THE EFFECT OF THREE TRYPTAMINE DERIVATIVES ON SEROTONIN METABOLISM *IN VITRO* AND *IN VIVO*

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Interest in inhibitors of monamine oxidase activity has recently been revived because of the use of such compounds as iproniazid and α-methylphenylethyl hydrazine hydrochloride (JB 516) (Biel *et al.*, 1958) in the treatment of various types of mental disease. The efficacy of these compounds has been attributed to their ability to cause serotonin and/or catecholamines to accumulate in the brain.

The effect of three tryptamine derivatives, namely, 5-hydroxy-α-methyltryptamine creatinine sulphate, α - methyltryptamine and α-ethyltryptamine hydrochloride on the synthesis and breakdown of serotonin *in vitro* and *in vivo* has been compared. These compounds were prepared by Drs. R. V. Heinzelman and J. Szmuszkoviez and Mr. W. C. Anthony of the Chemistry Department of The Upjohn Company.

METHODS. Monamine oxidase (MAO) activity was measured by the manometric method of Blaschko *et al.* (1937) using guinea pig liver as a source of enzyme. The substrates were present in a final concentration of 6.2×10^{-3} M. Inhibitors were tested in various concentrations in order that the I50 could be determined.

5-Hydroxytryptophan (5HTP) decarboxylase activity was measured by the method of Clark *et al.* (1954) using pork kidneys as the source of enzyme.

To test for inhibition of monamine oxidase activity *in vivo*, rats were given the compound being investigated, by either the oral or intraperitoneal route and, at certain specified intervals after dosing, the rats were decapitated and enzyme activity determined on the homogenized brain and liver by the manometric method. The amounts of liver and brain used were 200 and 400 mg, respectively, in a final volume of 2 ml.

Inhibition of 5-hydroxytryptophan decarboxylase activity *in vivo* was determined by observing whether the compound inhibited the diarrhea in mice which followed the administration of 5-hydroxytryptophan (1 mg/mouse). This diarrhea was attributed by Woolley (1958) to the formation of serotonin and was blocked in his experiments by BAS, a serotonin antagonist. In our experiments inhibitors of the decarboxylase also prevented the diarrhea.

RESULTS. The effect of the three tryptamine derivatives under study as well as iproniazid and amphetamine on monamine oxidase activity using various substrates is shown in table 1. It will be seen that the 5-hydroxyindole was the least potent of the three compounds. The other two indoles were comparable in activity to iproniazid. α-Methyltryptamine appeared to inhibit the oxidation of serotonin by competing with the substrate for the enzyme. This was shown by the increase in inhibition when the concentration of substrate was decreased and is presented graphically in figure 1.

The effect of the compounds on 5-hydroxytryptophan decarboxylase activity is shown in table 2. Here the change from methyl to ethyl substitution in the α-position caused a quite marked decrease in effect, the α-methyl compound inhibiting 50% at 10^{-3} M while the α-ethyl compound had no effect at 10^{-2} M. Hydroxylation at position 5 had little effect on activity, the results being similar with α-methyl and 5-hydroxy-α-methyltryptamines. These two compounds were somewhat more inhibitory than iproniazid.

The tryptamine derivatives were also tested on glutamic decarboxylase activity of rat brain (Roberts and Frankel, 1951). This enzyme, like 5-hydroxytryptophan decarboxylase, requires pyridoxal phosphate as coenzyme. In these experiments there was no inhibition in the presence of excess coenzyme and only a slight inhibition (around 15% for the tryptamine derivatives as well as iproniazid) without added coenzyme in a concentration of 10^{-3} M. Under the latter conditions this enzyme was not operating at maximum activity. Contrariwise, under the conditions in which the effect on 5-hydroxytryp-

TABLE 1

Effect of tryptamine analogues on monamine oxidase activity

The results are expressed as I50, the molar concentration of compound required to cause an inhibition of 50%.

