Br. J. Pharmac. (1971), **43**, 732–738.

Effect of *p*-nitromethylamphetamine on biogenic amines and their amino-acid precursors in rat brain

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Summary

1. Low doses of *p*-nitromethylamphetamine caused small increases in the concentrations of brain noradrenaline and dopamine in the rat; a dose of 60 mg/kg however, caused a decrease in the concentrations of both amines. *p*-Nitromethylamphetamine caused behavioural hyperexcitability only at doses which approximated to half the LD50 (68 mg/kg).

2. *p*-Nitromethylamphetamine potentiated the action of 4,α-dimethyl-*m*-tyramine in depleting brain noradrenaline. This suggests that it may affect brain noradrenaline concentrations by utilizing a reserpine resistant uptake mechanism.

3. *p*-Nitromethylamphetamine decreased the concentration of brain 5-hydroxytryptamine.

4. Changes in the blood and brain concentrations of tyrosine and γ-amino-n-butyric acid concentration in the brain could not be correlated with the changes in brain amines. However, a rise in the concentration of brain tryptophan appeared to be correlated with the fall in brain 5-hydroxytryptamine.

Introduction

In a previous study a comparison was made between the effects of (+)-amphetamine, (+)-methylamphetamine and *p*-bromomethylamphetamine on the gross behaviour of rats and on the biogenic amine concentration in the brain (Leonard & Shallice, 1971). The results of this study suggested that the potent psychotomimetic drug, p-bromomethylamphetamine, has biochemical actions which are different from those of the CNS stimulant drugs (+)-amphetamine and (+)-methylamphetamine and that such differences may explain their pharmacological effects in man.

Knoll (1970) has reported that the psychotomimetic activity of the phenylethylamines is enhanced by substitution at the *para* and *meta* positions of the benzene ring. To test his hypothesis, and to extend the findings of our previous investigation, it was of interest to see whether *p*-nitromethylamphetamine produced neurochemical changes which resembled those produced by *p*-bromomethylamphetamine.

Methods

Specific pathogen-free female rats (90–110 g) of the Alderley Park strain were used. *p*-Nitromethylamphetamine (V61) (1 ml/kg body weight) was injected intra-

peritoneally ; the control groups were injected with equivalent volumes of distilled water. The rats were killed by decapitation at different times after injection.

Effect of p-*nitromethylamphetamine on the concentration of amino-acids in brain and on biogenic amine concentrations in brain*

Groups of five rats were treated with *p*-nitromethylamphetamine (1, 10 and 60 mg/kg) and killed 0·5, 1·0, 2·0 and 4·0 h later. When the concentrations of serum tryptophan and tyrosine were determined, blood was collected from the severed 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 10% (w/v) trichloracetic acid and 0·4 N perchloric acid and centrifuged at approximately 800 *g* for 10 minutes. Portions of the supernatant were used for the determination of tyrosine concentration by the fluorimetric method of Waalkes & Udenfriend (1957), as modified by Udenfriend (1962), and tryptophan by the fluorimetric method of Hess & Udenfriend (1959), as modified by Guroff, King & Udenfriend (1961).

The brains, minus cerebella, were quickly removed and dissected into two equal parts along the mid-line. They were then weighed and kept on ice until homogenized in a 'Silverson' homogenizer, with 5 ml of the perchloric acid: trichloracetic acid mixture. The homogenates were then centrifuged for 15 min at approximately 800 *g* and portions of the supernatant were removed for the determination of tyrosine and tryptophan concentrations by the methods cited above. An additional portion of the supernatant was taken for the determination of γ-amino-*n*-butyric acid (γABA) using the fluorimetric method of Uchida & O'Brien (1964).

5-Hydroxytryptamine (5-HT) was determined in half-brains, prepared as described above, by the fluorimetric methods of Snyder, Axelrod & Zweig (1965) and Welch & Welch (1969) as modified by Leonard & Shallice (1971).

In experiments in which catecholamine concentrations were determined, groups of four rats were injected intraperitoneally with *p*-nitromethylamphetamine (0·5, 1·0, 10, 30 and 60 mg/kg). Noradrenaline and dopamine were determined in homogenates of whole brain (minus the cerebellum) by the solvent extraction method of Welch & Welch (1969).

