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LONG-LASTING DEPLETIONS OF STRIATAL DOPAMINE AND LOSS OF DOPAMINE UPTAKE SITES FOLLOWING REPEATED ADMINISTRATION OF METHAMPHETAMINE

GEORGE C. WAGNER, GEORGE A. RICAURTE, LEWIS S. SEIDEN*, CHARLES R. SCHUSTER, RICHARD J. MILLER and JOHN WESTLEY

Department of Biopsychology, (G.A.R., L.S.S. and R.J.M.) Department of Pharmacological and Physiological Sciences, (C.R.S.) Department of Psychiatry and (J.W.) Department of Biochemistry, The University of Chicago, Chicago, Ill. 60637 (U.S.A.)

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SUMMARY

Repeated administration of high doses of methamphetamine produced long-term decreases in dopamine (DA) levels and in the number of DA uptake sites in the rat striatum. These two effects were dose-related and did not appear to be due to the continued presence of drug in striatal tissue. Long-lasting depletions induced by methamphetamine were selective for striatal DA neurons since norepinephrine (NE) levels in all of the rat brain regions examined were not changed on a long-term basis by methamphetamine treatments. Supersensitivity of DA receptors did not accompany the loss of striatal DA and its uptake sites.

INTRODUCTION

Repeated administration of high doses of methamphetamine to monkeys was shown to produce depletions of caudate dopamine (DA) and midbrain and frontal cortex norepinephrine (NE) which lasted for as long as 6 months after discontinuation of the drug[13]. Recently, a similar finding was obtained in two additional species. Rats and guinea pigs subjected to high doses of methamphetamine exhibited a substantial depletion of caudate dopamine two weeks after drug administration had been stopped[15]. Currently, little is known about the neurochemical processes underlying these depletions. However, a recent report indicates that almost 4 months after a period of continuous amphetamine administration, caudate tyrosine hydroxylase activity was decreased. Also, that 6 days after the amphetamine treatment, striatal dopaminergic fibers had a swollen appearance as revealed by histofluorescent studies[6].

* To whom correspondence and reprint requests should be addressed.

Together these observations raise the question of whether amphetamines in high doses produce permanent toxic effects on central dopaminergic neurons.

The aim of this study was to characterize the long-term effects of methamphetamine treatments on central catecholaminergic neurons in rats. The principal finding is that high-dose (50 and 100 mg/day × 4) methamphetamine treatments result in a long-term loss of DA and its uptake sites in the rat striatum.

METHODS

Animals

Male, Sprague–Dawley rats (Holtzman, Madison, Wisc.) weighing initially approximately 250 g were used in all experiments. Rats were housed individually with free access to food (Teklad) and water. Colony room lights were automatically turned on at 06.00 and off at 18.00 h and temperature was maintained at 22 ± 1 °C. For all experiments, s.c. injections were given twice a day at 08.00 and 17.00 h using a 25 mg/ml solution of methamphetamine hydrochloride dissolved in physiological saline. Dose was adjusted by changing the volume of the 25 mg/ml methamphetamine solution that was injected. Sweetened condensed milk (Borden) was used as a dietary supplement to overcome the decrease in food intake produced by methamphetamine. Rats that died while being treated with methamphetamine (approximately 50% mortality at the highest daily doses described below) were replaced with matched rats in order to complete the group.

Dissection technique

Rats were killed by decapitation, their brains were quickly removed and then dissected over ice in the following manner. To obtain striatum, two transverse sections were made: the first posterior to the olfactory bulb and the second anterior to the optic chiasm. From this slice of tissue, the anterior striatum (containing portions of the caudate, globus pallidus and putamen) was obtained by dissecting away the cortical and septal parts of the slice, using the corpus callosum, septum and anterior commissure as landmarks. The rest of the brain (for level studies only) was further dissected according to the method of Glowinski and Iversen[7] to yield portions of the pons-medulla, midbrain and telencephalon. For uptake studies, striatal tissue was used immediately. For receptor binding and level studies, the tissue was stored in liquid nitrogen until assayed.

