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RELATIVE ACTIVITY OF SOME INHIBITORS OF MONO-AMINE OXIDASE IN POTENTIATING THE ACTION OF TRYPTAMINE IN VITRO AND IN VIVO

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Several known inhibitors of mono-amine oxidase (iproniazid, isocarboxazid, nialamide, phenelzine, pheniprazine and tranylcypromine) were tested for their ability to (i) inhibit the mono-amine oxidase activity of a rat brain mitochondrial preparation *in vitro*; (ii) potentiate the action of tryptamine on the isolated rat fundal strip preparation; and (iii) potentiate the acute toxicity of tryptamine in mice. There was some correlation between the order of potency of the drugs in the three tests, particularly in inhibiting the enzyme activity in the Warburg and in the tryptamine toxicity test in mice. Exceptions to this were isocarboxazid which had unexpectedly high activity on the rat fundal strip preparation, and tranylcypromine which was devoid of tryptamine-potentiation action on the rat fundus preparation although it inhibited rat brain mono-amine oxidase *in vitro* and potentiated the action of tryptamine *in vivo*. Tranylcypromine was considerably less active in inhibiting the mono-amine oxidase of rat fundus than rat brain tissue *in vitro*, while iproniazid and isocarboxazid had about the same potency on the enzyme from the two tissues.

When investigating the action of various analogues of tryptamine on the isolated rat fundal strip preparation, Vane (1959) observed that the addition to the organ bath of the mono-amine oxidase inhibitor β-phenylisopropylhydrazine caused a potentiation of the action of tryptamine while having no effect on the contractions due to 5-hydroxytryptamine. This was explained as being due to the penetration of the tryptamine but not 5-hydroxytryptamine inside the cell where it could be inactivated by mono-amine oxidase.

In view of the current interest in drugs that effect the metabolism of catechol and indole amines, and the possible use of these agents in the treatment of psychiatric disorders, we have attempted to develop simple pharmacological procedures for evaluating the mono-amine oxidase inhibitory activity of new drugs by assessing their ability to potentiate some actions of tryptamine.

Several known inhibitors of mono-amine oxidase were compared for their ability to (i) inhibit the mono-amine oxidase activity of a rat brain mitochondrial preparation *in vitro*; (ii) potentiate the action of tryptamine on the isolated rat fundal strip preparation; and (iii) potentiate the acute toxicity of tryptamine in mice.

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METHODS

Preparation of mono-amine oxidase from rat brain. A method similar to that described by Pletscher & Gey (1958) and Davison (1957) was used. The enzyme was prepared from the brains of freshly killed rats. The mixture of cell nuclei, mitochondria and microsomes obtained by differential centrifugation of the brain homogenates was used as the source of enzyme.

Preparation of mono-amine oxidase from rat fundus. It was difficult to obtain a satisfactory enzyme preparation from this tissue by grinding or homogenization, and therefore the following procedure was used. The fundi from a number of rats were cut into pieces and treated with a 10% solution of cetrimide for about 5 min. The tissue was then thoroughly washed in distilled water. About 0.2 g of fundal tissue treated in this manner was used in micro-flasks. In some experiments brain tissue was treated in a similar manner as a comparison.

Determination of enzyme inhibition. Tyramine or tryptamine (9×10^{-3} M) was used as substrate, and the rate of oxygen uptake at 37° C in a Warburg apparatus determined as a measure of enzyme activity. Test drugs were incubated with the enzyme preparation under standard conditions for 30 min before the addition of substrate. A thermobarometer and flasks containing enzyme preparation, substrate but no inhibitor were used as controls in all experiments. Flasks containing enzyme preparation, inhibitor but no substrate were included when required. In the experiments using micro-flasks the rate of oxygen uptake by tissue preparation plus inhibitor was determined for 20 min before tipping in the substrate. The effect of various concentrations of the test drug on the activity of the enzyme preparation was determined. From a plot of the percentage inhibition against log concentration, the concentration of test drug required, under these conditions, to reduce the activity of the enzyme preparation by 50% (I 50 value) was determined.