Effect of p-*nitromethylamphetamine on the depletion of brain catecholamines by 4-α-dimethyl*-m-*tyramine (H77/77)*

Groups of five rats were treated at 0 and 2 h with H77/77 alone or in combination with *p*-nitromethylamphetamine; the latter drug was injected 30 min before H77/77 administration. The dose schedule for this experiment was: Group 1, control group (distilled water); Group 2, H77/77 alone (2 × 12·5 mg/kg, i.p.); Group 3, *p*-nitromethylamphetamine alone (60 mg/kg, i.p.); Group 4, H77/77 plus *p*-nitromethylamphetamine.

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

The statistical significances of differences between results obtained from control and drug-treated animals were assessed using Student's *t* test.

Results

Behavioural effects

Doses of *p*-nitromethylamphetamine greater than 1 mg/kg caused salivation, piloerection and slight excitement in the rats. When doses of 30 mg/kg or above were used, the animals became hyperactive and hyperthermic and also, aggressive when disturbed. With all doses, the changes observed were most pronounced 2 h after the drug administration. The excitement and aggressiveness observed after administration of the higher doses of *p*-nitromethylamphetamine was followed by exhaustion. When the drug treated rats were aggregated, tonic convulsions and death occurred in approximately 30% of rats given 60 mg/kg of the drug.

Effects of p-*nitromethylamphetamine on some brain and blood amino-acid concentrations and on brain 5-HT concentrations*

Although *p*-nitromethylamphetamine (60 mg/kg) had no significant effect on brain tyrosine concentrations, serum tyrosine was reduced throughout the experimental period by all doses of the drug (Table 1).

The changes in brain tryptophan appeared to be dose dependent; the lowest dose (1 mg/kg) caused a decrease and the highest dose (60 mg/kg) an increase in concentration of this amino-acid (Table 1). In contrast, a reduction in the concentration of serum tryptophan was seen after all doses of the drug used.

Only the highest dose of the drug (60 mg/kg) caused any change in the concentration of γABA in the brain. This occurred 4 h after the drug had been administered.

TABLE 1. *Effect of* p-*nitromethylphenylamphetamine on the concentrations of some amino-acids in brain and serum*

Duration of treatment	0 h	0·5 h	1 h	2 h	4 h
Dose (mg/kg, i.p.)			Brain tyrosine		
60	27·5±0·86	26·6±0·35	27·0±0·71	25·9±0·71	27·8±0·67
			Serum tyrosine		
60	26·3±1·33	13·66±0·51‡	13·84±0·34‡	15·77±0·71‡	18·6±0·28‡
10	29·9±1·01	17·20±0·82‡	22·0 ±1·05‡	21·60±0·77‡	21·4±0·39‡
1	26·1±2·08	20·0±1·49†	21·3 ±1·74	18·9 ±1·12‡	18·8†±2·22†
			Brain tryptophan		
60	2·37±0·103	2·54±0·046	2·82±0·09‡	2·60±0·164	2·61±0·117
10	2·55±0·089	2·47±0·078	2·97±0·096	2·81±0·035	2·93±0·274
1	2·10±0·036	2·14±0·95	2·00±0·136	1·78±0·046‡	1·95±0·076
			Serum tryptophan		
60	20·6±1·37	12·9±0·61‡	14·2±1·55‡	15·7±0·74‡	18·6±1·19
10	23·2±0·85	14·3±0·66‡	21·2±1·86	24·7±1·73	20·2±1·82
1	25·9±1·63	15·7±0·51‡	17·7±1·36	16·5±1·03*	23·4±1·24
			Brain γABA		
60	469±11·2	465±15·6	491±143	471±23·8	509±112†
10	451±25·6	455±19·9	588± 6·7	455±27·6	455±25·6
1	472±26·0	454± 8·4	488±10·6	485±17·5	505±17·3

Results expressed as μg/g wet weight or μg/ml serum. All results expressed as mean ± S.E.M. for five rats per group. Significance of difference from observation at 0 h. *$P<0·05$; †$P<0·025$; ‡$P<0·01$ (Student's *t* test).

p-Nitromethylamphetamine (10 or 60 mg/kg) significantly reduced the concentration of 5-HT in the rat brain (Table 2).

