Biochemical Pharmacology, Vol. 30, No. 12, pp. 1543–1549, 1981.
Printed in Great Britain.

0006-2952/81/121543-07 $02.00/0
Pergamon Press Ltd.

CATECHOLAMINE LEVELS AND TYROSINE HYDROXYLASE ACTIVITIES IN RAT BRAIN REGIONS AFTER CHRONIC TREATMENT WITH, AND WITHDRAWAL OF, METHAMPHETAMINE

MARIE E. BARDSLEY* and HERMAN S. BACHELARD†‡

Department of Biochemistry, University of Bath, Bath, U.K. and †Biochemistry Department, St. Thomas's Hospital Medical School, London SE1 7EH, U.K.

(*Received* 20 *August* 1980; *accepted* 30 *January* 1981)

Abstract—Groups of rats were treated with methamphetamine chronically for a period of 30 days, during which time food consumption, drinking rates and body weights were monitored. Rats which remained anorexic during the chronic treatment showed after 15 and 30 days significant decreases in dopamine and noradrenaline levels, and also of tyrosine hydroxylase activities in all brain regions studied. Tyrosine hydroxylase activity recovered after 36 hr of withdrawal of the drug in regions rich in catecholamine-containing nerve terminals (striatum, nucleus accumbens and amygdala) but recovery was only partial in those regions which contain the cell bodies of major catecholamine pathways (tuberculum olfactorium, hypothalamus, pons and medulla). No recovery was detected in the cerebral cortex. Rats which appeared to overcome the anorexic effects of methamphetamine (as assessed by body weights, food and water intake) showed no such changes.

INTRODUCTION

It is now generally accepted that acute and chronic treatments with amphetamines can result in decreased tyrosine hydroxylase activities. This has been shown to occur *in vivo* [1] and *in vitro* [2, 3]. Studies on slices from the striatum incubated *in vitro* indicated inhibition by amphetamine to be 'non-competitive' with respect to tyrosine [3], but the effect may not be due to a direct effect on the hydroxylase since the enzymic activity was not modified by amphetamine when tested on homogenates or on partially purified preparations [2, 4, 5]. The decrease in striatal tyrosine hydroxylase activity was blocked by pretreatment with chlorpromazine or bicuculline [6]. Chronic treatment with methamphetamine (10 mg/kg body weight) also resulted in decreased tyrosine hydroxylase activity in the caudate nucleus; this could be prevented by pretreatment with haloperidol and chlorpromazine [7–9]. Such results on chronic treatment suggest that mediation of dopaminergic or Gaba receptors might be involved in the response of the hydroxylase.

Although the responses of the enzyme to chronic treatment with methamphetamine seem reasonably clear, there is some considerable variation in the results [7, 8, 10, 11]. Further, little knowledge seems to be available on the effects on catecholamine metabolism of withdrawal of amphetamines after chronic treatment, analogous to previous studies on carbohydrate metabolism [12].

We therefore set out to study the effects of chronic treatment with, and withdrawal of, methamphetamine on catecholamine levels and tyrosine hydroxylase activities in various regions of rat brain *in vivo*. The body weights and food and water consumption of the rats were monitored throughout the experimental periods.

The regions studied were: cerebral cortex, tuberculum olfactorium, striatum, nucleus accumbens, hypothalamus, amygdala, pons and medulla.

METHODS

Materials

DL-Methamphetamine hydrochloride, 6,7-dimethyl-5,6,7,8-tetrahydropterin ($DMPH_4$), dopamine hydrochloride, noradrenaline bitartrate, 3-methoxytyramine hydrochloride, normetanephrine hydrochloride, bovine serum albumin, tyrosine and Tris base (Trizma) were obtained from Sigma (London) Chemical Co. Ltd, Poole, Dorset, U.K. L-[side-chain-2,3-^{3}H]tyrosine (specific activity 13 Ci/mmol), L-3,4,-dihydroxy [ring 2,5,6-^{3}H]phenylalanine ([^{3}H]DOPA, specific activity 1.0 Ci/mmol) and S-adenosyl-L-[methyl-^{3}H]methionine were obtained from the Radiochemical Centre, Amersham, U.K. The alumina was a Merck product and NSD 1055 (*m*-hydroxy-*p*-bromo benzyloxyamine) was obtained from Sandev Ltd, Gilston Park, Harlow, Essex.

