AMPHETAMINE-LIKE ACTIVITY OF β-PHENETHYLAMINE AFTER A MONOAMINE OXIDASE INHIBITOR *IN VIVO*

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β-Phenethylamine possesses marked amphetamine-like effects which are demonstrable in animals pre-treated with a monoamine oxidase inhibitor. Like amphetamine, β-phenethylamine induces an increase of coordinated spontaneous motility in mice, anorexia in rats and dogs, hyperthermia in mice and rats, and exhibits a difference in lethality between isolated and aggregated mice. These effects are seen with similar doses of β-phenethylamine or amphetamine. But, unlike amphetamine, β-phenethylamine does not increase coordinated spontaneous motility in rats.

β-PHENYLETHYLAMINE has weak sympathomimetic activities (Bovet and Bovet-Nitti, 1948) which can best be demonstrated in animals pretreated with a monoamine oxidase inhibitor (Griesemer, Barsky, Dragsted, Wells and Zeller, 1954; Rebhun, Feinberg and Zeller, 1954; Bachtold and Pletscher, 1957). Under these conditions the amine can also induce amphetamine-like symptoms of central stimulation.

The present experiments were designed to evaluate the amphetamine-like effects of β-phenethylamine on a quantitative basis, by measuring some activities considered to be characteristic of amphetamine. Enhanced spontaneous coordinated motility, anorexigenic effect, hyperthermic activity and increased mortality in aggregated situations have been investigated.

METHODS

Animals. The animals used were adult mongrel dogs, Wistar rats weighing 100 to 200 g. and Swiss mice weighing 18 to 20 g. Anorexigenic activity was studied in male rats and in dogs of both sexes. Female rats and mice were used in the study of locomotor activity and hyperthermia. During experiments the animals were kept in a semi-dark and quiet room, the temperature of which was maintained from 20 to 22°.

Drugs. Iproniazid phosphate (Hoffmann La Roche) was used as a monoamine oxidase inhibitor. Subcutaneous treatment with iproniazid was given 24 hr. before the injection of β-phenethylamine hydrochloride at a dose of 200 mg./kg. (rats and mice) or 129 mg./kg. (dogs). These doses produce long, intensive inhibition of monoamine oxidases.

(±)-Amphetamine sulphate (Recordati) and β-phenethylamine hydrochloride (Hoffmann La Roche) were used. These compounds were administered subcutaneously over a range of doses to allow accurate representation of the activity pattern. An isotonic solution of sodium chloride was used for control injection.

Hyperthermia. Groups of six rats or mice were fasted for 12 hr. and were placed in cages measuring 45 × 40 cm. Rectal temperature was

measured hourly during five successive hr. by means of an Electric Universal Thermometer of the TE2 Ellab type. Amphetamine or phenethylamine was injected after the basal temperature had been taken twice. Animals which had an abnormal basal temperature were discarded.

Spontaneous activity. The method used was similar to that described by Dews (1953) for the study of spontaneous coordinated motility in mice.

Five mice or 4 rats were used at the same time in each experiment. The cages for mice measured 20 × 30 cm., and those for rats 45 × 30 cm. To avoid disturbing effects, infra-red photo-cells were used and the recording counters were installed in an adjacent room. Animals were injected subcutaneously 30 min. before being placed in the activity cages, spontaneous motility was recorded for a 15 min. period. Control experiments were made at random during the series of tests. At least 20 animals were used at each dose level.

Anorexigenic activity in rats. Animals were trained to take food during 8 hr. out of 24. They were kept in individual cages and generally developed a consistent habit of food intake within two weeks. On the day of the experiment a weighed meal was given to each animal immediately after treatment, and the amount of food intake was registered hourly for 4 hr.

Anorexigenic activity in dogs. A technique similar to that described by Di Ferrante and Longo (1953) was employed. Fifteen dogs were trained to eat a standard meal during a single 30 min. period every day at the same time in the morning. The drugs were injected an hr. before the feeding time. The meal was then offered hourly in order to estimate the presence and duration of the anorexia. The anorexigenic action of drugs was evaluated by considering only those dogs which had refused meals.

