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Uptake and storage of catecholamines in rabbit brain after chronic reserpine treatment

A. DAHLSTRÖM, K. FUXE, B. HAMBERGER AND T. HÖKFELT

The brains of rabbits treated chronically with small doses of reserpine have been examined by the histochemical fluorescence method for dopamine, noradrenaline and 5-hydroxytryptamine. Measurements were made 4 and 24 hr after the last reserpine injection both *in vivo* and *in vitro*. Evidence has been obtained that the small functionally-important pool of amine can be directly visualized in the fluorescence microscope. It was found to be present intraneuronally, probably localized to amine storage granules. The degree of functional recovery was correlated with the recovery, as shown by the increased fluorescence, of a small intraneuronal pool of amine and with the ability of amine storage granules to take up monoamines.

STRONG support has been obtained, in biochemical studies (Häggendal & Lindqvist, 1963, 1964) on brains of chronically reserpine-treated animals, for the concept that there exists a small, labile store of monoamine which is important for the immediate function of the monoaminergic neuron, whereas the largest store is not so important. Thus, the recovery of this small pool—but not of the normal amine levels—may be related to recovery of normal behaviour of the rabbit previously given reserpine. In the sympathetic nervous system it has been shown that the recovery from the pharmacological effects of reserpine coincide with the recovery of the ability of adrenal medullary granules (Lundborg, 1963; Carlsson, Jonasson & Rosengren, 1963) and peripheral adrenergic tissues (Andén, Magnusson & Waldeck, 1964; Andén & Henning, 1966) to take up noradrenaline. These findings are consistent with the view that the refilling of a small labile pool is—partially if not mainly—responsible for the early recovery of monoaminergic neurotransmission.

The purpose of the present investigation was to demonstrate the cellular localization of the small amounts of catecholamines and 5-hydroxytryptamine (5-HT) found in brains of chronically reserpine-treated rabbits (Häggendal & Lindqvist, 1963; 1964) 24 hr after the last injection. The presence of catecholamines and 5-HT was demonstrated using the histochemical fluorescence method (see Hillarp, Fuxe & Dahlström, 1966; Corrodi & Jonsson, 1967). Furthermore, *in vitro* studies on brain slices from these rabbits were made to see if the time-course of recovery of this small, labile pool of amines could be correlated with that of the ability of the central catecholamine terminals to take up and store noradrenaline.

Experimental

MATERIAL AND METHODS

Albino rabbits of 1·5–2·5 kg were used. Ten rabbits were treated with daily injections of reserpine (Serpasil) for 4–8 weeks (0·2 mg/kg, s.c.). Five of these rabbits were killed 4 hr, and the rest 24 hr after the last injection by an intravenous injection of air. Large parts of the telencephalon (including the caudate nucleus and putamen, the septal area and the

From the Department of Histology, Karolinska Institutet, Stockholm, Sweden.

hippocampal formation), of the diencephalon (mainly the hypothalamus and the subthalamus) and of the mesencephalon (mainly the tegmentum) and also the pons and the medulla oblongata were dissected out. The brain pieces were freeze-dried and treated with formaldehyde gas for 1 hr, embedded and sectioned as previously described (Dahlström & Fuxe, 1964; Hamberger, Malmfors & Sachs, 1965).

From the same animals the slices were made from the neocortex, the caudate nucleus, putamen, the hypothalamus, the vas deferens and/or submaxillary gland. The slices from each area of all the rabbits were incubated either with (-)-α-methylnoradrenaline (Corbasil), 0·1 and 1 μg/ml, or (—)-noradrenaline (0·1 and 1 μg/ml) for 20 min (Hamberger & Masuoka, 1965; Hamberger, 1967). After incubation the slices were freeze-dried (Thieme, 1965) and treated with formaldehyde gas as for the rest of the brain specimens.

Results

HISTOCHEMISTRY

4 *hr after the last reserpine injection*

In vivo. Practically no catecholamine and 5-HT nerve terminals were observed in the pieces studied except in two of the animals in the brains of which a number of weak to medium green-fluorescent catecholamine nerve terminals were observed, scattered mainly in the lateral hypothalamus and the retrochiasmatic area. The dopamine, noradrenaline and 5-HT cell bodies in the brain showed only a very weak to weak green or yellow fluorescence respectively.

In vitro. Previous to incubation practically no dopamine, noradrenaline or 5-HT terminals were observed though certain individual differences were found. After incubation with α-methylnoradrenaline (0·1 and 1 μg/ml) catecholamine nerve terminals with a medium to strong intensity could be observed in all these areas. If, instead, noradrenaline was administered to the incubation bath, higher concentrations (1 μg/ml) had to be used and even then only a weak green fluorescence could be observed in the catecholamine nerve terminals.

24 *hr after the last reserpine injection*

In vivo. The number and intensity of the noradrenaline nerve terminals in the hypothalamus had increased considerably in two out of five rabbits, most of the terminals having a very weak to weak green fluorescence intensity. In the brains of the other rabbits no certain signs of increase in fluorescence were observed. In the two rabbits mentioned above, weakly fluorescent catecholamine terminals could be seen also in the lower brain stem (e.g. in the ventral part of the griseum centralis, the dorsal motor nucleii of the vagus nerves and the nuclei of the tractus solitarii) and the dopamine nerve terminals of the caudate nucleus and putamen showed up with a distinct fluorescence. The varicose appearance of the terminals was the same as in normal animals. However, in none of the animals was fluorescence found in the noradrenaline nerve terminals of, for example,

the neocortex and the reticular formation. No 5-HT terminals were detected in any part of the brain studied.

