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The recovery of noradrenaline in adrenergic nerve terminals of the rat after reserpine treatment*

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Tissue concentrations of endogenous noradrenaline in heart, submaxillary gland, and gastrocnemic muscle have been examined after one large dose of reserpine (10 mg/kg) to rats. After the initial depletion of the amine, the concentration started to rise between 24 and 36 h. For about one week thereafter the amine recovery proceeded comparatively fast, then the rate of the recovery slowed. Between the 4th and the 6th weeks there was a pronounced drop in the noradrenaline concentration in all three tissues, apparently beginning in the 4th week with a maximal decrease of about 20% in the 5th week after reserpine. Thereafter the concentrations increased to approach normal about 6 weeks after reserpine. These results are discussed in relation to the axonal down-transport of newly formed amine storage granules and to the life-span of these granules in the nerve terminals. The different parts of the noradrenaline recovery curve appeared to reflect the axonal down-flow of granules. A theoretical recovery curve was calculated, based on granular transport. This curve was similar to the observed recovery curve. The claim is made that the recovery of adrenergic function and noradrenaline levels after reserpine is due to a down-transport of newly formed, amine storage granules to the nerve terminals. There seems little need for the theory that the storage function reappears in old, reserpine-blocked granules, as a mechanism for noradrenaline recovery after a large dose of reserpine.

Reserpine decreases the tissue levels of 5-hydroxytryptamine (Shore, Silver & Brodie, 1955; Pletscher, Shore & Brodie, 1955) and of catecholamines (Bertler, Carlsson & Rosengren, 1956; Carlsson & Hillarp, 1956). The block in adrenergic transmission after reserpine is most probably a result of the depletion of noradrenaline in the adrenergic neurons (cf. Carlsson, 1965). This depletion is considered to be the result of a long lasting block of the amine storage mechanism in the amine storage granules (see for example: Iversen, 1967; Andén, Carlsson & Häggendal, 1969) while the amine uptake mechanism at the level of the nerve membrane (the "membrane pump") appears to be unaffected (Malmfors, 1965; Hamberger, 1967).

After the initial depletion of the amines by reserpine, the tissue concentrations recover slowly (Carlsson, Rosengren & others, 1957; cf. Carlsson, 1965) and appear to reach normal some weeks after one large dose of the drug (Häggendal & Lindqvist, 1963, 1964; Dahlström & Häggendal, 1966a). Noradrenaline recovery in adrenergic neurons has been observed in the perikarya, long before the amine could be found in the nerve terminals (Dahlström & Fuxe, 1965; Dahlström, Fuxe & Hillarp, 1965;

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Norberg, 1965; Dahlström, 1967). The amine storage granules are probably formed in the perikarya, and transported rapidly down the axons to the nerve terminals, where they have a life-span of several weeks (Dahlström & Häggendal, 1966b, 1967, 1970). Therefore, it was suggested that the recovery of tissue noradrenaline concentrations after one large dose of reserpine, was due mainly to the down-transport of newly formed amine granules to the nerve terminals (Dahlström & Häggendal, 1966a), rather than to a return of the storage function in the old granules in the nerve terminals (e.g. Alpers & Shore, 1969).

Since reserpine, on account of its amine-depleting effect, is often used as a tool, it appears to be of importance to study in detail the recovery curve for endogenous noradrenaline after reserpine depletion. The question of whether the recovery of transmission, of the capacity of the tissues to take up and retain exogenous noradrenaline, and of the endogenous noradrenaline concentrations, depend upon restitution of the function of the old amine granules in the nerve terminals, or on the transport of newly formed granules to the nerve terminals after reserpine, appears difficult to answer until a detailed picture of the reserpine recovery has been obtained. This paper reports an attempt to do this for endogenous noradrenaline.

