent of potassium turnover is unaffected by axotomy (McLennan, 1953).

These experiments, then, show that changes in the anabolism and catabolism of acetylcholine are associated with section of the postganglionic axons of sympathetic ganglia. The question of their relation, if any, to the failure of ganglionic transmission must, however, await further investigation.

SUMMARY

1. The total cholinesterase content of axotomized ganglia is reduced to about one-half of that found in normal tissue. This represents a decrease in 'true' cholinesterase, since 'pseudo' cholinesterase does not appear to be present in this tissue.

2. The synthesis of acetylcholine *in vitro* is unaffected by axotomy, provided that the ganglia are incubated in a medium containing a low (3 m.moles/l.) concentration of potassium. If the potassium concentration in the medium is raised to 27 m.moles/l. promoting a great increase in the synthesis by normal ganglia, no such increase is observed with the axotomized ganglia.

3. These findings are discussed in relation to the observed failure of transmission through the ganglion consequent upon axotomy.

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