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# Some neurochemical effects of amphetamine, methylamphetamine and *p*-bromomethylamphetamine in the rat

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## Summary

1. Low doses of D-amphetamine increased brain noradrenaline concentrations in the rat; doses greater than 5 mg/kg, however, caused a decrease. Methylamphetamine also showed this dual effect, but a reduction in brain noradrenaline concentration only occurred when doses greater than 10 mg/kg were administered. *p*-Bromomethylamphetamine did not significantly reduce brain noradrenaline concentrations even at a dose of 60 mg/kg. The order of potency in reducing the concentration of noradrenaline correlated with the central stimulant effects; D-amphetamine produced the greatest and *p*-bromomethylamphetamine the least increase in motor activity.

2. D-Amphetamine and D-methylamphetamine potentiated the action of 4,$\alpha$-dimethyl-*m*-tyramine (H77/77) in depleting brain noradrenaline; the greatest potentiation was produced by D-amphetamine. This suggests that the phenylethylamines may affect brain noradrenaline concentrations by acting on the reserpine resistant uptake mechanism.

3. Differences were found in the effect of the three drugs on brain dopamine concentrations; D-amphetamine caused a decrease while *p*-bromomethylamphetamine caused an increase. Methylamphetamine had no effect on the concentration of dopamine. Only *p*-bromomethylamphetamine significantly reduced the depletion of brain dopamine concentrations caused by H77/77.

4. Methylamphetamine and *p*-bromomethylamphetamine reduced the concentration of 5-hydroxytryptamine (5-HT) in the brain; administration of the same dose of D-amphetamine did not change the concentration of 5-HT.

5. Changes in the blood and brain concentrations of tyrosine and tryptophan, and in the concentration of $\gamma$-amino-*n*-butyric acid in the brain could not be correlated with the changes observed in the concentrations of biogenic amines in the brain.

## Introduction

Previous 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 (Moore & Lariviere, 1963; Baird & Lewis, 1964; McLean & McCartney, 1961). From such studies, it would appear that amphetamine reduces the concentration of nor-

adrenaline in the brain by increasing the release of this amine from neurons (Carlsson, Lindquist, Dahlström, Fuxe & Masuoka, 1965), by inhibiting the uptake of the amine into storage sites (Iversen, Axelrod & Glowinski, 1967), by acting on adrenoceptive receptors within the brain (Vane, 1960 ; Smith, 1965) and thereby inhibiting the further synthesis of the amine by a feed-back mechanism (Littleton, 1967), or by a combination of these effects.

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 (Conell, 1958 ; Tolentino, 1957). These effects are shared to some extent by the structurally related drug, methylamphetamine. In contrast, *p*-bromomethylamphetamine has only very slight central stimulant properties, but is a potent psychotomimetic agent even after a single administration (Knoll, Vizi & Ecseri, 1966).

Because of the contrast between the structural similarities of these phenylethylamines and their pharmacological effects, it was of interest to compare the effects of these drugs on biogenic amines and their precursor amino-acids in the brain. In this way it might be possible to see whether the stimulant and the psychotomimetic effects have a different biochemical bases.

## Methods

Specific pathogen-free rats of either sex and of the Wistar strain (90–120 g) were used. Immediately after intraperitoneal injection with the drug or vehicle (distilled water), the rats were caged in groups of five until killed. The pharyngeal temperatures were recorded using an electrical thermistor (Light & Co., Brighton, Sussex) just before the rats were decapitated.

### *Effect of phenylethylamine derivatives on some amino-acids in brain and blood and on biogenic amines in brain*

Groups of five rats were treated with the phenylethylamine derivatives (at a dose of 1 and 5 mg/kg) and killed 30, 60, 90, 120, 180, 240 and 300 min later. When serum amino-acids were determined, blood was collected from the cut jugular veins and carotid arteries, allowed to clot and then centrifuged at approximately 800 *g* for 10 minutes.

Portions of the sera were diluted 1 : 10 with a 1 : 1 (v/v) mixture of 0·4 N perchloric acid and 10% trichloracetic acid and then centrifuged at approximately 800 *g* for 10 minutes. Portions of the supernatant were used for the determination of tyrosine by the method of Waalkes & Udenfriend (1957), as modified by Udenfriend (1962), and tryptophan by the method of Hess & Udenfriend (1959) as modified by Guroff, King & Udenfriend (1961). The brains, minus the cerebellum, were removed and dissected into two equal portions along the mid line. They were then weighed and kept on ice until homogenized in 5 ml of the 1 : 1 (v/v) perchloric acid : trichloracetic acid mixture in a Silverson homogenizer. The homogenate was then centrifuged for 10 min at approximately 800 *g* and portions of the supernatant fractions were removed for the determination of tyrosine and tryptophan by the same methods used for determining these amino-acids in serum. An additional portion of the supernatant was taken for the determination of γ-amino-*n*-butyric acid (γABA) by the fluorimetric method of Uchida & O'Brien (1964).

