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EFFECTS OF CHRONIC ADMINISTRATION OF DRUGS WHICH MODIFY NEUROTRANSMITTER RE-UPTAKE, STORAGE AND TURNOVER ON LEVELS OF TYROSINE AND TRYPTOPHAN HYDROXYLASE IN RAT BRAIN

EDWARD C. HULME, RENA HILL, MARCEL NORTH and MICHAEL R. KIBBY

Department of Biochemistry, University of Strathclyde, Glasgow G1 1XW, Scotland

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Abstract—Oral ingestion of *d*-amphetamine at a rate of 11·7 ± 2 mg/kg/24 hr (mean ± S.D.) failed to significantly alter fore- and midbrain tyrosine hydroxylase activities, measured *in vitro*, with respect to control activities after 3 or 7 days pretreatment, or hindbrain levels of the enzyme after 3 days pretreatment. Similarly, prolonged pretreatment with reserpine (1 mg/kg/24 hr), the dopamine-β-hydroxylase inhibitor FLA-63 (25 mg/kg/24 hr) or the DOPA decarboxylase inhibitor NSD1015 (25 mg/kg/24 hr) did not change fore- and midbrain levels of the enzyme, either when these drugs were given alone, or in combination with *d*-amphetamine. However, fore- and midbrain tyrosine hydroxylase levels in animals pretreated for 7 days with amphetamine alone were significantly enhanced in comparison with animals pretreated with reserpine, or an amphetamine/reserpine combination. The same trend may have been observable after 3 days, but failed to attain significance within this period.

d-Amphetamine, reserpine and an amphetamine/reserpine combination failed to alter fore- and midbrain tryptophan hydroxylase levels after 7 days pretreatment, and neither amphetamine nor an amphetamine/NSD1015 combination altered hindbrain levels of the enzyme. Seven days pretreatment with the tricyclic antidepressant drugs imipramine and iprindole (10 mg/kg/48 hr) exerted no effect on fore- and midbrain tryptophan hydroxylase levels.

These results indicate that hydroxylase levels in central monoamine neurones, unlike sympathetic postganglionic neurones, appear to be relatively insensitive to agents which lead to prolonged alteration of neurotransmitter turnover rates. This may reflect differences between peripheral and central monoamine neurones in terms of both anatomical and functional relationships.

IT HAS been known for some time that a chronic increase in the rate of catecholamine (CA) turnover within the sympathetic nervous system leads to an associated induction of the neurotransmitter-synthesising enzymes tyrosine hydroxylase and dopamine-β-hydroxylase. Such observations have been made both in sympathetic ganglia, and in the chromaffin cells of the adrenal medulla. Conversely, any reduction of the turnover rate or CA in these structures is accompanied by a decrease in the levels of the above enzymes.[1–9]

The extension of these observations to the central nervous system has been limited,[10,11] and little is known of the response of tryptophan hydroxylase to factors which modify amine turnover rates.

The present work was designed to test the hypothesis that a prolonged change in the turnover rate of the functional pool of CA or 5HT in the central nervous system produces a change in the activity of the corresponding synthesising enzymes.

Certain drugs have been used as pharmacological tools for this purpose. Substantial evidence exists to suggest that one of the major effects produced by *d*-amphetamine is an increased rate of turnover of the functional pools of neurotransmitter in central adrenergic and dopaminergic neurones. The drug causes preferential release of newly-synthesised CA from brain slices.[12] Its microiontophoretic application to noradrenergic brain-stem neurones mimics the effect of iontophoretically applied noradrenaline (NA), and this effect is abolished after depletion of CA stores by treatment with reserpine, or inhibition of tyrosine hydroxylase with α-methyl-*p*-tyrosine, or inhibition of dopamine-β-hydroxylase with FLA-63.[13] Low doses of *d*-amphetamine enhance central CA turnover, particularly that of striatal dopamine (DA).[14] Finally, the dependence of the central activating effects of *d*-amphetamine on uninterrupted CA synthesis is demonstrated by the antagonism of tyrosine hydroxylase and dopamine-β-hydroxylase inhibition on the behavioural stimulation produced by the drug.[15,16]