Catecholamine level studies

Catecholamine (CA) tissue concentrations were determined fluorometrically after ion-exchange chromatography using the method of Bertler et al.[1] and Carlsson and Waldeck[2].

To determine the dose-dependency of DA depletions produced by methamphetamine, different daily doses (12.5, 25.0, 50.0 or 100.0 mg/kg) were administered to groups of 8 rats for 4 days. Rats were killed two weeks after the last injection and CA levels were determined.

To determine the effects of daily dose vs overall dose, 3 groups of 10 rats received

daily doses of 12.5, 25.0 or 50.0 mg/kg for 40, 20 or 10 days respectively such that the total dose for each rat equaled 500 mg/kg. Ten additional rats received vehicle for forty days. Rats were killed two weeks after the last injection and CA levels were determined.

To determine the duration of dopamine depletions, 3 groups of 10 rats received 50 mg/kg for 30 days. Ten additional rats received daily injections of vehicle. Rats were killed 2, 4 or 8 weeks after the last injection and CA level determinations were then conducted.

Uptake studies

Rats, in groups of 6, received a total of 12.5, 25.0, 50.0 or 100.0 mg/kg/day of methamphetamine (in 2 doses/day) for 4 days; 24 rats received vehicle injections only. Rats were sacrificed 2–3 weeks after the last injection and assayed according to the method of Snyder and Coyle[14], with minor modifications. After the dissection, striatal tissue from two rats (approximately 50 mg/rat) was pooled, weighted and then homogenized in 50 vols. (w/v) of ice-cold 0.32 M sucrose. A crude synaptosomal preparation was obtained by centrifuging the homogenate at $1000 \times g$ for 10 min. 0.2 ml aliquots of the supernatant were added over ice to tubes containing 3.8 ml of a Krebs–Ringer phosphate buffer (pH 7.4) that contained in final concentartions: 118 mM NaCl, 16.2 mM Na_3PO_4, 4.7 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4 \cdot 7H_2O$, 1.1 mM ascorbic acid, 11.1 mM glucose, 1.3 mM EDTA, 1.25×10^{-4} M pargyline. The buffer also contained an equimolar mixture of cold and tritiated dopamine at different concentrations. Samples (except blanks) were incubated at 37 °C for 5 min, returned to ice and the filtered through GF/A filter discs. Filters were washed 3 times with physiological saline and measured for radioactivity by liquid scintillation spectroscopy at an efficiency of 13–22 %. Assays were performed in sextuplicate at each DA concentration with half of the tubes serving as blanks. Blanks consisted of samples kept at 0–4 °C during the 5 min incubation at 37 °C. Uptake of [^{3}H]DA was expressed as [^{3}H]DA dpm/mg/prot./5 min after correction for uptake at 0–4 °C. Samples were not pre-incubated.

Computer simulation studies

For exploring the qualitative kinetic properties of transport systems that result in accumulation of labeled materials, some computer-based simulation studies were carried out. A program representing both entry and exit processes as simple diffusion was based on the following equation:

$$C_t = C_{t-1} + k_{in} X - \frac{k_{out} C_{t-1}}{P}$$

where C_t is internal concentration at time t, X is external concentration, P is the internal pool size and k_{in} and k_{out} are rate constants for diffusion.

A second program, representing both entry and exit processes as mediated by enzyme-like carriers, was based on the following equation:

$$C_t = C_{t-1} + \frac{V_{in}S}{K_{in} + X} - \frac{V_{out}\ C_{t-1}}{(K_{out} + C_{t-1})\ P}$$

where V and K are kinetic coefficients analogous to the maximum velocity and Michaelis constant of one-substrate enzyme kinetics. These equations were used iteratively with very small time increments to generate time courses that illustrate the qualitative behavior of these accumulation models with various combinations of concentration, pool size and kinetic coefficients.

