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The recovery of the capacity for uptake-retention of [^{3}H]noradrenaline in rat adrenergic nerves after reserpine

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The uptake retention of [^{3}H]noradrenaline (2·5 μg/kg, i.v., 30 min before death) in rat salivary glands was studied at different times after reserpine treatment (10 mg/kg, i.p.). The effect of removing the cervical superior ganglion 12 h before death on the recovery of the [^{3}H]noradrenaline uptake-retention capacity after reserpine was also investigated. The ganglionectomy was unilateral, and the contralateral side was always preganglionically denervated. In glands with uninterrupted postganglionic adrenergic nerves the onset of recovery of the [^{3}H]noradrenaline retention capacity occurred 24–36 h after reserpine. Normal contents were found on the second to third days. Between day 3 and 6 a possible overshoot of [^{3}H]noradrenaline content, followed by normal and subnormal contents (7–21 days) were recorded. Ganglionectomy, 12 h before death, markedly delayed the recovery of [^{3}H]noradrenaline retention capacity. Both the recovery curve for [^{3}H]noradrenaline retention in glands with intact postganglionic nerves, and the effect of ganglionectomy on the [^{3}H]noradrenaline retention capacity, were clearly related to the relative number of new functioning amine granules that are transported via the axons to the nerve terminals at different times after the reserpine-pretreatment. The results indicate that young amine granules, recently transported to the nerve terminals via the axons, have the greatest capacity to take up and retain [^{3}H]noradrenaline. The half-life of this capacity in the young granules appears to be about 12 h. Since published results indicate that [^{3}H]noradrenaline is initially taken up in the "small easily releasable pool" of transmitter, we suggest that young amine granules are of the greatest importance for adrenergic function, *i.e.* that they are particularly active in taking up recaptured noradrenaline, in the synthesis, and in the release of this transmitter.

An axoplasmic flow of material, *inter alia* amine granules, is necessary for the recovery of both the [^{3}H]noradrenaline uptake-storage capacity and for the return of endogenous noradrenaline in nerve terminals to normal levels after reserpine treatment (Häggendal & Dahlström, 1971a, b). The recovery of *endogenous noradrenaline* to normal levels requires several weeks (cf. Häggendal & Dahlström, 1971a), whilst the capacity of the tissues to retain [^{3}H]noradrenaline is normal within 2–3 days after reserpine (Andén, Magnusson & Waldeck, 1964; Iversen, Glowinski & Axelrod, 1965; Andén & Henning, 1966; see also Häggendal & Dahlström, 1970a).

An overshoot in the amounts of noradrenaline accumulating above a 6 h ligation of the sciatic nerve, which probably reflects the number of amine granules transported distally per unit of time, has been observed during the third to sixth day after reserpine treatment (Dahlström & Häggendal, 1969). If the capacity of the tissues to retain [^{3}H]noradrenaline is associated mainly with the new, recently arrived

granules as suggested earlier (Dahlström & Häggendal, 1966; Häggendal & Dahlström, 1970a), this overshoot in the number of new granules reaching the nerve terminals would be expected to influence the pattern of the [^{3}H]noradrenaline retention during this interval. The present work was undertaken to test this hypothesis.

MATERIAL AND METHODS

Male albino rats of the Sprague-Dawley strain (200–250 g) were used. The rats were given one injection of reserpine (10 mg/kg, i.p.) 18, 24, 36 h, 2, 3, 4, 5, 6, 7, 9, 11, 13, 15, 17 and 21 days before death. Twelve h before the injection of (−)-[^{3}H]noradrenaline (^{3}H-NA) (2·5 μg/kg, i.v., specific activity 2·34 Ci/mmol, Amersham) the animals, including controls, were operated upon under ether anaesthesia. The cervical superior ganglion was removed unilaterally (alternating sides), and the cervical sympathetic trunk was cut on the contralateral side (denervated and decentralized sides, respectively). The isotope was injected intravenously, in about 200 μl, during an interval of between 2–3 s. The rats were decapitated 30 min after the ^{3}H-NA injection and the submaxillary glands were excised. One group of animals received atropine (1 mg/kg, i.v.) 15 min before the injection of ^{3}H-NA to decrease the range of variations of the values as suggested by Almgren (1970). Operated and unoperated rats without reserpine treatment were always used as controls.