There is also evidence to suggest that the level of activity in central catecholaminergic neurones can modulate serotonergic activity,[17] and amphetamines are known to enhance 5HT turnover rates in the central nervous system.[18] An attempt to study the effects of modification of the rate of turnover of 5HT with rather greater specificity has been made by the use of the tricyclic antidepressant drug imipramine. This drug differentially inhibits 5HT uptake by brain slices with respect to NA,[19] and reduces central 5HT turnover when administered both acutely,[20] and chronically.[21]

We have therefore studied the effects of chronic administration of *d*-amphetamine, and of the amphetamine antagonists reserpine, FLA-63,[16] and the aromatic amino acid decarboxylase inhibitor 3-hydroxybenzyl hydrazine (NSD1015)[22] on central levels of tyrosine and tryptophan hydroxylase levels, and of prolonged dosing with imipramine on tryptophan hydroxylase activity. *d*-Amphetamine, and amphetamine antagonists were adminstered both separately and in combination.

In addition, we felt that it would be of interest to compare the possible effects of imipramine, and the tricyclic antidepressant drug iprindole on central tryptophan hydroxylase levels. The latter is a far less potent inhibitor of 5HT uptake by brain slices than the former,[24] but both compounds appear to have similar clinical profiles as far as antidepressant activity is concerned.[25]

A previous study[11] has reported the effects of chronic administration of methamphetamine on tyrosine hydroxylase activity in crude homogenates of brainstem, and of striatal tissue, but it has been pointed out[23] that activity measured under these conditions may well reflect the physical state of the enzyme, rather than the amount of enzyme protein present. A solubilised preparation of tyrosine hydroxylase has therefore been used in these studies.

There is a substantial discrepancy between the long time-course of relief of depression during treatment with tricyclic antidepressants,[26] and the rapid expression of their effects on 5HT turnover rates in brain, suggesting that there may be no immediate link between the two effects, a suggestion which is supported by the effectiveness of iprindole, a rather poor inhibitor of monoamine uptake by brain tissue, as

an antidepressant. If, however, the development of the clinical effects of these drugs does involve a modification of central serotonergic activity, either as an indirect effect of a change in 5HT turnover rates, or as an effect of some other mechanism, a change in the activities of enzymes involved in the metabolism of 5HT, which might be expected to have a rather similar time-course, could provide a valuable biochemical correlate. We have investigated this possibility in the case of tryptophan hydroxylase.

MATERIALS AND METHODS

Animals. The animals used in this series of experiments were male Sprague–Dawley rats of the Holtzman strain, weighing 219 ± 10 g (mean ± S.D.), housed at 22° under uniform conditions of lighting, at a population density of 4–6 per cage.

Drug administration schedules. *d*-Amphetamine sulphate was administered at a concentration of 100 μg/ml in the animals' drinking water, in an attempt to produce a fairly uniform dosage rate. This concentration of the drug had no perceptible taste. Preliminary experimentation established that the mean 24 hourly rate of amphetamine ingestion reached a steady value within a few hours, and showed no tendency to systematic variation within the longest pretreatment time used (7 days). The mean rate of amphetamine consumption for rats presented with 100 μg/ml *d*-amphetamine sulphate, and laboratory chow *ad lib.*, was 11·7 ± 2·0 mg/kg/24 hr (mean ± S.D., 13 measurements).

Reserpine (1 mg/kg/24 hr) and FLA-63 (25 mg/kg/24 hr) were injected intraperitoneally (i.p.) as fine sonicated suspensions in 1% (w/v) gum tragacanth in isotonic saline. NSD1015 (25 mg/kg/24 hr), imipramine–HCl (10 mg/kg/48 hr) and iprindole–HCl (10 mg/kg/48 hr) were injected in solution in isotonic saline. Volumes injected were 0·2 ml. Control animals were injected with vehicle only. Injections were performed daily at 16.00 hours.

In order to compensate for the well-known hypodipsic and anorexic effects of *d*-amphetamine, control animals, and animals receiving amphetamine antagonists, either alone or in combination with amphetamine, were pair-fed and watered with animals receiving amphetamine alone or, failing this, with that group having the lowest mean rate of water and food consumption. Animal weights were monitored at the commencement and termination of each experiment on a group basis. Both amphetamine-treated animals and pair-fed controls tended to lose about 5 per cent of their body weight over a 3-day period, whilst animals fed and watered *ad lib.* tended to increase in weight by a similar amount.

