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CENTRAL STIMULANT ACTIONS OF α-ALKYL SUBSTITUTED TRYPTAMINES IN MICE

BY

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Much interest has been centred on the part played by 5-hydroxytryptamine in the actions of the central nervous system since it was shown that changes in the amount of this amine in the brain accompany the effects of many drugs. The central actions of 5-hydroxytryptamine cannot easily be studied by observing the effects due to its injection, since it does not readily penetrate to the brain from the circulation (Udenfriend, Bogdanski & Weissbach, 1957a). Effects may, however, be produced indirectly by administering the natural precursor of 5-hydroxytryptamine, 5-hydroxytryptophan, particularly when enzymic destruction of the 5-hydroxytryptamine formed *in situ* has been prevented by administration of monoamine oxidase inhibitors (Bogdanski, Weissbach & Udenfriend, 1958). Vane (1959) suggested that interesting central effects might be produced by tryptamine derivatives lacking the hydroxyl group which prevents 5-hydroxytryptamine from penetrating membranes, and also protected from the action of monoamine oxidase by suitable substitution. Among the substances suggested, he included α-methyltryptamine.

Vane, Collier, Corne, Marley & Bradley (1961) have since reported on a number of central actions of α-methyltryptamine and α-ethyltryptamine, and both compounds have been the subject of other recent biochemical and pharmacological studies (for example, Greig, Walk & Gibbons, 1959; Gey & Pletscher, 1962). In mice, α-methyltryptamine causes marked stimulation characterized by increased locomotor activity, hyperthermia, mydriasis and generalized body tremor. Since the tremor resembles that produced by 5-hydroxytryptophan after treatment with monoamine oxidase inhibitors, which response has already been used in this laboratory as a means of measuring the degree of enzyme inhibition (Lessin, 1959), attention was paid principally to this effect and quantitative studies were based mainly on its measurement. Preliminary reports of this work have been made in 1961 and 1962 to the British Pharmacological Society (Lessin & Parkes) and to the 22nd International Congress of Physiological Sciences at Leiden, 1962 (Parkes, Lessin & Long, 1962).

METHODS

Body tremor. This was measured by placing mice in canisters mounted on gramophone pick-ups, the output of which was amplified and the integrated output was recorded by a low-inertia motor operating a counter (Lessin, 1959; Parkes & Lessin, 1960); each count was made for 1 min.

Rectal temperature. This was measured with a thermistor probe as described by Lessin & Parkes (1957). Mice were maintained at stated ambient temperatures, either under an infrared lamp or in a thermostatically controlled cupboard.

Pupil diameter. This was measured by the method of Pulewka (1932).

5-Hydroxytryptamine levels. These were estimated in brains, removed from mice immediately after killing, by a modification of the method of Bogdanski, Pletscher, Brodie & Udenfriend (1956). The mouse was killed, its brain was removed and homogenized in 3 ml. of distilled water. The homogenate was transferred to a McCartney bottle containing 2 g of sodium chloride, 1 ml. of borate buffer (*p*H 9.6), and 10 ml. of washed butanol. The stoppered bottle was shaken for 15 min and then centrifuged. The butanol layer was transferred to another bottle and washed three times with 8 ml. of salt-saturated borate buffer (*p*H 9.6). 7 ml. of the butanol was transferred to a further bottle containing 2 ml. of 0.01 N-hydrochloric acid with 12 ml. of washed petroleum ether (boiling point, 60 to 80° C). The stoppered bottle was shaken for 5 min and centrifuged. The ether layer was removed.

In order to remove interference by substituted tryptamines such as α-methyltryptamine, the aqueous phase was made alkaline by the addition of 1.5 ml. of sodium chloride-saturated buffer (*p*H 9.6) and washed three times with 5 ml. of diethyl ether. After centrifugation, 2 ml. of the aqueous phase were transferred to a quartz cuvette containing 0.2 ml. of 10% formic acid. Fluorescence was measured at 340 mμ, the activation wavelength being 295 mμ. Standards and reagent blanks were also extracted in the same way

The compounds studied were DL-α-methyltryptamine, αα-dimethyltryptamine and DL-*N*-α-dimethyltryptamine (Fig. 1).

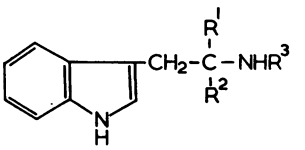

Fig. 1. Formulae of tryptamine derivatives referred to in text. α-Methyltryptamine: $R^1=CH_3$, $R^2=R^3=H$; αα-dimethyltryptamine: $R^1=R^2=CH_3$, $R^3=H$; *N*α-dimethyltryptamine: $R^1=R^3=CH_3$, $R^2=H$.

RESULTS

Stimulant actions

These, as exemplified by the responses to α-methyltryptamine and *N*α-dimethyltryptamine, took the form of general body tremor, heightened locomotor activity, mydriasis and hyperthermia. They were characterized by gradual onset and considerable duration (Fig. 2), and all effects showed regression upon dose (Fig. 3). High doses caused over-stimulation, exhaustion and collapse leading to death. The stimulant effects closely resembled those produced by tryptamine in mice previously treated with monoamine oxidase inhibitors. The time course of the stimulant effects of the substituted tryptamines was similar to that of the concentration of the compounds in the brain (Figs. 2 and 4). αα-Dimethyltryptamine caused only mild stimulation at near lethal doses.

The stimulant actions of α-methyltryptamine and *N*α-dimethyltryptamine resembled those of amphetamine, except that the substituted tryptamines caused tremor as their most obvious sign, which is not produced by amphetamine in mice, while effects upon locomotor activity were not so conspicuous as with amphetamine; also, aggressiveness and fighting were never seen with the tryptamine derivatives. The mydriatic effects of α-methyltryptamine, unlike that of amphetamine, could be produced only by injection, the compound being quite ineffective when applied to the cornea, or even when injected into the anterior chamber of the rabbit eye. A further difference concerned the well-known effect of grouping upon the toxicity of amphetamine; the toxicity of α-methyltryptamine and *N*α-dimethyltryptamine was unaffected by this (Table 1).

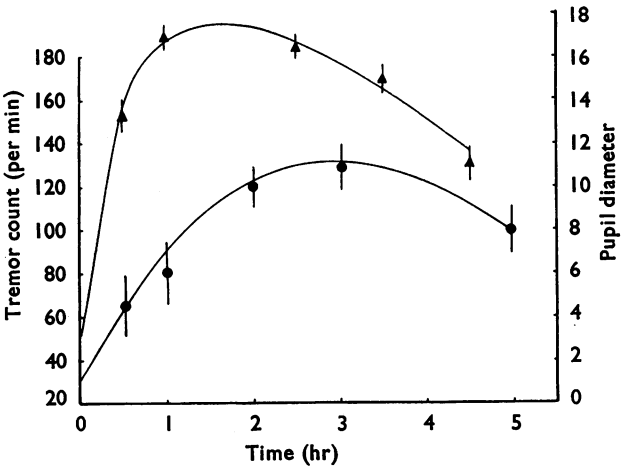

Fig. 2. Time course of tremors and pupillary dilatation in mice injected intraperitoneally with α-methyltryptamine hydrochloride. ●, Tremors after 25 mg/kg; ▲, pupillary dilatation (arbitrary units) after 20 mg/kg. Vertical lines are standard errors in this and subsequent Figures.

