Br. J. Pharmac. (1986), **88**, 285–290

Stereoselective accumulation of hydroxylated metabolites of amphetamine in rat striatum and hypothalamus

D.F.H. Dougan, A.M. Duffield*, P.H. Duffield & D.N. Wade

Department of Clinical Pharmacology and Toxicology, St Vincent's Hospital, Darlinghurst, N.S.W. 2010, Australia and Biomedical Mass Spectrometry Unit*, University of New South Wales, Kensington, N.S.W., 2033, Australia

1 The stereoselective accumulation of α-methyl-*p*-tyramine (AMPT) and α-methyl-*p*-octopamine (AMPO) in rat striatum and hypothalamus after acute and chronic administration of the (+)-and (−)-isomers of amphetamine (Amphet) and the acute administration of (+)- and (−)-AMPT has been investigated by chemical ionization gas chromatography mass spectrometry (c.i.g.c.m.s.).

2 Two h after the administration of (+)- or (−)-AMPT (5 mg kg^{-1} i.p.), the concentrations of the isomers in striatal tissue were approximately equal; 18 h later, the concentration of the (+)-isomer was 10 times that of the (−)-isomer.

3 The concentrations of AMPO in the striatum and hypothalamus 20 h after administration of (+)-AMPT were 68 ng g^{-1} and 484 ng g^{-1} respectively. After the administration of the (−)-isomer of AMPT, small quantities of AMPO were detected in both brain areas.

4 Twenty h after the last of 7 daily injections of (+)-Amphet (5 mg kg^{-1}, i.p.), the concentration of AMPO in the hypothalamus was 5.4 times the concentration at 20 h after one injection. In the striatum, the corresponding ratio for AMPO was 3.5 and for AMPT was 2.5.

5 These data indicate that, although both isomers of AMPT formed from Amphet administered systemically, cross the blood brain barrier, the (+)-isomers AMPT and AMPO are preferentially stored in striatal and hypothalamic aminergic nerve terminals.

6 The accumulations of AMPT and AMPO in rat striatum and hypothalamus after chronic administration of Amphet demonstrates that these metabolites persist in neuronal storage in these brain areas for days after administration. The half-lives of (+)-AMPT and (+)-AMPO in striatal neuronal storage, calculated from this data, were 1.5 days and 2.5 days, respectively. The corresponding half-life for hypothalamic (+)-AMPO was 7 days.

7 These findings suggest the involvement of accumulated AMPT and AMPO in the development of behavioural augmentation to repeated injections of Amphet (Randrup & Munkvad, 1970).

Introduction

The effects of amphetamine (Amphet) on locomotor and stereotype behaviour are generally accepted as being mediated by an indirect action on dopaminergic and noradrenergic neuronal systems in the brain (Kuczenski, 1983). In the rat, 60% of Amphet is metabolized by aromatic hydroxylation to α-methyl-*p*-tyramine (AMPT) (Smith & Dring, 1970), a compound which, in turn, is metabolized by the enzyme dopamine-β-hydroxylase (DBH) to α-methyl-*p*-octopamine (AMPO) (Axelrod, 1954; Sjoerdsma & von Studnitz, 1963). The contributions of these metabolites to the pharmacological actions of Amphet remain controversial.

It is generally assumed that AMPT formed mainly in the periphery does not contribute to the actions of Amphet as past work has suggested that the blood brain barrier effectively limits sufficient systemically formed AMPT from gaining access to catecholaminergic neurones in the central nervous system (Kuhn *et al.*, 1978). However, it has been demonstrated that these compounds can be sequestered in aminergic nerve terminals (Lewander, 1971; Jori *et al.*, 1979)

where they displace both dopamine and noradrenaline from neuronal sites (Taylor & Sulser, 1973). In *in vitro* studies, Cho *et al.* (1975) have shown that AMPT mimics some of the effects of Amphet. Further, Taylor & Sulser (1973) have demonstrated Amphet-like behavioural effects after intraventricular administration of (+)- and (−)-AMPT and AMPO.

