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EFFECT OF FOUR AMPHETAMINES ON BRAIN BIOGENIC AMINES AND THEIR METABOLITES

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Abstract—The effect of D-amphetamine, D-methylamphetamine, *p*-nitro and *p*-bromomethylamphetamine on the release and turnover (as assessed by synthesis blockade) of brain noradrenaline and 5-HT have been studied in the rat. All the amphetamines increased the turnover of brain 5-HT but their mechanism of action in reducing the level of this amine appeared to differ. Thus methylamphetamine and *p*-nitromethylamphetamine potentiated the effect of 4-methyl-α-ethyl-meta-tyramine in depleting brain 5-HT, whereas the other two amphetamines were without effect. Similarly both methyl and *p*-nitromethylamphetamine significantly decreased the turnover of brain noradrenaline whereas the other amphetamines were without effect. The effect of these drugs on the noradrenaline, dopamine, 5-HT, normetanephrine and 5-hydroxyindole acetic acid concentration in the cortex, middle brain and caudal brain regions were also studied. *p*-Bromomethylamphetamine appeared to be unique in that it only affected the normetanephrine and 5-hydroxyindole acetic acid content of the middle brain region. The other amphetamines affected the metabolism of the biogenic amines in at least two of the three brain regions studied. These findings are discussed with respect to the different behavioural effects produced in animals by these drugs.

SEVERAL investigators have studied the effects of D-amphetamine on the metabolism of brain catecholamines in order to elucidate the biochemical mechanisms which underly the central stimulant properties of this drug.[1–3] The effects of this drug on adrenergic processes within the brain would appear to be complex.[4–8] In addition to its stimulant properties which are thought to be mediated by central adrenergic mechanisms, the chronic administration of high doses of D-amphetamine produces psychotomimetic effects in man.[9,10] These effects are shared to some extent by the structurally related drug, D-methylamphetamine, which has a similar effect on brain catecholamines to D-amphetamine.[11,12]

Knoll and his co-workers,[13,14] have investigated the effect of a large number of substituted amphetamines on the behaviour of rats, cats and rabbits and compared their effects with those of LSD. In their studies[14] they found that *p*-bromomethylamphetamine, which produces only slight behavioural stimulation even in high doses, has a profile quantitively similar to LSD. *p*-Nitromethylamphetamine had a somewhat similar effect.[14] Knoll[14] postulated that the psychotomimetic effects of the amphetamines are associated with substitution in the para- or meta-position of the benzene ring.

In previous studies, Leonard and Shallice[11,15] compared the effects of D-amphetamine, D-methylamphetamine, *p*-bromomethylamphetamine and *p*-nitromethylamphetamine on the concentration of catecholamines and 5-hydroxytryptamine (5-HT) in the rat brain. The present study was undertaken to extend the scope of the investigation into the mode of action of these four phenylethylamines (amphetamines).

In this way it was hoped to determine whether the stimulant and the psychotomimetic effects have a different biochemical basis.

MATERIALS AND METHODS

Specific pathogen free female rats (90–120 g) of the Alderley Park strain were used. The drugs, which were readily soluble in water, were administered intraperitoneally in a volume of 1 ml/kg body weight. Following injection, the rats were kept five to a cage of dimensions 28 × 35 × 15 cm.

Effects on the depletion of brain catecholamines by α-methyl-metatyrosine (αMMT)

It has been shown that αMMT depleted brain catecholamines by forming metaraminol which competitively displaces the amines from their storage sites[16,17] Previous investigations had shown that four structurally unrelated hallucinogenic drugs potentiated the depleting action of αMMT,[18] and therefore it was of interest to see whether the phenylethylamines understudy could be differentiated by their action on αMMT.