Drug pretreatment schedules were commenced at 16.00 on day 1, and terminated at 9.00 on day 4 or day 8. Animals were killed, and assays performed, in random order.

Preparation of tissue extracts. Rats were sacrificed by stunning, followed by decapitation, and the brain rapidly removed and dissected on an ice-cold plate. Hydroxylase activities were assayed in two brain subsections. The first of these, consisting of striatum, hippocampus, hypothalamus, thalamus, and midbrain, had a mean weight of 0·79 ± 0·1 g, and contained 70 per cent of the total brain tyrosine hydroxylase activity, in good agreement with previously-published figures.[27]

According to the latter, and anatomical data concerning the distribution of monoaminergic neurones in rat brain,[28] the majority of this activity should have been contributed by tyrosine hydroxylase located in synaptic terminals, and axons,

about 50 per cent of it being attributable to the dopaminergic terminals of the caudate nucleus. This subsection of brain also contains about 80 per cent of the total brain content of tryptophan hydroxylating activity.[29]

The second subsection of brain assayed, designated "hindbrain" was designed to contain the cell bodies of the catecholaminergic neurones, while as far as possible excluding the synaptic terminals, and consisted of the medulla and pons, together with a piece of tissue contained between, and ventral to a horizontal plane through the Sylvian aqueduct, and the 1950 plane of König and Klippel,[30] excluding any pieces of hippocampus and cortex. This subsection had a mean weight of 0·24 ± 0·037 g, and accounted for 10 per cent of the total tyrosine hydroxylating activity of the brain, again in good agreement with previously published data.

There was no tendency for the weights of either of these two brain subsections to vary in response to the drug treatments used in the current series of experiments.

Tissue samples were homogenised in 1 ml of ice-cold Tris·Cl buffer, containing 0·2 per cent w/v of Triton X-100, in a Potter Elvehjem homogeniser with a rotating teflon pestle, and centrifuged at 20,000 *g* for 10 min at 0°. In the case of the fore and midbrain subsection, the pellet was thoroughly resuspended in 1·0 ml of the same buffer, and recentrifuged, the supernatant being added to the supernatant from the first centrifugation. Supernatants were assayed for tyrosine and tryptophan hydroxylase activity as described below, and were found to contain more than 95 per cent of the tyrosine hydroxylase activity of the tissue homogenised. On occasion, enzyme samples were dialysed at 4° against two 1 litre changes of Tris·Triton buffer, supplemented with 10 ml/l. of β-mercaptoethanol, and re-assayed.

Tyrosine hydroxylase assay. Tyrosine hydroxylase activity was assayed according to a modification of the method of Nagatsu *et al.*[31] The assay mixture contained 20 μmoles of Tris·Cl, 200 nmoles of tetrahydrobiopterin, or 6, 7-dimethyl-5,6,7,8-tetrahydropterin ($DMPH_4$), 20 nmoles of $FeSO_4$, 100 nmoles of pargyline, 2 μl of β-mercaptoethanol, 5×10^5 dis/min of [3,5-^{3}H]-*l*-tyrosine (33, or 42 Ci/m-mole), and 50 μl of enzyme sample at a pH of 6·2, and in a final volume of 0·25 ml. Duplicate incubations were carried out for 60 min at 37°. The reaction was stopped by the addition of 0·75 ml of 2·5 per cent w/v trichloracetic acid, and precipitated protein removed by centrifugation after cooling in ice for 15 min. 0·5 ml aliquots of the supernatant were applied to 5 × 0·6 cm columns of Amberlite IR120 (H^+ form), and tritiated water eluted with 5 ml of distilled water, amino acids and amines being retained by the ion-exchange resin. Formation of acidic labelled products was prevented by the presence of the monoamine oxidase inhibitor in the incubation medium. One ml samples of the eluate were mixed with 10 ml of scintillation fluid, and counted, as described below. Tritiated tyrosine was purified immediately before use by chromatography on a small column of Amberlite IR120 (H^+ form).