This evidence suggests that the accumulations of AMPT and AMPO in rat brain after systemic administration of Amphet should be examined further. Accumulation of the isomers of AMPT and AMPO into adrenergic neurones may help explain phenomena observed after repeated administration of Amphet, such as behavioural augmentation (Segal & Mandell, 1974; Browne & Segal, 1977).

In the present study, the extent of accumulation of the isomers of AMPT and AMPO into rat brain striatal and hypothalamic neurones, after acute and chronic administration of the isomers of Amphet and acute administration of the isomers of AMPT, has been investigated.

Methods

Injections

Rats (male, Fullinsdorf, 250–350 g) were injected with either the (+)- or (−)-isomers of Amphet. SO_4 (5 mg kg^{-1} i.p.) or the (+)- or (−)-isomers of AMPT.HCl (5 mg kg^{-1} i.p.) either 2 or 20 h before decapitation. In addition (+)- or (−)-Amphet was administered every 24 h for 7 days, the last injection being 20 h before decapitation.

Assay for AMPT and AMPO

Rats were first stunned, then decapitated. The hypothalamus and striatum were dissected from ice-cold brains and then homogenized in 0.4 M $HCLO_4$ (2 ml) containing the deuterated internal standards (AMPT-[$^2H_9$] and AMPO-[$^2H_9$]. The supernatant was prepared for gas chromatographic chemical ionization mass spectrometric assay of AMPT and AMPO as described previously for octopamine and tyramine (Duffield *et al.*, 1981). These α-methylated phenolamines were then derivatized in ethyl acetate with pentafluoropropionic anhydride ($(PFP)_2O$) at 60°C for 30 min (Duffield *et al.*, 1981).

Instrumentation

Gas chromatographic separations were carried out using a glass U-shaped column (length 1.8 m, i.d. 2 mm) containing 3% OV-17 on Gas Chrom (100–120 mesh), maintained at 130°C for 1 min after injection and then programmed at 12°C min^{-1}. Methane served as the g.c. carrier gas (flow = 20 ml min^{-1}) and c.i. reactant gas (ion source pressure = 0.8–0.9 Torr). The retention time for AMPT-$(PFP)_2$ (m/z 444) and its deuterated analogue (m/z 453) was 3.0 min. For AMPO-$(PFP)_3$ (m/z 442) and its deuterated analogue (m/z 451), the corresponding retention time was 2.1 min. Instrumental analyses were conducted with a Finnigan 3200 Chemical Ionization Quadrupole g.c.m.s. system (San Jose, California, U.S.A.) interfaced to the same manufacturer's Incos Model 2300 Data System. Selected ion monitoring (s.i.m.) was used to record the ion contents of AMPT-$(PFP)_2$ and AMPT-[$^2H_9$]-$(PFP)_2$ (m/z 444 and m/z 453) and AMPO-$(PFP)_3$ and AMPO-[$^2H_9$]-$(PFP)_3$ (m/z 442 and m/z 451), respectively. The method is capable of measuring 20 pg of these compounds injected onto the g.c. column.

Spectra

The positive ion methane chemical ionization mass spectra of AMPO-$(PFP)_3$ contains the following m/z values (> 1% relative abundance): 442 $(MH\text{-}PFPOH)^+$ (100), 428 (7), 165 (3). For AMPT-$(PFP)_2$, the following m/z values (> 1% relative abundance) were observed: 444 $(MH)^+$ (100), 280 (12), 190 (4).

Stereochemical impurities

The isomeric purity of the (+)- and (−)-isomers of Amphet and AMPT administered to rats was determined by reacting each compound with trifluoroacetyl-(−)-prolyl chloride (Gordis, 1966). The resulting diasterioisomers were separated and estimated by methane c.i.g.c.m.s. using selected ion monitoring (Wiecek *et al.*, 1979).

Adjustment of AMPO concentrations to account for isomeric impurity

The administered isomers were all contaminated with small amounts of their opposite enantiomers. Thus, (+)-Amphet contained 1.6% of the (−)-isomer. To adjust the experimental results for this isomeric impurity, the levels of AMPO were multiplied by 100/98.4. Since the contribution of the (−)-isomer to the production of AMPO was very small in comparison to the (+)-isomer, no further adjustment was made in this instance. Similarly, (+)-AMPO concentrations obtained after administering (+)-AMPT (which contained 14% (−)-AMPT) were adjusted by multiplying by 100/86.