Effect on catecholamine concentrations in the brain

p-Nitromethylamphetamine appeared to have a biphasic effect on the concentration of brain noradrenaline; after the lower doses (0·1–10 mg/kg) the small changes seen were all increases whereas after a dose of 60 mg/kg there was a reduction (Table 3). The most obvious effect of *p*-nitromethylamphetamine on the concentration of cerebral dopamine was a reduction after a dose of 60 mg/kg. Small increases in the concentration of dopamine were seen after doses of 10 and 30 mg/kg (Table 3).

Effect on the depletion of brain catecholamines by H77/77

The degree of depletion of brain noradrenaline observed after administration of H77/77 was increased by administration of 60 mg/kg *p*-nitromethylamphetamine.

TABLE 2. *Effect of* p-*nitromethylamphetamine on the concentration of 5-hydroxytryptamine in the rat brain*

Duration of treatment	0 h	0·5 h	1 h	2 h	4 h
Dose (mg/kg, i.p.)					
60	0·50±0·021	0·41±0·018‡	0·37±0·021‡	0·35±0·020‡	0·41±0·020‡
10	0·56±0·031	0·55±0·017	0·50±0·012*	0·46±0·020†	0·38±0·006‡
1	0·53±0·015	0·51±0·016	0·53±0·013	0·54±0·026	0·52±0·018

Results expressed as μg/g wet weight for five animals per group. Each result represents the values obtained from two separate experiments. All results expressed as mean ± S.E.M. Significance of difference from observations at 0 h. * $P<0·05$; † $P<0·025$; ‡ $P<0·01$ (Student's *t* test).

TABLE 3. *Effect of* p-*nitromethylamphetamine on brain noradrenaline and dopamine concentrations*

	Duration of treatment									
	0 h	0·5 h	1 h	2 h	4 h	0 h	0·5 h	1 h	2 h	4 h
Dose (mg/kg, i.p.)	Noradrenaline					Dopamine				
60	0·38 ±0·035	0·32 ±0·021	*0·29 ±0·028	0·34 ±0·036	†0·27 ±0·02	0·86 ±0·03	†0·74 ±0·02	‡0·62 ±0·02	‡0·65 ±0·02	‡0·63 ±0·02
30	0·40 ±0·006	0·44 ±0·02	0·42 ±0·012	0·41 ±0·02	0·39 ±0·006	0·79 ±0·06	*0·99 ±0·05	0·80 ±0·02	0·94 ±0·06	0·81 ±0·03
10	0·37 ±0·02	*0·46 ±0·03	0·37 ±0·02	0·36 ±0·03	0·36 ±0·02	0·83 ±0·02	*0·91 ±0·01	0·81 ±0·02	0·77 ±0·03	0·83 ±0·01
1	0·38 ±0·03	0·46 ±0·03	0·40 ±0·03	0·45 ±0·02	0·41 ±0·03	0·83 ±0·03	0·89 ±0·01	0·81 ±0·01	0·99 ±0·02	0·99 ±0·03
0·5	0·42 ±0·02	0·45 ±0·03	0·44 ±0·02	*0·49 ±0·03	*0·49 ±0·02	0·89 ±0·03	0·94 ±0·05	0·90 ±0·04	0·88 ±0·05	0·91 ±0·05

Results expressed as μg/g wet weight for five animals per group. All results expressed as mean ± S.E.M. Significance of difference from result at 0 h: * $P<0·05$; † $P<0·025$; ‡ $P<0·01$ (Student's *t* test).

However, no potentiation of the depletion of brain dopamine by H77/77 was observed (Table 4).

Discussion

p-Nitromethylamphetamine has a spectrum of pharmacological activity which appears to be intermediate between that of methylamphetamine and *p*-bromomethylamphetamine (Leonard & Shallice, 1971). *p*-Nitromethylamphetamine has a dose dependent biphasic effect on the concentration of brain noradrenaline. It also increases the depletion of brain noradrenaline, without affecting the depletion of brain dopamine, following the administration of H77/77. In these respects it closely resembles the action of methylamphetamine on brain monoamine concentrations. H77/77, in depleting brain catecholamines, is thought to utilize a transport mechanism in the membrane of the noradrenaline containing nerves which is resistant to the action of reserpine (Carlsson, Fuxe, Hamberger & Malmfors, 1969). As the depletion of noradrenaline caused by H77/77 is potentiated by administration of *p*-nitromethylamphetamine, it would suggest that similar to (+)-amphetamine and methylamphetamine (Leonard & Shallice, 1971), *p*-nitromethylamphetamine may act in the same way as H77/77. However, *p*-nitromethylamphetamine also reduces the concentration of brain 5-HT and serum tryptophan and in this respect resembles *p*-bromomethylamphetamine. There would seem to be a correlation between the fall in 5-HT and the rise in its precursor amino-acid in the brain which suggests that *p*-nitromethylamphetamine could be affecting the synthesis of this amine.

