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THE INHIBITION OF AMINE OXIDASE AND THE CENTRAL STIMULATING ACTION OF THE STEREOISOMERIC AMPHETAMINES AND 1-PHENYLETHYLAMINES

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The stereoisomers of amphetamine and 1-phenylethylamine have been studied in the rat both as central stimulants and as inhibitors of amine oxidase from brain, liver, and kidney. There was no correlation between these two effects; thus it is unlikely that the central stimulating action of amphetamine is due to inhibition of amine oxidase.

It is known that species differences exist in the pattern of substrate and inhibitor specificities Pratesi and Blaschko (1959) showed that, for a substrate as well as for an inhibitor, the presence of a centre of asymmetry in the molecule may confer different affinities (or rates of oxidation) on the two enantiomorphs, and also that this depends on the source of the enzyme.

Mann and Quastel (1940) suggested that central stimulation by amphetamine might be due to inhibition of amine oxidase. However, although in rabbit and man (+)-amphetamine (dexamphetamine) is a stronger stimulant than the (−) form, Pratesi and Blaschko (1959) have shown that the two stereoisomers are equally active as inhibitors of rabbit liver enzyme.

To test the idea that amphetamine owes its analeptic properties to its inhibitory action on amine oxidase, it was clearly necessary to use amine oxidase from the same species as that used for the pharmacological experiments. It was also desirable to use amine oxidase from the brain in preference to liver, because the patterns of substrate and inhibitor specificities might vary in different organs.

The present paper describes studies in the rat on dexamphetamine and (−)-amphetamine and (+)- and (−)-1-phenylethylamine as central stimulants and as inhibitors of amine oxidase from different organs (brain, liver, kidney). We studied 1-phenylethylamine because Pratesi and Blaschko (1959) found it to be a stronger inhibitor of the rabbit and cat liver enzyme than amphetamine; moreover 1-phenylethylamine had not yet been studied as a central stimulant. We have also studied the activity of dexamphetamine and (−)-amphetamine on amine oxidase from rabbit brain.

METHODS

The two amphetamines and 1-phenylethylamines used in our experiments were prepared by Dr. A. La Manna of our Institute. All these substances were obtained as the hydrochlorides. The signs (+) and (−) refer to the rotation of the hydrochlorides in aqueous solution. In some pharmacological experiments we have also used the sulphate of dexamphetamine.

Toxicity was studied in mice weighing 20 to 25 g. by injecting aqueous solution of the substances intraperitoneally. The animals were placed in groups of two or three in glass cages and observed for 24 hr.

Central stimulant activity was studied by measuring the duration of central depression in rats treated with chloral hydrate; the substance under examination was injected 5 min. after the beginning of the depression.

The sources of amine oxidase were rat liver, brain, and kidney, and rabbit brain. The fresh tissue was ground with sand; 2 ml. of 0.067 M-sodium phosphate buffer at *p*H 7.4 were added for each 1 g. of fresh tissue and the sand was then removed by centrifugation. Sometimes the rabbit brain was homogenized in a Potter's tube with twice its volume of buffer. The preparations were dialysed for 4.5 hr. against 0.067 M-sodium phosphate buffer and then overnight against tap water; one tenth volume of 0.2 M-sodium phosphate buffer was finally added.

Enzymatic activity was measured manometrically. The manometer flasks contained 2.0 ml. of fluid and 0.3 ml. of N-KOH. The temperature was 37° and the gas phase was O_2. Tyramine hydrochloride was always used as substrate at a final concentration of 0.01 M. The inhibitor was tipped from the side arm together with the substrate at the zero time.

RESULTS

Toxicity.—The results obtained by intraperitoneal administration of (+)- and (−)-1-phenylethylamine to mice are shown in Table I.

TABLE I

LETHAL EFFECTS OF (+)- AND (−)-1-PHENYLETHYLAMINE IN MICE

The drugs were injected intraperitoneally. The values represent number of mice killed and number of mice injected.