Compound	Substrate			
	Serotonin	Epinephrine	Tyramine	Tryptamine
5-Hydroxy-α-methyltryptamine creatinine sulphate	5×10^{-3}	3×10^{-3}	3×10^{-3}	2×10^{-2}
α-Methyltryptamine	1×10^{-4}	1×10^{-3}	2×10^{-3}	1×10^{-3}
α-Ethyltryptamine HCl	3×10^{-4}			
Iproniazid phosphate	3×10^{-4}	4×10^{-4}		
l-Amphetamine sulphate	14% at 10^{-3} M	1×10^{-3}		

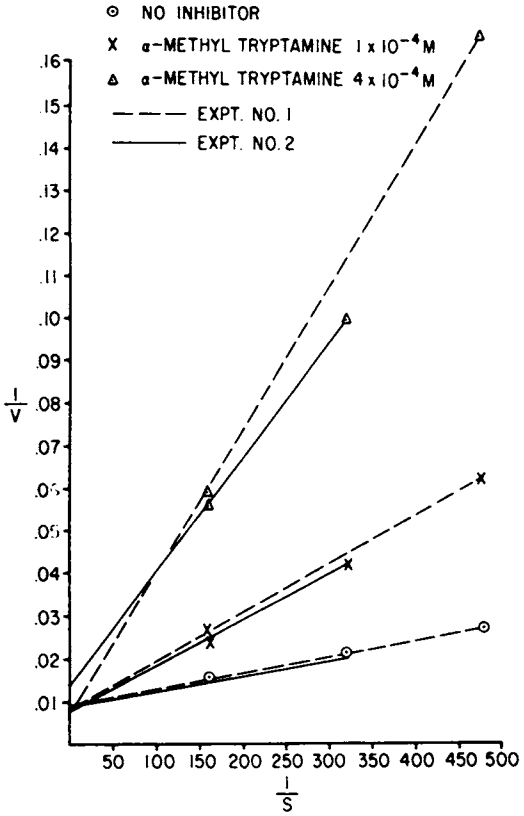

FIG. 1. The reciprocal of the velocity is plotted against the reciprocal of the substrate concentration (Lineweaver and Burk, 1934).

tophan decarboxylase was measured, the concentration of coenzyme was optimal. Thus under conditions for optimal activity, 5-hydroxytryptophan decarboxylase is more sensitive to the tryptamine derivatives than is glutamic decarboxylase.

TABLE 2

Effect of tryptamine analogues on 5-hydroxytryptophan decarboxylase activity

Compound	% Inhibition at 10^{-2} M	I50
5-Hydroxy-α-methyltryptamine creatinine sulphate	—	7×10^{-4}
α-Methyltryptamine	—	1×10^{-3}
α-Ethyltryptamine HCl	0	—
Iproniazid phosphate	65	5×10^{-3}
l-Amphetamine sulphate	0	—

The effect of the compounds on monamine oxidase activity *in vivo* is shown in table 3. The results correlated with the results *in vitro* in that α-methyl and α-ethyltryptamines inhibited both brain and liver monamine oxidase activity while 5-hydroxy-α-methyltryptamine had no inhibitory effect in the doses given. It may also be seen that all of these compounds stimulated the endogenous respiration of brain. The effect of the tryptamine compounds on monamine oxidase was reversible since 24 hours after administration the activity of the enzyme had returned to the control level.

The effect of the tryptamines on 5-hydroxytryptophan decarboxylase activity *in vivo* is shown in table 4. In this test both α-methyltryptamine and 5-hydroxy-α-methyltryptamine inhibited the diarrhea following 5-hydroxytryptophan. However, α-ethyltryptamine had no effect unless very large doses were given, which correlated with the lack of effect of this compound on 5-hydroxytryptophan decarboxylase *in vitro*. Iproniazid in a dose of 325 mg/kg, i.p., also decreased the diarrhea following 5-hydroxytryptophan.

TABLE 3

Effect of tryptamine analogues administered in vivo on monamine oxidase activity

The compounds were administered intraperitoneally and at the times specified, the rats were decapitated and monamine oxidase activity measured on the homogenized brain and liver.