Product formation was directly proportional both to time and to enzyme concentration over the range used in these assays. Tyrosine hydroxylase activity was completely dependent on the presence of a reduced pteridine cofactor. The apparent K_m's of the enzyme for $DMPH_4$, and tetrahydrobiopterin were 500, and 417 μM respectively. The K_m for tyrosine under our assay conditions was in excess of 200 μM, and hence there was no significant variation in apparent enzyme activity (in the sense of dis/min of product formed/hr/mg protein) with tyrosine specific activity over the range which might be expected to result from changes in endogenous tyrosine levels.

Nevertheless, particularly when pretreating with drugs such as NSD1015 known to cause substantial increases in brain tyrosine levels,[22] enzyme samples were re-assayed after dialysis for 24 hr under the conditions described in the previous section. Enzyme samples supplemented with a concentation of β-mercaptoethanol equivalent to that present in the dialysis buffer, and stored at 4°, tended to lose about 30 per cent of their activity within 24 hr. This inactivation was prevented by dialysis. However, dialysis in no case altered the tyrosine hydroxylase activity of enzyme samples derived from treated animals relative to those derived from controls.

Concentrations of NA and DA in the incubation media were of the order of 5×10^{-7} M, which are well below those at which they are effective tyrosine hydroxylase inhibitors.

Enzyme activities measured by the method described generally exceeded blank values, which were of the order of 1000 dis/min by a factor of about 10.

Tryptophan hydroxylase assay. Tryptophan hydroxylase activity was assayed by a modification of the method of Sanders-Bush *et al.*[32] The assay mixture contained 12·5 μmoles of Tris–Cl, 250 nmoles of 5HT (creatinine sulphate complex), 25 nmoles of pyridoxal phosphate, 250 nmoles of $DMPH_4$, 100 nmoles of tranylcypromine, 25 nmoles of $FeSO_4$, 2·5 μl of β-mercaptoethanol, 10^7 dis/min of [G-^{3}H]-*l*-tryptophan (1 Ci/mmol) and 50 μl of enzyme preparation, at pH 7·7 and in a final volume of 0·3 ml. Duplicate incubations were carried out in air at 37° for 60 min, following which assay mixtures were diluted to 1·3 ml with distilled water, cooled to 0°, and rapidly applied to 5 × 0·6 cm columns of Zeo-Karb 226 (100–120 mesh, K^+ form), or Amberlite CG50 (100–120 mesh, K^+ form), and washed on with 2 ml of distilled water. Unchanged tryptophan was washed off the columns with 50 ml of distilled water, and labelled serotonin eluted with 5 ml of 4 M acetic acid.

The recovery of serotonin fluorescence was measured for each sample, and used to compensate for fluctuations occurring during the isolation procedure. Aliquots (2 ml) were withdrawn from the eluates, mixed with 14 ml of scintillator, and counted, as described below.

[^{3}H]-*l*-Tryptophan was purified immediately before use by passage through a small column of Zeo-Karb 226 (K^+ form).

Formation of [^{3}H]-serotonin was directly proportional to time, and to enzyme concentration over the range used in these assays. The apparent K_m of the enzyme for tryptophan was 475 μM, and hence there was no discernible dependence of apparent enzyme activity on tryptophan specific activity over the range of variation which would be expected from fluctuations in endogenous tryptophan concentrations. Enzyme activity was inhibited in the absence of O_2. Serotonin was the only radioactive amine detectable in the incubation medium described above by descending chromatography on cellulose phosphate paper according to the method of Butterworth.[33]

Enzyme activities measured by this method were generally about 5 times the blank values obtained by omission of the enzyme from the incubation mixture, which were about 1000 dis/min.

Miscellaneous. The scintillation fluids used throughout this series of experiments consisted of 2 parts of toluene to 1 part of Triton X-100 (v/v), and contained 5 g of 2,5-diphenyloxazole, and 0·5 g of 1,5-bis[2-(5-phenyloxazolyl)] benzene/litre. Samples were counted in a Packard Tri-Carb 4000, or a Nuclear Chicago Isocap

liquid scintillation spectrometer. Variations in counting efficiency were corrected by the external standard channels ratio method.

