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THE EFFECTS OF α-ALKYL SUBSTITUTED TRYPTAMINES ON 5-HYDROXYTRYPTAMINE UPTAKE BY BLOOD PLATELETS

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In an accompanying paper (Lessin, Long & Parkes, 1965) a hypothesis has been proposed to account for some central stimulant effects of α-alkyl substituted tryptamines. The evidence there presented was interpreted as suggesting that these effects depend on two properties: reversible inhibition of monoamine oxidase, and interference with uptake and storage of 5-hydroxytryptamine in the brain, so that a greater proportion of the amine exists in the " free " or pharmacologically active state. Evidence for effects on amine distribution in the brain is not readily obtainable but properties of agents which affect amine uptake by tissues may be detected using blood platelets. The results of such a study with the tryptamine derivatives are presented in this paper.

Preliminary communications of these results have been made to the Biochemical Society (Long & Lessin, 1962) and to the British Pharmacological Society (Lessin & Long, July 1961).

METHODS

In vitro *studies*

Ox blood was collected into polyethylene bottles containing 10% v/v of disodium edetate solution (1% in 0.7% saline). The blood was rapidly cooled in ice and kept between 0 and 5° C during the processing, which was carried out within 2 hr. To each 100 ml. of blood was added 50 ml. of buffered saline solution (platelet medium) as used by Sano, Kakimoto & Taniguchi (1958). The diluted blood was centrifuged at 1,500 revs/min for 5 min using 100-ml. plastic centrifuge cups (M.S.E. refrigerated centrifuge, Head No. 6887) and the upper layer was removed. It was then recentrifuged under the same conditions in order to remove contaminating red cells. The volume of platelet-containing plasma obtained in this way was about 30% of the total liquid volume and contained 70% of the platelets present in the original blood.

For uptake experiments, 9 ml. of this material was used and all compounds added were contained in 1 ml. of platelet medium; siliconed glassware was used throughout. After incubation at 37° C in air for 60 min the tubes were centrifuged at 0° C (3,000 revs/min) for 30 min and the supernatant fluid was discarded. The platelet pellet was suspended in platelet medium, recentrifuged and the supernatant fluid was again discarded. The pellet was then suspended in 2.5 ml. of 0.02 N-hydrochloric acid and left for 30 min, centrifuged and the supernatant fluid treated directly with 1-nitroso-2-naphthol and sodium nitrite according to the colorimetric procedure for 5-hydroxytryptamine (Udenfriend, Weissbach & Clark, 1955).

The initial apparent 5-hydroxytryptamine content of 9 ml. of platelet-rich plasma was 15 to 40 μg and the increase after incubation with 5-hydroxytryptamine ranged from 10 to 30 μg. Platelets were used within

24 hr of obtaining the blood; this was found necessary, since the ability of platelets to concentrate 5-hydroxytryptamine declined with time.

Monoamine oxidase activity was measured manometrically using an enzyme preparation from mouse brain with 5-hydroxytryptamine as substrate (2×10^{-3} M).

For identification of indoles present in platelets, extracts were prepared by homogenizing the platele. pellet with 0.2 ml. of 2 N-hydrochloric acid, lyophilizing the extracts and redissolving the residue in water Paper chromatograms were run in a butanol/acetic acid/water mixture (4 : 1 : 1) and indoles were revealed by spraying with dimethylaminocinnamaldehyde (Harley-Mason & Archer, 1958).

In vivo *studies*

Mice (30 to 35 g) were used for the *in vivo* studies and blood was withdrawn from them during light ether anaesthesia. It was found essential to withdraw blood from mice by heart puncture since this was the only method which gave samples containing the full complement of platelets (1.4×10^6/mm^3). About 1 to 2 ml. of blood could be taken using No. 20 short-bevel needles and siliconed syringes. The 5-hydroxytryptamine and test compounds were administered intraperitoneally, blood being withdrawn after an appropriate interval. Platelets were prepared and 5-hydroxytryptamine was estimated by the method described in the previous section.

Values for 5-hydroxytryptamine are expressed as μg/ml. of whole blood or $\mu g/8 \times 10^8$ platelets. In this study several strains of mice were used and found to differ in their platelet content of 5-hydroxytryptamine.

RESULTS

In vitro *studies*

The results were obtained by testing each compound at four concentrations for effects on the uptake of 5-hydroxytryptamine and the molar concentrations required for 50% inhibition of uptake were read from curves plotted from the results. The tryptamine derivatives studied did not interfere with the colorimetric method of estimation and chromatographic experiments showed that these compounds were not present in the platelets at the end of incubation.

