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THE RELATIVE ACTIVITIES OF SOME TRYPTAMINE ANALOGUES ON THE ISOLATED RAT STOMACH STRIP PREPARATION

BY

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The relative potencies of analogues of tryptamine and 5-hydroxytryptamine have been determined on the rat fundus preparation. This tissue had an amine oxidase activity, which, in the homogenate, was able to inactivate both tryptamine and 5-hydroxytryptamine to about the same degree. Amine oxidase inhibitors potentiated the action of tryptamine and many analogues on the isolated rat fundus preparation, but not the action of 5-hydroxytryptamine or of other hydroxytryptamines. This suggested that, in the isolated organ, the amine oxidase was unable to inactivate 5-hydroxytryptamine, but could inactivate tryptamine, 5-methoxytryptamine and many others. These results may be explained if it is supposed that tryptamine entered the cell, but because of the polar hydroxyl group 5-hydroxytryptamine did not. This hypothesis is supported by the oil/water partition coefficients. The structure/activity of the various tryptamine derivatives is discussed in the light of this assumption.

Variations in the structure of tryptamine and 5-hydroxytryptamine have mainly been made in a search for strong antagonists to 5-hydroxytryptamine. Much less work has been published on the relative potency of tryptamine derivatives as mimics of 5-hydroxytryptamine. Erspamer (1952a) and Freyburger, Graham, Rapport, Seay, Govier, Swoap, and Van der Brook (1952) studied the LD50 values of various substituted tryptamines. Erspamer (1952b, 1954) also tested analogues of tryptamine on the oestrous rat uterus preparation. Quadbeck and Röhm (1954) used the jejunum of the guinea-pig to study 5 - methoxy - and 5 - chloro - tryptamine, and Armstrong (1958) has tested tryptamine analogues for their ability to produce pain.

The rat stomach strip preparation (Vane, 1957) is extremely sensitive to 5-hydroxytryptamine-like compounds and has been used to study the relative potencies of substituted tryptamines, as they became available.

METHODS

The rat stomach strip was prepared as described by Vane (1957). It was mounted in a 5 ml. organ bath and bathed in Tyrode solution at 37°. Hyoscine hydrobromide (10^{-7}) was added to the Tyrode solution to minimize any acetylcholine-like effect and to reduce baseline irregularities. The movements of the muscle were recorded, not with a spring lever as originally described, but with a pendulum lever (Paton, 1957) of identical characteristics. The drugs were added to the organ bath in not more than 0.4 ml. saline, and the contraction of the stomach strip was allowed to develop for 90 sec. The bath was then washed out by overflow and the muscle stretched for 30 sec. The next dose of drug was added 2 or 3 min. later, giving a full cycle time of 4 or 5 min. In some experiments, the muscle was allowed to relax without additional stretching to obtain information on the duration of action of the compounds. A slow drip of Tyrode through the organ bath was maintained between each addition of drug. A vibrator mounted on the rim of the organ bath reduced friction effects between the writing point and the smoked paper.

The relative activities of the analogues of tryptamine are expressed in terms of molar potencies equiactive with 5-hydroxytryptamine (=1). For example, the contraction produced by a suitable dose of the test drug was bracketed between two doses of 5-hydroxytryptamine. This was repeated at a second concentration: from these values the number of nanograms of the test drug to give an equivalent contraction to 1 ng. 5-hydroxytryptamine was calculated. This relative activity was then recalculated as the number of moles of the test drug equiactive to one mole of 5-hydroxytryptamine. Thus, an

equipotent molar ratio of 100 means that 100 molecules of the test drug produce the same contraction as 1 molecule of 5-hydroxytryptamine. Most compounds were tested on at least two preparations. In some experiments, the relative potencies were determined both before and after the addition of an amine oxidase inhibitor to the bathing solution.

Spinal cats were prepared by the method of Kosterlitz, Krayer, and Matallana (1955). The blood pressure was recorded from a cannula in the left carotid artery with a mercury manometer.

Amine Oxidase Determination

The fundal portions of the stomachs from 24 rats were homogenized in 0.067 M sodium phosphate buffer of *p*H 7.4. The homogenate was dialysed against several changes of a large volume of phosphate buffer for some hours to reduce the normal respiratory activity. It was then diluted so that 1.5 ml. of the suspension contained the equivalent of 1 rat fundus. 1.5 ml. of the suspension was pipetted into each manometer flask. The centre well contained 0.2 ml. of 20% KOH solution dispersed over a piece of filter paper. The side arm contained 0.3 ml. of the substrate made up to give a final concentration when tipped of 0.0075 M. The gas space was filled with oxygen and the oxygen consumption of the preparation measured manometrically for 1 hr. One manometer was used as a thermobarometer and control readings were taken for flasks containing homogenate but no substrate. This procedure is an adaptation of the technique of Barlow, Blaschko, Himms, and Trendelenburg (1955).

Measurement of Dissociation Constants

All solutions were made up using distilled water, reboiled and cooled under a stream of nitrogen. 10 ml. of a 0.02 M solution of the amine was pipetted into a small tube and 1.0 ml. of 0.1N-HCl added to bring the *p*H down to below *p*H 2.0. The electrodes of a Pye *p*H meter, connected to an ink recorder, were lowered into the solution, together with two lengths of syringe needle tubing. Nitrogen was bubbled through one of these at a rate which gave adequate stirring and the other was connected to a micrometer syringe containing 5N-NaOH (carbonate free). The syringe was driven at a constant rate by a velodyne motor at a speed to deliver 0.02 ml. of alkali/min. As the *p*H of the solution was continuously recorded by the pen writer on moving paper, the titration curve was drawn directly, and the pK_a values were estimated from this curve.

Oil/Water Partition Coefficients

Olive oil B.P. was shaken with a buffer solution of *p*H 7.0 to remove any acidity. The emulsion was separated by centrifugation and the oil was washed in a similar manner with distilled water. Tryptamine hydrochloride and 5-hydroxytryptamine creatinine sulphate were dissolved in Tyrode solution. 100 ng. of 5-hydroxytryptamine base/ml., 4 μg. of tryptamine base/ml. and equal volumes of the amine solution and olive oil were shaken together for 15 min. The phases were separated and the aqueous phase assayed for 5-hydroxytryptamine or tryptamine content. An aliquot of the oil phase was then shaken for 30 min. with twice its volume of 0.3N-HCl to extract the amine from the oil. After separation, the aqueous phase was neutralized with 0.3N-NaOH and assayed for 5-hydroxytryptamine or for tryptamine. The concentration of amine in both water and oil was estimated in this way to eliminate the possibility of destruction or inactivation.