MATERIALS AND METHODS

Male albino rats of the Sprague-Dawley strain (200–250 g) were used. The animals were given one single dose of reserpine (Serpasil, 2·5 mg ampoules) (10 mg/kg, i.p.) 12, 18, 24, 36 h, 2, 3, 4, 5, 6, 7, 9, 11, 13 days, 2, 3, 4, 5, and 6 weeks before decapitation. After the reserpine injection the rats were kept at 23–25°, except for the first 6–8 h, during the phase of leakage of amines, when the temperature was 15–17°. which kept mortality to about 1%. The rats were kept 5 in each cage and received food and water freely. Control rats were similarly treated in every way except that they were not given reserpine. At death, heart, submaxillary glands and gastrocnemius muscles were excised, immediately weighed, frozen in dry ice, and kept at — 70° until assay.

The heart, salivary glands and the muscles were homogenized in 10 ml 0·4N perchloric acid (PCA, ice-cooled) with 20 mg of ethylenediamine tetra-acetate (EDTA) and 10 mg of ascorbic acid. After homogenization, using an Ultra-Turrax homogenizer (Janke & Kunkel), and centrifugation, the extracts were purified on columns of strong cation exchange resins (Dowex 50 W-X4). The noradrenaline was estimated by the modified trihydroxyindole fluorometric method (Häggendal, 1963) in an Aminco-Bowman spectrophotofluorometer. In every series of estimations two samples of normal tissues, with known amounts of added noradrenaline (0·1 μg), were included to check recovery.

RESULTS

General observations

After reserpine, body and individual tissue weights were reduced for about one week compared to the controls probably because of diarrhoea, salivation and reduced food and water intake. During the second day after reserpine the rats recovered from the sedation and food and water were taken. Reserpine causes changes in e.g. heart muscle (cf. Zaimis, 1961) which may contribute to the weight reductions observed, although no difference in distribution or amount of nerve terminals has

been observed in reserpine-treated rats compared to normal rats (see e.g. Malmfors, 1965; Hamberger, 1967). Therefore, the figures for noradrenaline concentrations in the different tissues after reserpine were corrected for the weight losses, to avoid falsely high figures, especially during the initial period after the reserpine.

Tissue noradrenaline concentrations

The recovery of noradrenaline in the tissues followed a multiphasic course. As seen from Fig. 1d the concentrations were very low 12–24 h after reserpine in the three tissues (Section I in Fig. 2). Between 24 and 36 h there was a clear increase in all three tissues. After 36 h the concentrations increased rapidly with a smooth gradient, up to approximately day 7 after reserpine (Fig. 1a–c; Section II in Fig. 2).

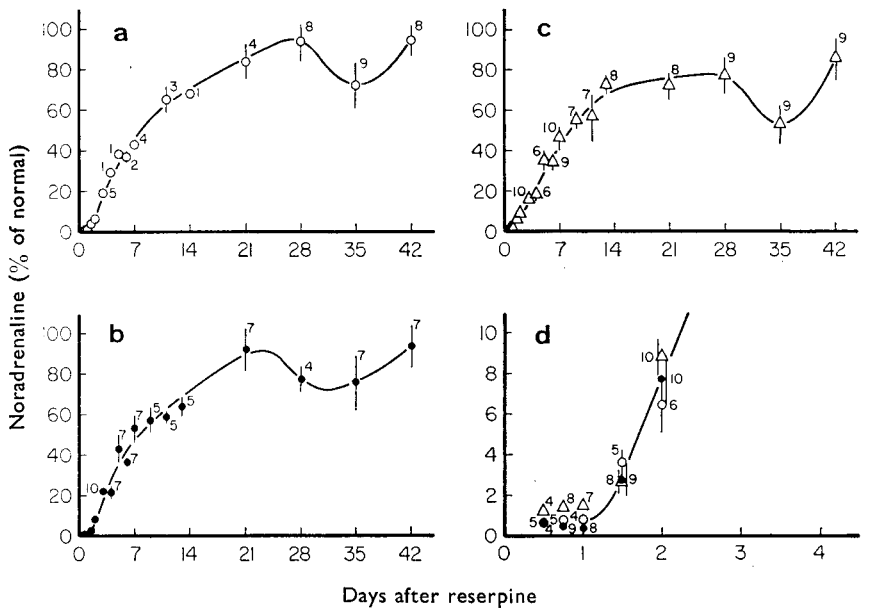

FIG. 1. The course of recovery of endogenous noradrenaline after reserpine treatment (10 mg/kg, i.p.) in different tissues of the rat: (a) gastrocnemius muscle, (b) submaxillary gland, (c) heart. The noradrenaline values are expressed in per cent of the values in control rats, killed and assayed parallelly. Corrections for weight reductions in reserpine-treated rats have been performed. The vertical bars indicate the s.e., and the small figures indicate number of experiments. (d) The noradrenaline concentrations in heart (△), salivary gland (●), and gastrocnemius muscle (○) during the early period after the reserpine injection. In all three tissues the concentrations start to increase between 24 and 36 h after reserpine (details from a–c).