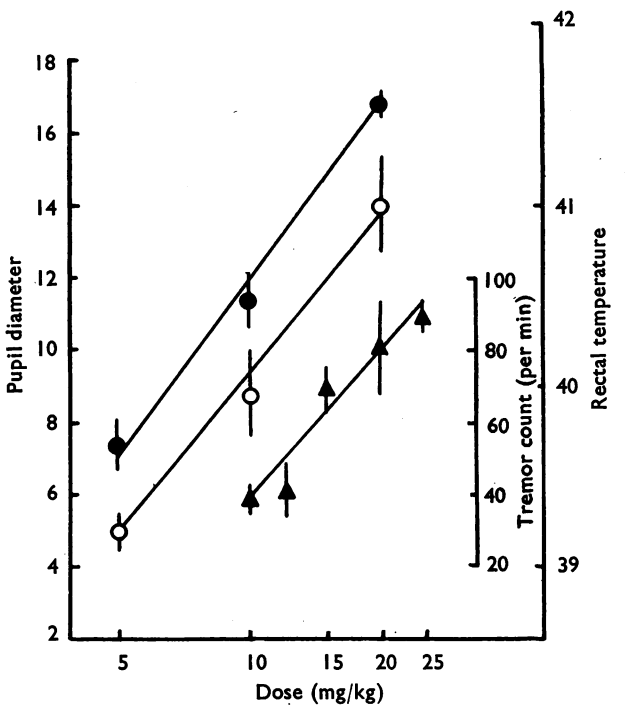

Fig. 3. Dose/response curves for pupillary dilatation, rectal temperature (at ambient temperature of 28° C) and tremors in mice injected intraperitoneally with α-methyltryptamine hydrochloride. Calculated values are: ●, pupillary dilatation (arbitrary units), b=15.6±4.3, n=24; ○, rectal temperature, b=3.0±1.1, n=30; and ▲, tremors, b=131.4±28.6, n=181.

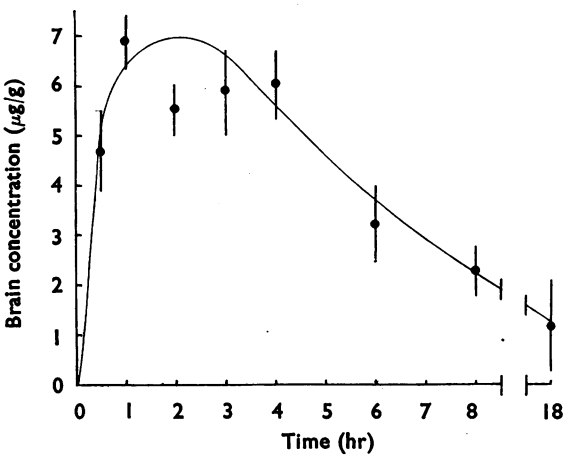

Fig. 4. Brain levels of α-methyltryptamine in mice killed at various intervals after intraperitoneal injection of 25 mg/kg of α-methyltryptamine hydrochloride at zero time.

TABLE 1

ACUTE TOXICITY OF TRYPTAMINE DERIVATIVES TO MICE KEPT SINGLY AND IN GROUPS

Injections were intraperitoneal. Grouped mice were ten per cage

Compound	Dose (mg/kg)	Mortality	
		Grouped	Singly
α-Methyltryptamine	160	5/10	7/10
*N*α-Dimethyltryptamine	125	6/10	6/10

Interaction with reserpine

When given to mice sedated by reserpine, α-methyltryptamine, αα-dimethyltryptamine and *N*α-dimethyltryptamine could arouse them as amphetamine could, restoring body temperature (Fig. 5) and overcoming ptosis temporarily. In animals treated with low doses of these tryptamine derivatives, normally sedative doses of reserpine produced characteristic stimulant effects (Fig. 6). In this, they resembled inhibitors of monoamine oxidase, such as the hydrazides or harmaline, except that the stimulant effects lasted for only a few hours, after which the normal reserpine-induced sedation supervened (Fig. 7). After apparent recovery from reserpine, the sensitivity of the animals to the stimulant effects of these agents was greatly increased (Table 2). It should be noted that a similar increase in sensitivity was also observed, in mice after recovery from reserpine, with 5-hydroxytryptophan and tryptamine after treatment with a monoamine oxidase inhibitor (Parkes & Lessin, 1960).

α-Methyltryptamine and *N*α-dimethyltryptamine could also prevent the facilitation of leptazol convulsions in mice by reserpine (Table 3). In this, the drugs resemble other inhibitors of monoamine oxidase and differ from amphetamine (Lessin & Parkes, 1959).

Activity as monoamine oxidase inhibitors

α-Methyltryptamine, α-ethyltryptamine and *N*α-dimethyltryptamine inhibit monoamine oxidase *in vitro* and *in vivo* (Grieg *et al.*, 1959; Gey & Pletscher, 1962; Tedeschi, Tedeschi,

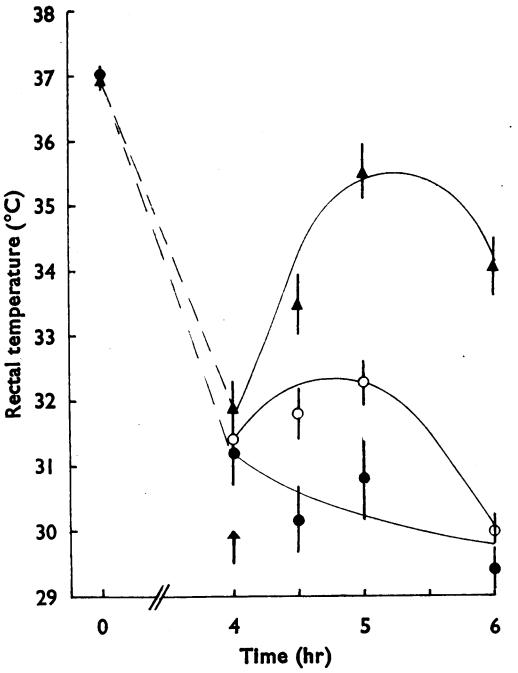

Fig. 5. Rectal temperatures of mice previously treated with reserpine (2 mg/kg, intraperitoneally) at zero time, and, at arrow, injected with *N*α-dimethyltryptamine (5 or 10 mg/kg, subcutaneously). Room temperature, 22° C. ●, Controls; ○, *N*α-dimethyltryptamine (5 mg/kg); and ▲, *N*α-dimethyltryptamine (10 mg/kg).

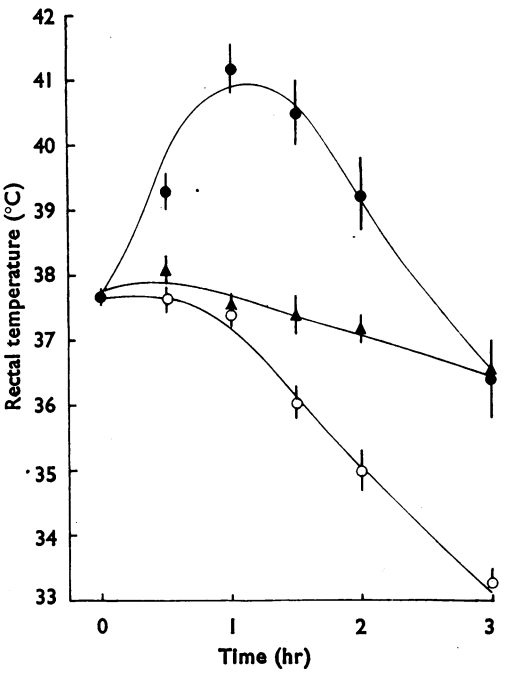

Fig. 6. Rectal temperatures of mice injected with reserpine (2 mg/kg, intraperitoneally) at zero time 15 min after α-methyltryptamine hydrochloride (5 mg/kg, intraperitoneally). Room temperature, 22° C. ▲, Untreated mice; ○, reserpine; and ●, reserpine after α-methyltryptamine.

