The transport and life-span of amine storage granules in bulbospinal noradrenaline neurons of the rat

In previous studies on peripheral sympathetic adrenergic nerves, accumulations of noradrenaline in distorted axons were seen proximal to a ligation with the use of fluorescence histochemistry (Dahlström & Fuxe, 1964a; Dahlström, 1965; Kapeller & Mayor, 1967). Convincing evidence appears to exist supporting the view that these accumulations are signs of a rapid and steady proximo-distal transport of amine storage granules down the axons (Dahlström & Häggendal, 1966 a; 1967). The rate of this transport was found to vary in different mammals, ranging between 2–10 mm/h. The storage granules are manufactured in the nerve cell bodies and transported via the axons to the nerve terminals, where they function as factories and stores for the transmitter. Their life-span in the varicosities of the nerve terminals has been calculated as about 5 weeks (rat) to 10 weeks (cat) (Dahlström & Häggendal, 1966a).

In the central nervous system (CNS) widespread systems of noradrenaline-, dopamine- and 5-hydroxytryptamine (5-HT)-containing neurons have been described (Dahlström & Fuxe, 1964b). The spinal cord has been shown to contain noradrenaline and 5-HT-containing nerve terminals and axons, the cell bodies of the neurons being situated in the medulla oblongata (Dahlström & Fuxe, 1965). After a transection of the spinal cord, the monoamines below the section disappeared within a few days (Andén, Häggendal & others, 1964). Above the transection, increases in noradrenaline content were observed biochemically (Häggendal & Magnusson, unpublished observation) and, in the same year, fluorescence histochemical studies revealed accumulations of noradrenaline and 5-HT within bulgy, enlarged axons proximal to the cut (Dahlström & Fuxe, 1964c). The same phenomenon also occurred in other monoamine neurons in the CNS after lesions (see *e.g.* Andén, Dahlström & others, 1966).

Thus, in the CNS also, a proximo-distal transport of granules in all probability occurs. We have now attempted to estimate the rate of this transport of noradrenaline storage granules in the spinal cord, and to calculate their life-span.

In normal rats most noradrenaline in the spinal cord is located in the nerve terminals and only a small amount is found in the axons (Dahlström & Fuxe, 1965). Accumulations of noradrenaline above a section would therefore probably be difficult to demonstrate, since the net increase would probably be comparatively small. From several studies it is known that two days after the administration of one large dose of reserpine (giving a long-lasting or even irreversible block of granular storage mechanisms, Dahlström & Häggendal, 1966b) the nerve terminals are still depleted of noradrenaline, while the cell bodies and axons have already recovered (Dahlström, Fuxe & Hillarp, 1965; Dahlström & Fuxe, 1965). In the peripheral nervous system it was observed that the amounts of noradrenaline accumulated above a 6 hr ligation of the sciatic nerve had returned to about normal levels on the second to third day after the reserpine treatment. Therefore, all rats used in the present experiments were given reserpine (10 mg/kg i.p.) 4 days before death.

The spinal cord of one group of rats was sectioned at the level of Th 6–7 under ether anaesthesia 12 or 24 h before death. After death one cm of the spinal cord just above the transection was dissected and assayed (2 or 4 together) for noradrenaline content (Häggendal, 1963). Another group of animals in which the spinal cord was not sectioned, had one cm parts of the thoracic cord dissected, pooled (5–10 together), and analysed for noradrenaline.

The noradrenaline content in the one cm part rose steadily from $3{\cdot}5 \pm 0{\cdot}55$ ng in unoperated rats, to $4{\cdot}4 \pm 0{\cdot}15$ ng 12 h after section and to $6{\cdot}0 \pm 0{\cdot}30$ ng in rats

transected 24 h earlier (mean values ± standard errors, n = 4). The gradual increase of noradrenaline (Fig. 1) followed a curve similar to the one observed for sciatic nerve (Dahlström & Häggendal, 1966a). However, the inclination of the curve was less for the spinal cord than for the sciatic nerve, indicating a slower rate of transport of the amine granules. For the spinal cord this rate of transport was calculated to be 0·7 mm/h. Thus, it seems that the transport of noradrenaline granules in the central noradrenaline neurons is much slower than in the peripheral sympathetic neuron (5 mm/h in the rat).

As seen from the data, about 2·5 ng of noradrenaline, probably stored within granules, was transported down the spinal cord each 24 h. To get information on the approximate number of nerve terminals these transected axons would have to supply with granules, the spinal cord below the level of Th 6 was assayed in normal, untreated rats. The noradrenaline content in this part of the spinal cord was found to be 78 ± 6·8 ng (mean ± s.e. n = 7). According to the calculations made in experiments with the sympathetic neuron system of the sciatic nerve (see Dahlström & Häggendal, 1966a) the life-span of amine storage granules in the spinal cord was found to be about 31 days (compare 35 days in the peripheral nervous system).