Protein concentrations were measured by the method of Lowry *et al.*[34]

The statistical significance of differences between data obtained from variously pretreated groups of animals was assessed by the performance of a one way analysis of variance, followed, when the value of F obtained was significant at $P = 0.05$ by two-tailed t-tests between individual groups.

Results throughout have been expressed in terms of enzyme activity/g wet wt of tissue sample, and have been given as a percentage of control values to exclude small day to day variations. The pattern of results was not altered by expression in terms of protein concentration in the assay mixture.

Drugs, biochemicals and radiochemicals. [3,5-^{3}H]-*l*-Tyrosine (33 and 42 Ci/mmole). and [G-^{3}H]-*l*-tryptophan (1 Ci/m-mole) were obtained from the Radiochemical Centre, Amersham, Bucks., U.K. $DMPH_4$ was obtained from Sigma (London). 5HT (creatinine sulphate complex) was obtained from Koch-Light Laboratories Ltd. *d*-Amphetamine sulphate was supplied by Halewood Chemicals Ltd. NSD1015 was the product of Smith and Nephew Research Ltd. FLA-63 was the gift of Astra Chemicals Ltd. Iprindole HCl was the gift of John Wyeth & Brother Ltd., Maidenhead, Bucks., U.K. Tetrahydrobiopterin was the gift of Dr. R. Long of Roche Products. Tranylcypromine, imipramine-HCl and pargyline were the gifts of Dr. A. L. Green of this department.

RESULTS

Effects of amphetamine and reserpine on food and water intake. Preliminary experimentation established that the rate of *d*-amphetamine ingestion attained during pretreatment was sufficient to cause strong anorexia and hypodipsia (Table 1), and that reserpine significantly antagonised the former ($P < 0.005$) but not the latter ($P > 0.05$) effect.

TABLE 1. EFFECTS OF AMPHETAMINE, RESERPINE AND AMPHETAMINE/RESERPINE COMBINATIONS ON FOOD AND WATER CONSUMPTION*

Drug pretreatment	Water consumption (ml/rat/24 hr)	Food consumption (g/rat 24 hr)
Control	39·5 ± 1·3(14)	19·25 ± 0·95(8)
Reserpine	38·4 ± 2(11)	16·4 ± 0·6(5)
Amphetamine	25·6 ± 1·2(13)†	8·1 ± 0·48)†
Amphetamine + reserpine	28·4 ± 2(11)†	12·6 ± 1·2(5)‡

* Reserpine (1 mg/kg/24 hr) was injected in suspension in 0·2 ml of 1 per cent gum tragacanth in isotonic saline. *d*-Amphetamine sulphate was present at a concentration of 100 μg/ml in drinking water. Non-reserpinised rats were injected with vehicle only. Each observation consists of the mean 24-hourly rates of food and water consumption of a group of six animals. Results are presented as mean ± S.E.M. Figures in parentheses indicate the number of observations from which the mean is calculated. Food and water consumption rates for groups of animals subjected to a given drug pretreatment did not vary significantly from day to day over a 7 day period.

† $P < 0.001$ with respect to controls. ‡ $P < 0.005$ with respect to controls and amphetamine + reserpine group.

Tyrosine hydroxylase levels. Pretreatment for a 3-day period with *d*-amphetamine sulphate led to small increases in tyrosine hydroxylase activities in the fore- and midbrain structures assayed in four out of five experiments, which varied from 5 to 42 per cent. In the remaining experiment, in which groups of four rather than six animals were used, a non-significant decrease of 9 per cent was observed. Analysis of the pooled data revealed that any increase in fore- and midbrain tyrosine hydroxylase levels produced by pretreatment with *d*-amphetamine for a 3-day period just failed to reach significance at the 5 per cent level ($0{\cdot}05 < P < 0{\cdot}07$, Table 2).