Isolated rat fundal strip preparation. In vitro experiments in the rat fundal strip preparation were carried out using the method described by Vane (1957). An isotonic lever of approximately 14:1 magnification was used. Drugs were added to the organ bath (15 ml.) in a volume of approximately 0.2 ml. The tryptamine was allowed to act for 1.5 min, the bath washed out, and 30 sec later washed out again. The tissue was then allowed to rest for 3 min. The interval between successive doses of tryptamine was thus 5 min. Inhibitors of mono-amine oxidase and other test drugs were added to the bath immediately after the second washing. The heights of the contractions produced by a series of concentrations of tryptamine were determined before and at intervals following the addition to the bath of various concentrations of the mono-amine oxidase inhibitor. The potentiating action of the drugs increased with the length of time they were in the organ bath. For comparative purposes, the potentiation observed after the drug had been in the bath for 45 min was determined, as this was found to be the time requirement for maximal effect with most drugs. Dose-response curves for the action of tryptamine before and after the addition of the test drug were plotted. Since these were approximately parallel it was permissible to evaluate the dose-ratios, that, is the ratio of the concentrations of tryptamine required to produce equal effects before and after addition of the test drug to the organ bath. The log of the dose-ratio was then plotted against the log of the concentration of the test drug.

In other experiments rats were injected subcutaneously with a test drug and killed 4 to 5 hr later. Fundal strips were prepared and set up in the usual manner. The heights of the recorded contractions due to various doses of tryptamine were compared with those from untreated animals.

Potentiation of tryptamine toxicity in mice. The acute subcutaneous toxicity of tryptamine was found to increase considerably if the mice were pretreated with an inhibitor of mono-amine oxidase. Groups of 10 mice were given subcutaneously various doses of the test compounds, and 18 hr later were injected subcutaneously with tryptamine at a dose (250 mg/kg) which caused no deaths in unpretreated controls. Mortalities were recorded 18 hr after the tryptamine injections and the LD50's determined from a plot of probit mortality against log dose. These experiments were carried out at an environmental temperature of 26 to 28° C. In the experiments with tranylcypromine, the tryptamine was administered 2 hr instead of 18 hr after the inhibitor.

Drugs. The following known inhibitors of mono-amine oxidase were used: Iproniazid phosphate (Roche Products), isocarboxazide (Roche Products), nialamide (Pfizer), phenelzine dihydrogen sulphate (W. R. Warner & Co.), tranylcypromine sulphate (Smith, Kline & French) and pheniprazine hydrochloride.

RESULTS

Mono-amine oxidase inhibitory activity in vitro

Rat brain. Table 1 lists the concentration of drug (I 50) which was required to produce 50% inhibition of rat brain mono-amine oxidase using tyramine as substrate. Johnston (personal communication) has confirmed that with three of the inhibitors

TABLE 1

RELATIVE ACTIVITY OF VARIOUS DRUGS IN INHIBITING MONO-AMINE OXIDASE *IN VITRO* AND POTENTIATING THE ACTION OF TRYPTAMINE *IN VITRO* AND *IN VIVO*

Figures in brackets refer to the number of experiments. (i) In the manometric experiments the inhibitors were incubated for 30 min with the brain mitochondrial preparation before addition of the substrate (tyramine 9×10^{-3} M). Oxygen uptake was corrected for thermobarometer changes and uptake by the preparation without substrate. Values quoted are the mean I 50 values (concentration required to produce 50% inhibition) with the standard deviation. (ii) The concentration of drug (EC) required to produce a fourfold potentiation of the action of tryptamine on the isolated rat fundal strip preparation. (iii) The dose of compound (ED) which, when given 18 hr previously, caused 50% mortality in mice receiving 250 mg/kg of tryptamine subcutaneously. Tranylcypromine was given 2 hr before the tryptamine

