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HISTOCHEMICAL STUDIES ON THE EFFECT OF (+)-AMPHETAMINE, DRUGS OF THE IMIPRAMINE GROUP AND TRYPTAMINE ON CENTRAL CATECHOLAMINE AND 5-HYDROXYTRYPTAMINE NEURONS AFTER INTRAVENTRICULAR INJECTION OF CATECHOLAMINES AND 5-HYDROXYTRYPTAMINE

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Kjell FUXE and Urban UNGERSTEDT, *Histochemical studies on the effect of (+)-amphetamine, drugs of the imipramine group and tryptamine on central catecholamine and 5-hydroxytryptamine neurons after intraventricular injection of catecholamines and 5-hydroxytryptamine*, European J. Pharmacol. 4 (1968) 135-144.

Intraventricular injection of catecholamines and 5-hydroxytryptamine were performed in rats. The effect of various types of systematically administered psychoactive drugs was studied (with the help of the histochemical fluorescence method) on the uptake and accumulation of these amines in the catecholamine and 5-hydroxytryptamine neurons, which occurred in those parts of the neurons lying close to the ventricles. The results indicate that desipramine and protriptyline cause a selective competitive blockade of the amine uptake at the nerve cell membrane of the noradrenaline neurons whereas imipramine appears to block amine uptake at the cell membrane of the 5-hydroxytryptamine neurons in a competitive manner. Evidence was obtained that amphetamine probably releases mainly extragranular amines from central dopamine and noradrenaline neurons. The results with tryptamine indicate that this drug in relatively high doses may have a presynaptic action on the 5-hydroxytryptamine neurons probably by increasing the release of 5-hydroxytryptamine over the cell membrane.

Rat brain
Central catecholamine and 5-hydroxytryptamine neurons
Fluorescence microscopy
Intraventricular injections of monoamines
Imipramine
Protriptyline
Tryptamine
Amphetamine

1. INTRODUCTION

In previous fluorescence microscope studies (Fuxe and Ungerstedt, 1966, 1967a, b) evidence was obtained that dopamine (DA), noradrenaline (NA), α-methyl-DA and α-methyl-NA, when administered intraventricularly in low doses (0.2–2 μg), are taken up selectively by the parts of the catecholamine (CA) neurons lying close to the ventricles and the ventral part of the subarachnoidal space. Intraventricularly administered 5-hydroxytryptamine (5-GT) (1–5 μg) was also taken up and accumulated selectively in the parts of the central 5-HT neurons present in the zone mentioned above. This paper deals with the effect of drugs on the reserpine resistant intraneuronal accumulation of intraventricularly administered CA and 5-HT as studied with the histochemical fluorescence method for the demonstration of CA and 5-HT (see Hillarp, Fuxe and Dahlström, 1966; Corrodi and Jonsson, 1967).

2. MATERIAL AND METHODS

150 female, Sprague-Dawley rats (body weight 150 g) were used. 20 μl of DA, (−)-NA, (−)-α-methyl-NA or 5-HT in a Krebs-Ringer bicarbonate medium was injected into the lateral ventricle in doses ranging

between 1–5 μg as described previously (Fuxe and Ungerstedt, 1966). Body temperature was always checked and maintained at +37°C.

All the rats were pretreated with reserpine (10 mg/kg, i.p., 8–12 hr before killing). The rats receiving DA, NA or 5-HT were also pretreated with nialamide (100 mg/kg (DA, NA) or 500 mg/kg (5-HT), i.p.) 3 hr (DA, NA) or 30 min (5-HT) before the intraventricular injection. The amine used was injected 30 min before killing except in the rats of groups 3 and 4. In the latter groups it was given 1¼ hr before killing. All doses are given as the base.

