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Relative activities on and uptake by human blood platelets of 5-hydroxytryptamine and several analogues

G. V. R. BORN, KANCHANA JUENGJAROEN AND F. MICHAL

M.R.C. Thrombosis Research Group, Department of Pharmacology, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN

Summary

1. The specificity of platelet receptor sites for 5-HT uptake and for the rapid morphological change and aggregation was investigated with 5-hydroxytryptamine (5-HT) and seventeen analogues as well as with some antagonists of 5-HT.

2. The analogues, with the exception of 5-hydroxy-N'N'-dibutyltryptamine, caused the rapid morphological change in platelets. In concentrations below those needed to produce the agonistic action (viz. 0·05–2·0 μM), these analogues themselves inhibited competitively the shape change caused by 5-HT.

3. The velocity of change in shape caused by 5-HT was reduced in low Na media.

4. Ten analogues produced platelet aggregation; three of these, viz. 5-methoxy-α-methyltryptamine, 5-hydroxy-α-methyltryptamine and 5-hydroxy-N'N'-diisopropyltryptamine), were approximately equipotent with 5-HT. Six analogues did not induce platelet aggregation.

5. All the analogues which prevented the initial change in shape of platelets caused by 5-HT also inhibited its aggregating effect, apparently competitively with low K_i values (0·02–1·6 μM).

6. As with the inhibition of shape change, the inhibition of aggregation shows relatively low structural specificity of the receptor site.

7. Methysergide was a potent inhibitor of shape change and aggregation ($K_i \sim 0{\cdot}03$ μM); imipramine was much less inhibitory ($K_i \sim 5$–10μM).

8. Only one analogue (5-hydroxy-α-methyltryptamine) was taken up like 5-HT by platelets. All the other analogues inhibited the uptake of 5-HT by platelets ($K_i = 0{\cdot}2$–$2{\cdot}7$ μM).

9. Methysergide was a weak inhibitor of 5-HT uptake ($K_i \sim 125$ μM) whereas imipramine was very effective ($K_i \sim 0{\cdot}3$ μM).

10. Our results show that the initial change in shape of platelets is required for and precedes aggregation. The structural specificity of the platelet receptor concerned with shape change and aggregation caused by 5-HT appears low whereas the uptake mechanism is a highly specific one. The uptake probably proceeds through more than one step, the relationship between the steps is not yet clear.

Introduction

Since the discovery (Rand & Reid, 1951) that the blood platelets of many mammalian species including man contain 5-hydroxytryptamine (5-HT) in comparatively high concentrations, much work has been done to find out how the amine comes to be there and what its biological function is (for reviews see Erspamer, 1966; Pletscher, 1968). The evidence indicates that platelets have a mechanism for the active transport of 5-HT through the outer membrane (Born & Gillson, 1959) and another mechanism for accumulating 5-HT (Hardisty & Stacey, 1955) in cytoplasmic organelles in association with ATP (Born, 1956a, 1958; Born, Ingram & Stacey, 1958; Baker, Blaschko & Born, 1959; Tranzer, Da Prada & Pletscher, 1966; Da Prada & Pletscher, 1968; Pletscher, Da Prada & Tranzer, 1969). 5-HT can also move slowly across the platelet membrane by passive diffusion, either in the cold (Born & Bricknell, 1959) or after its release from the organelles by reserpine or by phenylalkylamines (Hughes & Brodie, 1959).

When platelets are present in clotting plasma they lose some of their ATP and most of their 5-HT at the same time (Born, 1956b, 1958), apparently in a specific release reaction which can be induced by thrombin (Grette, 1962) as well as by some other agents which cause platelets to aggregate (for review see Mustard & Packham, 1970).

5-HT can aggregate the platelets of several species including man (Mitchell & Sharp, 1964; Mills, 1970) but not of others, for example guinea-pig and horse (Sinakos & Caen, 1967). This aggregation is unusual in that it increases with 5-HT concentration only up to a certain value (in man *ca.* 10 μM) and decreases with higher concentrations. Recent *in vitro* and *in vivo* experiments had suggested that 5-HT initiates aggregation by reacting with the same type of receptor which mediates its active transport through the platelet membrane (Baumgartner & Born, 1968, 1969; Baumgartner, 1969). This work suggested the possibility that 5-HT has a regulatory function in the ability of platelets to aggregate on which their haemostatic effectiveness depends (Michal & Penglis, 1969).

The specificity and some chemical properties of receptors can be established by their reactions with analogues of the physiological agonists and with specific antagonists. Such observations have shown that the 5-HT receptor of nerve cells differs from that of smooth muscle cells (Gaddum & Picarelli, 1957). The 5-HT receptor on smooth muscle has been further characterized by comparing the contractor activities of analogues of 5-HT and tryptamine with their ability to penetrate through the cell membrane (Vane, 1959; Born, 1962a; Handschumacher & Vane, 1967; Born, 1970b). Little is known about the nature of the 5-HT receptor on platelets. The receptor which mediates the aggregation of sheep platelets by 5-HT is blocked by lysergic acid diethylamide but not by morphine, atropine or cocaine (Michal, 1969) so that, to this extent, the receptor resembles that of smooth muscle cells and not that of nerve cells. This paper reports the effects of a considerable number of analogues of 5-HT and tryptamine as agonists, partial agonists and antagonists on the rapid change in shape (Macmillan & Oliver, 1965; Born, 1970a) and aggregation of platelets and on their uptake of 5-HT. The results indicate the degree of specificity of the actions of 5-HT on platelets and of the uptake mechanism for it. For comparison the contractor potency of the analogues on a sensitive smooth muscle preparation was determined.

Methods

Preparation of platelet-rich plasma

Blood was obtained from healthy volunteers of either sex. A stainless steel needle was inserted into the antecubital vein and the freely flowing blood was collected in polycarbonate centrifuge tubes containing 0·1 vol of a 3·8% solution of trisodium citrate. The blood was gently mixed with the citrate by inverting the tubes 3 times against polycarbonate lids and centrifuged at room temperature for 15 min at 250 g to sediment the red cells. The supernatant platelet-rich plasma was transferred to a siliconized glass bottle and kept at room temperature for 4–5 h during which samples were used for experiments. The concentration of platelets was determined by counting under a phase-contrast microscope. An experiment showed that, under these conditions, the rate of uptake of 5-HT by platelets in platelet-rich plasma 1 h and 6 h after bleeding was nearly the same.

Platelet change in shape

The method used for obtaining photometric records of the rapid change in shape without aggregation of platelets was as recently described (Born, 1970a).

Platelet aggregation

The aggregation of platelets in plasma was measured with a photometric method (Born, 1962b). A 1 ml sample was warmed at 37° C for 5 min in a siliconized glass tube, which was then placed in the cell compartment of the aggregometer also maintained at 37° C (Mills & Roberts, 1967). Light transmittance through the tube was recorded continuously on a 10 mV recorder (W. & W. Electronics, Basel) at a chart speed of 3 cm/minute. Reagents to produce concentrations up to 100 μM were added with microsyringes (Samplejector, Guild Corporation) in a volume up to 10 μl. The rate of platelet aggregation was measured as the tangent to the steepest slope in the light transmittance records during aggregation and expressed as aggregation velocity in cm/min (Baumgartner & Born, 1968).

