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SELECTIVE (+)-AMPHETAMINE NEUROTOXICITY ON STRIATAL DOPAMINE NERVE TERMINALS IN THE MOUSE

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1 Infusion of large doses of (+)-amphetamine continuously for 7 days by means of osmotic minipumps caused a long-lasting reduction of endogenous dopamine levels, dopamine nerve terminals demonstrated histochemically and [^{3}H]-noradrenaline uptake *in vitro* in the striatum of mice.

2 The effect was dose-dependent, fully developed after 4 days and selective for striatal dopamine up to a dose of (+)-amphetamine of 25 μg/h. Higher doses, which produced increased mortality, also affected dopamine levels in the olfactory tubercle as well as noradrenaline in several regions.

3 Fluorescence histochemical studies using the Falck-Hillarp technique disclosed catecholamine accumulations in the striatum after (+)-amphetamine; a sign of neurotoxic damage. No effects on the dopamine cell bodies were noted. There were also no indications of neurotoxic damage to noradrenaline or 5-hydroxytryptamine neurones induced by (+)-amphetamine.

4 Large doses of (−)-amphetamine were without effect, demonstrating that the long-lasting impairment of transmitter uptake-storage mechanism in striatal dopamine nerve terminals is selective for (+)-amphetamine.

5 There was a slow gradual recovery of endogenous dopamine and [^{3}H]-noradrenaline uptake in the striatum with time, which was almost complete 6 months after the (+)-amphetamine administration.

6 The results give further evidence for the view that (+)-amphetamine in large doses can have a selective neurotoxic action on a vulnerable population of a dopamine nerve terminals in the striatum. The results suggest in addition that there is a slow regrowth and regeneration with time of damaged dopamine nerve terminals.

Introduction

Several recent studies have indicated that (+)-amphetamine in large doses can have a neurotoxic action on central dopamine neurones of the rat (Ellison, Eison, Huberman & Daniel, 1978; Fuller & Heinrick-Luecke, 1980), mouse (Steranka & Sanders-Bush, 1980; Nwanze & Jonsson, 1981) and cat (Trulson & Jacobs, 1979). The evidence include long-lasting reductions of endogenous dopamine, [^{3}H]-dopamine uptake and tyrosine hydroxylase activity as well as histochemical findings of swollen dopamine axons. Based on similar evidence it has been suggested that methamphetamine after repeated injection might also be toxic to neostriatal dopaminergic fibres (Hotchkiss, Morgan & Gibb, 1979; Wagner, Ricaurte, Seiden, Schuster, Miller & Westley, 1980; Hotchkiss & Gibb, 1980; Lorez 1981). Although the data available are strongly indicative of a neurotoxic action of (+)-amphetamine on striatal dopaminergic neurones, little is known as to specificity in action, level of damage and degree of irreversibility. In view of the interest in the neurotoxicity of amphetamine, we have carried out a systematic study of the acute and long-term effects of (+)- and (−)-amphetamine on the dopaminergic neurones in the CNS of mice, employing neurochemical and histochemical methods. Part of the present study has been communicated in a preliminary form (Nwanze & Jonsson, 1981).

Methods

Male albino mice (N.M.R.I., 25–30 g, Anticimex, Sollentuna) were used in the present experiments. (+)- or (−)-amphetamine was administered continuously for various periods of time (2–7 days) by means of osmotic minipumps (Alzet; capacity 240 μl and delivery rate 1 μl/h). The amphetamine filled (solvent 0.9% w/v NaCl solution) pumps were placed

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subcutaneously at the back under light ether anaesthesia and removed for termination of drug infusion after various periods of time. Controls were treated in the same way except that the osmotic pumps were filled with saline or the mice sham-operated only. The animals were kept in separate cages during the drug infusion in rooms with controlled temperature, humidity and dark-light schedule (10/14 h). The mice had free access to food and water. The body weight was recorded before killing which was carried out by cervical dislocation. The CNS was rapidly dissected out and placed in cold (+4°C) physiological saline for a few minutes awaiting the regional dissection which is schematically illustrated in Figure 1. From the spinal cord a piece of about 1 cm was taken, constituting the lower and the upper segments of the thoracic and lumbar spinal cord respectively.

Monoamine assay

Endogenous noradrenaline (NA), dopamine and 5-hydroxytryptamine (5-HT) was determined by liquid chromatography with electro-chemical detection (LCEC) according to Keller, Oke, Mefford & Adams (1976). For details of the assay procedure for catecholamines, see Jonsson, Hallman, Mefford & Adams (1980) and for 5-HT, see Ponzio & Jonsson (1979). The values were expressed as ng/g wet weight of the tissue and corrected for recovery, based on internal standard measurements.