Treatment of animals

The rats (Remote Wistar males) were 30–35 days old on commencement of treatment; they were housed in groups of five under constant conditions, i.e. light–dark cycles (12 hr), temperature (21–23°) and diet (Oxoid 41B). Three experimental animal groups were set up as follows:

(I) The 'chronically treated' received DL-methamphetamine twice a day (every 12 hr)by i.p. injec-

* Current address: Biochemistry Department, Trinity College, Dublin 2, Ireland.

‡ Author to whom correspondence should be addressed.

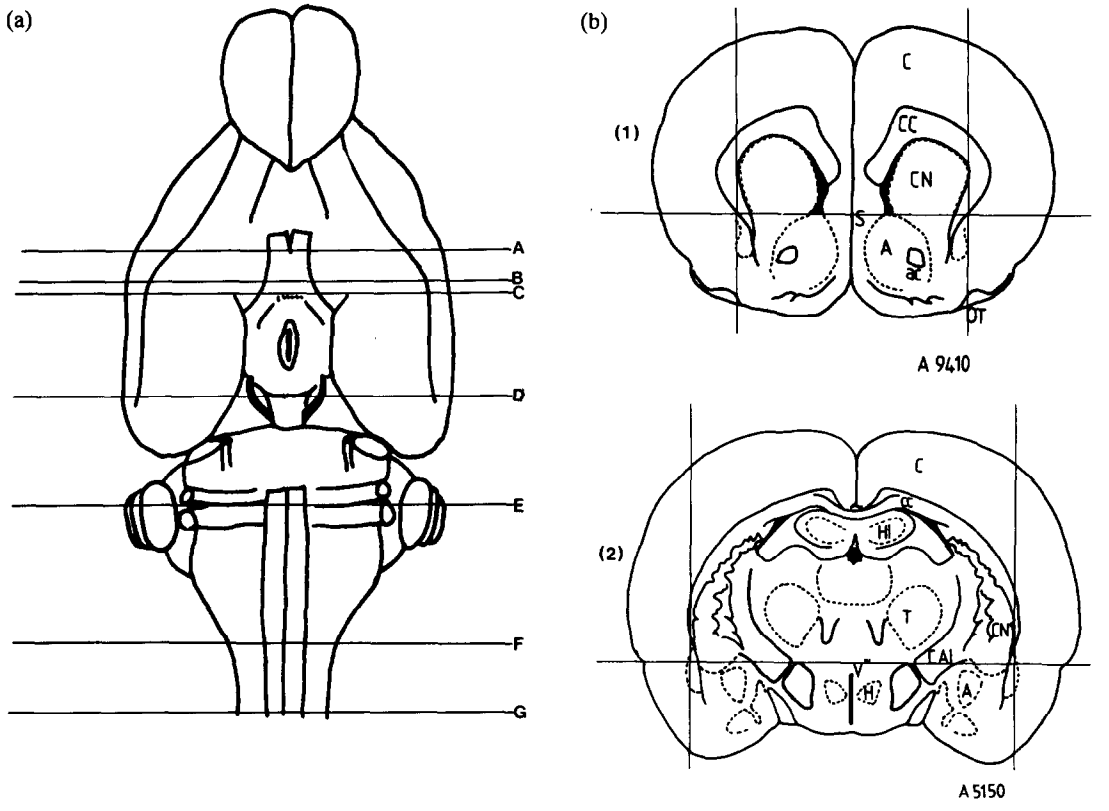

Fig. 1. Dissection technique used. (a) Ventral view of the brain. Vertical sections were made at positions A–G. (b) Sections from cuts A and B (1) and C and D (2) respectively (the relevant references to the Konig & Klippel [24] stereotaxic rat brain atlas are given). Cuts were made at the positions shown. 1. Shows the dissection of the accumbens and striatum (caudate nucleus). 2. Shows the dissection of the hypothalamus and amygdala. When the cuts shown were completed the section containing the hypothalamus and amygdaloid complex was turned to the ventral view when the demarcation of the hypothalamus could be seen and the hypothalamus cut out.

Key: T—thalamic nuclei, CN—caudate nucleus, CC—corpus callosum, HI—hippocampus, H—hypothalamus, ac—accumbens, CAI—capsule interna, OT—olfactory tract, V^{III}—third ventricle, A—amygaloid nuclei.

Cuts E, F, G show the dissection of the pons and medulla, respectively.

tion. The initial dose was 5 mg/kg per 24 hr, increasing to 10 mg/kg per 24 hr after 5 days and to 15 mg/kg per 24 hr after 10 days. The rats were then maintained at this dose for a total period of 30 days. This drug regime was based on that used in a previous study [12].

(II) The 'withdrawn' rats were treated identically to those in the 'chronically treated' group except that they received saline injections 24 hr prior to death.

