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# THE EFFECT OF NERVE STIMULATION ON THE AXONAL TRANSPORT OF NORADRENALINE AND DOPAMINE-β-HYDROXYLASE

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1 A method for stimulating the lumbar sympathetic outflow from the spinal cord of the rat is described which does not require artificial respiration of the animal.

2 In some, but not all experiments continuous stimulation at 2 Hz or intermittently at 10 Hz accelerated the rate at which noradrenaline and dopamine-β-hydroxylase accumulated central to a ligature on the sciatic nerve by approximately 40%.

3 It is concluded that, although nervous activity is not necessary for axonal transport of transmitter granules in sympathetic neurones, intense nervous activity may accelerate the rate of granule transport.

## Introduction

Noradrenaline-containing granules appear to be assembled in the perikaryon of sympathetic neurones and transported to the nerve terminals by a fast axonal transport process (see Dahlström, 1971 for review). Thus dense-core granules can be seen to accumulate central to a ligature placed on a nerve (Kapeller & Mayor, 1967; Geffen & Ostberg, 1969) and noradrenaline (NA) levels central to a ligature increase linearly over a period of 48 h (Dahlström & Häggendal, 1966). Dopamine-β-hydroxylase (DBH) (EC 1.14.2.1), which catalyses the final step in the synthesis of NA, is a constituent of granules and accumulates at a ligature at a rate similar to NA (Laduron & Belpaire, 1968; Brimijoin, 1972; Coyle & Wooten, 1972). In the work described here we have attempted to assess the effect of nerve stimulation on the rate of transport of transmitter granules in sympathetic neurones by studying the effect of stimulating the lumbar sympathetic outflow on the accumulation of NA and DBH proximal to a ligature placed on the rat sciatic nerve.

## Methods

The method used for stimulating the lumbar sympathetic outflow was an adaptation of the method of Gillespie & Muir (1967). These authors used pithed rats and inserted a steel stimulating rod through one orbit and down the vertebral canal, but in the present experiments the rod was inserted posteriorly into the vertebral canal after exposure of the cord at T13-L1. This obviated the need to respire the animal artificially and in our hands gave a much higher survival rate.

Rats (Sprague-Dawley, 300-350 g) were anaesthetized with urethane (1.4 g/kg s.c.). The sciatic nerve on each side was ligatured at mid-femur level with 4/0 silk thread. A midline incision was then made through the skin of the back and the vertebral canal at T13-L1 was exposed by means of a dental drill. A stimulating electrode consisting of an approximately 15 cm length of stainless steel wire (17 swg) was inserted posteriorly into the vertebral canal and passed down until its end abutted against the first coccygeal vertebra. A length of Teflon tubing was slipped down the electrode to the point where it entered the vertebral canal thus insulating segments cranial to L1 from electrical stimulation. An indifferent electrode was placed beneath the skin of the abdomen. At the end of the experiment the position of the electrodes in the vertebral canal was checked by X-ray photography.

Monophasic square-wave pulses (10 V, 1 ms) of various frequencies were applied to the electrodes by a Palmer stimulator and the strength and duration of the pulses were monitored on an oscilloscope. Up to ten rats could be connected in parallel to this system. Front and hind legs were secured firmly with string to prevent excess movement during stimulation. In control animals electrodes were placed in the vertebral canal but no stimuli were applied.

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Following stimulation for an appropriate period the animals were killed and a 2.5 mm portion of each sciatic nerve central to and including the ligature was removed and stored at −20°C until assayed for NA and DBH. Zero-time values were obtained by assaying 2.5 mm sections of nerve from normal animals.

NA was assayed by the method of Saelens, Schoen & Kovacsics (1967) as modified by Iversen & Jarrott (1970). DBH was assayed by a modification of the method of Molinoff, Weinshilboum & Axelrod (1971) as previously described (Keen & McLean, 1974).

## Results

Preliminary experiments showed that the rate of accumulation of NA and DBH central to a ligature placed on the sciatic nerve was not affected by urethane anaesthesia or by placing an electrode in the vertebral canal. Since the latter presumably abolished spontaneous activity in the autonomic efferents of the sciatic nerve this is consistent with the finding of Geffen & Rush (1968) that decentralization did not affect the rate of transport of NA in the splenic nerve.

The results of ten independent experiments, involving 162 determinations, in which an electrode placed in the vertebral canal was stimulated at various frequencies are shown in Table 1, expressed as a percentage of values in control animals for that experiment. The overall mean values for the accumulation of NA and DBH in control animals were 347 ± 27 pg/h and 21.7 ± 2.1 pmol tyramine/5 min incubation per h respectively.