5-Hydroxytryptamine was determined in half brains, prepared as described above, by a modification of the methods of Snyder, Axelrod & Zweig (1965) and Welch & Welch (1969). The half brains were each homogenized in 2 ml cold 0·01 N hydrochloric acid, together with 5 g sodium chloride and 20 ml cold *n*-butanol. The homogenate was then shaken mechanically for 30 min and centrifuged at approximately 1,000 *g* for 10 minutes. A 15 ml portion of the butanol phase was taken and added to 30 ml *n*-heptane and 3 ml of 0·05 M sodium phosphate buffer (pH 7) and shaken mechanically for 15 minutes. After centrifugation, the organic phase was removed by aspiration and a 2 ml portion of the phosphate buffer was added to 0·3 ml of 0·1 M ninhydrin in water in a test tube. The tube was placed in a water bath at 65° C for 30 min, removed and allowed to stand at room temperature (21° C) for 60 minutes. Fluorescence was measured by means of a Farrand spectrophotofluorimeter (activation and fluorescence wavelengths were 385 and 490 nm, respectively).

For the experiments in which noradrenaline and dopamine were determined the treatment times were extended so that one group was killed at 6 h and a further group at 16 h after injection. Noradrenaline and dopamine were determined using homogenates of the whole brain by the method of Welch & Welch (1969). This method consists of homogenizing the brain in 0·01 N HCl and extracting the homogenate with *n*-butanol saturated with sodium chloride. The butanol phase is then shaken with *n*-heptane and phosphate buffer (pH 7) which removes the catecholamines from the organic solvent. The hydroxyindole fluorophors were developed in a 1·0 ml portion of the phosphate buffer using iodine as the oxidant.

### *Effect of different doses of phenylethylamines on brain catecholamine concentrations*

Groups of rats were treated for 120 min with 0·5, 1·0, 5·0, 10·0, 30·0 and 60·0 mg/kg (*p*-bromomethylamphetamine only) of the phenylethylamines. This treatment time coincided with the time taken for the drugs to have a maximal effect on brain catecholamine concentrations. After decapitation, the brains were assayed for noradrenaline and dopamine by the method of Welch & Welch (1969).

### *Effect of phenylethylamines on depletion of brain catecholamines caused by 4,α-dimethyl-*m*-tyramine (H77/77)*

Carlsson, Fuxe, Hamberger & Malmfors (1969) reported that H77/77 displaces brain catecholamines, particularly noradrenaline, and utilizes the reserpine resistant uptake mechanism to bring about this displacement. The effect of H77/77 together with the phenylethylamines was therefore studied to see whether the drugs affected brain catecholamine concentrations by acting on the reserpine resistant uptake mechanism.

Groups of rats were treated at 0 and 2 h with H77/77 alone or in combination with one of the phenylethylamines. The phenylethylamine was injected 30 min before each H77/77 treatment. The dose schedule for this experiment was: group 1, distilled water only; group 2, H77/77 only ($2 \times 12{\cdot}5$ mg/kg intraperitoneally); group 3, H77/77 + a phenylethylamine ($2 \times 1$, 5 or 10 mg/kg intraperitoneally); group 4, phenylethylamine alone.

Two hours after the last injection of H77/77, the animals were decapitated and the catecholamines estimated on the brains by the method of Welch & Welch (1969).

Drugs used were: D-amphetamine sulphate; D-methylamphetamine hydrochloride; *p*-bromomethylamphetamine hydrochloride. All doses reported refer to the salts; the drugs were injected intraperitoneally in a volume of 1 ml/kg body weight. Control groups were injected with an equivalent volume of distilled water. The significance of the results was assessed using Student's *t*-test.

## Results

### *Behavioural effects produced by the phenylethylamines*

Doses of D-amphetamine above 1 mg/kg produced excitement, tremor, head shaking, piloerection, salivation and hyperthermia. At a dose of 10 mg/kg, excitement was followed by exhaustion and loss of spatial orientation. Hyperthermia was greatest at a dose of 30 mg/kg; three animals given this dose died before the end of the 2 h experimental period. The symptoms produced by D-methylamphetamine were qualitatively similar to those produced by amphetamine but were less severe. In animals treated with *p*-bromomethylamphetamine there was some excitement, salivation and hyperthermia at doses of 30 and 60 mg/kg. Head shaking, and aggressiveness when disturbed, were more pronounced with this drug than with the other amphetamines. Doses of 10 mg/kg and lower produced only slight effects.

### *Effect on some blood and brain amino-acids and on brain 5-hydroxytryptamine concentrations*

*Tyrosine.* Table 1 shows that all three phenylethylamines at doses of 5 mg/kg reduced both serum and brain tyrosine concentrations throughout the 2 h period of observation during which the symptoms were most pronounced. At doses of 1 mg/kg, D-amphetamine and D-methylamphetamine produced small falls in the concentration of brain tyrosine only at 3 and 4 h, respectively, after injection. In contrast, *p*-bromomethylamphetamine caused a fall in the concentration of brain tyrosine for most of the experimental period.

The effects of D-amphetamine and D-methylamphetamine at a dose of 1 mg/kg, on serum tyrosine concentrations were biphasic; the concentration was elevated initially and fell after 2 hours. *p*-Bromomethylamphetamine (1 mg/kg) had no effect on the serum tyrosine concentration.