Between day 7 and the third to fourth week the concentrations increased more slowly (Section III in Fig. 2). During the fourth to sixth week there was a drop (Section IV in Fig. 2) in all three tissues (Fig. 1a–c). The maximal decrease was about 20% 5 weeks after reserpine. This decrease was statistically significant when the values from all three tissues were taken together ($P < 0{\cdot}025$). Thereafter the levels slowly increased and appeared to reach normal levels about 6 weeks after the reserpine.

The different sections (I–IV) of the recovery curve will be discussed below and related to possible mechanisms in the adrenergic neuron.

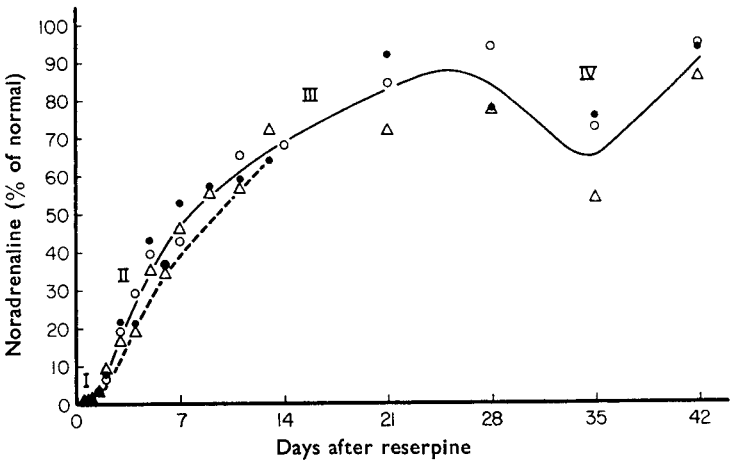

FIG. 2. The noradrenaline recovery in peripheral tissues of the rat after one large dose of reserpine (10 mg/kg, i.p.). The values from Fig. 1a–c have been indicated, with the same symbols and the curve for recovery in the three tissues, taken together, has been drawn. I–IV indicate different parts of the recovery curve, used for convenience in the discussion of the curve. The concentrations at 5 weeks are significantly lower than the values at 4 weeks and 6 weeks, when values from all 3 tissues are taken together ($P < 0{\cdot}025$). The theoretical curve for recovery, calculated on basis of axonal transport of granules, is indicated (- - - -). The values used for the calculations are indicated in Fig. 3. A correction of the values by 25% was made (see discussion).

DISCUSSION

Section I

During this time the endogenous noradrenaline concentrations are very low, resulting from the blockage of the noradrenaline storage mechanism in the amine granules (cf. Carlsson, 1965). Within this time the adrenergic transmission is also blocked, and exogenous noradrenaline (^{3}H-NA) is not retained in the tissues (Muscholl, 1960; Andén, Magnusson & Waldeck, 1964; Iversen, Glowinski & Axelrod, 1965; Häggendal & Dahlström, 1970). All these functions appear to be dependent on intact amine storage granules (e.g. Häggendal & Malmfors, 1969; Jonsson, Hamberger & others, 1969; for review see Andén & others, 1969).

One function so far observed to be undestroyed during this period is the synthesis of noradrenaline in the granules (Kirshner, 1962; Glowinski, Iversen & Axelrod, 1965). Furthermore, a so-called reserpine resistant uptake mechanism has been discussed (see Andén & others, 1969) but for the recovery of endogenous noradrenaline and of nerve function after reserpine *in vivo* this mechanism appears to be of little or no importance.