[^{3}H]-noradrenaline uptake in vitro

Standardized slices were prepared by punching out discs (diameter 1.5 mm) from the *nucleus caudatus putamen* (striatum, rostral part) of 0.5 mm thick frontal sections made by freehand. Four discs were taken from each animal and they were incubated in 0.05 μM [^{3}H]-NA in Krebs-Ringer bicarbonate buffer (pH 7.4) containing 10 μM pargyline for 10 min at +37°C (Lidbrink & Jonsson, 1974). After termination of the incubation the discs were briefly rinsed in cold buffer and solubilized (0.5 ml Protosol, NEN). After addition of toluene phosphor, radioactivity taken up and retained in the discs was determined by liquid scintillation spectrometry with a counting efficiency of about 28%. The [^{3}H]-NA uptake values were expressed as nCi/disc and corrected for 'extraneuronal' uptake by subtracting uptake values obtained by performing identical incubations at +4°C.

Gradient centrifugations

Striata from two mice were homogenized in 1.4 ml cold 0.25 M sucrose using a glass-homogenizer with a teflon pestle running at a speed of 1,000 rev/min (8–10 strokes). The original homogenate was centrifuged at 3,000 *g* for 10 min. The supernatant (containing synaptosomes) was diluted with Krebs-Ringer buffer (pH 7.4) and after addition of [^{3}H]-NA (final concentration 0.1 μM) the mixture was incubated at +37°C for 20 min. The incubation was terminated by adding cold Krebs-Ringer buffer and the solution centrifuged at 15,000 *g* for 20 min. All the subsequent procedures were carried out at +4°C. The pellet was gently resuspended in 0.2 ml 0.25 M sucrose and layered on top of a linear, continuous sucrose gradient (5 ml) ranging from 0.3 M to 1.6 M sucrose. The gradients were centrifuged in a swing-

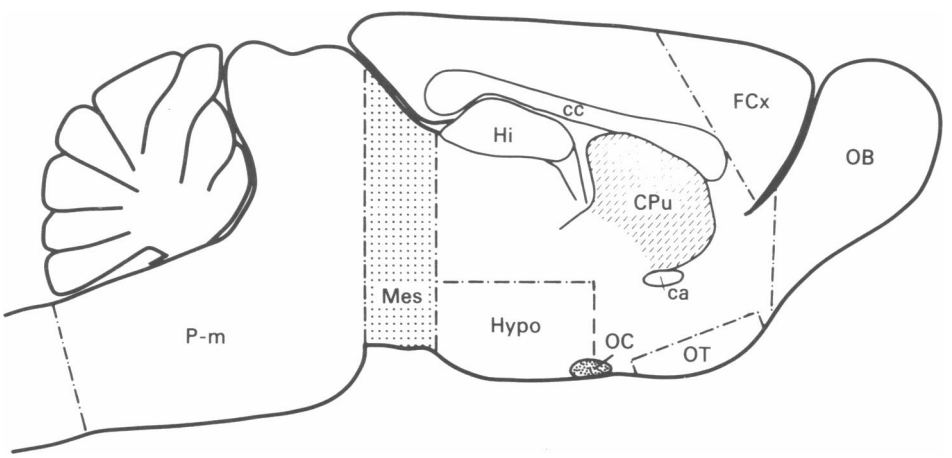

Figure 1 Schematic representation of adult mouse brain (near midsagittal plane) with cutting lines (- · - ·-) indicated for the regional dissection. OB = olfactory bulb; FCx = frontal cortex; OT = olfactory tubercle; CPu = nuc. caudatus putamen = striatum: Hypo = hypothalamus; Hi = hippocampus; Mes = mesencephalon; P-m = pons-medulla; OC = optic chiasm; ca = anterior commisure; cc = corpus callosum.

out rotor at 32,000 *g* for 60 min and thereafter fractionated (18 fractions/gradient). For further details on the gradient centrifugation procedure, see Pycock & Jonsson (1974). The collected fractions were then extracted with 0.1 M perchloric acid (PCA) and extracts subjected to an Al_2O_3 adsorption-desorption purification procedure (see Keller *et al.*, 1976). The absorbed catecholamines were eluted with 0.5 ml 0.1 N PCA, of which one aliquot was taken for radioactivity determination by liquid scintillation spectrometry and one aliquot taken for catecholamine assay employing LCEC (see above).

Fluorescence histochemistry

The spinal cord (thoracic-lumbar region) and brain (divided in four parts) were rapidly frozen in liquid propane cooled by liquid nitrogen and freeze-dried (Olson & Ungerstedt, 1970). The freeze-dried specimen were exposed to formaldehyde gas of optimum humidity (+80°C for 60 min) and further processed for the fluorescence histochemical demonstration of biogenic monoamines according to the method of Falck and Hillarp (see Corrodi & Jonsson, 1967; Fuxe, Hökfelt, Jonsson & Ungerstedt, 1970). Serial sectioning was carried out and every tenth section (10 μm) preserved, embedded in Entellan (Merck) and analysed by fluorescence microscopy.

Drugs and substances used

The following were used: [^{3}H]-noradrenaline HCl (12 Ci/mmol, NEN); (+)-amphetamine sulphate (Sigma); (−)-amphetamine sulphate (SKF); pargyline HCl (Sigma); α-methyl-dopamine (MSD); 5-HT creatinine sulphate (Regis).