TABLE 2. EFFECTS OF (a) AMPHETAMINE, RESERPINE AND AMPHETAMINE/RESERPINE COMBINATIONS (b) FLA-63 AND AMPHETAMINE/FLA-63 COMBINATIONS AND (c) NSD1015 AND AMPHETAMINE/NSD1015 COMBINATIONS ON LEVELS OF TYROSINE HYDROXYLASE ACTIVITY IN FORE- AND MID-BRAIN, EXCLUDING CORTEX*

	Tyrosine hydroxylase activity (% of control)	
	Durations of pretreatment	
Drug pretreatment	3 days	7 days
Control	100 ± 4·3 (24)	100 ± 5 (6)
Reserpine	98·2 ± 6·4 (20)	92 ± 3 (6)
Amphetamine	112·7 ± 5·3 (25)	106 ± 3 (6)†
Amphetamine + reserpine	101·3 ± 5·1 (20)	93 ± 4 (6)
Control	100 ± 11 (6)	
FLA-63	76 ± 16 (5)	
Amphetamine + FLA-63	103 ± 12 (5)	
Control	100 ± 6 (4)	100 ± 7 (4)
NSD1015	98 ± 8 (4)	76 ± 8 (3)
Amphetamine + NSD1015	86 ± 9 (4)	82 ± 5 (3)

* Reserpine (1 mg/kg/24 hr), FLA-63 (25 mg/kg/24 hr) and NSD 1015 (25 mg/kg 24 hr) were administered by i.p. injection as described in materials and methods. *d*-Amphetamine sulphate was administered orally. In each experiment groups of animals were pair fed and watered with the group having the lowest rate of water and food consumption. These were (a) the amphetamine-treated group (b) the FLA-63/amphetamine group and (c) the NSD1015/amphetamine group. Results are given as mean ± S.E.M. In case (a), observations from five separate 3 day pretreatment experiments have been normalised and pooled by division of individual observations by the mean activity of the corresponding control group. Numbers in parentheses indicate the number of observations in each group.

† $P < 0{\cdot}01$ with respect to reserpine group; $P < 0{\cdot}05$ with respect to amphetamine + reserpine group.

In no case, therefore, did pretreatment with amphetamine, and amphetamine antagonist or a combination of the two significantly alter fore- and midbrain levels of tyrosine hydroxylase, with respect to control levels, whether the duration of pretreatment was 3 or 7 days (Table 2). This lack of effect of amphetamine was confirmed in two experiments in which groups of six amphetamine-treated animals were compared with pair-fed controls, without being subjected to the stress of intraperitoneal injection. Similarly, 3 days of pretreatment with amphetamine, or an amphetamine/NSD1015 combination exerted no effect on hindbrain tyrosine hydroxylase

TABLE 3. EFFECTS OF AMPHETAMINE, AND AMPHETAMINE/NSD1015 COMBINATIONS ON *in vitro* LEVELS OF TYROSINE HYDROXYLASE ACTIVITY IN HINDBRAIN*

Drug pretreatment	Tyrosine hydroxylase activity (% of control)
Control	100 ± 8 (6)
Amphetamine	92 ± 4 (6)
Amphetamine + NSD1015	110 ± 10 (5)

* NSD1015 (25 mg/kg/24 hr) was administered by i.p. injection, and *d*-amphetamine sulphate orally, as described in materials and methods. The control group, and the group treated with the amphetamine/NSD1015 combination were pair-fed and watered with the amphetamine group. Duration of pretreatment was 3 days. Results are expressed as a percentage of mean of control activity ± S.E.M. Figures in parentheses indicate the number of observations in each group.

activity (Table 3). However, significant variation appeared to develop amongst fore- and midbrain tyrosine hydroxylase levels of animals treated for 7 days with amphetamine, reserpine, and an amphetamine/reserpine combination (Table 2). The enzyme activity measured for the amphetamine-treated group is significantly higher than that measured for the reserpine-treated group ($P < 0.01$), and for the group treated with the drug combination ($P < 0.05$). The same trend may have been observable after 3 days of pretreatment, but in this case it failed to reach significance. The interpretation of this effect is not obvious, and the situation may have been obscured by the extreme debilitation of animals after prolonged reserpinisation or amphetamine treatment.