Groups of five rats were treated at 0 hr with 300 mg/kg αMMT alone or simultaneously with one of the phenylethylamines. D-amphetamine and D-methylamphetamine were given in doses of 1 and 10 mg/kg whereas *p*-bromo and *p*-nitromethylamphetamine were given in doses of 1 and 60 mg/kg. The animals were killed 3 hr later. Noradrenaline and dopamine were determined in whole brain by the solvent extraction method of Welch and Welch.[19] The dose schedule for this experiment was:

Group 1. control group (distilled water)
Group 2. αMMT alone (300 mg/kg i.p.).
Group 3. the phenylethylamine alone (1 mg/kg i.p.).
Group 4. αMMT + the phenylethylamine (300 + 1 mg/kg).
Group 5. a phenylethylamine alone (10 or 60 mg/kg).
Group 6. αMMT + the phenylethylamine (300 + 10 or 60 mg/kg).

Effect on the depletion of brain catecholamines by α-methyl-para-tyrosine (αMPT). Spector and co-workers[20] reported that αMPT depletes brain catecholamines by inhibiting tyrosine hydroxylase. As this enzyme is rate limiting in the synthesis of these amines it is possible to get an approximate assessment of whether or not a compound is affecting their rate of turnover.

αMPT (300 mg/kg i.p.) was injected into groups of rats alone and together with the phenylethylamines. The dosage schedule was the same as that given for the αMMT experiment. Some 3 hr after injection the rats were decapitated and the catecholamines determined by the method of Welch and Welch.[19]

Effect on the depletion of brain 5-HT by parachlorophenylalenine (PCPA). Koe and Weisman[21] demonstrated that PCPA impairs the synthesis of 5-HT by inhibiting tryptophan hydroxylase activity. As this enzyme forms the rate limiting step in the synthesis of 5-HT it is possible to get an approximate assessment of whether the phenylethylamine is affecting the turnover of this amine.

Groups of five rats were injected at 0 hr with PCPA alone (400 mg/kg i.p.) or simultaneously with the phenylethylamines (10 mg/kg). The phenylethylamine was again injected 4·5 hr later and the animals killed by decapitation 9 hr after the first injection. One group of rats was also injected with the phenylethylamine alone at 0

and 4·5 hr. 5-HT was determined in homogenates of the whole brain by the fluorimetric method of Bogdanski *et al.*[22]

The effect on the depletion of brain 5-HT by 4-methyl-α-ethyl-meta-tyramine (H75/12). Carlsson and co-workers[23] have shown that H75/12 displaces brain 5-HT and possibly utilises the reserpine resistant uptake mechanism to bring about this displacement. The effect of H75/12 together with the phenylethylamines was therefore studied to see whether the drug affected brain 5-HT concentrations by acting on the reserpine resistant uptake mechanism.

H75/12 was injected at 0 and 2 hr at a dose of 25 mg/kg and the animals killed by decapitation 2 hr after the second dose. The phenylethylamines were injected at dose of 10 mg/kg 30 min before the administration of each dose of H75/12. Some group of rats were also injected with the phenylethylamine alone (2 × 10 mg/kg). 5-HT was estimated in the whole brain by the fluorimetric method of Snyder, Axelrod and Zweig[24] and Welch and Welch,[19] as modified by Leonard and Shallice.[15]

The effect on the catecholamine and indolealkylamine content of three anatomical areas of the rat brain. In these experiments, the effect of the phenylethylamine was investigated in order to localize approximately their site of action.

Groups of five rats were injected intraperitoneally with the phenylethylamines (10 mg/kg) and killed 2 hr later. This corresponded to the period for peak effect of the drugs on brain amines. Following decapitation, the brains were removed and dissected on a cooled glass plate into the cortex, middle brain and caudal brain. The cerebellum was discarded. The middle brain region consisted of the hippocampus, striatum, thalamus, hypothalamus and infundibulum. The caudal brain consisted of the pons, medulla, tegmentum and colliculus; the cortex included the olfactory lobes. The brain regions were immediately frozen on solid carbon dioxide then weighed and homogenized in 9 ml of 0·01 N HCl containing 0·2 ml 10% (w/v) ethylenediamine tetracetic acid (as the disodium salt). The homogenate was centrifuged for 15 min at approximately 800 *g*, and 4·5 ml of the supernatant, plus the pellet, was extracted with salt saturated *n*-butanol: noradrenaline, dopamine and 5-hydroxyindole acetic acid were then determined by the method of Welch and Welch.[19] 5-HT was determined in a portion of the butanol extract as described above for the H75/12 experiment.