*Tryptophan.* At a dose of 5 mg/kg, all three drugs increased the concentration of tryptophan in the brain (Table 2). However, there were differences in the effects of these drugs on the serum tryptophan concentrations. D-Amphetamine caused a biphasic change; a reduction in the concentration of tryptophan in serum occurred initially which changed to a significant increase at the end of the 4 h experimental period. In contrast, D-methylamphetamine did not significantly alter the concentrations of serum tryptophan. *p*-Bromomethylamphetamine significantly reduced the serum tryptophan concentration from 3 h after the drug had been administered until the end of the experimental period.

TABLE 1. *Effect of some phenylethylamines on brain and serum tyrosine concentrations*

| Treatment time<br>Drug and dose | 0 | 0·5 h | 1 h | 1·5 h | 2 h | 3 h | 4 h | 5 h |
|---|---|---|---|---|---|---|---|---|
| | | | | Brain tyrosine | | | | |
| D-Amphetamine (5 mg/kg) | 21·9±1·01 | 22±1·23 | 20·4±0·977 | §16·1±1·17 | §14·6±0·244 | *18·6±1·74 | 20·1±1·02 | |
| D-Methylamphetamine (5 mg/kg) | 22·6±0·958 | 22·2±1·48 | †19·3±0·54 | §17·9±0·879 | §16·9±0·428 | 20±1·56 | 20·9±0·77 | |
| *p*-Bromomethylamphetamine (5 mg/kg) | 22·8±1·32 | 20·2±1·42 | 22·3±1·05 | 21·3±0·473 | §15·8±0·543 | 21·7±0·963 | 20·8±1·03 | 16±0·84 |
| | | | | Serum tyrosine | | | | |
| D-Amphetamine (5 mg/kg) | 20·8±1·2 | §13·5±0·411 | §14·6±0·286 | §10·9±0·835 | §11·7±0·822 | 18·6±1·01 | 21·3±0·528 | |
| D-Methylamphetamine (5 mg/kg) | 22·3±0·79 | §17·7±0·757 | §15·7±0·38 | §14·2±0·95 | §16·2±0·681 | *20·1±0·853 | 20·8±0·417 | |
| *p*-Bromomethylamphetamine (5 mg/kg) | 21·1±1·23 | 18·4±0·678 | 18·9±1·10 | †17·1±0·307 | †17·1±0·39 | ‡15·6±0·88 | ‡16·6±0·763 | §13·1±0·655 |
| | | | | Brain tyrosine | | | | |
| D-Amphetamine (1 mg/kg) | 23·4±1·30 | | 24·6±2·08 | | 21·5±2·46 | *18·4±0·495 | 19·9±0·840 | |
| D-Methylamphetamine (1 mg/kg) | 17·3±0·348 | | 17·1±1·34 | | 17·7±0·744 | 14·8±1·19 | ‡13·1±0·981 | |
| *p*-Bromomethylamphetamine (1 mg/kg) | 23·9±0·910 | | 23·3±0·766 | | *21·5±1·05 | ‡20·1±0·770 | †21·2±0·127 | |
| | | | | Serum tyrosine | | | | |
| D-Amphetamine (1 mg/kg) | 21·6±0·489 | | †23·8±0·777 | | †19·6±0·290 | ‡18·2±0·568 | §15·8±0·469 | |
| D-Methylamphetamine (1 mg/kg) | 16·0±0·741 | | *18·2±0·636 | | 16·3±0·952 | 15·8±0·578 | ‡13·0±0·376 | |
| *p*-Bromomethylamphetamine (1 mg/kg) | 23·2±2·02 | | 24·0±0·892 | | 21·4±0·791 | 22·0±0·898 | 22·8±1·29 | |

Results expressed as μg/g wet weight or μg/ml serum. All results expressed as mean ± standard error of the mean for five animals per group. Significance expressed as * $P<0.05$, † $P<0.025$, ‡ $P<0.01$, § $P<0.005$.

TABLE 2. *Effect of some phenylethylamines on brain and serum tryptophan concentrations*