^{3}H-NA was separated by column chromatography and measured by liquid scintillation counting (Waldeck, 1968). By addition of antioxidants and of 10 μg of cold noradrenaline the ^{3}H-NA was protected during estimation. The contamination with metabolites of ^{3}H-NA was much reduced by the column step. The results were expressed in d/min per gland, and not per g of tissue, since the weight of the tissue is altered after reserpine treatment (cf. Häggendal & Dahlström, 1971a).

RESULTS

The ^{3}H-NA content per denervated or decentralized salivary gland of the reserpine treated rats were expressed in % of the ^{3}H-NA content in denervated or decentralized control glands, respectively, from non-reserpinized rats. In control rats, the ^{3}H-NA contents in the ganglionectomized glands and in the decentralized glands were not significantly different (Table 1).

Atropine appeared to decrease the ^{3}H-NA values in control glands, but the reduction (about 30%) was only significant in the decentralized glands. After reserpine there was no consistent or significant difference between the atropine or non-atropine treated glands. However, there did seem to be a reduced variation of the ^{3}H-NA uptake after atropine pretreatment (Table 1), in agreement with Almgren (1970) who suggests this to be due to an anti-acetylcholine effect of atropine.

The ^{3}H-NA content of the glands was very low 30 min after ^{3}H-NA was given to rats (i.v.) dosed with reserpine 18 h before. On the decentralized side, the ^{3}H-NA content started to rise 24–36 h after the single injection of reserpine. After a rapid increase, normal levels were reached around the third day. Some of the values appeared to overshoot between the third and the 8th day after reserpine, with a maximum at about 4 days, but this trend was not significantly different from control values. Following this period a *decline* was observed and apparently subnormal values of ^{3}H-NA were obtained which at 11 and 13 days, taken together, were significantly lower than the values at days 4 to 7, taken together ($P < 0{\cdot}005$). There-

after the values appeared to increase, and normal values were observed by about 3 weeks.

On the ganglionectomized side, the ^{3}H-NA content followed a different course during the first 7 to 9 days of recovery. Thus, up to about 7 days after reserpine the content increased more slowly than in decentralized glands. The ^{3}H-NA levels reached almost normal values 6 to 7 days after reserpine. Thereafter the levels decreased and during days 11 and 13 were significantly lower than controls (11 and 13 days values taken together; $P < 0{\cdot}005$). They then slowly approached normal by about 3 weeks after reserpine.

The differences between decentralized and ganglionectomized tissues regarding their capacity for uptake-retention of ^{3}H-NA after reserpine are plotted in Fig. 3a. The difference appeared to be maximal at day 4, and was statistically significant from day 2 to day 7, except at day 5 (see Table 1 and discussion).

DISCUSSION

The results obtained exhibit a wide scatter (Table 1), which is often seen in *in vivo* isotope experiments of this type (see *e.g.* Almgren, 1970) despite a great care to decrease variations due to technical reasons. The intravenous injection of ^{3}H-NA was standardized as far as possible with respect to volume and time of injection. Contamination of blood containing the isotope is unlikely, since the half-life of circulating exogenous noradrenaline is short, and at 30 min after an injection the blood level is about zero (see *e.g.* Iversen, 1967). Despite this scatter a definite pattern for the ^{3}H-NA retention after reserpine may be discerned. This includes a rapid increase of ^{3}H-NA contents to normal and possibly higher, with a maximum around day 4; this is followed by a significant decline with a minimum at 11 to 13 days, and a slow return to normal. This change will be discussed below with respect to axonal transport of newly formed granules which shows a similar pattern after reserpine (Dahlström & Häggendal, 1969).

