J. Pharm. Pharmac., 1969, **21**, 633–638 *Received June* 16, 1969

Recovery of noradrenaline in adrenergic axons of rat sciatic nerves after reserpine treatment

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The recovery of noradrenaline in adrenergic axons of the rat sciatic nerve after a single dose of reserpine (10 mg/kg i.p.) has been studied in unligated nerves and nerves ligated for 6 h. In unligated nerves the recovery at 24 h after reserpine was about 14% of normal. The noradrenaline content then slowly rose to reach about normal concentrations 6–7 days after reserpine injection. In nerves ligated 6 h before death, about 8·0 ng of noradrenaline accumulated proximal to the ligation in normal animals. At 6 and 12 h after reserpine about 4% of normal amounts of noradrenaline were found. Thereafter the amount of accumulated noradrenaline rapidly increased to about normal levels on day 2 after reserpine. At this time the content in unligated nerves was only about 45% of normal unligated nerve. On days 3–5 after reserpine, supranormal accumulations of noradrenaline were found (statistically highly significant), having a maximum at day 4 of about 145% of normal. At this time the noradrenaline content in unligated nerve was only about 80% of normal. The results may indicate an increased synthesis and increased rate of downtransport of amine storage granules during the early recovery phase after reserpine. This phenomenon may be part of a feed-back mechanism operating after depletion of the transmitter in the nerve terminals.

The accumulation of noradrenaline above a constriction of peripheral adrenergic nerves has been studied both histochemically (Dahlström & Fuxe, 1964; Dahlström, 1965; Kapeller & Mayor, 1966) and quantitatively (Dahlström & Häggendal, 1966a, 1967). The rapidly occurring accumulation has been interpreted to arise from a piling up above the lesion of amine storage granules, synthesized in the nerve cell body and transported proximo-distally in the axon (Dahlström & Häggendal, 1966a, 1967; Dahlström, 1966). The minimal effective rate of this transport and the average life-span of the granules in the terminals have for the rat been calculated to be about 5 mm/h, and several weeks, respectively (Dahlström & Häggendal, 1966a).

The catecholamine-depleting effect of reserpine has been demonstrated to be due to a blockage of the catecholamine-storage mechanism in the amine storage granules of central and peripheral catecholamine-containing neurons (Carlsson, Hillarp & Waldeck, 1963; Carlsson, 1965). This blockage is long-lasting, and after high doses of reserpine it even seems to be irreversible, since recovery to normal levels takes several weeks in peripheral tissues and in the central nervous system (Dahlström & Häggendal, 1966b). With the use of the histochemical fluorescence method of Hillarp, Falck and coworkers (for ref. and description see Corrodi & Jonsson, 1967) it has been demonstrated that the very first reappearance of noradrenaline after

reserpine depletion occurs in the nerve cell body in a zone around the nucleus (Carlsson, Falck & Hillarp, 1962; Dahlström, Fuxe & Hillarp, 1965; Dahlström, 1967). At about 24 h after the reserpine treatment most nerve cell bodies have about normal levels of catecholamine-fluorescence, several, however, having supranormal amounts of catecholamine. This overshooting is more pronounced at 36–48 h and can then be seen in large numbers of cells (Dahlström & others, 1965). In the non-terminal axons noradrenaline-fluorescence has been demonstrated about 3–4 cm away from the perikarya as early as 15–18 h after the injection of the drug. At 30–36 h the recovery has proceeded further, permitting about "normal" amounts of noradrenaline to accumulate above a ligation made 1 h before death (Dahlström, 1967).

Since the histochemical method used for the above experiments is only semi-quantitative (see Corrodi & Jonsson, 1967) the present biochemical study was undertaken to follow more in detail, and quantitatively, the reappearance of noradrenaline in the sciatic nerve of the rat. Of special interest was the question whether the supranormal levels of noradrenaline seen in the nerve cell bodies during the recovery phase was reflected in the axons.