	Inhibition of rat brain mono-amine oxidase *in vitro*		Tryptamine potentiation of rat fundus		Tryptamine potentiation in mice	
Drug	I 50 (μM) (i)	Potency ratio	EC (μM) (ii)	Potency ratio	ED (mg/kg) (iii)	Potency ratio
Iproniazid	16±0·1 (20)	1·0	4·7±0·3 (8)	1·0	55±10 (3)	1·0
Nialamide	7·8±0·5 (2)	1·9	0·18±0·1 (4)	26	4±0·5 (3)	14
Isocarboxazid	4·8±0·9 (4)	3·1	0·05±0·02 (8)	94	8±2 (1)	7
Phenelzine	0·9±0·1 (2)	18	0·09±0·02 (4)	55	4±1 (1)	14
Pheniprazine	0·53±0·02 (2)	31	0·13±0·04 (6)	36	1·0±0·2 (2)	55
Tranylcypromine	0·36 (1)	45	Inactive from 0·6–50 μM (6)	—	1·3±0·5 (2)	42

used in this work (phenelzine, isocarboxazid and tranylcypromine) the potency ratios are not significantly altered if tryptamine rather than tyramine is used as substrate, although the absolute I 50 values change.

Rat fundus. Cetrimide was found to be a very useful agent for obtaining an enzyme preparation from the fundal tissue. Cetrimide is known to disrupt the permeability of the membrane of some cells so that molecules of small molecular weight easily pass in or out of the cell (Hotchkiss, 1946). The rate of oxidation of tryptamine by fundal tissue treated in this manner was found to be 116±3 μl. oxygen/g tissue/hr. This figure agrees well with the figure of 124 μl. oxygen/g/hr quoted by Vane (1959) using an enzyme preparation obtained by homogenization and dialysis of the fundal tissue. With brain tissue treated with cetrimide in an identical manner the rate of oxygen uptake using tryptamine as substrate was 450± 10 μl. oxygen/g tissue/hr, which agrees reasonably with the figure of 380±60 μl. oxygen/g/hr obtained using the mitochondrial preparation. In the presence of various concentrations of three amine oxidase inhibitors these rates of oxygen

uptake were reduced (Table 2). Whereas both iproniazid and isocarboxazid inhibited both the brain and fundus preparations to an approximately equal extent, tranylcypromine was considerably more active as an inhibitor of the brain preparation. Furthermore, the slope of the percentage inhibition-tranylcypromine concentration curve was much flatter for fundal tissue than for brain tissue.

TABLE 2

RELATIVE EFFECTIVENESS OF TRANYLCYPROMINE, IPRONIAZID, AND ISOCARBOXAZID IN INHIBITING THE MONO-AMINE OXIDASE ACTIVITY OF RAT BRAIN AND FUNDAL TISSUE *IN VITRO*

Brain and fundal tissue were treated in an identical manner, with cetrimide, thoroughly washed, and incubated with phosphate buffer in manometric flasks. The inhibitors were incubated with the tissue preparations for 30 min before addition of tryptamine (9×10^{-3} M). The rate of oxygen uptake (corrected for no-substrate uptake) was 450 ± 10 µl. oxygen/g tissue/hr for the brain preparation and 116 ± 3 µl. oxygen/g tissue/hr for the fundal tissue. Figures are the mean values together with the standard deviation. * Single experiments

Drug	Molar concentration	% inhibition of tryptamine oxidation	
		Brain	Fundus
Tranylcypromine	2×10^{-7}	33 ± 12	0
	8×10^{-7}	61 ± 3	0
	3×10^{-6}	95 ± 1	26 ± 3
	3×10^{-5}	100	54 ± 4
	1×10^{-4}	—	$63+3$
Iproniazid	2×10^{-5}	26*	29 ± 1
	8×10^{-5}	63 ± 3	53 ± 2
Isocarboxazid	10^{-5}	14 ± 2	11*
	4×10^{-5}	46 ± 5	47*

Potentiation of the action of tryptamine on the isolated rat fundal strip preparation

In vitro *experiments.* Fig. 1 is part of the record of an experiment showing the increased sensitivity of the rat fundal strip preparation to the action of tryptamine caused by the addition of isocarboxazid to the organ bath. It will be noticed that this effect increased with time, and for comparative purposes the potentiation produced after the test drug had been in the bath for 45 min was used. When the log of the dose-ratio was plotted against log of the concentration of mono-amine oxidase inhibitor straight lines were obtained (Fig. 2), and these were approximately parallel for the drugs tested. This enabled potency ratios to be determined by comparing the concentrations of the test drug required to produce a fourfold increase in the sensitivity of the rat fundal strip (Table 1).