Results

Effects of various doses of (+)-amphetamine on regional catecholamine levels

Analysis of the effect of varying the dose of (+)-amphetamine infused over one week on the regional catecholamine levels demonstrated that practically no effects on the NA levels in any region studied were observed after infusion of (+)-amphetamine up to 37 μg/h (Figure 2). Infusion of 62 μg/h led to very marked NA depletions in all regions studied, except the olfactory tubercle. The NA depletion was most pronounced in frontal cortex and spinal cord. As to the effects on the dopamine levels, only two regions were observed to be significantly affected, the olfactory tubercle and the striatum. In these two regions a clear dose-response relationship was found and the dopamine stores in the striatum were the most sensitive to the depleting effect of (+)-amphetamine. Infusion of the drug up to 17 μg/h resulted in no change in dopamine concentration, while 25 μg/h produced about 20% dopamine depletion in the olfactory tubercle and about 50% reduction in the striatum. The highest dose tested caused an almost complete dopamine depletion in the latter region, while dopamine concentrations were depleted by about half in the olfactory tubercle. It was found, however, that doses above 25 μg/h led to an increased mortality and after infusion of 62 μg/h more than 50% of the animals died within a couple of days after starting the infusion. The effects of these higher doses are therefore difficult to judge. For the rest of the studies an infusion dose of 25 μg/h was chosen, no mortality being observed.

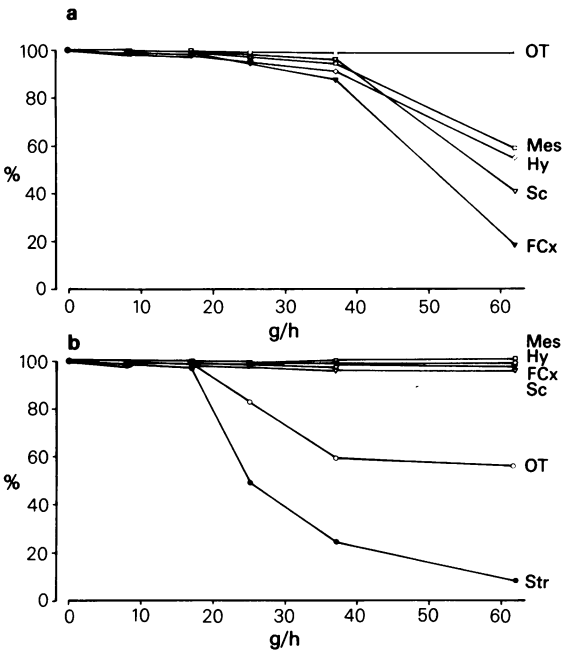

Figure 2 Effects of various doses of (+)-amphetamine administered continuously using osmotic minipumps for 7 days on the regional noradrenaline (a) dopamine (b) levels in mice. The pumps delivered 6.3, 12.5, 25, 37 or 62 μg/h. The mice were killed 8 days after the start of the (+)-amphetamine infusion. Each point represents the mean of 4 determinations except for 62 μg/h where $n = 2$, expressed as % of control for respective catecholamine and region. FCx = frontal cortex; Str = striatum; OT = olfactory tubercle; Hy = hypothalamus; Mes = mesencephalon; Sc = spinal cord.

Acute and long-term effects of (+)-amphetamine

The effect of varying the period of (+)-amphetamine infusion (25 μg/h, 2–7 days) on the regional