Simultaneous recording of platelet aggregation and platelet change in shape

The initial rapid change in shape of platelets exposed to 5-HT or analogues was also measured without inhibiting their subsequent aggregation by recording the changes in light scattered by the suspension of platelets (Michal & Born, 1971). Platelet-rich plasma was diluted with 0·154 M NaCl (0·5 ml PRP and 0·5 ml saline) in a siliconized glass cuvette and the changes caused by the addition of 5-HT were measured simultaneously by recording the changes in light transmitted through the suspension (aggregation) and light scattered at right angles to the incident beam (shape change). Because EDTA was absent from the sample, the initial rapid decrease in scattered light was followed by an increase in transmitted light; these changes gave a quantitative measure of the rapid shape change and aggregation in the same sample of platelet suspension.

Inhibition of 5-HT uptake by its analogues

Uptake of 5-HT was estimated from radioactivity measurements. 5-HT creatinine sulphate was used in which the β-carbon atom of the side chain was labelled with ^{14}C. Platelet-rich plasma was incubated at 37° C in the small polypropylene centri-

fuge tubes of the Eppendorf Microfuge centrifuge with radioactive 5-HT without or with the analogues at different concentrations. The final volume in each tube was 1 ml. After incubation for 3 min the tubes were centrifuged in the Eppendorf centrifuge at 12,000 *g* for 30 seconds. The supernatant platelet-free plasma was decanted and the inside of the tube above the platelet pellet was dried with filter paper. The pellet was resuspended in 0·2 ml water and frozen and thawed twice to lyse the platelets and to release their 5-HT. The lysate was soaked up on glass paper which was transferred into a counting vial; the centrifuge tube was mopped out with another paper which was added to the first in the vial. Each vial received 15 ml diotol scintillation fluid and radioactivities were estimated in a Tricarb scintillation spectrophotometer (Packard Model 3375).

The sedimented platelets were not washed after centrifugation so that the extracellular 5-HT was included in the radioactivity measurements. The volume of plasma trapped in these platelet pellets was about 1 μl/10^8 platelets. The amounts of 5-HT or its analogues present in this volume of plasma were subtracted when their uptake by the platelets was calculated.

Estimation of uptake of 5-HT analogues by fluorimetry

Uptake of some analogues by platelets was estimated by measuring the increase in the fluorescence of extracts of platelets which had been incubated with the analogues under the same conditions used for estimating 5-HT uptake. Platelet-rich plasma was incubated at 37° C and the analogue was added usually at the concentration that caused maximal aggregation of platelets, that is 10 μM. After incubation the plasma was centrifuged and the supernatant decanted. The sedimented platelets were lysed in 1 ml of distilled water and a protein-free extract was prepared for fluorimetry (Crosti & Lucchelli, 1962) but without the addition of concentrated HCl. All the substances emitted maximal fluorescence at a wave length of 350 nm when activated at 275 nm in weakly acid or neutral solution; in strong acid the fluorescence was weaker (see also Bowman, Caulfield & Udenfriend, 1955; Udenfriend, Weissbach & Clark, 1955). Fluorescence was measured in an Aminco Bowman Spectrophotofluorometer.

Activities of 5-HT analogues on smooth muscle

The rat fundus strip preparation (Vane, 1957) was mounted in a 10 ml organ bath bathed in Krebs solution at 37° C; movements of the muscle were recorded with an auxotonic lever (Paton, 1957). Substances were added to the bath in not more than 0·5 ml saline and the contraction of the strip was allowed to develop for 60 or 90 seconds.

The activities of the analogues were compared with that of 5-HT in terms of percentage of 5-HT activity (5-HT=100). A potency of 100% means that one molecule of the test compound had the same contractor activity as one molecule of 5-HT. The activities of tryptamine and other lipid-soluble analogues on smooth muscle are increased in the presence of an amine oxidase inhibitor (Vane, 1959). Therefore, the activities of some of the analogues on platelets and on smooth muscle were determined in the absence and presence of the amine oxidase inhibitor phenylisopropyl hydrazine (PIH).

Materials

5-Hydroxytryptamine (3′-^{14}C) creatinine sulphate was purchased from the Radiochemical Centre, Amersham (specific activity 56 Ci/mol), 5-hydroxytryptamine creatinine sulphate from May & Baker. Analogues of 5-hydroxytryptamine or tryptamine, most of them as the hydrochloride unless otherwise stated were obtained as follows: N′N′-diethyltryptamine, N′-ethyltryptamine, 5-hydroxy-N′N′-dipropyltryptamine, 5-hydroxy-N′N′- diisopropyltryptamine and 5-hydroxy-N′N′-diisobutyltryptamine were kindly given by Drs. R. B. Barlow and Khan ; α-methyltryptamine by Dr. A. Spinks ; 5-hydroxy-N′N′-dimethyltryptamine as oxalate salt by K. & K. Laboratories ; 5-methoxy-α-methyltryptamine, 5-hydroxy-α-methyltryptamine as dipicrate salt, 5-chloro-α-methyltryptamine, 7-chloro-α-methyltryptamine and N′N′-dimethyltryptamine from I.C.I. 4-Hydroxytryptamine and 6-hydroxytryptamine both as creatinine sulphate were given by Professor V. Erspamer. The purity of these analogues was confirmed by thin layer chromatography. 2-Bromo-lysergic acid diethylamide (BOL 148) and methysergide bimaleate were gifts from Sandoz Limited. Phenylisopropyl hydrazine hydrochloride (PIH) from Parke-Davis Ltd. Imipramine hydrochloride from Geigy. Tryptamine from B.D.H. Adenosine diphosphate, adenosine triphosphate and adenosine from Sigma London, Ltd.

Results

Change in shape : 5-hydroxytryptamine

5-Hydroxytryptamine added to platelet-rich plasma causes a rapid change in the morphology of the platelets which could be demonstrated optically like that produced by ADP. A typical record is shown in Fig. 1: it was produced by adding 5-HT (1×10^{-5}M) to a mixture of 0·1 ml citrated platelet-rich plasma and 0·9 ml tris-buffered saline which contained EDTA (final concentration 4×10^{-3}M) to prevent aggregation ; the final concentration of platelets was $9{\cdot}8 \times 10^{7}$/ml. The optical record consisted of a fast phase during which light transmission decreased rapidly for about 5 seconds. This fast phase was taken to indicate the velocity of the reaction underlying the shape change and was measured by the time slope of the trace. The fast phase merged into a slow phase during which light transmission decreased much less rapidly for at least 15 minutes. This slow phase was again measured by the slope of the trace. The records produced by 5-HT differed from those produced by ADP (Born, 1970a) in two ways: (1) the fast phase was slower than that produced by ADP ; and (2) the fast phase merged into the slow phase without the interposition of a maximum and a plateau seen on ADP records. The slope of the slow phase produced by 5-HT was similar to that produced by ADP. Similar records of the shape change were obtained with citrated platelet-rich plasma of rabbits.

The velocity of the fast phase increased with increasing 5-HT concentrations from 10^{-6} to 10^{-5}M ; with higher concentrations the velocity diminished again (Fig. 2). Thus, 5-HT had a self-inhibitory effect on the velocity of the shape change as it has on that of aggregation (Baumgartner & Born, 1968, 1969). The velocity of the slow phase was not affected by variations in 5-HT concentration.