Tranylcypromine is not included in Fig. 2, because, although concentrations ranging from 0.02 to 50 µM were used, there was no reproducible increase in the sensitivity of the rat fundal strip preparation to the action of tryptamine. With the higher concentrations of the drug a transient increase in sensitivity was sometimes observed followed by a decrease in sensitivity.

Amongst the other drugs tested for their effect on the responses to tryptamine on this preparation were cocaine hydrochloride, dexamphetamine sulphate, imipramine hydrochloride and pyrogallol. Cocaine (0.13 to 1.3 µM) produced no effect at low concentrations and only a slight inhibition of the tryptamine responses at the high ones. Dexamphetamine sulphate in concentrations less than 0.25 µM

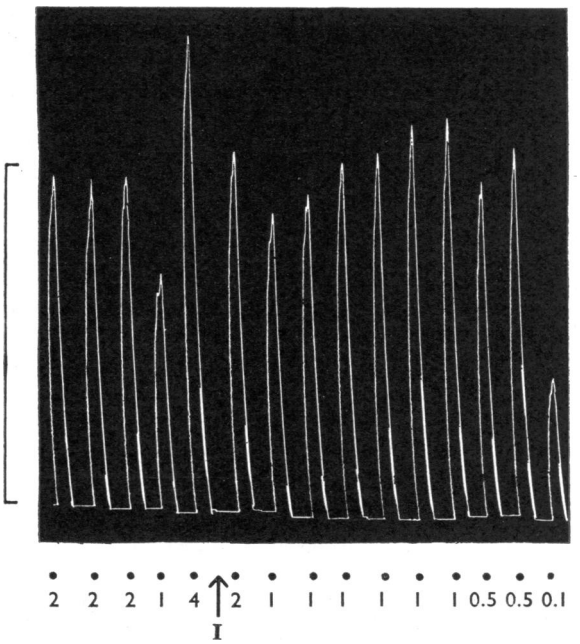

Fig. 1. Contractions of the isolated rat fundal strip preparation produced by tryptamine. The addition of isocarboxazid (0·01 μg/ml.) to the bath caused a progressive increase in the sensitivity of the preparation to tryptamine. Tryptamine added at intervals of 5 min. At black dots tryptamine in the number of μg indicated was added to the bath. At I, isocarboxazid (0·01 μg/ml.) added to bath and subsequently added after each washing out. Vertical scale, 10 cm.

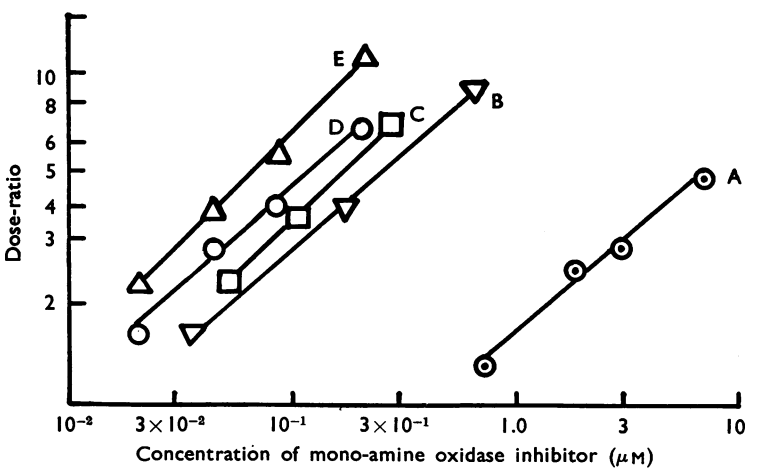

Fig. 2. Dose-response curves for the potentiation produced by various inhibitors of mono-amine oxidase of the action of tryptamine on the isolated rat fundus preparation. The dose-ratio is the ratio of the concentration of tryptamine required to produce equal responses of the preparation before and 45 min following the addition of the test drug to the organ bath. Ordinate: log dose-ratio. Abscissa: log concentration of mono-amine oxidase inhibitor (μM). A=iproniazid; B=nialamide; C=pheniprazine; D=phenelzine; E=isocarboxazid.

had no effect on the tryptamine responses. At concentrations greater than 25 μM dexamphetamine produced contractions of the fundus preparation. Imipramine hydrochloride at concentrations of 0.03 to 0.3 μM had no effect, whereas concentrations of 3 μM produced inhibition of the tryptamine responses. Pyrogallol (0.4 to 8 μM) did not modify the tryptamine responses.