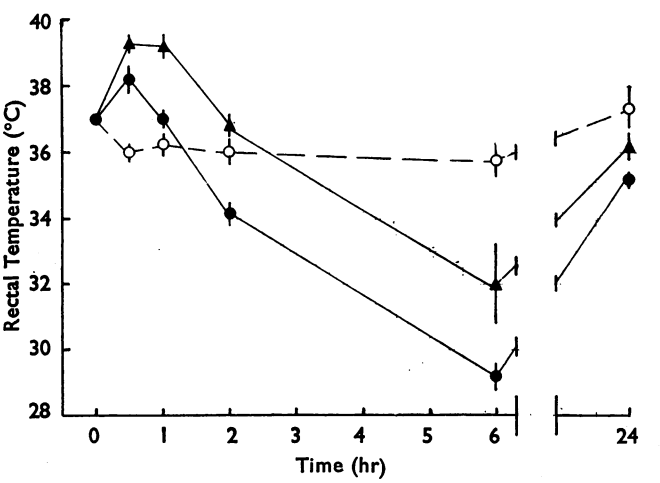

Fig. 7. Rectal temperatures of mice injected with reserpine (2 mg/kg, intraperitoneally) alone (●) and 30 min after harmaline hydrochloride (10 mg/kg, intraperitoneally; ○) or α-methyltryptamine hydrochloride (25 mg/kg, intraperitoneally; ▲). Room temperature, 22° C.

TABLE 2

INCREASE IN SENSITIVITY TO STIMULANT TREATMENTS OF MICE PREVIOUSLY TREATED WITH RESERPINE (2 MG/KG, INTRAPERITONEALLY) 3 TO 5 DAYS EARLIER

Potency ratios give the response after reserpine relative to that without reserpine, with ranges in brackets

Stimulant treatment	Effect measured	No. of mice	Potency ratio
5-Hydroxytryptophan (4 hr after irreversible monoamine oxidase inhibitor)	Tremors	80	1·25 [(1·08–1·47)
Tryptamine (1 hr after irreversible monoamine oxidase inhibitor)	Pupillary dilatation	60	2·7 (1·5–5·0)
α-Methyltryptamine	Pupillary dilatation	60	2·06 (1·88–2·4)
	Tremors	72	2·2 (1·8–2·6)

TABLE 3

EFFECT OF TRYPTAMINE DERIVATIVES AND AMPHETAMINE UPON FACILITATION OF CONVULSIONS DUE TO LEPTAZOL BY RESERPINE

Treatment was by intraperitoneal injection 30 min before infusion of leptazol. Leptazol was infused intravenously in a concentration of 4 mg/ml. at 0·3 ml./min, and the values are means and standard errors of the volumes given up to the time of terminal extensor spasm. Reserpine was injected intravenously in a dose of 2 mg/kg, 4 to 6 hr before the leptazol

Treatment	Volume (ml.) of leptazol infused for: Controls	Reserpine treated	
None	0·615±0·05	0·23±0·04	$P<0·001$
α-Methyltryptamine (25 mg/kg)	0·62 ±0·04	0·59±0·062	
	$P>0·9$		
	$P>0·7$		
None	0·594±0.035	0·365±0.035	$P<0·01$
Nα-Dimethyltryptamine (50 mg/kg)	0·65 ±0·03	0·615±0·053	
	$P>0·3$		
	$P>0·6$		
None	0.615±0.05	0·23±0.04	$P>0·9$
Amphetamine sulphate (10 mg/kg)	0·36 ±0·037	0·28±0·03	
	$P<0·02$		
	$P>0·2$		

TABLE 4

ACTIVITIES AS POTENTIATORS OF TREMORS DUE TO 5-HYDROXYTRYPTOPHAN IN MICE (MONOAMINE OXIDASE INHIBITION *IN VIVO*)

Injections were intraperitoneal

Compound	Effective dose range (mg/kg)	Relative activity
Harmaline	1–10	100
α-Methyltryptamine	1–10	100
Nα-Dimethyltryptamine	1–10	100
αα-Dimethyltryptamine	5–50	20

Fowler, Green & Fellows, 1962). Inhibitors of this enzyme permit 5-hydroxytryptophan in small doses to cause tremors (Lessin, 1959), and both α-methyltryptamine and Nα-dimethyltryptamine, when followed by 5-hydroxytryptophan, caused tremors in mice in doses less than those required for their production by the compounds when given alone. αα-Dimethyltryptamine was also active in this way, although only weakly stimulant alone (Table 4).

Interaction with other monoamine oxidase inhibitors

Mice were treated with single doses of irreversible monoamine oxidase inhibitors, such as 1-L-α-alanyl-2-isopropylhydrazide hydrochloride (Ro 4-1340) sufficient to cause almost complete block of the enzyme as estimated biochemically. All three tryptamine derivatives showed an increased stimulant activity in such mice, whether measured as tremors, mydriasis or hyperthermia (Fig. 8).

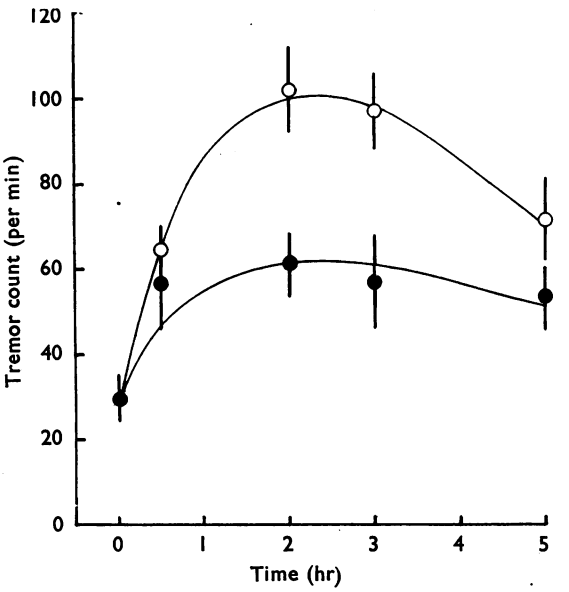

Fig. 8. Tremors at various intervals after α-methyltryptamine hydrochloride (10 mg/kg, intraperitoneally) in mice injected 1 hr previously with 1-L-α-alanyl-2-isopropylhydrazide hydrochloride (50 mg/kg, intraperitoneally; ○) and in mice not so previously treated (●).