(III) The control animals received saline injections over the same time period.

All three groups were killed 12 hr after their last injection. The weights, drinking rates and food consumption were measured at the same time each day (12.00–13.00 hr).

Dissection

The rats were killed by decapitation, the brains dissected over ice and the regions were frozen in liquid nitrogen. The regions were then weighed and stored in liquid nitrogen until analysis. The time from decapitation to the stage where all regions had been frozen in liquid nitrogen was 4–5 min.

Figure 1 shows the dissection technique employed.

Tyrosine hydroxylase assay

Tyrosine hydroxylase activity was measured by a modification of the method described by Hendry and Iversen [13]. The assay is based on the conversion of [side-chain-^{3}H]tyrosine to [side-chain-^{3}H]3,4-dihydroxyphenylalanine ([side-chain-^{3}H]DOPA). Further breakdown of the DOPA to dopamine was prevented by the presence of the aromatic amino acid decarboxylase inhibitor, NSD 1055 (NSD 1055 did not affect tyrosine hydroxylase activity). The tissue was homogenized in 5 or 10 vol (depending on the brain region) of 5 mM Tris–HCl buffer, pH 6.0, at 4°.

Tissue homogenate (10 μl) was added to tubes in an ice-bath. The blank tubes contained either 10 μl of homogenizing buffer or 10 μl of homogenate. Several tubes containing 10 μl of [^{3}H]DOPA (20 μCi/ml) served to monitor [^{3}H]DOPA recovery. Then 10 μl of the 'substrate–cofactor mix' was added to all tubes except the DOPA recovery tubes and the homogenate blanks. The 'substrate–cofactor mix' was made up by mixing equal volumes of a solution containing 4 mM $DMPH_4$, 1.3 M mercaptoethanol,

0.4 mg/ml NSD 1055 and 0.4 mM tyrosine in 0.8 M potassium phosphate buffer, pH 6.0, with 300 μCi/ml of L-[side-chain-^{3}H]tyrosine in 3 mM Tris–HCl buffer, pH 8.6. 2-Iodotyrosine was added to the 'substrate–cofactor mix' to a final concentration of 0.1 mM for the homogenate blanks.

On adding the 'substrate–cofactor mix', each tube was mixed carefully and incubated for 15 min in a shaking water bath at 37°. The final concentrations of substrate and cofactor were higher than previously reported [13]. They are not as high as would be ideal in terms of the K_m values, but provided in our system the best compromise between concentrations required for enzyme saturation and concentrations giving substrate-type inhibition. Final concentrations used in this study were 0.1 mM tyrosine and 1 mM-DMPH$_4$. The reaction was terminated by the addition of 200 μl of 0.4 M perchloric acid containing L-DOPA (2 μg/ml).

The [^{3}H]DOPA formed was separated from any unchanged [^{3}H]tyrosine by the adsorption of DOPA onto alumina columns. The alumina was prepared by washing repeatedly with distilled water to remove fine particles, then resuspended in 0.5 M potassium phosphate buffer, pH 7.4. Pasteur pipettes were plugged with cotton wool and filled with alumina to a height of 1 cm. The pipettes were then attached to glass reservoirs and washed with 5 mM Tris–HCl buffer, pH 8.6.

Before pouring onto the column, the contents of each tube were neutralized by adding 3.8 ml of a solution containing 50 mM Tris–HCl buffer, pH 8.6, 75 mM NaOH and 50 mM EDTA. The columns were subsequently washed with 40 ml of 5 mM Tris–HCl buffer, pH 8.6; the [^{3}H]DOPA was eluted with 1.5 ml of 1 M acetic acid into scintillation vials. Triton X-100–toluene scintillant (12 ml; 3:7 v/v) containing 0.5% (w/v) 2,5-diphenyloxazole was added and the samples were counted in a Packard Tri-Carb scintillation counter (3385). Units of enzymic activity, measured in duplicate, are expressed as nmol/min at 37°; we were unable to detect activities below 1.5×10^{-2} units.

Assays of noradrenaline and dopamine

The method used is based on the conversion of noradrenaline and dopamine by catechol-*O*-methyl transferase (EC2.1.1.6), in the presence of [^{3}H]S-adenosyl methionine to labelled normetanephrine and 3-methoxytyramine, respectively. The products were separated either by t.l.c. or by descending paper chromatography, and counted [14, 15].