| Pretreatment time | 0 | 0–5 h | 1 h | 1·5 h | 2 h | 3 h | 4 h | 5h |
|---|---|---|---|---|---|---|---|---|
| | Brain tryptophan | | | | | | | |
| Drug and dose | | | | | | | | |
| D-Amphetamine (5 mg/kg) | 2·5±0·132 | 2·8±0·175 | §3·2±0·126 | 2·4±0·175 | 2·6±0·212 | §3·4±0·083 | §3·4±0·221 | |
| D-Methylamphetamine (5 mg/kg) | 2·8±0·087 | §3·6±0·162 | §3·6±0·13 | ‡3·3±0·107 | §3·5±0·076 | *3·3±0·259 | §4·2±0·319 | |
| *p*-Bromomethylamphetamine (5 mg/kg) | 2·3±0·06 | *2·9±0·393 | §2·8±0·116 | 2·2±0·164 | §2·9±0·137 | 2·4±0·104 | 2·4±0·134 | 2·2±0·118 |
| D-Amphetamine (1 mg/kg) | 2·52±0·110 | | 2·07±0·332 | | 2·41±0·389 | 2·48±0·105 | 2·56±0·091 | |
| D-Methylamphetamine (1 mg/kg) | 3·57±0·264 | | 3·04±0·238 | | 3·18±0·229 | 2·97±0·152 | 3·24±0·326 | |
| *p*-Bromomethylamphetamine (1 mg/kg) | 2·57±0·102 | | 2·4±0·061 | | †2·03±0·129 | 2·23±0·223 | 2·40±0·162 | |
| | Serum tryptophan | | | | | | | |
| D-Amphetamine (5 mg/kg) | 12·6±0·781 | §8·34±0·759 | §7·52±0·499 | 10·4±1·29 | 10·7±0·66 | §17·7±1·31 | 16·7±0·301 | |
| D-Methylamphetamine (5 mg/kg) | 16·7±0·899 | 14·7±0·728 | 14·7±0·286 | 15·9±1·23 | 18·2±0·816 | 16·2±0·389 | 17·1±0·497 | |
| *p*-Bromomethylamphetamine (5 mg/kg) | 14·2±1·56 | 13·5±0·965 | 15·8±0·942 | 17·4±0·533 | 17·2±0·299 | †9·71±0·773 | †9·35±0·834 | †9·24±0·621 |
| D-Amphetamine (1 mg/kg) | 12·6±1·13 | | 14·0±0·843 | | 12·5±0·489 | 14·4±0·495 | 13·5±1·11 | |
| D-Methylamphetamine (1 mg/kg) | 16·6±0·487 | | 16·4±1·82 | | 16·3±1·40 | 19·8±1·81 | 18·7±0·937 | |
| *p*-Bromomethylamphetamine (1 mg/kg) | 16·9±1·13 | | *13·8±0·729 | | 14·0±1·56 | 15·2±0·961 | 17·6±1·07 | |

Results, expressed as μg/g wet weight or μg/ml serum, for five animals per group. All results expressed as mean ± standard error of the mean. Significance expressed as shown in Table 1.

TABLE 3. *Effect of some phenylethylamines on γ-amino-n-butyric acid (γABA) concentrations in the brain*

| Pretreatment time | Brain γABA 0 | 0·5 h | 1 h | 1·5 h | 2 h | 3 h | 4 h | 5 h |
|---|---|---|---|---|---|---|---|---|
| Drug and dose | | | | | | | | |
| D-Amphetamine (5 mg/kg) | 336±23·1 | ‡436±7·75 | §483±16·8 | ‡432±18·7 | 366±18·2 | 326±16·9 | 329±27·4 | |
| D-Methylamphetamine (1 mg/kg) | 359±26·2 | ‡453±28·2 | 364±12·6 | 420±7·46 | ‡449±7·49 | 430±1·1 | 302±4·93 | |
| *p*-Bromomethylamphetamine (5 mg/kg) | 318±10·8 | 347±26·3 | 359±38·5 | ‡268±19·5 | §235±7·14 | 356±21·2 | 351±9·45 | 329±11·3 |
| D-Amphetamine (1 mg/kg) | 423±16·1 | | 398±11·7 | | 417±20·8 | 417±14·5 | 425±19·5 | |
| D-Methylamphetamine (1 mg/kg) | 415±11·5 | | ‡314±26·6 | | §255±29·9 | §288±21·9 | *354±8·48 | |
| *p*-Bromomethylamphetamine (1 mg/kg) | 491±39·8 | | †401±21·1 | | †401±18·1 | †399±13·5 | †381±19·2 | |

Results expressed as μg/g wet weight. All results, expressed as mean ± standard error of the mean, are for five animals per group. Significance expressed as shown in Table 1.

TABLE 4. *Effect of some phenylethylamines on the concentration of 5-hydroxytryptamine in the brain*

| Pretreatment time | 0 h | 0·5 h | 1 h | 1·5 h | 2 h | 3 h | 4 h |
|---|---|---|---|---|---|---|---|
| Drug and dose | | | | | | | |
| D-Amphetamine (5 mg/kg) | 0·656±0·044 | 0·658±0·027 | 0·621±0·06 | 0·795±0·099 | 0·788±0·077 | 0·788±0·124 | 0·643±0·075 |
| D-Methylamphetamine (5 mg/kg) | 0·659±0·029 | 0·682±0·027 | *0·591±0·011 | ‡0·563±0·023 | 0·543±0·013 | 0·668±0·086 | ‡0·855±0·091 |
| *p*-Bromomethylamphetamine (5 mg/kg) | 0·572±0·057 | 0·654±0·145 | 0·672±0·065 | 0·53±0·015 | 0·734±0·113 | ‡0·32±0·03 | 0·317±0·051 |
| D-Amphetamine (1 mg/kg) | 0·44±0·029 | | 0·45±0·043 | | 0·42±0·011 | 0·47±0·039 | 0·43±0·042 |
| D-Methylamphetamine (1 mg/kg) | 0·86±0·07 | | 0·77±0·085 | | 0·82±0·058 | 0·82±0·091 | 0·82±0·067 |
| *p*-Bromomethylamphetamine (1 mg/kg) | 0·50±0·039 | | 0·61±0·096 | | *0·70±0·054 | 0·50±0·058 | 0·50±0·035 |

Results, expressed as μg/g wet weight, for five animals per group. Each result represents the mean of two observations. All results expressed as mean ±standard error of the mean. Significance expressed as shown in Table 1.

