Br. J. Pharmac. Chemother. (1967), **29**, 105–118.

THE RELATIONSHIP BETWEEN THE PENETRATION OF TRYPTAMINE AND 5-HYDROXYTRYPTAMINE INTO SMOOTH MUSCLE AND THE ASSOCIATED CONTRACTIONS

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(*Received November* 10, 1966)

On the isolated rat stomach strip preparation the contractions induced by tryptamine are potentiated approximately twenty-fold by amine oxidase inhibitors, whereas those produced by 5-hydroxytryptamine (5-HT) are unaffected. It has been suggested (Vane, 1959) that this difference in action is due to the penetration of tryptamine into the cell so that it can be destroyed by amine oxidase, but that the lack of penetration of 5-hydroxytryptamine makes its action independent of amine oxidase inhibitors. Other possible explanations are either that tryptamine and 5-HT act on two different types of receptors (Woolley & Shaw, 1957; Barlow, 1961); or that 5-HT is metabolized by a route which is unaffected by amine oxidase inhibitors (McIsaac & Page, 1959; Weissbach, Lovenberg, Redfield & Udenfriend, 1961).

Previously the entrance of 5-HT into smooth muscle has been studied for periods from 10 min to 3 hr (Born, 1962). In the present work, radioactive tryptamine and 5-HT were used so that the entrance of these substances into smooth muscle could be measured at times (30 sec to 3 min) during which the contractions of the muscle developed. In other experiments the influence of amine oxidase inhibitors and of 5-HT antagonists were determined. A preliminary report of this work has been presented (Handschumacher & Vane, 1963).

METHODS

The rat stomach strip was prepared as described by Vane (1957) and suspended in a 5 ml. organ bath at 37° C. The movements of the muscle against an initial load of 2 *g* were recorded either auxotonically on smoked paper with a pendulum lever (Paton, 1957) magnifying 16 times or isometrically using a transducer and a pen-recorder. For measuring the rate of contraction, diffusion delays in the bathing fluid were minimized by changing the entire contents of the bath within 0.5–2 sec, as follows: the drug was mixed with fresh bathing solution in a 20 ml. syringe attached to the bottom inlet of the bath and submerged in the outer temperature-controlled water bath. When the syringe was discharged its contents displaced the fluid from the organ bath; sufficient fluid was used to ensure more than complete displacement of the contents of the bath. Drugs were added

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every 4 or 5 min and were in contact with the tissue for 60–90 sec; the bath was then washed out and the tissue allowed to relax. When maximal isometric contractions were measured, the tissue was repeatedly washed for 30 min between doses of drugs.

Other isolated tissues were used including the guinea-pig taenia coli, the rat uterus (taken from rats injected with 50 μg stilboestrol 24 hr previously) and the guinea-pig ileum. These preparations were suspended in a bath of appropriate size and the movements were recorded auxotonically with a pendulum lever on smoked paper.

Solutions and drugs. Compositions of solutions, gassing mixtures and the resultant pH values are shown in Table 1. The bathing solutions were gassed both in the bath and in the reservoir. In all experiments where the pH of the solutions was changed, samples of the solution were taken for accurate measurement of the pH. Throughout the text, the nominal values of 6.6, 7.4 and 8.6 will be used. When rat stomach strips were bathed in solutions of different pH the changes were made in random order. In all experiments not involving carbamyl choline, hyoscine hydrobromide (10^{-7} g/ml) was added to the bathing solution to reduce base-line irregularities.

TABLE 1
COMPOSITION OF SOLUTIONS, GASSING MIXTURES AND RESULTANT pH VALUES

Bathing solution	Composition (g/l. H_2O)		Gassed with	pH	
				Measured	Nominal value used in text
Krebs	NaCl	6·9	5% CO_2	7·4	7·4
	KCl	0·35	95% O_2		
	$CaCl_2.6H_2O$	0·55			
	KH_2PO_4	0·16			
	$MgSO_4.7H_2O$	0·29			
	Glucose	1·0	100% O_2	8·5 (but precipitation)	
	$NaHCO_3$	2·1			
Tyrode	NaCl	8·0	5% CO_2	7·0–7·4	7·4
	KCl	0·2	95% O_2		
	$CaCl_2.6H_2O$	0·4			
	$MgCl_2.6H_2O$	0·021			
	NaH_2PO_4	0·05			
	Glucose	1·0	100% O_2	8·6–8·9	8·6
	$NaHCO_3$	1·0			
Modified Tyrode (differences from Tyrode underlined)	NaCl	8·6	5% CO_2	6·4–6·6	6·6
	KCl	0·2	95% O_2		
	$CaCl_2.6H_2O$	0·4			
	$MgCl_2.6H_2O$	0·021			
	NaH_2PO_4	0·05	100% O_2	8·6–8·8	8·6
	Glucose	1·0			
	$NaHCO_3$	0·2			

The following drugs were used: ^{14}C-5-hydroxytryptamine creatinine sulphate (5-hydroxy-3-indolyl-(ethyl-2-amine-1-^{14}C)creatinine sulphate monohydrate; (11.4 mc/mM, Radiochemical Centre, Amersham); tryptamine-2-^{14}C bisuccinate (1.3 mc/mM, New England Nuclear Corporation). Stock solutions were made in isotonic saline and kept frozen and all doses refer to the free base. Phenylisopropyl hydrazine hydrochloride (PIH) (2×10^{-7} g/l.) or bromolysergic acid diethylamide (Brom-LSD) (5×10^{-7} g/l.) were added where indicated to the bathing solutions. Carbamyl choline chloride and α-methyl tryptamine hydrochloride were also used in some experiments.