Toxicity. Mice and rats were aggregated in groups of 10 in cages measuring 15 × 40 cm. (mice) or 25 × 40 cm. (rats). Comparative studies in isolated conditions were made by placing animals in individual cages. Drugs were injected subcutaneously and mortality was recorded 24 hr. later. At least 10 animals were used at each dose level. The calculations were made according to the method of Litchfield and Wilcoxon (1949).

TABLE I

ANOREXIGENIC ACTIVITY OF PHENETHYLAMINE HYDROCHLORIDE IN NORMAL OR IPRONIAZID PRE-TREATED RATS

Number of animals	Iproniazid (24 hr. before) mg./kg./s.c.	Phenethylamine mg./kg./s.c.	Mean average food intake ± s.e. after injection			
			1 hr.	2 hr.	3 hr.	4 hr.
10	Controls		6·7 ± 0·66	9·0 ± 0·11	10·6 ± 0·09	12·4 ± 0·12
10	—	10	6·1 ± 0·58	8·6 ± 0·61	9·7 ± 0·66	12·5 ± 0·10
34	200	—	5·2 ± 0·40	6·6 ± 0·40	8·1 ± 0·44	9·5 ± 0·56
5	200	5	0·2 ± 0·19	2·0 ± 0·99	3·4 ± 0·99	4·2 ± 1·0
8	200	2·5	0·6 ± 0·37	4·1 ± 0·54	5·5 ± 0·97	6·3 ± 1·0
8	200	1·25	1·8 ± 0·58	4·7 ± 0·84	6·2 ± 0·70	8·1 ± 0·43
8	200	0·62	2·8 ± 0·81	5·2 ± 0·99	7·1 ± 0·76	9·8 ± 0·84

RESULTS

Hyperthermia. Phenethylamine caused hyperthermia only in animals pre-treated with iproniazid (Fig. 1 and 2) and was more active in rats than in mice. In rats phenethylamine produced greater hyperthermia than did amphetamine. In mice this effect was short-lasting and was followed by hypothermia.

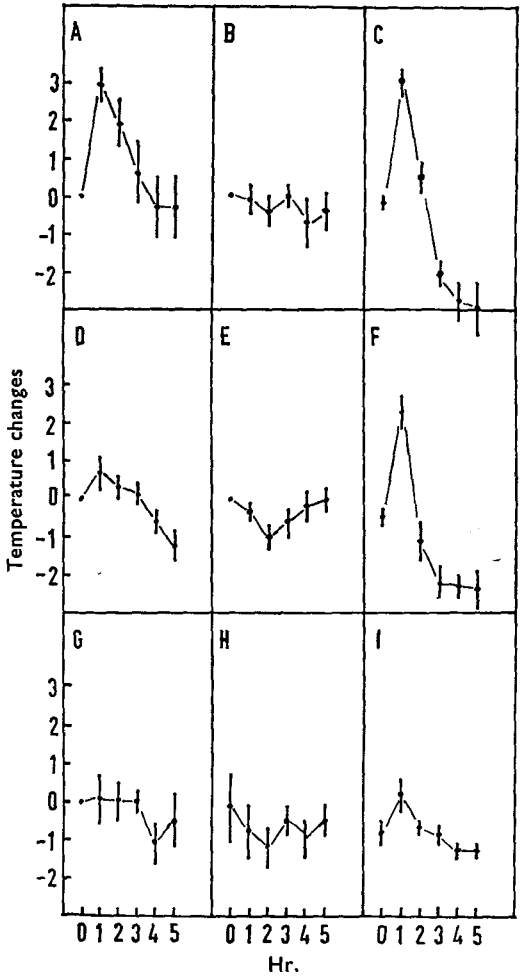

FIG. 1. Hyperthermic effect in mice. Each point on the curves represents the mean temperature of 12–18 mice. Vertical lines indicate the standard error. A, D, G, Amphetamine (10, 5, 2·5 mg./kg., s.c. respectively). C, F, I, Iproniazid (200 mg./kg., s.c.) 24 hr. before phenethylamine (40, 20, 10 mg./kg., s.c. respectively). B. Phenethylamine (40 mg./kg., s.c.), E. No treatment. H. Iproniazid (200 mg./kg., s.c.) 24 hr. before beginning temperature readings.