Tryptophan hydroxylase levels. Tables 4 and 5 present comparable data for tryptophan hydroxylase levels in fore- and midbrain, and in hindbrain. Pretreatment for 7 days with amphetamine, reserpine, or a combination of the two drugs failed to alter fore- and midbrain enzyme levels (Table 4), and as in the case of tyrosine hydroxylase, neither amphetamine nor an amphetamine/NSD1015 combination influenced hind-

TABLE 4. EFFECTS OF (a) AMPHETAMINE, RESERPINE, AND AMPHETAMINE/RESERPINE COMBINATIONS AND (b) IPRINDOLE AND IMIPRAMINE ON *in vitro* LEVELS OF TRYPTOPHAN HYDROXYLASE ACTIVITY IN FORE- AND MIDBRAIN, EXCLUDING CORTEX*

Drug pretreatment	Tryptophan hydroxylase activity (% of control)
Control	100 ± 11 (6)
Reserpine	79 ± 12 (6)
Amphetamine	83 ± 11 (6)
Reserpine + amphetamine	91 ± 12 (6)
Control	100 ± 9 (4)
Iprindole	100 ± 14 (4)
Imipramine	95 ± 6 (4)

* Reserpine (1 mg/kg/24 hr) was administered by i.p. injection, and *d*-amphetamine sulphate orally, as described in materials and methods. The control group, and groups receiving reserpine, and amphetamine + reserpine were pair-fed and watered with the group receiving amphetamine alone. Imipramine·HCl (10 mg/kg/48 hr) and iprindole·HCl (10 mg/kg/48 hr) were given by i.p. injection, as described in materials and methods. Duration of pretreatment was 7 days in both experiments. Results are expressed as % of the mean activity of the appropriate control group. ± S.E.M. Figures in parentheses indicate the number of observations in each group.

TABLE 5. EFFECTS OF AMPHETAMINE AND AMPHETAMINE/NSD1015 COMBINATIONS ON *in vitro* LEVELS OF TRYPTOPHAN HYDROXYLASE ACTIVITY IN HINDBRAIN*

Drug pretreatment	Tryptophan hydroxylase activity (% of control)
Control	100 ± 12 (6)
Amphetamine	91 ± 14 (6)
Amphetamine + NSD1015	105 ± 6 (5)

* NSD1015 (25 mg/kg/24 hr) was administered by i.p. injection, and *d*-amphetamine sulphate orally, as described in materials and methods. The control group and the group treated with amphetamine + NSD1015 were pair-fed and watered with the amphetamine-treated group. Duration of pretreatment was 3 days. Results are expressed as a percentage of the mean activity of the control group + S.E.M. Figures in parentheses indicate the number of observations in each group.

brain levels (Table 5). Similarly, chronic administration of the two tricyclic antidepressant drugs imipramine and iprindole (7 days pretreatment) had no significant effect on and midbrain tryptophan hydroxylase activity (Table 4).

DISCUSSION

The results presented here indicate the existence of marked differences between central monoamine neurones and sympathetic postganglionic neurones in respect of their responses to prolonged alteration of neurotransmitter turnover rates.

Prolonged treatment with *d*-amphetamine sulphate, an agent known to increase central CA turnover rates and which probably exerts its primary effect on the functional pool of transmitter in central CA neurones, had no marked effect on brain tyrosine hydroxylase levels, although there may have been a slight trend towards increased levels of the enzymes in fore- and midbrain after a short (3-day) period of pretreatment. Similarly, treatment with agents known to antagonise the central activation produced by *d*-amphetamine failed to influence tyrosine hydroxylase levels when comparisons were carried out with control groups of animals, although some evidence was obtained for a significant enhancement of levels of the enzyme in the brains of animals treated with a prolonged period with *d*-amphetamine as opposed to reserpine, or an amphetamine/reserpine combination. It is probable, however, that these effects are attributable to, or at least were profoundly modified by, the stress produced by extended treatment with the above drugs.

Central tryptophan hydroxylase levels appear to show a comparable insensitivity to changes in transmitter turnover rate. Imipramine exerts a relatively specific inhibitory effect on the reuptake of 5HT into synaptic terminals, and reduces turnover of the amine in central serotonergic neurones, probably acting primarily on the functional pool of transmitter since its effects are accompanied by a reduction in the firing rate of raphe neurones.[42] However, prolonged treatment with this drug failed to alter fore- and midbrain trytophan hydroxylase activity, measured *in vitro*. This lack of response of the level of the enzyme alteration of transmitter turnover rate is confirmed by the lack of effect of amphetamine, either in fore- and midbrain, or in hindbrain, and of reserpine in fore- and midbrain.