p-Nitromethylamphetamine is dissimilar to both methylamphetamine and *p*-bromomethylamphetamine in that it causes a decrease in the concentration of brain γABA, has no effect on the concentration of serum tyrosine and causes a dose dependent biphasic change in the concentrations of brain tryptophan and dopamine. Furthermore, hyperactivity and other sympathomimetic effects were clearly observed only at doses of *p*-nitromethylamphetamine which approximated to half the LD50 dose (Knoll, 1970). In contrast, methylamphetamine caused behavioural stimulation at doses which are low relative to its LD50 dose, whereas little excitement was observed after administration of *p*-bromomethylamphetamine in doses greater than half the LD50 dose.

Knoll (1970) made a systematic study of the effect of chemical substitution in the benzene ring of methylamphetamine, on the behaviour of rodents, rabbits and cats. From these, and previous investigations (Knoll, Vizi & Ecsery, 1966), he has concluded that substitution at the *para* or *meta* positions of the benzene ring is a require-

TABLE 4. *Effect of p-nitromethylamphetamine on the depletion of brain catecholamines by* 4-α *dimethyl-*m-*tyramine (H77/77)*

Treatment	Dose (mg/kg, i.p.)	Noradrenaline (μg/kg)	Dopamine (μg/kg)
Control	—	0·32±0·007	0·48±0·020
H77/77 alone	2×12·5	0·20±0·009*	0·36±0·027*
p-Nitromethylamphetamine alone	2×60	0·19±0·009*	0·34±0·006*
p-Nitromethylamphetamine+H77/77	2×(60+12·5)	0·14±0·013*†	0·32±0·019*

Results expressed as mean ± S.E.M. for five animals per group. *P* values (Student's *t* test). *Difference from control $P<0·001$; † Difference from H77/77 $P<0·001$.

ment for an amphetamine derivative to possess psychotomimetic activity. He found, for example, that only derivatives which were substituted at these positions caused the hissing reaction in cats which is a characteristic of LSD treated animals. In contrast, neither methylamphetamine alone, nor its derivatives substituted in the ortho position, caused such effects.

In spite of apparent similarities in behavioural effects, there are differences between the neurochemical effects of these phenylethylamines. LSD decreases the concentration of brain noradrenaline and increases that of 5-HT, presumably by affecting the turnover of these amines (Freedman, 1961 ; Diaz, Ngai & Costa, 1968 ; Andén, Corrodi, Fuxe & Hökfelt, 1968 ; Leonard & Tonge, 1969 ; Tonge & Leonard, 1969). In contrast, methylamphetamine and its ring-substituted derivatives, whilst decreasing the concentration of brain noradrenaline, possibly by increasing its turnover (Leonard, unpublished), either have no effect or decrease the concentration of brain 5-HT. It is open to speculation whether or not the psychotomimetic effect of drugs such as LSD are due to the direct stimulation of 5-HT receptors, as has been proposed by Andén and co-workers (1968) because the gross behavioural changes caused by structurally dissimilar hallucinogenic drugs cannot be antagonized by pretreating the experimental animals with *p*-chlorophenylalanine (Tonge & Leonard, 1969), a drug which preferentially reduces the concentration of brain 5-HT (Koe & Weisman, 1966). However, Knoll (1970) found that this drug blocked the characteristic behavioural effects of both LSD and *p*-bromomethylamphetamine.

On the present evidence there appears to be little correlation between the effects of the phenylethylamines on the concentrations of monoamines in the brain and their effects on behaviour. Such differences may be resolved when it is possible to devise a behavioural model from which one can reliably predict whether or not the administration of a compound will induce hallucinogenic activity in man.

The authors wish to thank Dr. E. S. Vizi for supplying the sample of *p*-nitromethylamphetamine used in this study.

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(*Received August* 4, 1971)