After homogenization of the brain regions in 5 mM Tris–HCl buffer, pH 6.0, at 4°, an aliquot was removed for the assay of tyrosine hydroxylase and for a protein determination. Perchloric acid (1 M), containing 1 mM EDTA and 10 mM ascorbic acid, was added to a known volume of homogenate to give a final concentration of 0.1 M perchloric acid. The homogenates were mixed and centrifuged in a microfuge (Beckman microfuge B) at 8730 *g* (11,600 rpm) for 3 min at 4°. The supernatant was decanted and stored at −20° overnight. Internal standards were added to the homogenate immediately after the aliquot necessary to measure protein and tyrosine hydroxylase activity had been taken.

Protein determination

The Lowry protein assay [16] was used to measure protein concentration. The standard protein used was bovine serum albumin.

Statistical treatment

The numbers of animals providing each result were normally five and never less than four. Tests for significance are based on the two-tailed Student *t*-test.

RESULTS

Figures 2–5 show the weights and food consumption of the two sets of experimental animal groups;

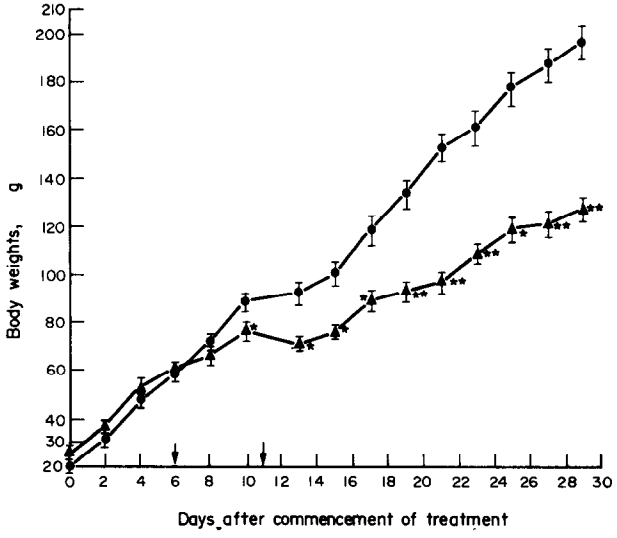

Fig. 2. Body weights of 'chronically treated' (▲) and control (●) groups from set I during the 30 days of treatment. The arrows on the abscissa denote an increase in the dose of methamphetamine administered. The error bars denote the S.E.M. Significant differences from control values: *$P < 0.05$, **$P < 0.0001$.

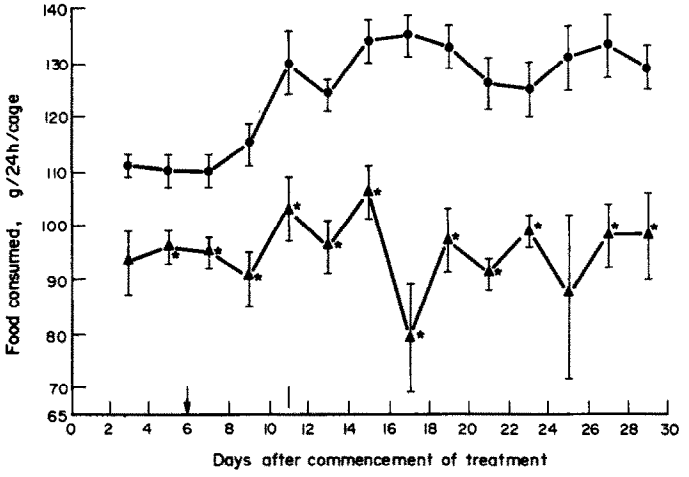

Fig. 3. Food consumption of 'chronically treated' (▲) and control (●) groups from set I during the 30 days of treatment with methamphetamine. Details as for Fig. 2.

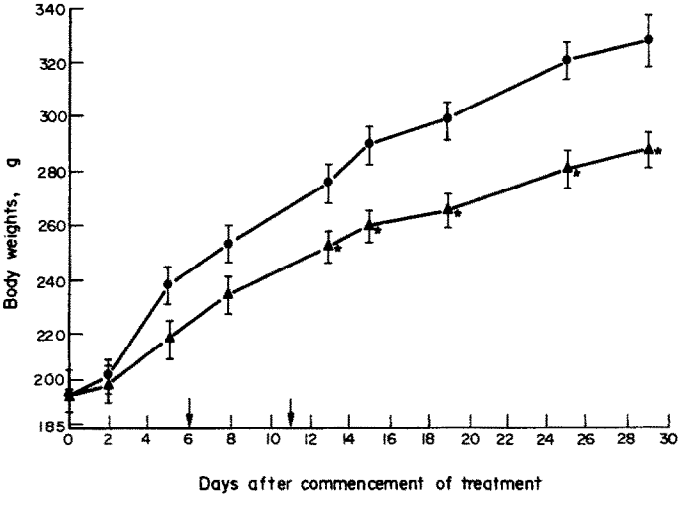

Fig. 4. Body weights of 'chronically treated' (▲) and control (●) groups from set II during the 30 days of treatment with methamphetamine. Details as for Fig. 2.