Group 1. The effects of the following drugs were studied on the reserpine-resistant accumulation of intraventricularly administered DA, NA and α-methyl-NA (in doses of 1–5 μg) in the central DA and NA neurons: *desipramine* (15 rats; a single dose of 25–50 mg/kg, i.p., 30 or 60 min before the intraventricular injection, or two doses of 50 and 25 mg/kg, given i.p., 3 and 1 hr respectively before the intraventricular injection), *protriptyline* (4 rats; 25 mg/kg, i.p., 1 hr before killing), (+)-*amphetamine* (12 rats; 1.0, 2 and 10 mg/kg, i.p., 15 and 30 min before the intraventricular injection), *imipramine* (4 rats; 30–50 mg/kg, i.p., 30 min before the amine injection), *Lu 3–010* (a bicyclic phtalane derivative with thymoleptic properties; see Petersen and Möller-Nielsen (1966); 4 rats; 25 mg/kg given i.p. 3 and 1 hr before injection), *tryptamine* (4 rats; 25 mg/kg, i.p., 30 min before the amine injection). The 3 latter drugs were given only when α-methyl-NA was injected.

Group 2. The effects of the following drugs were studied on the reserpine-resistant accumulation of intraventricularly administered 5-HT (1–5 μg) in the central 5-HT neurons: *desipramine* (4 rats; two doses of 50 and 25 mg/kg given i.p. 3 and 1 hr respectively before the intraventricular injection), (+)-amphetamine (4 rats; 1 and 10 mg/kg, i.p., 40 min before the intraventricular injection), *tryptamine* (9 rats; 5, 25 and 50 mg/kg, i.p., 20 min before the intraventricular injection), *imipramine* (12 rats; 30–50 mg/kg, i.p., 40 min before the amine injection), *lysergic acid diethylamide* (4 rats; LSD, 2 mg/kg, i.p., 40 min before the intraventricular injection).

Group 3. The effects of the following drugs were studied on the release of the reserpine-resistant accumulation of intraventricularly administered α-methyl-NA (2 μg, given 1¼ hr before killing) in the central DA and NA neurons: *desipramine* (8 rats; 25 mg/kg, i.p., 1 hr before killing), *protriptyline* (4 rats; the same dose and times as for desipramine) and (+)-*amphetamine* (8 rats; 1 and 10 mg/kg, i.p., 1 hr before killing).

Group 4. The effects of the following drugs were studied on the release of the reserpine-resistant accumulation of intraventricularly administered 5-HT (5 μg, given 1¼ hr before killing): *tryptamine* (4 rats; 5 and 25 mg/kg, i.p., 15 min after intraventricular injection) and *imipramine* (4 rats; 30–50 mg/kg, i.p., 15 min after the intraventricular injection).

All the rats were killed by decapitation under light chloroform anaesthesia. The telencephalon, diencephalon, mesencephalon, pons and the medulla oblongata were dissected out, rapidly frozen, reacted for one hour with formaldehyde gas generated from paraformaldehyde which had been stored at 50–90% humidity, embedded in paraffin and mounted as previously described in detail (Dahlström and Fuxe, 1964; Hamberger, Malmfors and Sachs, 1965). For the observation of the 5-HT nerve terminals an improved modification of the histochemical fluorescence method was used (Fuxe and Jonsson, 1967). The fluorescence microscopy was performed independently by the two investigators using coded preparations. The investigators were kept uninformed about the code until the data had been collected. Whenever a decreased fluorescence intensity was observed in the nerve terminals of experimental animals compared to controls in groups 3 and 4 this probably indicates an increased rate of disappearance of the amine from these terminals.

Drugs: The following drugs were used: (+)-Amphetamine sulphate (commercial); desipramine hydrochloride (Pertofran, J.R.Geigy Ltd.); dopamine hydrochloride (commercial); 5-hydroxytryptamine hydrochloride (commercial); imipramine hydrochloride (Tofranil, Geigy); Lu 3-010 hydrochloride (H. Lundbeck and Co Ltd.); D-lysergic acid diethylamide tartrate (Sandoz Ltd.); α-methyl-dopamine hydrochloride (Hassle Ltd.); α-methyl-noradrenaline hydrochloride (Corbasil, Hoechst); nialamide (Niamidal, the Swedish Pfizer Ltd.); protriptyline hydrochloride (dr. C.A.Stone, Merck Institute for Therapeutic Research); reserpine (Serpasil, The Swedish Ciba Ltd.); tryptamine hydrochloride (commercial).