In vivo/in vitro *experiments.* Fig. 3 shows the recorded heights of the contractions due to various concentrations of tryptamine of the fundal strips prepared from normal and drug-treated rats. Under these conditions tranylcypromine caused a

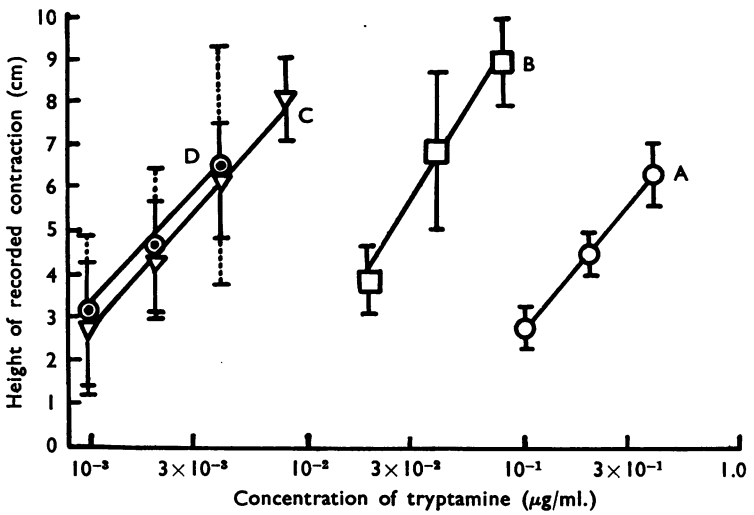

Fig. 3. Dose-response curves of the sensitivity to tryptamine of strips of fundus removed from rats injected 4 to 5 hr previously with 5.0 mg/kg subcutaneously of various mono-amine oxidase inhibitors. Ordinate: height of recorded contraction of tissue (cm; 14 : 1 magnification). Abscissa: concentration of tryptamine (μg/ml.) in organ bath (log scale). The vertical lines are the standard deviations about the mean. A=controls; B=iproniazid; C=isocarboxazid; D=tranylcypromine.

marked increase in sensitivity of the fundal strip. This increase in sensitivity was greater than that produced by the same dose of iproniazid. Since the effect of only one dose of inhibitor was studied in this manner, it is not possible to compare potencies. However, the results show that, whereas tranylcypromine does not potentiate the action of tryptamine in the isolated organ bath experiments, potentiation considerably greater than that produced by the same dose of iproniazid is obtained if the compound is injected into the rat before removal of the fundus.

Potentiation of tryptamine toxicity in mice

The acute subcutaneous LD50 of tryptamine hydrochloride in mice kept at an environmental temperature of 28 to 30° C was found to be about 500 mg/kg, death occurring without any marked symptoms of central stimulation. After subcutaneous administration of a mono-amine oxidase inhibitor, however, the LD50 was reduced, the extent of this reduction depending on the dose of inhibitor. For example, 18 hr after the administration of 1/10th the LD50 of nialamide or phenelzine, the

LD50 of tryptamine was reduced from 500 to 85 mg/kg. Furthermore, the mice showed pronounced symptoms of central stimulation, including tremor, convulsions, and marked agitation suggesting that the increased toxicity might be linked with a central action of the tryptamine. There was, however, no increase in co-ordinated motor activity such as is seen after amphetamine administration.

Fig. 4 shows plots of the percentage mortality due to 250 mg/kg tryptamine in mice 18 hr after the administration of various inhibitors of mono-amine oxidase. Although all these lines are not parallel, for comparative purposes the dose of each mono-amine oxidase inhibitor required to produce 50% mortality due to the administration of tryptamine was determined, and the results are set out in Table 1. In this test tranylcypromine substantially potentiated the action of tryptamine.