Drug	Dose			
	200 mg./kg.	250 mg./kg.	275 mg. kg.	300 mg./kg.
(+)-1-Phenylethylamine hydrochloride ..	0/4	0/6	4/6	5/6
(−)-1-Phenylethylamine hydrochloride ..	0/4	0/6	3/6	5/6

The acute toxicities of (+)- and of (−)-1-phenylethylamine are almost equal. With doses higher than 250 mg./kg. bodyweight, signs of motor excitement appeared, which intensified as the dose increased and which were more evident with (−)-1-phenylethylamine. Lethal doses caused death within a few minutes; with sublethal doses motor excitement subsided within a few hours.

Analeptic Action.—The 1-phenylethylamines and amphetamines were compared. The results are shown in Table II.

With chloral hydrate, the duration of central depression (the interval between the disappearance and reappearance of the righting reflex) was easy to determine. Differences with (+)- and (−)-1-phenylethylamine were not significant, whereas dexamphetamine was at least twice as active as (−)-amphetamine. (+)- and (−)-1-Phenylethylamine were five to six times less potent than dexamphetamine sulphate (at a dose level of 20 mg./kg.).

TABLE II

CENTRAL STIMULATING ACTION OF (+)- AND (−)-1-PHENYLETHYLAMINE AND OF DEXAMPHETAMINE AND (−)-AMPHETAMINE IN RATS

Chloral hydrate (300 mg./kg. intraperitoneally) was given in all experiments. All other drugs were injected subcutaneously. The dose of the drug marked with an asterisk has been calculated as if it were the sulphate. Narcosis time denotes the interval between the disappearance and the reappearance of the righting reflex.

Drug	Dose (mg./kg.)	No. of Rats	Narcosis Time	
			Mean and S.E. of Mean (min.)	% of Controls
Chloral hydrate alone ..		8	92±2·6	100
(−)-1-Phenylethylamine hydrochloride	100	6	68±5·9	73
(−)-1-Phenylethylamine hydrochloride	150	8	55±3·5	60
(+)-1-Phenylethylamine hydrochloride	100	6	75±3·5	81
(+)-1-Phenylethylamine hydrochloride	150	8	64±1·9	69
Dexamphetamine sulphate	20	8	67±2·0	72
Chloral hydrate alone ..		8	119±6·8	100
Dexamphetamine sulphate	20	7	56±2·5	48
,, ,,	10	7	97±7·3	81
(−)-Amphetamine hydrochloride*	40	9	66±7·0	55

Action on Amine Oxidase.—With enzyme preparations from rat liver, different inhibitor concentrations have been tested. The results obtained, expressed as the % inhibition of O_2 consumption 15 and 30 min. after the experiment had started, are shown in Table III.

TABLE III

INHIBITION OF AMINE OXIDASE FROM RAT LIVER

The substrate was 0·01 M-tyramine. Values are means of 3 to 5 experiments.

Inhibitor	Final Concentration (M)	% Inhibition of O_2 Uptake	
		After 15 min.	After 30 min.
Dexamphetamine ..	0·01	67	70
(−)-Amphetamine ..	0·01	52	51
(+)-1-Phenylethylamine	0·01	72	70
(−)-1-Phenylethylamine	0·01	24	30
Dexamphetamine ..	0·0033	52	52
(−)-Amphetamine ..	0·0033	31	32
(+)-1-Phenylethylamine	0·0033	53	51
(−)-1-Phenylethylamine	0·0033	12	15
Dexamphetamine ..	0·0017	46	47
(−)-Amphetamine ..	0·0017	23	26
(+)-1-Phenylethylamine	0·0017	46	44
(−)-1-Phenylethylamine	0·0017	5	8
Dexamphetamine ..	0·001	34	35
(−)-Amphetamine ..	0·001	20	22
(+)-1-Phenylethylamine	0·001	31	29
(−)-1-Phenylethylamine	0·001	2	2

Fig. 1 shows the time courses of oxidation using the four inhibitors at 0.01 M concentration. At this concentration dexamphetamine was more active than the (−) isomer; an equal inhibition (52%) was obtained with 0.01 M-(−)-amphetamine and 0.0033 M-dexamphetamine. (+)-1-Phenylethylamine was more active than (−)-1-phenylethylamine; the difference of activity between these two antipodes was even greater than for amphetamine because equal inhibitory effects (30%) were obtained with 0.01 M-(−)-1-phenylethylamine and 0.001 M-(+)-1-phenylethylamine. There were no differences between dexamphetamine and (+)-1-phenylethylamine in equimolecular concentrations.