3. RESULTS

Group 1. With DA, NA or α-methyl-NA (1–2 μg) alone a partial to marked restoration of fluorescence was observed in the CA nerve terminals and cell bodies present close to the ventricles and the ventral part of the subarachnoidal space in a zone about 200–400 μ wide (a gradient was present from the ependyma into the underlying brain tissue). Green fluorescent pericytes and endothelial cells were observed in the capillary walls. The zone, in which restoration of the CA neurons could be observed, was most prominent close to the site of injection, where most of the CA nerve terminals showed a strong intensity. With a higher dose (5 μg), the restoration was more marked in all parts of the CA neurons present in this zone. The same was true also for the pericytes and the endothelial cells of the capillary walls, and furthermore the background fluorescence was increased. However, there was no apparent increase in thickness of the zone.

Pretreatment with desipramine (especially in the highest doses) or protriptyline partly inhibited the reserpine-resistant accumulation of NA, α-methyl-NA or DA in the *NA* nerve terminals (figs. 1 and 2), but not in *DA* cell bodies and terminals which showed a normal accumulation of intraventricularly injected amine. The degree of blockade was independent of the CA used but dependent on the distance from the ependyma and the distance from the site of injection. Furthermore, the effect was dose-dependent. Protriptyline was found to be more potent than desipramine. In agreement with the above mentioned statements the blockade was most pronounced in the NA nerve terminals present just beneath the fourth ventricle and the subarachnoidal space of the medulla oblongata and pons, where the concentration of injected amine in the cerebrospinal fluid can be expected to be low. However, in many rats a clear blockade of amine accumulation also occurred in the NA nerve terminals surrounding the third ventricle, whereas usually little or no blockade was observed in the NA nerve terminals of the hippocampal formation and the septal area where the concentration of injected amine should be high. Furthermore, the degree of blockade was less pronounced or absent

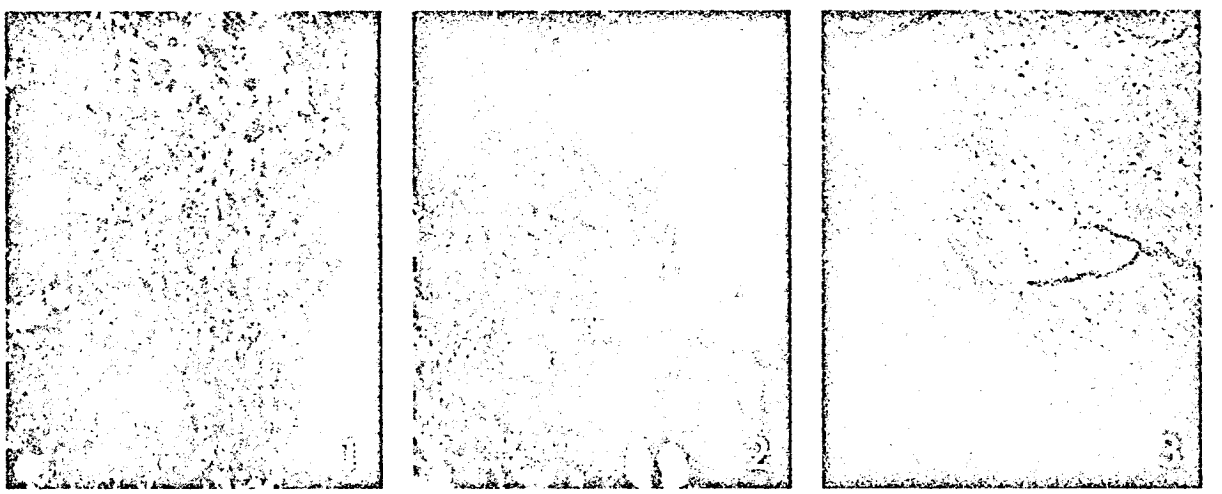

Fig. 1. The ventricular area of the third ventricle (to the right) of a reserpine-pretreated rat 30 min after an intraventricular injection of α-methyl-NA (5 μg). There is a marked accumulation of amine in the NA nerve terminals present in this area. X 200.