I. In the decentralized gland three parts of the ^{3}H-NA uptake-storage curve can be distinguished.

1. *The onset of the recovery of the* ^{3}H-NA *retention.* The onset of recovery occurred between 24 and 36 h after reserpine (Fig. 1). This is in accordance with previous studies, where partial recovery of the ^{3}H-NA retention capacity had occurred between 30 and 48 h after reserpine (Andén & others, 1964; Iversen & others, 1965; Häggendal & Dahlström, 1970a). In this period both the endogenous noradrenaline contents (Häggendal & Dahlström, 1970a; 1971a) and the transmission (Andén & others, 1964; Andén & Henning, 1966; Almgren & Lundborg, 1971) begin to recover; also the newly formed functioning amine granules have been estimated to arrive in the nerve terminals during this time. These granules are formed after reserpine administration and can be observed as reserpine-depletable noradrenaline fluorescence, initially in the cell bodies about 12 to 15 h after reserpine, and a few hours later in the adrenergic axons (see Dahlström, 1967; Dahlström & Häggendal, 1969).

2. *The rapid increase to a maximum of* ^{3}H-NA *retention.* After the onset of recovery of ^{3}H-NA uptake-storage capacity (occurring between 24–36 h) the ^{3}H-NA content increased rapidly in the glands, and approached normal about the 3rd day and then appeared to be above normal (Fig. 1) for days 4–7, although the difference from control was not significant (Table 1). (The possibility of a two-peak recovery

Table 1. *The ^{3}H-NA content (d/min) in rat salivary glands after the i.v. injection of 2·5 μg/kg (−)-^{3}H-NA (specific activity 2·34 Ci/mmol, Amersham) 30 min before death to reserpine-pretreated rats (10 mg/kg, i.p.).* Both the control rats and the reserpine-treated rats were operated upon 12 h before death (unilateral removal of the cervical superior ganglion and contralateral decentralization of this ganglion). One group of rats (II) received atropine (1 mg/kg, i.v.) 15 min before the ^{3}H-NA injection. The other group (I) was not given atropine. T indicates total, i.e. groups I and II taken together. The values are expressed in % (mean ± s.e. of the ^{3}H-NA content in decentralized and ganglionectomized control glands respectively (d/min, mean ± s.e. are given within brackets). In the right column, the difference between the decentralized and ganglionectomized side, calculated for each rat, is given. (The degree of significance between the differences in reserpine rats and that in control rats was calculated according to Student's *t*-test, n.s. indicates no significance: $P > 0{\cdot}05$).

Time after reserpine	Group	n	Decentralized (A)	Ganglionectomized (B)	Difference between A and B	
0 h	I	21	100·0 ± 14·48 (34·897 ± 5·054)	100·0 ± 12·28 (31·389 ± 3·856)	0·0 ± 6·41	
	II	16	100·0 ± 6·02 (25·133 ± 1·513)	100·0 ± 6·76 (27·540 ± 1·862)	2·0 ± 4·69	
	T	37	100·0 ± 8·52	100·0 ± 7·47	0·9 ± 4·12	
18 h	I	4	1·0 ± 0·07	1·3 ± 0·15	−0·3 ± 0·11	
	II	5	3·2 ± 1·10	1·9 ± 0·33	1·3 ± 0·8·0	
	T	9	2·2 ± 0·70	1·6 ± 0·21	0·6 ± 0·51	(n.s.)
24 h	I	3	1·1 ± 0·17	1·5 ± 0·49	−0·4 ± 0·32	
	II	5	6·9 ± 2·20	4·7 ± 1·23	2·2 ± 1·02	
	T	8	4·7 ± 1·69	3·5 ± 0·95	1·2 ± 0·78	(n.s.)
36 h	I	4	28·0 ± 9·75	17·9 ± 7·07	10·2 ± 3·19	
	II	5	10·8 ± 1·22	5·8 ± 0·56	5·1 ± 1·03	
	T	9	18·5 ± 5·04	11·1 ± 3·60	7·3 ± 1·67	(n.s.)
48 h	I	4	86·0 ± 12·56	32·0 ± 5·43	54·0 ± 16·80	
	II	5	48·6 ± 19·03	41·0 ± 16·90	7·6 ± 16·04	
	T	9	65·2 ± 13·04	37·0 ± 9·31	28·2 ± 13·60	($P<0{\cdot}025$)
3 days	I	9	97·0 ± 21·11	37·1 ± 4·45	59·9 ± 22·98	
	II	5	101·8 ± 16·62	36·0 ± 6·66	65·8 ± 13·17	
	T	14	98·7 ± 14·39	36·7 ± 3·57	62·0 ± 15·12	($P<0{\cdot}001$)
4 days	I	4	124·5 ± 31·09	85·3 ± 23·37	39·3 ± 13·27	
	II	5	137·8 ± 25·93	35·2 ± 7·68	102·6 ± 18·37	
	T	9	131·9 ± 18·80	57·4 ± 13·59	74·4 ± 15·71	($P<0{\cdot}001$)
5 days	I	2	117·5 ± 12·50	72·5 ± 41·50	45·0 ± 29·00	
	II	5	85·0 ± 9·54	73·6 ± 11·74	12·0 ± 10·33	