In all experiments, the significance of the results was assessed using Student's *t*-test. Deviations are expressed as standard errors of the mean (S.E.M.).

RESULTS

The effects of the 4 phenylethylamines on the gross behaviour of the rats has been described in detail elsewhere.[11,15] Briefly, all the drugs produced behavioural stimulation, salivation and hyperthermia. The severity of these affects depended on the dose and nature of the drug used. D-amphetamine and methylamphetamine caused maximal sympathomimetic effects in relatively low doses (5 mg/kg), whereas dopamine and *p*-bromomethylamphetamine caused slight behavioural excitation only in high doses (30–60 mg/kg).

Effect on the depletion of brain catecholamines by α-methyl-meta-tyrosine (*αMMT*). At both 1 and 60 mg/kg, *p*-nitromethylamphetamine potentiated the depletion of brain noradrenaline by αMMT. In contrast, amphetamine, methylamphetamine and *p*-bromomethylamphetamine had no effect on the action of αMMT (Table 1). None of

TABLE 1. THE EFFECT OF SOME PHENYLETHYLAMINES ON THE DEPLETION OF BRAIN CATECHOLAMINES CAUSED BY α-METHYL-META-TYROSINE (αMMT)

Treatment	Dose (mg/kg)	Noradrenaline (μg/g)	Dopamine (μg/g)
Control		0·40 ± 0·043	0·60 ± 0·046
αMMT	300	0·12 ± 0·012‡	0·02 ± 0·047‡
D-Amphetamine	1	0·26 ± 0·023†	0·48 ± 0·027*
D-Amphetamine +αMMT	1 + 300	0·15 ± 0·019‡	0·18 ± 0·021‡
D-Amphetamine	10	0·27 ± 0·007†	0·63 ± 0·051
D-Amphetamine +αMMT	10 + 300	0·11 ± 0·008‡	0·21 ± 0·037‡
D-Methylamphetamine	1	0·34 ± 0·025	0·43 ± 0·021‡
D-Methylamphetamine +αMMT	1 + 300	0·17 ± 0·025‡	0·27 ± 0·039‡
D-Methylamphetamine	10	0·31* ± 0·037	0·64 ± 0·029
D-Methylamphetamine +αMMT	10 ± 300	0·14 ± 0·016‡	0·22 ± 0·009‡
Control		0·35 ± 0·009	0·72 ± 0·014
αMMT	300	0·17 ± 0·010‡	0·32 ± 0·011‡
p-Bromomethylamphetamine	1	0·36 ± 0·013	0·78 ± 0·014
p-Bromomethylamphetamine+αMMT	1 + 300	0·16 ± 0·009‡	0·36 ± 0·011‡
p-Bromomethylamphetamine	60	0·28 ± 0·016	0·80 ± 0·026
p-Bromomethylamphetamine+αMMT	60 + 300	6·15 ± 0·004‡	0·36 ± 0·013‡
p-Nitromethylamphetamine	1	0·34 ± 0·018	0·78 ± 0·010
p-Nitromethylamphetamine +αMMT	1 + 300	0·11§ ± 0·005‡	0·26 ± 0·010‡
p-Nitromethylamphetamine	60	0·24 ± 0·026‡	0·72 ± 0·010
p-Nitromethylamphetamine +αMMT	60 + 300	0·13§ ± 0·004‡	0·32 ± 0·015‡

Results expressed as mean ± standard error of the mean for five animals per group. Significance of difference between control and experimental groups expressed as * $P < 0·05$; † $P < 0·01$; ‡ $P < 0·001$. § Difference between group treated with αMMT alone and αMMT + *p*-nitromethylamphetamine significant at $P < 0·01$.