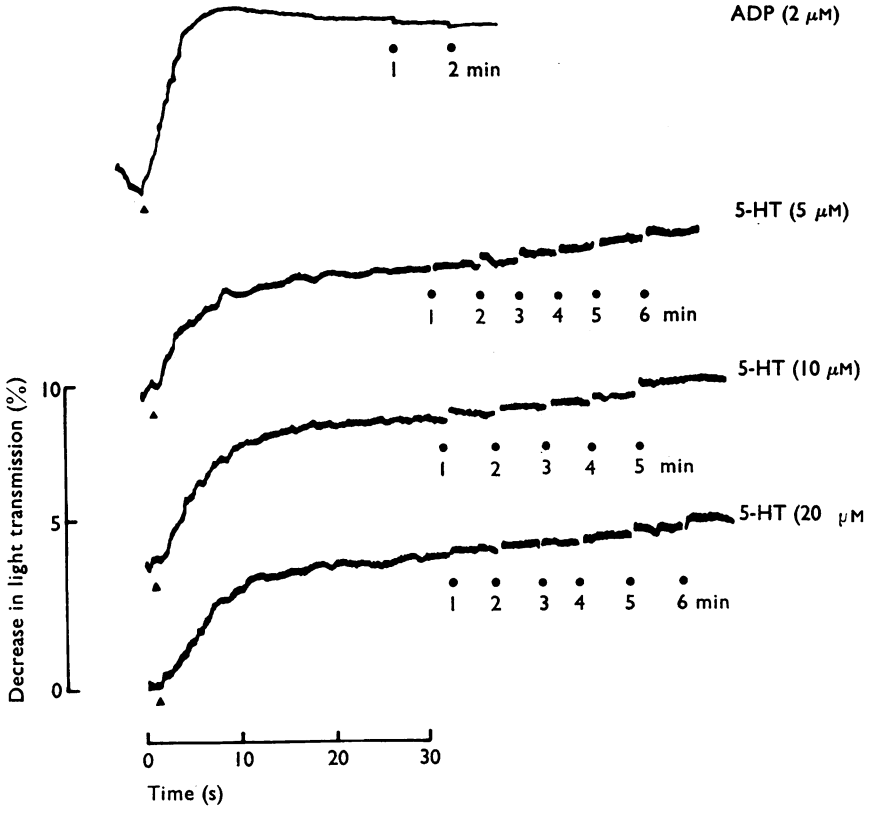


FIG. 1. Change in light transmission in a suspension of human platelets (0·1 ml platelet-rich plasma and 0·9 ml EDTA-Tris buffered saline) containing $9{\cdot}8\times10^7$ platelets per ml caused by adding 5-HT or ADP as indicated by triangles. Concentrations of each agonist are shown beside each trace.

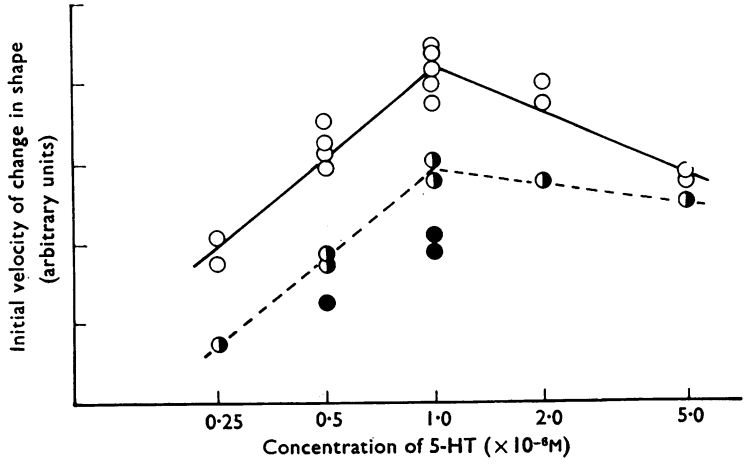


FIG. 2. Initial velocity of the rapid change in shape of platelets caused by increasing concentrations of 5-HT alone and in the presence of N'N'-dipropyltryptamine. The morphological change was measured by recording changes in light transmission through a platelet suspension (0·1 ml platelet-rich plasma and 0·9 ml EDTA-Tris buffered saline) containing $9{\cdot}34\times10^7$ platelets per ml. The inhibitor was incubated for 2 min in the platelet suspension before 5-HT was added. ○, 5-HT alone; ◑, 5-HT with N'N'-dipropyltryptamine (1 μM); ●, 5-HT with N'N'-dipropyltryptamine (2 μM).

Analogues

The specificity of the shape changing effect of 5-HT was investigated by observing whether it was produced by various analogues of 5-HT and tryptamine. All of the seventeen tested analogues of 5-HT and tryptamine except one, viz. 5-hydroxy-N'N'-dibutyltryptamine produced a morphological reaction similar to that of 5-HT. The velocity of the reaction caused by the analogues, like that caused by 5-HT itself, always increased with concentrations up to about $1–2 \times 10^{-5}$M and decreased with higher concentrations. The maximal velocity varied from 0·35–1·5 times that of 5-HT, showing that the activities of the different analogues were rather similar. These results suggest that the receptor on human platelets which is responsible for the shape changing action of 5-HT is not very specific in its structural requirements.

Inhibition

The shape change produced by 5-HT was inhibited by all the eleven analogues (Table 1) which were examined for this effect. The inhibitory concentrations were below those in which these analogues themselves brought about the shape change. Figure 2 shows that the velocity of the change in shape produced by 5-HT was considerably diminished by N'N'-dipropyltryptamine ; the log dose-response curves were parallel, suggesting that this substance inhibited competitively, with a K_i of about 2 μM.

To establish such dose-response curves, several velocity measurements had to be made with 5-HT in the absence and presence of inhibitor. With a high proportion of plasmas, the effectiveness of 5-HT diminished rapidly, which limited the number of reliable measurements with these plasmas. Log dose-response curves were established ; the K_i values (Table 1) were all below, and indeed remarkably similar, indicating again the low specificity of this 5-HT receptor.

The change in shape induced by ADP was not affected by 5-HT or its analogues, confirming that the receptor for ADP is different from that for 5-HT.

The change in shape produced by 5-HT was strongly inhibited by the 5-HT antagonists methysergide (K_i=0·03 μM) and less strongly by imipramine ($K_i \approx$5·0 μM). The inhibition by methysergide increased with time up to about 8 min, whereas that of imipramine did not.

The change in shape induced by 5-HT was inhibited by adenosine or 2-chloroadenosine. At similar shape change velocities, the inhibitory effect of these substances was less against 5-HT than against ADP. For example, 2-chloroadenosine (5×10^{-5}M) inhibited the velocity with 5-HT by 72% and that with ADP by 90% ; even at $2 \times 10–^{5}$M the effect of ADP was still inhibited by 82%.

The change in shape caused by 5-HT, like that caused by ADP (Born, 1970a) was inhibited also by ATP. For example, when added 1 min before the agonists, ATP (1×10^{-4}M) inhibited the velocity produced by 1×10^{-5}M 5-HT by 46%. Interestingly, the same concentration of ATP caused a smaller inhibition when the concentration of 5-HT was halved.

The optical effect of the shape change was inhibited also when the antagonists were added after the agonist 5-HT ; this is shown for methysergide as well as for 2-chloroadenosine in Fig. 3.