Penetration experiments. Twelve rat stomach strips were prepared at one time and a glass weight equivalent to a load of 2 *g* when in the solution was tied on the free lower end. The strips were suspended in 400 ml. or more of the appropriate gassed bathing solution at 37° C for a period of 3–4 hr. When used, phenylisopropyl hydrazine or bromolysergic acid diethylamide was added to the bathing solution 1 hr before the tissues were taken for study. For measurement of the entrance

of the radioactive compound a stomach strip was transferred into 20 ml. of the corresponding bathing solution containing the radioactive amine in a test tube. After the appropriate time the tissue was removed, blotted lightly on filter paper and weighed. The time required to remove the tissue and blot excess fluid from it did not exceed 5 sec. The strip was then ground in 4 ml. of 80% ethanol with an all glass Potter-Elvejhem homogenizer. The total time interval between removal of the strip from the radioactive solution and grinding was usually between 45 and 60 sec and never exceeded 90 sec. After centrifugation to remove the insoluble material, the total radioactivity and that associated with metabolite present in the supernatant fluid were assayed. The percentage conversion to metabolites was determined by applying 1 ml. of the supernatant solution to a column containing 2 ml. of Zeocarb 225A resin (60–100 mesh) in the hydrogen form ; the column was washed with 2 ml. of 80% ethanol. This method removed unchanged tryptamine or 5-HT by adsorption to the resin and allowed the passage of any neutral or acidic metabolites into the effluent. A 0.4 ml. sample of this effluent was assayed for radioactivity.

Monoamine oxidase activity at three pHs. Rat stomach strips were ground in 10 vol. of *tris*-hydroxymethylaminomethane (tris)-phosphate buffer (0.01 M, pH 7.5) using a glass homogenizer, and the homogenate was strained through gauze to remove large particles. The incubation mixture consisted of 3.9 ml. of tris-phosphate buffer (0.1 M, pH 6.5, 7.5 or 8.5), 0.1 ml. of tryptamine 2-^{14}C (200 μg/ml.) and 1 ml. of homogenate. The mixture was shaken at 37° in air ; 1 ml. samples were removed after 0, 10, 20 and 30 min and mixed with 3 ml. of absolute ethanol to stop the enzymic action. These samples were warmed to 60°, and centrifuged to remove the coagulated protein. Two millilitres of the supernatant solution was passed through columns of Zeocarb 225A and the amount of metabolites assayed as above.

Oil–water distribution coefficients. Ethyl acetate was equilibrated with tris-phosphate buffers (0.1 M with respect to tris and phosphate) at pH 6.6, 7.4 and 8.6. Five millilitres of this ethyl acetate was then shaken with 5 ml. of the corresponding buffer solution containing ^{14}C-tryptamine or ^{14}C-5-HT (2 μg/ml.). Samples of the ethyl acetate phase and aqueous phase were removed for the determination of radioactivity.

Determination of radioactivity. Samples (0.1 to 0.4 ml.) of all aqueous or alcoholic extracts were mixed in low-potassium glass vials or polyethylene vials with 10 ml. of a toluene-ethanol scintillation fluid of the following composition: absolute ethanol, 330 ml. ; toluene, 660 ml. ; 1,4-tris/2-(5-phenyl-oxazolyl)/benzene, 33 mg ; 2,5-diphenyl oxazole, 2.7 g ; Cab-O-Sil, 30 g. The radioactivity was then assayed in a Liquid Scintillation Beta-Spectrometer (Packard Instrument Co.). Whenever practicable, all samples were counted for sufficient time to give a standard error not in excess of $\pm 5\%$. In all experiments internal standards were included in the determination of radioactivity ; quenching of the fluorescence did not exceed 5% of the total radioactivity.

Chromatography. Six stomach strips were exposed to radioactive tryptamine (2 μg/ml. 1.5×10^4 cpm/ml.) for 3 min in modified Tyrode solution bubbled with oxygen at 37°. They were removed, blotted and ground in 80% ethanol as described above. A second group of six stomach strips were treated in the same way except that the bathing solution contained phenylisopropylhydrazine (2×10^{-7} g/ml.). Five millilitres of extract from each group was passed through Zeocarb 225A columns to remove unchanged tryptamine. A second 5 ml. from each group was passed through a column containing 2 ml. of Dowex 1X4 (100–200 mesh, formate form) ; this adsorbed all the radioactivity. The tryptamine was eluted with 10 ml. of 0.1 N formic acid ; under these conditions the metabolites remained on the column. The Zeocarb effluent and the Dowex eluate were separately evaporated to dryness below 30° and extracted with 2 ml. of 0.1 N formic acid ; 0.2 ml. of these extracts were chromatographed with reference spots of tryptamine and indole acetic acid on Whatman No. 1 paper with isopropyl alcohol: $NH_4OH : H_2O$ (100 : 15 : 10) as a solvent. The areas corresponding to tryptamine and indole acetic acid were located by spraying with Ehrlich's reagent (Smith, 1958). The chromatograms were cut into bands and assayed directly for radioactivity in 20 ml. of scintillation fluid.