Reserpine, used as a tool in this study, may, however, to some extent influence the transport of the granules. During the accumulation period, that is, the fourth day after resperpine administration, there may be a slightly increased rate of accumulation, as seems to be the case above a constriction of peripheral adrenergic nerves (Dahlström & Häggendal, to be published). The increase appears, however, to be about 140% of normal.

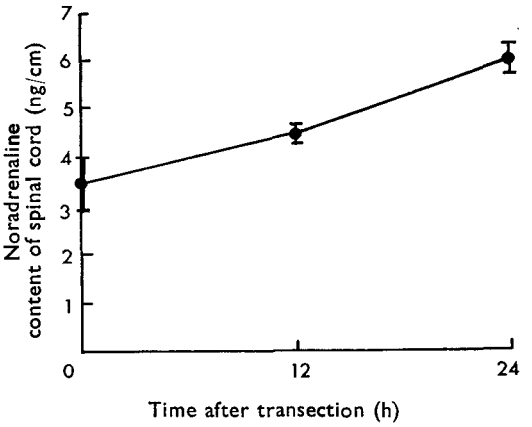

FIG. 1. The accumulation of noradrenaline in the 1 cm part of thoracic spinal cord just above a transection at the level of Th 6-7. All the animals were killed on the fourth day after a single dose of reserpine (10 mg/kg, i.p.). The values are given in means ±, indicated by vertical bars. Four estimations were made in all cases.

It was earlier found that after one large dose of reserpine, the recovery of noradrenaline to 100% of normal levels was completed after about 5 weeks in the rat and after about 7 weeks in the rabbit, both in peripheral tissues and in the CNS (Dahlström & Häggendal, 1966b). Since the recovery followed an approximately straight line with time, and since the time needed for full recovery coincided with the calculated life-span of granules in the peripheral adrenergic neurons in both species, it was suggested that a large dose of reserpine blocked the storage mechanism of the granules irreversibly and that the down transport of newly synthesized granules (from the cell body to the terminals) was essential for recovery of noradrenaline levels in the

terminals. Since the recovery time after reserpine was the same in the CNS as in the peripheral adrenergic neurons, it was suggested that in the CNS also the granular life-span was about 5 weeks. Thus, the results obtained in the present experiments are in agreement with earlier findings.

It may be mentioned that preliminary experiments with the accumulation of 5-HT in the spinal cord have revealed a higher rate of transport of presumably 5-HT-storing granules than of noradrenaline granules in reserpinized rats.

The present experiments, together with previous ones indicate that the life-span of the amine storage granules together with the size of the nerve terminal net of the neurons constitute important factors in regulating the rate of formation and the rate of proximo-distal transport of amine storage granules in both central and peripheral adrenergic neurons.

This study was supported by the Swedish State Medical Research Council (B68-14x-166-04 and B68-14x-2207-02), and by grants from the Faculty of Medicine, University of Göteborg. For skilful technical assistance we are indebted to Miss Birgitta Parkner.

Department of Pharmacology,
Department of Histology,
University of Göteborg,
Göteborg, Sweden.

C. JAN HÄGGENDAL
ANNICA B. DAHLSTRÖM

October 10, 1968

REFERENCES

ANDÉN, N.-E., DAHLSTROM, A., FUXE, K., LARSSON, K., OLSEN, L. & UNGERSTEDT, U. (1966). *Acta physiol. scand.*, **67**, 313–326.

ANDÉN, N.-E., HÄGGENDAL, J., MAGNUSSON, T. & ROSENGREN, E. (1964). *Ibid.*, **62**, 115–118.

DAHLSTRÖM, A. (1965). *J. Anat.*, **99**, 677–689.

DAHLSTRÖM, A. & FUXE, K. (1964a). *Z. Zellforsch.*, **62**, 602–607.

DAHLSTRÖM, A. & FUXE, K. (1964b). *Acta physiol. scand.*, **62**, Suppl. 1, 232.

DAHLSTRÖM, A. & FUXE, K. (1964c). *Ibid.*, **60**, 293–294.

DAHLSTRÖM, A. & Fuxe, K. (1965). *Ibid.*, **64**, *Suppl.* 247, 1–36.

DAHLSTRÖM, A., FUXE, K. & HILLARP, N.-Å. (1965). *Acta pharmac. tox.*, **22**, 277–292.

DAHLSTRÖM, A. & HÄGGENDAL, J. (1966a). *Acta physiol. scand.*, **67**, 278–288.

DAHLSTRÖM, A. & HÄGGENDAL, J. (1966b). *J. Pharm. Pharmac.*, **18**, 750–752.

DAHLSTRÖM, A. & HÄGGENDAL, J. (1967). *Acta physiol. scand.*, **69**, 153–157.

HÄGGENDAL, J. (1963). *Ibid.*, **59**, 242–254.

KAPELLER, K. & MAYOR, D. (1967). *Proc. Roy., Soc*, B., 282–292.