Despite the apparent completeness of the enzyme block due to a single dose of an irreversible monoamine oxidase inhibitor, such treatment alone never caused stimulation in the mice used in this work. Repeated doses of inhibitor at intervals of 1 or 1.5 hr, however, resulted in the appearance of signs of stimulation in mice after 6 to 8 hr (Table 5) including hyperactivity, hyperthermia, tremor and mydriasis. Mice treated with three doses of 75 mg/kg of pargyline (Taylor, Wykes, Gladish & Martin, 1960) at intervals of 1.5 hr

TABLE 5

STIMULANT EFFECTS PRODUCED IN MICE BY SIX INTRAPERITONEAL INJECTIONS AT HOURLY INTERVALS WITH 50 MG/KG OF THE MONOAMINE OXIDASE INHIBITOR, 1-L-α-ALANYL-2-ISOPROPYLHYDRAZIDE HYDROCHLORIDE

Each value is the mean with standard error for ten mice, measured 1 hr after the last injection

Condition	Mean tremor count	Body temperature (° C)	Pupil diameter (scale units)
Treated	43·1±5.3	38·36±0·25	11·2±0·96
Control	8·0±1·5	35·94±0·24	3·5±0·30
	$P<0·001$	$P<0·001$	$P<0·001$

showed threshold signs of stimulation, and the tryptamine derivatives, when given to these mice, were stimulant in very small doses. Their activity in this situation represented at least a ten-fold increase with α-methyltryptamine and *N*α-dimethyltryptamine (Fig. 9). αα-Dimethyltryptamine, however, now had a stimulant activity which approached that of the other two derivatives, representing more than a fifty-fold increase over its activity in normal mice. Harmaline also acquired a stimulant activity after this treatment (Table 6).

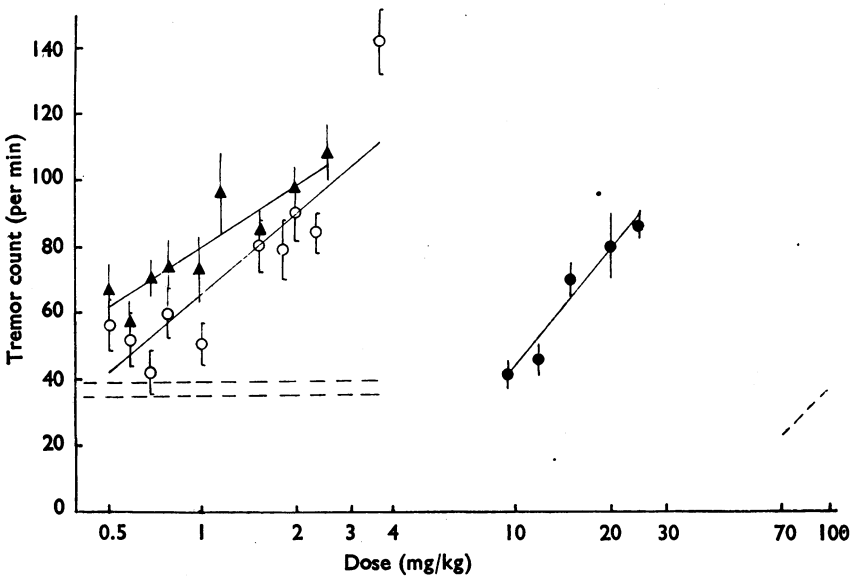

Fig. 9. Activity of α-methyltryptamine hydrochloride and αα-dimethyltryptamine hydrochloride in causing tremors in mice previously treated with three injections of pargyline hydrochloride (75 mg/kg, intraperitoneally) at 1.5-hr intervals, compared with their activities in mice not so previously treated. The tryptamine derivatives were injected 1.5 hr after the third dose of pargyline and tremors were counted 1.5 hr later. The broken lines enclose the 95% probability limits of the mean tremor count for mice treated only with pargyline, as described above (37.0±2.0, n=162). ▲, α-Methyltryptamine after pargyline (b=61.0±17.0, n=336); ○, αα-dimethyltryptamine after pargyline (b=78.7±23.0, n=194); ●, α-methyltryptamine alone (from Fig. 3; b=131.4±28.6, n=181); and – – –, αα-dimethyltryptamine alone.

TABLE 6

STIMULANT ACTIVITY OF TRYPTAMINE DERIVATIVES AFTER REPEATED DOSES OF PARGYLINE

The doses quoted caused equal tremor counts 1·5 hr after intraperitoneal injection. Pargyline was given in three intraperitoneal injections of 75 mg/kg each

Amine	Dose (mg/kg) of amine: Without treatment	After treatment	Potentiation of stimulant activity
α-Methyltryptamine	20	1	20
αα-Dimethyltryptamine	Little activity at 100	1.5	>65
*N*α-Dimethyltryptamine	20	1	20
Harmaline	Inactive at 100	5	20

Interaction with inhibitors of aromatic aminoacid decarboxylase

After inhibition of monoamine oxidase by treatment with a hydrazide, such as 1-L-α-alanyl-2-isopropylhydrazide hydrochloride (Ro 4-1340), mice were injected with various inhibitors of aromatic aminoacid decarboxylase, α-methyldopa (Smith, 1960), 1-*p*-dimethylaminobenzyl-2-DL-serylhydrazide (Ro 4-3156) (Pletscher, Gey & Burkard, 1965), or 1-*p*-dimethylaminobenzyl-2-(5-methylisoxazol-3-ylcarbonyl)hydrazide (Ro 5-1025). The tremors, due to subsequent treatment with 5-hydroxytryptophan, were reduced or delayed in onset by all these agents, as has also been described for α-methyldopa by Corne, Pickering & Warner (1963) (Fig. 10). The decarboxylase inhibitors were also found to

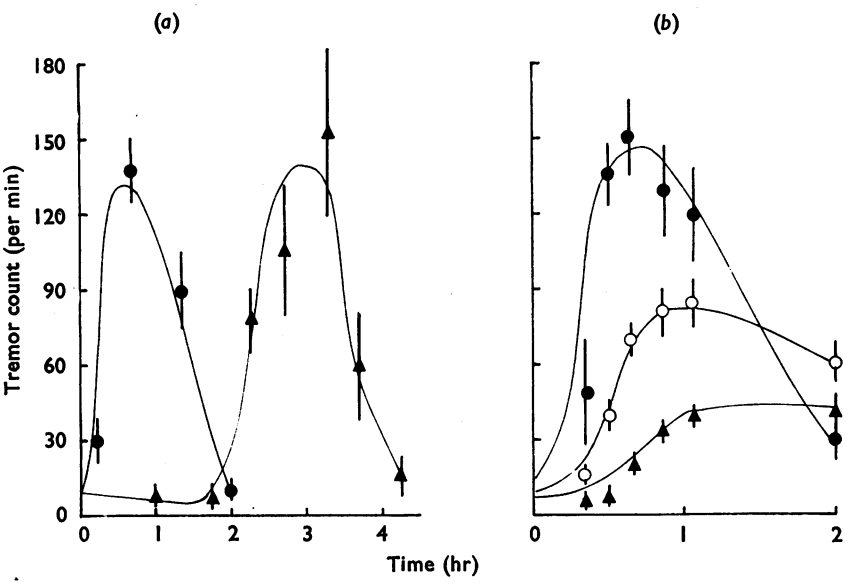

Fig. 10. Tremors produced by 5-hydroxytryptophan (50 mg/kg, intraperitoneally). (*a*) ●, 5 hr after iproniazid phosphate (100 mg/kg, intraperitoneally); ▲, 5 hr after iproniazid phosphate and 30 min after 1-*p*-dimethylaminobenzyl-2-(5-methylisoxazol-3-ylcarbonyl)hydrazide (100 mg/kg, subcutaneously). (*b*) ●, 1 hr after 1-L-α-alanyl-2-isopropylhydrazide hydrochloride (50 mg/kg, intraperitoneally); ○, 1 hr after the last drug and 10 min after α-methyldopa (100 mg/kg, subcutaneously); ▲, 1 hr after 1-L-α-alanyl-2-isopropylhydrazide hydrochloride and 10 min after α-methyldopa (200 mg/kg, subcutaneously).