Compound	Dose mg/kg (mmol/kg)	Interval Between Dosing and Decapitation Minutes	Tissue	O_2 Consumed in 60 Min		Difference (b) − (a) or MAO Activity	Decrease in MAO Activity %
				(a) Tissue alone	(b) Tissue and serotonin		
None	—	—	Brain	24	93	69	—
5-Hydroxy-α-methyltryptamine creatinine sulphate	350 (0.88)	15	Brain	35	98	63	No inhibition
		30	Brain	45	116	71	No inhibition
		60	Brain	58	134	76	No inhibition
		120	Brain	50	130	80	No inhibition
None	—	—	Liver	35	130	95	—
5-Hydroxy-α-methyltryptamine creatinine sulphate	350 (0.88)	15	Liver	27	125	98	No inhibition
		30	Liver	30	127	97	No inhibition
		60	Liver	50	148	98	No inhibition
		120	Liver	47	153	106	No inhibition
None	—	—	Brain	17	106	89	—
5-Hydroxy-α-methyltryptamine creatinine sulphate	350	15	Brain	50	130	80	No inhibition
		30	Brain	55	143	88	No inhibition
		60	Brain	60	132	72	No inhibition
		120	Brain	60	150	90	No inhibition
None	—	—	Liver	40	135	95	—
5-Hydroxy-α-methyltryptamine creatinine sulphate	350	15	Liver	48	150	102	No inhibition
		30	Liver	59	163	104	No inhibition
		60	Liver	58	167	109	No inhibition
		120	Liver	55	161	106	No inhibition
None	—	—	Brain	20	110	90	—
α-Methyltryptamine	45 (0.26)	15	Brain	41	90	49	46
		30	Brain	46	67	21	77
None	—	—	Liver	25	133	108	—
α-Methyltryptamine	45 (0.26)	15	Liver	26	60	34	69
		30	Liver	33	73	40	63
None	—	—	Brain	17	106	89	—
α-Methyltryptamine	45	15	Brain	20	95	75	17
		60	Brain	60	105	45	50
		120	Brain	72	102	30	66
None	—	—	Liver	55	145	90	—
α-Methyltryptamine	45	15	Liver	54	74	20	78
		60	Liver	59	99	40	56
		120	Liver	42	109	67	26
None	—	—	Brain	51	115	64	—
α-Ethyltryptamine HCl	45 (0.20)	15	Brain	63	124	61	5
		30	Brain	85	132	47	27
		60	Brain	100	144	44	31
None	—	—	Liver	40	156	116	—
α-Ethyltryptamine HCl	45 (0.20)	15	Liver	36	93	57	51
		30	Liver	32	83	51	56
		60	Liver	48	99	51	56

TABLE 3—*Continued*

Compound	Dose mg/kg (mmol/kg)	Interval Between Dosing and Decapitation Minutes	Tissue	O_2 Consumed in 60 Min		Difference (b) − (a) or MAO Activity	Decrease in MAO Activity %
				(a) Tissue alone	(b) Tissue and serotonin		
None	—	—	Brain	51	115	64	—
α-Ethyltryptamine HCl	90 (0.40)	15	Brain	65	85	20	69
		30	Brain	70	78	8	86
		60	Brain	86	88	2	97
None	—	—	Liver	33	152	119	—
α-Ethyltryptamine HCl	90 (0.40)	15	Liver	41	83	42	65
		30	Liver	24	76	52	56
		60	Liver	44	86	42	65

TABLE 4

Effect of the tryptamine analogues on the diarrhea in mice following the administration of 5-hydroxytryptophan

Mice were injected intraperitoneally with the compound to be tested. Thirty minutes later 5-hydroxytryptophan (1 mg/mouse) was injected.

Compound	Dose mg/kg (mmol/kg)	No. of Mice	Interval Between 5HTP Administration and Onset of Diarrhea (min)	Severity of Diarrhea	Remarks
None	—	4	3.7	+++	
5-Hydroxy-α-methyltryptamine creatinine sulphate	419 (1.05)	2	—	—	No diarrhea in 30 min
5-Hydroxy-α-methyltryptamine creatinine sulphate	84 (0.2)	2	—	—	No diarrhea in 30 min
None	—	3	4.9	+++	
5-Hydroxy-α-methyltryptamine creatinine sulphate	35 (0.09)	3	—	±	1 Animal had mild diarrhea after 10.5 min; 2 animals no diarrhea in 30 min
None	—	4	5.8	+++	
α-Methyltryptamine	15 (0.086)	2	—	—	No diarrhea in 30 min; mice very excited
α-Methyltryptamine	3 (0.02)	2	8.3	+	
α-Methyltryptamine	3 (0.02)	2	—	—	No diarrhea in 30 min
None	—	4	6.6	+++	
α-Ethyltryptamine hydrochloride	84 (0.37)	2	—	—	No diarrhea in 30 min
α-Ethyltryptamine hydrochloride	17 (0.07)	2	5.8	+++	
None	—	4	3.6	+++	
α-Ethyltryptamine hydrochloride	15 (0.07)	2	8.8	++	Mice were stimulated after 5HTP
α-Ethyltryptamine hydrochloride	3 (0.01)	2	4	+++	

The doses of compound used in the *in vivo* experiments are not necessarily the minimum effective doses.