Histology. Samples of stomach strips were taken at the beginning and the end of the incubation periods at both pH 6.6 and 8.6. Histological preparations were kindly made for us by Dr. John Walters of the Pathology Department of the Royal College of Surgeons of England.

Equiactive molar ratios. Throughout this paper, the term " ratio " is used to mean the ratio between the molar doses of tryptamine and 5-hydroxytryptamine required to produce equal contractions of the tissue. This was usually determined at a high and a low dose level. Since tryptamine was always less active than 5-HT, a ratio of 100 means that 100 molecules of tryptamine were needed to produce the same response as that obtained with one molecule of 5-HT.

RESULTS

The relative activities of tryptamine and 5-HT on the rat stomach strip bathed in Tyrode solution varied from 400 (Vane, 1959) to 933 (Barlow & Khan, 1959). In the present experiments Krebs solution bubbled with 95% O_2, 5% CO_2 (pH 7.4) was used first as a bathing medium and the average ratio was 88 (six experiments). This lower ratio may have been due either to the different ionic compositions of the bathing fluids or to the difference in pH. To distinguish between these possibilities, the ratio between the equipotent activities of tryptamine and 5-HT was determined in Krebs solution bubbled with 95% O_2 and 5% CO_2 (pH 7.4) and was found to be 60. When the same solution was bubbled with 100 O_2 (pH 8.6), the ratio increased to 400. Since the higher pH caused precipitation of insoluble salts from the Krebs solution, the same strip was then bathed in Tyrode solution when the ratios changed to 100 at pH 7.4 and 600 at pH 8.6. Thus the change in ratio was associated with change of pH and not with the change of ionic composition of the two fluids. Figure 1 shows a typical experiment. First at pH 7.4, the ratio between tryptamine and 5-HT was 120. A few minutes after introducing Tyrode at pH 8.6, the sensitivities changed and the new ratio after the preparation had stabilized was 600. Subsequently, with the same strip bathed in modified Tyrode solution at pH 6.6, the ratio was 50. In some experiments, such as this one, 5-HT became more effective at the raised pH but in all experiments tryptamine was less active at the higher pH. This reduction in the activity of tryptamine was usually by four- to ten-fold and was the main effect seen when the pH was raised from 7.4 to 8.6. At the lowered pH of 6.6 with modified Tyrode the sensitivity of the tissue to both tryptamine

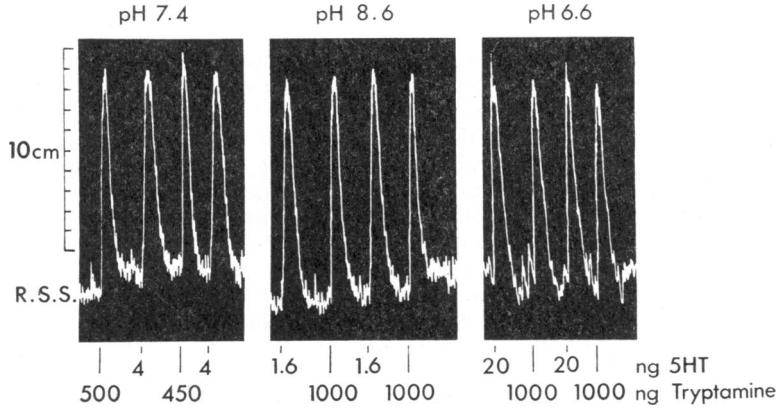

Fig. 1. Rat stomach strip bathed in modified Tyrode solutions. At pH 7.4 (first panel), 4ng 5-HT induced a response equivalent to that of 480–500ng tryptamine, giving a ratio of 120. At pH 8.6 (second panel) the ratio was 600 whereas at pH 6.6 it was 50. Time in min; vertical scale: 10 cm.

and 5-HT was decreased. This decrease in sensitivity (Fig. 1) may have been caused by a decreased ability of the tissue to contract, since contractions produced by carbamyl choline were affected in a similar manner. The results of 11 experiments in which the pH was changed are shown in Table 2.

TABLE 2

EQUIACTIVE MOLAR RATIOS FOR TRYPTAMINE AND 5-HT ON THE RAT STOMACH STRIP AT DIFFERENT pH VALUES

Experiments 1–6 were with auxotonic and 7–11 with isometric contractions

	Ratio		
Experiment	pH 6·6	pH 7·4	pH 8·6
1		55	330
2*	50	120	600
3	100		800
4	80		400
5	75	200	1,000
6	60		500
7	67	154	670
8		100	800
9		94	940
10		100	1,000
11	67	166	332
Mean	71	123	670

* Experiment illustrated in Fig. 1.