Spontaneous motility. Phenethylamine produced an increase of coordinated spontaneous motility in mice pre-treated with iproniazid

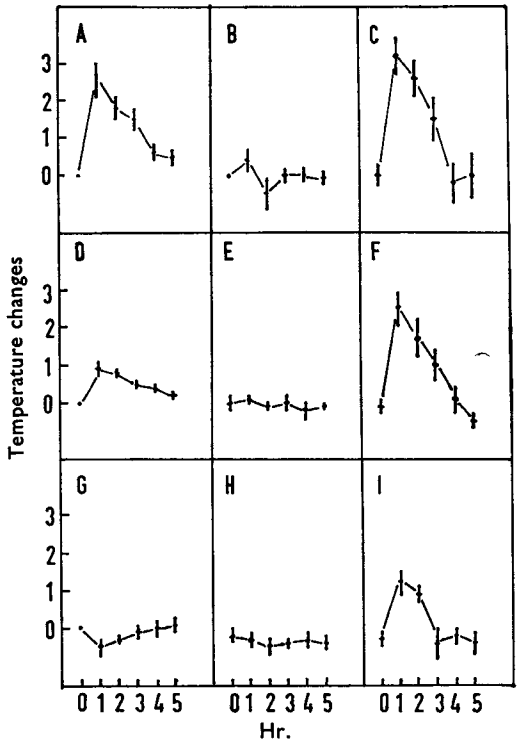

FIG. 2. Hyperthermic effect in rats. Each point on the curves represents the mean temperature of 12–18 rats. Vertical lines indicate the standard error. A, D, G, Amphetamine (10, 5, 2·5 mg./kg., s.c. respectively). C, F, I, Iproniazid (200 mg./kg., s.c.) 24 hr. before phenethylamine (10, 5, 2·5 mg./kg., s.c. respectively). B. Phenethylamine (50 mg./kg., s.c.). E. No treatment. H. Iproniazid (200 mg./kg., s.c.) 24 hr. before beginning temperature readings.

and showed a dose-effect relationship similar to that seen with amphetamine (Fig. 3). This effect was not observed, however, in rats. In this species only symptoms of excitement were evident without any increase of co-ordinated motility. This unexpected negative finding was verified repeatedly at doses of phenethylamine ranging from 1 to 50 mg./kg.

Anorexia. Phenethylamine caused anorexia in animals pretreated with iproniazid; it was more active than amphetamine in rats (Tables I–II),

TABLE II

ANOREXIGENIC ACTIVITY OF AMPHETAMINE SULPHATE IN RATS

Number of animals	Amphetamine mg./kg./s.c.	Mean average food intake ± s.e. after injection			
		1 hr.	2 hr.	3 hr.	4 hr.
49	Controls	6·2 ± 0·35	7·5 ± 0·37	8·9 ± 0·34	10·4 ± 0·42
9	5	1·5 ± 0·58	2·5 ± 0·60	3·0 ± 0·57	5·2 ± 0·93
13	2·5	2·3 ± 0·36	3·6 ± 0·88	6·0 ± 0·61	7·3 ± 0·48
13	1·25	3·0 ± 0·42	4·0 ± 0·69	6·0 ± 0·81	8·1 ± 1·06
13	0·62	4·0 ± 0·61	6·0 ± 0·61	8·0 ± 0·85	10·0 ± 0·96