Materials

All drugs were added to the organ bath in a neutral saline solution. Most of the tryptamines were as hydrochlorides; the exceptions are designated in the acknowledgments. Throughout the paper, names for compounds have been used which indicate their relationship to the parent compound, rather than the more exact but less informative chemical name. The chemical structures and names used are all shown in Table II. Both iproniazid phosphate and phenyl-*iso*propylhydrazine hydrochloride were used as amine oxidase inhibitors. Where concentrations or doses are given, the tryptamines are expressed in terms of base. All other compounds are in terms of salt.

RESULTS

All the amines tested caused contraction of the stomach strip. The relative activities varied from greater than 30,000 for indolemethylamine, which was the weakest, up to 1.5 for 5-hydroxy-α-methyltryptamine, one of the strongest. The log dose/response curves for the more active of the compounds were compared with that for 5-hydroxytryptamine and found to be parallel over the range of dose studied. Because of the high magnification of the lever and the length of the strip of tissue, the full dose/response curve was not examined. This may account for the differences in relative activities shown in this paper and that of Barlow and Khan (1959).

Another factor which may cause variation in the potency of these amines is the ability of amine oxidase in the tissue to limit the concentration of active compound at the receptor site. Using the analogy of potentiation of the activity of acetylcholine on smooth muscle by the inhibition of cholinesterase with neostigmine, the activity of the tryptamines was studied in the presence of amine oxidase inhibitors. With iproniazid (10^{-5}) or in later experiments with phenyl*iso*propylhydrazine (Horita, 1958) in the Tyrode, it was found that the action of many of the tryptamines was greatly potentiated. Fig. 1 illustrates this and shows that the potency of tryptamine can be increased more than forty times by an amine oxidase inhibitor. At the same time, it must be

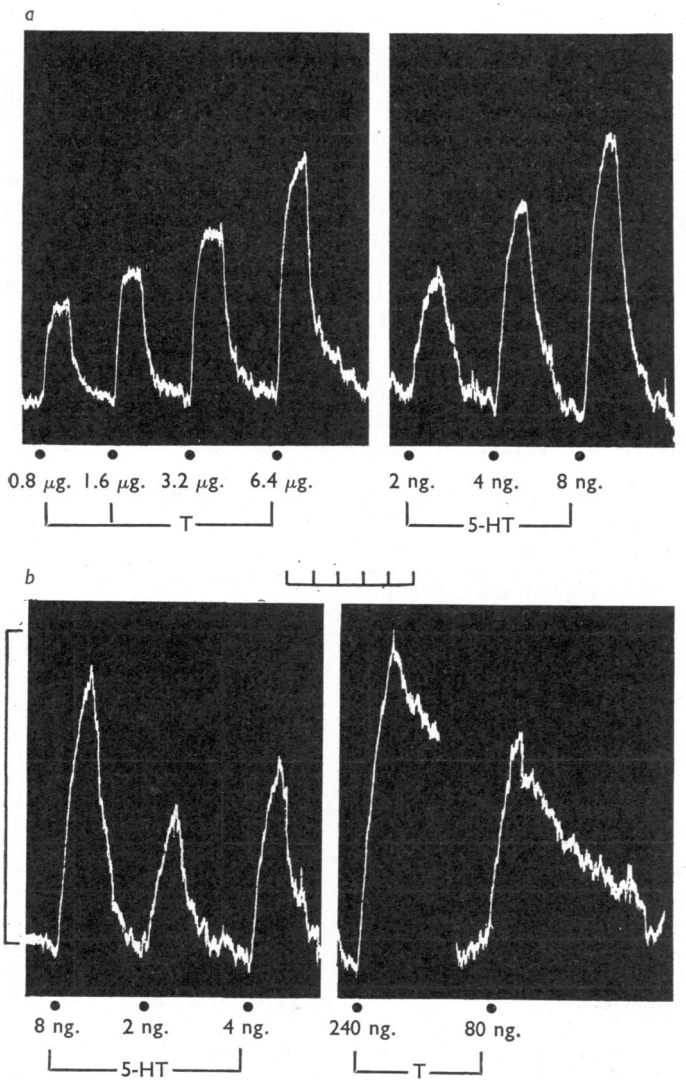


FIG. 1.—The response of the isolated rat stomach strip preparation to 5-hydroxytryptamine and tryptamine. (*a*) Responses to tryptamine (*T*) (Table II, No. 1) and 5-hydroxytryptamine (*5-HT*). (*b*) 45 min. after the addition of phenylisopropylhydrazine (0·1 μg./ml.) to the Tyrode solution. The response to 5-hydroxytryptamine is unchanged, but that to tryptamine is potentiated, 80 ng. eliciting a contraction equivalent to more than 3·2 μg. before amine oxidase inhibition. Note also the slowness of relaxation after washing out the tryptamine compared to (*a*). Time, min. Vertical scale, 10 cm.

noted that the potency of 5-hydroxytryptamine was unaffected by inhibition of amine oxidase. This differential potentiation was always obtained. In some experiments there was a gradual increase in sensitivity to all drugs including 5-hydroxytryptamine, but the addition of an amine oxidase inhibitor did not noticeably accelerate this process for 5-hydroxytryptamine. In view of the well-substantiated fact that amine oxidase preparations studied by manometric techniques oxidize both tryptamine and 5-hydroxytryptamine at about the same rate (Blaschko, 1952; Freyberger *et al.*, 1952), this result was puzzling. To confirm the presence of an amine oxidase-like enzyme in the rat fundus, and to study its characteristics, rat fundi were finely ground up and the ability of this material to oxidize tyramine and some of the tryptamines was measured manometrically. The results are shown in Table I. It can be seen that an enzyme with the accepted characteristics of amine oxidase was present in the tissue. It oxidized tyramine at a fairly fast rate, and tryptamine, 5-hydroxytryptamine, and 5-methyltryptamine at about 60% of this rate. It had no action on 5-hydroxy-α-methyltryptamine and was completely inhibited by iproniazid (10^{-5}). The initial substrate concentration was 0.0075 M or between 1.0 and 1.5 mg./ml. for the amines used (as base). One fifth to one third of the total amount of amine was oxidized by the enzyme in 1 hr., a rate of about 6 μg./rat fundus/min. for tryptamine and 5-methyltryptamine and 7 μg./rat fundus/min. for 5-hydroxytryptamine. Thus, if the highly artificial conditions in a Warburg flask can be compared with those in an isolated organ bath, it is possible to say that the amount of amine oxidase present in a rat fundus was more than enough to account for the potentiation of the activity of tryptamine by amine oxidase inhibitors. The immunity of 5-hydroxytryptamine from attack by amine oxidase in the organ bath when it can be oxidized at about the same rate as tryptamine by the tissue homogenate is still unexplained.