TABLE 2. EFFECT OF SOME PHENYLETHYLAMINES ON THE DEPLETION OF BRAIN CATECHOLAMINES BY α-METHYL-PARA-TYROSINE (αMPT)

Treatment	Dose (mg/kg)	Noradrenaline (μg/g)	Dopamine (μg/g)
Control		0·38 ± 0·010	0·80 ± 0·033
αMPT	300	0·24 ± 0·021‡	0·39 ± 0·014‡
D-Amphetamine	1	0·38 ± 0·039	0·70 ± 0·043†
D-Amphetamine +αMPT	1 + 300	0·24 ± 0·009‡	0·43 ± 0·036‡
D-Amphetamine	10	0·31 ± 0·015	0·89 ± 0·040
D-Amphetamine +αMPT	10 + 300	0·25 ± 0·039*	0·35 ± 0·025‡
D-Methylamphetamine	1	0·52 ± 0·021‡	0·83 ± 0·065
D-Methylamphetamine +αMPT	1 + 300	0·30 ± 0·041	0·46 ± 0·026‡
D-Methylamphetamine	10	0·28 ± 0·015*	0·69 ± 0·029*
D-Methylamphetamine +αMPT	10 + 300	0·36‖ ± 0·022	0·34 ± 0·039‡
Control		0·36 ± 0·010	0·58 ± 0·035
αMPT	300	0·26 ± 0·027‡	0·21 ± 0·014‡
p-Bromomethylamphetamine	1	0·45 ± 0·038*	0·57 ± 0·024
p-Bromomethylamphetamine+αMPT	1 + 300	0·23 ± 0·031	0·29 ± 0·068‡
p-Bromomethylamphetamine	60	0·40 ± 0·013†	0·53 ± 0·025
p-Bromomethylamphetamine+αMPT	60 + 300	0·30 ± 0·016*	0·26 ± 0·024*
p-Nitromethylamphetamine	1	0·36 ± 0·020	0·62 ± 0·059
p-Nitromethylamphetamine +αMPT	1 + 300	0·33 ± 0·040	0·28 ± 0·039*
p-Nitromethylamphetamine	60	0·40 ± 0·044	0·56 ± 0·063
p-Nitromethylamphetamine +αMPT	60 + 300	0·40§ ± 0·029	0·29 ± 0·077*

Results expressed as mean ± standard error of the mean, for five animals per group. Significance expressed as shown in Table 1. ‖ Significance of difference, between αMPT treated group and that treated with αMPT + methylamphetamine significant at $P < 0·01$; § Significance of difference $P < 0·05$.

the phenylethylamines significantly affected the depletion of brain dopamine caused by αMMT.

Effect on the depletion of brain catecholamines by α-methyl-para-tyrosine (αMPT). Both methylamphetamine (10 mg/kg) and *p*-nitromethylamphetamine (60 mg/kg) significantly antagonized the depletion of brain noradrenaline by αMPT (Table 2). Neither amphetamine nor *p*-bromomethylamphetamine had any effect on the action of this drug on brain noradrenaline; none of the phenylethylamines affected the depletion of brain dopamine caused by αMPT.

Effect on the depletion of brain 5-HT by para-chlorophenylalanine (PCPA). The depletion of brain 5-HT was significantly potentiated by all the phenylethylamines (Table 3).

TABLE 3. EFFECT OF SOME PHENYLETHYLAMINES ON THE DEPLETION OF BRAIN 5-HT CAUSED BY PARA-CHLOROPHENYLALANINE (PCPA)