Our experiments have, therefore, failed to demonstrate any clear effect of pharmacological agents known to alter rates of amine turnover within the central nervous system on the levels of the corresponding synthetic enzymes. These findings are broadly

in agreement with results reported by Besson *et al.*[35] during the preparation of this manuscript. These workers also failed to demonstrate any effect of chronic administration of amphetamine on *in vitro* tyrosine hydroxylase activity, either in hindbrain, or in forebrain structures, and detected no significant effect of reserpine on enzyme levels in striatum or cortex. They report, however, a small (25 per cent) induction of tyrosine hydroxylase activity in brainstem by reserpine, in agreement with a previously published report.[10]

In the light of our findings, and those of the above workers, it seems probable that the inhibition of tyrosine hydroxylase activity in crude homogenates of striatal tissue observed after chronic administration of methamphetamine[11] is related to the increase in the proportion of enzyme activity in the striatum found in a particulate as opposed to a soluble form, which has been reported to result from treatment with this drug,[23] rather than to a decrease in absolute tissue levels.

There are differences between the functional roles played by sympathetic postganglionic neurones, and central adrenergic and serotonergic neurones. The sympathetic postganglionic neurone can be considered to fulfil an effector function, whereas a large proportion of central monoamine neurones are likely to be involved as elements in feedback loops, and can thus be thought of as having an essentially regulatory function. A number of recent findings emphasise the possible regulatory importance of interaction between neurones characterised by different varieties of neurotransmitter. The involvement of catecholamine neurones in the modulation of cholinergic transmission is suggested by the observations that intraventricular injection of NA, or of DA on a chronic basis, or prolonged administration of *d*-amphetamine increase choline acetylase levels in brain.[36, 39] The existence of interactions of the converse type is suggested by the effects of a variety of cholinergic agonists and antagonists on central CA turnover rates.[40, 41] Observations of this type have led to the proposal that the nigro-striatal DA neurones participate in a feedback loop involving a cholinergic component.[41]

Rather more direct evidence for the involvement of negative feedback effects in regulating the activity of central serotonergic neurones has been inferred from the reduction in the firing rates of raphe neurones which results from the intrasynaptic accumulation of 5HT after blockade of reuptake of the amine following treatment with imipramine.[42]

Induction of tyrosine hydroxylase and dopamine-β-hydroxylase by pharmacological agents in sympathetic ganglia is contingent upon an increase in the level of electrical activity incident on the postganglionic cell.[7] In the case of central monoamine neurones, however, the response of this parameter to drugs such as *d*-amphetamine which alter the distribution of neurotransmitter within, or its release from the synaptic terminals might be strongly influenced by participation in feedback loops of the type discussed above, which might well tend to negate the primary drug effect. In this case, it may be unwise to draw too facile an analogy between sympathetic postganglionic neurones, and central monoamine neurones.

Another series of experiments by the present authors* indicate that intraventricular injections of cyclic-AMP, cyclic-AMP analogues or phosphodiesterase inhibitors do not appear to cause a significant induction of central tyrosine hydroxylase. This again contrasts with the situation reported to exist in sympathetic ganglia,[43, 44] and

* E. C. Hulme and M. R. Kibby, manuscript in preparation.

may be indicative of a fundamental difference beween sympathetic postganglionic neurones and central catecholamine neurones in terms of their mechanisms for regulating the levels of neurotransmitter-synthesising enzymes.

The failure of iprindole to influence central tryptophan hydroxylase levels is not perhaps surprising in view of the lack of effect of imipramine. In searching for a neurochemical correlate of the development of antidepressant activity during treatment with these drugs it may once more be appropriate to give consideration to the possible importance of interaction between serotonergic and non-serotonergic neurones. Thus, the tricyclic antidepressant amitryptiline has been shown to enhance central choline acetylase activity, when administered chronically.[45] The possibility that actions of this kind may provide the key to the clinical efficacy of these drugs awaits further investigation.

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