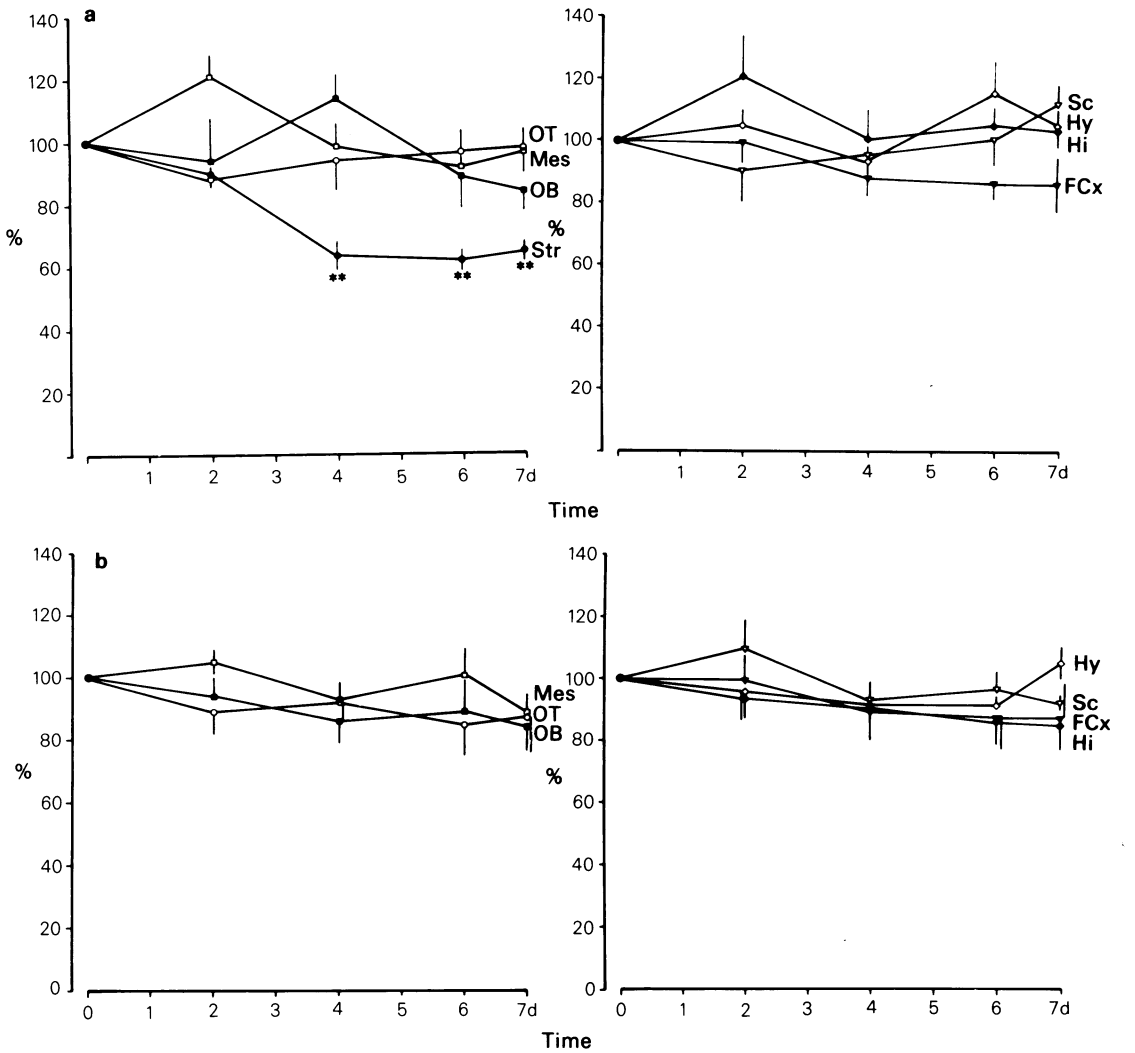

Figure 3 Effects of (+)-amphetamine continuously administered (25 μg/h) for 2, 4, 6 and 7 days on the regional dopamine (a) and noradrenaline (b) levels. The mice were killed 24 h after the termination of the (+)-amphetamine infusion. Each point represents the mean of 4 determinations, expressed as % of control for respective catecholamine and region; vertical lines indicate s.e.mean. Abbreviations as in Figure 2. OB = olfactory bulb. ** = $0.001 > P > 0.01$ (Student's *t* test).

catecholamine levels was investigated 24 h after termination of the infusion (Figure 3). No significant effects on the NA levels in any region were observed at any of the time periods studied. The dopamine levels were also unaffected in most regions and a significant dopamine depletion was only observed in the striatum. There was a small but not statistically significant dopamine reduction (−10%) after (+)-amphetamine infusion for 2 days, while the maximal depletion was achieved after infusion for 4 days. There was a tendency for a dopamine increase (+20%; $P < 0.1$) in the mesencephalon after (+)-amphetamine infusion for 2 days, whereas prolonging the infusion period further led to a normalizing of the dopamine levels.

Analysis of the long-term effects of a one week (+)-amphetamine infusion (25 μg/h) on the endogenous dopamine levels and [^{3}H]-NA uptake *in vitro* in the striatum demonstrated a gradual recovery of both parameters with time (Figure 4). The recovery was fairly slow, but appeared to be somewhat more rapid for the [^{3}H]-NA uptake and after 8 weeks

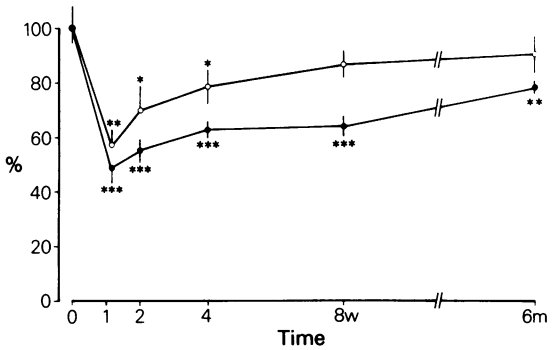

Figure 4 Effects of continuous (+)-amphetamine infusion (25 μg/h) for 7 days on the long-term effects (8 days–6 months; w = week; m = month) on the endogenous dopamine (●) and [^{3}H]-noradrenaline (○) uptake *in vitro* in the striatum of mice. Each point represents the mean of 4 determinations, expressed as % of vehicle treated control; vertical lines indicate s.e.mean. ** = $0.001 > P > 0.01$; *** = $P < 0.001$ (Student's *t* test).

the uptake values were not significantly different from control. The same pattern of recovery was observed with dopamine, although the reduction (−22%) was still significant 6 months after the (+)-amphetamine infusion. No significant changes of the catecholamine levels were found in other regions studied at any time-interval.