Fig. 2. The same area and treatment as described in the text to fig. 1 with the exception that the rat has also been pretreated with desipramine (3 and 1 hr before killing with a dose of 50 mg/kg and 25 mg/kg, i.p.). There is only a partial blockade of the accumulation of the amine in the NA nerve terminals. X 200.

Fig. 3. The periventricular part of the septal area (left) and nuc. caudatus putamen (right) of a rat treated with reserpine-nialamide. No specific fluorescence is observed in the NA and DA nerve terminals of these areas. X 80.

Fig. 4. The same area as in legend to fig. 3. The rat has been pretreated with reserpine-nialamide and then received an intraventricular injection of NA (2 μg). The amine is accumulated in the NA nerve terminals (septal area, left) and the DA nerve terminals (nuc. caudatus putamen, right) respectively. X 80.

Fig. 5. Same area and treatment as in legend to fig. 4 with the exception that the rat has been pretreated with amphetamine (10 mg/kg, i.p.) 30 min before the amine injection. There is a partial reduction in the accumulation of amine within both the NA and DA nerve terminals which is seen as a decrease in the intensity of the fluorescence. X 80.

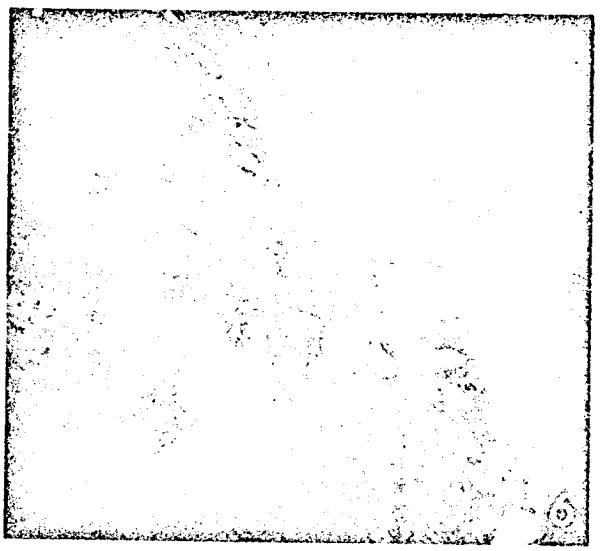

Fig. 6. The periventricular part of the pons of a reserpine-nialamide pretreated rat after an intraventricular injection of 5-hydroxytryptamine (5 μg). There is an accumulation of 5-HT within 5-HT cell bodies and cells of the capillary walls (↑). X 200.

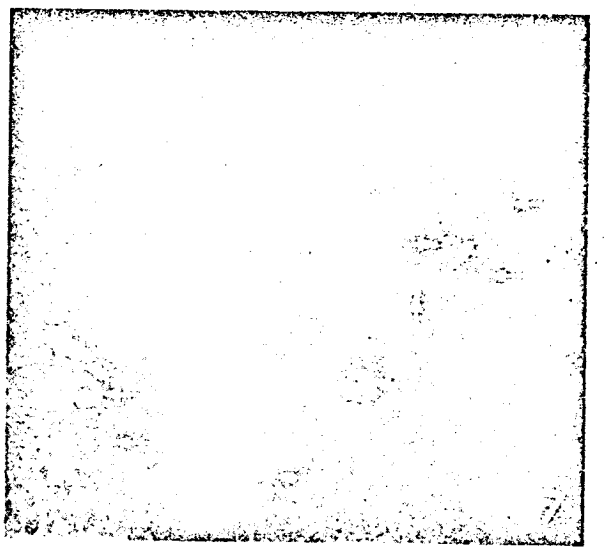

Fig. 7. Same area and treatment as described in legend to fig. 6 with the exception that the rat has been treated with imipramine (30 mg/kg, i.p.) 30 min before the amine injection. There is a partial blockade of the accumulation of amine within the 5-HT cell bodies (↑) and also within the pericytes. X 200.

with the highest dose (5 μg) as compared to the lower doses (1–2 μg) of amine. Lu 3-010 caused a similar degree of blockade as desipramine whereas imipramine and tryptamine were not able to block the accumulation of amine to any observable degree in the NA neurons. As mentioned above the accumulation of CA in the DA neurons (e.g. the DA nerve terminals of the median eminance and the nuc. caudatus putamen, and the DA cell bodies of the arcuate nucleus) was not affected by pretreatment with desipramine, protriptyline, imipramine or Lu 3-010. There was usually only a slight reduction in the fluorescence of the cells of the capillary walls after pretreatment with the imipramine-like drugs.