The potentiation of the action of tryptamine, but not 5-hydroxytryptamine, on the stomach strip by the addition of amine oxidase inhibitors afforded a means of studying the characteristics of the enzyme in the whole tissue. Table II compares the equipotent molar ratios of the

TABLE I

THE OXIDATION OF TRYPTAMINE AND DERIVATIVES AND TYRAMINE BY A HOMOGENATE OF RAT FUNDUS

The respiration of the enzyme without substrate has been subtracted from all the values. The average value was 20 μl. O_2/g. of tissue/hr. The addition of iproniazid (10 μg./ml.) completely inhibited the oxidation of all the substrates.

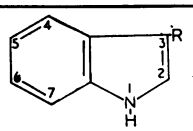
Substrate	O_2 Consumption (μl./g. of tissue/hr.)	Substrate (as Base) Oxidized, Calculated from Average Wet Weight of Fundus of 400 mg. (μg./rat fundus/min.)
Tyramine	207	8·4
5-Hydroxytryptamine	142	7·4
Tryptamine	124	5·9
5-Methyltryptamine	112	5·8
5-Hydroxy-α-methyltryptamine	0	0

tryptamines both before and after inhibition of amine oxidase. For the series of tryptamines unsubstituted in the indole ring, the potentiation of activity by inhibition of amine oxidase fitted well with the established ideas about the enzyme. For instance, the introduction of a methyl group on the α-carbon of the side-chain prevented oxidation by amine oxidase. Thus, when the enzyme was uninhibited, α-methyltryptamine (Table II, No. 4) has more than 10 times the potency of tryptamine (No. 1). However, after inhibition of the enzyme the contraction due to α-methyltryptamine remained the same height, but the potency of tryptamine increased, so that tryptamine was the more potent. Similarly, the introduction of two methyl, ethyl or propyl groups on the terminal amino group (Nos. 10, 11, and 12) apparently increased the potency, but that this increase in potency was only due to a decreasing action of amine oxidase was confirmed by repeating the experiments in the presence of iproniazid or phenyl*iso*propylhydrazine.

TABLE II

EQUIPOTENT MOLAR RATIOS OF TRYPTAMINE DERIVATIVES ESTIMATED ON THE ISOLATED RAT STOMACH STRIP, BEFORE AND AFTER AMINE OXIDASE INHIBITION

The numerals in brackets refer to the number of experiments on each compound. 5-Hydroxytryptamine was taken as unity.



No.	Ring Substituent	Side-chain R	Trivial Name Used in Text and Systematic Chemical Name	Normal (A) Equipotent Molar Ratio. Mean ± S.E. (No. of Tests)	After Amine Oxidase Inhibition (B) Equipotent Molar Ratio. Mean ± S.E. (No. of Tests)	Potentiation (A/B)
1	—	$-CH_2-CH_2-NH_2$	Tryptamine 3-(2-Aminoethyl)indole	408 ± 49 (15)	32 ± 4·8 (11)	12·7
2	—	$-CH_2-NH_2$	Indolemethylamine 3-Aminomethylindole	29,000 (2)	33,000 (2)	0·9
3	—	$-CH_2-CH_2-CH_2-NH_2$	Homotryptamine 3-(3-Aminopropyl)indole	1,920 (2)	460 (2)	4·2
4	—	$-CH_2-CH(CH_3)-NH_2$	α-Methyltryptamine 3-(2-Aminopropyl)indole	31 ± 5·7 (5)	40 (2)	0·8
5	—	$-CH_2-CH(C_2H_5)-NH_2$	α-Ethyltryptamine 3-(2-Aminobutyl)indole	4,600 (2)	—	—
6	—	$-CH_2-C(CH_3)_2-NH_2$	$\alpha\alpha$-Dimethyltryptamine 3-(2-Amino-2 : 2-dimethylethyl)indole	1,400 (1)	—	—
7	—	$-CH_2-CH_2-NH(CH_3)$	*N'*-Methyltryptamine 3-(2-Methylaminoethyl)indole	1,120 ± 192	126 (1)	8·9
8	—	$-CH_2-CH_2-NH(C_2H_5)$	*N'*-Ethyltryptamine 3-(2-Ethylaminoethyl)indole	250 (2)	170 (1)	1·5
9	—	$-CH_2-CH_2-NH(C_3H_7)$	*N'*-Propyltryptamine 3-(2-Propylaminoethyl)indole	330 (2)	330 (1)	1·0
10	—	$-CH_2-CH_2-N(CH_3)_2$	*N'N'*-Dimethyltryptamine 3-(2-Dimethylaminoethyl)indole	196 ± 30 (3)	110 (1)	1·8
11	—	$-CH_2-CH_2-N(C_2H_5)_2$	*N'N'*-Diethyltryptamine 3-(2-Diethylaminoethyl)indole	83 (2)	87 (1)	1·0
12	—	$-CH_2-CH_2-N(C_3H_7)_2$	*N'N'*-Dipropyltryptamine 3-(2-Dipropylaminoethyl)indole	34 (2)	41 (1)	0·8
13	2-CH_3	$-CH_2-CH_2-N(CH_3)_2$	2 : *N'N'*-Trimethyltryptamine 3-(2-Dimethylaminoethyl)-2-methylindole	1,200 (1)	—	—

TABLE II (*continued*)