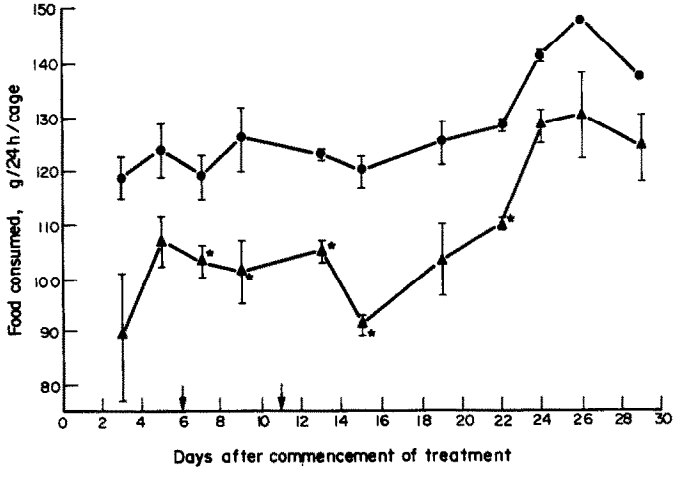

Fig. 5. Food consumption of 'chronically treated' (▲) and control (●) groups from set II during the 30 days of treatment with methamphetamine. Details as for Fig. 2.

since these two sets were run at different times, the experimental animals were compared only with the control group which was being treated simultaneously. The graphs show that the first set (I) did not overcome the anorexic effects of methamphetamine and were only about 60% of the control weights by the end of the experimental period (Fig. 2). Their food consumption (Fig. 3) was also significantly lower (75% of the control values). The second set (II) apparently did overcome the anorexic effects of the drug. The weights of the chronically treated animals were about 90% of their respective controls (Fig. 4) and their food consumption was barely affected (over 90% of control, Fig. 5). Similarly, the drinking rates of the first set (I) were significantly lower than the controls throughout, whereas there was no difference in the second set (II).

Set I, which showed the slower weight increase, with lower rates of consumption of food and water (above), reacted much more adversely to handling, compared with the more 'tolerant' set II, or to both control groups. Although this aspect of their behaviour was not part of our experimental design, and so was not assessed quantitatively, we gained the clear impression that the set I experimental group was under considerably more 'stress' than set II (see Discussion).

Tyrosine hydroxylase activity in the 'chronically treated' set I rats, which did not appear to overcome the anorexic effects of methamphetamine, was after 15 days of treatment lower in all eight regions analysed (Fig. 6a). At this time the 'withdrawn' group showed a partial recovery in the tuberculum olfactorium and complete recovery in the striatum and hypothalamus. After 30 days of treatment (Fig. 6b) the activity of tyrosine hydroxylase in all eight brain regions of the 'chronically treated' group of rats was below the sensitivity of the assay (see Methods). The apparent absence of significant enzymic activity shown in Fig. 6(b) was not due to problems of assay, since these were performed in parallel with control samples of the same brain regions.

The 'withdrawn' rats showed complete recovery of enzymic activity in the striatum, accumbens and amygdala, with partial recovery in the tuberculum