TABLE 1. *Inhibition by analogues and by methysergide and imipramine of the uptake of 5-HT and its effect on change in shape and aggregation of platelets*

Analogue No.	Substance: Ring substitution	Side chain	Name	Apparent *Ki* (μM) for Change in shape by 5-HT: Individual	Mean	Aggregation by 5-HT: Individual	Mean	Uptake of 5-HT: Individual	Mean
	5-HT analogues								
27	5-OH	$-CH_2-CH_2-N(CH_3)_2$	5-Hydroxy-N′N′-dimethyltryptamine	1·0, 1·0	1·0	0·1, 0·4	0·25	0·5	0·5
28	5-OH	$-CH_2-CH_2-N(C_2H_5)_2$	5-Hydroxy-N′N′-diethyltryptamine	0·5	0·5	0·1, 0·2	0·15	2·0	2·0
29	5-OH	$-CH_2-CH_2-N(C_3H_7)_2$	5-Hydroxy-N′N′-dipropyltryptamine	0·1	0·1	0·02	0·02	0·2	0·2
32	5-OH	$-CH_2-CH_2-N(CH(CH_3)_2)_2$	5-Hydroxy-N′N′-diisopropyltryptamine	0·05	0·05	0·1	0·1	0·1	0·1
33	5-OH	$-CH_2-CH_2-N(CH(CH_3)C_4H_9)(C_4H_9)$	5-Hydroxy-N′N′-dibutyltryptamine	0·3, 0·5	0·4	0·2, 0·2, 0·3	0·2	0·6, 0·6, 0·9	0·7
	Tryptamine homologues								
1	—	$-CH_2-CH_2-NH_2$	Tryptamine	0·4, 0·8, 0·2, 1·1	0·6	0·1, 0·2, 0·2, 0·3	0·2	2·0, 3·0, 3·0	2·7
10	—	$-CH_2-CH_2-N(CH_3)_2$	N′N′-Dimethyltryptamine	1·0, 0·6, 0·7	0·8	0·1, 0·2, 0·4, 0·4	0·3	1·5	1·5
11	—	$-CH_2-CH_2-N(C_2H_5)_2$	N′N′-Diethyltryptamine	0·7	0·7	0·2, 0·5, 0·6	0·4	1·0, 2·0, 1·0, 1·5, 1·0	1·3
12	—	$-CH_2-CH_2-N(C_3H_7)_2$	N′N′-Dipropyltryptamine	0·5, 1·0, 0·5 3·8, 4·0, 2·0	2·0	0·03, 0·1 2·0, 3·5, 2·5	1·6	0·4, 0·5, 0·5	0·5
	Other analogues								
8	—	$-CH_2-CH_2-NH(C_2H_5)$	N′-Ethyltryptamine	0·6, 3·0, 2·5	2·0	0·9, 1·3	1·1	1·0	1·0
35	7-Cl	$-CH_2-CH(CH_3)-NH_2$	7-Chloro-α-methyltryptamine	0·5, 0·5	0·5	0·3	0·3	0·5	0·5
	Methysergide		1-Methyl lysergic acid butanolamide	0·03	0·03	0·03	0·03	120, 130	125
	Imipramine		N-(α-dimethylaminopropyl) aminodibenzene	5·0	5·0	10·0	10·0	0·3	0·3

Effect of sodium and potassium concentrations on the change in shape caused by 5-HT

The uptake of 5-HT by platelets, like many other uptake processes in different cells (Stein, 1967), requires sodium (Sneddon, 1969). It was of interest, therefore, to find out whether the reaction of 5-HT with platelets, which is responsible for

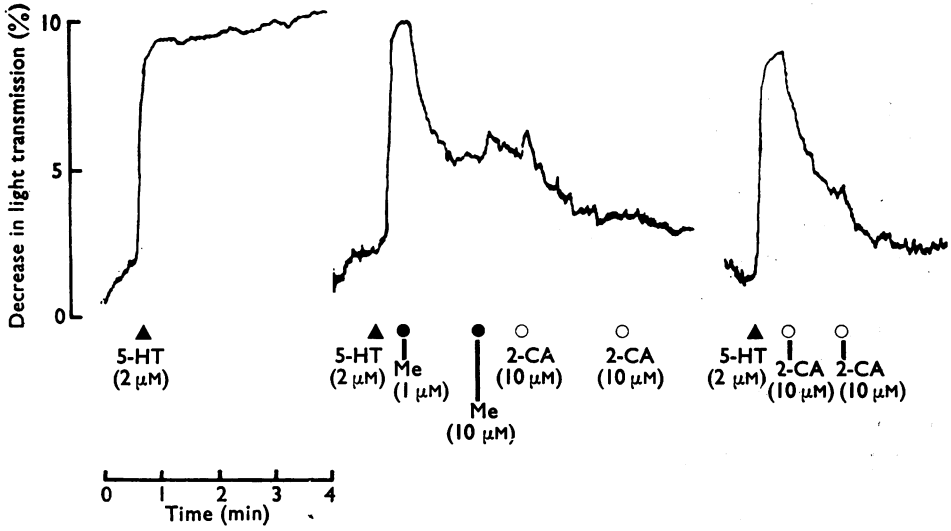


FIG. 3. Effect of methysergide (Me) and 2-chloroadenosine (2-CA) on the rapid change in shape of platelets caused by 5-HT. The suspension of platelets contained 0·1 ml platelet-rich human plasma and 0·9 ml EDTA-Tris buffered saline.

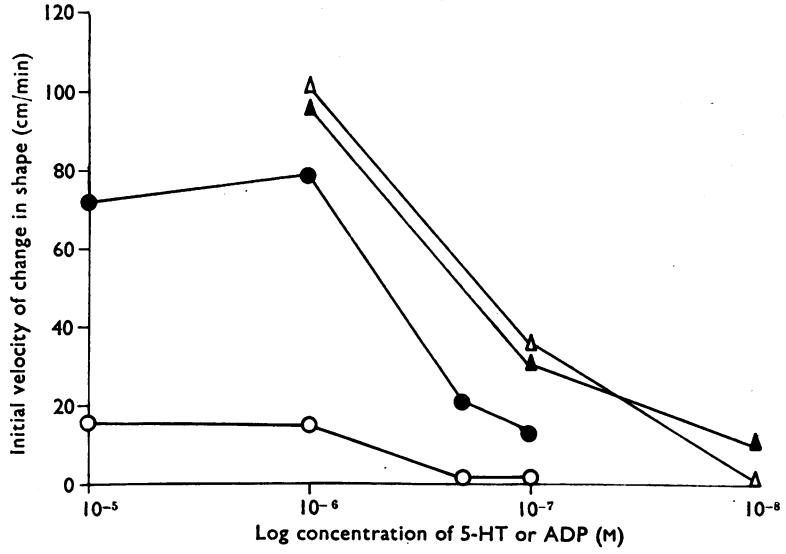


FIG. 4. Initial velocity of the rapid shape change in platelets suspended in media of low and normal Na concentration (see text). Responses to added ADP: ▲, normal Na concentration; △, low Na concentration. Responses to added 5-HT: ●, normal Na concentration; ○, low Na concentration.

their change in shape, also requires Na. The demonstration of this presented a technical problem, because almost all alterations in the normal environment of platelets cause them to change their shape. For that reason it was impossible to provide the cells with an environment low in Na by centrifuging platelet-rich plasma and resuspending the platelets in Na-free artificial media. Furthermore, human platelets maintain their normal disc shape less well than rabbit platelets. For that reason, rabbit platelets were used in the following experimental technique.

Citrated plasmas were chosen in which the platelet concentration was particularly high, that is, greater than 10^9 platelets/ml. One sample of such a plasma was diluted 1 in 20 with Tris-EDTA-saline and another with the same solution except that it contained choline chloride instead of NaCl and that it was adjusted to pH 7·4 with Tris instead of with NaOH. In this way the concentration of Na in the medium was reduced from about 130 to about 7·5 mM. The velocities of the shape change produced by different concentrations of ADP were very similar in both normal and low Na media, whereas the velocities produced by 5-HT were much smaller in low than in normal Na (Fig. 4).

