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CHOLINE ACETYLASE IN ANTERO- AND RETRO-GRADE DEGENERATION OF A CHOLINERGIC NERVE

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Feldberg (1943), and later Banister & Scrase (1950), showed that after denervation the superior cervical ganglion gradually loses its ability to synthesize acetylcholine. Neither of the methods used in these investigations permitted direct measurement of choline acetylase activity. Even though that employed by Banister & Scrase was very sensitive, the rate-determining factor in the production of ACh in their system need not have been choline acetylase; equally it could have been the activity of the enzymes which are necessary to produce acetyl coenzyme A, the substrate required by choline acetylase. The problem is resolved in the present experiments by using a system in which acetyl CoA is provided separately (Hebb, 1955). This method permits a direct measurement of ChA (choline acetylase) activity and we have been able to determine how the ChA of the cervical sympathetic nerve is affected by degenerative section.

METHODS

Fifteen Welsh mountain sheep (thirteen male castrates age 2½–8 months, wt. 15–36 kg, two ewes approximate age 4 years, wt. 29 and 30·5 kg) and two cats (both female, age 2 years, wt. 1·8 and 2·5 kg) were used in the experiments. Chronic and acute operations on the cats were done, as described by Banister & Scrase (1950). In both chronic and acute operations on sheep, induction with pentobarbitone ('Nembutal', Abbott's veterinary solution, 0·28–0·6 ml/kg intravenously) was followed by cyclopropane anaesthesia. The tissues were removed for analysis at the acute operation between 2 and 28 days after section of the nerve. In nine sheep the cervical sympathetic nerve on one side (eight on the right and one on the left) was divided at the caudal pole of the superior cervical ganglion; while in four others the sections were performed in the mid-cervical region through a paramedial incision (only on the right side).

Measurement of choline acetylase activity. The ganglia and nerves removed from the animal were treated as follows: outer coverings of connective tissue were stripped off as completely as possible; the nervous tissue was cut into smaller pieces; and these were macerated in a mortar in two successive washes of cold (− 10° C) dry acetone (c. 150 ml.), which with the tissue (40–160 mg) was filtered off through a small Buchner funnel (one layer of Whatman no. 30 paper). In early experiments it was found that large losses of enzyme occurred if the tissue was ground too finely. For this reason we used the pestle only to flatten and break up the tissue enough for the drying action of the acetone to be effective. The tissue residue recovered from the filter was

dried *in vacuo* (at 300 mm Hg below atmospheric pressure) over P_2O_5 for 4–5 hr. It was then weighed and made up in cysteine-saline (3 mg L-cysteine/ml.) in a concentration of 25 mg acetone powder/ml. The tissue-saline suspension was kept at −17° C for 1–4 days. Before incubation the frozen extracts were thawed and briefly centrifuged (1550 g for 3 min).

In a number of experiments duplicate incubations to determine choline acetylase activity were done, using two separate systems for synthesis in the presence of either acetate or citrate (Hebb, 1955). Later a modification combining these methods was tried, and since it was slightly more sensitive it was adopted as a routine. In the modified system the incubation (at 37° C) was done in two steps:

(1) An initial incubation from 10 to 15 min of the following:

1. 70 units crude yeast CoA (Hebb, 1955).
2. 0·8 ml. of a reaction mixture made up as follows: 23·8 ml. K-ATP originally containing 540 mg Ba-ATP; 4 ml. 4% choline; 2·2 ml. 24% KCL; 2 ml. 0·5% eserine sulphate.
3. 0·1 ml. 2·4% $MgCl_2.6H_2O$.
4. 0·2 ml. 6·3% Na citrate.
5. 0·1 ml. 0·5% Na_2HPO_4.
6. 0·1 ml. 2·7% Na acetate.$3H_2O$.
7. 0·1 ml. 6% L-cysteine HCl (neutralized with KOH).
8. 0·10–0·12 ml. aged frozen liver enzyme (Kaplan & Lipmann, 1948).
9. Water to bring total volume to 2·1 ml.