14	5-OH	$-CH_2-CH_2-NH_2$	5-Hydroxytryptamine 3-(2-Aminoethyl)-5-hydroxyindole	1·0	1·0	1·0
15	4-OH	$-CH_2-CH_2-NH_2$	4-Hydroxytryptamine 3-(2-Aminoethyl)-4-hydroxyindole	1·8 (2)	2·0 (2)	0·9
16	6-OH	$-CH_2-CH_2-NH_2$	6-Hydroxytryptamine 3-(2-Aminoethyl)-6-hydroxyindole	460 (2)	560 (2)	0 8
17	6-OCH_3	$-CH_2-CH_2-NH_2$	6-Methoxytryptamine 3-(2-Aminoethyl)-6-methoxyindole	1,520 (2)	950 (2)	1·6
18	5 : 6-$(OCH_3)_2$	$-CH_2-CH_2-NH_2$	5 : 6-Dimethoxytryptamine 3-(2-Aminoethyl)-5 : 6-dimethoxyindole	300 (2)	147 (2)	2·0
19	5-OCH_3	$-CH_2-CH_2-NH_2$	5-Methoxytryptamine 3-(2-Aminoethyl)-5-methoxyindole	20±4 (4)	1·7±0·2 (4)	11·8
20	5-OCH_3	$-CH_2-CH(CH_3)-NH_2$	5-Methoxy-α-methyltryptamine 3-(2-Aminopropyl)-5-methoxyindole	2·9±0·6 (3)	2·5 (2)	1·2
21	5-Cl	$-CH_2-CH_2-NH_2$	5-Chlorotryptamine 3-(2-Aminoethyl)-5-chloroindole	100 (2)	7·6 (2)	13·2
22	5-CH_3	$-CH_2-CH_2-NH_2$	5-Methyltryptamine 3-(2-Aminoethyl)-5-methylindole	184±42 (3)	9 (1)	20·0
23	5-CH_3	$-CH_2-CH(CH_3)-NH_2$	5 : α-Dimethyltryptamine 3-(2-Aminopropyl)-5-methylindole	14±3·3 (3)	16 (1)	0·9
24	5-OH	$-CH_2-CH(CH_3)-NH_2$	5-Hydroxy-α-methyltryptamine 3-(2-Aminopropyl)-5-hydroxyindole	1·4±0·1 (7)	2·0 (2)	0·7
25	5-OH	$-CH_2-CH(C_2H_5)-NH_2$	α-Ethyl-5-hydroxytryptamine 3-(2-Aminobutyl)-5-hydroxyindole	7·0 (1)	—	—
26	5-OH	$-CH_2-CH_2-NH(CH_3)$	5-Hydroxy-*N'*-methyltryptamine 5-Hydroxy-3-(2-methylaminoethyl)indole	6·3 (2)	9·5 (1)	0·7
27	5-OH	$-CH_2-CH_2-N(CH_3)_2$	5-Hydroxy-*N'N'*-dimethyltryptamine 3-(2-Dimethylaminoethyl)-5-hydroxyindole	10±3·6 (3)	8·0 (1)	1·2
28	5-OH	$-CH_2-CH_2-N(C_2H_5)_2$	*N'N'*-Diethyl-5-hydroxytryptamine 3-(2-Diethylaminoethyl)-5-hydroxyindole	20 (1)	20 (1)	1·0
29	5-OH	$-CH_2-CH_2-N(C_3H_7)_2$	5-Hydroxy-*N'N'*-dipropyltryptamine 3-(2-Dipropylaminoethyl)-5-hydroxyindole	4·0 (2)	6·0 (2)	0·7
30	5-OH	$-CH_2-CO_2H$	5-Hydroxyindoleacetic acid 5-Hydroxyindol-3-ylacetic acid	Inactive	—	—
31	5-OCH_3	$-CH_2-CO_2H$	5-Methoxyindoleacetic acid 5-Methoxyindol-3-ylacetic acid	,,	—	—

For tryptamine derivatives with a hydroxyl group on the indole ring, the results were altogether different. Inhibition of amine oxidase did not potentiate the action of these compounds. An increase in the activity of 4-, 5-, and 6-hydroxytryptamine and the *N'*-substituted 5-hydroxytryptamines has never been observed after amine oxidase inhibition. On the other hand, the actions of methoxytryptamines (Fig. 2), 5-chlorotryptamine, and 5-methyltryptamine are all potentiated by amine oxidase inhibition (Table II, Nos. 17, 18, 19, 21, and 22).

The inability of the amine oxidase in the rat stomach preparation to act on the hydroxytryptamines suggested that a diffusion barrier existed which allowed tryptamine, but not 5-hydroxytryptamine, to pass. As the ability of this barrier to distinguish between tryptamine and 5-hydroxytryptamine might depend upon the physicochemical properties of the compounds, the

TABLE III

*p*K VALUES AND OIL/WATER SOLUBILITY COEFFICIENTS

The estimated *p*K values are assignable as follows: pKa_1, creatinine; pKa_2, terminal amino group; and pKa_3, ring hydroxyl group. The partition coefficient (concentration in oil/concentration in water) is given at the lowest initial concentration in the Tyrode solution to give easily assayable values. (100 ng. of 5-hydroxytryptamine base/ml. and 4 μg. of tryptamine base/ml.). The total recovery from both phases was 95% for 5-hydroxytryptamine and 116% for tryptamine.

No.		pKa_1	pKa_2	pKa_3	Partition Coefficient
1	Tryptamine hydrochloride	—	10·2	—	0·92
14	5-Hydroxytryptamine creatinine sulphate ..	4·9	10·0	11·1	0·055
27	5-Hydroxy-*N'N'*-dimethyltryptamine hydrochloride	—	9·8	11·2	
20	5-Methoxy-α-methyltryptamine hydrochloride	—	10·3	—	
12	*N'N'*-Dipropyltryptamine hydrochloride	—	8·6	—	
	Creatinine hydrochloride ..	4·9	—	—	