Amphetamine in the highest dose used was able to block partially the accumulation of CA (1–2 μg) in *both* the DA and NA cell bodies and terminals in all parts of the zone surrounding the ventricles and the ventral part of the subarachnoidal space (figs. 3, 4 and 5). There was a clear, although less marked, blockade of accumulation in most of the DA and NA neurons, e.g. those of the hypothalamus, also with the higher doses of CA and also close to the site of injection. Thus, in the presence of high exogenous concentrations of amines amphetamine was clearly more effective than desipramine in blocking the accumulation of amines in the NA neurons. The low dose of (+)-amphetamine was usually ineffective in blocking the accumulation of CA in the CA neurons. There was a clear to marked blockade of accumulation of fluorescence in the cells of the capillary walls after the highest dose of amphetamine.

Group 2. Pretreatment with desipramine or amphetamine did not to any observable degree block reserpine-resistant accumulation of 5-HT (1–5 μg) in the central 5-HT neurons regardless of whether the terminals in the septal area or in the medulla oblongata were studied. However, the accumulation of yellow fluorescence in the cells of the capillary walls was partly blocked by amphetamine. Tryptamine, on the other hand, was found to block partially not only the accumulation of fluorescence in the cells of the capillary walls but also the accumulation of 5-HT (5 μg) in 5-HT nerve terminals, e.g. those of the periventricular nuclei of the hypothalamus and those of the dorsal motor nucleus of the vagus. However, this effect could only be observed in some rats (4 out of 7) and with the highest doses. Close to the site of injection

Table 1

The *in vivo* effect of drugs on the accumulation of monoamines in central DA, NA and 5-HT neurons after intraventricular amine injections to reserpine-nialamide pretreated rats *.

Neurons containing:		DA	NA	5-HT
Administered amine:		DA, NA, α-methyl-NA	DA, NA, α-methyl-NA	5-HT
Drug	Dose (mg/kg)			
imipramine	50	0	0	–
desipramine	25	0	–	0
Lu 3-010	25	0	–	0
protriptyline	25	0	– –	0
amphetamine	10	–	– –	0
tryptamine	25	0	0	(–)
LSD	2			0

0 = no effect
– = decreased degree of accumulation
+ = increased degree of accumulation
one minus or plus = small but clearly observable effect
two minuses or pluses = moderate effect
three minuses or pluses = marked effect
The number of + or – signs is not quantitatively related.

* When the α-methylated compounds were used, no nialamide pretreatment was necessary.

Table 2

The *in vivo* effect of drugs on the disappearance rate of reserpine-resistant accumulations of monoamine in central DA, NA and 5-HT neurons after intraventricular amine injections to reserpine-nialamide pretreated rats.

Neurons containing:		DA	NA	5-HT
Administered amine:		α-methyl-NA	α-methyl-NA	5-HT
Drug	Dose (mg/kg)			
imipramine	50			0
desipramine	25	0	(+)	
protriptyline	25	0	(+) or +	
amphetamine	10	+++	+++	
tryptamine	25			+

0 = no effect
+ = increased release
See also text to table 1.

Fig. 8. Tuberculum olfactorium of a reserpine-nialamide pretreated rat 1 hr after an intraventricular injection of NA (2 μg). There is an accumulation of amine within the DA nerve terminals in this area and also within the NA vasomotor nerve terminals. X 200.

Fig. 9. Same area and treatment as in fig. 8 with the exception that the rat has been treated with amphetamine (10 mg/kg, i.p.) 15 min after the amine injection. There is a markedly increased rate of amine disappearance from the DA nerve terminals after treatment with this drug. The rate of fluorescence disappearance from the vasomotor nerve terminals, however, is not clearly affected. X 200.

