Recovery of the amine uptake-storage mechanism in nerve granules after reserpine treatment: inhibition by axotomy

After a large dose of reserpine, the nerve function recovers centrally (Häggendal & Lindqvist, 1963; 1964) and peripherally (Andén, Magnusson & Waldeck, 1964; Andén & Henning, 1966) 2 or 3 days later while the monoamine levels are still very low. There is, however, a rather sudden rise in the ability of the amine granules to take up and retain amines at the time of functional recovery; it increases to almost normal levels in the adrenal medulla (Lundborg, 1963; Carlsson, Jonason & Rosengren, 1963) but only partially in the sympathetic nerves (Andén & others, 1964; Andén & Henning, 1966; Lundborg & Stitzel, 1968) and in the brain (Glowinski, Iversen & Axelrod, 1965). Two hypotheses have been put forward to explain this recovery of the nerve function and the uptake-storage mechanism despite low endogenous amine levels: (1) transport from the cell bodies to the nerve terminals of newly synthesized granules unaffected by reserpine (Dahlström, Fuxe & Hillarp, 1965; Dahlström & Häggendal, 1966; 1969), and (2) disappearance of minute amounts of very highly bound reserpine from the amine granules (Alpers & Shore, 1969). The hypotheses can be differentiated in an experiment involving interruption of the connections between the cell bodies and the terminals (axotomy). Such an investigation can probably only be made on central monoamine neurons since their terminals, in contrast to those of the peripheral neurons, do not start to degenerate until more than two days after axotomy. For example, the monoamine levels in the caudal spinal cord do not change during the first three days after axotomy of the bulbospinal monoamine neurons to that region by spinal cord transection (Carlsson, Falck & others, 1964; Andén, Häggendal & others, 1964).

Male Sprague-Dawley rats, 180–230 g, had spinal cord transection in the mid-thoracic region during ether anaesthesia. Reserpine was given intraperitoneally in a dose of 5 mg/kg. Careful attempts were made to keep the rectal temperature at 37°. Usually it was only 34–36° in the reserpine treated and transected rats, or 1–2° lower than in the controls, and attempts to elevate it resulted in increased mortality. The peripheral but not the central L-3,4-dihydroxyphenylalanine (L-dopa) decarboxylase activity was inhibited by N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl)hydrazine (Ro 4–4602 50 mg/kg, i.p. 4½ h before death) (Bartholini & Pletscher, 1968). In this way, there was an increase in the amount of [^{3}H]noradrenaline formed and accumulated in the central nervous system from ^{3}H-L-dopa (5 μg/kg, i.v. 4 h before death, 1 μg/ml, ring 2,5,6-^{3}H, about 30 Ci/mM, Radiochemical Centre in Amersham). Each experimental group consisted of 4–5 rats. The tissue [^{3}H]-

Table 1. *Concentrations of* [^{3}*H*]*noradrenaline in different parts of the rat central nervous system 4 h after treatment with* [^{3}*H*]L-*dopa* (5 μg/kg., i.v., 30 min after Ro 4–4602 50 mg/kg, i.p.). At different times before death, mid thoracic transection of the spinal cord was made or reserpine 5 mg/kg was injected i.p., or both. Number of experiments in parentheses. Values in % ± s.e. of those in the first column. Actual concentrations (ng/g ± s.e.) of [^{3}H]noradrenaline within brackets in the first column

Part of the CNS	Section 50 h No reserpine	Section 50 h Reserpine 6 h	Section 50 h Reserpine 50 h	No section Reserpine 50 h	Section 6 h No reserpine
Caudal half of the spinal cord	100·0% (5) [0·071 ± 0·0069]	3·8 ± 0·84 (5)	7·8 ± 1·36 (5)	84·8 ± 34·67 (2)	98·7 (1)
Cranial half of the spinal cord	100·0% (5) [0·174 ± 0·0219]	1·6 ± 0·33 (5)	12·7 ± 0·74 (5)	28·7 ± 7·62 (2)	82·1 (1
Whole brain	100·0% (5) [0·149 ± 0·0172]	2·8 ± 0·61 (5)	10·6 ± 0·78 (5)	23·0 ± 7·91 (2)	70·6 (1)

noradrenaline was determined by liquid scintillation counting after cation exchange chromatography and freeze-drying (Carlsson & Waldeck, 1963).

The results (Table 1) were calculated in each experiment as per cent of those in the sectioned but not reserpine-treated group. Reserpine treatment 6 h before death caused a pronounced reduction of the amount of [^{3}H]noradrenaline accumulated in all parts of the central nervous system. If reserpine had been given 50 instead of 6 h before death to spinal rats, the [^{3}H]noradrenaline was increased in the brain by about 4 times and in the cranial half of the sectioned cord by about 8 times. In the caudal half of the cut spinal cord, the [^{3}H]noradrenaline was increased about twice. The differences between the means of the 50 and 6 h groups were significant at the 0·1% level in the brain and the cranial half of the spinal cord and at the 5% level in the caudal half (Student's *t*-test).