In most experiments the accumulation of both

**Table 1** The effect of nerve stimulation on the accumulation of noradrenaline (NA) and dopamine-β-hydroxylase (DBH) in ligatured sciatic nerves

| *Expt* | *Time (h)* | *Rate (Hz)* | *Stimulation* | | *Noradrenaline* | | P | *Dopamine-β-hydroxylase* | | P |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Duration (s)* | *Stimuli/min* | *Control* | *Stimulated* | | *Control* | *Stimulated* | |
| 1 | 6 | 2 | continuous | 120 | 100 ± 15 (5) | 154 ± 30 (4) | NS | 100 ± 7 (4) | 157 ± 23 (4) | NS |
| 2 | 6 | 2 | continuous | 120 | 100 ± 8 (8) | 144 ± 16 (8) | < 0.05 | 100 ± 10 (7) | 123 ± 13 (8) | NS |
| 3 | 6 | 10 | continuous | 600 | 100 ± 12 (8) | 94 ± 23 (10) | NS | 100 ± 14 (8) | 102 ± 10 (10) | NS |
| | | 2 | continuous | | | 114 ± 5 (6) | NS | | 111 ± 16 (6) | NS |
| 4 | 6 | 30 | 1 | 30 | 100 ± 25 (7) | 90 ± 14 (8) | NS | | | |
| 5 | 6 | 30 | 1 | 30 | 100 ± 42 (4) | 255 ± 62 (4) | NS | | | |
| 6 | 11 | 10 | 2 | 20 | 100 ± 6 (6) | 137 ± 9 (4) | < 0.01 | | | |
| 7 | 6 | 10 | 2 | 20 | 100 ± 11 (6) | 141 ± 10 (10) | < 0.05 | | | |
| | | 20 | 1 | 20 | | 145 ± 28 (9) | NS | | | |
| 8 | 6 | 2 | 10 | 20 | 100 ± 28 (4) | 136 ± 19 (10) | NS | 100 ± 12 (8) | 114 ± 12 (10) | NS |
| | | 10 | 2 | 20 | | 180 ± 20 (10) | < 0.05 | | 176 ± 25 (10) | < 0.05 |
| 9 | 6 | 10 | 2 | 20 | 100 ± 3 (9) | 83 ± 4 (6) | < 0.05 | 100 ± 16 (6) | 100 ± 10 (6) | NS |
| 10 | 6 | 2 | 10 | 20 | 100 ± 5 (4) | 107 ± 15 (4) | NS | 100 ± 21 (4) | 120 ± 24 (4) | NS |

Rats were anaesthetized and their sciatic nerves ligatured. The lumbar outflow was then stimulated at the rate shown and either continuously or intermittently at minute intervals for the duration shown. At the end of the experiment the segment of nerve proximal to the ligature was assayed for NA and DBH and the accumulation calculated by substracting values obtained for corresponding segments of non-ligatured nerves. Accumulation expressed as % of the mean value for control animals in that experiment. Each value mean with s.e. mean (number of determinations). *P*, significance of difference from control by *t*-test.