Since changing the pH from 8.6 to 7.4 gave a potentiation of the effects of tryptamine qualitatively similar to inhibition of its metabolism at pH 8.6 by phenylisopropyl hydrazine (Vane, 1959), the effects of phenylisopropyl hydrazine were determined at the different pHs. The equipotent molar ratios of tryptamine and 5-HT were found to be 160 at pH 7.4 and 400 at pH 8.6. After inhibition of amine oxidase by phenylisopropyl hydrazine the ratio at pH 8.6 was 30 and at pH 7.4 was 25. Thus the large change in the ratio with pH was almost eliminated after inhibition of amine oxidase. With the tryptamine analogue, α-methyl tryptamine, a type of compound which is not a substrate of amine oxidase (Blaschko, 1952), it was possible to confirm the role of metabolism in these changes of ratio with pH. Thus at pH 8.6 the tryptamine/5-HT ratio was 330 whereas the α-methyl tryptamine/5-HT ratio was 15. The Tyrode was then changed to pH 7.4; the tryptamine/5-HT ratio was 55 and the α-methyl tryptamine/5-HT ratio was 25. These effects were reversible and, on returning to pH 8.6, the tryptamine/5-HT ratio again rose to 350 but that of α-methyl tryptamine/5-HT was again 15.

The effects of antagonists on the actions of tryptamine and 5-HT were also studied at the different pHs, since Barlow & Khan (1959) had shown that Brom-LSD antagonized 5-HT more than tryptamine on rat stomach strips. The change in ratio with pH was eliminated when Brom-LSD was present in concentrations of 10^{-8} g/l. Although the effects of 5-HT and tryptamine were greatly antagonized their activity ratio was reduced to about 30 and became independent of pH. A possible explanation for this result was found in the tissue concentrations of the amines (see below).

The equiactive molar ratios between tryptamine and 5-HT were also determined on the rat uterus, guinea-pig ileum, and the guinea-pig taenia coli in Tyrode solution at

different pHs (Table 3). The results were qualitatively similar to those obtained with the rat stomach strip. Since the changes in ratio produced by changes in pH or by inhibition of monoamine oxidase activity were greatest in the rat stomach strip, this tissue was used to determine the penetration of 5-HT and tryptamine. To simplify the experimental procedures, the distribution of tryptamine and 5-HT was measured only at pH 6.6 and 8.6. All strips were preconditioned by long incubation in the appropriate bathing solutions to simulate conditions in the isolated organ bath.

TABLE 3

EFFECTS OF pH ON EQUIACTIVE MOLAR RATIO BETWEEN TRYPTAMINE AND 5-HT ON DIFFERENT ISOLATED ORGANS

Organ	pH 6·6	pH 7·4	pH 8·6
Guinea-pig ileum	400		1,500
Guinea-pig taenia coli	50		150
Rat uterus		20	50
		30	150

Penetration of amines into tissues

The recovery of radioactivity was established as follows: (a) a stomach strip was ground as described and ^{14}C-tryptamine (8 μg, 50,000 counts/min) was added. After centrifugation, assay of the supernatant fluid gave recovery of 102%. (b) When the supernatant was passed through a Zeocarb column 98% of the radioactivity was removed from the solution. In similar experiments 99% of added 5-HT (8 μg) was removed by passage through the column. (c) The major delay between removal of the stomach strip from the bathing solution and inactivation of the enzymes by grinding in ethanol was the process of weighing the sample. When this was eliminated the same metabolism of tryptamine (50%) was observed. (d) A sample of bath fluid was assayed for radioactivity before and after exposing it to the stomach strips for 3 min; no metabolic products of tryptamine or 5-HT were detected in the bath fluid.

The identity of the metabolite formed within the tissue when tryptamine was exposed to stomach strips was established as follows. Particle-free supernatants prepared as described above from six stomach strips exposed to tryptamine (2 μg/ml.) for 3 min were combined. A 5 ml. sample was passed through a column of Dowex 1 X 4. This absorbed all of the radioactivity, which was then eluted with 0.1 N formic acid to remove tryptamine. This solution was evaporated to dryness and chromatographed on paper. The metabolites of tryptamine were prepared for paper chromatography by passing another 5 ml. of the particle-free supernatant through a Zeocarb 225A column and washing with 80% ethanol. The eluate was evaporated, redissolved and chromatographed. The only radioactive peak found in the extraction for tryptamine corresponded to a marker spot of tryptamine on the paper; similarly, the radioactivity in the metabolite was associated entirely with the spot corresponding to indole acetic acid. In the presence of phenylisopropyl hydrazine (2×10^{-7}) insufficient metabolite was present to be characterized and 93% of the radioactivity in the extract was associated with tryptamine.

The entry of 5-HT and tryptamine into the stomach strips at both pH values is expressed on the ordinates of the histograms (Figs. 2 and 3) as a percentage equilibrium with the total tissue water. Thus, 100% on the graph would indicate a concentration of

amine or its metabolites in the whole tissue equivalent to the concentration present in the bathing solution. Similarly, a figure of 20–30% might be considered to be equivalent to filling of the extracellular space since inulin penetration fell within this range. Each value represented in the figure is derived from at least two experiments in each of which determinations were made on duplicate stomach strips.