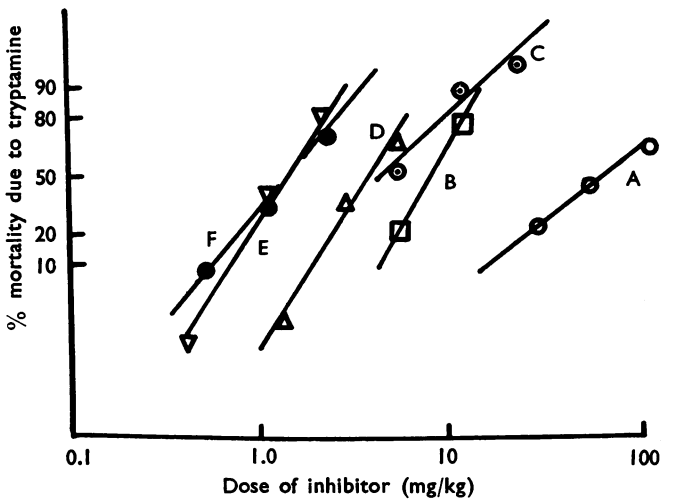

Fig. 4. Dose-response curves for the potentiation produced by various inhibitors of mono-amine oxidase of the acute toxicity of tryptamine in mice. Test drugs (with the exception of tranylcypromine) were administered subcutaneously 18 hr before tryptamine (250 mg/kg subcutaneously). Tranylcypromine was given 2 hr before the tryptamine. Ordinate: % mortality following tryptamine administration (probability scale). Abscissa: dose of mono-amine oxidase inhibitor (mg/kg). A=iproniazid; B=isocarboxazid; C=nialamide; D=phenelzine; E=phenelprazine; F=tranylcypromine.

In similar experiments the acute subcutaneous toxicity of tyramine and β-phenylethylamine was also greater in mice pretreated with inhibitors of mono-amine oxidase. The mice showed symptoms of central excitation, there was an increase in locomotor activity, vocalization and a series of escape and defence encounters similar to those seen in mice after amphetamine and described by Chance (1946). The excitement was quite different from that produced by tryptamine in mice similarly pretreated with a mono-amine oxidase inhibitor.

Comparison of the relative activities of the drugs in the three tests

The correlation between the order of activity of the compounds in the three tests was not close (Table 1) although fairly good correlation was obtained between the

inhibition of rat brain mono-amine and tryptamine potentiation in mice. Two results required explanation: (a) the complete lack of activity of tranylcypromine, and (b) the high activity of isocarboxazid, on the rat fundal strip preparation.

If the mechanism proposed by Vane (1959) for the potentiating action of mono-amine oxidase inhibitors on the rat fundal strip preparation is correct, then these differences might be attributable to one or both of two factors: (i) differences in the sensitivity to inhibitors between the mono-amine oxidase prepared from the rat fundus tissue and that from rat brain ; and (ii) differences in the ability of the drugs tested to penetrate into the cell of the fundal tissue and reach the enzyme.

The difference in enzyme sensitivity mentioned in (i) might be due to (a) a difference in the nature of the mono-amine oxidase in the two tissues, or (b) metabolic alteration of some of the drugs by one tissue preparation but not by the other.

The data set out in Table 2 show that there is a marked difference in the sensitivity of rat brain and fundal tissue to tranylcypromine, whilst there is little difference in sensitivity to iproniazid and isocarboxazid.