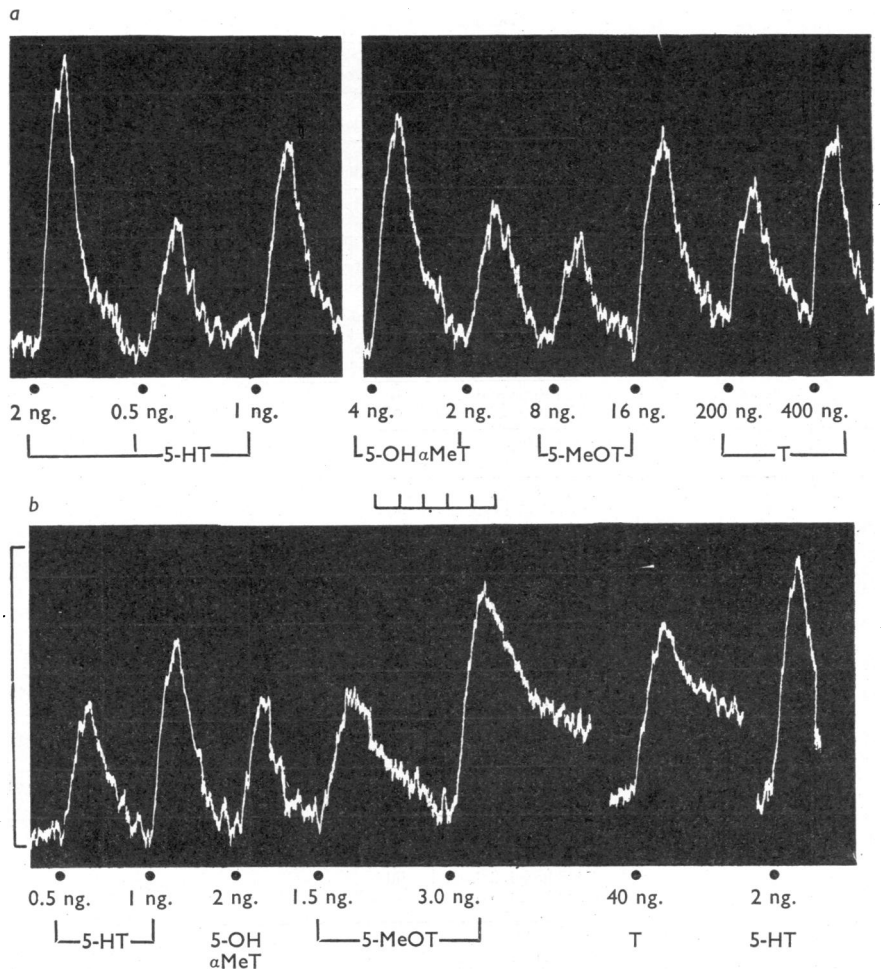


FIG. 2.—(*a*) Responses of the isolated stomach strip preparation to 5-hydroxytryptamine (***5-HT***), 5-hydroxy-α-methyltryptamine (***5-OHαMeT***, No. 24), 5-methoxytryptamine (***5-MeOT***, No. 19) and tryptamine (***T***, No. 1). (*b*) 60 min. after addition of phenyl*iso*propylhydrazine (0·1 μg./ml.) to the Tyrode solution. The contractions produced by ***5-HT*** and ***5-OHαMeT*** are unchanged. The effects of ***5-MeOT*** and ***T*** are potentiated in magnitude and duration. Time, min. Vertical scale, 10 cm.

dissociation constants and the oil/water partition coefficients were determined for representative members of the series (Table III). The *p*K values showed that the terminal amino group was almost completely ionized at physiological *p*H, but the ring hydroxyl group was in the unionized form. That the ring hydroxyl group contributed a great deal of oil insolubility to the molecule was shown by a comparison of the oil/water partition coefficients for tryptamine and 5-hydroxytryptamine at a low concentration. Tryptamine was almost as soluble in the oil as in the aqueous phase, but 5-hydroxytryptamine had a coefficient of 0.055, meaning that only 55 parts enter the oil phase out of every 1,000 in the aqueous phase.

Duration of Action

The rate of relaxation of the stomach strip after washing out the active drug probably depends upon the rate of detachment of the drug from the receptor site. This may be influenced by at least three factors, the affinity of the drug for the receptor, the rate of diffusion of the uncombined drug away from the receptor and destruction of the drug by an enzyme.

Figs. 1 and 2 show that after enzyme inhibition the contraction produced by tryptamine took much longer to pass off. Fig. 3 also illustrates that tryptamines which cannot be attacked by amine oxidase had a much longer duration of action than structurally similar compounds oxidizable by the

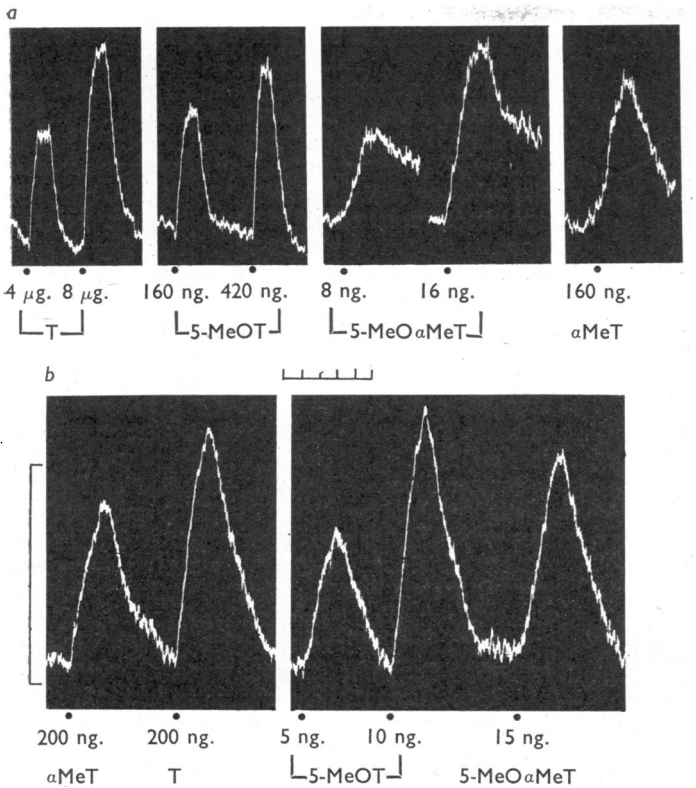


FIG. 3.—(*a*) Contractions of the isolated rat stomach strip preparation produced by tryptamine (***T***, No. 1), 5-methoxytryptamine (***5-MeOT***, No. 19), 5-methoxy-α-methyltryptamine (***5-MeOαMeT***, No. 20) and α-methyltryptamine (***αMeT***, No. 4). Note that the last two drugs produce effects which are much longer in duration. (*b*) A similar comparison, on a different preparation. Phenyl*iso*propylhydrazine had been added to the Tyrode solution 75 min. beforehand. The effects are comparable in duration, and tryptamine and 5-methoxytryptamine are now slightly more active than their α-methyl analogues. Time, min. Vertical scale, 10 cm.

enzyme. On the other hand, the relaxation of the tissue after 5-hydroxytryptamine was fairly rapid, relaxation after 5-hydroxy-α-methyltryptamine was also rapid and amine oxidase inhibitors did not affect the rate of relaxation after either substance (Fig. 2).

Blood Pressure

Intravenous injections of 5-hydroxytryptamine, 5-methoxytryptamine and tryptamine were compared for their pressor effects in the spinal cat, using infrequent injections to avoid tachyphylaxis. After the injection of either iproniazid or phenyl*iso*propylhydrazine, the effect of 5-hydroxytryptamine did not change substantially. The effects of both tryptamine and 5-methoxytryptamine were potentiated both in magnitude and in duration of action (Fig. 4).

DISCUSSION

In the Warburg flask, both 5-hydroxytryptamine and tryptamine are good substrates for liver amine oxidase. It has now been shown that the rat fundus, when finely ground up, contained an enzyme which behaved like amine oxidase and oxidized both 5-hydroxytryptamine and tryptamine. It also broke down 5-methyltryptamine. Other tryptamines with substituents in the 5-position of the indole ring should also be substrates for amine oxidase, but it was not possible to study these because of the limited quantity of the compounds available.

If the enzyme present in the homogenate is also active in the whole tissue, then inhibition of the enzyme should lead to a potentiation of the action of substances normally oxidized by it. Such a potentiation is well established for the action of acetylcholine on smooth muscle after inhibition of cholinesterase. It has also been suggested that the potentiation of the action of adrenaline-like compounds by ephedrine and cocaine may be due to amine oxidase inhibition (see Blaschko, 1952, 1954), but this hypothesis has to be reconsidered in the light of the recent work of Burn and Rand (1958), who postulate that substances such as ephedrine bring about their sympathomimetic effects through the release of noradrenaline.