Fig. 10. Periventricular part of the fourth ventricle at the level of the vestibular nuclei of a reserpine-nialamide pretreated rat 1 hr after an intraventricular injection of 5-HT. There is an accumulation of 5-HT within very fine 5-HT nerve terminals (appearing as very fine dots) lying just under the ependyma and within pericytes. X 120.

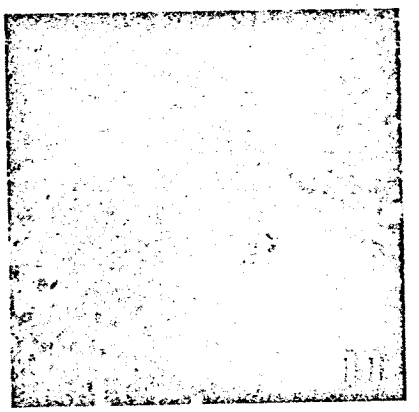

Fig. 11. Same area and treatment as described in legend to fig. 10 with the exception that the rat has been treated with tryptamine (50 mg/kg, i.p.) 15 min after the amine injection. There is a clearly increased rate of amine disappearance from the 5-HT nerve terminals and also from the cells of the capillary walls. X 200.

(e.g. in the septal area) no blockade could be observed in any rat probably due to the high 5-HT concentrations in these areas. No certain blockade could be observed with the lowest dose (5 mg/kg). Imipramine especially in the highest dose used was found to block partially the accumulation of 5-HT in the 5-HT neurons (figs. 6 and 7). With the low dose, effects were observed only in 2 rats out of 4. The effects were mainly observed in the 5-HT nerve terminals and cell bodies present just beneath the floor of the fourth ventricle and the aqueductus Sylvii. LSD in the dose used did not influence the degree of accumulation of fluorescence in the 5-HT neurons.

Group 3. Amphetamine caused a rapid and marked

release of the reserpine-resistant accumulation of α-methyl-NA in both the NA and DA cell bodies, non-terminal axons and terminals (figs. 8 and 9). The effects were also present with a dose of 1 mg/kg. After desipramine and protriptyline no increased rate of disappearance of fluorescence was observed in the *DA* neurons. In the *NA* neurons, the rate of disappearance of fluorescence after these drugs especially after protriptyline was increased only in 10 rats out of 20 and only in areas far away from the site of injection e.g. in the medulla oblongata. Thus, the effects of desipramine and protriptyline were much less marked when given after the intraventricular injection then when they were administered before it, whereas the opposite seems to be true for amphetamine.

Group 4. Tryptamine was found to enhance the rate of disappearance of fluorescence from the 5-HT neurons (figs. 10 and 11). This effect occurred although to a lesser extent with the lowest dose. The fluorescence in the pericytes was affected in a similar way. Imipramine did not enhance the rate of disappearance of 5-HT from the 5-HT neurons. The results are summarized in tables 1 and 2.

4. DISCUSSION

The results indicate that tricyclic and bicyclic thymoleptic drugs such as desipramine, protriptyline and Lu 3-010 are able to block partially the reserpine-resistant uptake-concentration mechanism of the central NA neurons (cf. Carlsson et al., 1966; Hamberger, 1967), but not to any certain degree that of the central DA and 5-HT neurons. Thus, it was found that the reserpine-resistant accumulation of intraventricularly administered NA, α-methyl-NA or DA was partly blocked in the NA neurons after pretreatment with these drugs especially with protriptyline. However, imipramine did not cause any observable degree of blockade of this mechanism in the NA neurons, which is in agreement with the relatively poor blocking action of this drug observed in other studies (Carlsson et al., 1968).