The effect of (+)-amphetamine infusion (25 μg/h, 7 days) on the sedimentation characteristics of striatal dopamine synaptosomes was investigated employing continuous sucrose density gradients ranging from 0.3 M to 1.6 M sucrose (Figure 5). Endogenous dopamine or prelabelling with [^{3}H]-NA were analysed as markers for dopamine synaptosomes. (+)-Amphetamine treatment caused a marked reduction (−60%) of both parameters in the synaptosomal region equilibrating at about 1.1–1.2 M sucrose. The reduction was similar when measuring dopamine and [^{3}H]-NA. The distribution of both endogenous dopamine and prelabelled [^{3}H]-NA in the sucrose gradient was heterogeneous to a certain degree in the synaptosomal region and the reduction after (+)-amphetamine was not completely symmetrical.

Fluorescence histochemistry

Twenty-four hours following infusion of (+)-amphetamine (25 μg/h) for one week there was a marked reduction of the dopamine fluorescence in the striatum (Figures 6a and b). In agreement with the biochemical data, it was observed that the reduction of dopamine fluorescence intensity was clearly more pronounced after infusion of 37 μg/h than after 25 μg/h. It was further noted that the dopamine reduction was not evenly distributed throughout the striatum. The decrease in dopamine fluorescence was most pronounced in the dorsal parts (see Figure 6c, while the dopamine fluorescence in the ventral parts of the striatum as well as in the olfactory tubercle and nuc. accumbens was not notably affected (Figure 6e). In the striatum it was observed that concomitant with the reduction in dopamine there appeared increased accumulations of catecholamine fluorescence (Figure 6d), which displayed a fluorescence morphology similar to, though less pronounced than, the transmitter accumulations seen in axons of monamine neurones after administration of monoamine

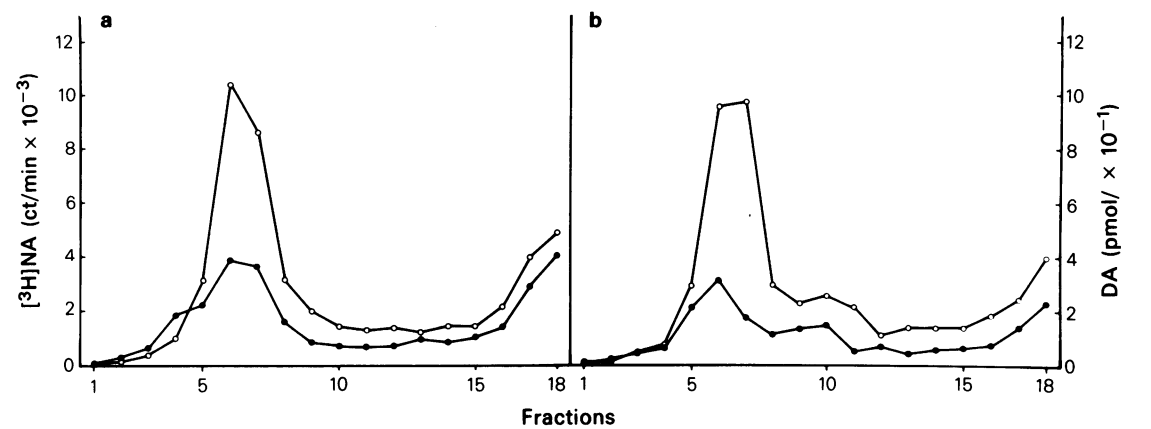

Figure 5 Effects of continuous (+)-amphetamine infusion (25 μg/h) for 7 days on the distribution of endogenous dopamine (DA) (b) and [^{3}H]-noradrenaline ([^{3}H]-NA) (a) taken up in the synaptosomal fraction in a linear, continuous sucrose density gradient ranging from 0.3 M to 1.6 M (fractions 1–18) sucrose and centrifuged at 32000 *g* for 60 min. The mice were killed 1 day after termination of the (+)-amphetamine infusion. Each point is the mean of 2 determinations; (○) control; (●) (+)-amphetamine.