Brown and Gillespie (1957) have suggested that the tissue receptors for noradrenaline were also responsible for its destruction. That this concept cannot hold for tryptamine receptors is shown by the fact that blocking amine oxidase activity substantially increased the action of tryptamine in the rat fundus. Indeed, this potentiation after enzyme inhibition is the first clear evidence that amine oxidase limits the amount of the active substance reaching the receptor site in the whole tissue. This potentiation, sometimes by more than twentyfold and comparable in magnitude to acetylcholine potentiation after cholinesterase inhibition, provided an opportunity to study the interrelation between the activity of a drug on the rat fundus and the role of amine oxidase in modifying this activity. A comparison of the relative activities of tryptamine (No. 1) and

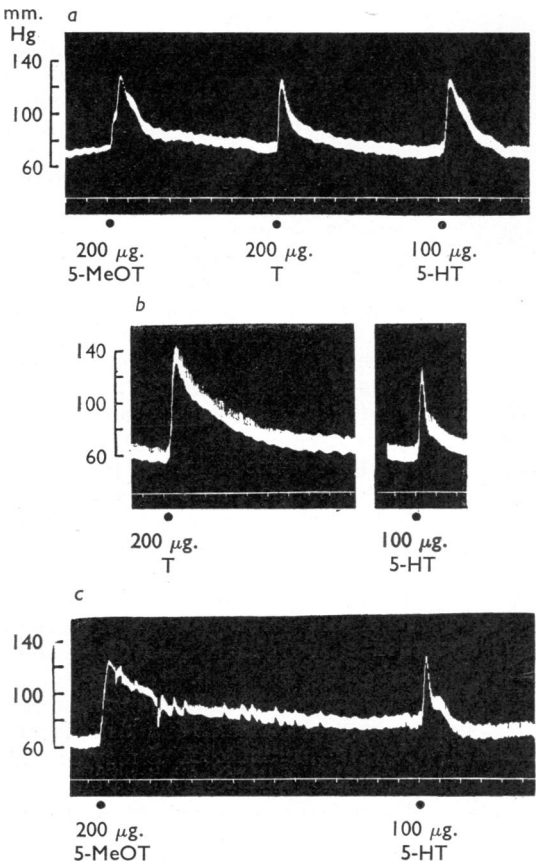


FIG. 4.—Blood pressure recording from a spinal cat (3·3 kg.). (*a*) The effects of 5-methoxytryptamine (***5-MeOT***, No. 19), tryptamine (***T***, No. 1) and 5-hydroxytryptamine (***5-HT***). (*b*) After phenyl*iso*propylhydrazine (1 mg./kg.). Note the potentiation and prolongation of the tryptamine effect. A second dose of phenyl*iso*propylhydrazine (mg./kg.) was given before the 5-hydroxytryptamine injection. (*c*) After a third dose of phenyl*iso*propylhydrazine (1 mg./kg.). Note the prolongation of the effect of ***5-MeOT***. Time, min.

α-methyltryptamine (No. 4) before and after enzyme inhibition afforded a good example. It is known that an α-methyl group in the side-chain prevents oxidation by amine oxidase. The fact that in the normal fundus α-methyltryptamine was 10 times more active than tryptamine can be explained by the action of amine oxidase in limiting the concentration of tryptamine around the receptor site. The true potency of these two substances was only seen, therefore, after amine oxidase inhibition, when tryptamine was found to be slightly more active than α-methyltryptamine. This means that at the muscle receptor tryptamine is more potent than α-methyltryptamine, but that without amine oxidase inhibition the concentration of tryptamine at the receptor is kept at a low level by destruction. In fact in the absence of amine oxidase activity, tryptamine was the most potent of all the tryptamines studied in which there were no substituents on the indole ring (Table II). None of the other compounds "fits" the receptor as well as does tryptamine, but they appear more potent when amine oxidase is active because they are oxidized to a lesser degree. An estimate of the ability of amine oxidase to inactivate these compounds in the isolated organ is shown in Table II by the degree of potentiation after enzyme inhibitors. It is interesting to note that the substitution of the hydrogen atoms of the terminal amino group by alkyl groups is as effective in preventing oxidation by the enzyme as is the introduction of a methyl group on the α-carbon atom. The *N'N'*-dimethyl and *N'N'*-diethyl derivatives (Nos. 10 and 11) are, however, less active at the muscle receptor: it is not until the terminal amino group is loaded with two propyl groups (No. 12) that the potency returns almost to match tryptamine itself.

Having found that tryptamine was limited in its action by the amine oxidase present in the tissue, and that this could be overcome either by the introduction of a methyl group on the α-carbon atom or of two propyl groups on the terminal amino group without much loss in inherent activity, it was expected that the same would hold true for 5-hydroxytryptamine. It was therefore puzzling to find that 5-hydroxy-α-methyltryptamine (No. 24) and 5-hydroxy-*N'N'*-dipropyltryptamine (No. 29) were less active than 5-hydroxytryptamine. An explanation is found in the observation that inhibition of amine oxidase in the fundus strip does not appreciably potentiate the action of 5-hydroxytryptamine. Because of the gradual increase in general sensitivity of the rat fundus to all drugs as the experiment proceeds, a slight potentiation of the action of 5-hydroxytryptamine could easily be missed. Such a potentiation would show itself, however, as a value of less than 1.0 in Table II. 5-Hydroxy-α-methyltryptamine (No. 24), for example, appears less active in comparison with 5-hydroxytryptamine after amine oxidase inhibition (potentiation factor = 0.7). Using 5-hydroxy-α-methyltryptamine as the reference standard, 5-hydroxytryptamine could be said to be increased in potency after amine oxidase inhibition by a factor of 1.4. This is the maximum possible potentiation from the values in Table II, and will be regarded as insignificant for the purpose of this discussion.

In contrast to the lack of potentiation of 5-hydroxytryptamine on the fundus strip after amine oxidase inhibition, the finely ground-up preparation oxidized 5-hydroxytryptamine at about the same rate as tryptamine. The only conclusion that can be drawn is that in the whole preparation the tissue receptors and the amine oxidase are spatially arranged so that tryptamine reaches both but 5-hydroxytryptamine only reaches the receptors. This would account for the inability of structural modification and amine oxidase inhibition to increase the potency of 5-hydroxytryptamine.