The degree of blockade obtained with these drugs was probably dependent on the amine concentrations in the cerebrospinal fluid surrounding the various areas of the periventricular and subarachnoidal zone studied. If a low dose of amine was used there was a relatively marked blockade of accumulation of fluorescence in the parts of the zone localized at a distance from the ependyma or the ventral surface of the brain and present in areas far away from the site of injection (e.g. the medulla oblongata). In the hypothalamus, the effect was usually less marked or absent and in the hippocampal formation and septal area there was no blockade. With the higher doses of amine used, desipramine and the other imipramine-like drugs had little blocking action, and effects were also slight in the medulla oblongata and pons. From *in vitro* studies on brain slices (Hamberger, 1967) it has been possible to obtain evidence for a competitive blockade of the reserpine-resistant uptake concentration mechanism in the central NA neurons after desipramine. The present results from *in vivo* studies with these drugs are in agreement with Hamberger's results (1967). Thus, the blockade will be overcome where the amine concentrations are high, when the amines can compete with these drugs for the uptake sites at the nerve cell membrane. The results obtained in this study seem to explain the findings (Glowinski, Axelrod and Iversen, 1966) that the accumulation of intraventricularly administered H^3-NA was markedly blocked by desipramine in the medulla oblongata, whereas the blockade of accumulation of H^3-NA was almost insignificant in e.g. the hippocampal formation. In agreement with the present findings, no blockade of accumulation of H^3-NA was observed in the striatum.

The fact that desipramine and protriptyline caused only a slightly increased rate of disappearance of reserpine-resistant accumulations of α-methyl-NA in NA nerve terminals indicates that desipramine and protriptyline have little releasing action on such (probably extragranular) accumulations in the central NA neurons. The effects observed are probably due to the blockade of the uptake-concentration mechanism at the cell membrane (see above), since some of the CA leaking out over the nerve cell membrane cannot be pumped in again. In the peripheral adrenergic neurons, however, this effect of desipramine is much more marked (Malmfors, 1965). The present results favouring a selective action of desipramine, protriptyline and Lu 3-010 on the NA neurons of the central nervous system underline and extend the results from previous studies (see Carlsson et al., 1966; Fuxe,

Hamberger and Malmfors, 1966, 1967; Hamberger, 1967).

Amphetamine in the doses used is able to cause a partial blockade of the reserpine-resistant accumulation of intraventricularly administered NA, α-methyl-NA and DA in both the DA and NA neurons. This blocking action of an amphetamine was partly dependent on the exogenous amine concentrations in the region studied. However, it was evident that the intraventricularly administered amines could not compete as efficiently with amphetamine as with desipramine or protriptyline.

Studies on the blockade of accumulation of intraventricularly administered H^3-NA in normal animals by amphetamine also indicate that the blockade is most marked in regions far away from the site of injection, e.g. in the medulla oblongata (Glowinski et al., 1966) where the exogenous amine concentrations are probably low. The complete lack of blockade by amphetamine of the H^3-NA accumulation in the hippocampus observed by these authors may at least partly be due to a high degree of competition. The fact that these authors could not observe any blockade of accumulation of tritiated catechols after H^3-NA in the striatum of normal rats amphetamine, is difficult to understand in view of the clearcut blockade obtained in the present study of the reserpine-resistant accumulation of CA in the nuc. caudatus putamen by amphetamine. The reserpine-resistant accumulation of 5-HT in the 5-HT neurons was not affected to any observable degree by amphetamine or by desipramine in the doses used.

Amphetamine was found to be more potent as a releaser than as a blocker of the reserpine-resistant accumulation of α-methyl-NA within both the DA and NA neurons. Thus, this releasing effect was observed with a dose of 1 mg/kg, which could not clearly block the accumulation of intraventricularly administered CA in DA and NA neurons. Furthermore, even a dose of 2 mg/kg of amphetamine given 15 minutes *before* the amine injection was relatively ineffective, indicating that even in the presence of relatively high brain amphetamine concentrations at the moment of intraventricular amine injection (Valzelli, Consolo and Morpurgo, 1967) blockade of amine uptake into the CA nerve terminals could not be clearly observed. In view of these results and the well-known sympathomimetic activities of amphetamine it is likely that the release of "extragranular" amines from DA and NA neurons may be the primary action of amphetamine. Evidence along these lines has also been obtained in studies with α-methyl-*m*-tyramine and related compounds, which deplete central CA probably by a displacing action (Carlsson, Fuxe and Ungerstedt, 1968). However, with the highest doses of amphetamine used in the present study, there may also exist a competitive blockade of the reserpine-resistant uptake-concentration mechanism in the central DA and NA neurons. Such an action could contribute to the decreased reserpine-resistant accumulation of intraventricularly administered CA in the CA neurons observed after high doses of amphetamine, although here also the releasing action of amphetamine is probably of primary importance.