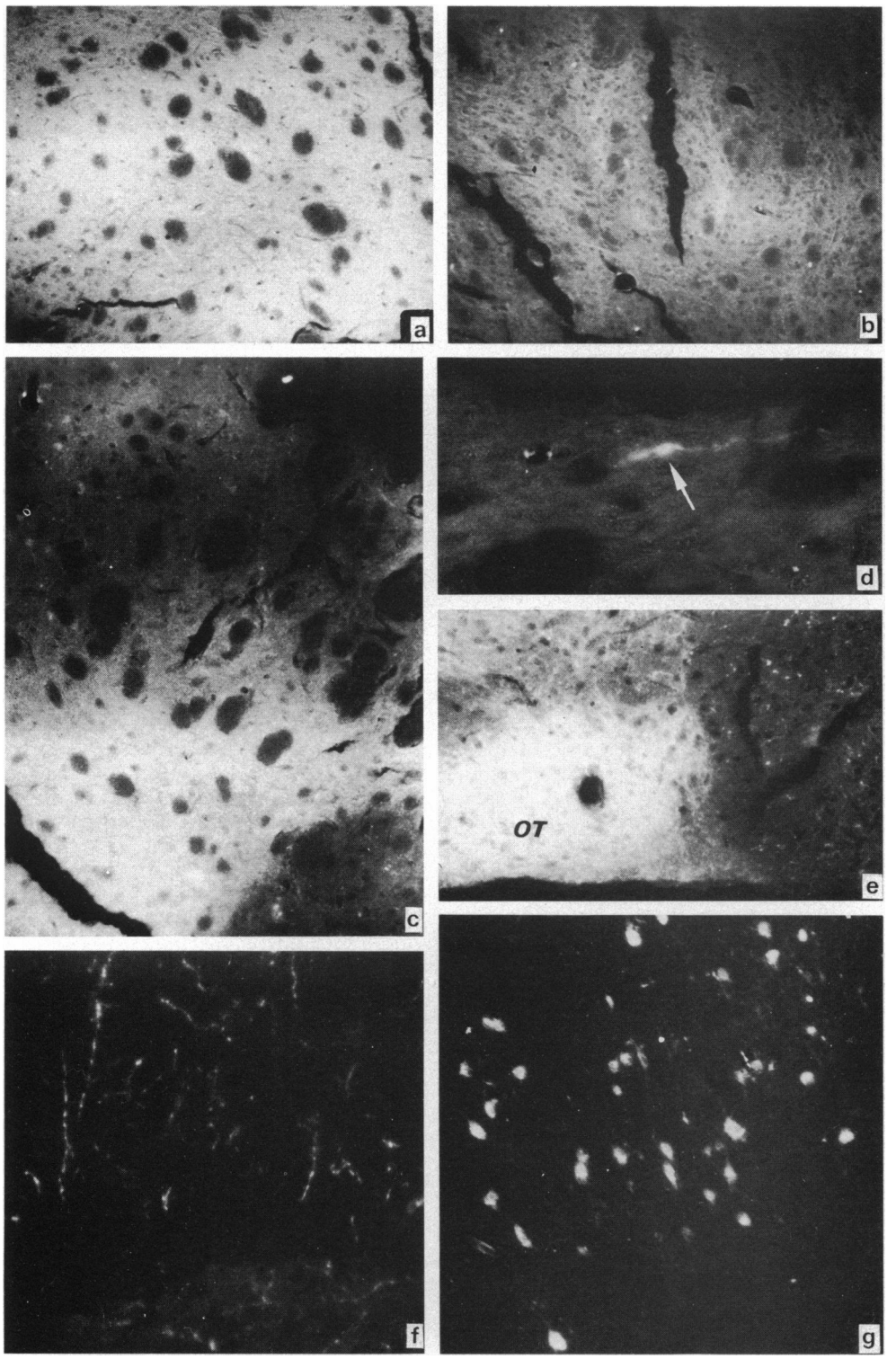
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Figure 6 Fluorescence histochemical demonstration of catecholamines in mouse brain according to the method of Falck and Hillarp. (a) Control, showing intense dopamine fluorescence in the striatum (× 146); (b)-(g) (+)-amphetamine (25 μg/h, 7 days; killed on 8th day); (b) marked reduction of dopamine fluorescence in the striatum (× 146); (c) striatum, reduction of dopamine fluorescence is heterogeneous with a more pronounced depletion in the dorsal aspect of the striatum (× 146); (d) striatum, accumulation of catecholamine fluorescence in a swollen axon (→; × 238); (e) intense dopamine fluorescence in the olfactory tubercle showing an unaffected fluorescence morphology (× 146); (f) intensely fluorescent NA nerve terminals with an unchanged appearance innervating the hippocampus (× 238); (g) intensely fluorescent dopaminergic perikarya belonging to the A9-cell group in the substantia nigra. The fluorescence of the dopaminergic perikarya is not notably altered after (+)-amphetamine (× 238).

neurotoxins (see Ungerstedt, 1968; Jonsson, 1981). The (+)-amphetamine administration did not alter the catecholamine innervation pattern, either in respect of the number of nerve terminals or the fluorescence intensity, in *i.a.* the cerebral cortex, cerebellum, hippocampus (Figure 6f), hypothalamus and the spinal cord. The external layer of the median eminence, known to be innervated mainly by dopaminergic nerve terminals (Jonsson, Fuxe & Hökfelt, 1972), thus displayed an unaltered fluorescence morphology after (+)-amphetamine. The same was the case for the dopamine cell-bodies (A8, A9 and A10; according to Dahlström & Fuxe, 1964) in the mesencephalon (Figure 6g). There were no clear signs of catecholamine accumulation in the vicinity of the dopaminergic perikarya and along the dopaminergic axon bundle forming the nigro-striatal and mesocortical pathways. The possibility that single accumulations escaped detection, however, cannot be excluded. Clear-cut signs of catecholamine accumulations were thus observed only in the striatum. As to the NA and 5-HT perikarya in the brain stem, no notable changes in fluorescence morphology could be observed and no transmitter accumulations in the 5-HT or NA axons could be found with the technique used.