The inhibitions after 15 and 30 min. were almost equal. Fig. 1 shows that, at least for the duration of our experiments, the relationship between O_2 consumption and time was linear, both in presence of substrate only and in the presence of both tyramine and inhibitor.

To study organ specificity, work on rat liver was extended to brain and kidney preparations. The inhibitors were used in two concentrations, 0.01 M and 0.001 M (Table IV). For enzymes obtained from brain and kidney we again observed that dexamphetamine was a stronger inhibitor than (−)-amphetamine, and that (+)-1-phenylethylamine was more active than (−)-1-phenylethylamine. With brain as well as with liver amine oxidase, dexamphetamine and (+)-1-

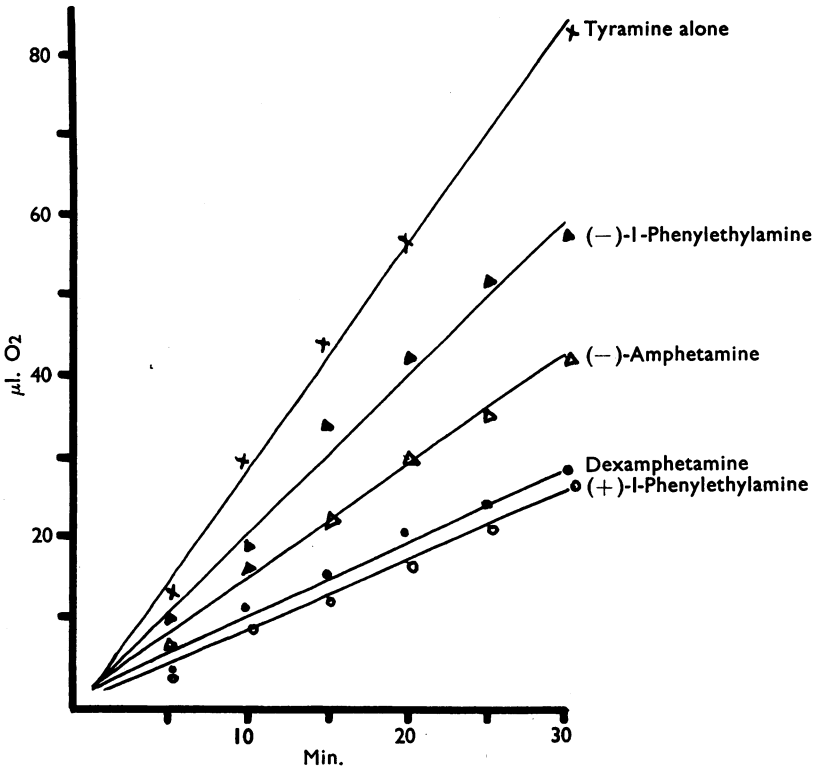

FIG. 1.—Inhibition of amine oxidase by dexamphetamine, (−)-amphetamine, and by (+)- and (−)-1-phenylethylamine (0.01 M). Rat liver was used as the source of enzyme, and tyramine as substrate. Ordinate, oxygen uptake; abscissa, time.