At a dose of 1 mg/kg, only *p*-bromomethylamphetamine significantly reduced the concentration of tryptophan in brain and serum; at this dose D-amphetamine and D-methylamphetamine had no significant effect.

*γ-Amino-n-butyric acid.* At a dose of 5 mg/kg, D-amphetamine and D-methylamphetamine produced a significant increase in brain γABA concentrations; *p*-bromomethylamphetamine reduced the concentration of γABA in the brain initially and then caused an increase after 3 h and 4 h (Table 3). Doses of 1 mg/kg of D-methylamphetamine and *p*-bromomethylamphetamine caused a reduction in the brain γABA concentration for the duration of the experiment. D-amphetamine had no effect.

*5-Hydroxytryptamine.* D-Methylamphetamine, at a dose of 5 mg/kg, produced a biphasic change in the brain 5-HT concentration, which was reduced at 1 h and 1·5 h (when the symptoms were most obvious) and was increased 4 h after the drug had been injected (Table 4). *p*-Bromomethylamphetamine reduced the concentration of 5-HT 3 h and 4 h after the drug had been administered. D-Amphetamine did not affect the concentration of 5-HT in the brain.

The phenylethylamines had little or no effect on the concentration of this 5-HT when they were injected at a dose of 1 mg/kg.

### *Effect on brain catecholamines*

*Noradrenaline.* At a dose of 1 mg/kg, D-amphetamine significantly elevated brain noradrenaline concentrations for most of the experimental period (Fig. 1). However, 16 h after this dose of D-amphetamine had been administered, the brain noradrenaline concentrations were significantly reduced. *p*-Bromomethylamphetamine significantly raised the concentrations of brain noradrenaline for approximately 6 h after the drug had been administered. D-Methylamphetamine, at a dose of 1 mg/kg, did not significantly alter brain noradrenaline concentrations.

*Dopamine.* D-Amphetamine, at a dose of 1 mg/kg, caused a significant increase in the concentration of brain dopamine up to 4 h after the drug had been administered (Fig. 1). At this same dose, *p*-bromomethylamphetamine increased the concentration of dopamine in the brain 6 h after administration of the drug. D-Methylamphetamine (1 mg/kg) produced a biphasic change in the concentration of this amine, the concentration being significantly increased at 1 h and reduced at between 6 and 16 h after administration (Fig. 1).

### *Effect of different doses of phenylethylamines on brain catecholamine concentrations*

*Noradrenaline.* Two hours after treatment with the lowest doses used (0·5 and 1·0 mg/kg), both D-amphetamine and D-methylamphetamine increased the concentration of noradrenaline in the brain, but the concentration of this amine was reduced when higher doses of the drugs were administered (Fig. 2). In contrast, *p*-bromomethylamphetamine did not produce a significant reduction in the concentration of brain noradrenaline even at a dose of 60 mg/kg.

*Dopamine.* D-Amphetamine caused a significant reduction in the concentration of brain dopamine when doses of 10 mg/kg or greater were administered (Fig. 2); in contrast, methylamphetamine at doses up to 10 mg/kg increased the concentration of dopamine, while *p*-bromomethylamphetamine had no significant effect.

### *Effect of phenylethylamines on depletion of brain catecholamines caused by 4,α-dimethyl-*m*-tyramine (H77/77)*

The results are summarized in Table 5. At a dose of 10 mg/kg, both D-amphetamine and D-methylamphetamine potentiated the depletion of brain noradrenaline caused by H77/77; lower doses of the drugs had no effect on the action of H77/77. Neither D-amphetamine nor D-methylamphetamine significantly affected the depletion of brain dopamine caused by H77/77. In contrast, *p*-bromomethylamphetamine significantly antagonized the depletion of dopamine caused by this drug.

The experiment was repeated using animals which had been pretreated with 1 mg/kg of H77/77 instead of 12·5 mg/kg used in the previous experiment. Doses