Table 1—*continued.*

Time after reserpine	Group	n	Decentralized (A)	Ganglionectomized (B)	Difference between A and B	
	T	7	94·3 ± 9·31	73·3 ± 12·15	21·4 ± 11·31	(n.s.)
6 days	I	8	106·9 ± 17·07	97·4 ± 16·38	9·5 ± 15·03	
	II	5	130·0 ± 29·77	87·6 ± 20·93	46·4 ± 13·40	
	T	13	115·77 ± 15·13	93·6 ± 12·43	23·7 ± 11·45	($P<0.025$)
7 days	I	4	110·3 ± 27·32	89·3 ± 15·17	21·0 ± 16·35	
	II	5	122·8 ± 28·1	89·4 ± 29·9	33·4 ± 10·70	
	T	9	117·2 ± 18·63	89·3 ± 16·93	27·9 ± 9·00	($P<0.01$)
9 days	I	9	88·1 ± 19·95	99·8 ± 18·6	—11·7 ± 7·44	
	II	5	88·8 ± 21·91	60·8 ± 5·05	28·0 ± 21·23	
	T	14	88·4 ± 14·50	85·9 ± 12·90	2·5 ± 9·96	(n.s.)
11 days	II	5	70·2 ± 8·24	71·4 ± 8·54	—12·0 ± 1·83	(n.s.)
13 days	I	8	75·8 ± 15·89	59·9 ± 11·97	15·9 ± 14·50	
	II	5	76·4 ± 5·03	53·0 ± 12·62	23·4 ± 15·25	
	T	13	76·0 ± 9·69	57·2 ± 8·53	18·8 ± 10·53	(n.s.)
15 days	I	4	93·0 ± 12·45	92·3 ± 12·91	0·8 ± 5·72	(n.s.)
17 days	I	5	76·1 ± 2·38	84·4 ± 12·99	—7·8 ± 14·27	(n.s.)
21 days	I	5	114·4 ± 20·62	94·2 ± 8·78	20·2 ± 13·75	
		4	94·8 ± 8·09	86·3 ± 4·82	8·5 ± 9·31*	(n.s.)

* if n = 4.

cannot be excluded, since the 5th day value of atropine pretreated rats was rather low, whilst the non-atropinized rats show a high value. The reason for this difference at 5 days after reserpine is unknown). During the same period after reserpine an overshoot has previously been observed in the amounts of endogenous noradrenaline, accumulating above a 6 h ligation of the sciatic nerve (Dahlström & Häggendal, 1969) (see Fig. 2, hatched line).

3. *The decline to subnormal and return to normal levels of* ^{3}H-NA *retention.* About day 9, the content of ^{3}H-NA in the glands started to decrease to subnormal values with a return to normal 3 weeks after reserpine. Within this period (8th to 13th day studied, see Fig. 2, hatched line) the amounts of amine granules transported down the axons were significantly subnormal (Dahlström & Häggendal, 1969), while normal transport was found 3 weeks after reserpine (unpublished). Thus, the overall pattern for ^{3}H-NA retention in adrenergic nerve terminals and the down-transport of new granules appear to be similar after reserpine administration (Fig. 2).