In other experiments the concentration of Na was reduced by dialysing platelet-rich plasma against ten volumes of isotonic choline chloride solution which also contained citrate at the same concentration as the plasma. With plasma containing 124 mM NaCl, the concentration of Na was reduced by about 75% in one hour. The plasma was then diluted 1 in 20 with the Na-free solution already described which brought the Na concentration down to 1–2 mM. In this medium, the effect of 5-HT on platelet shape was abolished but could be restored by adding low concentrations of NaCl ; the effect of ADP was only slightly diminished.

When KCl was added to platelet-rich plasma, the velocity of the shape change was increased.

Aggregation

5-Hydroxytryptamine

The optical effects associated with aggregation by 5-HT have already been described for human (Mitchell & Sharp, 1964), sheep (Michal & Penglis, 1969), and for rabbit platelets (Baumgartner & Born, 1969). The main differences from the effect of ADP are that the velocities with 5-HT are less at the same concentrations and that 5-HT concentrations above about 5×10^{-5}M are inhibitory. Furthermore, the velocity of aggregation by 5-HT is more dependent on temperature than that by ADP (Fig. 5).

Analogues

The aggregating activities of the seventeen analogues were compared with the activity of 5-HT by determining the maximal response for each substance. The results are shown in Table 2 in which these activities are compared also with the contractor activities of the substances on the rat stomach strip. 5-Methoxy-α-methyltryptamine was more potent, and two other analogues were almost as potent, as 5-HT itself (Fig. 6), with similar slopes and self-inhibition at concentrations above about 10 μM.

A second group of seven analogues contained both ring-substituted substances, including 5-OH derivatives, and derivatives of tryptamine. These analogues also caused aggregation but much less strongly than those in the first group, and their log dose-response curves were much less steep. These also showed self-inhibition above about 10 μM. A third group of six analogues was inactive; three of these were tryptamines, two were 5-hydroxy and one a 6-hydroxy derivative.

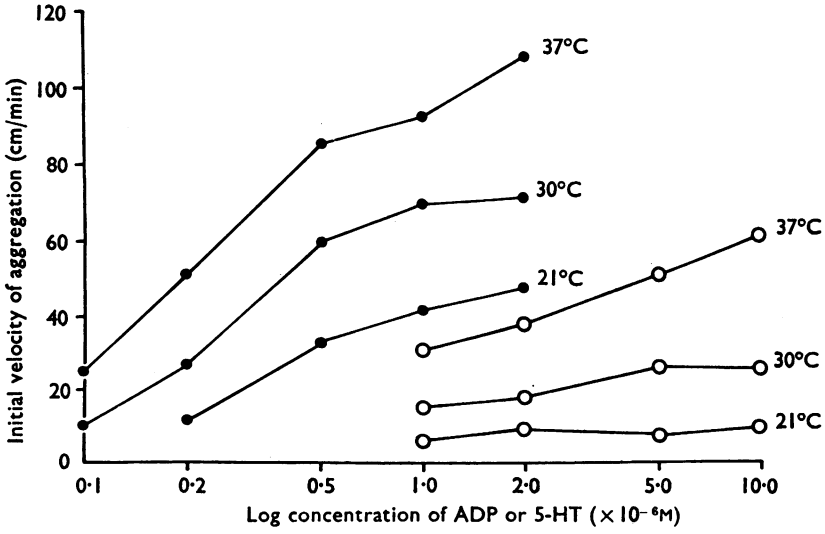


FIG. 5. Initial velocity of platelet aggregation in human citrated platelet-rich plasma in response to added ADP or 5-HT measured at 37° C, 30° C and 21° C. ●, ADP; ○, 5-HT.

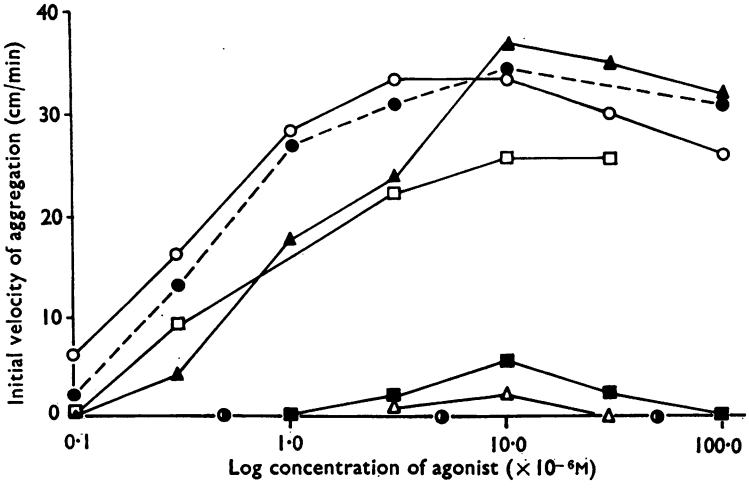


FIG. 6. Log dose-response curves for the velocity of platelet aggregation caused in human, citrated platelet-rich plasma by 5-HT and several analogues: 5-hydroxy-N'N'-diisopropyltryptamine, 5-hydroxy-α-methyltryptamine, 5-methoxy-α-methyltryptamine, 5-hydroxy-N'N'-dipropyltryptamine, and 5-hydroxy-N'N'-diethyltryptamine. α-Methyltryptamine did not cause aggregation. ●, 5-HT; ▲, 5-hydroxy-N'N'-diisopropyltryptamine; □, 5-hydroxy-α-methyltryptamine; ○, 5-methoxy-α-methyltryptamine; △, 5-hydroxy-N'N'-dipropyltryptamine; ■, 5-hydroxy-N'N'-diethyltryptamine; ◑, α-methyltryptamine.

TABLE 2. *Comparative activities of 5-HT and its analogues in causing* (1) *change in shape,* (2) *aggregation of platelets and* (3) *contraction of smooth muscle*

Analogue No. taken from Vane (1959)	Substance: Ring substitution	Substance: Side chain	Substance: Name	Change in shape	Aggregation	Potentiation of ADP-induced aggregation	Smooth muscle contraction: Present results	Smooth muscle contraction: Vane (1959)
A. *Comparatively strong aggregating agents*								
14	5-OH	$-CH_2-CH_2-NH_2$	5-Hydroxytryptamine	100	100		100	100
20	5-OCH_3	$-CH_2-CH(CH_3)-NH_2$	5-Methoxy-α-methyltryptamine	87–107	105, 105, 106, 100	101	50	33
24	5-OH	$-CH_2-CH(CH_3)-NH_2$	5-Hydroxy-α-methyltryptamine	100, 86, 77, 96	95, 82, 82, 101, 103	101	50	67
32	5-OH	$-CH_2-CH_2-N(CH(CH_3)_2)_2$	5-Hydroxy-N′N′-diisopropyltryptamine	92, 100	90, 110, 92	94	10	—
B. *Comparatively weak aggregating agents*								
1	—	$-CH_2-CH_2-NH_2$	Tryptamine	53, 97	50, 37, 17, 51, 31	41	1·1	0·25
10	—	$-CH_2-CH_2-N(CH_3)_2$	N′N′-Dimethyltryptamine	—	35	—	—	0·5
12	—	$-CH_2-CH_2-N(C_3H_7)_2$	N′N′-Dipropyltryptamine	67, 43	33, 18	30	2	2·94
15	4-OH	$-CH_2-CH_2-NH_2$	4-Hydroxytryptamine	89, 52	33	67	50	55·5
28	5-OH	$-CH_2-CH_2-N(C_2H_5)_2$	N′N′-Diethyl-5-hydroxytryptamine	85	21, 29, 41, 23	55	3·8	5·0
29	5-OH	$-CH_2-CH_2-N(C_3H_7)_2$	5-Hydroxy-N′N′-dipropyltryptamine	67, 37	99, 58, 44, 38, 23	69	9·1	2·5
34	5-Cl	$-CH_2-CH(CH_3)-NH_2$	5-Chloro-α-methyltryptamine	100, 120	0, 30, 24	75	5·5	—
C. *Inactive as aggregating agents*								
4	—	$-CH_2-CH(CH_3)-NH_2$	α-Methyltryptamine	80, 81	No aggregation	13	4·0	3·2
8	—	$-CH_2-CH_2-NH(C_2H_5)$	N′-Ethyltryptamine	64	No aggregation	13	—	0·4
11	—	$-CH_2-CH_2-N(C_2H_5)_2$	N′N′-Diethyltryptamine	34	No aggregation	12	1·1	1·2
27	5-OH	$-CH_2-CH_2-N(CH_3)_2$	5-Hydroxy-N′N′-dimethyltryptamine	150, 152	No aggregation	11	10	10
16	6-OH	$-CH_2-CH_2-NH_2$	6-Hydroxytryptamine	50, 83	No aggregation	15	—	0·22
33	5-OH	$-CH_2-CH_2-N(C_4H_9)_2$	5-Hydroxy-N′N′dibutyltryptamine	Negative	No aggregation	—	—	—