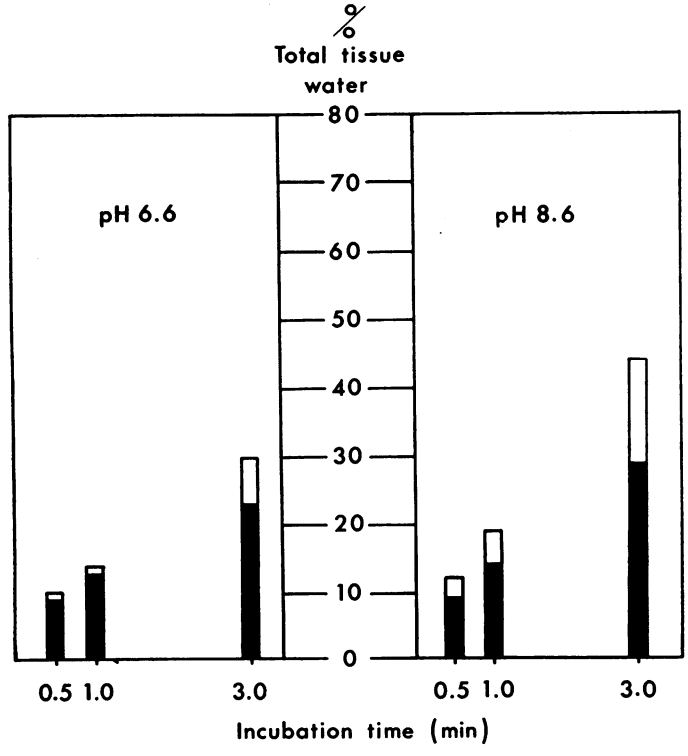

Fig. 2. The entry of 5-hydroxytryptamine into the rat stomach strip. The black columns represent the percentage filling of total tissue water with 5-HT, where 100% represents equilibrium with that in the bathing fluid (2 μg/ml). The open columns represent amounts of metabolized amine. At pH 6.6 the entry is slightly less than at pH 8.6 and so is the metabolism. White column = metabolized amine ; black column = unchanged amine.

Several points emerge from these results. For 5-HT (Fig. 2): (1) In all the experiments, the amount of unchanged 5-HT accounted for the greater part of the radioactivity in the stomach strip. (2) The concentration of *unchanged* 5-HT in the strip never exceeded that which would have been expected if this compound had entered only the extracellular space ; this confirms the results of Born (1962). (3) Higher pH caused only slightly greater levels of total radioactivity in the tissue, an increase mainly attributable to metabolites. (4) At 3 min there was almost twice as much 5-HT in the tissue as at 1 min.

The results obtained with tryptamine (Fig. 3) contrast strikingly with those obtained with 5-HT. (1) At both the low and high pH, the rate of entry of tryptamine was greater than that of 5-HT, but after 3 min, the amount of tryptamine in tissue was only slightly