There are at least two ways in which orientation of the receptors and the enzyme might account for the results. First, it could be postulated that there are different receptors for tryptamine and for 5-hydroxytryptamine. The tryptamine receptors would have to be devoid of enzyme in the vicinity. In considering this hypothesis, it must be remembered that substances such as 5-methoxytryptamine (No. 19), 5-chlorotryptamine (No. 21) and 5-methyltryptamine (No. 22) are all potentiated in their action in the presence of an amine oxidase inhibitor, or by the introduction of a methyl group on the α-carbon atom. It would indeed be strange if all of these 5-substituted compounds combined preferentially with "tryptamine receptors" and not with "5-hydroxytryptamine receptors."

The second hypothesis is much more attractive and involves only a diffusion barrier which excludes 5-hydroxytryptamine but allows tryptamine to penetrate. The simplest explanation is that the cell surface is the diffusion barrier and that the amine oxidase is contained inside the cell.

There is abundant evidence that, in general, amine oxidase is an intracellular enzyme associated with cytoplasmic granules (Blaschko, 1952) and there is no reason to suppose that the rat fundus differs from other tissues in this respect. The *p*K values for 5-hydroxytryptamine, which agree with those published by Rapport, Green, and Page (1948), show that the ring hydroxyl group is only ionized to the extent of 0.005% at physiological *p*H. Nevertheless, the polar properties of the unionized ring hydroxyl group substantially alter the physical characteristics of tryptamine-like substances (compare benzene and phenol), as is shown by the low oil/water partition coefficient of 5-hydroxytryptamine as compared with tryptamine. Meyer (1899) and Overton (1901) first used the olive oil/water partition coefficient as a guide to the penetration of substances into the cell. It is now generally accepted that drugs with high fat solubility enter the cell easily, and that those with low fat solubility do not unless a special transport mechanism exists (see Brodie and Hogben, 1957).

It would be expected then, simply on the basis of fat solubility, that tryptamine would enter the cell far more easily than 5-hydroxytryptamine, unless an active transport mechanism exists for 5-hydroxytryptamine. Born (1958) has shown that platelets, which store 5-hydroxytryptamine in cytoplasmic granules, must have an active transport mechanism, but this seems to be a specialized case. All other evidence points to the fact that 5-hydroxytryptamine has low lipid solubility and does not penetrate cells. For instance, when administered parenterally, 5-hydroxytryptamine does not cross the blood-brain barrier unless huge doses are used (Brodie and Hogben, 1957). In contrast, central disturbances similar to those caused by mescaline and lysergic acid diethylamide have been reported after doses of 1 mg./kg. of *N'N'*-dimethyltryptamine and *N'N'*-diethyltryptamine (Szára, 1957), so these substances must cross the blood-brain barrier.

If it is accepted that tryptamine enters cells and 5-hydroxytryptamine does not, the results presented in this paper are explicable. The contraction of the rat fundus is brought about by a surface action of tryptamine-like compounds, an action not dependent upon cell penetration. At the same time, those tryptamine derivatives which can enter the cell and which are inactivated by the intracellular amine oxidase are normally limited in their action because the surface concentration is constantly being reduced by intracellular inactivation. Substances with a polar hydroxyl group attached to the indole ring, such as 4-, 5- and 6-hydroxytryptamine, do not enter the cell and are therefore immune from the action of amine oxidase. Inhibition of amine oxidase will not potentiate their action on the rat fundus. If, however, the fundus is homogenized and the integrity of the cell destroyed, then the amine oxidase can act on 5-hydroxytryptamine. In substances in which the polar nature of the hydroxyl group is removed, such as 5-methoxytryptamine, cell permeability is restored and the action of such compounds on the rat fundus is once more limited by the intracellular enzyme.

The differences in the persistence of the tryptamine analogues after washing out the bath are also interesting. First, only those which can penetrate the cell have a prolonged action after the drug has been removed from the bath. The relaxation rate of the fundus is similar after either 5-hydroxytryptamine or 5-hydroxy-α-methyltrypt-

amine (No. 24). Secondly, of those drugs which penetrate the cell, only those which are unaffected by amine oxidase activity produce a persistent contraction (Figs. 1, 2, and 3). Thirdly, if the amine oxidase within the cell is inhibited, then all the compounds which diffuse inside have a similar persistent action. It would seem, therefore, that in order for a drug to produce a contraction of the rat fundus which persists after removal of the drug from the bath, it must diffuse into the cell and be protected from the action of the intracellular enzyme responsible for inactivation.

This theory of selective membrane permeability as an explanation of the results presented in this paper seems to be the most reasonable. However, the cell membrane itself is not the only possible diffusion barrier, for the cytoplasmic particles may also present a diffusion barrier to polar molecules. Indeed, it would be difficult to explain how a population of cytoplasmic granules can exhibit both amine oxidase activity and a capacity to store 5-hydroxytryptamine if some intracellular diffusion barrier was not present.

The rôle of amine oxidase in the body has been enigmatic for many years. It was first thought to be the mechanism for inactivation of adrenaline and, later, noradrenaline. However, the inability to potentiate the actions of adrenaline and noradrenaline in the whole animal by amine oxidase inhibitors (Corne and Graham, 1957) has made this rôle doubtful. When 5-hydroxytryptamine was identified both as a naturally occurring substance and as a substrate for amine oxidase, it was generally thought that a new and more likely rôle had been discovered. Even so, the actions of 5-hydroxytryptamine in the whole animal are not potentiated by inhibition of the enzyme, although the stores of 5-hydroxytryptamine in bound form increase (Udenfriend, 1958). The significance of amine oxidase as an intracellular enzyme and of the possible natural substrates being excluded from the cell has, in general, been ignored. Presented in this context, the function of amine oxidase would seem to be directed towards preventing unnatural but active compounds from having an action on, and being stored within, the cell. This concept of amine oxidase as a protective enzyme, to reduce the concentration of unwanted but active compounds around the extracellular receptors has been discussed by Blaschko (1952). It becomes much stronger if the amine oxidase is unable to inactivate the naturally occurring substrate, 5-hydroxytryptamine, because of a diffusion barrier. This is certainly true for the isolated rat fundus. The degree and duration of action of both tryptamine (No. 1) and 5-methoxytryptamine (No. 19) are also potentiated in the spinal cat by amine oxidase inhibition, whereas the action of 5-hydroxytryptamine is not. If it is generally true for all tissues, then a relatively low conversion of injected or endogenous 5-hydroxytryptamine into urinary 5-hydroxyindoleacetic acid would be expected. Keglević-Brovet, Supek, Kveder, Iskrić, and Kečkeš (1958) have shown that only about 20% of injected labelled 5-hydroxytryptamine is excreted in the form of 5-hydroxyindoleacetic acid, a figure similar to that arrived at by Erspamer (1956). Further work on this point is needed. In the whole animal, for instance, it would be expected that for any given effect of 5-hydroxytryptamine which is limited by the action of amine oxidase, the effect of 5-hydroxy-α-methyltryptamine (No. 24) or 5-hydroxy-*N'N'*-dipropyltryptamine (No. 29) would be much greater.