The content of [^{3}H]noradrenaline formed and accumulated 50 h after reserpine treatment was greater in all parts of the central nervous system of intact rats than in rats with a spinal cord transection, showing that the uptake-storage mechanism could recover at least as much in the caudal spinal cord as in the other parts between 6 and 50 h after reserpine treatment. The difference between the intact and spinal animals was also seen as a more marked return of, e.g., spontaneous motility, reactivity to stimuli and eyebulb protrusion. It was possibly owing to the better condition of the intact rats.

The amount of [^{3}H]noradrenaline accumulated in the caudal half of the cut spinal cord was about the same if the operation was performed 6 or 50 h before death. Therefore it is unlikely that degeneration of the descending bulbospinal neurons to the caudal part of the spinal cord was responsible for the smaller recovery of the uptake-storage mechanism in that region between 6 and 50 h after reserpine treatment.

In conclusion, axotomy markedly inhibited the recovery of the uptake-storage mechanism in the caudal spinal cord after reserpine treatment. Therefore, formation and down transport of new granules appear to be the most important factor in recovery from reserpine, although restoration of old granules probably contributes to recovery.

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Cardiac catecholamine levels and blood pressure after chronic treatment with β-adrenergic blocking agents

The mechanism of the hypotensive effect observed clinically after chronic treatment with β-adrenergic blocking agents is not yet elucidated. Blockade of the sympathetic supply to the heart (Prichard & Gillam, 1966), direct or centrally mediated vasodilatation (Waal, 1966) and a reduction of cardiac output as a result of a decreased heart rate (Frölich, Tarazi & others, 1968) were suggested as possible explanations.

Some hypotensive agents such as reserpine and guanethidine are believed to elicit their effects partially by depleting noradrenaline in the peripheral adrenergic nerve endings. In this study experiments were performed to establish whether chronic treatment with β-adrenergic blocking agents would cause changes in endogenous catecholamine levels in the heart, brain and spleen of normotensive, non-anaesthetized rats, and whether there would be any correlation between these changes and the systolic blood pressure.

Wistar rats (150 g to 175 g) (6 rats for each drug) were injected intraperitoneally daily with 3 mg/kg of propranolol, 5 mg/kg of Kö 592 [1-(3-methylphenoxy)-2-hydroxy-3-isopropylaminopropan] or 10 mg/kg of INPEA [1-(*p*-nitrophenyl)-2-isopropylaminoethanol hydrochloride] for 4 weeks; the dose of each drug was doubled for the subsequent 5 weeks. Concurrently, controls (6 rats) were injected intraperitoneally with 0·5 ml of physiological saline.

Indirect systolic blood pressure was measured weekly (18–20 h after administration of β-adrenergic blocking agents) from the tail of the non-anaesthetized rat by the use of an occluding cuff and a pneumatic pulse transducer connected to an electrosphygmograph, and registered on a Grass polygraph by means of a transducer-monitor coupler (E and M Physiograph Instrumentation, Houston, Texas, U.S.A.). This method was reported to be in good agreement with the direct measurements of blood pressure (Maistrello & Matscher, 1969; Baum & Rowles, 1969).

It was established in preliminary experiments that a single dose of propranolol (6 mg/kg), Kö 592 (10 mg/kg) or INPEA (20 mg/kg) administered intraperitoneally produced on the average a 79, 64 or 74% blockade respectively of the positive chronotropic effects elicited by 1 μg/kg of isoprenaline when the latter was administered intraperitoneally 1 h after β-adrenergic blocking agents. After 18–20 h the blockade was 58, 61 or 63% respectively.

The animals were killed after 9 weeks; the heart, brain and spleen were dissected and placed in liquid nitrogen. Catecholamines were extracted from tissue with acidified n-butanol (Maickel, Cox & others, 1968) and determined by the method of Anton & Sayre (1962). Their amount is expressed in ng/g of tissue and corrected for standard recoveries which ranged from 84 to 92% (average 86%).

Table 1 demonstrates that catecholamine content of the heart was significantly reduced after treatment with propranolol, Kö 592 and INPEA by 51, 32 and 56% respectively. The reduction was significantly greater in INPEA- and propranolol-treated rats than in those treated with Kö 592. Brain catecholamine content was increased by 29% in Kö 592 treated rats, but reduced by 14% in the INPEA treated group. No significant changes were observed after propranolol treatment.