Section II

Onset of noradrenaline recovery. Fig. 1d shows the onset of increase in tissue noradrenaline concentrations which occurred between 24 and 36 h after reserpine in all three tissues. Previously Andén & others (1964) had not found any increase in rat peripheral organs until 48–72 h after reserpine. This difference may be due to a more sensitive assay and more material were used by us. In the early part of this period the recovery not only of endogenous noradrenaline concentrations, but also of transmission and the capacity to retain small amounts of ^{3}H-NA begins. Thus, between 30 and 48 h after reserpine, nerve function had partially recovered and so

also had the rat heart and femoral muscle capacity to take up and store ^{3}H-NA (Andén & others, 1964). Iversen & others (1965) also observed a partial recovery of ^{3}H-NA uptake capacity in rat heart at 36–48 h. Both ^{3}H-NA retention capacity and endogenous noradrenaline concentrations begin to recover 24–36 h after reserpine in the rat (Häggendal & Dahlström, 1970). As these three functions depend on functioning amine storage granules, these are probably present 24 and 36 h after reserpine in the adrenergic nerve terminals.

Two main possibilities may be discussed for the onset of recovery of these three functions: the first is that the old reserpine-blocked granules may recover their functions, the second is that new functioning granules, formed in the cell bodies *after* the reserpine administration, have been transported to the nerve terminals during this time. The first possibility has been suggested by many authors, lately by Alpers & Shore (1969) based partly on their own results (on the rate of disappearance of [^{3}H]reserpine from peripheral tissues after a small i.v. dose) and partly on the results from Carlsson, Rosengren & others (1957) which indicated that noradrenaline recovery in rabbit heart appears to be completed within 14 days after reserpine. Later, Häggendal & Lindqvist (1964) showed rabbit heart noradrenaline to approach normal levels 5 weeks after reserpine with 2 week values at about 70% of normal (see also Carlsson, 1965). The present findings show that full recovery of noradrenaline concentrations in rat peripheral tissues after reserpine does not occur until after about 6 weeks.

In support of the second possibility, new, functioning, noradrenaline-containing granules are most probably being transported down adrenergic axons in the sciatic nerve, as early as 15—18 h after reserpine (Dahlström, 1967, Dahlström & Häggendal, 1969), and would reach the nerve terminals in the hind limb skeletal muscle around 24 h after reserpine. Thus, there is a good correlation in time between the calculated arrival of new, functioning granules in the nerve terminals and the onset of recovery of transmission, [^{3}H]noradrenaline retention capacity and endogenous noradrenaline levels.

Further support for this second possibility has recently been obtained. Interruption of the axoplasmic flow of material including amine storage granules for 12 h by axotomy caused a delay in the recovery of the tissue capacity to retain small amounts of ^{3}H-NA (Häggendal & Dahlström, 1970) and also the endogenous noradrenaline concentrations after reserpine (Dahlström & Häggendal, unpublished). Since no sign of nerve terminal degeneration was observed and the influence of impulse activity was minimized (by preganglionic denervation), interruption in axoplasmic flow is probably the main reason. A similar delay after axotomy in the onset of increase in the concentration of endogenous noradrenaline in the spinal cord after reserpine has been demonstrated (Dahlström & Häggendal, unpublished). Furthermore, transsection of the spinal cord delayed the recovery of ^{3}H-NA formation and storage after ^{3}H-L-dopa administration in reserpine pretreated rats (Andén & Lundborg, 1970).

Progress of noradrenaline recovery. The increase in endogenous noradrenaline in the nerve terminals during section II of the recovery curve (Fig. 2) was relatively fast, and reached about 50% of control values after 7 days. The accumulation of noradrenaline above a ligation of the sciatic nerve at different intervals after reserpine treatment has been reported (Dahlström & Häggendal, 1969) and Fig. 3 is based on Fig. 1 in that paper. The figures used were obtained by subtraction of the amount

of noradrenaline found in 1 cm of unligated nerve from that found in 1 cm above a ligation, made 6 h before death. Fig. 3 thus shows the amount of noradrenaline transported to the 1 cm nerve above the ligation during 6 h. This would reflect the number of noradrenaline-containing amine granules that, after formation in the cell bodies, are transported along the nerve during the 6 h after ligation. Fig. 3 shows that during the third to sixth day after reserpine the amount of noradrenaline (in granules) transported to the 1 cm of nerve just above the ligation is supranormal, reaching a maximum of about 160% of the control values. After the seventh day, the amounts decrease again, being about 90% of normal during the 9th to 13th (latest period studied) days.