II. To study further the importance of axonal down-transport of new granules for ^{3}H-NA retention, its content in salivary glands was studied 12 h after ganglionectomy, *i.e.* interrupted transport.

In the ganglionectomized glands the ^{3}H-NA retention was significantly lower than in the decentralized glands at 2–7 days, and was most marked during the 3rd to 5th day (Fig. 1 and 3a).

Axotomy may induce several events of importance for the ^{3}H-NA uptake-storage

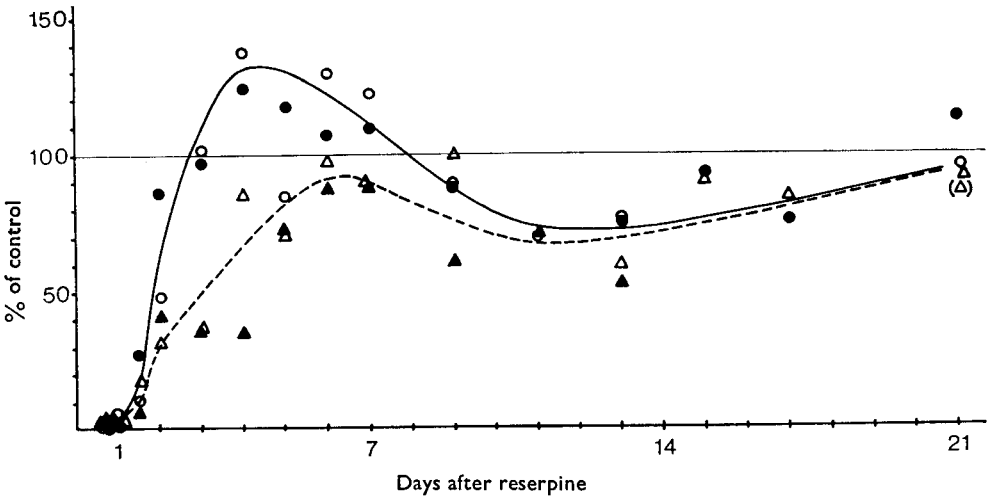

FIG. 1. The uptake-retention of ^{3}H-NA in rat salivary glands after reserpine treatment (10 mg/kg, i.p.). 12 h before the injection of (—)-^{3}H-NA (2·5 μg/kg, i.v.) the cervical superior ganglion was decentralized unilaterally and removed on the contralateral side. Decentralized side ———; ganglionectomized side – – – –. Some rats were given atropine (1 mg/kg, i.v.) 15 min before the ^{3}H-NA injection: ○ atropine-treated, decentralized side; △ atropine-treated, ganglionectomized side, ● no atropine, decentralized side; ▲ no atropine, ganglionectomized side. Mean values are indicated. For s.e. see Table 1.

in the nerve terminals, *e.g.* (i) Block of the propagation of nerve impulses: in the study this is unlikely to explain the difference between the two curves, since the decentralized glands were also lacking normal impulse activity. (ii) Degeneration of the nerve terminals: an unlikely explanation in this case, since nerve terminals of normal appearance were seen by a histochemical fluorescence technique in rat iris (Malmfors & Sachs, 1965) and submaxillary gland (Dahlström, unpublished) 12 h after ganglionectomy, although the possibility that reserpinized rats could be more sensitive to axotomy, has not been excluded. Furthermore, membrane pump inhibitors were still effective, indicating an intact nerve terminal membrane 12 h