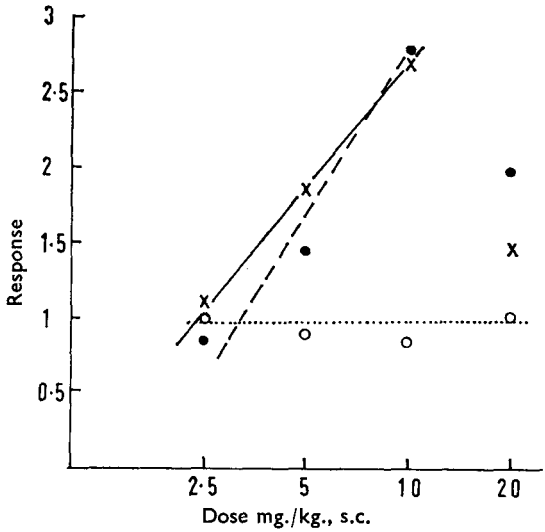

FIG. 3. The effect of phenethylamine hydrochloride on the coordinated activity of normal (○ ····· ○) and of iproniazid pretreated mice (● — — ●) and the effect of amphetamine sulphate on the coordinated activity of normal mice (X——X). Ordinated response expressed as ratio of count after drug to count of controls on same day.

less active than amphetamine in dogs when in animals pretreated with iproniazid, 129 mg/.kg. s.c., phenethylamine, 1–1·69 mg./kg. s.c., delayed food intake 2–10 hr. while amphetamine alone, 1–1·69 mg./kg. s.c., delayed it 3–20 hr.

TABLE III

LETHALITY OF PHENETHYLAMINE HYDROCHLORIDE IN NORMAL OR IPRONIAZID PRETREATED MICE IN AGGREGATED OR ISOLATED SITUATIONS

Iproniazid (24 hr. before) mg./kg./s.c.	Isolated		Aggregated		Increased toxicity potency ratio
	LD50 mg./kg./s.c.	Slope	LD50 mg./kg./s.c.	Slope	
—	420 (280–630)	1·7 (1·0–2·89)	420 (271–651)	1·85 (1·02–3·33)	Not significant
200	290 (250–336)	1·26 (1·06–1·48)	28 (14–53)	2·86 (1·68–4·86)	10·35 (5·4–19·9)
Increased toxicity potency ratio	Not significant		15 (6·9–32·2)		

Toxicity. The data obtained are summarised in Tables III–V. The LD50 of phenethylamine alone was the same in both isolated and aggregated mice and was not significantly different in isolated and aggregated rats. Toxicity increased after treatment with iproniazid. The enhancement was not significant in isolated mice but consisted of a 15-fold increase in aggregated animals. The increase of toxicity in rats was 32-fold in isolated, and 52-fold in aggregated animals. The enhancement of

TABLE IV

LETHALITY OF PHENETHYLAMINE HYDROCHLORIDE IN NORMAL OR IPRONIAZID PRE-TREATED RATS IN AGGREGATED OR ISOLATED SITUATIONS

Iproniazid (24 hr. before) mg./kg./s.c.	Isolated		Aggregated		Increased toxicity potency ratio
	LD50 mg./kg./s.c.	Slope	LD50 mg./kg./s.c.	Slope	
—	750 (614–915)	1·34 (1·13–1·58)	470 (356–620)	1·57 (1·20–2·04)	Not significant
200	23 (12–41)	2·23 (1·11–4·46)	9 (5·3–15·3)	4·3 (1·16–15·9)	2·5 (1·16–5·60)
Increased Toxicity potency ratio	32·6 (17–60)		52·2 (29–93·9)		

TABLE V

LETHALITY OF AMPHETAMINE SULPHATE IN AGGREGATED OR ISOLATED SITUATIONS

Animals	Isolated		Aggregated		Increased toxicity potency ratio
	LD50 mg./kg./s.c.	Slope	LD50 mg./kg./s.c.	Slope	
Mice	205 (164–256)	1·41 (1·14–1·73)	15·5 (12–20)	1·45 (1·20–1·74)	13·2 (9·4–18·4)
Rats	37 (30–45·5)	1·38 (1·10–1·72)	20·5 (17·6–23·7)	1·27 (0·99–1·62)	1·80 (1·38–2·34)

toxicity of phenethylamine in aggregated animals is therefore more marked in mice than in rats.