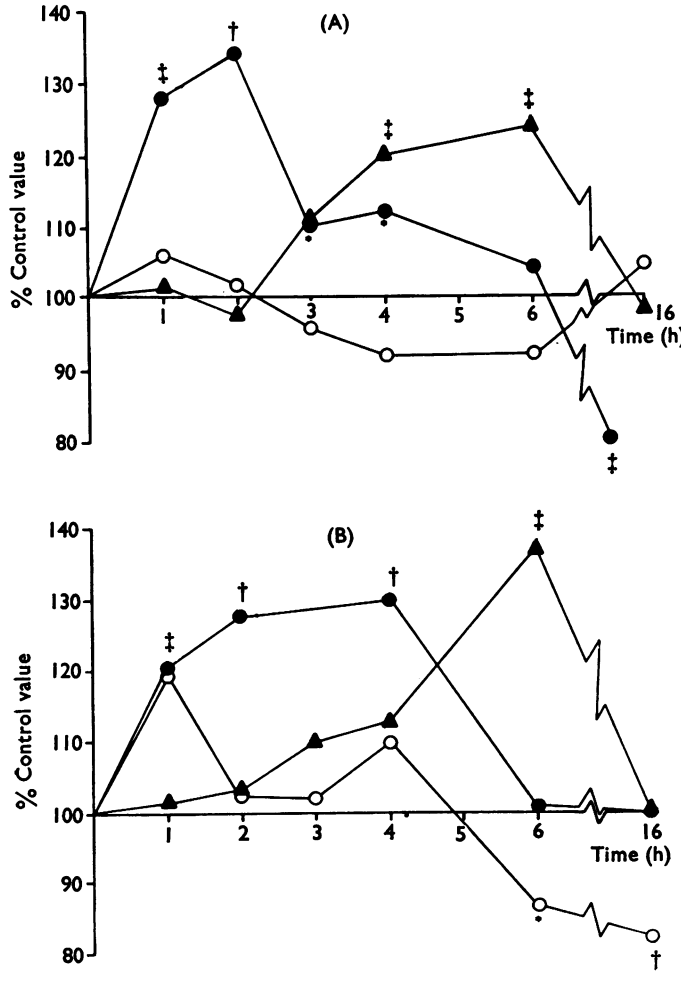


FIG. 1. Time courses of effects of D-amphetamine, D-methylamphetamine and *p*-bromomethylamphetamine on brain catecholamine concentrations. (A), Brain noradrenaline; (B), brain dopamine. Animals were injected with a dose of 1 mg/kg of the amphetamine. Each point, expressed as a percentage of the control value, represents the mean of at least five animals. The significance of the difference from the control animals is shown by $*P<0.05$, $\dagger P<0.01$, $\ddagger P<0.001$. ●——●, Amphetamine; ○——○, methylamphetamine; ▲——▲, *p*-bromomethylamphetamine. Control values for noradrenaline were 0·58±0·012 μg/g; and for dopamine, 0·94±0·076 μg/g. These values represent the mean ±S.E.M.