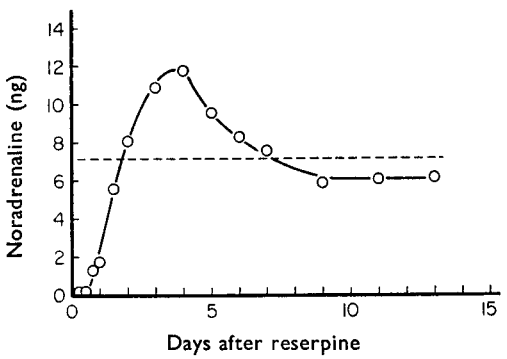

FIG. 3. The noradrenaline amounts transported to the 1 cm part of the rat sciatic nerve just above a 6 h ligation, made at different time intervals after reserpine (10 mg/kg, i.p.). This noradrenaline is probably located within amine granules, transported to this nerve part during 6 h. The curve is based on Fig. 1 in Dahlström & Häggendal (1969) and obtained by subtraction of the amounts of the amine in 1 cm of unligated nerves from those found proximal to a 6 h ligation, at different times after reserpine. The noradrenaline values are given in % of the amounts transported to the 1 cm part of sciatic nerve above a 6 h ligation in normal animals.

Thus, the rise in noradrenaline concentration in the nerve terminals during the first week after reserpine (Section II) not only appears to begin somewhat later than the period after reserpine (15–18 h), when noradrenaline-storing amine granules have reappeared in cell bodies and axons, but also, the recovery appears to be fastest in section II of the curve, when the down-transport of noradrenaline-containing granules has reached normal and supra-normal levels. The close relation between these curves indicates that the noradrenaline recovery in the nerve terminals during this period may mainly depend on axonal transport of new, functioning amine granules to the nerve terminals (see below).

Within Section II, the capacity for ^{3}H-NA retention, and the response to nerve stimulation in the nerve terminals also increases. During the second to fourth day, ^{3}H-NA retention after small intravenous doses approaches normal (Andén & others, 1964; Iversen & others, 1965; Häggendal & Dahlström, 1970). Normal response to nerve stimulation, obviously requiring only a small proportion of normal transmitter stores (Häggendal & Lindqvist, 1963, 1964; Andén & others, 1969), was also observed in this time period (Andén & others, 1964). As with endogenous noradrenaline, the capacity for ^{3}H-NA retention, and the response to nerve stimulation increase markedly in this period, when the down-transport of new amine granules is supranormal, it is possible that all these three parameters are linked to the new granules arriving at the

nerve terminals. Since endogenous noradrenaline concentrations at this time are still low (40–50% of normal), while the capacity for ^{3}H-NA retention and transmission appears to be normal, granules of different ages may have different properties (cf. Dahlström & Häggendal, 1970; Häggendal & Dahlström, 1971).

If the increase in noradrenaline concentrations in section II of the recovery curve is due to the arrival in the nerve terminals of newly formed amine granules, a theoretical calculation of the amines recovery, based on axonal transport of granules, should give a curve similar to that of section II. A calculation was undertaken: (*a*) based on the values from Fig. 3 the amount of adrenaline in granules transported down the axons to the nerve terminals per day was calculated; (*b*) as the amount of the amine normally present in the nerve terminals of the adrenergic axons in the sciatic nerve is known (900 ng, Dahlström & Häggendal, 1966b, probably mainly stored in amine granules cf. Iversen, 1967; Andén & others, 1969), the values obtained can be expressed as a percent increase in noradrenaline per day. The amine granules in the sciatic nerve contain less noradrenaline than they do when approaching the nerve terminals, both in normal and reserpine-pretreated rats (Dahlström, Häggendal, Larsson & Magnusson, unpublished) [this is supported by De Potter, Chubb & De Shaepdryver (1970) who found that nerve trunk granules contained less noradrenaline than nerve terminal granules, compared to their protein content]; it was calculated that the granules in the sciatic nerve contained at least 25% less of the amine than granules in the nerve terminals. For this reason, the figures from Fig. 3 were increased by 25%, to correct for the noradrenaline content in the granules when they had reached the nerve terminals. The theoretical curve for recovery, obtained by these calculations (Fig. 2), together with the curve for the assayed amine recovery are rather similar and suggest that there is little requirement for a restitution of old granules to explain the recovery of noradrenaline after reserpine.