There was a recovery of the intensity of the dopamine fluorescence in the striatum with time, although at 8 weeks (the longest time-interval studied) after (+)-amphetamine a moderate but consistent reduction of the dopamine fluorescence intensity could still be seen. The general impression was also that there was a reduction of the number of fluorescent catecholamine accumulations in the striatum produced by (+)-amphetamine at this time.

Effect of (−)-amphetamine on the regional catecholamine levels

Two doses of (−)-amphetamine were tested; 25 or 37 μg/h for 7 days (Table 1). There was no increased mortality after these doses, in contrast to (+)-amphetamine where 25–30% of the animals died before one week after infusion of 37 μg/h. The catecholamine concentrations were not altered significantly in most regions, even after infusion of the larger dose. In none of the experiments was any significant effect on the striatal dopamine levels observed. Some minor catecholamine depletions (about 25% or less) were observed. Eighteen days after infusion of the larger (−)-amphetamine dose the only region which displayed any significant change ($P < 0.05$) was the frontal cortex, where a reduction of approx. 25% in dopamine and 20% in noradrenaline concentrations was found.

Effect of (+)-amphetamine on the regional 5-hydroxytryptamine

The (+)-amphetamine infusion (25 μg/h) for 7 days and analysis one day later led to significant 5-HT increases in some regions (hippocampus, mesencephalon, pons-medulla, spinal cord), although quantitatively not very marked, except in the hippocampus where the increase in 5-HT was about 50% (Table 2).

Discussion

In agreement with previous studies, the present study showed that large doses of (+)-amphetamine can produce a marked and long-lasting reduction of striatal dopamine concentration, demonstrated both histochemically and neurochemically, concomitant with an impairment of the uptake mechanism of dopaminergic nerve terminals, as reflected by a reduction of [^{3}H]-NA uptake (Ellison *et al.*, 1978; Fuller & Heinrick-Luecke, 1980; Steranka & Sanders-Bush, 1980). The dopamine depletion was accompanied by the appearance of intensely fluorescent catecholamine accumulations in the striatum, which is probably related to an accumulation of dopamine transmitter in axons proximal to damaged dopaminergic nerve terminals. Similar observations have been made by Ellison *et al.* (1978) and Lorez (1981). This type of axonal transmitter accumulation is consistently observed after terminal degeneration induced by monoamine neurotoxins (see Jonsson, 1981) and the present results are therefore strongly indicative of a dopaminergic nerve terminal damage and degeneration produced by (+)-amphetamine.

Table 1 Effects of (−)-amphetamine infusion on the regional catecholamine levels in mice

Treatment/Region	Control NA	Control DA	(−)-Amphetamine NA	(−)-Amphetamine DA	% change NA	% change DA
25 μg/h 7 days (8 days):						
Frontal cortex	402±15	46±5.8	342±11*	42±5.2	−15	—
Striatum	—	15.593±1220	—	14.173±755	—	—
37 μg/h 7 days (8 days):						
Olf. bulb	262±38	268±11	222±22	192±17*	—	−28
Frontal cortex	355±54	44±4.2	269±33	43±3.4	—	—
Striatum	—	13.131±525	—	12.281±278	—	—
Olf. tubercle	333±47	7.027±519	273±34	7.263±1252	—	—
Hippocampus	373±28	16±0.4	320±31	15±0.8	—	—
Hypothalamus	2028±212	484±65	1573±167	484±48	—	—
Mesencephalon	955±41	518±36	752±63*	414±15*	−22	−20
Pons-medulla	739±59	71±6.8	566±34*	62±7.3	−24	—
Sp. cord	416±83	43±4.1	381±60	43±6.0	—	—
37 μg/h 7 days (18 days):						
Olf. bulb	241±21	232±28	210±21	251±47	—	—
Frontal cortex	464±27	49±2.5	378±30*	37±1.9*	−18	−26
Striatum	—	13.940±1932	—	13.350±982	—	—
Olf. tubercle	401±66	7.502±696	267±13	7.536±262	—	—
Hypothalamus	1937±90	664±56	1782±165	655±29	—	—
Mesencephalon	846±25	557±28	772±74	509±24	—	—

Mean ± s.e.mean ($n = 4$), expressed as ng/g.
* = $0.05 > P > 0.01$ (Student's t test).

The fluorescence histochemical data demonstrated furthermore that the dopamine depleting effect of (+)-amphetamine was not even throughout the striatum, but the dorsally located DA terminals appeared to be most sensitive. This would indicate that there is within the striatum a population of preferentially vulnerable dopaminergic nerve terminals. The present subcellular distribution data could also be interpreted to support this view.