In the case of the administered (−)-isomers, the contribution of the (+)-isomeric impurity to the production of AMPO was substantial. The (−)-Amphet used contained 9.6% of the (+)-isomer. The (+)-Amphet contribution to the AMPO levels was

calculated by determining 9.6% of the corresponding AMPO concentrations obtained after (+)-Amphet administration (adjusted as described above). This was subtracted from the levels of AMPO obtained experimentally after (−)-Amphet administration to give the level of AMPO formed from the (−)-isomer. Further refinements did not significantly alter the concentration obtained.

Similarly, adjustment for the 2% of (+)-AMPT found in the administered (−)-AMPT was carried out by subtracting 2% of the corresponding AMPO concentrations observed after (+)-AMPT administration (adjusted as described above) from the AMPO concentrations measured after (−)-AMPT administration.

Linearity of AMPO production with dose of (+)-Amphet, or (+)-AMPT was assumed. Kuhn *et al.* (1978) demonstrated linearity between (+)-Amphet concentration and AMPO production in brain slice experiments.

Estimation of the terminal half-lives of α-methylphenolamines using data on their accumulation after repeated dosing

If uptake and distribution phases are negligible, during repeated dosing, the ratio of the concentrations of a drug in a single compartment at the same time interval after the 1st and Nth dose is:

$$\frac{C_N}{C_1} = \frac{1\text{-}e^{-Nk\tau}}{1\text{-}e^{-k\tau}} \qquad (1)$$

where C_1 and C_N are the concentrations of the drug at the same time after the 1st and Nth doses, k is the rate constant of elimination and τ is the dosing interval (Gibaldi & Perrier, 1982). An approximate estimate of k for each α-methylphenolamine in either striatum or hypothalamus was made by substituting the ratio of its concentrations 20 h after the 7th and 1st daily doses of (+)-Amphet (Tables 1 and 2) in equation 1.

The corresponding estimates of the terminal half-lives were calculated from:

$$\text{half-life} = 0.693/k \qquad (2)$$

Statistics

Results are given as the mean and s.e.mean of at least 6 results. They were analysed by means of Student's two-tailed t test.

Materials

Dexamphetamine.SO_4 ((+)-Amphet) was obtained from USV, Australia, and (−)-amphetamine.SO_4 ((−)-Amphet) from Riker Laboratories, Australia. The (+)- and (−)-isomers of AMPT.HBr (*p*-hydroxyamphetamine.HBr) were procured from Smith, Kline and French, Philadelphia, Pa., U.S.A. AMPO.HCl (*p*-hydroxynorephedrine.HCl) was purchased from Aldrich Chemical Company, Milwaukee, Wisconsin, U.S.A. The deuterated internal standards AMPT-[$^2H_9$] and AMPO-[$^2H_9$] were prepared as described by Duffield *et al.* (1983).

Results

Each of the isomers of Amphet and AMPT administered in these experiments was found to contain, by methane c.i.g.c.m.s. and selected ion monitoring, a small amount of the opposite enantiomer. (+)-Amphet contained 98.4% of (+)-Amphet and 1.6% of the (−)-isomer. (−)-Amphet consisted of 90.4% and 9.6% of the (−)-and (+)-isomers, respectively. (+)-AMPT was made up of 86.0% of the (+)-isomer and 14.0% of the (−)-isomer, 98% of the (−)-AMPT consisted of the (−)-isomer and the (+)-isomer made up 2%.

Using these data, the experimental determinations of (−)-AMPO derived from each of these isomers was adjusted to account for its enantiomeric impurity. No corrections for stereo-chemical impurities were made to brain concentrations of AMPT because the adjustments were small and made no difference to the conclusions drawn from the results.