DA receptor binding assays

A group of 18 rats was treated with 100 mg/kg/day of methamphetamine for 4 days; 18 additional rats were treated with vehicle only. Groups of 6 vehicle-treated rats were killed 2, 4 or 8 weeks after the last injection. Binding assays were performed with [^{3}H]spiroperidol following the procedure of Creese and Snyder[5], with minor modifications. Membrane homogenates were prepared using striatal tissue from two rats such that the final tissue concentration was from 3 to 8 mg wet weight/ml. Tubes containing 100 μl [^{3}H]spiroperidol (final concentration ranging from 0.15 to 2.4 nM), 100 μl of (+)- or (—)butaclamol (final concentration 1 μM) received 1.8 ml of the membrane homogenate and were then incubated for 15 min at 37 °C. Membranes were collected on GF/B filters, washed 3 times with 4 mls of 0.05 M Tris buffer (pH 7.7) and bound radioactivity was determined as in uptake experiments. Specific binding was defined as the difference between radioactivity bound in the presence of (—)butaclamol minus that bound in the presence of (+)butaclamol. Specific binding ranged from 60 to 80% of the radioactivity bound in the presence of (—)butaclamol. Assays were performed in sextuplicate with half of the tubes containing (—)butaclamol and the other half containing (+)butaclamol. Specific [^{3}H]spiroperidol binding was expressed as pmol/mg protein/15 min.

Protein determinations

Protein was determined according to the method of Lowry[9].

Data analysis

Kinetic data were processed by use of a Hewlett–Packard 2000 C computer with a BASIC program for iterative fitting to the best rectangular hyperbola, following the suggestions of Wilkinson[16] and Cleland[3] for proper statistical evaluation of such data. Equilibrium binding data were analyzed in the same way with final conversion to the Scatchard plot form[12]. Weighted averages were computed with a BASIC program that uses reciprocal variances as the weighting factors.

Statistics

Results were analyzed using a two-tailed Student's *t*-test.

Drugs and materials

DA[ethyl-1-^{3}H] (19.7 Ci/mmol) and spiroperidol [1-phenyl-4-^{3}H] (23.2 Ci/mmol) (new England Nuclear, Boston, Mass.) dopamine hydrochloride and bovine

serum albumin (Sigma, St. Louis, Mo.) were used. Butaclamol enantiomers were a gift from Ayerst Research Laboratories (Montreal, Que.). Methamphetamine hydrochloride was supplied by the National Institute of Drug Abuse, (Bethesda, Md.). GF/A and GF/B filters (Whatman, N.J.) and Quantafluor (Mallinkrodt, St. Louis, Mo.) were also used.

RESULTS

Catecholamine levels

Dose-dependency. Four-day regimens of different doses of methamphetamine influenced levels of DA in rat caudate in a dose-dependent fashion. Two weeks after termination of the drug injection period, only the two higher doses of the four methamphetamine doses tested (12.5, 25.0, 50.0 and 100.0 mg/kg) produced significant decreases in caudate DA (Table I). None of the methamphetamine regimens produced changes in NE levels in any of the brain regions assayed.

Daily dose vs total dose. When daily dose of methamphetamine was varied but total dose was kept constant (by administering 12.5, 25.0 and 50.0 mg/kg for 40, 20 and 10 days, respectively), caudate DA depletions were seen to depend more on daily dose magnitude than on the total dose (Table II). Only rats receiving the higher daily doses (25 and 50 mg/kg) exhibited significantly decreased levels of caudate DA even though the 12.5 mg/kg group eventually received the same total dose. NE levels in all the brain regions examined were unaffected by any of the drug regimens.

Depletion duration. Rats given 50 mg/kg/day of methamphetamine for 30 days and killed 2, 4 or 8 weeks after the last injection all showed significant caudate DA depletions (Table III). After 8 weeks there was no apparent trend towards recovery of normal DA levels. NE levels were normal at all post-drug waiting periods tested.