Treatment	Dose (mg/kg)	5HT (μg/g)
Control		0·59 ± 0·035
PCPA	400	0·35 ± 0·042‡
D-Amphetamine alone	2 × 10	0·40 ± 0·034‡
D-Amphetamine + PCPA	2 × 10 (+ 400)	0·18§ ± 0·027
D-Methylamphetamine alone	2 × 10	0·45 ± 0·016‡
D-Methylamphetamine + PCPA	2 × 10 (+ 400)	0·14§ ± 0·022‡
Control		0·55 ± 0·062
PCPA	400	0·37 ± 0·036†
p-Bromomethylamphetamine alone	2 × 10	0·31 ± 0·016‡
p-Bromomethylamphetamine + PCPA	2 × 10 (+ 400)	0·19§ ± 0·023
p-Nitromethylamphetamine alone	2 × 10	0·25 ± 0·014‡
p-Nitromethylamphetamine + PCPA	2 × 10 (+ 400)	0·13§ ± 0·036

Results expressed as mean ± standard error of the mean, for five animals per group. Significance expressed as in Table 1. § Significance of difference between the group treated with PCPA alone and that treated with PCPA + the amphetamine significant at $P < 0·001$.

TABLE 4. EFFECT OF SOME PHENYLETHYLAMINES ON THE DEPLETION OF BRAIN 5-HT INDUCED BY 4-METHYL-α-ETHYL-META-TYRAMINE (H75/12)

Treatment	Dose (mg/kg)	5HT (μg/g)
Control		0·48 ± 0·007
H75/12	2 × 25	0·41 ± 0·013‡
D-Amphetamine alone	2 × 10	0·49 ± 0·027
D-Amphetamine + H75/12	2 × (10 + 25)	0·39 ± 0·025‡
D-Methylamphetamine alone	2 × 10	0·42 ± 0·009‡
D-Methylamphetamine + H75/12	2 × (10 + 25)	0·3§ ± 0·009‡
Control		0·46 ± 0·010
H75/12	2 × 25	0·35 ± 0·013‡
P-Bromomethylamphetamine alone	2 × 10	0·39 ± 0·015‡
P-Bromomethylamphetamine + H75/12	2 × (10 + 25)	0·33 ± 0·013‡
P-Nitromethylamphetamine alone	2 × 10	0·30 ± 0·006‡
P-Nitromethylamphetamine + H75/12	2 × (10 + 25)	0·27§ ± 0·007‡

Results expressed as mean ± standard error of the mean for five animals per group. Significance expressed as shown in Table 1. Significance of difference between the group treated with H75/12 + the amphetamine shown by § $P < 0·001$.

Effect on the depletion of brain 5-HT by 4 methyl α-ethyl-meta-tyramine (H75/12). Both methylamphetamine and *p*-nitromethylamphetamine potentiated the depletion of 5-HT caused by H75/12; amphetamine and *p*-bromomethylamphetamine did not affect this depleting effect (Table 4).

Effect on brain catecholamines and indolealkylamines in three brain areas. The results of this experiment are summarised in Table 5.

Normetanephrine. None of the phenylethylamines significantly affected the concentration of this metabolite in the cortex. However, amphetamine, *p*-nitro and *p*-bromomethylamphetamine significantly elevated the normetanephrine content of the middle brain region; none of the phenylethylamines affected the normetanephrine content of the caudal brain.

Noradrenaline. Both amphetamine and methylamphetamine reduced the concentration of this amine in both the cortex and caudal brain. Methylamphetamine produced a fall in the noradrenaline content of the middle brain region. *p*-Bromo and *p*-nitromethylamphetamine had no effect on the concentration of this amine in any of the regions.

Dopamine. Both amphetamine and methylamphetamine reduced the dopamine concentration in the cortex and caudal brain. In addition, methamphetamine reduced the dopamine content in the middle brain area. *p*-Nitromethylamphetamine significantly increased the concentration of dopamine in both the cortex and middle brain whereas *p*-bromomethylamphetamine did not alter the dopamine content of any of the brain regions.

5-HT. d-Amphetamine, methylamphetamine and *p*-nitromethylamphetamine reduced the 5-HT concentration in the middle brain region. However, *p*-nitromethylamphetamine elevated the concentration of this amine in the caudal brain whereas the other phenylethylamines had no effect.