As an indication of the ability of isocarboxazid, tranylcypromine and iproniazid to penetrate into the cell of the rat fundal tissue when added to the Tyrode solution, we measured their partition ratios between chloroform and Tyrode solution. It has been shown that the barrier which separates plasma from the gastro-intestinal tract has the characteristics of the lipoid membrane, and is preferentially permeable to the oil-soluble undissociated forms of drugs (Brodie & Hogben, 1957 ; Shore, Brodie & Hogben, 1957 ; Hogben, Tocco, Brodie & Schanker, 1959). The results obtained are set out in Table 3. Isocarboxazid had a partition ratio approximately

TABLE 3

RELATIVE POTENCIES OF VARIOUS DRUGS (IPRONIAZID=1) IN INHIBITING THE MONO-AMINE OXIDASE OF RAT FUNDUS *IN VITRO*, IN POTENTIATING THE ACTION OF TRYPTAMINE ON THE RAT FUNDAL STRIP PREPARATION, AND THEIR PARTITION COEFFICIENTS BETWEEN CHLOROFORM AND TYRODE SOLUTION (*p*H 7·4)

Drug	Inhibition of fundal mono-amine oxidase *in vitro*	Partition coefficient chloroform/ Tyrode	Tryptamine potentiation on rat fundus
Iproniazid	1	0·9	1
Isocarboxazid	1·8	100	94
Tranylcypromine	2·2	3·8	Nil

100 times that of iproniazid, suggesting that the high relative activity of the former drug in potentiating the action of tryptamine on the rat stomach strip preparation might be due to its greater ease in penetrating into cells. However, tranylcypromine also had a fairly high partition ratio (3.8), suggesting that this drug could also penetrate inside the cells of the fundal tissue.

The experiments mentioned above suggested that the lack of activity of tranylcypromine in potentiating the action of tryptamine on the isolated rat fundus preparation was related to a lack of sensitivity of the mono-amine oxidase in this tissue to inhibition by tranylcypromine *in vitro*. However, it was also possible that the potentiating action of inhibitors of mono-amine oxidase might involve some quite unspecific action such as alteration of permeability of the cells of the fundal

tissue. In order to investigate this and to attempt to establish a direct correlation between inhibition of the amine oxidase of the rat fundus in the isolated organ bath and increased sensitivity to tryptamine, the following experiments were carried out.

The degree of potentiation produced by various drugs of the action of tryptamine on the rat fundal strip in the isolated organ bath was determined in the usual manner. The strip was then removed, cut into pieces and its mono-amine oxidase activity determined in the Warburg apparatus using tryptamine as substrate. Strips of fundal tissue whose sensitivity to tryptamine had been determined but which had not been treated with an inhibitor of mono-amine oxidase were used as controls. Results of these experiments are set out in Table 4. There was a reasonably good

TABLE 4

CORRELATION BETWEEN THE INCREASED SENSITIVITY OF RAT FUNDAL STRIP TO THE ACTION OF TRYPTAMINE AND INHIBITION OF THE MONO-AMINE OXIDASE OF THIS TISSUE

The inhibitors were added to the isolated organ bath and the tryptamine potentiation determined in the usual way. The fundal tissue was then removed, treated with cetrimide, and the mono-amine oxidase activity measured in the Warburg apparatus using tryptamine as substrate. Tranylcypromine (10 μM) reduced the sensitivity of the isolated fundal strip

	Concentration in organ bath μM	Tryptamine potentiation (dose-ratio)	% inhibition of rat fundal mono-amine oxidase
Iproniazid	2·8	2·0	8
	5·6	3·5	53
	11·2	5·2	90
Isocarboxazid	0·022	2·3	44
	0·044	3·8	52
	0·09	5·9	96
Tranylcypromine	0·02	Nil	Nil
	0·08	Nil	Nil
	0·32	Nil	Nil
	2·0	Nil	Nil
	10·0	Nil	31

correlation between the inhibition of the mono-amine oxidase of the fundal tissue and tryptamine-potentiating action, suggesting that these two effects were causally related. With tranylcypromine, inhibition of mono-amine oxidase was only observed at the highest concentration tested at which a slight reduction in the sensitivity of the fundal strip to tryptamine was recorded.

DISCUSSION

The results presented in this paper suggest that testing drugs for their ability to potentiate the action of tryptamine both *in vitro* and *in vivo* may be a useful method of examining potential mono-amine oxidase inhibitors. Tedeschi, Tedeschi & Fellows (1959) have used the potentiation of the convulsant action of tryptamine in rats as a method of assaying mono-amine oxidase inhibitors, while Sjoerdsma, Oates, Zaltzman & Udenfriend (1959) suggest that the assay of urinary tryptamine may be taken as an index of mono-amine oxidase inhibition in man.