As reported by Stacey (1961), the rate of 5-hydroxytryptamine uptake was almost independent of concentration over a wide range (Fig. 1). The tryptamine derivatives had a

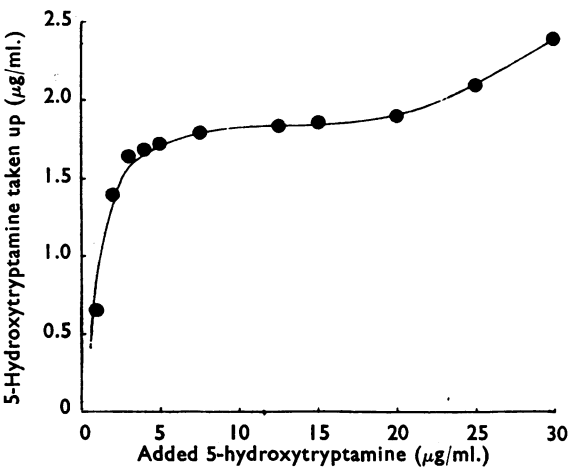
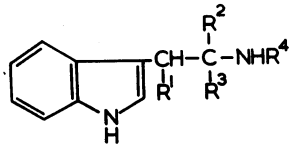


Fig. 1. Relationship between uptake of 5-hydroxytryptamine by ox blood platelets, and concentration of the amine in the incubation medium. Each point represents the mean of two determinations.

TABLE 1

COMPARISON BETWEEN THE ACTIVITY OF SUBSTITUTED TRYPTAMINES AS REVERSIBLE INHIBITORS OF MONOAMINE OXIDASE *IN VITRO* AND THEIR ABILITY TO BLOCK PLATELET UPTAKE OF 5-HYDROXYTRYPTAMINE



Compound	Substituent				Molar concentration/or 50% inhibition of	
	R^1	R^2	R^3	R^4	Monoamine oxidase	5-Hydroxytryptamine uptake
α-Methyltryptamine	H	CH_3	H	H	10^{-5}	6×10^{-5}
*N*α-Dimethyltryptamine	H	CH_3	H	CH_3	$2{\cdot}5\times10^{-5}$	6×10^{-5}
αα-Dimethyltryptamine	H	CH_3	CH_3	H	1.1×10^{-4}	10^{-4}
ααβ-Trimethyltryptamine	CH_3	CH_3	CH_3	H	8×10^{-4}	$1{\cdot}5\times10^{-4}$

marked effect in inhibiting 5-hydroxytryptamine uptake but their activities in this respect varied much less than their ability to inhibit monoamine oxidase (Table 1).

When inhibition of uptake by a fixed concentration of drug was measured using various concentrations of 5-hydroxytryptamine, results characteristic of competitive inhibition were obtained (Fig. 2). Thus, the percentage inhibition of uptake increased as the 5-hydroxytryptamine concentration was decreased.

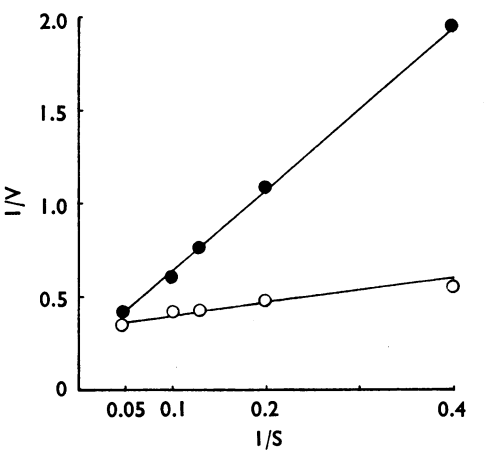


Fig. 2. Competitive inhibition of 5-hydroxytryptamine uptake by α-methyltryptamine (4×10^{-5} M, ●) and in the absence of an inhibitor (○). V=rate of 5-hydroxytryptamine uptake; S=5-hydroxytryptamine concentration in medium (μg/ml.).

It was observed that 5-hydroxy-α-methyltryptamine, a compound with pharmacological properties similar to those of 5-hydroxytryptamine (Vane, 1959), was also taken up by platelets and the colorimetric method used did not distinguish between these two compounds. The 5-hydroxy-α-methyltryptamine appeared to be taken up by the same mechanism as that which transports 5-hydroxytryptamine (Table 2) and could be found by

TABLE 2

APPARENT 5-HYDROXYTRYPTAMINE CONCENTRATION OF PLATELETS INCUBATED WITH 5-HYDROXY-α-METHYLTRYPTAMINE, 5-HYDROXYTRYPTAMINE OR A MIXTURE OF BOTH AMINES

Apparent platelet amine contents of incubates are calculated as 5-hydroxytryptamine

Additions to incubation medium	Apparent platelet amine content of incubate (μg)
—	19
5-Hydroxytryptamine (10 μg/ml.)	33
5-Hydroxy-α-methyltryptamine (10 μg/ml.)	30
5-Hydroxytryptamine (10 μg/ml.) + 5-hydroxy-α-methyltryptamine (10 μg/ml.)	30

paper chromatography in extracts of washed platelets. This uptake was antagonized by inhibitors in the same way as was that of 5-hydroxytryptamine (Fig. 3).