The LD50 of amphetamine in both isolated and aggregated mice and in isolated rats was similar to the corresponding LD50 of phenethylamine in mice pre-treated with iproniazid (Table V). No significant increase in toxicity of amphetamine was seen in aggregated rats.

DISCUSSION

The data reported indicate that phenethylamine, in animals pre-treated with iproniazid, and amphetamine are analogous to each other, both qualitatively and quantitatively. Phenethylamine causes anorexia, hyperthermia and enhanced toxicity in aggregated animals. In addition, these effects are induced by doses similar to the doses of amphetamine producing the same effects.

It is known that phenethylamine, in contrast to amphetamine, is rapidly inactivated by monoamine oxidases (Blaschko, 1952). The fact that, after the inhibition of these enzymes, phenethylamine is similar to amphetamine, both qualitatively and quantitatively, constitutes a further indication that the methyl group on the α-carbon atom of the ethylamine side-chain is not essential for amphetamine-like activity. This methyl group, which differentiates the structure of amphetamine from that of phenethylamine, is however important in preventing inactivation of the molecule by monoamine oxidases. Similar conclusions have been reached. recently by Van der Schoot, Ariëns, van Rossum and Hurkmans (1962).

As far as the spontaneous co-ordinated motility is concerned, it appears to be increased by phenethylamine, after a monoamine oxidase inhibitor,

in mice, but not in rats. The rats were clearly excited but not in a co-ordinated manner. It is difficult to explain this unexpected negative result since, in rats, the other effects of amphetamine and phenethylamine are strikingly similar.

On the other hand, iproniazid pre-treatment does not alter the experimental conditions necessary for the demonstration of an increased motor activity, since the amphetamine effect alone or after iproniazid treatment, changes only in intensity and not in quality.

It may be supposed that in rats, after monoamine oxidase inhibition, phenethylamine is transformed into a metabolite which interferes with the co-ordinated motor exciting activity. For instance, phenethylamine is known to be transformed into β-hydroxy-β-phenethylamine by dopamine β-oxidase (Pisano, Creveling and Udenfriend, 1960); this compound may interfere with some of the actions of β-phenethylamine. Preliminary results obtained in this laboratory seem to be consistent with this hypothesis.

References

Bachtold, H. and Pletscher, A. (1957). *Experientia*, **15**, 163–165.
Blaschko, H. (1952). *Pharmacol. Rev.*, **4**, 415–458.
Bovet, D. and Bovet-Nitti, F. (1948). *Médicaments du Système Nerveux Végétatif*, pp. 74–77, Bale: S. Karger.
Dews, P. B. (1953). *Brit. J. Pharmacol.*, **8**, 46–48.
Di Ferrante, N. and Longo, V. G. (1953). *Farmaco, Ed. Sci.*, **8**, 16–21.
Griesemer, E. C., Barsky, J., Dragsted, C. A., Wells, J. A. and Zeller, E. A. (1954). *Proc. Soc. exp. Biol., N.Y.*, **84**, 699–701.
Litchfield, J. T. and Wilcoxon, F. (1949). *J. Pharmacol.*, **96**, 99–113.
Pisano, J. J., Creveling, C. R. and Udenfriend, S. (1960). *Biochim. Biophys. Acta*, **43**, 566–568.
Rebhun, J., Feinberg, S. M. and Zeller, E. A. (1954). *Proc. Soc. exp. Biol., N.Y.* **87**, 218–220.
Van der Schoot, J. B., Ariëns, E. J., van Rossum, J. M. and Hurkmans, J. A. Th. M. (1962). *Arzneimit.-Forsch.*, **12**, 902–907.