AMPT was found in the striatum 2 h and 20 h after a single injection of (+)-AMPT or (−)-AMPT (5 mg kg^{-1}) (Table 1). At 2 h, the concentration of the (+)-isomer was similar to that observed for the (−)-isomer. At 20 h, the concentration of the (+)-isomer was 10 times greater than the (−)-isomer. Further, 20 h after both acute and the last of 7 daily injections of (+)- and (−)-Amphet (5 mg kg^{-1} i.p.), greater concentrations of AMPT are found in the striatum after the (+)-isomer than the (−)-isomer (Table 1). In the hypothalamus, much lower concentrations of both (+)- and (−)-AMPT were found 2 h after the administration of (+)- or (−)-AMPT (5 mg kg^{-1}), respectively (Table 1). Eighteen h later, no AMPT could be detected in the hypothalamus.

AMPO was clearly demonstrated in both hypothalamus and striatum 2 h and 20 h after acute administration and 20 h after the last of 7 daily injections of (+)-Amphet (5 mg kg^{-1} i.p.) (Table 2). In contrast, the hypothalamic and striatal concentrations of (−)-AMPO following administration of (−)-Amphet (5 mg kg^{-1} i.p.) were zero (Table 2).

AMPO was also clearly demonstrated to be present in hypothalamic tissue 2 and 20 h after the administration of (+)-AMPT (Table 2). However, in contrast to the (−)-isomer of Amphet, administration of the (−)-isomer of AMPT (5 mg kg^{-1} i.p.) resulted in measura-

Table 1 Concentration of α-methyl-*p*-tyramine (AMPT) in rat brain regions at different times following the administration (i.p.) of the stereoisomers of AMPT (5 mg kg^{-1}) and amphetamine (Amphet, 5 mg kg^{-1})

		*AMPT**		
Region	*Treatment*	*2 h after 1 injection*	*20 h after 1 injection*	*20 h after 7 daily injections*
Striatum	(+)-AMPT	253 ± 56	148 ± 60	ND
	(−)-AMPT	224 ± 42	14.2 ± 4.6	ND
	(+)-Amphet	29 ± 3	14 ± 1	35 ± 4
	(−)-Amphet	16 ± 2	2.2 ± 0.1	5.7 ± 0.3
Hypothalamus	(+)-AMPT	22 ± 3	<1	ND
	(−)-AMPT	46 ± 3	<1	ND
	(+)-Amphet	<1	<1	<1
	(−)-Amphet	<1	<1	<1

*Concentrations are expressed in ng g^{-1} of wet tissue and are the mean and s.e.mean of at least 4 results.
ND – not determined.

ble quantities of AMPO in both striatum and hypothalamus at 2 and 20 h after the injection.

Chronic administration of the isomers of Amphet (5 mg kg^{-1} i.p.) lead to considerable accumulation of its hydroxylated metabolites in the brain. Thus, 20 h after the last of 7 daily injections of (+)-Amphet, the striatal concentration of (+)-AMPT was 35 ng g^{-1}, a 2.5 fold increase over its concentration 20 h after one injection. Though the levels were much lower, the (−)-isomer of AMPT accumulated similarly (ratio 2.6). For striatal (+)-AMPO, the corresponding ratio was 3.5 and for hypothalamic (+)-AMPO, it was 5.4

Discussion

Studies using [^{3}H]-AMPT (Groppetti & Costa, 1969; Kuhn *et al.*, 1978) or chemical assay methods including mass fragmentography (Cattabeni *et al.*, 1973; Danielson & Boulton, 1976) have demonstrated that (+)-Amphet is hydroxylated in rat liver to form AMPT which, in turn, is metabolized by the enzyme dopamine β-hydroxylase (EC 1.14.2.1, DBH) in aminergic nerve terminals to AMPO. The present work, using a highly sensitive and specific method of estimation (c.i.g.c.m.s.) has confirmed these previous

Table 2 Concentrations of α-methyl-*p*-octopamine (AMPO) in rat brain regions at different times following the administration (i.p.) of the stereoisomers of α-methyl-*p*-tyramine (AMPT, 5 mg kg^{-}) and amphetamine (Amphet, 5 mg kg^{-1})