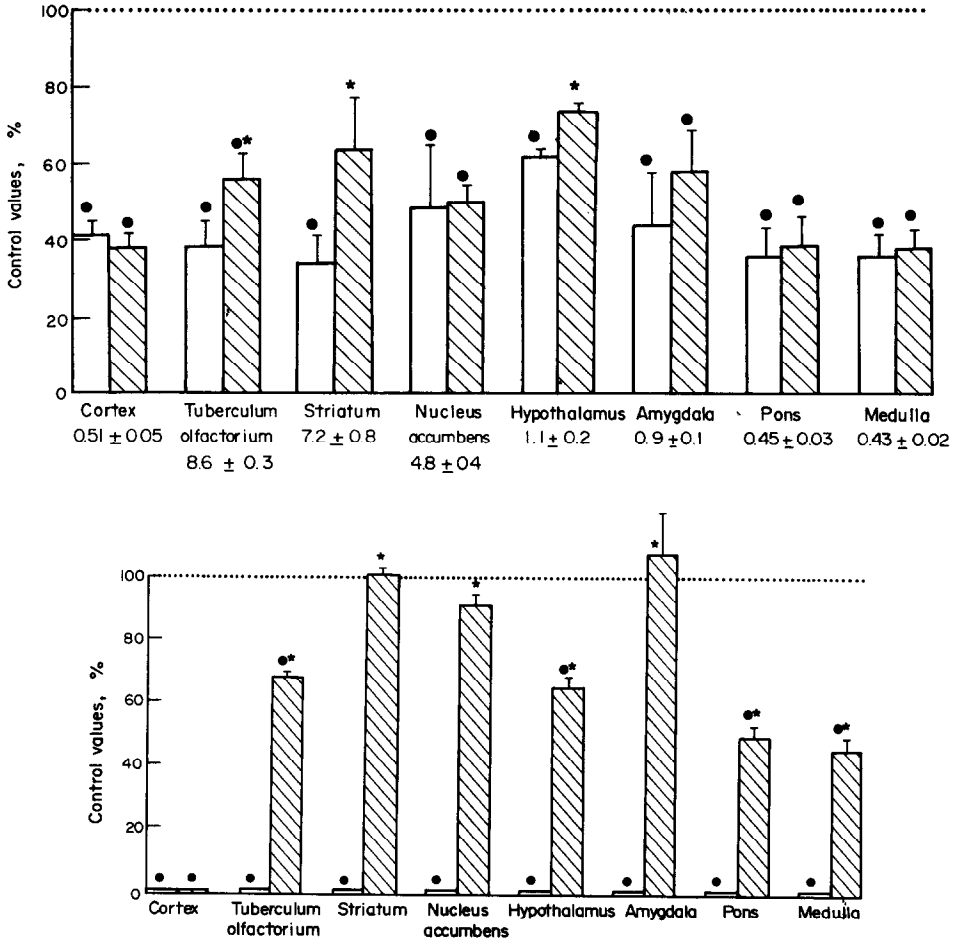

Fig. 6. Tyrosine hydroxylase activity in the 'chronically treated' (□) and 'withdrawn' (▨) rats in experimental set I after 15 days (a) and 30 days (b) of chronic treatment with methamphetamine. The activities are expressed as a percentage of control activities; the activities of the control samples (nmol/min per g fresh weight ±S.D.) appear below each column. The bars denote S.D. ●—Values are significantly different from the control group ($P < 0.01$). *—Values for the withdrawn group are significantly different from the chronic group ($P < 0.01$).

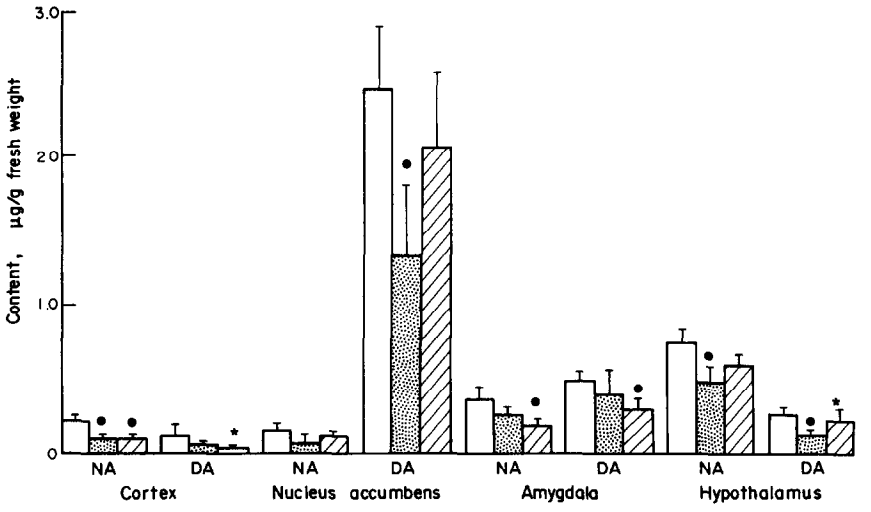

Fig. 7. Contents of dopamine and noradrenaline in rat brain regions of control (□), 'chronically treated' (▨) and 'withdrawn' (▨) rats of set I after 30 days of treatment. The error bars are S.D. ●—Values significantly different from the control group ($P < 0.01$). *—Values for the withdrawn group significantly different from the chronic group ($P < 0.01$).

olfactorium, hypothalamus, pons and medulla (Fig. 6b). It is noticeable that the recovery was lowest in those regions which contain the cell bodies of the major noradrenergic and dopaminergic pathways.