Tryptamine only causes a slight, if any, blockade of the reserpine-resistant accumulation of 5-HT in the 5-HT neurons, but when given after the 5-HT injection it clearly increased the rate of 5-HT disappearance from the 5-HT neurons. The tryptamine concentration at the moment of the intraventricular injection was probably of the same order in the two types of experiments since the monoamine oxidase was inhibited in these experiments and thus the major metabolic pathway of tryptamine had been blocked. This action although reduced was also observed with a dose of 5 mg/kg. Tryptamine may thus have a presynaptic action on the 5-HT neurons by e.g. releasing "extragranular" 5-HT.

Imipramine, on the other hand, influenced only the accumulation of 5-HT, which was partly blocked. Since the degree of blockade was highly dependent on the exogenous 5-HT concentrations in a way similar to that observed after desipramine and protriptyline in the case of the NA neurons, the results indicate, that imipramine may be a competitive blocker of the reserpine-resistant uptake-concentration mechanism in the 5-HT neurons *in vivo* (see also Carlsson et al., 1968). It is obvious that this effect of the antidepressant drug, imipramine, can be of importance for its antidepressant properties, especially since its blocking actions on the NA neurons seem to be poor Furthermore chronic treatment with imipramine has given similar results (unpublished data). *In vitro* studies on brain slices have revealed that incubation with imipramine, desipramine or tryptamine will block the uptake of added C^{14}-5-HT to a similar de-

gree (Blackburn, French and Merrills, 1967) which is in marked contrast to the present *in vivo* findings. The present results support recent evidence that demethylation of imipramine is not of importance for the mental or antitetrabenazine effects of imipramine (Michaelis and Stille, 1968). Thus, these authors found in experiments with labelled imipramine in rats that unchanged imipramine is mainly responsible for the radioactivity measured in the brain and could not detect any desipramine in these brains. In this connection it should once more be pointed out that desipramine did not block to any observable degree the uptake and accumulation of 5-HT in the 5-HT neurons, suggesting that the site of action of imipramine is different from that of desipramine.

It should be pointed out that amphetamine and tryptamine and to a somewhat lesser degree also desipramine and protriptyline decreased the degree of reserpine resistant accumulation of amines in the pericytes and possibly also endothelial cells of the capillary walls after intraventricular injection of DA, NA, α-methyl-NA or 5-HT. These *in vivo* results are in agreement with those of Hamberger (1967) who observed reserpine-resistant accumulation of CA in these cells in *in vitro* studies on brain slices provided that the monoamine oxidase had been inhibited. In these studies it was similarly found that the CA accumulation was blocked by desipramine, cocaine and amphetamine and it was also found to be energy-dependent. Thus, from the present and previous experiments (see Hamberger, 1967) it seems likely that this uptake mechanism in the cells of the capillary walls is specific and may play a role not only in the blood-brain barrier for CA (see also Bertler et al., 1966) and 5-HT but also in the inactivation of CA and 5-HT released from the specific monoamine nerve terminals of the brain. Thus, the amines diffusing away from the synaptic cleft can be taken up in the pericytes, which lie on the outer surface of the capillary vessels, where they are broken down by the monoamine oxidase to the acid metabolites.

The results presented support the view that there exists a reserpine-resistant uptake concentration mechanism in all parts of the DA, NA and 5-HT neuron. The studies with imipramine-like drugs, amphetamine and tryptamine, indicate, however, that they have different properties in some respects. Furthermore, direct evidence has been given that the antidepressant drugs desipramine and protriptyline *in vivo* are able to increase the NA concentrations at the central NA receptor sites by causing a selective competitive blockade of the uptake of NA into the NA neurons. But, evidence has been obtained on the other hand, that the antidepressant drug imipramine *in vivo* mainly causes an increase of the 5-HT concentrations at central 5-HT receptor sites by causing a competitive blockade of the uptake concentration mechanism of the 5-HT neurons. These results point to a role of both NA and 5-HT neurons in the regulation of the effective state.

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