In vivo *studies*

The 5-hydroxytryptamine content of platelets after intraperitoneal injection of the amine was examined in mice, groups of animals being sampled after 1, 2, 4 and 18 hr. Table 3

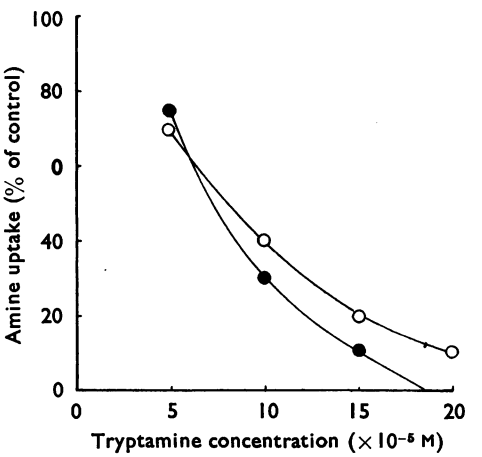


Fig. 3. The inhibition of the uptake of 5-hydroxy-α-methyltryptamine and 5-hydroxytryptamine by tryptamine. ○, 5-Hydroxytryptamine (10 μg/ml.); ●, 5-hydroxy-α-methyltryptamine (10 μg/ml.).

TABLE 3

EFFECT OF VARYING THE TIME INTERVAL BETWEEN INJECTION OF 5-HYDROXYTRYPTAMINE CREATININE SULPHATE AND TAKING BLOOD SAMPLES IN MICE

The amine was injected in a dose (of the salt) of 25 mg/kg, intraperitoneally, and blood concentrations refer to the base. Numbers of mice are given in brackets

Time after injection (hr)	Blood 5-hydroxytryptamine (μg/ml.)
0 (controls)	7·3±0.4 (10)
1	10·0±0·4 (3)
2	12·5±0·9 (6)
4	13·4±0·6 (6)
18	11·3±0·7 (6)

shows that the greatest increase in platelet 5-hydroxytryptamine content over that of control animals occurred at 2 and 4 hr after injection.

The effect of various doses of 5-hydroxytryptamine on the platelet amine level 4 hr after administration is shown in Table 4. The uptake of 5-hydroxytryptamine appeared to be independent of the dose, within the range 5 to 45 mg/kg. Furthermore, no regression upon dose was apparent in these experiments, even when mice were previously treated with the monoamine oxidase inhibitor 1-L-α-alanyl-2-isopropylhydrazide hydrochloride (Ro 4-1340) (Table 4). However, when the 5-hydroxytryptamine precursor, 5-hydroxytryptophan

TABLE 4

PLATELET AMINE LEVELS AFTER VARIOUS DOSES OF 5-HYDROXYTRYPTAMINE IN MICE WITH AND WITHOUT PREVIOUS TREATMENT BY A MONOAMINE OXIDASE INHIBITOR

All samples were taken 4 hr after intraperitoneal injection of 5-hydroxytryptamine. Treatment refers to intraperitoneal injection of 1-L-α-alanyl-2-isopropylhydrazide hydrochloride (50 mg/kg), 1 hr before the 5-hydroxytryptamine. Numbers of mice are given in brackets

Dose of 5-hydroxytryptamine (mg/kg)	Blood 5-hydroxytryptamine (μg/ml.) in mice	
	Not treated	Treated
0	5·9±1·8 (4)	5·1±0·4 (5)
5	10·1±0·3 (5)	9·1±0·8 (4)
15	9·6±0·2 (6)	7·3±0·6 (7)
45	11·9±1·1 (4)	—

(10 to 90 mg/kg), was administered, regression of platelet amine upon dose of the amino acid became apparent. This was particularly striking in animals previously treated with the monoamine oxidase inhibitor (Fig. 4).

The aromatic aminoacid decarboxylase inhibitor, β-3,4-dihydroxyphenyl-α-methylalanine, was effective in preventing the rise in platelet content of 5-hydroxytryptamine produced by injected 5-hydroxytryptophan (Table 5).