		*AMPO**		
Region	*Treatment*	*2 h after 1 injection*	*20 h after 1 injection*	*20 h** after 7 daily injections*
Striatum	(+)-AMPT	36 ± 10	68 ± 21	ND
	(−)-AMPT	2.3 ± 0.6	1.6 ± 0.6	ND
	(+)-Amphet	5 ± 1	8 ± 1	28 ± 3
	(−)-Amphet	0	0	0
Hypothalamus	(+)-AMPT	396 ± 97	484 ± 107	ND
	(−)-AMPT	17 ± 2	11 ± 3	ND
	(+)-Amphet	38 ± 6	48 ± 4	257 ± 13
	(−)-Amphet	0	0	0

*Concentrations are expressed in ng g^{-1} of wet tissue and are the mean and s.e.mean of at least 6 results.
ND – not determined.
**20 h after the last of 7 daily injections.

observations and extended them by demonstrating the stereo-selective accumulation and metabolism of (+)- and (−)-AMPT in both rat striatal and hypothalamic tissues. Further, it has demonstrated that accumulated AMPT and AMPO persist in the striatum and hypothalamus for days.

Thus, although the striatal concentrations of both the (+)- and (−)-isomer of AMPT were equal 2 h after administration of the isomers, by 20 h the striatal concentration of the (+)-isomer (148 ng g^{-1}) was 10 times that of the (−)-isomer (14.2 ng g^{-1}, Table 1). This indicates that, after systemic administration, both the (+)- and (−)-isomers of AMPT cross the blood brain barrier but suggest that the (+)-isomer is preferentially stored in the striatum.

Jori *et al.* (1979), using chemical and electrolytic lesions, have clearly demonstrated that AMPT is stored in dopaminergic nerve endings. Further, in *in vitro* experiments, AMPT uptake in striatal slices is inhibited by dopamine, Amphet and cocaine indicating that the dopamine uptake system is involved in the accumulation of AMPT (Cho *et al.*, 1975). The present findings suggest that the accumulation of AMPT in rat striatal dopaminergic neurones is highly stereo-selective for the (+)-isomer.

The original observations of Goldstein & Anagnoste (1965), using radiochemical methods, suggested that the (−)-isomer of AMPT derived from (−)-Amphet was not a substrate for DBH in the brain. Using the c.i.g.c.m.s. assay described in this paper (sensitivity 20 pg), no (−)-AMPO was detected in either hypothalamic or striatal tissue after the administration of (−)-Amphet. However, after the administration of (−)-AMPT (5 mg kg^{-1} i.p.), AMPO was detected in rat hypothalamus and striatum (Table 2).

At 2 h after administration of AMPT, the concentration of AMPO derived from the (−)-isomer was 1/20 that formed from the (+)-isomer. This demonstrates that the (−)-isomer of AMPT is a substrate for DBH, although, compared to the (+)-isomer, a relatively poor one (Table 2). The presence of stereoselective uptake for AMPT into striatal tissue demonstrated in the present work raises the possibility that poor uptake into aminergic neurones may be one of the reasons that (−)-AMPT is poorly converted to (−)-AMPO by DBH. By 20 h, the level of AMPO formed from (−)-AMPT had decreased to less than 1/40 that formed from (+)-AMPT suggesting that (−)-AMPO is also less readily taken up into aminergic neurones than (+)-AMPO.

Determinations of the half-lives of (+)-AMPT and (+)-AMPO in rat brain have been made. After intraventricular administration of (+)-AMPT and (+)-AMPO, their half-lives in whole brain were calculated to be 1.3 h and 2.1 h, respectively (Taylor & Sulser, 1973). These were estimated from data collected over a period of 5 h after administration. Lewander (1971) determined the half-life of (+)-AMPO in whole brain, after the administration of (+)-AMPT (20 mg kg^{-1} i.p.), to be 20–24 h. This determination was made with data collected over 24 h after administration.