The activities of 5-HT itself are designated 100. The platelets were human and the smooth muscle was rat stomach strip.

All of the analogues which were able to cause aggregation also induced the shape change (Table 2). Of the compounds incapable of causing aggregation, four still induced the shape change; another, N'N'-diethyltryptamine, caused the shape change with only one out of five different persons' plasmas; and one, 5-hydroxy-N'N'-dibutyltryptamine, never caused the shape change.

Inhibition of aggregation

All the analogues which prevented the initial shape change of platelets caused by 5-HT also inhibited its aggregating effect and this inhibition, like that of shape change, was apparently competitive (Fig. 7). The inhibitory activities are expressed as K_i values in Table 1; the K_i values were all low (0·02–2·7 μM) and these substances are, therefore, potent inhibitors of aggregation. As with the inhibition of shape change, the inhibition of aggregation shows relatively little structural specificity of the receptor.

Analogues in the second group of Table 2, which themselves caused aggregation, inhibited 5-HT aggregation if their concentrations were below those which cause shape change or aggregation. This indicates that the analogues in this group were able to occupy the 5-HT receptor and to prevent the access of 5-HT to it. The dual action of these analogues may be responsible for the shallow log dose-response curves of their agonistic effect.

These analogues in the third group of Table 2, which themselves did not cause aggregation, prevented change in shape and aggregation in concentrations which were below those effective in causing shape change. The log dose-response curves for the inhibition of aggregation, as of change in shape (Fig. 2), were parallel.

Methysergide was the most potent inhibitor of aggregation by 5-HT and inhibited competitively. Imipramine was much less inhibitory than methysergide and the inhibition was not competitive.

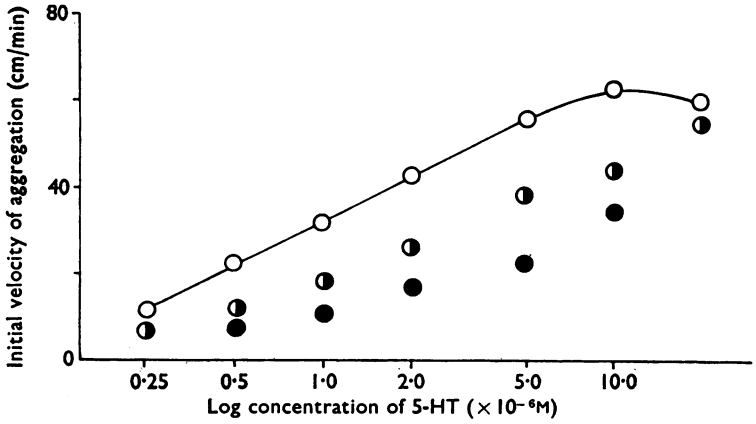
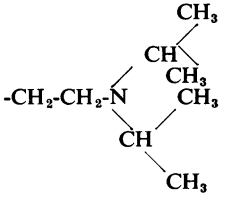


FIG. 7. Initial velocity of aggregation in a suspension of human platelets (0·5 ml platelet-rich plasma and 0·5 ml saline) caused by 5-HT alone and in the presence of N'N'-diethyltryptamine. The inhibitor was added 3 min before 5-HT; temperature was maintained at 37° C. ○, 5-HT alone; ◑, 5-HT with N'N'-diethyltryptamine (0·2 μM); ●, 5-HT with N'N'-diethyltryptamine (0·5 μM).

TABLE 3. *Uptake of 5-HT and its analogues by human platelets estimated by spectrophotofluorimetry*

No.	Substance: Ring substitution	Substance: Side chain	Substance: Name	Uptake ($\times 10^{-9}$ mol/10^8 platelets $\times$ h): Without PIH*	Uptake ($\times 10^{-9}$ mol/10^8 platelets $\times$ h): With PIH
14	5-OH	$-CH_2-CH_2-NH_2$	5-Hydroxytryptamine	1·03	—
24	5-OH	$-CH_2-CH(CH_3)-NH_2$	5-Hydroxy-α-methyltryptamine	0·85	1·25
20	5-OCH_3	$-CH_2-CH(CH_3)-NH_2$	5-Methoxy-α-methyltryptamine	0	0·07
32	5-OH		5-Hydroxy-N′N′-diisopropyltryptamine	0	0·11
29	5-OH	$-CH_2-CH_2-N(C_3H_7)_2$	5-Hydroxy-N′N′-dipropyltryptamine	0·08	—
4	—	$-CH_2-CH(CH_3)-NH_2$	α-Methyltryptamine	0·06	0·11
1	—	$-CH_2-CH_2-NH_2$	Tryptamine	0·01	0·05

* PIH = Phenylisopropylhydrazine HCl.

Uptake

Comparison with 5-HT uptake

Human platelets take up 5-HT by a mechanism which causes it to be highly concentrated in intracellular storage granules and which is highly specific for 5-HT as far as the naturally occurring amines are concerned (for reviews, see Pletscher, 1968; Born, 1969); adrenaline is also taken up but much more slowly and concentrated far less (Born & Smith, 1970). It was of particular interest, therefore, to see which of our structural analogues of 5-HT could be taken up by platelets by a process similar to that for 5-HT.

Table 3 shows that only one analogue, 5-hydroxy-α-methyltryptamine, was taken up like 5-HT, in that their concentrations in the platelets after 1 h were similar. This was one of the three analogues which were about as effective in causing aggregation as 5-HT itself. In the presence of a monoamine oxidase inhibitor the uptake of this analogue was increased a little. In contrast, the two other analogues which caused aggregation like 5-HT were not taken up.

Neither tryptamine nor three analogues which, like it, were weak aggregating agents, were taken up by the concentrative mechanism; after 1 h the platelets contained only very small amounts which were slightly increased in the presence of an amine oxidase inhibitor (Table 3).

Inhibition of 5-HT uptake

The initial velocity of the uptake of radioactive 5-HT was determined in the absence and presence of sixteen analogues, as well as of methysergide or imipramine.