TABLE IV
INHIBITION OF AMINE OXIDASE FROM RAT LIVER, BRAIN, AND KIDNEY

Drug	Final Concentration (M)	% Inhibition of O_2 Uptake					
		Liver		Brain		Kidney	
		After 15 min.	After 30 min.	After 15 min.	After 30 min.	After 15 min.	After 30 min.
Dexamphetamine	0·01	67	70	71·5	69	79	83
(−)-Amphetamine	0·01	52	51	32	37·5	50	58
(+)-1-Phenylethylamine	0·01	72	70	74·5	73·5	58	62
(−)-1-Phenylethylamine	0·01	24	30	59	56·5	15	31
Dexamphetamine	0·001	34	35·5	39	39	34	41
(−)-Amphetamine	0·001	20	22	21	19	14	17
(+)-1-Phenylethylamine	0·001	31	29·5	28	26	22	22
(−)-1-Phenylethylamine	0·001	2	2	14	12	7	9

phenylethylamine were almost equally potent; with kidney amine oxidase dexamphetamine caused a slightly stronger inhibition.

In the experiments on the rabbit brain, only dexamphetamine and (−)-amphetamine were used as inhibitors. The results, expressed as % inhibition of O_2 consumption, were (average values): 0.01 M dexamphetamine, 80%; 0.01 M (−)-amphetamine, 66%; 0.001 M dexamphetamine, 36%; 0.001 M (−)-amphetamine, 22%.

On the basis of these results it follows that dexamphetamine was slightly more active than (−)-amphetamine.

DISCUSSION

To investigate whether inhibition of amine oxidase and central stimulation by amphetamine run parallel, both actions were studied in one and the same animal species using the enantiomorphic pairs of the two substances, amphetamine and 1-phenylethylamine. Reports of the action of 1-phenylethylamine are limited to the description of its pressor activity, which is about 1/1,000 that of adrenaline (Barger and Dale, 1910). Our experiments show that 1-phenylethylamine had pharmacological characteristics different from those of amphetamine.

The behaviour of the animals treated with 1-phenylethylamine was characterized by signs of

motor excitement only if lethal or nearly lethal doses were administered. The lethal dose was similar whether the animals were alone or in groups (J. H. Burn, personal communication). Spontaneous activity, however, was increased by doses of amphetamine well below lethal dose; the presence of other animals in the cage during the experiment greatly increased toxicity (Bovet and Bovet-Nitti, 1948).

The results of the biochemical experiments with rat liver as the source of amine oxidase were clearly at variance with those obtained in pharmacological experiments; dexamphetamine and (+)-1-phenylethylamine were equally active as amine oxidase inhibitors but differed widely in their central effects, while (+)- and (−)-1-phenylethylamine were equi-active pharmacologically but had very different activities as inhibitors of amine oxidase.

The results obtained with enzyme preparations from kidney and brain confirmed the conclusions reached with the enzyme from liver; they excluded organ specificity, at least within the range of the experiments carried out, and this extends observations by Blaschko and Himms (1955).

The results obtained with preparations of rabbit brain demonstrated a higher activity of dexamphetamine than of the (−) form, a difference of the same order as that found in the pharmacological experiments. Pratesi and Blaschko (1959), using rabbit liver as source of amine oxidase, found that the two stereoisomers of amphetamine were equally active as inhibitors. It is interesting that dexamphetamine, which in our experiments on rat liver proved to be as strong an inhibitor as (+)-1-phenylethylamine, appears to be more active on amine oxidase from guinea-pig liver than on that from rabbit liver (Pratesi and Blaschko, 1959). Such findings emphasize the dependence of stereospecificity on animal species.

A correlation between central stimulant action of amphetamine and inhibition of amine oxidase had been proposed by Mann and Quastel (1940) on the basis of the observation that the sequence of activity of amphetamine and three other phenylisopropylamines was the same for the effect on O_2 consumption of brain slices, for the anti-amine oxidase effect, and for the pharmacological action. Activity *in vivo* may be partly determined by unknown factors, but the lack of correlation between the inhibition of amine oxidase and the pharmacological effects of dexamphetamine and (+)-1-phenylethylamine and a similar lack of correlation between the two pairs of enantiomorphs can hardly be reconciled with the idea that central stimulation by amphetamine is due to its activity as an inhibitor of amine oxidase.

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