TABLE I

Regional brain CA levels in rats receiving different daily doses of methamphetamine hydrochloride for 4 days

Values reported are means in μg/g tissue $\pm$ S.E.M. Rats were killed two weeks after the last injection. DA, dopamine; NE, norepinephrine.

	n	*Caudate DA (μg/g)*	*Telencephalon NE (μg/g)*	*Midbrain NE (μg/g)*	*Pons-medulla NE (μg/g)*
Vehicle	8	9.57 $\pm$ 0.88	0.21 $\pm$ 0.02	0.56 $\pm$ 0.10	0.56 $\pm$ 0.04
12.5 mg/kg/day D-methamphetamine	8	9.67 $\pm$ 0.41	0.23 $\pm$ 0.01	0.66 $\pm$ 0.04	0.49 $\pm$ 0.03
25 mg/kg/day D-methamphetamine	8	7.84 $\pm$ 0.90	0.24 $\pm$ 0.01	0.58 $\pm$ 0.03	0.50 $\pm$ 0.04
50 mg/kg/day D-methamphetamine	8	5.81 $\pm$ 0.80*	0.20 $\pm$ 0.03	0.55 $\pm$ 0.06	0.56 $\pm$ 0.09
100 mg/kg/day D-methamphetamine	8	4.40 $\pm$ 0.67**	0.23 $\pm$ 0.02	0.67 $\pm$ 0.06	0.50 $\pm$ 0.05

* $P < 0.05$ vs both vehicle and 12.5 mg/kg day groups; ** $P < 0.05$ vs 25 mg/kg group.

TABLE II

Regional brain CA levels in rats treated with 12.5, 25.0, and 50.0 mg/kg/day of methamphetamine hydrochloride for 40, 20 or 10 days, respectively

Values reported are means in μg/g tissue ± S.E.M. Rats were killed two weeks after the last injection. DA, dopamine; NE, norepinephrine. (The total dose for each group was 500 mg/kg).

	n	*Caudate DA (μg/g)*	*Telencephalon NE (μg/g)*	*Midbrain NE (μg/g)*	*Pons-medulla NE (μg/g)*
Vehicle	10	9.42 ± 0.63	0.27 ± 0.06	0.61 ± 0.10	0.59 ± 0.06
12.5 mg/kg/day D-methamphetamine for 40 days	10	8.27 ± 0.73	0.28 ± 0.02	0.57 ± 0.06	0.63 ± 0.04
25 mg/kg/day D-methamphetamine for 20 days	10	6.94 ± 0.90*	0.35 ± 0.06	0.58 ± 0.05	0.64 ± 0.03
50 mg/kg/day D-methamphetamine for 10 days	10	5.06 ± 0.46*	0.29 ± 0.02	0.63 ± 0.04	0.66 ± 0.04

* $P < 0.05$ vs vehicle group.

TABLE III

Regional brain catecholamine levels in rats allowed 2, 4 or 8 weeks waiting periods after receiving 50 mg/kg/day of methamphetamine hydrochloride for 30 days

Values reported are means in μg/g tissue ± S.E.M. DA, dopamine; NE, norepinephrine.

	n	*Caudate DA (μg/g)*	*Telencephalon NE (μg/g)*	*Midbrain NE (μg/g)*	*Pons-medulla NE (μg/g)*
Vehicle	10	8.26 ± 0.59	0.38 ± 0.02	0.67 ± 0.04	0.61 ± 0.14
50 mg/kg/day D-methamphetamine 2 weeks	10	3.62 ± 0.47*	0.37 ± 0.03	0.66 ± 0.03	0.50 ± 0.04
50 mg/kg/day D-methamphetamine 4 weeks	10	5.27 ± 0.60*	0.38 ± 0.06	0.71 ± 0.09	0.62 ± 0.05
50 mg/kg/day D-methamphetamine 8 weeks	10	4.72 ± 0.36*	0.37 ± 0.02	0.65 ± 0.09	0.71 ± 0.04

* $P < 0.05$ vs vehicle group.