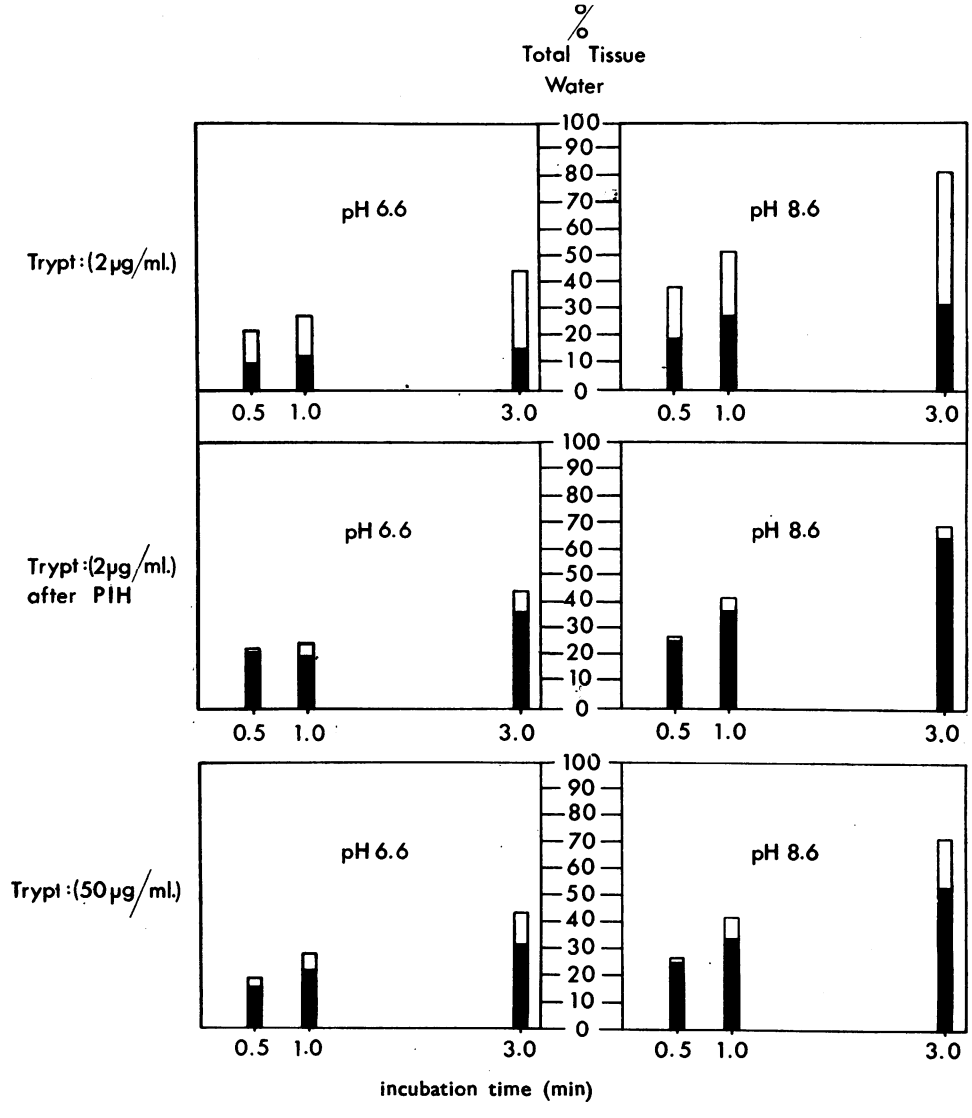

Fig. 3. The entry of tryptamine into the rat stomach strip. Details as in Fig. 2. The top two histograms show that much of the tryptamine which enters the tissue is metabolized. More enters at pH 8.7 than at pH 6.6. The middle two histograms show that in the presence of an amine oxidase inhibitor (P.I.H.=phenyl isopropyl hydrazine, 2×10^{-7}) the breakdown of tryptamine is virtually abolished. The lower two histograms show that breakdown is also proportionally inhibited when a high concentration (50 μg/ml.) of tryptamine is used. White column = metabolized amine ; black column = unchanged amine.

more than at 1 min. (2) The amount of tryptamine represented a considerably smaller portion of the total tissue radioactivity than with 5-HT. (3) The total radioactivity in the tissue was much more than that required to fill the extracellular space. (4) Although the rate of entry of total radioactivity was very rapid at the higher pH, the amount of unchanged tryptamine in the tissue was approximately equivalent to that expected after the extracellular space was filled. This suggested that the rate of entry into the cell was slower than the rate of metabolism. (5) The metabolism of tryptamine was markedly reduced in the presence of phenylisopropyl hydrazine (2×10^{-7}). Considerable amounts of intact tryptamine must have been inside the cells under these conditions. (6) Although changes in concentration of tryptamine did not materially affect the rate of entry into the tissue, when the concentration was very high (50 μg/ml.) the degree of metabolism was considerably reduced.

To determine whether the amount of radioactivity in the rat stomach strip increased greatly after 3 min the entry of 5-HT and tryptamine was measured also after 10 min incubation. At pH 6.6 the total radioactivity (including that due to metabolites) represented penetration of 57% of the tissue water for 5-HT and 62% for tryptamine. At pH 8.6 the figures were 76% for 5-HT and 73% for tryptamine. Thus, the values for

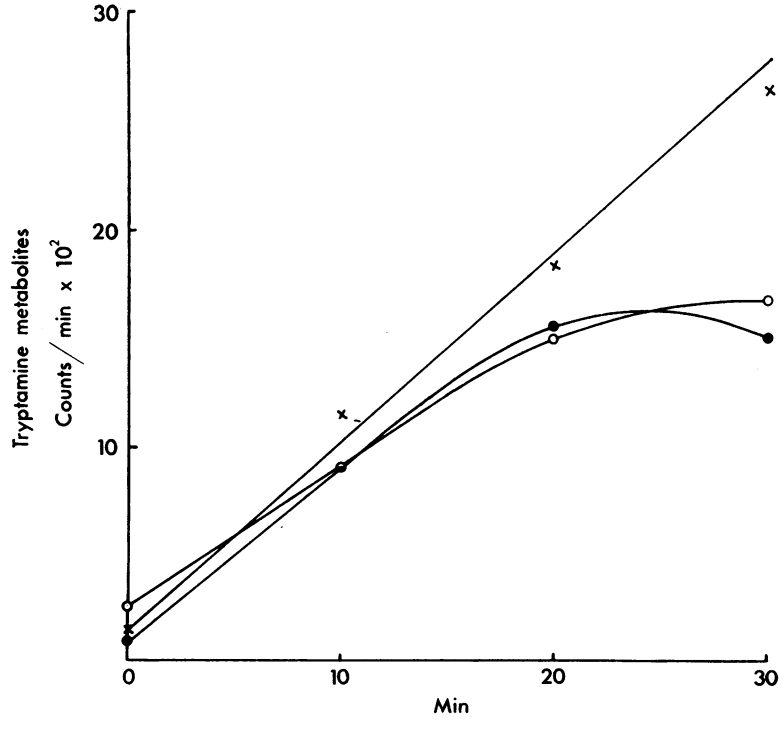

Fig. 4. Effect of pH on breakdown of tryptamine. The initial rate of oxidation of tryptamine by enzyme prepared from rat stomach strips was virtually unaffected by pH: × = pH 8.5, ○ = pH 7.5, ● = pH 6.5. For details see Methods.

tryptamine (Fig. 3) at 3 min are almost equilibrium figures and those for 5-HT at 3 min (Fig. 2) are approaching equilibrium figures.