(2) The addition of 0·4 ml. extract of test tissue and incubation for 1 hr at 37° C. The ACh formed at the end of incubation was assayed on the frog rectus abdominis muscle, using precautions described by Feldberg & Hebb (1947).

RESULTS

The results are shown in the accompanying graph in which the ChA activity of the superior cervical ganglion and of the cervical sympathetic nerve on the operated side is expressed as a percentage of the values of the corresponding structure on the unoperated side in the same animal. In control experiments it was found that the difference between the two cervical sympathetic nerves or the two superior cervical ganglia of the same individual did not exceed 20%, a difference which could represent the sum of the errors of estimation. Larger differences were found when the cervical sympathetic nerves and the superior cervical ganglia of one animal were compared with those of another. In the thirteen sheep the values found for ChA activity in normal (i.e. non-denervated) ganglia varied from 5 to 10 mg ACh synthesized/g dried tissue/hr at 37° C. The range of values for the unsectioned cervical sympathetic nerve trunk was from 4·2 to 9 mg. The variations observed appeared to be related to differences in structure, particularly the amount of connective tissue within the nerve trunk and ganglia.

The graph shows that within 48 hr of section of the cervical sympathetic a significant fall of ChA activity occurred in the superior cervical ganglion. The fall continued steadily for 5–6 days when the activity was about 5% of the control tissue; then there was a further slower loss and at 3 and 4 weeks after section the values were only 0·7–1·5 and 0–1·5% respectively. The loss of activity from the denervated superior cervical ganglion 1 week or longer

after section was found to be about the same whether the point of section was immediately caudal to the lower pole of the ganglion or much lower in the neck, some 12–15 cm farther away from the ganglion. It may also be seen from the graph that in the group of experiments in which the nerve was sectioned at the lower point the loss of ChA from the nerve trunk beyond the section was equivalent to the loss of enzyme from the ganglion—that is, all parts of the nerve distal to the section were equally affected.

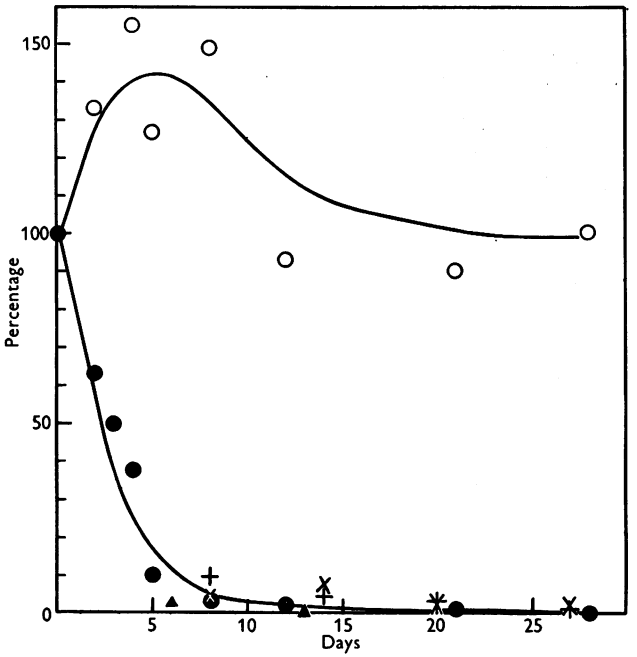

Fig. 1. Graph showing ChA activity of cervical sympathetic nerves and S.C.G. (superior cervical ganglion) at zero time and for 2–28 days following section of the cervical sympathetic nerve (1) immediately caudal to the S.C.G., and (2) in the lower cervical region. *Ordinate*: ChA activity, yield of ACh from sectioned nerves expressed as a percentage of the yield from the corresponding structure on the unoperated side of the same animal. *Abscissa*: time in days from date of operation. (1) Section of cervical sympathetic nerve in sheep immediately caudal to S.C.G. ●, ChA activity of S.C.G.; ○, ChA activity of nerve trunk central to point of section. (2) Section of cervical sympathetic nerve in sheep in lower cervical region. ×, ChA activity of S.C.G.; +, ChA activity of nerve trunk lying between section and S.C.G.; ▲, values for ChA activity of cat S.C.G. after section of cervical sympathetic nerve in mid-cervical region.