The fluorescence histochemical analysis did not indicate any signs of damage of the dopaminergic perikarya in the mesencephalon or dopaminergic axons outside the striatum. Consistent with this a gradual recovery of endogenous dopamine, dopamine fluorescence and the [^{3}H]-NA uptake capacity was observed and this recovery was almost complete 6 months after the (+)-amphetamine administration. The slow, gradual reappearance of the

Table 2 Effects of (+)-amphetamine (25 μg/h, 7 days) on the regional 5-hydroxytryptamine levels in mice.

Region	Control	(+)-Amphetamine	% change
Frontal cortex	238±24	271±11	—
Hippocampus	126±13	185±11**	+47
Striatum	348±35	402±18	—
Hypothalamus	420±44	503±38	—
Mesencephalon	435±17	559±23*	+29
Pons-medulla	229±25	279±6.6*	+22
Spinal cord	273±20	353±20*	+29

The mice were killed 8 days after the start of the (+)-amphetamine infusion.
Mean ± s.e.mean of 4 determinations expressed as ng/g.
* = $0.05 > P > 0.01$; ** = $0.001 > P > 0.01$ (Student's t test).

dopamine parameters is probably related to a regrowth and regeneration of damaged dopaminergic nerve terminals. Further support for this view is the disappearance of the (+)-amphetamine-induced catecholamine accumulations in the striatum in the chronic state. A regeneration of damaged nerve terminals after a local denervation in the innervation area has been noted previously for monamine neurones (Nakai, Jonsson & Kasamatsu, 1981; see also Björklund & Stenvi, 1979). The present observations do not allow any definite conclusion as to whether or not the observed recovery is due to a true regenerative sprouting or a collateral sprouting of intact dopaminergic terminals or due to both processes. It was noted that there was a more rapid recovery of the capacity of the uptake mechanism than that of the endogenous dopamine stores (see Figure 4). This finding is also consistent with the regeneration hypothesis in view of previous observations showing regenerating fibres to have a low transmitter content (Jonsson & Sachs, 1972) due to a lag in the normalization of the transmitter storage capacity.

Concerning the specificity of action of (+)-amphetamine on striatal dopaminergic nerve terminals, the neurochemical data point to a relative specificity. Keeping the amount of (+)-amphetamine infused (25 g/h; 7 days) at a level that avoided mortality, it is clear that the long-lasting effects on dopamine uptake-storage mechanisms are rather specifically confined to a preferentially vulnerable population of dopaminergic nerve terminals localized in the dorsal parts of the striatum. The reason for this preferential regional effect within the striatum is at present unknown, although it could possibly be related to different pharmacokinetics of (+)-amphetamine regionally, different changes in blood-flow (see Lavyne, Koltun, Clement, Rosene, Pickren, Zervas & Wurtman, 1977) and/or alterations in blood-brain permeability leading to regional differences in (+)-amphetamine distribution (see Kuhn & Schanberg, 1978; Domer, Sankar, Cole & Wellmeyer, 1980). It has previously been reported that multiple injections of (+)-amphetamine or methamphetamine can have long-lasting effects on 5-HT neurones (Trulson & Jacobs, 1979; 1980; Hotchkiss *et al.*, 1979; Hotchkiss & Gibb, 1980; Ricuarte, Schuster & Seiden, 1980), but in agreement with Steranka & Sanders-Bush (1981), Steranka (1981) and Lorez (1981), no indications of any neurotoxic effects on 5-HT neurones were noted in the present study. The reason for this discrepancy is not clear, but might be related to species and/or strain differences as well as differences in the specificity of action between (+)-amphetamine and methamphetamine. The present findings of increased 5-HT levels after (+)-amphetamine could indicate a functional alteration of the 5-HT neurones.

The present study also demonstrated a stereoselective effect for (+)-amphetamine since no effects on the striatal dopamine levels were noted after infusion of large doses of (−)-amphetamine. Similar findings have recently been reported for the rat (Steranka, 1981). Consistent with this is the earlier finding that (+)-amphetamine has a markedly preferential acute action on dopaminergic neurones (Bunney, Walters, Kuhar, Roth & Aghajanian, 1975; Kuczenski & Segal, 1975). It was noted in the present study, that (−)-amphetamine produced a small but significant long-lasting reduction of both dopamine and NA in the frontal cortex, but more work is needed to elucidate whether or not this is related to a neurotoxic effect.

The present observations indicate that the long-lasting, probably neurotoxic, effects on striatal dopaminergic nerve terminals is specific for (+)-amphetamine and in view of its known preferential dopamine releasing effect, it is reasonable to assume that the neurotoxic action is mediated via a direct action on the dopaminergic nerve terminals. Recent results of Fuller & Heinrick-Luecke (1980) are in agreement with this view. These authors demonstrated that a dopamine uptake inhibitor was able to prevent the long-term (+)-amphetamine-induced depletion. This would further imply that the neurotoxicity of (+)-amphetamine is elicited via an action on the carrier of the dopamine uptake mechanism localized at the axonal membrane. However, further studies are needed to elucidate how such an action can lead to perturbation of dopamine uptake-storage mechanisms and damage of dopaminergic nerve terminals in the striatum.

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