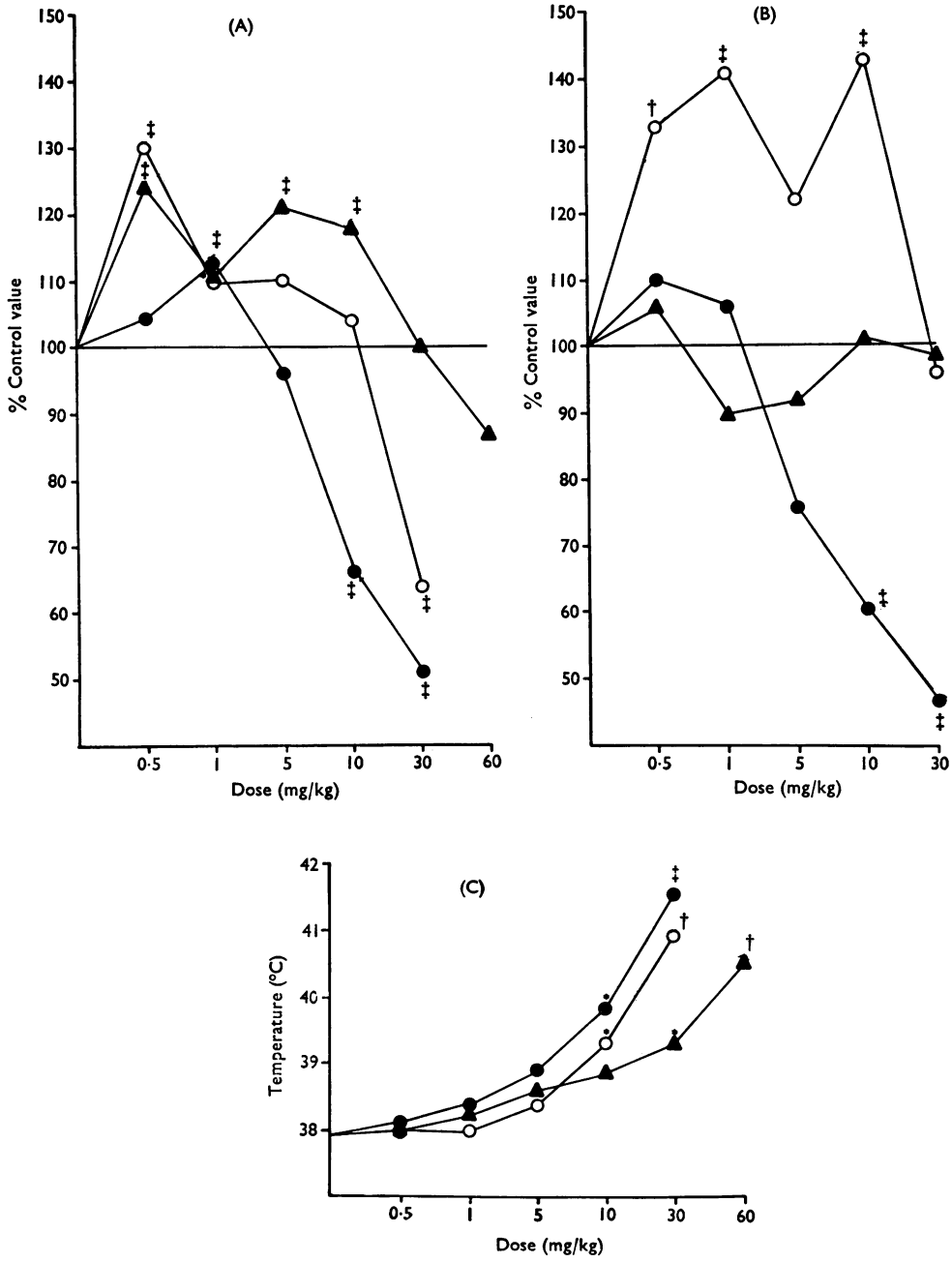


FIG. 2. Effect of different doses of D-amphetamine, D-methylamphetamine and *p*-bromomethylamphetamine on brain catecholamine concentrations and pharyngeal temperatures. (A), Brain noradrenaline; (B), brain dopamine; (C), pharyngeal temperature. All animals were killed 2 h after the drug had been administered. Each point, expressed as a percentage of the control value for changes in catecholamine levels, represents the mean of at least five animals. The significance of the difference from the control animals is shown by $*P<0{\cdot}05>0{\cdot}025$, $\dagger P<0{\cdot}01>0{\cdot}005$, $\ddagger P<0{\cdot}001$. ●——●, Amphetamine; ○——○, methylamphetamine; ▲——▲, *p*-bromomethylamphetamine. Control values for noradrenaline were $0{\cdot}56\pm0{\cdot}012$ μg/g, and for dopamine were $0{\cdot}92\pm0{\cdot}084$ μg/g. These values represent the mean ± S.E.M.

TABLE 5. *Effect of some phenylethylamines on the depletion of brain catecholamines caused by* 4,α-*dimethyl-m-tyramine* (*H*77/77)

| Treatment | Dose (mg/kg) | Noradrenaline (μg/g) | Significance from control | Significance from H77/77 alone | Dopamine (μg/g) | Significance from control | Significance from H77/77 alone |
|---|---|---|---|---|---|---|---|
| Control | | 0·58±0·012 | | | 0·94±0·084 | — | — |
| H77/77 alone | 2×12·5 | 0·37±0·054 | <0·0005 | — | 0·66±0·047 | <0·01 | — |
| D-Amphetamine alone | 2×10 | 0·36±0·032 | <0·0005 | — | 0·74±0·022 | <0·05 | — |
| D-Amphetamine+H77/77 | 2×(10+12·5) | 0·22±0·015 | <0·0005 | <0·0125 | 0·66±0·112 | <0·01 | n.s. |
| D-Methylamphetamine alone | 2×10 | 0·46±0·026 | <0·01 | — | 1·04±0·030 | n.s. | — |
| D-Methylamphetamine+H77/77 | 2×(10+12·5) | 0·25±0·012 | <0·0005 | <0·025 | 0·67±0·061 | 0·0125 | n.s. |
| *p*-Bromomethylamphetamine | 2×10 | 0·65±0·05 | <0·1 | — | 1·04±0·099 | n.s. | — |
| *p*-Bromomethylamphetamine +H77/77 | 2×(10+12·5) | 0·33±0·031 | <0·0025 | n.s. | 0·79±0·049 | <0·05 | <0·05 |

Results expressed as mean ± standard error of the mean, for five animals per group. Each result represents the mean of two observations. n.s. = not statistically significant at the 5% level.

of the depleting agent lower than 1 mg/kg had no effect on brain noradrenaline concentrations. The concentration of noradrenaline in the brain was reduced by 24% after administration of 1 mg/kg of H77/77, whereas a dose of 12·5 mg/kg reduced the concentration of this amine by 36%. The effect of this lower dose of H77/77 on the concentration of noradrenaline in the brain was potentiated by both amphetamine and methylamphetamine ($2 \times 10$ mg/kg) but not by *p*-bromomethylamphetamine.

## Discussion

In this and in other investigations, D-amphetamine has been shown to lower the brain content of noradrenaline in rats (McLean & McCartney, 1961 ; Moore & Lariviere, 1963) and mice (Moore, 1963 ; 1964). This effect, which is shared by methylamphetamine, could be due to the drug affecting the synthesis, storage or metabolism of the amine.

D-Amphetamine, D-methylamphetamine and *p*-bromomethylamphetamine all significantly reduced the concentration of tyrosine in both the serum and brain. This could account for a reduction in the net synthesis of noradrenaline. However, should such a mechanism of action be of primary importance, there are several anomalies which must be considered. All three drugs increase the concentration of brain dopamine, and, in the lower doses used, the concentrations of brain noradrenaline. Methylamphetamine increased the concentration of dopamine at all doses used. As only 1–2% of brain tyrosine is converted to dopamine and noradrenaline (Schubert, Nyback & Sedvall, 1970), it would appear that the changes in brain tyrosine which follow administration of the phenylethylamines are not of decisive importance in determining the changes which occur in the catecholamine concentrations. A possible explanation for the rise in brain dopamine concentration and the reduction in the concentration of noradrenaline in the brain caused by D-amphetamine and D-methylamphetamine is that these drugs inhibit dopamine $\beta$-oxidase activity. DL-Amphetamine inhibits this enzyme *in vitro* (Goldstein & Contrera, 1961). However, the concentration of amphetamine required to produce an inhibition of dopamine $\beta$-oxidase was high and other investigators have not found evidence to substantiate such an inhibitory effect *in vivo* (Littleton, 1967). It seems unlikely, therefore, that the amphetamines produce the observed changes in brain catecholamine concentrations by a direct action on the mechanisms concerned with the synthesis of these amines.