In this project, repeated daily dosing with (+)-Amphet leads to dramatic increases in the striatal and hypothalamic concentrations of these metabolites. A comparison of the concentrations of striatal (+)-AMPT at 20 h after one dose and 20 h after the last of 7 daily doses suggests that the half-life in the striatum of that left 20 h after administration (terminal half-life) is much longer than those calculated for whole brain over a short period of time.

Using these results in the formula of Gibaldi & Perrier (1982), the terminal half-life of (+)-AMPT in the striatum was calculated to be of the order of 1.5 days. Using the corresponding data from Table 2, the terminal half-lives of (+)-AMPO in the striatum and hypothalamus are approximately 2.5 and 7 days, respectively. These long terminal half-lives suggest that a fraction of the α-methylated phenolamines which enters or is formed in these brain areas persists in neuronal storage for days.

The long terminal half-lives of α-methylated-phenolamines in striatum and hypothalamus and their resultant accumulation after chronic administration of (+)-Amphet suggest they may play a role in behavioural augmentation of Amphet. However, both the isomers of Amphet elicit stereotype behaviour (Browne & Segal, 1977). Therefore, the comparative lack of AMPO after administration of either (−)-Amphet or (−)-AMPT (Table 2) suggests that the α-methyloctopamines are not important in eliciting stereotype behaviour. In the case of AMPT, the present study clearly shows that it is stereoselectivity accumulated into the striatum (Table 1). Twenty h after the administration of (+)-Amphet (5 mg kg^{-1} i.p.), the striatal concentration of (+)-AMPT was 6 times the concentration of (−)-AMPT following (−)-Amphet (5 mg kg^{-1} i.p.) administration (Table 1). Browne & Segal (1977) have shown approximately equal increases in stereotype behaviour in rats given either (+)-Amphet (2.5 mg kg^{-1} i.p.) or (−)-Amphet (12 mg kg^{-1} i.p.). Also, intraventricular administration of (+)- and (−)-AMPT has been shown to elicit stereotype behaviour in rats (Taylor & Sulser, 1973). Further, in this laboratory, we have demonstrated that pretreatment of rats with (+)-AMPT (10 mg kg^{-1} i.p.) 24 h before administration of Amphet (4 mg kg^{-1} i.p.) enhances the stereotype behaviour induced by Amphet (unpublished observations). These findings suggest a role for the α-methyltyramines in stereotype behaviour.

In summary, the accumulation and persistence of hydroxylated metabolites of Amphet in aminergic neurones in rat striatum and hypothalmus indicates

that these metabolites may have a role in eliciting the enhanced behavioural responses caused by the repeated administration of either (+)- or (−)-Amphet.

The much more favourable accumulation of (+)-AMPO over (−)-AMPO suggests the α-methyloctopamines play, at most, a minor role. However, the effectiveness of (+)- and (−)-Amphet in eliciting behavioural augmentation parallels the levels of accumulation of their metabolites (+)-AMPT and (−)-AMPT, respectively. Both the α-methyltyramines may contribute to the eliciting of behavioural augmentation.

Financial support from the National Health & Medical Research Council of Australia is gratefully acknowledged.

References

AXELROD, J. (1954). Studies on sympathomimetic amines II. The biotransformation and physiological disposition of d-amphetamine, d-p-hydroxyamphetamine and d-methamphetamine. *J. Pharmac. exp. Ther.*, **110**, 315–326.

BROWNE, R.G. & SEGAL, D.S. (1977). Metabolic and experiential factors in the behavioral response to repeated amphetamine. *Pharmac. Biochem. Behav.*, **6**, 545–552.

CHO, A.K., SCHAEFFER, J.C. & FISCHER, J.F. (1975). Accumulation of 4-hydroxyamphetamine by rat striatal homogenates. *Biochem. Pharmac.*, **24**, 1540–1542.

CATTABENI, F., RACAGNI, G. & GROPPETTI, A. (1973). Use of mass fragmentography to detect subcellular localization of drug metabolites acting as false transmitters. In *Advances in Biochemical Psychopharmacology. Vol. 7*, ed. Costa, E. & Holmstedt, B. pp. 45–55. New York: Raven Press.