Further support for the "new granule" theory is given by Mueller & Shideman (1968) who found that protein synthesis inhibitors markedly delayed the recovery of endogenous noradrenaline concentrations after reserpine. It appears likely that protein synthesis inhibition affects the formation of new granules in the perikarya more than it blocks the restitution of the storage mechanism in the old granules.

Section III

During this period, from about the second to third week inclusive, the recovery of noradrenaline begins to slow. Fig. 3 shows that the period of overshooting in the amount of produced and down-transported granules, is followed by a period of subnormal production and transport of granules during the second week (7–13 days after reserpine). The decrease in the rate of noradrenaline recovery thus occurred when signs of a decreased down-transport of granules were observed. We therefore suggest that the two phenomena are intimately related.

Section IV

A clear-cut drop in the noradrenaline concentrations was observed in all tissues between the fourth and the sixth weeks. Already during the fourth week the levels start declining. The drop is statistically significant when the values from the three times are taken together, and has regularly been observed in experiments over three years at all seasons. A similar, but earlier drop in noradrenaline concentrations has also been found in the brain (unpublished observation) during the recovery after

reserpine. At present, it is difficult to explain this drop on basis of the theory that noradrenaline recovery is due to an "awakening" of old, blocked granules. With the "new granule" theory, however, this drop may be explained thus: the amine storage granules in the nerve terminals appear to have a life-span of 3–4 weeks, in their capacity to store endogenous noradrenaline (Dahlström & Häggendal, unpublished) taking into consideration the degree of noradrenaline loading of the axonal granules compared to the nerve terminal granules. A supranormal quantity of noradrenaline granules appears to reach the nerve terminals during the first week after reserpine, and these granules have a life-span for storage of 3–4 weeks. Since the amount of granules transported to the nerve terminals per time unit at this time (about 4 weeks) is probably normal or subnormal, the loss of an unusually large number of noradrenaline-containing granules cannot be compensated for immediately. The result will be a drop in the concentration of noradrenaline, followed by gradual return to normal at about 6 weeks after reserpine.

The present study demonstrates that the recovery in nerve terminals of endogenous noradrenaline concentrations after reserpine (10 mg/kg, i.p.) follows a multiphasic course. The levels start to increase after 24 h while new, functioning amine granules are thought to be transported down the rat adrenergic axons somewhat earlier (18 h) (Dahlström, 1967; Dahlström & Häggendal, 1969). Therefore, when experimentally functioning amine granules are not wanted in the nerve terminals (and the tool for this is often reserpine) the reserpine-pretreatment should not be earlier than about 15 h before the start of the experiment, to avoid the possibility that some newly formed functioning amine granules may have reached the nerve terminals by axonal transport.

The effects on concentrations of endogenous noradrenaline in the adrenergic neuron persist up to at least 6 weeks after the reserpine injections. The variations in concentrations after reserpine appear to be well correlated to the down-transport of amine storage granules and the properties of these granules. It is evident that the local synthesis of noradrenaline in the nerve terminals is essential, not only for the economy of the transmitter stores in the normal condition, but also for the amine's recovery after reserpine. However, noradrenaline synthesis, without functioning storage sites in the down-transported amine granules, is unlikely to be in itself responsible for the amine's recovery after reserpine. As proposed earlier (Dahlström & Häggendal, 1966a) after one large dose of reserpine the recovery of the concentrations of endogenous noradrenaline, and also transmission, and the capacity to take up and store small amounts of ^{3}H-NA are now suggested to be mainly accounted for by the axonal transport of new amine granules, formed in the perikarya after the reserpine injection.

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