The loss of ChA activity from the superior cervical ganglion of two cats, one tested 6 and the other 13 days after denervation, was of the same order as the loss observed in sheep ganglia for similar periods of degeneration. Thus the results confirmed those described earlier by Banister & Scrase (1950), and

showed that the reduced ACh synthesis observed by these authors in denervated ganglia could be wholly accounted for as a loss of ChA activity. Though in their experiments the enzymic formation of acetyl CoA might also have been affected, the change in ChA by itself could have brought about the reduced rate of synthesis which they found.

In contrast to the effects produced by section on the distal part of the nerve, the first effect on the proximal end was a rise in its ChA content. Expressed in terms of the yield of ACh/g acetone-dried tissue/hr, the largest increase observed was about 55%. In fact, however, the total amount of enzyme in the proximal portion of the nerve may have been more than the graph indicates since the dry weight of tissue per unit length of nerve also increased initially: in one experiment it doubled, in others the change was less than this. Enlargement of the nerve and a rise in ChA activity were both observed only during the first few days after section of the nerve. During the second, third and fourth weeks of degeneration there was no significant difference in the dry weight or in the ChA content of the proximal part of the sectioned nerve as compared with the corresponding part of the unsectioned nerve.

DISCUSSION

That nearly all the ChA should disappear from the distal part of a sectioned nerve is not surprising since it has for some time been thought that the source of the ChA which is found throughout a cholinergic neurone is the cell body. On this view the enzyme in the axon is in transit from the cell body where it is manufactured to the endings where it is used. While our experiments furnish no proof for the idea, it is for the present the most reasonable way of explaining our results: on the one hand, the fall of enzyme in the distal part of the nerve is in effect a cutting off of the supply of newly manufactured enzyme; on the other hand, the initial rise in the ChA content of the proximal part of the nerve may represent a piling up of enzyme in the axon due to its continued production in the cell at a time when it can no longer be utilized.

A somewhat similar situation has been recorded by Sawyer (1946). He showed that true cholinesterase accumulated in the nerve stump on the central side of section in the cut sciatic nerve of the guinea-pig. Its concentration in the fresh nerve tissue increased to about 300% within 10–14 days after operation. On the other side of the section, however, the true cholinesterase fell to about 40% of its control value. Sawyer concluded that the greater part of the enzyme in the proximal regenerating stump must have been secreted by the axis cylinders. Again it seems a reasonable inference that the enzyme which is so secreted was originally derived from the cell body.

It is now well known that acetylating enzymes depend for their activity upon the presence of acetyl CoA. The production of acetyl CoA is an enzymic process, and in the earlier experiments of Banister & Scrase (1950) its produc-

tion from citrate in the presence of CoA might have been the rate-limiting factor which determined the yield of ACh. In other words, their experiments did not determine whether anterograde degeneration was associated with a loss of ChA, or a loss of the enzymes which can produce acetyl CoA. The present results, however, are in reasonably good agreement with theirs; and since the method we have used is a method of measuring ChA activity independently of other enzyme changes it may be concluded that in their experiments as well as in our own the observed fall in ACh synthesis is a measure of the ChA activity lost by the nerve.

SUMMARY

1. Measurement of the choline acetylase of cervical sympathetic nerves and superior cervical ganglia in sheep after chronic degenerative section of the cervical sympathetic nerve leads in the distal part of the nerve to a fall in its choline acetylase content.

2. The residual enzyme activity 1 week after section is about 5% of the control and at 4 weeks is between 0 and 1·5% of the control.

3. The proximal part of the nerve behaves quite differently. Here the enzyme content is higher than normal during the first few days after section; then for a subsequent period of 12 days to 3 weeks it remains at about the same level as the enzyme in the control unsectioned nerves.

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