DANIELSON, T.J. & BOULTON, A.A. (1976). Distribution and occurrence of amphetamine and p-hydroxyamphetamine in tissues of the rat after injection of d-amphetamine sulphate. *Eur. J. Pharmac.*, **37**, 257–264.

DUFFIELD, P.H., DOUGAN, D.F.H., WADE, D.N. & DUFFIELD, A.M. (1981). A chemical ionization gas chromatographic mass spectrometric assay for octopamine and tyramine in rat brain. *Biomed. Mass Spectrom.*, **8**, 170–173.

DUFFIELD, P.H., DOUGAN, D.F.H., WADE, D.N., LOW, G.K.-C. & DUFFIELD, A.M. (1983). A negative ion chemical ionization GCMS assay for octopamine, tyramine and their α-methylated analogs in regions of rat brain after administration of amphetamine. *Spectros. Int. J.*, **2**, 311–317.

GIBALDI, M. & PERRIER, D. (1982). In *Pharmacokinetics*. pp. 113–143. New York: Marcel Dekker, Inc.

GOLDSTEIN, M. & ANAGNOSTE, B. (1965). The conversion *in vivo* of d-amphetamine to (+)-p-hydroxynorephridrine. *Biochim. biophys. Acta*, **107**, 166–168.

GORDIS, E. (1966). Gas chromatographic resolution of optical isomers of microgram samples of amphetamine. *Biochem. Pharmac.*, **15**, 2124–2126.

GROPPETTI, A. & COSTA, E. (1969). Tissue concentrations of p-hydroxynorephidrine in rats injected with d-amphetamine: effect of pretreatment with desipramine. *Life Sci.*, **8**, 653–665.

JORI, A., CACCIA, S., GUISO, A., BALLABIO, M. & GARATTINI, S. (1979). Selective storage of p-hydroxy-d-amphetamine in the dopaminergic nerve terminals. *Biochem. Pharmac.*, **28**, 1205–1207.

KUCZENSKI, R. (1983). Biochemical actions of amphetamine and other stimulants. In *Stimulants: Neurochemical, Behavioral and Clinical Perspectives*. ed. Creese, I. pp. 31–61. New York: Raven Press.

KUHN, C.M., SCHANBERG, S.M. & BREESE, G.R. (1978). Metabolism of amphetamine in rat brain tissue. *Biochem. Pharmac.*, **27**, 343–351.

LEWANDER, T. (1971). Displacement of brain and heart noradrenaline by p-hydroxynorephidrine after administration of p-hydroxyamphetamine. *Acta pharmac. tox.*, **29**, 20–32.

RANDRUP, A. & MUNKVAD, I. (1970). Biochemical, anatomical and psychological investigations of stereotyped behaviour induced by amphetamines. In *Amphetamines and Related Compounds*. ed. Costa, E. & Garattini, S. pp. 217–230. New York: Raven Press.

SEGAL, D.S. & MANDELL, A.J. (1974). Long-term administration of d-amphetamine: progressive augmentation of motor activity and stereotypy. *Pharmac. Biochem. Behav.*, **2**, 249–255.

SJOERDSMA, A. & VON STUDNITZ, W. (1963). Dopamine-β-oxidase activity in man, using hydroxyamphetamine as substrate. *Br. J. Pharmac. Chemother.*, **20**, 278.

SMITH, R.L. & DRING, L.G. (1970). Patterns of metabolism of β-phenyisopropylamines in man and other species. In *Amphetamines and Related Compounds*. ed. Costa, E. & Garattini, S. pp. 121–139. New York: Raven Press.

TAYLOR, W.A. & SULSER, F. (1973). Effects of amphetamine and its hydroxylated metabolites on central noradrenergic mechanisms. *J. Pharmac. exp. Ther.*, **185**, 620–632.

WIECEK, C., HALPERN, B., SARGESON, A.M. & DUFFIELD, A.M. (1979). The determination of steric purity of amines and amino acids by gas chromatography and mass spectrometry. *Org. Mass Spectrom.*, **14**, 281–285.

(Received November 18, 1985.
Accepted December 16, 1985.)