The catecholamine and 5-HT cell bodies usually showed some recovery, especially in those rabbits which showed increased amounts of catecholamine nerve terminals. Large individual differences were observed, however, among the cell bodies within each of the various monoamine cell groups, probably reflecting different rates of granule formation (Dahlström, Fuxe & Hillarp, 1965).

In vitro. The slices from the hypothalamus and the caudate nucleus and putamen, but not those from the neocortex, showed weakly fluorescent catecholamine nerve terminals even before incubation with the amines. At this time interval, uptake in catecholamine terminals was found after incubation with α-methylnoradrenaline (1 and 0·1 μg/ml) just as after 4 hr. Also after noradrenaline (0·1 μg/ml) a clear uptake was found in catecholamine terminals but not in non-terminal axons. A comparison of the 4 and 24 hr tissue sections showed the main difference to be that a clear uptake occurred in catecholamine terminals after incubation with noradrenaline (0·1 μg/ml) in the second but not in the first instance.

BEHAVIOUR

Four hr after the last reserpine injection the rabbits exhibited a fully developed reserpine syndrome (see Carlsson, 1966), whereas after 24 hr the animals usually showed normal gross behaviour. The rabbits with functional recovery were the same animals showing the best recovery of catecholamine fluorescence with increased amounts of fluorescent dopamine and noradrenaline nerve terminals.

Discussion

The present findings support the view that the small pool of dopamine and noradrenaline, which was discovered by Häggendal & Lindqvist (1964) in the brains of chronically reserpine-treated rabbits, is present in various dopamine and noradrenaline nerve terminal systems respectively. The fact that no recovery was observed in the 5-HT nerve terminals 24 hr after injection may be due to technical reasons since the 5-HT nerve terminals are very thin, the fluorescence is highly ultraviolet-sensitive, and the fluorescence yield for 5-HT is considerably less than for catecholamine (Dahlström & Fuxe, 1964; Corrodi & Jonsson, 1967); also the labile 5-HT fraction may well lie in 5-HT nerve terminals. Individual variations were observed in the degree of recovery of the catecholamine nerve terminals 24 hr after reserpine treatment. Thus, in some of the rabbits it was not possible to see any recovery of fluorescence in the catecholamine nerve terminals in spite of good functional recovery. A probable explanation for this may be that the labile amine fraction in the rabbits with a poor recovery of fluorescence was too small to be visualized with the present technique. It is also possible that the receptor sites for the amines after chronic reserpine treatment have developed a supersensitivity for the amines according to the "law of denervation" (see Rosenbluth, 1949), which may contribute to the

functional recovery. Häggendal & Lindqvist (1964) have shown that the normetanephrine level in the brains of rabbits killed 24 hr after the final reserpine injection (at full functional recovery) was also lower than in normal rabbits. This may indicate that in reserpine-treated rabbits less noradrenaline is released by nervous activity than in normal rabbits.

The incubation experiments now reported provide evidence that the recovery of the small amine fraction within the specific catecholamine nerve terminals coincides with the ability of the catecholamine storage granules to take up and store low concentrations of noradrenaline; this noradrenaline was taken up to a greater extent by the catecholamine nerve terminals at 24 hr than at 4 hr after the last injection. This is in contrast to the results obtained with α-methylnoradrenaline (resistant to monoamine oxidase), which was taken up by the catecholamine nerve terminals to the same degree at both 4 and 24 hr after the last reserpine injection. Thus 24, but not 4, hr after reserpine administration low concentrations of noradrenaline can be taken up by the dopamine and noradrenaline nerve terminals and stored in a site protected from monoamine oxidase. Since no definite fluorescence was observed in the non-terminal axons of the catecholamine neurons after incubation with noradrenaline, even 24 hr after reserpine injection, this site may represent the catecholamine storage granules since very few of these are found in the non-terminal parts. On the other hand granules are highly concentrated in the varicosites, which represent the presynaptic structures (Fuxe, 1965; Hillarp & others, 1966). This view is also supported by the fact that it seems to be necessary for the noradrenaline to be present in the granules in order to be released by nerve impulses (Malmfors, 1965). At the present time it is not possible to decide whether the small amine fraction observed in the present experiments is located in the small ATP-free pool, like the one first discovered by Hillarp (1960) in the adrenal medullary granules, or not. Nor is it possible to decide whether this store lies in amine granules which previously have been blocked by reserpine and which regain their ability to take up and store catecholamines, or whether the store lies in newly formed granules, which have been produced in the cell bodies and rapidly transported to the terminals (Dahlström & others, 1965; Dahlström, 1967; Dahlström & Häggendal, 1966). This question remains till other experiments, e.g. axonal interruptions, have been made. It may be, however, that small repeated doses do not damage the granules in the same way as does a single high dose.

Taken together, the present results support the view that the small pool of dopamine, noradrenaline and 5-HT immediately important for function and which was discovered in brain by Häggendal & Lindqvist (1963, 1964), can be directly visualized within the various dopamine and noradrenaline nerve terminal systems of the brain. Also, functional recovery is correlated not only with restoration of a small, probably intragranular, pool of amine, but also in all probability with a partial recovery of uptake of noradrenaline into the amine storage granules. A similar, partial recovery of uptake has also been observed to be correlated with the recovery of function in the nictitating membrane of the cat after reserpine (Andén &

Henning, 1966). The fact that at the time of full functional recovery there is only a partial recovery of uptake of amine in the adrenergic nerve terminals (Andén & Henning, 1966) and in the central catecholamine nerve terminals, seems to favour the possibility that newly formed amine storage granules transported along the axon (Dahlström, 1967) are the structures into which the amines are taken.

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