Material and methods

Male albino rats of the Sprague-Dawley strain (200–250 g) were given one single dose of reserpine (Serpasil, 2·5 mg ampoules) intraperitoneally (10 mg/kg) 6, 12, 18, 24, 36 h, 2, 3, 4, 5, 6, 7, 9, 11 and 13 days before death according to Tables 1 and 2. During the time between the reserpine injection and killing the rats, they were kept at a temperature of 23–25°. In half of the animals the sciatic nerve was ligated bilaterally under ether anaesthesia 6 h before death. In the rest of the rats the nerves were used unligated. The rats were killed by a blow in the head, and the sciatic nerves dissected out immediately. In the *ligated group* the proximal 1 cm part of the nerve above the ligation was taken out and assayed in groups of 4 to 8. Nerves from normal rats given no drug, and with bilateral 6 h ligations were also collected and assayed at every experimental series. In the *unligated rats* about 3 cm of the nerve, from the dorsal exit through *foramen infrapiriformis* and distally, was dissected

Table 1. *The noradrenaline content in % (mean ± s.e.) of the normal value/cm unligated sciatic nerve of rat after reserpine treatment* (10 mg/kg). *The normal value* (100%) *corresponds to* 1·91 ± 0·08 ng/cm.

Time after reserpine treatment	Number of experiments	Amount of noradrenaline % of normal ± s.e.
0 h*	24	100 ± 4·3
18 h	1	11
24 h	7	14 ± 2·7
36 h	1	40
2 d	8	44 ± 6·6
3 d	14	72 ± 3·7
4 d	6	83 ± 5·7
5 d	6	72 ± 10·4
6 d	5	85 ± 9·4
7 d	6	103 ± 11·3
9 d	7	108 ± 18·7
11 d	8	92 ± 9·7
13 d	8	92 ± 4·4

* Normal rats not given reserpine.

Table 2. *The noradrenaline content in the proximal 1 cm part of the sciatic nerve of rat after ligation, in normal and reserpine-treated rats. All rats were ligated 6 h before death. The values are expressed in % (mean ± s.e.) of the noradrenaline accumulation estimated in normal, 6 h ligated nerves at every experimental series. The noradrenaline value for 6 h ligated normal nerves (100%) corresponds to 7·99 ± 0·36 ng/nerve.*

Time interval reserpine treatment—death	Number of experiments	Amount of noradrenaline accumulated in % of normal	Difference from normal levels (*P* values)
0 h*	26	100 ± 4·5	
6 h	2	4 ± 1·0	<0·001
12 h	3	4 ± 1·2	<0·001
18 h	8	16 ± 8·6	<0·001
24 h	7	22 ± 7·2	<0·001
36 h	8	69 ± 9·9	<0·001
2 d	9	96 ± 5·7	>0·5
3 d	9	133 ± 10·4	<0·001
4 d	10	145 ± 11·6	<0·001
5 d	11	120 ± 4·9	<0·01
6 d	9	108 ± 11·2	>0·25
7 d	9	105 ± 6·6	>0·5
9 d	10	88 ± 3·6	>0·1†
11 d	11	86 ± 3·9	<0·1†
13 d	10	87 ± 4·5	>0·1†

* Normal rats given no reserpine but ligated 6 h before death.

† When taken together, $P < 0{\cdot}025$.

bilaterally and assayed in groups of 4–8. Unligated nerves of normal rats were collected at every experimental series. At every experimental occasion 1–3 single observations were made in each time group. The noradrenaline values of the single observations were expressed in % of the mean noradrenaline value in the normal control group for each experimental occasion.

The method for bioassay used was the trihydroxyindole method as modified by Häggendal (1963). After the dissection, the nerves were either frozen in dry ice and stored at −70° until bioassay, or immediately put into ice-cooled 0·4 N perchloric acid (7 ml) with 20 mg of ethylenediamine tetra-acetate (EDTA) added. After homogenization, using an Ultra-Turrax (Janke & Kunkel) homogenizer, and centrifugation, the extracts were purified on columns of strong cation exchange resins (Dowex-W 50 X8). The fluorescence was measured in an Aminco-Bowman spectrophotofluorometer. In every experiment at least 2 samples containing 0·5 or 1 cm of normal nerve with an addition of known amounts of noradrenaline (50 or 100 ng) were included in the series to check the recovery through the estimation procedures. The recovery was about 85% in all series, and no correction was made for this.