Even though amine oxidase may be barred from oxidizing 5-hydroxytryptamine directly, it is interesting to speculate on the other possible inactivation processes. It has recently been shown that one of the first steps in the inactivation of adrenaline and noradrenaline is the methylation of one of the ring hydroxyl groups (Axelrod, Senoh, and Witkop, 1958 ; Axelrod and Tomchick, 1958). It is tempting to postulate that methoxylation enables the previously highly polar amine to penetrate the cell and there be oxidized by the intracellular amine oxidase. This sequence would explain why amine oxidase inhibitors do not potentiate the actions of adrenaline and noradrenaline in the body, for the methoxylated amine has much less activity than the parent compound (Gillespie, Evarts, Fleming, and Sjoerdsma, 1958).

A similar sequence is less likely for 5-hydroxytryptamine, because 5-methoxytryptamine is highly active. If 5-hydroxytryptamine were methoxylated and then inactivated by amine oxidase, inhibition of amine oxidase would lead to an accumulation of 5-methoxytryptamine and an apparent potentiation of the effects of 5-hydroxytryptamine. As this does not occur, it is unlikely that methoxylation plays an important rôle in 5-hydroxytryptamine metabolism. If an intermediate product is formed, it must be both more lipid soluble and substantially less active than 5-hydroxytryptamine.

The ability to modify the tryptamine or 5-hydroxytryptamine molecule in such a way as to preserve its activity but to make it resistant to attack by amine oxidase may have important ramifications. The central activity of tryptamine-like drugs has received much attention in the last

few years, but work has been hampered and hard to interpret because 5-hydroxytryptamine does not cross the blood-brain barrier. The use of an analogue of tryptamine, able to penetrate cells but resistant to amine oxidase activity, might give interesting results. 5-Methoxy-α-methyltryptamine (No. 20), *N'N'*-dipropyltryptamine (No. 12), and α-methyltryptamine (No. 4) are the most potent of these compounds. Similarly, if 5-hydroxytryptamine does have peripheral actions which are limited by amine oxidase activity, the α-methyl or *N'N'*-dipropyl derivatives of 5-hydroxytryptamine would be useful tools.

There are several other points of interest which emerge from a comparison of the activities and structures of these compounds. Methylation of the ring hydroxyl group, for instance, only reduced the potency by a factor of about two (after amine oxidase inhibition). Thus, 5-methoxytryptamine (No. 19) had an equipotent molar ratio of 1.7, compared to 5-hydroxytryptamine=1. 6-Hydroxytryptamine (No. 16) had an equipotent molar ratio of 560 and this was reduced to 950 for 6-methoxytryptamine (No. 17), again a ratio between the hydroxy and methoxy derivatives of 1.7. 5:6-Dimethoxytryptamine (No. 18) had an equipotent molar ratio of 147. Assuming that the hydroxy derivative would have a potency 1.7 times as great, then the equiactive molar ratio of 5:6-dihydroxytryptamine can be predicted to be 87. This does not support the speculation that 5:6-dihydroxytryptamine would be more potent than 5-hydroxytryptamine (Dalgliesh, 1958). Should a highly active compound containing two ring hydroxyl groups be made, 4:5-dihydroxytryptamine would be the more interesting, for 4-hydroxytryptamine (No. 15) had one half the activity of 5-hydroxytryptamine, whereas 6-hydroxytryptamine (No. 16) had only one-sixhundreth of the activity.

The importance of the substituent in the 5-position of the indole ring is shown by the fact that tryptamine has less activity than 5-hydroxy-, 5-methoxy-, 5-chloro-, or 5-methyl-tryptamine (Nos. 14, 19, 21, and 22). That 4-hydroxytryptamine should be of comparable activity to 5-hydroxytryptamine, whereas 6-hydroxytryptamine was so much weaker emphasizes again that the 4- and 5-position of the indole ring may be important points of attachment for tryptamine derivatives to the receptor site. At the other end of the molecule, the importance of the length of the side-chain is illustrated by comparison of the activities of indolemethylamine, tryptamine and homotryptamine (Nos. 2, 1, and 3). As would be expected, the order of potency is tryptamine, homotryptamine and, very much weaker, indolemethylamine. If the terminal amino group is a second point of attachment of the molecule to the receptor site, the rotation of the longer side-chain of homotryptamine will include this second point of attachment, whereas the side-chain of indolemethylamine will be too short.

Further information on the possible points of attachment of tryptamine derivatives to the receptor site can be adduced from a comparison of the activities of *N'N'*-dimethyltryptamine (No. 10) and 2:*N'N'*-trimethyltryptamine (No. 13). The introduction of the methyl group at the 2-position of the indole ring brings about a tenfold reduction in activity. It will also limit free rotation of the side-chain by steric hindrance. If the reduction in activity is assumed to be due to steric hindrance, then it can be deduced that the point of attachment of the terminal amino group is best fitted when the side-chain is rotated to a position fairly close to the indole ring, at a maximum distance away from the ring hydroxyl group. These two probable points of attachment of 5-hydroxytryptamine to the receptor site may not, however, be the only ones. The importance of the ring nitrogen, for instance, can only be assessed when simpler compounds which do not contain it are available.

Finally, by the use of amine oxidase inhibitors, it is now possible to distinguish biologically between 5-hydroxytryptamine and other tryptamine derivatives which may be present in tissue extracts.

This work was made possible by the gifts of many compounds otherwise unobtainable. I would particularly like to thank Dr. R. B. Barlow for compounds Nos. 7, 8, 9, 10, 11, 12, 13, 27, 28, and 29, all of which were used as the hydrochlorides except Nos. 10 (fumarate) and 27 (creatinine sulphate); Dr. G. Curzon for Nos. 19 (hydrochloride), 27 (base), 30 and 31; Dr. J. B. Jepson for Nos. 7, 8, 11, 17, and 18 as hydrochlorides, Nos. 2, 3, and 26 as hydrogen oxalates, Nos. 15 and 16 as creatinine sulphates, No. 6 as acetate; and Dr. A. Spinks of I.C.I. (Pharmaceutical Division) for Nos. 4, 5, 20, 21, 22, 23, 24, and 25 as hydrochlorides; Nos. 24 and 25 were also supplied as dipicrates. The synthesis of these I.C.I. compounds has been described by Young (1958).

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