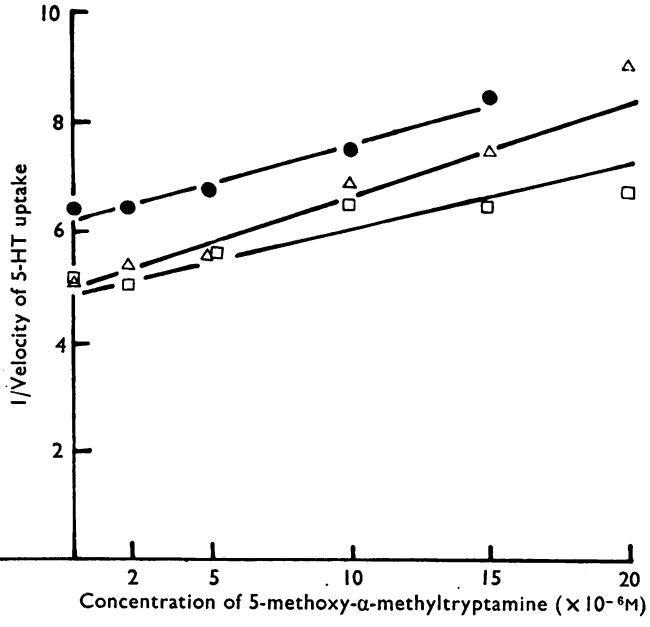


FIG. 8. Non-competitive inhibition by 5-methoxy-α-methyltryptamine of the uptake of 5-HT by human platelets. The uptake is expressed in nmol 5-HT per 3×10^8 platelets. ●, 5-HT (2 μM); △, 5-HT (10 μM); □, 5-HT (5 μM).

All of these analogues diminished the velocity. One of them, 5-methoxy-α-methyltryptamine, inhibited in a non-competitive manner (Fig. 8). Inhibition by the other fifteen analogues was apparently competitive (Fig. 9). The K_i values were remarkably similar, even for structurally quite dissimilar analogues (Table 1). The only hint of a structure-action relationship was in the series, tryptamine, N'N'-dimethyl-, N'N'-diethyl-, and N'N'-dipropyl-tryptamines, of which the first was the least and the last the most inhibitory, but only about 4 times more. Methysergide was a very weak inhibitor of 5-HT uptake, whereas imipramine inhibited it very effectively with a K_i value similar to those of the analogues. Preincubation of methysergide with platelet-rich plasma for 1–16 min before adding 5-HT did not influence its inhibitory activity on 5-HT uptake, but increased the inhibition of change in shape and aggregation (see above).

Discussion

Structure-activity relationships

The purpose of the experiments was to obtain information about the receptor or receptors for 5-HT on the surface of platelets and to compare it or them with the 5-HT receptors of other cell types. Other endogenous transmitter substances have at least two different types of receptor which can be distinguished by blockade with different antagonists, namely, the nicotinic and muscarinic receptors for acetylcholine and the α- and β-adrenoceptors for the catecholamines. For 5-HT too, at least two different types of receptors are known which are located respectively on nerve and smooth muscle cells (for review see Born, 1970b). Furthermore it cannot be assumed that receptors for 5-HT are the same in different species. Most is

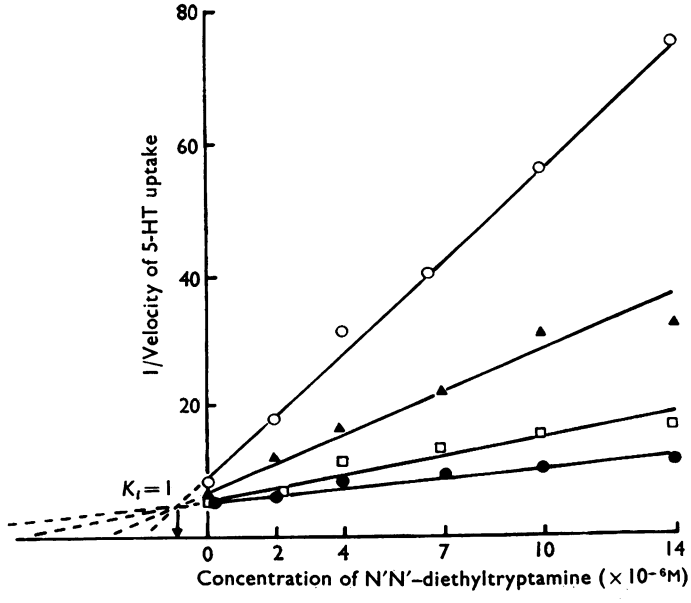


FIG. 9. Competitive inhibition by N'N'-diethyltryptamine of the uptake of 5-HT by human platelets. The uptake is expressed in nmol per 3×10^8 platelets. ○, 5-HT (1 μM); ▲, 5-HT (2 μM); □, 5-HT (5 μM); ●, 5-HT (10 μM).

known about the smooth muscle receptor for 5-HT on the fundal part of the rat stomach (Vane, 1959) and about that of the nerve cells of the guinea-pig ileum (Gaddum & Picarelli, 1957). We have used platelets mainly from man and, less, from the rabbit.

Change in shape and aggregation

Aggregation by 5-HT, as by ADP (Born, 1970a), is invariably preceded by a change in platelet shape. The question is, therefore, whether the shape change is necessary for aggregation to occur. The relevant results are set out in Table 2. Sixteen analogues caused a change in shape like 5-HT itself; only eleven of these also caused subsequent aggregation. One analogue, namely N'N'-diethyltryptamine, caused change in shape in only one out of five different people's plasmas and this analogue, as well as five others, never caused aggregation. It appears, therefore, that these substances cannot produce aggregation unless preceded by the shape change but that, on the other hand, the shape change can be produced without being necessarily followed by aggregation.

Evidently, aggregation depends not only on the occurrence of the shape change but also on something else which is either not provided or blocked by the actions

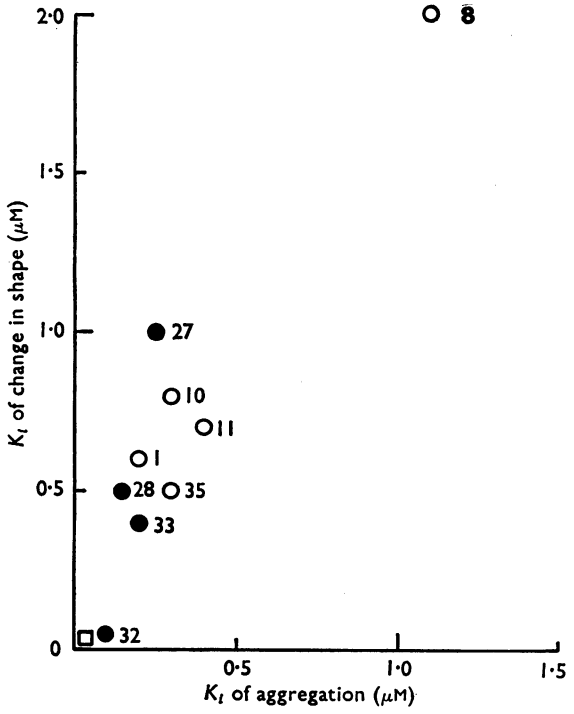


FIG. 10. Relationship between the inhibitory values (K_i) for the inhibition of change in shape (ordinate) and aggregation (abscissa) induced by 5-HT in human platelet-rich plasma. Analogues of 5-hydroxytryptamine and tryptamine are numbered as in Table 1. Values of K_i for imipramine and for compound 12 are: K_i aggregation=10 and 1·6; K_i change in shape= 5 and 2 respectively. The coefficient of correlation between the values for inhibition of aggregation and inhibition of change in shape is 0·93. ●, Analogues of 5-HT; ○, analogues of tryptamine; □, methysergide.

of these particular tryptamine derivatives. Up to now, the only way in which it was possible to prevent aggregation following the shape change was by the removal of ionic calcium with EDTA.