The concentrations of noradrenaline and dopamine were determined in four brain regions from the rats in this first experimental set I. The results, shown in Fig. 7, indicate that chronic methamphetamine treatment appeared to cause some decrease in noradrenaline and dopamine in all regions; this was statistically significant for noradrenaline levels in the cortex, dopamine levels in the accumbens and both noradrenaline and dopamine levels in the hypothalamus. On withdrawal from methamphetamine there was a recovery of dopamine levels in the accumbens, and complete or partial recovery of dopamine and noradrenaline, respectively, in the hypothalamus. In the cortex and amygdala either no change or a further depletion in noradrenaline and dopamine levels was observed.

Similar measurements in the second set of animals in which 'tolerance' to the methamphetamine-induced anorexia was observed (set II) showed no significant changes in either tyrosine hydroxylase activity or noradrenaline and dopamine levels in both the 'chronically treated' and 'withdrawn' rats (Fig. 8). The results of Fig. 8 were more variable than those described above (this is shown in the heights of the error bars); tyrosine hydroxylase activities, in contrast to those of Fig. 6 which were

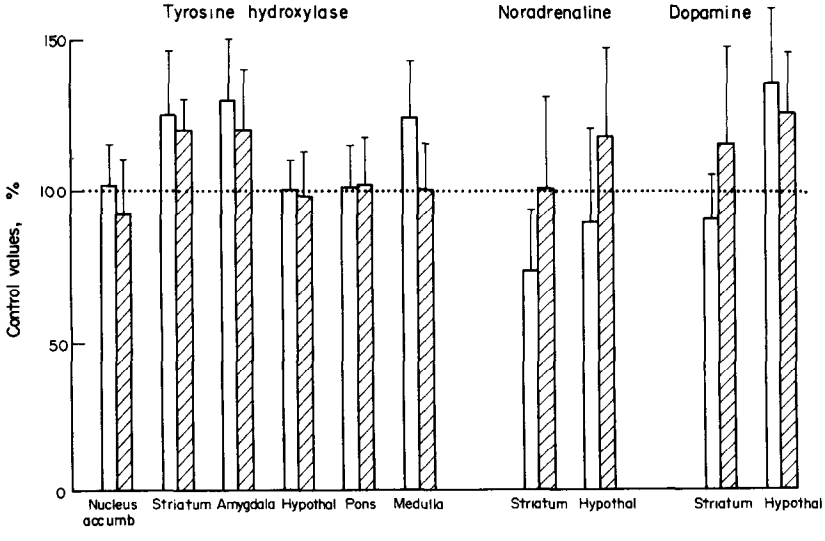

Fig. 8. Tyrosine hydroxylase activities, and contents of dopamine and noradrenaline, in rat brain regions of 'chronically treated' (□) and 'withdrawn' (▨) rats of set II after 30 days of treatment. Details are as for Fig. 6. No value is significantly different from control values ($P > 0.05$).

decreased, tended to be elevated but were not statistically significant.

DISCUSSION

The results presented here show that animals on the same drug regime may show profoundly different biochemical effects after chronic treatment with methamphetamine. Rats which remained anorexic on such treatment showed a considerable depletion in tyrosine hydroxylase activity after 15 and 30 days of treatment, with recovery of the activity in some but not all regions on withdrawal. The results showed that no significant recovery was seen in the cell-body-containing regions, but only in those rich in dopaminergic or noradrenergic nerve terminals; this would seem to indicate an activation of pre-existing enzyme as opposed to an increased synthesis, since synthesis of the enzyme occurs mainly in cell bodies from where it is transported to the nerve terminals by axonal flow [17]. The changes in noradrenaline and dopamine levels do not parallel those in tyrosine hydroxylase activity; one possible explanation for this is that there is a lag period between increased enzyme activity and the replenishing of the noradrenaline and dopamine stores. The results obtained from the second experimental set of animals show that if the rats develop 'tolerance' to the methamphetamine-induced anorexia, there are no such changes in brain catecholamine metabolism.

Therefore, there seems to be a correlation between 'tolerance', as measured by the ability to overcome the effects of methamphetamine-induced anorexia, and brain catecholamine metabolism. That the two sets of animals were on the same drug regime emphasizes the importance of using a behavioural criterion when measuring biochemical changes on long-term drug administration.