reduce or delay the onset of stimulation due to repeated dosage with irreversible monoamine oxidase inhibitors (Fig. 11) but had no influence on the stimulation resulting from injection of tryptamine (Fig. 12). Tremors caused by α-methyltryptamine and *N*α-dimethyltryptamine were, however, delayed or reduced by previous treatment with all the decarboxylase inhibitors (Fig. 13), though the mydriasis and hyperthermia due to the tryptamine derivatives were not affected.

Effects on brain levels of 5-hydroxytryptamine

Injection of animals with 5-hydroxytryptophan results in elevation of 5-hydroxytryptamine levels in the brain (Udenfriend *et al.*, 1957a). Mice treated with an irreversible

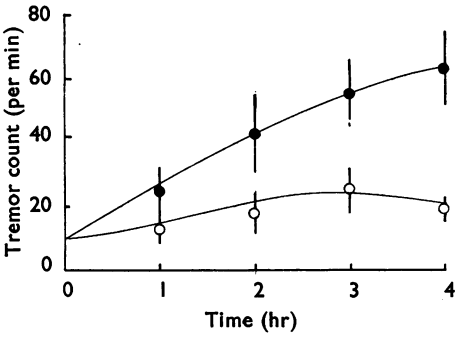

Fig. 11. ●, Tremor counts in mice at intervals after injection of the last of three doses of pargyline hydrochloride (100 mg/kg, intraperitoneally at 2-hr intervals). ○, Tremor counts in mice treated as above but receiving also α-methyldopa (100 mg/kg, intraperitoneally with each dose of pargyline).

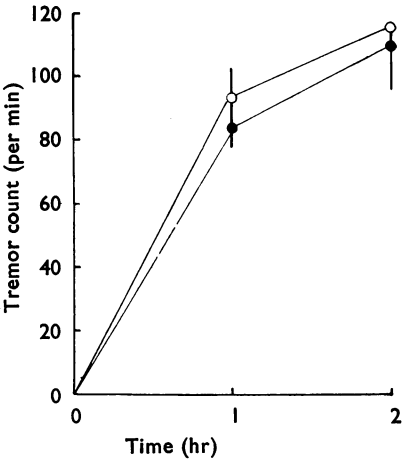

Fig. 12. Tremor counts at intervals after tryptamine hydrochloride (30 mg/kg, intraperitoneally) in two groups of mice treated 1 hr previously with 1-L-α-alanyl-2-isopropylhydrazide hydrochloride (50 mg/kg, intraperitoneally), one group (○) being treated with α-methyldopa (100 mg/kg, intraperitoneally) 30 min before injection of tryptamine.

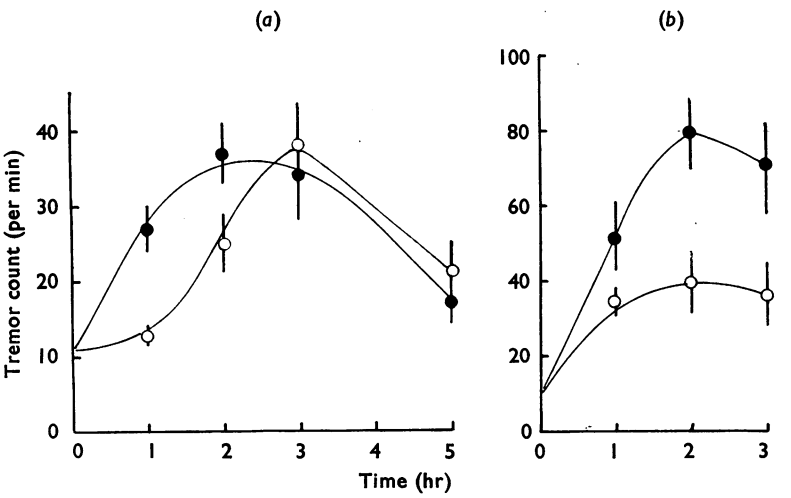

Fig. 13. Effects of aromatic amino acid decarboxylase inhibitors upon tremors produced by tryptamine derivatives. (*a*) Two groups of mice treated with *N*α-dimethyltryptamine (10 mg/kg, intraperitoneally at zero time), one group (○) having received 1-*p*-dimethyliminobenzyl-2-DL-serylhydrazide (25 mg/kg, subcutaneously) 15 min earlier. (*b*) Two groups of mice treated with α-methyltryptamine hydrochloride (20 mg/kg, intraperitoneally at zero time), one group (○) having received α-methyldopa (100 mg/kg, subcutaneously) 15 min earlier.

monoamine oxidase inhibitor were injected with 5-hydroxytryptophan and killed immediately after measurement of the resulting tremors. 5-Hydroxytryptamine was estimated in the brain of each mouse and was expressed as μg/g wet wt. Brain 5-hydroxytryptamine levels and tremor counts rose and fell in a similar manner with time after 5-hydroxytryptophan injection (Fig. 14). Correlation was shown between individual tremor counts and corresponding brain 5-hydroxytryptamine levels (Fig. 15). This relationship shows that no detectable tremors occurred with brain 5-hydroxytryptamine levels less than 75% above control values (1 μg/g). In the mice used, this elevation required the injection of at least 200 mg/kg of 5-hydroxytryptophan intraperitoneally, which was the minimum dose causing detectable tremor unless preceded by an inhibitor of monoamine oxidase, when as little as 10 mg/kg produced tremor and resulted in a level of 1.75 μg/g of 5-hydroxytryptamine.

The reduction in 5-hydroxytryptophan tremors caused by treatment with decarboxylase inhibitors was shown to be paralleled by a lessened increase in brain 5-hydroxytryptamine, the relationship between the two values remaining unaltered (Fig. 16).