When the tissues were preincubated for 1 hr with Brom-LSD (0.5 μg/ml.), a concentration sufficient to give at least 100-fold antagonism of the response to 5-HT, there was no effect on the rates of penetration of 5-HT or tryptamine into the tissue, at concentrations of either 2 μg or 50 μg/ml. of these amines.

Effects of pH on amine oxidase activity and lipid solubility of amines

To determine whether the changes in the tryptamine response induced by changes in pH were associated with a change in the activity of monoamine-oxidase within the tissue, the experiment presented in Fig. 4 was performed. No differences were apparent in the initial reaction rates. However, after 30 min the activity at pH 6.5 and 7.5 had decreased, possibly because of inactivation of the enzyme.

Since the entrance of tryptamine into the rat stomach strip was highly dependent upon pH, the distribution coefficients of both tryptamine and 5-HT were determined between ethyl acetate as a lipid phase and a tris-phosphate buffer at pH 6.6, 7.4 and 8.6. The oil or lipid solubility of 5-HT was very much less than that for tryptamine at all pH values (Table 4).

TABLE 4

ETHYL ACETATE/WATER DISTRIBUTION COEFFICIENTS FOR 5-HT AND TRYPTAMINE AT DIFFERENT pHs

	pH 6·6	pH 7·4	pH 8·4
5-HT	0·008	0·014	0·029
Tryptamine	0·034	0·066	0·198

Determination of the relative contraction rates at different pH values

Using a fast paper speed, the rate of the contraction of the rat stomach strip was recorded with submaximal concentrations of 5-HT and tryptamine at both pH 7.4 and 8.6 (Table 5). At both pH values, the initiation of the contraction occurred much more rapidly with tryptamine than with 5-HT. At the lower pH value, however, the contractions induced by both of these amines were slower in onset than at the higher pH level.

TABLE 5

COMPARISON OF RATES OF ISOMETRIC CONTRACTION OF THE RAT STOMACH STRIP WITH 5-HT AND TRYPTAMINE

The times (in sec) are given to develop 10, 50 and 90% of the full contraction. The values in the table are averages obtained from three experiments

	pH 7·4			pH 8·6		
	Time to develop (sec)			Time to develop (sec)		
	10%	50%	90%	10%	50%	90%
5-HT	32	48	60	18	40	50
Tryptamine	18	22	37	10	22	33

Pathology

Portions of the rat stomach strips were taken immediately after suspension in the bathing fluid and after 4 hr in the solution at either pH 6.6 or 8.6. Histological examination by routine paraffin techniques and staining with haematoxylin and eosin showed no difference in the appearance of the tissues at either pH despite the long periods of bathing.

DISCUSSION

It is generally assumed that for sub-maximal contractions of smooth muscle, receptor activation is proportional to the tension developed. To interpret our results it is also necessary to assume that tryptamine and 5-HT diffuse uniformly and with equal velocity through the extracellular water of the tissues and that neither is concentrated specifically by the cells during the time of development of contraction. Born (1962) showed that the isolated taenia coli of the guinea-pig could concentrate 5-HT from the bathing medium, but that this was an extremely slow process, unlikely to have an influence during the 40–70 sec that it takes for full contractions to develop. Furthermore our results show that, for both 5-HT and tryptamine, the amount penetrating the tissue is not much more after 10 min than after 3 min. Indeed, the fact that the full contraction develops in 1 min or less suggests that diffusion of both substances throughout the extracellular fluid is very rapid.

Figure 5 shows a model consistent with our experimental results. 5-HT, which is relatively insoluble in lipids, penetrates into the cells little or not at all during the

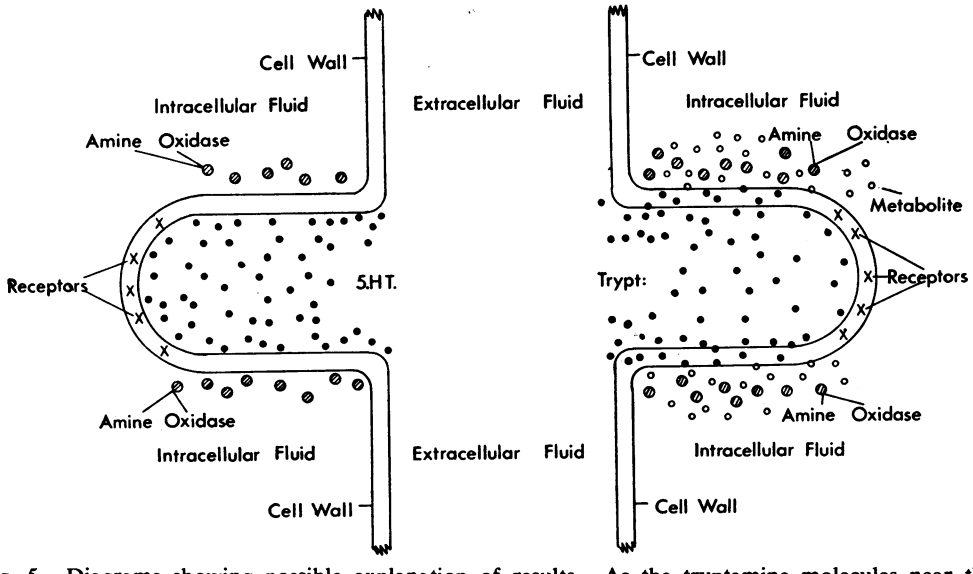

Fig. 5. Diagrams showing possible explanation of results. As the tryptamine molecules near the receptors, the concentration decreases because of diffusion into the cell. Prevention of this diffusion by lower pH, or inhibition of amine oxidase would increase the concentration of tryptamine at the receptor. Since 5-HT is excluded from the cell, these functions do not influence its action.