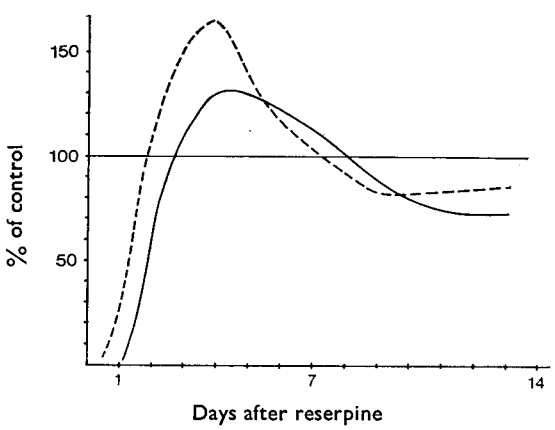

FIG. 2. The recovery curve for ^{3}H-NA uptake-retention capacity in decentralized rat salivary glands after reserpine treatment (from Fig. 1) shown together with the curve for noradrenaline accumulation in 6 h ligated rat sciatic nerves at different times after reserpine treatment (hatched line). This curve is based on results given in Dahlström & Häggendal (1969) and indicates in all probability the relative amounts of new amine storage granules that are transported distally in the axons to the nerve terminals per unit of time after reserpine.

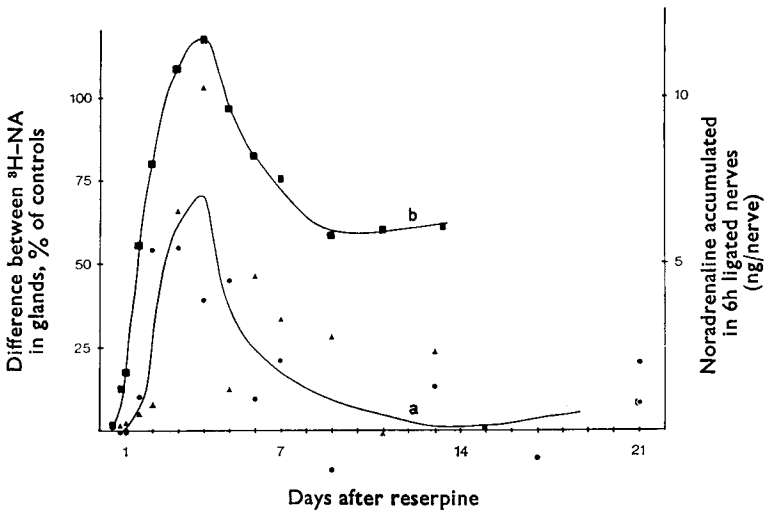

FIG. 3. (a) The differences between the ^{3}H-NA content between decentralized and ganglionectomized rat salivary glands at different times after reserpine treatment (10 mg/kg, i.p.). The values are derived from the curves in Fig. 1 and from Table 1, and are expressed in % of control glands. ● no atropine pre-treatment, and ▲ atropine-pretreated rats.

(b) The relative amounts of noradrenaline accumulated above a 6 h ligation of rat sciatic nerves at different times after reserpine treatment (10 mg/kg, i.p.). The values are derived from Fig. 1 in Dahlström & Häggendal, 1969. The curve indicates in all probability the relative amounts of functioning noradrenaline-containing amine storage granules that are transported distally in the adrenergic axons at different times following reserpine treatment.