DA uptake

Rats treated with 50 mg/kg/day of methamphetamine for 4 days and assayed 2–3 weeks later, displayed a significant decrease in DA uptake site number (V_{max}), (Fig. 1). The K_m of residual sites for DA was not changed. Only the two higher (50 and 100 mg/kg) daily doses of methamphetamine produced significant decreases in V_{max} (Table IV).

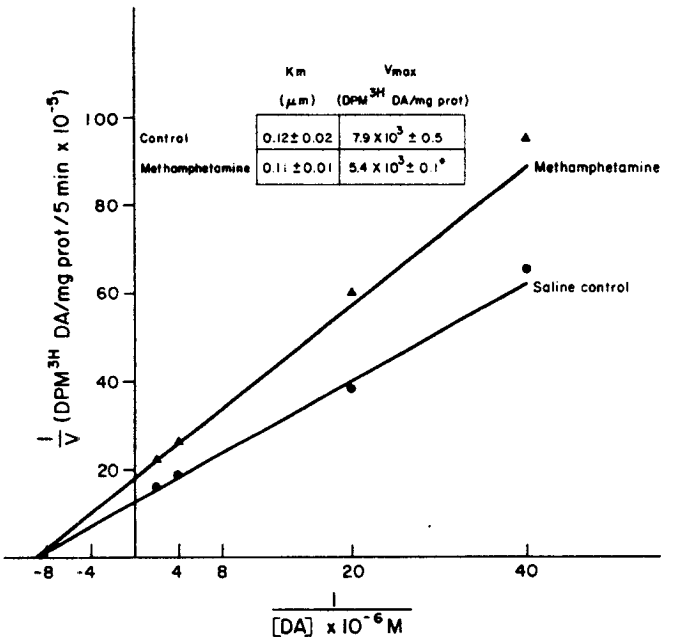

Fig. 1. Double-reciprocal plot of [^{3}H]dopamine uptake by rat striatal homogenates 2–3 weeks after the high dose methamphetamine treatment (50 mg/kg/day for 4 days). Dopamine uptake was determined at dopamine concentrations ranging from 0.25 to 5.0 μM. The K_m values of saline treated (0.11 μM) and methamphetamine treated (0.18 μM) were not significantly different. The difference in dopamine uptake site density (V_{max}) is significant ($P < 0.05$). Data are from one representative experiment replicated twice.

TABLE IV

Dose dependency of the loss of striatal DA uptake sites following methamphetamine treatments

Values shown are the mean ± S.E.M. per cent decreases in V_{max} (total number of DA uptake sites) produced by each of the 4 day methamphetamine regimens. Each mean per cent decrease in V_{max} represents the weighted average of 3 individual V_{max} per cent changes produced by a given methamphetamine daily dose. Reciprocal variances of individual V_{max} values were used as weighting factors. Uptake assays were performed 2–3 weeks after drug was discontinued.

Methamphetamine daily dose (mg/kg)	*n*	*Mean per cent decrease in V_{max} (W-mean ± S.E.M.)*
12.5	3	2.0 ± 4.4
25	3	1.7 ± 3.6
50	3	21.7 ± 2.3
100	3	44.0 ± 6.7

Computer simulation

Time course simulations were carried out for an accumulation model that represents both entry and exit processes as occurring by simple diffusion. The ratio of entry and exit constants and the internal pool size were varied. Similar studies were undertaken for a model in which entry and exit are mediated by enzyme-like carriers.

In this case the varied parameters were pool size, the ratio of the limiting velocities for entry and exit, the ratio of Michaelis constants for entry and exit, and the ratio of external concentration to the Michaelis constant for entry.

The time courses generated by these simulation studies were subjected to analysis by the same procedures used in the experimental studies reported here. The results obtained are summarized in the practical generalizations that follow. None of these results are altered when 'mixed' systems of diffusion in one direction and mediation in the other are considered: (a) the initial velocity of accumulation in such systems is insensitive to changes in either the size of the internal pool or the kinetic parameters of any countervailing system for transporting the labeled substance out; (b) changes in pool size or output kinetic parameters large enough to affect practical velocity estimates also result in obvious nonlinearities in isotope accumulation with time, that is, the velocity estimates are then clearly not true initial velocities.