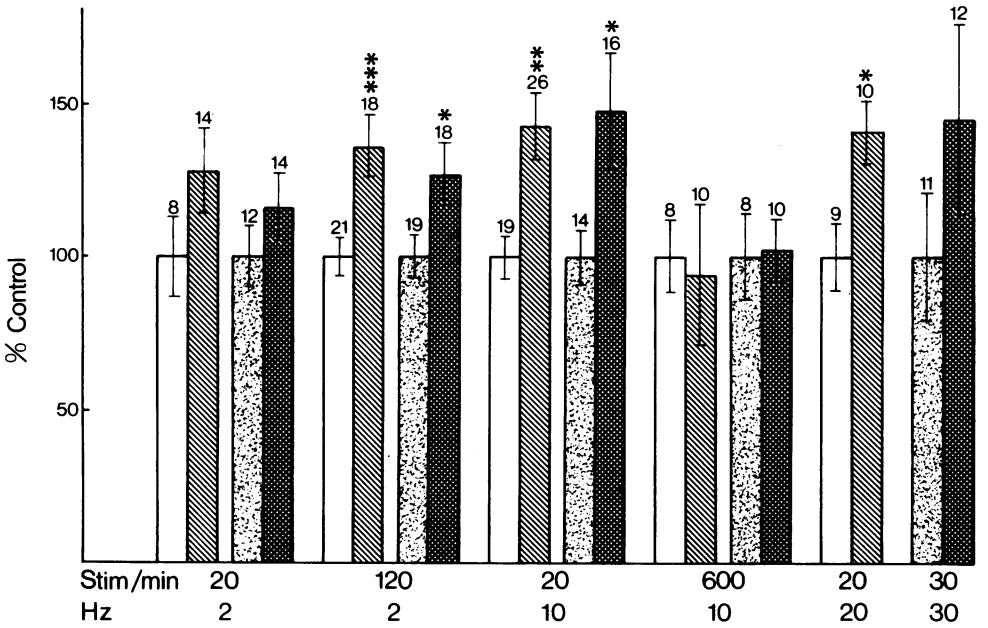


**Fig. 1** Effect of electrical stimulation on accumulation of noradrenaline (NA) and dopamine-β-hydroxylase (DBH) in ligatured sciatic nerves of rat. Open columns, NA in control; diagonally hatched columns, NA in stimulated nerve; stippled columns, DBH in control; cross-hatched columns, DBH in stimulated nerve. Vertical lines indicate s.e. mean. Summary of results from experiments in which the stated number of stimuli were applied every min for 6 h at either 2 Hz or 10 Hz to the lumbar outflow. Full data are presented in Table 1. $n$ = number of determinations. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.005$ by $t$-test analysis (where data from two or more experiments have been combined the number of degrees of freedom has been appropriately reduced).

NA and DBH was greater in stimulated animals than in controls but, owing to the variability of the preparation, this effect was in many cases not significant. Because of this variability and the large number of animals required we did not feel justified in extending these experiments further. Figure 1 shows the results collected together according to the types of stimulation applied.

It may be seen that continuous stimulation at 2 Hz and intermittent stimulation of 10 Hz increased accumulation of both NA and DBH by approximately 40%.

## Discussion

Nervous activity is not necessary for axonal transport of NA because decentralization does not affect the rate of transport in cat splenic nerves (Geffen & Rush, 1968) and transport continues at a normal rate in hypogastric nerves isolated *in vitro* (Banks, Mayor, Mitchell & Tomlinson, 1971a). There is however evidence that axonal transport of certain compounds is enhanced under conditions of intense nervous activity. The transport of [$^{14}$C]-glutamate in frog and snail neurones is increased by electrical stimulation (Kerkut, Shapira & Walker, 1967) and the transport of NA in rat sciatic nerves is accelerated during recovery from reserpine (Dahlström & Häggendal, 1969). In the CNS, transport of [$^{3}$H]-dopamine is also enhanced after reserpine treatment (Fibiger & McGeer, 1973), whilst osmotic stimuli, suckling and parturition stimulate the axonal transport of neurohypophysial hormones in rat pituitary tracts (Norström & Sjöstrand, 1972a,b).

The rate of spontaneous activity in adrenergic nerves rarely exceeds 10/s (Folkow, 1952; Celander, 1954) and so we chose 2 Hz and 10 Hz for most of the experiments reported here. Despite considerable variability in individual experiments there was overall an increase of approximately 40% in the accumulation of both NA and DBH after continuous stimulation at 2 Hz or intermittent stimulation at 10 Hz.

During their passage down the axon adrenergic granules are not fully loaded (de Potter, 1971) and so an increased rate of accumulation of NA proximal to a ligature could be explained either by increased loading of granules with NA or by the transport of a greater number of normally-loaded granules. The fact that in stimulated animals the rates of accumulation of NA and DBH were increased to a similar extent suggests that the latter was the case. This could be due either to granules being transported at a faster speed or to an increased number of granules per unit cross section of axon being transported at the same speed, two possibilities between which the present experiments do not differentiate.

Axonal transport of adrenergic granules is blocked by colchicine (Dahlström, 1968) and the extent to which colchicine and vinblastine block NA transport correlates well with their effect on microtubules (Banks, Mayor & Tomlinson, 1971b), suggesting that microtubules are important for the transport process. Microtubular protein is transported at a rate of 2 mm/day (James & Austin, 1970; Karlsson & Sjöstrand, 1971) whereas granules are transported at 120 mm/day (Dahlström & Häggendal, 1966). Hence if the granules are associated with microtubules they must move relative to them. Schmitt (1968) suggested that granules might move along microtubules by a Ca-ATPase-activated sliding filament mechanism analogous to the actin-myosin system in skeletal muscle. If this were so one might expect that changes in intracellular Ca concentration resulting from increased nerve activity could influence the granule-microtubule interaction. However marked changes in Ca concentration in hypogastric nerves *in vitro* have little effect on granule accumulation (Banks, Mayor & Mraz, 1973). Since minor disruption of microtubule structure is able to influence noradrenaline transport (Banks *et al.*, 1971b) it may be that increases in transport require the formation of increased numbers of microtubules within the axon. This would be especially likely if increased transport involved an increase in the number of granules per unit cross-section of the nerve as discussed above. Such a proliferation of microtubules has been observed in rat pituitary stalk following osmotic stress (Grainer & Sloper, 1972).

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