DISCUSSION. The inhibitory effects of α-methyl and α-ethyltryptamine on monamine oxidase activity *in vitro* were comparable to that exerted by iproniazid. The I50's of the two indole compounds were 1×10^{-4}, 2.6×10^{-4} and that of iproniazid 2.5×10^{-4} M with serotonin as substrate and under our experimental conditions. The tryptamine compounds differed from iproniazid in that their effects were reversible in a few hours in our experiments while the enzyme is inhibited for several days after iproniazid (Davison, 1957). Of the indole compounds tested, α-methyltryptamine was the most potent inhibitor; introduction of substituents in the ring or on the side chain decreased activity. Inhibition by α-methyltryptamine differed from that by amphetamine in that the indole compound was more inhibitory with serotonin as substrate than with epinephrine while the reverse was true for amphetamine (table 1). If these differences also occur *in vivo* one would expect α-methyltryptamine to be more effective in causing an accumulation of serotonin than of epinephrine or other catecholamines whereas the reverse would be true of amphetamine.

α-Methyl and α-ethyltryptamine inhibited monamine oxidase *in vivo* when administered either intraperitoneally or orally. This was shown by the decreased rate of metabolism of serotonin by excised brain or liver of an animal treated with these tryptamine compounds. The inhibition of enzyme activity by brain also indicated that these compounds crossed the blood-brain barrier. Further evidence of central action was the marked irritability and restlessness, sensitivity to noise and Straub tail produced in rats and mice on either oral or intraperitoneal administration of these compounds. In mice this was especially noticeable after administration of 5-hydroxytryptophan together with the monamine oxidase inhibitors.

A hitherto unexplainable finding was the increased endogenous oxygen consumption (without substrate) by brain from the rats treated with the tryptamine compounds. This also occurred with brains from rats treated with several other monamine oxidase inhibitors, namely, methoxyphenamine hydrochloride (Orthoxine), amphetamine and iproniazid. This effect did not occur with liver.

α-Methyltryptamine and 5-hydroxy-α-methyltryptamine also inhibited 5-hydroxytryptophan decarboxylase while α-ethyltryptamine had no effect at a concentration of 10^{-2} M. This enzyme is considerably less sensitive to α-methyl and α-ethyltryptamine than is monamine oxidase, however.

In vivo α-methyl and 5-hydroxy-α-methyltryptamine inhibited 5-hydroxytryptophan decarboxylase activity as determined by the diarrhea test of Woolley (1958) described above. Very large doses of α-ethyltryptamine also showed some inhibition, although this compound did not inhibit at a concentration of 10^{-2} M *in vitro*. This effect may be due to an antiserotonin action quite unrelated to an action on decarboxylase activity. Iproniazid also inhibited the 5-hydroxytryptophan induced diarrhea in mice. In these experiments the metabolite was exogenously supplied and the site (or sites) of its metabolism is not known.

Of the two enzymes investigated the predominant action of α-methyl and of α-ethyltryptamine *in vivo* is probably on monamine oxidase. With these compounds monamine oxidase is inhibited in both brain and liver, and with both compounds there is a quite marked central stimulation in rats and mice. Conversely with 5-hydroxy-α-methyltryptamine there was no inhibition of monamine oxidase *in vivo* and no evidence of any central nervous system stimulation.

Other indole compounds have been described which inhibited monamine oxidase in concentrations comparable to those of the indoles herein described (Freter *et al.*, 1958). Some of these compounds also inhibited 5-hydroxytryptophan decarboxylase activity to some extent but in general were considerably less effective as inhibitors of this enzyme than of monamine oxidase.

SUMMARY

α- Methyltryptamine and α-ethyltryptamine inhibited MAO activity both *in vitro* and *in vivo*. *In vitro* the activities of these indoles were of the same order as that of iproniazid. *In vivo* they differed from iproniazid in that their effect was reversible. 5-Hydroxy-α-methyltryptamine was less active *in vitro* than the two other indoles tested and showed no inhibition *in vivo* in the

dose used. α-Methyl and α-ethyltryptamine, but not 5-hydroxy-α-methyltryptamine, caused an central stimulation of rats and mice. This effect correlated with the ability to inhibit MAO. α-Methyltryptamine and 5-hydroxy-α-methyltryptamine inhibited 5-hydroxytryptophan decarboxylase *in vitro* and *in vivo* as determined by Woolley's diarrhea test in mice. Although it is possible for α-methyltryptamine to inhibit both MAO and 5-hydroxytryptophan decarboxylase *in vivo* the observed effects would indicate that MAO is predominantly affected.

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