5-hydroxyindole acetic acid. Apart from amphetamine, all the phenylethylamines increased the concentration of this metabolite in the caudal brain. *p*-Bromomethylamphetamine also increased the concentration of 5-hydroxyindole acetic acid in the middle brain region.

DISCUSSION

In a previous study[11,15] it was shown that the order of potency for the 4 amphetamines in reducing the concentration of noradrenaline correlated with their central stimulant effects. Thus, D-amphetamine which produced the greatest depletion of brain noradrenaline, caused the greatest increase in motor activity and *p*-bromomethylamphetamine, which elevated brain noradrenaline levels, caused the least. Furthermore these differences between the predominately stimulant amphetamines and *p*-bromomethylamphetamine, were even more apparent in animals which had been treated with 4-α-dimethyl-meta-tyramine (H77/77). H77/77 depletes brain noradrenaline by a mechanism similar to that described for H75/12.[25] Whereas amphetamine, methylamphetamine and *p*-nitromethylamphetamine potentiated the depletion of brain noradrenaline by this compound, *p*-bromomethylamphetamine did not.

From all the studies of the effect of the four amphetamines on brain catecholamines it appears that only when these drugs are studied in combination with H77/77 is i possible to differentiate *p*-bromomethylamphetamine from the predominantly stimulant type. As H77/77 specifically depletes brain catecholamines by acting on the pum

TABLE 5. EFFECT OF SOME PHENYLETHYLAMINES ON BRAIN CATECHOLAMINES AND ON BRAIN INDOLE-ALKYLAMINES IN THREE AREAS OF THE RAT BRAIN

	Cortex					Middle brain					Caudal brain				
Drug and dose	NM	NA	DA	5HT	5HIAA	NM	NA	DA	5HT	5HIAA	NM	NA	DA	5HT	5HIAA
Control	0·31 ± 0·026	0·66 ± 0·074	1·07 ± 0·116	0·66 ± 0·093	0·38 ± 0·037	0·28 ± 0·007	1·06 ± 0·025	1·30 ± 0·105	0·61 ± 0·032	0·37 ± 0·081	0·42 ± 0·029	0·84 ± 0·131	0·74 ± 0·121	1·25 ± 0·261	0·90 ± 0·039
Amphetamine 10 mg/kg	0·30 ± 0·013	0·43 ± 0·025‡	0·67 ± 0·100†	0·53 ± 0·062	0·43 ± 0·046	0·32 ± 0·007‡	0·87 ± 0·118	1·05 ± 0·87	0·44 ± 0·070*	0·25 ± 0·033	0·56 ± 0·061*	0·39 ± 0·038†	0·49 ± 0·030	1·78 ± 0·150	0·90 ± 0·046
Methyl-amphetamine 10 mg/kg	0·29 ± 0·016	0·36 ± 0·029‡	0·60 ± 0·101‡	0·68 ± 0·062	0·46 ± 0·045	0·35 ± 0·050	0·70 ± 0·032†	1·01 ± 0·101*	0·47 ± 0·047†	0·26 ± 0·011	0·47 ± 0·056	0·43 ± 0·050‡	0·48 ± 0·067	1·24 ± 0·118	1·34 ± 0·047†
Control	0·30 ± 0·034	0·52 ± 0·012	0·93 ± 0·034	0·67 ± 0·047	0·35 ± 0·115	0·27 ± 0·012	0·99 ± 0·041	0·90 ± 0·027	0·80 ± 0·022	0·35 ± 0·055	0·40 ± 0·082	0·90 ± 0·046	0·84 ± 0·07	1·29 ± 0·044	1·60 ± 0·117
p-Bromomethyl-amphetamine 10 mg/kg	0·33 ± 0·074	0·56 ± 0·007	0·97 ± 0·026	0·72 ± 0·030	0·29 ± 0·026	0·38 ± 0·018‡	1·08 ± 0·043	1·06 ± 0·089	0·78 ± 0·042	0·49 ± 0·046‡	0·32 ± 0·017	1·30 ± 0·126	0·83 ± 0·060	1·40 ± 0·063	2·37 ± 0·332*
p-Nitromethyl-amphetamine 10 mg/kg	0·33 ± 0·034	0·64 ± 0·054	1·23 ± 0·082†	0·61 ± 0·021	0·27 ± 0·066	0·36 ± 0·028†	0·99 ± 0·038	1·37 ± 0·169†	0·68 ± 0·045†	0·36 ± 0·055	0·29 ± 0·017	0·96 ± 0·046	0·92 ± 0·055	1·59 ± 0·99†	2·54 ± 0·397*