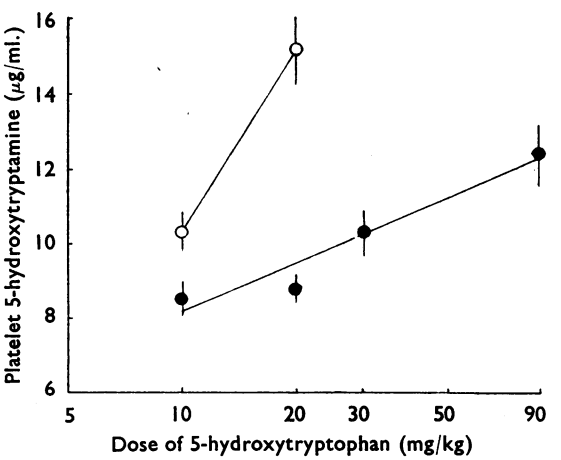


Fig. 4. Platelet 5-hydroxytryptamine levels 1 hr after various doses of 5-hydroxytryptophan injected into mice. ●, Animals not previously treated (b=4.2±1.8; n=29); ○, animals treated 1 hr earlier with 1-L-α-alanyl-2-isopropylhydrazide hydrochloride, 30 mg/kg, intraperitoneally (b=15.5±8.0; n=10).

TABLE 5

EFFECT OF β-3,4-DIHYDROXYPHENYL-α-METHYLALANINE UPON 5-HYDROXYTRYPTOPHAN-INDUCED INCREASE IN PLATELET CONTENT OF 5-HYDROXYTRYPTAMINE IN MICE

All injections were intraperitoneal. Blood samples were taken 1 hr after giving the amine precursor. 5-Hydroxytryptamine contents refer to μg of the base in 8×10^8 platelets. Numbers of mice are given in brackets

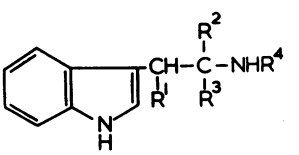
Treatment	Platelet 5-hydroxytryptamine content	*P*
Nil	2·6±0·2 (8)	<0·001
5-Hydroxytryptophan (50 mg/kg)	5·6±0·3 (8)	
α-Methyldopa (100 mg/kg) 5 min before 5-hydroxytryptophan (50 mg/kg)	3·9±0·3 (8)	<0·001

The effects of various tryptamine derivatives as *in vivo* inhibitors of 5-hydroxytryptamine uptake are shown in Table 6. The compounds were administered at various dose levels 1 hr before injection of 5-hydroxytryptamine (5 mg/kg) and the blood was collected 2 to 4 hr later. Chromatographic evidence suggested that the inhibition of uptake was not due to replacement of 5-hydroxytryptamine by the test compounds.

TABLE 6

THE EFFECT OF VARIOUS SUBSTITUTED TRYPTAMINES ON THE INCREASE OF PLATELET AMINE CONTENT 4 HR AFTER INTRAPERITONEAL INJECTION OF 5-HYDROXYTRYPTAMINE INTO MICE

Injections were intraperitoneal. Platelet 5-hydroxytryptamine contents refer to μg of the base in 8×10^8 platelets. Numbers of mice are given in brackets



Compound	Substituents R¹	R²	R³	R⁴	Dose (mg/kg)	Platelet 5-hydroxytryptamine content after: No treatment	Treatment	*P*
α-Methyltryptamine	H	CH_3	H	H	10	6·34±0·20 (19)	4·58±0·21 (13)	<0·05
					2·5	6·34±0·20 (19)	5·36±0·30 (6)	>0·05
αα-Dimethyltryptamine	H	CH_3	CH_3	H	5	6·33±0·20 (23)	5·17±0·40 (6)	<0·05
					1·25	6·33±0·20 (23)	6·58±0·80 (4)	>0·05
ααβ-Trimethyltryptamine	CH_3	CH_3	CH_3	H	40	5·24±0·25 (12)	4·01±0·25 (12)	<0·05
					20	5·24±0·25 (12)	4·27±0·20 (6)	<0·05
					10	5·24±0·25 (12)	4·83±0·18 (6)	>0·05

Injection of 5-hydroxy-α-methyltryptamine was followed by an increase in apparent 5-hydroxytryptamine content of the platelets which could be shown by paper chromatography to be due to the presence of the injected amine (Table 7). Significant inhibition of 5-hydroxytryptamine uptake was caused by the compounds studied, ααβ-trimethyltryptamine being the least active.