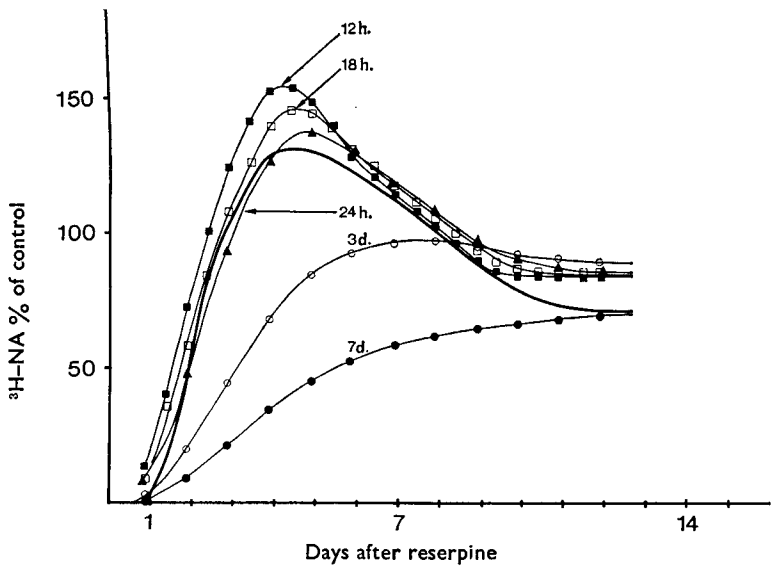

FIG. 4. Theoretical curves for the recovery of the capacity of uptake-retention of ^{3}H-NA in rat adrenergic nerve terminals after reserpine treatment. The curves are produced on the basis of assumptions indicated in the text, and are calculated for a theoretical half-life (T 1/2) for the capacity of the young amine granules to take up and store ^{3}H-NA of 12 h (■———■), 18 h (□———□), 24 h (▲———▲), 3 d (○———○) or 7 d (●———●). The empirical curve (from Fig. 1) for ^{3}H-NA retention capacity is also indicated (———). Both the theoretical and the empirical curves are expressed in % of control, *i.e.* the ^{3}H-NA content in decentralized glands of rats not given reserpine (see text).

after ganglionectomy, preventing the uptake of both exogenous noradrenaline (Malmfors & Sachs, 1965) and ^{3}H-NA (Häggendal & Dahlström, in preparation). Also, the glands in normal, non-reserpinized rats contained about the same amounts of ^{3}H-NA on the denervated and decentralized sides (Table 1). (iii) Arrest of axonal transport: probably the most important factor for explaining the difference between the two ^{3}H-NA retention curves is the arrest of the axonal transport of new amine storage granules after ganglionectomy. Fig. 3 shows the difference in ^{3}H-NA retention between decentralized and ganglionectomized glands at different times after reserpine. The levels appear to be most sensitive to axotomy during the 3rd to 4th days when the difference between the two sides is highly significant ($P < 0{\cdot}001$). This corresponds to the period of time when the amount of newly formed amine granules being down-transported distally in the axons per unit of time is particularly high (Fig. 3b). Later, transport is reduced, which may be the reason for the smaller effect of ganglionectomy on the total capacity of the tissue to retain ^{3}H-NA.

The similar shape of the curves for ^{3}H-NA retention in glands with an uninterrupted axoplasmic flow and for axonal down transport of new granules after reserpine treatment (Fig. 2, hatched line and Fig. 3b) seems to indicate that the newly transported granules are of importance for the recovery of the ^{3}H-NA uptake-storage capacity after reserpine.

III. To test this further, theoretical curves for the recovery of the ^{3}H-NA retention in the tissue after reserpine have been calculated based on the accumulation in the nerve terminals of newly formed and recently down-transported granules. The different curves were calculated on the assumption that the half-life (T 1/2) of the capacity of these new granules to take up and store ^{3}N-HA is 12, 18, 24 h, 3 or 7 days (Fig. 4).

The flow of newly formed granules at different times after reserpine is indicated in Figs 2 and 3b, which show the amount of endogenous noradrenaline in the granules that have accumulated above a 6 h ligation. The figure may be considered to reflect the relative differences in the number of new granules reaching the nerve terminals per unit of time after reserpine (cf. discussion in Dahlström & Häggendal, 1969; Häggendal & Dahlström, 1971a). The following assumptions have been made: (1) The flow of new granules (Q (τ)) is indicated by the amount of accumulated endogenous noradrenaline (Figs 2 and 3b). (2) A fraction (a) of the endogenous noradrenaline content in each granule can be exchanged by ^{3}H-NA, and this fraction is constant both in normal animals and at different times after reserpine. (3) The amount of exchangeable noradrenaline is zero in the nerve terminals 12 h after reserpine, and (4) The decay of the capacity of the recently down-transported young granules to take up and retain ^{3}H-NA is monoexponential with a half-life of T 1/2. The amount of exchangeable noradrenaline (M (t)) in the nerve terminals at a given time (t) can then be calculated from the formula

$$M(t) = a \cdot \int_0^t Q(\tau) \cdot e^{-k(t-\tau)} d\tau$$

$$\text{where } k = \frac{\ln 2}{T\ 1/2}$$

The integral was numerically calculated for different T 1/2 values using Simpson's

one-third rule. The figures were expressed in % of the M (t) values obtained when the value for normal axonal flow (*i.e.* without reserpine pretreatment) was used for the respective T 1/2 values.