Injection with single doses of monoamine oxidase inhibitors has been reported to increase brain levels of 5-hydroxytryptamine (Udenfriend, Weissbach & Bogdanski, 1957b). It is consistent with the relationship found here between 5-hydroxytryptamine levels and appearance of tremor, that overt stimulation did not result from such treatment, since the

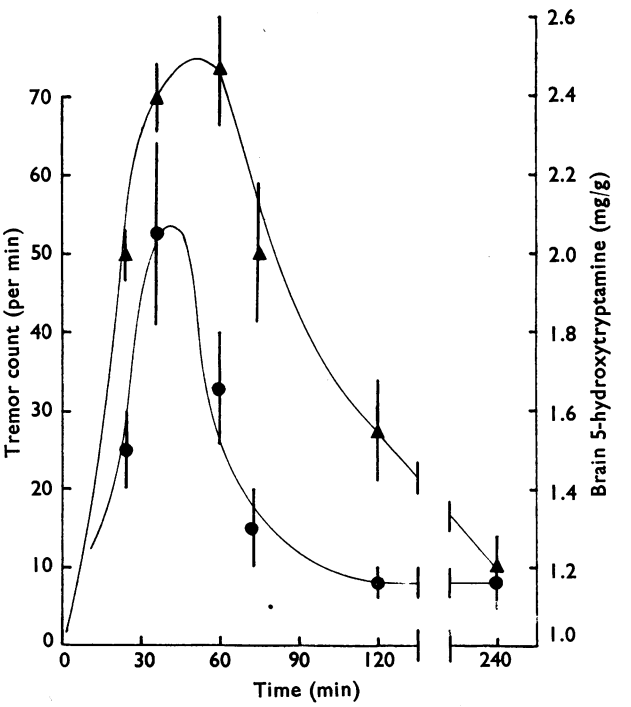

Fig. 14. Tremor counts (●), measured immediately before killing, and brain levels of 5-hydroxytryptamine (▲) in groups of mice killed at various intervals after 5-hydroxytryptophan (30 mg/kg, intraperitoneally) given 1 hr after 1-L-α-alanyl-2-isopropylhydrazide hydrochloride (30 mg/kg, intraperitoneally).

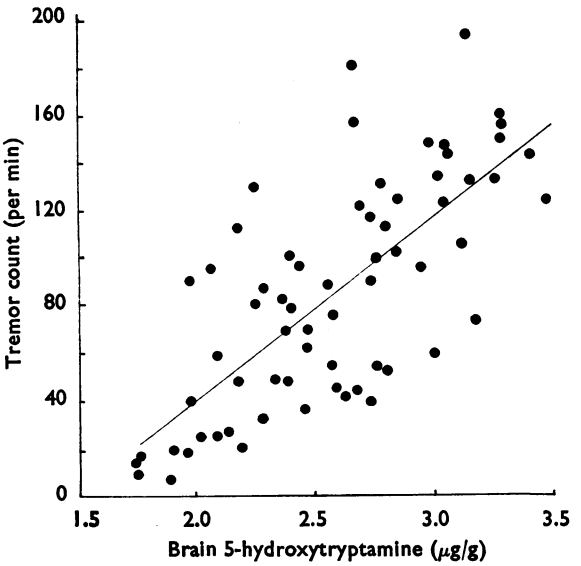

Fig. 15. Tremor count and brain level of 5-hydroxytryptamine, for each mouse, killed immediately after counting. The animals were given various doses of 5-hydroxytryptophan intraperitoneally, 1 hr after various doses of 1-L-α-alanyl-2-isopropylhydrazide hydrochloride, and tremors were measured 30 to 40 min later. Calculated relationship: r=0.72 ($P<0.001$), b=77.6±18.5, n=66.

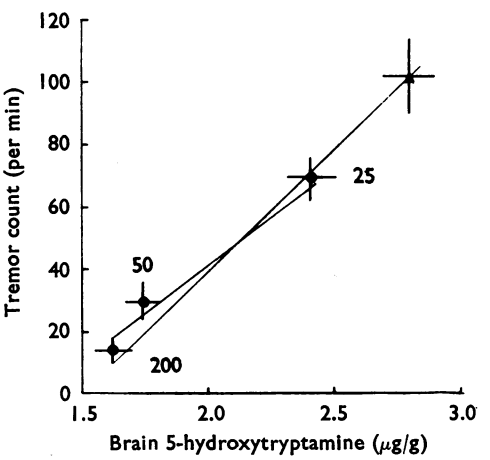

Fig. 16. The effect of various doses of α-methyldopa (values on graph, in mg/kg), subcutaneously, upon tremor count and brain level of 5-hydroxytryptamine (●) of mice, measured 30 to 40 min after 5-hydroxytryptophan (50 mg/kg, intraperitoneally) given 15 min after the α-methyldopa and 1 hr after 1-L-α-alanyl-2-isopropylhydrazide hydrochloride (50 mg/kg, intraperitoneally). The point ▲ represents the mean value for fourteen mice receiving no α-methyldopa, with its standard errors; the line passing through this point is the relation shown in Fig. 15. Relations calculated for the correlation between tremor and brain levels in the same mice after α-methyldopa are: r=0.86 ($P<0.001$), b=56.6±15.3, n=22.

5-hydroxytryptamine level found rarely reached 175% of the normal value (Table 7). Repeated doses of irreversible monoamine oxidase inhibitor, however, were shown to permit greater increase in 5-hydroxytryptamine levels and the appearance of tremor, the relation being similar to that found when 5-hydroxytryptophan was used (Fig. 17).

When given before 5-hydroxytryptophan, both irreversible monoamine oxidase inhibitors, such as 1-L-α-alanyl-2-isopropylhydrazide hydrochloride, and reversible inhibitors, such as harmaline, resulted in similar relations between 5-hydroxytryptamine levels and tremor (Fig. 17). When similar experiments were made, injecting the tryptamine derivatives before

TABLE 7

EFFECT OF SINGLE DOSES OF 1-L-α-ALANYL-2-ISOPROPYLHYDRAZIDE HYDROCHLORIDE (Ro 4-1340) IN CAUSING TREMORS AND RAISING THE LEVEL OF BRAIN 5-HYDROXYTRYPTAMINE IN MICE

Brain 5-hydroxytryptamine concentrations are given as μg/g wet weight. Values are means and standard errors

Dose of Ro 4-1340 (mg/kg)	Response after 2 hr		Response after 6 hr	
	Tremor count	5-Hydroxytryptamine (μg/g)	Tremor count	5-Hydroxytryptamine (μg/g)
100	10·2±2.3	1·59±0·06	9·6±2·2	1·48±0·04
200	12·6±4·6	1·59±0·05	24·1±3·8	1·74±0·05

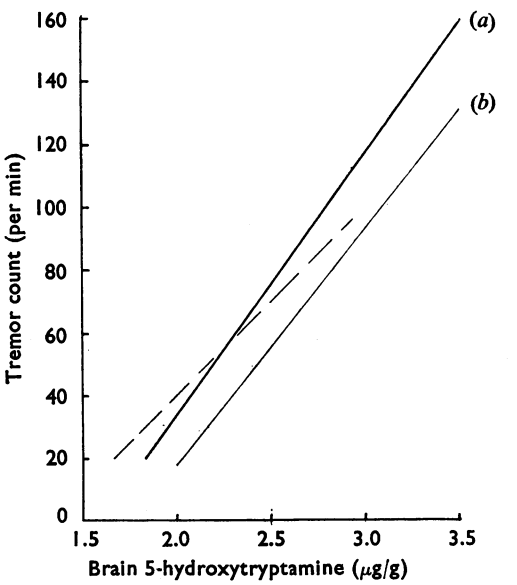

Fig. 17. Correlations for tremor counts and brain levels of 5-hydroxytryptamine in mice treated with: (*a*) 1-L-α-alanyl-2-isopropylhydrazide hydrochloride followed by 5-hydroxytryptophan (from Fig. 15); n=66, b=77.6±18.5 (*b*) Harmaline hydrochloride, various intraperitoneal doses, followed 10 min later by 5-hydroxytryptophan (50 mg/kg, intraperitoneally); n=40, b=72.3±29.7. Interrupted line: repeated treatment with 1-L-α-alanyl-2-isopropylhydrazide hydrochloride: typically, 100 mg/kg intraperitoneally, overnight, and three doses of 50 mg/kg intraperitoneally at intervals of 2 hr. Tremors were measured and the mice were killed for brain level determinations 3 hr after the last dose; n=31, b=53.8±22.4.