Another possible mode of action is that these drugs affect the storage of catecholamines. D-Amphetamine inhibits the *in vitro* accumulation of noradrenaline into both noradrenergic and dopaminergic nerve terminals (Haggendal & Hamberger, 1967), possibly by inhibiting the uptake of the amine and also by increasing the release of the accumulated amine (Carlsson & Waldeck, 1966 ; Anden, Corrodi & Fuxe, 1968). A similar explanation has been given for the effect of amphetamine and methylamphetamine on the uptake of $^3$H-noradrenaline *in vivo* (Iversen *et al.*, 1967).

More direct evidence for the mode of action of amphetamine, and of the other phenylethylamines which were studied, comes from the effect of these drugs on the depletion of brain catecholamines by H77/77. This compound, in producing a

depletion in cerebral catecholamines, utilizes a transport mechanism which is resistant to the action of reserpine (Carlsson *et al.*, 1969). As the depletion of noradrenaline caused by H77/77 is potentiated by amphetamine and methylamphetamine it would suggest that they act in the same way as the depleting agent. *p*-Bromomethylamphetamine differs in that it does not significantly potentiate this action of H77/77. It was of interest to find that only *p*-bromomethylamphetamine significantly reduced the depletion of brain dopamine by H77/77.

A further mechanism which must be considered concerns the effect of the phenylethylamines on the metabolism of the catecholamines. It is well established that amphetamine, and some of its halogen substituted derivatives, are inhibitors of monoamine exidase (MAO) both *in vitro* (Fuller & Walters, 1965 ; Parmar, 1966), and *in vivo* (Iversen *et al.*, 1967). However, unless it is postulated that the phenylethylamines affect brain catecholamine concentrations by two separate mechanisms (MAO inhibition at low doses which becomes masked by depletion of the catecholamines at higher doses of the drugs), it would appear that MAO inhibition is a relatively unimportant mechanism *in vivo*.

It is possible that the reduction in brain noradrenaline caused by amphetamine and methylamphetamine is the result of the drugs acting on adrenoceptors within the brain, and thereby reducing the further synthesis of the catecholamines by a feed-back mechanism. Such a view has been proposed by other investigators (Van Rossum, Van Der Schoot & Hurkmans, 1962; Smith, 1965). However, this explanation seems unlikely as drugs which block the synthesis of noradrenaline also completely antagonize the central effects of amphetamine (Weissman & Koe, 1965 ; Hanson, 1967). It would therefore appear that in order for amphetamine, and presumably the structurally related phenylethylamines, to produce their effects a small pool of noradrenaline must be present in the brain. The intensity of the central stimulant effects would thus depend on the degree of turnover of the catecholamines produced by the drug ; the greater the turnover the greater the reduction in brain catecholamine concentrations.

Both *p*-bromomethylamphetamine and methylamphetamine reduced brain 5-HT concentrations. This effect is also found when other halogen substituted amphetamine derivatives are used (Miller, Cox, Snodgrass & Maickel, 1970). However, at an equivalent dose, amphetamine did not affect the brain 5-HT concentration. Thus reduction in 5-HT is unlikely to be the result of the drugs affecting the concentration of tryptophan as the concentration of this precursor amino-acid is raised during the period when 5-HT concentrations are reduced. It is possible that the reduction in brain 5-HT is due to the drugs inhibiting tryptophan hydroxylase activity, although this would not appear to be a mechanism which is of primary importance in bringing about the reduction in brain 5-hydroxytryptamine for other halogen substituted amphetamines (Fuller, Hines & Mills, 1965 ; Pletscher, Da Prada, Burkard, Bartholini, Steiner, Bruderer & Bigler, 1966).

Considerable differences were found in the effect of the phenylethylamines on the concentration of γABA in the brain. At the highest dose used, amphetamine and methylamphetamine increased the concentration of this amino-acid, whereas *p*-bromomethylamphetamine produced a fall. At the lowest doses, both methylamphetamine and *p*-bromomethylamphetamine caused a fall in the concentration of γABA ; amphetamine had no effect. If γABA is an inhibitory

transmitter in the central nervous system, as has been suggested (Krnjevic & Schwartz, 1966), one might expect that the changes produced by the three phenylethylamines would be qualitatively similar, the change in the concentration of γABA being related to the severity of the stimulant effect. It would appear from this investigation that no such pattern exists.

The pattern of neurochemical changes produced by *p*-bromomethylamphetamine is quite dissimilar to that produced by other psychotomimetic drugs, for example LSD and mescaline. Thus, in a detailed study of the effects of four structurally unrelated hallucinogens on brain biogenic amines, it was found that all four drugs increased the turnover of noradrenaline but decreased that of 5-HT (Leonard & Tonge, 1969 ; Tonge & Leonard, 1969). The reasons for these differences between *p*-bromomethylamphetamine and the other hallucinogens are not apparent. Nevertheless, the present study does suggest that an analysis of some neurochemical parameters enables a separation to be made between a phenylethylamine with predominantly central stimulant effect from one with mainly psychotomimetic effects.

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