development of contraction. Because cell penetration is so small, several-fold changes in it will not have a great influence on the concentration of 5-HT at receptors on the cell membrane. Since the 5-HT concentration at the receptors is independent of cell penetration, it will also be independent of the activity of amine oxidase inside the cell and relatively independent of changes in pH. The lack of potentiation of 5-HT contractions by amine oxidase inhibitors (Vane, 1959) is also explained on this basis.

The slight increase in the total radioactivity entering the tissue at the higher pH, best seen after 3 min, might account for the slightly faster contraction but is mainly associated with an increase in the formation of 5-hydroxy indole acetic acid. Since the extracellular space of smooth muscle is approximately 30% of the tissue volume (Born, 1962; Goodford & Leach, 1966) all intracellular radioactivity is probably associated with metabolites of 5-HT. The decrease in contractions induced by 5-HT at lower pH values is probably a reflection of the decreased contractility of the muscle fibres *per se*, since a similar diminution of contraction was seen with the quaternary compound carbamyl choline.

Tryptamine, which is relatively soluble in lipid, penetrates considerably into the cells even while the contraction is developing, as shown by the high proportion of metabolite found at 0.5 and 1 min. At the higher pH, which increases the unionized species, penetration is faster still, which is reflected by the greater amount of radioactivity in the tissue. Most, if not all, of the intracellular radioactivity is in the form of indole acetic acid. This is consistent with the intracellular location of amine oxidase and suggests very rapid elimination of tryptamine in the intracellular fluid. This rapid removal from the area immediately adjacent to the receptor effectively limits the concentration of tryptamine at the receptor. Since a reduction in the pH results in reduced penetration into the cells and amine oxidase inhibitors prevent destruction within the cells, both of these conditions can be regarded as potentiating the actions of tryptamine by increasing its concentration at the receptors.

When amine oxidase activity is inhibited the cell presumably comes to contain intact tryptamine which can equilibrate with that in the bathing fluid. Since this equilibrium of intracellular and extracellular tryptamine concentration is associated with a sustained isometric contraction these results do not lend support to receptor theories based on a net flux of agonists across the membrane (Mackay, 1963).

Two anomalies that arose out of earlier work can now be explained. First, the differences in relative activities of tryptamine and 5-HT on the rat stomach strip of 400 (Vane, 1959) and 933 (Barlow & Khan, 1959) may have been caused by differences in pH of the bathing fluids, resulting in differences in the penetration of tryptamine. Secondly, there is a change in ratio from the high figure of several hundred to one to 30–40 : 1 in the presence of bromolysergic acid diethylamide and other tryptamine antagonists. This change in ratio is very similar to that produced by amine oxidase inhibitors, but Barlow (1961) showed that tryptamine antagonists had little or no activity as amine oxidase inhibitors in concentrations necessary to bring about the change in ratio. Another explanation might be that the antagonists interfere with the penetration of tryptamine into the cell, but the present results show that it is not so. However, a possible explanation arises from the observation that, with high concentrations of tryptamine, the percentage of the tryptamine within the tissue metabolized by the rat stomach strip is

much reduced. Under these conditions, the enzymatic potential for metabolism is probably saturated and a proportionally smaller amount of tryptamine is destroyed. In the presence of brom-LSD high concentrations of tryptamine have to be used to obtain a contraction, so the metabolism of tryptamine will be depressed. Since this is exactly similar to the situation with an amine oxidase inhibitor the ratio of 5-HT to tryptamine activity will change. Thus in the presence of brom-LSD two opposing factors are in play: the actions of tryptamine are reduced by antagonism at the receptor level, but they are also enhanced by the decrease in metabolism within the cell. With 5-HT, which is not metabolized inside the cell, the antagonism by brom-LSD will be greater.

SUMMARY

1. The time course of penetration and metabolism of tryptamine and 5-hydroxytryptamine in the rat isolated stomach strip have been measured under various conditions and related to the development of contractions.

2. 5-HT is relatively insoluble in lipid and during the time of development of contraction little 5-HT enters the cell.

3. Tryptamine is much more soluble in lipid and enters the cell during the development of contraction. That which enters is rapidly metabolized.

4. The breakdown of tryptamine inside the cell is abolished by an amine oxidase inhibitor.

5. Changes in pH of the bathing fluid substantially change the relative activities of tryptamine and 5-HT. This is probably due to changes in lipid solubility, leading to changes in cell penetration.

6. Brom-lysergic acid diethylamide does not influence entry of 5-HT and tryptamine into the extra- or intracellular water.

7. A general hypothesis is proposed to account for the results.

We are very grateful to Mr. Peter Gorinsky and Miss K. Giebler for technical assistance and to the U.S. Public Health Service for a grant (NB 04139).

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