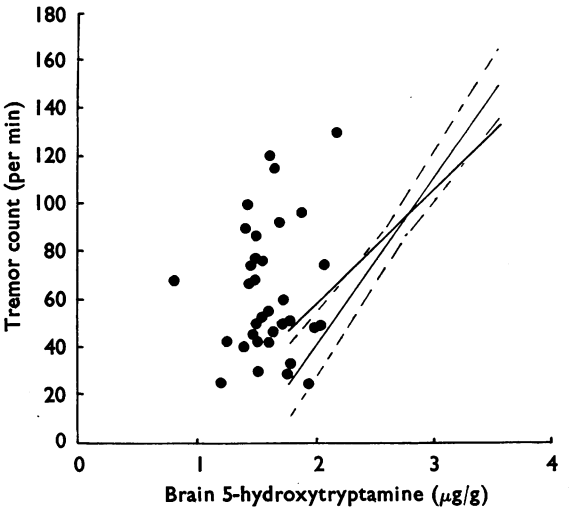

Fig. 18. Correlation between tremor count and brain level of 5-hydroxytryptamine (heavy line) in mice treated with various doses of 5-hydroxytryptophan, intraperitoneally, 30 min after α-methyltryptamine hydrochloride (10 mg/kg, intraperitoneally). n=15, b=56.6±25.0, r=0.6. The fine line is the relation found for 5-hydroxytryptophan after a hydrazide (from Fig. 15), with its fiducial limits (broken lines). The scattered points are individual tremor counts and brain levels of 5-hydroxytryptamine for mice treated only with α-methyltryptamine hydrochloride (30 to 60 mg/kg, intraperitoneally) and measured 1.5 hr after injection.

5-hydroxytryptophan and taking care to extract out these tryptamines from the brain tissue when estimating 5-hydroxytryptamine, the relations between 5-hydroxytryptamine levels and tremor were again similar to those found with other tremor-producing combinations (Fig. 18). Injection of α-methyltryptamine alone in doses sufficient to cause stimulation, however, was followed by only little increase in brain 5-hydroxytryptamine, as was also found by Gey & Pletscher (1962), and the tremor counts showed no correlation with this (Fig. 18).

DISCUSSION

The central stimulant actions of α-methyltryptamine and *N*α-dimethyltryptamine show points of resemblance to those of amphetamine and methamphetamine which may be related to the common structural feature of the two pairs of compounds, which are aromatic isopropylamines. The differences are, however, perhaps of more significance, as distinguishing the indolyl- from the phenylalkylamines. Being tryptamine derivatives, α-methyltryptamine and *N*α-dimethyltryptamine might be expected to show stimulant properties resembling those of tryptamine, but which would be apparent without prior inhibition of monoamine oxidase, since the α-methyl group protects them from oxidation by this enzyme. In fact, whereas tryptamine and its derivatives do cause similar stimulant effects, the evidence presented shows that their modes of action differ.

That the actions of these compounds are not simply those of protected tryptamines is suggested first by the results with decarboxylase inhibitors, which delay or reduce the tremors due to α-methyltryptamine and *N*α-dimethyltryptamine while having no effect on

those caused by tryptamine after monoamine oxidase inhibition. This effect of decarboxylase inhibitors argues against any explanation of the actions of the tryptamine derivatives as due to direct stimulation, as concluded by Vane *et al.* (1961), and suggests that the action may be mediated by a product of aminoacid decarboxylation, such as 5-hydroxytryptamine. Further evidence for their action not being exerted directly is the enhancement of the stimulant effects of the tryptamine derivatives by previous treatment with irreversible monoamine oxidase inhibitors, for the compounds themselves are not substrates for the enzyme.

An alternative explanation accounts for the stimulant properties of these tryptamine derivatives by their property of inhibiting monoamine oxidase, stimulation being the result of accumulating stimulant amines (Greig *et al.*, 1959). However, other reversible monoamine oxidase inhibitors, such as harmaline, which appear more potent than the tryptamine derivatives from *in vitro* measurements (Long, unpublished) do not show comparable stimulant properties. (Although harmaline causes tremor in mice, this is qualitatively distinguishable from that of 5-hydroxytryptophan and the tryptamines and, moreover, is not accompanied by other signs of stimulation.) Against this, it should be stressed that it is not reliable to argue from *in vitro* values for the potency of reversible inhibitors to activities they may show *in vivo*. Direct evidence of their enzyme inhibitory potency *in vivo* cannot be obtained, because dilution of tissue homogenates and addition of substrate in the course of estimating enzyme activity will encourage reversal of any inhibition which has been produced in the tissue *in vivo*. Gey & Pletscher (1962) showed that, in causing elevation of brain 5-hydroxytryptamine levels, the tryptamine derivatives were less effective than harmaline and they doubted that their stimulant action could be accounted for in this way. It has here been shown that the rise in 5-hydroxytryptamine levels which the derivatives produce in the brain is insufficient to reach the minimum associated with stimulant effects when produced by other means.

Vane *et al.* (1961) have argued that monoamine oxidase inhibition is unlikely to be responsible for the stimulant properties of α-alkyltryptamines, since prior treatment with large doses of an irreversible inhibitor does not reduce their activity. (Indeed, it is shown here, and also suggested by the results given by Vane *et al.* (1961), that the activity of these compounds is enhanced by such treatment.) This principle, of blocking an action completely with one drug in order to assess its importance in causing the effect of another, is unreliable when applied to enzyme activities, since it is practically impossible to abolish all activity by the use of an inhibitor, however potent (Long, unpublished). Many enzymes are present in excess of the minimum required to deal with usual amounts of substrate (see Pletscher *et al.*, 1965, for references) and the small residuum after massive inhibition may still be sufficient to metabolize the amounts of substrate normally present. Moreover, regeneration of enzyme commences very soon after " irreversible " inhibition (Long, unpublished). What is more important, further inhibition now becomes more significant than before. A concentration of inhibitor causing 50% reduction of the total enzyme activity may leave substrate turnover unchanged, but the same concentration, producing 50% reduction of a residual activity which is already only 0.1% of the total, may greatly reduce turnover rate.

This explains the stimulant effectiveness of repeated doses of monoamine oxidase inhibitors, the first of which, although causing no stimulation, may leave enzyme activity too low for all but the most sensitive means of biochemical detection. It also suggests that the

powerful stimulant activity of harmaline and the tryptamine derivatives in mice previously treated with several doses of irreversible inhibitor is not necessarily inconsistent with an explanation in terms of monoamine oxidase inhibitory properties. In this situation, however, $\alpha\alpha$-dimethyltryptamine approaches α-methyltryptamine in potency, although it is considerably weaker as a monoamine oxidase inhibitor, both as estimated *in vitro* (Long, unpublished) and from elevation of brain 5-hydroxytryptamine levels *in vivo* (Gey & Pletscher, 1962), while both drugs appear more active stimulants than the more potent inhibitor, harmaline. Further, the temporary reversal of the action of reserpine in mice previously treated with the tryptamine derivatives stands in contrast to the sustained prevention of reserpine sedation with the reversible monoamine oxidase inhibitor, harmaline, even though the intensity of the stimulant phase is greater with the tryptamines.