A possible contributory factor to an interpretation of these results can be found by comparing them with those reported by Ellison *et al.* [11]. They showed that when pellet implantation was used as the vehicle for long-term administration of (+)-amphetamine, there was damage to the nerve fibres in the caudate and a corresponding drop in tyrosine hydroxylase activity. However, when the same calculated dose was given intraperitoneally, there was no decrease in tyrosine hydroxylase activity. This shows that the biochemical effects of a continuous intoxication with amphetamine are radically different from sporadic intoxication. These data and the observation that 'stress' releases stored amphetamine from the adipose tissue of rats that have been chronically pretreated with amphetamine [S. Sparber, personal communication] suggests that if the animals in set I were more 'stressed' than those in set II, the 'effective dose' of circulating amphetamine may have been higher and the rats intoxicated for a longer period of time. It is not possible to state with certainty that set I animals were more 'stressed' since this aspect was not part of our experimental design, as has often been the case in biochemical studies; however, we could not fail to observe that set I animals during handling showed more aggression, in that they reacted violently to handling, in comparison to set II or both control groups.

Stress has been shown to correlate with changes in amine metabolism *per se* [18, 19], and to increase the effects of methamphetamine on amine depletion [20–24].

One explanation for the results obtained from experimental Set I is that they were subjected to more amphetamine and/or intoxicated for a longer period of time owing to an increased level of 'stress'. The results of these experiments demonstrate that it may no longer be adequate to report only the drug regime and corresponding biochemical changes in the brain without an independent behavioural assessment.

Acknowledgements—We are grateful to the University of Bath and to the Department of Education (Ireland) for financial support to M.E.B.

REFERENCES

1. F. Javoy, Y. Agid, D. Bouvet and J. Glowinski, *J. Pharm. Pharmac.* **26**, 179 (1974).
2. M. J. Besson, A. Cheramy, C. Gauchy and J. Musacchio, *Eur. J. Pharmac.* **22**, 181 (1973).
3. M. J. Besson, A. Cheramy and J. Glowinski, *J. Pharmac. exp. Ther.* **177**, 196 (1971).
4. S. P. Bagchi and P. L. McGeer, *Life Sci.* **3**, 1195 (1964).
5. E. G. McGeer and P. L. McGeer, *Can. J. Biochem.* **45**, 115 (1967).
6. J. E. Harris and R. J. Baldessarini, *J. Pharm. Pharmac.* **25**, 755 (1973).
7. H. C. Fibiger and E. G. McGeer, *Eur. J. Pharmac.* **16**, 176 (1971).
8. L. Y. Koda and J. W. Gibb, *J. Pharmac. exp. Ther.* **185**, 42 (1973).
9. M. K. Buening and J. W. Gibb, *Eur. J. Pharmac.* **26**, 30 (1974).
10. E. C. Hulme, R. Hill, M. North and M. R. Kibby, *Biochem. Pharmac.* **23**, 1393 (1974).
11. G. Ellison, M. S. Eison, H. S. Huberman and F. Daniel, *Science* **201**, 276 (1978).
12. D. M. Manning, R. H. C. Strang and H. S. Bachelard, *Biochem. Pharmac.* **23**, 1265 (1974).
13. I. A. Hendry and L. L. Iversen, *Brain Res.* **29**, 159 (1971).
14. A. C. Cuello, R. Hiley and L. L. Iversen, *J. Neurochem.* **21**, 1337 (1973).
15. N. E. Bardsley and H. S. Bachelard, *Cell. Molec. Biol.* **27**, 71–76 (1981).
16. O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. Randall, *J. biol. Chem.* **193**, 265 (1951).
17. H. McIlwain and H. S. Bachelard, *Biochemistry and the Central Nervous System*, 4th Ed. Churchill-Livingstone, London (1971).
18. Y. Ishii, M. Homma and Y. Yoshikawa, *Neuropharmacology* **14**, 155 (1975).
19. G. Curzon, in *Chemical Communication within the Nervous System and its Disturbance in Disease* (Eds. A. Taylor and M. T. Jones), p. 45. Pergamon Press, Oxford (1978).
20. M. Beauvallet, B. M. Halpern, J. Fugazza and C. Drudi-Baracco, *Biochem. Pharmac.* **12**, Suppl. 8 (1962).
21. M. Beauvallet, J. Fugazza and M. Legrand, *Therapie* **22**, 1273 (1967).
22. K. E. Moore and E. W. Lariviere, *Biochem Pharmac.* **13**, 1283 (1963).
23. K. E. Moore and E. W. Lariviere, *Biochem. Pharmac.* **13**, 1098 (1964).
24. A. I. Salama and M. E. Goldberg, *Archs Int. Pharmacodyn. Ther.* **181**, 474 (1969).