TABLE 7

THE EFFECT OF 5-HYDROXY-α-METHYLTRYPTAMINE UPON APPARENT PLATELET LEVEL OF 5-HYDROXYTRYPTAMINE IN MICE

The compound was given 2 hr before amine determinations. Injections were intraperitoneal. Apparent 5-hydroxytryptamine concentrations refer to μg of the base in 8×10^8 platelets. Numbers of mice are given in brackets

Treatment	Apparent platelet 5-hydroxytryptamine	*P*
None	2·9±0·1 (6)	<0·05
5-Hydroxy-α-methyltryptamine (20 mg/kg)	4·3±0·3 (6)	

DISCUSSION

It has been demonstrated previously (Stacey, 1961) that tryptamine inhibits the uptake of 5-hydroxytryptamine by blood platelets *in vitro*. This property is also possessed by α-alkyl tryptamines, which behave as competitive inhibitors of amine uptake, both in ox platelets *in vitro* and *in vivo* in mice. Since the α-alkyl tryptamines can inhibit the uptake of 5-hydroxytryptamine in this situation, it is reasonable to suppose that such an action may be exerted in the central nervous system as required by the hypothesis put forward to account for the stimulant actions of these drugs in the previous paper (Lessin *et al.*, 1965).

Several interesting points emerge from the experiments on the uptake of 5-hydroxytryptamine by mouse platelets *in vivo*. Whereas the increase in platelet amine after injection of 5-hydroxytryptamine was independent of dose in the range studied and was unaffected by prior monoamine oxidase inhibition, the increase after 5-hydroxytryptophan was dose-dependent and considerably increased by monoamine oxidase inhibition. It may therefore be deduced that monoamine oxidase plays little part in the inactivation of injected 5-hydroxytryptamine. This is supported by several pharmacological studies which have failed to demonstrate potentiation of injected 5-hydroxytryptamine after inhibition of this enzyme (for example, Goldberg & Sjoerdsma, 1959).

The effect of β-3,4-dihydroxyphenyl-α-methylalanine in preventing the rise in platelet 5-hydroxytryptamine after administration of 5-hydroxytryptophan may be due to its activity as an aromatic amino acid decarboxylase inhibitor, although direct effects on amino acid transport (Smith, 1963) or amine storage are also possible.

SUMMARY

1. A number of α-alkyl substituted tryptamines inhibited uptake of 5-hydroxytryptamine by blood platelets *in vitro* and *in vivo*. The action *in vitro* was competitive.

2. Uptake of 5-hydroxytryptamine *in vivo* after injection of the amine was not increased after a monoamine oxidase inhibitor.

3. Uptake after injection of 5-hydroxytryptophan was greatly increased after a monoamine oxidase inhibitor and was reduced by α-methyldopa.

4. 5-Hydroxy-α-methyltryptamine both inhibited the uptake of 5-hydroxytryptamine and was itself taken up by platelets.

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

REFERENCES

GOLDBERG, L. I. & SJOERDSMA, A. (1959). Effects of several monoamine oxidase inhibitors on the cardiovascular actions of naturally occurring amines in the dog. *J. Pharmacol. exp. Ther.*, **127**, 212–218.

HARLEY-MASON, J. & ARCHER, A. A. P. G. (1958). Use of p-dimethylaminocinnamaldehyde as a spray reagent for indole derivatives on paper chromatograms. *Biochem. J.*, **69**, 60*P*.

LESSIN, A. W., LONG, R. F. & PARKES, M. W. (1965). Central stimulant actions of α-alkyl substituted tryptamines in mice. *Brit. J. Pharmacol.*, **24**, 49–67.

LONG, R. F. & LESSIN, A. W. (1962). Inhibition of 5-hydroxytryptamine uptake by platelets *in vitro* and *in vivo*. *Biochem. J.*, **82**, 4–5*P*.

SANO, I., KAKIMOTO, Y. & TANIGUCHI, K. (1958). Binding and transport of serotonin in rabbit blood platelets and the action of reserpine. *Amer. J. Physiol.*, **195**, 495–498.

SMITH, S. E. (1963). Uptake of 5-hydroxy[^{14}C]tryptophan by rat and dog brain slices. *Brit. J. Pharmacol.*, **20**, 178–189.

STACEY, R. S. (1961). Uptake of 5-hydroxytryptamine by platelets. *Brit. J. Pharmacol.*, **16**, 284–295.

UDENFRIEND, S., WEISSBACH, H. & CLARK, C. T. (1955). Estimation of 5-hydroxytryptamine (serotonin) in biological tissues. *J. biol. Chem.*, **215**, 337–344.

VANE, J. R. (1959). The relative activities of some tryptamine analogues on the isolated rat stomach strip preparation. *Brit. J. Pharmacol.*, **14**, 87–98.