The dependence of the stimulant actions of the tryptamine derivatives on a property other than, or perhaps in addition to, monoamine oxidase inhibition is further indicated by the relation found between intensity of tremor and brain content of 5-hydroxytryptamine. Whereas this was similar in all instances when tremor was caused by monoamine oxidase inhibitors in repeated doses or when followed by 5-hydroxytryptophan, and by the tryptamine derivatives also when followed by 5-hydroxytryptophan, tremors produced by the tryptamine derivatives alone were associated with little or no rise in brain 5-hydroxytryptamine. The evidence, therefore, suggests that, if the substituted tryptamines cause tremor through the mediation of a product of aminoacid decarboxylation, such as 5-hydroxytryptamine, they do so without noticeably increasing the level of this amine present in the brain.

We suggest that the tryptamine derivatives exert an action in addition to the inhibition of monoamine oxidase, by interfering with the distribution of amines, such as 5-hydroxytryptamine, between the " free " and " bound " states within the tissues, in the sense in which these terms were introduced by Brodie, Tomich, Kuntzman & Shore (1957), and resulting in a higher proportion of newly formed amine remaining in the " free " or active state. Evidence that such an action is exerted within the central nervous system would be difficult to obtain, but an action which appears relevant has been shown for the tryptamine derivatives on blood platelets where, as described in the following paper, they interfere with the uptake of 5-hydroxytryptamine both *in vitro* and *in vivo* (Lessin, Long & Parkes, 1965).

The proposed mode of action for the stimulant effects of the tryptamine derivatives in mice, as exemplified by that of causing tremor, is shown diagrammatically in Fig. 19. Both monoamine oxidase inhibition and interference with amine uptake are postulated as responsible for the accumulation of free 5-hydroxytryptamine leading to the production of tremor by these agents alone, the low effectiveness of $\alpha\alpha$-dimethyltryptamine in this respect being related to its poor activity on the enzyme. After a very high degree of monoamine oxidase inhibition by irreversible inhibitors, however, stimulant activity would be to a greater extent dependent on effectiveness in interfering with amine uptake, in which all three tryptamine derivatives show similar activity (Lessin *et al.*, 1965). The diagram shows how the sedative effect of reserpine is associated with amine depletion, while prevention of this sedation, or its conversion to stimulation, follows treatment with monoamine oxidase inhibitors, whereby the liberated amine is preserved in the free state, in accordance with the scheme put forward by Brodie & Shore (1957). In the presence of the tryptamine deriva-

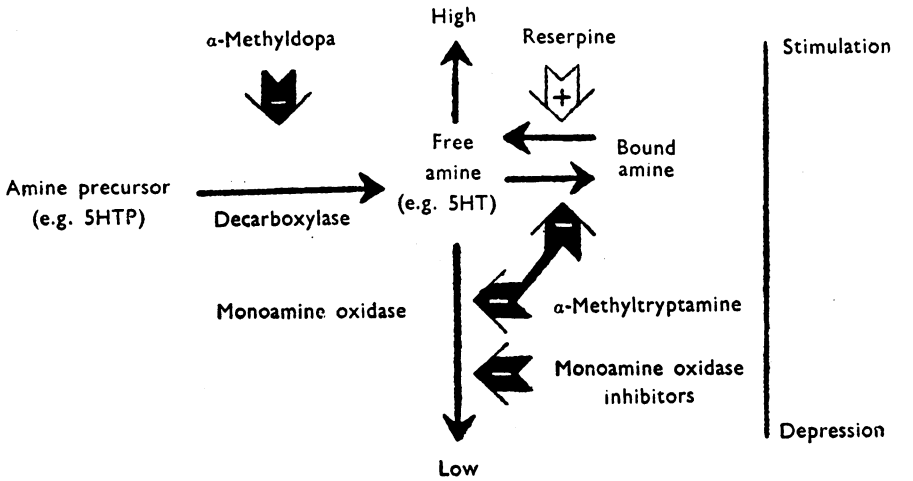

Fig. 19. Schematic representation of the relation between amine metabolism and stimulation, and the effects of various agents, incorporating the action suggested for the tryptamine derivatives. 5HT=5-hydroxytryptamine; 5HTP=5-hydroxytryptophan.

tives, reserpine results in more intense stimulation because, in addition to some inhibition of monoamine oxidase, interference with uptake further displaces equilibrium in favour of amine in the free state. It follows that the most powerful stimulant combination will be reserpine with a tryptamine derivative in mice previously treated with an irreversible monoamine oxidase inhibitor, and this was found to be so.

Vane *et al.* (1961) have objected to this proposed mechanism on the grounds that α-methyltryptamine is stimulant after reserpine, which, they say, would already have inhibited storage of amines. This objection might be valid only if the effect of the reserpine were complete. It is probable, however, that this is never so and that a situation exists similar to that described above regarding monoamine oxidase inhibition: namely that, even when amine levels have become undetectable, a residue remains which may be the subject of physiologically significant alteration. After reserpine depletion it has been shown that synthesis of 5-hydroxytryptamine continues and also that storage ability soon begins to regenerate (Brodie, Spector, Kuntzman & Shore, 1958; Dubnick, Leeson, Chessin & Scott, 1960; Brodie & Shore, 1957). In these circumstances displacement of equilibrium in favour of the free amine could well be particularly effective for stimulation, as suggested by the enhanced activity shown for tryptamine and 5-hydroxytryptophan, as well as the tryptamine derivatives.

SUMMARY

1. Central stimulant properties in mice are described for α-methyltryptamine, αα-dimethyltryptamine and *N*α-dimethyltryptamine. Those principally studied were tremor, hyperthermia and mydriasis.

2. Treatment with irreversible monoamine oxidase inhibitors potentiated the stimulant activity, which indicated there was no direct action resembling that of tryptamine as these amines were already protected from monoamine oxidase by their α-methyl substituent.

3. The compounds were themselves reversible inhibitors of monoamine oxidase and their potentiation by irreversible inhibitors was thought not to preclude this activity as the basis of a stimulant action exerted through the mediation of accumulating endogenous amines; the more completely an enzyme system is inhibited, the greater is the effect of further inhibition upon substrate turnover.

4. The interaction of the substituted tryptamines with reserpine, however, differed considerably from that of other monoamine oxidase inhibitors.

5. Mediation of the stimulant action by endogenous amines is suggested from the attenuation of tremor by inhibitors of aromatic decarboxylase.

6. Tremors caused by the tryptamines were not, however, associated with the rise in brain level of 5-hydroxytryptamine consistently found when tremors were caused by other monoamine oxidase inhibitors, with or without 5-hydroxytryptophan.

7. The tryptamines are thought to produce these effects by an alteration of the equilibrium between " free " and " bound " amines in the tissues in favour of the active state, possibly together with the inhibition of monoamine oxidase. The implications of this hypothesis are shown to correspond to the characteristics of the stimulant actions of the compounds.

The compounds studied were synthesized by Drs Cohen, Heath-Brown and Philpott (Roche Products Ltd.) in the course of work on a series of tryptamine derivatives. $N\alpha$-Dimethyltryptamine is described in British Patent No. 893,707 (1962).

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