Results are expressed as μg/g wet weight. All results expressed as mean ± standard error of the mean for five animals per group. Significance expressed as * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$. NM = Normetanephrine, NA = Noradrenaline, DA = Dopamine.

mechanism at the level of the neuronal cell membrane,[25] (a mechanism of action which is dissimilar to that for reserpine) it may be speculated that the pharmacological differences between these amphetamines may be due to their differences in action at the neuronal membrane.

In the present study no clear distinction emerged between the two types of phenylethylamine. Thus only *p*-nitromethylamphetamine potentiated the depletion of brain nor adrenaline by αMMT. Previous studies[18] had shown that structurally unrealted hallucinogenic drugs potentiated the depleting effect of αMMT and it was therefore anticipated that *p*-bromomethylamphetamine which has a behavioural profile in animals similar to LSD,[14] might have a similar effect. As *p*-bromomethylamphetamine has an effect on brain biogenic animals which is dissimilar to other known hallucinogenic drugs[18,27] it is possible that this amphetamine is not an hallucinogen. Preliminary studies in man (Knoll, personal communication) indicate that this suggestion is correct and emphasizes the difficulty in predicting from its effects on animal behaviour, whether a compound is hallucinogenic.

All four amphetamines significantly potentiated the depletion of brain 5-HT following the administration of parachlorophenylalanine. These results suggest that the amphetamines increase the turnover of this amine in the brain. This finding could account for the fact that apart from *p*-bromomethylamphetamine, the amphetamines reduce the concentration of brain 5-HT. However, from their effects on the depletion of brain 5-HT by H75/12 it is apparent that their action on the 5-HT containing neuronal membrane differs; only methylamphetamine and *p*-nitromethylamphetamine potentiated the depleting action of this compound. Carlsson and co-workers[23] have suggested that H75/12 acts on the 5-HT containing neuronal membrane in a similar way to the mechanism of action of H77/77 on the catecholamine containing neurone. Clearly it is not possible to differentiate between the pharmacological effects of these drugs from their actions on brain 5-HT containing neurones.

In an attempt to study the possible site of action of the phenylethylamines in more detail, studies were made of their effects on the biogenic amines and their metabolites in three main functional areas of the rat brain. In general, it would appear that whereas *d*-amphetamine and methylamphetamine predominantly affect the adrenergic system in most regions of the brain, the para substituted phenylethylamines have a much smaller effect at an equivalent dose. It is of interest that *p*-bromomethylamphetamine primarily affects amine metabolism in the middle brain region and has little effect on amines in the cortex or caudal brain. It can be speculated that as this brain region is concerned primarily with the control of emotional responses in mammals, such biochemical changes may be concerned with the specific behavioural effects of this drug in animal and man. Furthermore, the increase in the concentration of normetanephrine in this region of the brain indicates that the extra-neuronal metabolism of noradrenaline is increased; similarly the increase in 5-hydroxyindole acetic acid suggests that the rate of catabolism of 5-HT is also increased.

Because of the extreme anatomical and functional diversity of different regions of the brain, there is clearly a need to investigate changes in biogenic amines and their metabolites in more discrete areas. This has been emphasized by the recent findings of Ott, Schmitt, Pohle and Matthies[12] who showed that whereas methylamphetamine reduced the noradrenaline content in most regions of the rat brain, it increased the noradrenaline content of the tegmentum. Further studies will therefore be devoted to

an investigation of changes induced by the phenylethylamines in different regions of the rat brain.

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