It was also found that the attempted use of initial velocity estimates distorted by any of the causes in (b) gave rise to double reciprocal plots that tended to be nonlinear (concave from above). Finally, it was found that ignoring the systematic nonlinearity of the double reciprocal plot in such a case (that is, considering the curvature to be lost in the scatter of the experimental values), gave rise to derived values for Michaelis constants that tended to be altered more than those for the corresponding maximum velocities.

These considerations indicate that effects on either the pool size or the kinetic parameters of an exit transport system are an unlikely basis for explaining changes in initial velocity of accumulation. Furthermore, even if the limitations of practical experimental data cause initial velocity estimates to be distorted, the finding of an altered maximum velocity of accumulation, with a relatively unaltered Michaelis constant, is still not likely to be the result of changes in either pool size or output kinetics.

TABLE V

Specific binding of [^3H]spiroperidol to striatal membranes obtained from rats treated with 100 mg/kg/day of methamphetamine for 4 days

Rats were killed 2, 4 or 8 weeks after the last injection. K_d and B_{max} values were derived from Scatchard analyses of specific [^{3}H]spiroperidol binding at [^{3}H]spiroperidol concentrations ranging from 0.15 to 2.4 nM. None of the differences in dissociation constants (K_d) or receptor densities (B_{max}) between saline-treated and methamphetamine-treated rats were significant. Each of the values shown is the mean ± S.E.M. of 3 separate determinations.

Post-drug waiting period (weeks)		B_{max} *(fmol/mg prot.)*	K_d *(nM)*
2	saline	92 ± 8	0.24 ± 0.02
	methamphetamine	107 ± 11	0.19 ± 0.03
4	saline	127 ± 9	0.19 ± 0.04
	methamphetamine	116 ± 6	0.22 ± 0.03
8	saline	107 ± 12	0.21 ± 0.02
	methamphetamine	102 ± 14	0.23 ± 0.04

DA receptor binding assay

Rats treated with 100 mg/kg/day of methamphetamine for 4 days and allowed a 2, 4 or 8 week post-drug waiting period did not show any changes in either the number (B_{max}) or affinity (K_d) of [^{3}H]spiroperidol binding sites (Table V).

DISCUSSION

The striatal DA depletions observed in this study were dependent upon high daily doses of methamphetamine (Tables I and II). A lower daily dose, even when administered for a longer period of time so as to give the same cumulative dose, did not cause a significant DA depletion (Table II). Furthermore, DA depletions are seen to last 8 weeks beyond the termination of the drug treatment period (Table III). If residual drug or its metabolites were responsible for the long-lasting depletion (e.g. by causing continued release and/or blockade of reuptake of DA at the terminal), it would be expected that DA levels would gradually increase over the 8 week waiting period as residual compounds were cleared from the tissue. This, however, was not observed.

The lack of changes in NE levels following these drug treatments is consistent with the previously reported data on rats and guinea pigs[15]. Long-lasting NE depletions following methamphetamine administration were, however, observed in rhesus monkeys[13]. This discrepancy may be indicative of species or drug regimen differences.

Concomitant with the striatal DA depletions, a dose-related loss of DA uptake sites was observed (Table IV). Kinetic analysis of [^{3}H]DA uptake showed a decrease in V_{max} with no change in K_m. This indicates the reduction in [^{3}H]DA is due to decreased DA uptake site number and not to a decrease in uptake site affinity. Since previous studies have shown that decreases in CA uptake are reflective of VA terminal loss[5,11], these data are compatible with the hypothesis that there is DA terminal loss after high dose methamphetamine treatments. It is unlikely that the observed decrease in [^{3}H]DA uptake is due to residual methamphetamine or one of its metabolites acting as a competitive inhibitor of [^{3}H]DA uptake since a change in K_m is not observed. Also, as shown by the computer simulation studies, it is improbable that the reduction in [^{3}H]DA results from a releasing action exerted by residual drug or metabolites since such an effect would produce changes in K_m rather than V_{max}.