In Fig. 4 the theoretical curves for T 1/2 = 12 h, 18 h, 24 h, 3 days or 7 days are shown together with the experimentally obtained curve from Fig. 1. With respect to not only the maximum of the curve for ^{3}H-NA uptake-storage capacity, but also the inclination and decline of the curve, the theoretical curve for T 1/2 = 12 h appears to have the best congruence with the empirical curve. However, since the empirical curve is based on values which show a wide scatter, the figure of 12 h for the T 1/2 must be considered as approximate.

IV. The above discussion indicates that mainly the new amine granules are responsible for ^{3}H-NA uptake-retention in adrenergic nerve terminals, not only after reserpine pretreatment, but also in the normal animal. At present the possibility cannot be excluded that some other, extragranular, factor, causing a reappearance of the function of old granules and transported distally in the axons in parallel with the new amine granules, is responsible for the results.

The terms "young" and "old" as applied to granules imply the occurrence of an ageing process of the granules in the terminals. Data in support of an ageing process are, e.g., the finding of the release of granule proteins (chromogranin A and dopamine-β-hydroxylase) together with noradrenaline upon nerve stimulation (De Potter, De Schaepdryver & others, 1969; Geffen, Livett & Rush, 1969; Gewirtz & Kopin, 1970; Smith, De Potter & others, 1970). Perhaps by gradual, repeated losses of their protein content during release, the functional properties of the granules may change. Support for the existence of two types of noradrenaline storage particles has appeared lately: (i) Electronmicroscopically, a morphological heterogeneity of dense core vesicles (probably corresponding to noradrenaline storage vesicles, e.g., Hökfelt, 1968) has been observed. A preponderance of large dense core vesicles has been seen in cell bodies and axons above a constriction, whilst they are comparatively less numerous in nerve terminals. In contrast, the small type of vesicle constitutes most of the vesicle population in nerve terminals (Geffen & Ostberg, 1969; Hökfelt, 1969; Kapeller & Mayor, 1969; Fillenz, 1970). (ii) In sucrose gradient centrifugation studies two types of noradrenaline-storing particles with different density have been observed (e.g., Roth & others, 1968; De Potter, 1968; Hörtnagl, Hörtnagl & Winkler, 1969; De Potter, Chubb & De Schaepdryver, 1970; Lagercrantz, 1971). It is interesting that only the heavier particles seem to contain measurable amounts of dopamine-β-hydroxylase (e.g., De Potter, 1971).

Recently, De Potter & Chubb (1971) have recalculated the life-span of amine granules in adrenergic nerve terminals. These authors wanted to use a better marker for the granules than their noradrenaline content, since this may vary in different parts of the neuron; they chose dopamine-β-hydroxylase. Using the axonal transport and terminal content of this enzyme, they calculated a life-span of some 40 h. Since the less dense amine granules apparently contain little or none of this enzyme (De Potter, 1971), there may be two types of noradrenaline storage particles. However, it seems more likely that the two are related by an ageing process as discussed above. De Potter & Chubb (1971) estimated the life-span of dopamine-β-hydroxylase. It is interestingly close to our estimation of the T 1/2 for uptake-retention of ^{3}H-NA, a capacity probably connected with young amine granules.

If the hypothesis about "young" and "old" amine granules is true, it may also have implications for theories of different stores of transmitter in the terminals. Thus, "the small, easily releasable pool" may be mainly in young granules, whilst "the large, more stable pool" may be mainly in old amine granules. This has been discussed elsewhere (Dahlström & Häggendal, 1972; Häggendal & Dahlström, 1970b, 1971b).

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