The three test systems used in this investigation were of increasing complexity. Thus in the *in vitro* experiments carried out in the Warburg apparatus, there was probably little diffusion barrier between the test drug and the enzyme system. In the isolated organ-bath experiments, the inhibitor had first to penetrate into the cell before coming into contact with the amine oxidase. In the intact animal, the situation was still more complex since there are probably two or more diffusion barriers to cross, and the inhibitor may be metabolically altered or inactivated by other enzyme systems before reaching the intracellular mono-amine oxidase.

The activities of two drugs, isocarboxazid and particularly tranylcypromine, deserve special attention. Isocarboxazid was only about twice as potent as iproniazid in inhibiting the mono-amine oxidase activity of rat fundal tissue *in vitro* using tryptamine as substrate, but was some 70 times as potent in potentiating the action of tryptamine on this tissue in the isolated organ bath. In view of the high chloroform/buffer partition ratio of isocarboxazid, the simplest explanation of this high activity is that the drug penetrates into the cell of the fundal tissue more easily than does iproniazid. The possibility that the drug may be metabolized in the tissue to a more active substance has not been ruled out.

Iproniazid and isocarboxazid were about equiactive in inhibiting the mono-amine oxidase activity of rat brain and rat fundal tissue. Tranylcypromine was therefore of particular interest, for against the fundal enzyme it had only 1/100th of its activity against the brain amine oxidase. This may be due to a difference in the mono-amine oxidase in fundal and brain tissue. The lack of activity of tranylcypromine in potentiating the effect of tryptamine on the rat fundal preparation fits in well with this idea. In the high concentrations required to produce effective inhibition of the enzyme in the organ bath the drug appears to reduce the sensitivity of the preparation by some unspecific means. Barlow (1961) has already suggested that the mono-amine oxidase in rat fundus differs from that in guinea-pig liver.

Another explanation would be that tranylcypromine is metabolized *in vivo* to a more active substance and that the enzymes required for this are present in the brain preparation but not in the fundal tissue. This is supported by the experiments where prior injection of tranylcypromine to rats greatly increased the sensitivity of the fundus to tryptamine. This increase in sensitivity was larger than that produced by the same dose of iproniazid.

The two explanations of the results are not mutually exclusive ; there may be both a difference in the enzymes from brain and fundus and also some metabolism of tranylcypromine to a more active substance.

After inhibition of mono-amine oxidase, tyramine and phenylethylamine produced a central stimulation resembling that of amphetamine, whereas the central effects produced by tryptamine were of a different type. As amphetamine contains an alpha-methyl group it is not readily inactivated by mono-amine oxidase Similarly, when the amine oxidase is inhibited, tyramine and phenylethylamine are not readily inactivated, and will therefore have a prolonged action. Thus the resemblance between the central action of amphetamine and of tyramine or phenylethylamine after mono-amine oxidase inhibition may be due to a similar central sympatho-

mimetic action of these substances. Furthermore, the difference between tryptamine after mono-amine oxidase inhibition and amphetamine suggests that amphetamine and tyramine are acting on similar "adrenergic" receptors in the central nervous system, and that these are different from the receptors acted on by tryptamine.

The fact that tranylcypromine, a known inhibitor of mono-amine oxidase, does not potentiate tryptamine on the isolated rat fundal strip preparation *in vitro* limits the value of this procedure for evaluating potential inhibitors of mono-amine oxidase, although it gives useful information with many drugs. It must, of course, be considered as a complementary test to other procedures, such as the manometric method and the tryptamine toxicity test. It is possible that the fundal strip prepared from a rat pretreated with mono-amine oxidase inhibitors, as described above, would constitute a more satisfactory routine procedure for examining new compounds. Bearing these limitations in mind, the examination of drugs for their ability to potentiate the action of tryptamine both *in vitro* and *in vivo*, as described in this paper, may be a useful method of assessing pharmacologically the mono-amine oxidase inhibitory activity of new substances.

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