RESULTS

Unligated nerves

In the normal rat, 1 cm of the sciatic nerve was found to contain somewhat less than 2 ng/cm (1·91 ± 0·08). One day after reserpine treatment the nerve appeared to hold about 4% of the normal value (the fluorescence intensities of the samples, however, being very close to that of the blanks). During the following days the noradrenaline content rose, reaching about normal concentrations on the 6th to 7th

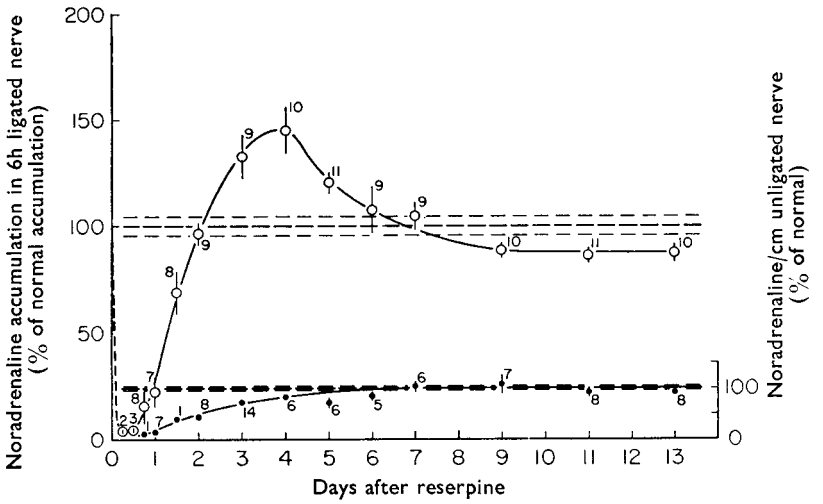

FIG. 1. The noradrenaline content in sciatic nerve of normal and reserpine-treated rats (one single dose of 10 mg/kg i.p.). The upper curve (○———○) indicates the accumulation of noradrenaline in the 1 cm part of nerve above a ligation performed 6 h before death. The values are expressed as % of the mean value for the noradrenaline amount found to accumulate in normal rat nerves above a 6 h ligation at every experimental occasion. 100% corresponds to 7·99 ng ± 0·36 (mean ± s.e., n = 26). The lower curve (●———●) indicates the noradrenaline content in unligated nerves, expressed as % of the content found in normal unligated nerves at every experimental occasion. The amount of noradrenaline/cm normal unligated nerve (100% ordinate to the right) corresponds to 1·91 ng/cm ± 0·08 (mean ± s.e., n = 24). The vertical bars represent the s.e., and the numerals indicate number of experiments.

day. As seen in Fig. 1 the levels remained around normal up to day 13 after the reserpine treatment.

Ligated nerves

In rats without reserpine treatment, about 8 ng of noradrenaline was found above a ligation made 6 h before death (7·99 ± 0·36 ng/cm). After reserpine treatment the amounts were strongly decreased during the first 12 h, but from 18 h and on the accumulated noradrenaline values rapidly reached normal values. A pronounced overshooting was observed between the 3rd and the 5th day (highly significant, $P < 0{\cdot}001$ and $P < 0{\cdot}01$, Table 2), reaching a maximum of about 45% above normal on the 4th day (see Table 2 and Fig. 1). At 9, 11 and 13 days after reserpine, the accumulated noradrenaline was slightly less than normal (86–88%, significant at $P < 0{\cdot}025$ when the 3 groups were taken together).