Finally, denervation supersensitivity of striatal DA receptors like that reported following large 6-hydroxydopamine-induced DA depletions (80–90% depletions)[4,10] was not observed 2, 4 or 8 weeks following the methamphetamine treatment (Table V). This may be due to the lower DA depletions reported in this study (about 40%).

In summary, high dose methamphetamine treatments produced a long-term loss of DA and DA uptake sites in the rat striatum. These deficits are probably not caused by residual drug or metabolites and may be indicative of nerve terminal loss.

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REFERENCES

1 Bertler, A., Carlsson, A. and Rosengren, E., A method for the fluorometric determination of adrenaline in tissue, *Acta physiol. scand.*, 44 (1958) 273–292.
2 Carlsson, A. and Waldeck, B., A fluorometric method for the determination of dopamine (3-hydroxytyramine), *Acta physiol. scand.*, 44 (1958) 293–298.
3 Cleland, W. W., The statistical analysis of enzyme kinetic data, *Advanc. Enzymol.*, 29 (1967) 1–32.
4 Creese, I., Burt, D. R. and Snyder, S. H., Dopamine receptor binding enhancement accompanies lesion-induced behavioral supersensitivity, *Science*, 197 (1977) 596–598.
5 Creese, I. and Snyder, S. H., ^{3}H-spiroperidol labels serotonin receptors in rat cerebral cortex and hippocampus, *Europ. J. Pharmacol.*, 49 (1978) 201–202.
6 Ellison, G., Eison, M. S., Huberman, H. S. and Daniel, F., Long-term changes in dopaminergic innervation of caudate nucleus after continuous amphetamine administration, *Science*, 201 (1978) 276–278.
7 Glowinski, J. and Iversen, L. L., Regional studies of catecholamines in the rat brain. I. The disposition of ^{3}H-norepinephrine, ^{3}H-dopamine, and ^{3}H-dopa in various regions of the brain, *J. Neurochem.*, 13 (1966) 665–669.
8 Jonsson, G., Pycock, C. H., Fuxe, K. and Sachs, C., Changes in the development of central noradrenalin neurons following neonatal administration of 6-hydroxydopamine, *J. Neurochem*, 22 (1974) 419–426.
9 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein measurements with Folin phenol reagent, *J. Biol. Chem.*, 193 (1951) 265–275.
10 Nagy, J. I., Lee, T., Seeman, P. and Fibiger, H. C., Direct evidence for presynaptic and postsynaptic dopamine receptors in the brain, *Nature (Lond.)*, 271 (1978) 278–281.
11 Sachs, C. and Jonsson, G., Effects of 6-hydroxydopamine on central noradrenaline neurons during ontogeny, *Brain Research*, 99 (1975) 277–291.
12 Scatchard, G., The attractions of proteins for small molecules and ions, *Ann. N.Y. Acad. Sci.*, 51 (1949) 660–672.
13 Seiden, L. S., Fischman, M. W. and Schuster, C. R., Long-term methamphetamine induced changes in brain catecholamines in tolerant rhesus monkeys, *Drug and Alcohol Dependence*, 1 (1975/76) 215–219.
14 Snyder, S. H. and Coyle, J. T., Regional differences in ^{3}H-norepinephrine and ^{3}H-dopamine uptake in rat brain homogenates, *J. Pharmacol. exp. Ther.*, 165 (1968) 78–86.
15 Wagner, G. C., Seiden, L. S. and Schuster, C. R., Methamphetamine induced changes in brain catecholamines in rats and guinea pigs, *Drug and Alcohol Dependence*, 4 (1979) in press.
16 Wilkinson, G. N., Statistical estimations in enzyme kinetics, *J. Biochem. (Tokyo)*, 80 (1961) 324.