DISCUSSION

There seems to be little doubt that the recovered noradrenaline, observed in the adrenergic axons of the sciatic nerve, is stored within amine storage granules, since tetrabenazine, a shortlasting blocker of the noradrenaline storage mechanism in the granules (Pletscher, Brossi & Gey, 1962; Häggendal, 1968), or a second injection of reserpine, can disperse the reappeared noradrenaline (Dahlström, 1967).

In earlier histochemical experiments it was found that no noradrenaline accumulated above a 1 h ligation in rats given reserpine (10 mg/kg) 6–12 h previously. At 15 h after reserpine some noradrenaline accumulated, thereafter the recovery proceeded rapidly, and normal fluorescence intensities were found by 30–36 h after

reserpine. In this quantitative study, accumulation of noradrenaline was traced as early as 18 h after reserpine, which is in agreement with the histochemical observations.

In the present experiment the amount of noradrenaline accumulated above a 6 h ligation was found to be about normal at day 2 after reserpine; unligated nerves at the same time held about 45% of normal. Three to 5 days after reserpine, supranormal accumulations of noradrenaline developed, while the noradrenaline content in unligated nerves was still below normal. According to earlier calculations on the rate of transport of granules, made under the presumption that the storage granules do not increase their content of noradrenaline during the time of accumulation (Dahlström & Häggendal, 1966a), these results would indicate an increased rate of transport of granules in the nerve during the 3rd to 5th day after reserpine. It does indeed seem as if the granules do increase to some extent their content of noradrenaline during the time of accumulation. However, this increase appears to be of about the same relative size in normal rats as in rats given reserpine 3 or 4 days beforehand (Dahlström & Häggendal, unpublished observations). Therefore, the supranormal levels of noradrenaline accumulation observed in this work may indicate an increased amount of downtransported granules, and thereby an increased synthesis of granules in the cell bodies, and also, an increased rate of the proximo-distal transport.

It is well-known that the recovery of noradrenaline to normal amounts after reserpine depletion takes a long time (cf. Carlsson, 1965). In a preliminary study, this time was found to be several weeks (Dahlström & Häggendal, 1966b) in the rat, approximately the same time as was calculated to be the average life-span of granules in the nerve terminals (Dahlström & Häggendal, 1966a). The recovery curve after the reserpine injection followed an approximately straight line, suggesting that a continuous, steady downtransport of newly formed granules from the nerve cell bodies is necessary for a complete recovery of noradrenaline levels in the nerve terminals after one high dose of the drug (Dahlström & Häggendal, 1966b). However, it has been observed that within the first week after the injection the recovery curve has a temporary steeper inclination (Häggendal & Dahlström, unpublished observations). The results of the present experiments suggest that this temporary increase in the rate of noradrenaline recovery in the nerve terminals may be due to an increased synthesis and downtransport of amine storage granules within this early time period.

It has been observed that increased nerve activity causes an increased synthesis of proteins in the perikarya (cf. Hydén, 1960). Since reserpine depletes the transmitter stores, transmission is blocked and the functional response to nerve activity abolished. It may be speculated that some feed-back mechanism may cause a compensatory increased impulse activity to the adrenergic neurons in reserpinized animals. This increased nerve activity could possibly induce an increased synthesis of the protein containing storage granules. Even if other mechanisms may explain the results, such an increased formation of granules as observed, may seem biologically adequate in a situation where the granules in the neuron are blocked, as for example exists after reserpine.

Acknowledgements

The present study has been supported by grants from the Swedish Medical Research Council (grants nr B69-14X-2207-03 and B69-14X-166-05 A) and by grants from the

Faculty of Medicine, University of Göteborg, Sweden. For generous supply of Serpasil we are indebted to the Swedish CIBA, Stockholm. The skilful technical assistance of Mr. Pär-Anders Larsson, Miss Ingalill Nordgren, Miss Birgitta Parkner and Miss Agneta Wilén is gratefully acknowledged. For preparation of figure, and statistical treatment of the material we are indebted to Research engineer Tor Magnusson.

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