We began the experiments with evidence that both change in shape and aggregation were consequences of the interaction between 5-HT and its active analogues with one type of receptor (Baumgartner & Born, 1969 ; Baumgartner, 1969). The assumption now receives support from the experiments in which the inhibitory potency of the different analogues was determined against the velocities of both shape change and aggregation caused by 5-HT itself. In Fig. 10 these inhibitory potencies are plotted against each other as K_i values ; they show a high degree of correlation ($r=0{\cdot}93$), indicating that both inhibitions increased in proportion to one another with increasing affinities of the analogues for the receptor. The figure also brings out two other points ; first, imipramine lies well off the regression line for the analogues, being twice as inhibitory towards shape change as towards aggregation ; the reason for this is not clear. Second, the regression line for the analogues is steeper than expected if the inhibitory effects were exactly equivalent. The explanation for this is probably the difference in time at which the two velocity measurements were made after the addition of 5-HT. 5-HT has a self-inhibiting effect which, at a given concentration, increases with time (see Baumgartner & Born, 1969 and this paper). This self-inhibition would manifest itself less strongly during the shape change measurements, which are made first, than during the aggregation measurements which are made some ten to thirty seconds later. If the 5-HT has already caused some inhibition the analogue inhibitors would be expected to be apparently more inhibitory on aggregation than on the change in shape.

Apart from the fact that some analogues did not cause aggregation, those that did could be divided into two groups which differed in the steepness of their log dose-response curves. The first group was made up of analogues, three in number, which gave log dose-response curves similar to that of 5-HT itself. All these analogues were substituted in the 5 position on the ring, two being hydroxy and the other methoxy. Two of them, including the methoxy, had their amino groups unsubstituted but the other was the N'N'-diisopropyl analogue of 5-HT. These analogues, again like 5-HT, were self-inhibitory in concentrations greater than about 10 μM.

Another group of eight analogues produced log dose-response curves which were much less steep than those of the first group but similar to each other. This group included tryptamine and two of its analogues as well as two analogues of 5-HT ; also 4-HT and two other analogues substituted with Cl in positions 5 or 7. For all these analogues the maximal velocities of aggregation were less than half of those produced by the first group. However, like the first group they all showed self-inhibition above about 10 μM.

Finally, six analogues never produced aggregation ; three of these were tryptamines, two 5-hydroxytryptamines, and the other was 6-hydroxytryptamine. Only one, 5-hydroxy-N'N'-dibutyltryptamine, was totally inactive as regards both shape change and aggregation.

The next question, therefore, is whether all the analogues with activity act on one type of receptor, presumably that for 5-HT itself, or on more than one type. Evidence in favour of only one receptor is when log dose-response curves are parallel, as in the first group. *Prima facie,* the flatter log dose-response curves of the second group would suggest an action on a different receptor. However, against this is

that (1) analogues in the second group were powerful antagonists of aggregation by 5-HT, with K_i values of between 0·02 and 2·7 μM ; (2) that their aggregating effect was blocked, like that of 5-HT, by methysergide ; and (3) that these analogues were self-inhibitory, again like 5-HT itself.

It seems, therefore, that all the active analogues cause change in shape and aggregation of platelets by acting on only one type of receptor. This receptor has some properties which are similar to the D receptor for 5-HT of other cells, particularly smooth muscle (Michal, 1969) ; thus, a potent antagonist is methysergide whereas cocaine is almost ineffective and morphine completely so.

This generalization leaves many problems unsolved. First, there were at least four analogues which caused the shape change but not aggregation. Because this separation of effects was so strange, we confirmed the absence of aggregation under the light microscope. The shape changing activity of these analogues was inhibited also by methysergide. This means that these particular substances also reacted with the 5-HT receptor. Therefore, the reaction of an agonist or partial agonist with this receptor is usually but not invariably associated with the change which is responsible for aggregation. One analogue which caused change in shape but not aggregation was α-methyltryptamine, the 5-hydroxy analogue of which was particularly potent in causing both change in shape and aggregation. This would suggest that the 5-hydroxy (or 5-methoxy) group is essential for the aggregating effect, at least for a log dose-response relationship like that for 5-HT.

It can be concluded that the receptor which mediates the effects of 5-HT on the platelets is remarkable for its low structural specificity. A possible explanation for the total inactivity of 5-hydroxy-N'N'-diisopropyl tryptamine is that it has the longest aliphatic chains. Apart from these comments, the results are such that no clear-cut conclusions can be arrived at about structure-activity relationships.

Uptake

The uptake of 5-HT by platelets consists of more than one step, the relationship between which is not yet clear (Born & Gillson, 1959 ; Pletscher, 1968). First the amine reacts with a receptor on the platelet. Then it is transported through the membrane, apparently by a carrier system which requires ATP ; and finally it is bound to ATP in characteristic cytoplasmic granules which are formed only when 5-HT is available (Pletscher, Da Prada & Tranzer, 1969).

Out of six analogues with which uptake was determined only one, 5-hydroxy-α-methyltryptamine, was taken up by and concentrated in platelets like 5-HT. The other two analogues which were as potent as 5-HT in causing shape change and aggregation were not taken up at all as determined fluorimetrically in the absence of an amine oxidase inhibitor. In the presence of such an inhibitor, very small amounts of these and of the other analogues could be demonstrated fluorimetrically in the platelets, but these amounts were similar to those for tryptamine which is taken up only by diffusion and not concentrated in platelet granules (Stacey, 1961). These observations suggest that the 5-HT uptake mechanism is highly specific and requires both the 5-hydroxy group and no substitution on the amino group. Whether the specificity requirements are for the movement through the membrane or for the formation of the granules, or for both, has still to be established.

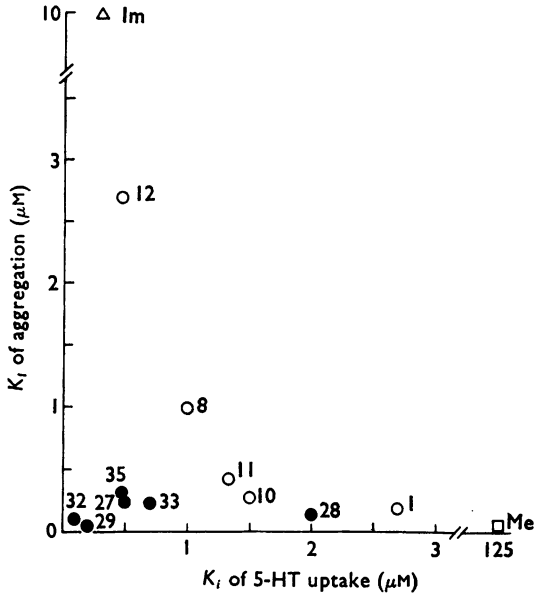


FIG. 11. Relationship between the inhibitory values (K_i) for the inhibition of aggregation (ordinate) induced in human platelet-rich plasma by 5-HT and for the inhibition of 5-HT uptake (abscissa). Analogues of 5-HT and tryptamine are numbered as in Table 1. ●, Analogues of 5-HT; ○, analogues of tryptamine. Im, Imipramine; Me, methysergide.

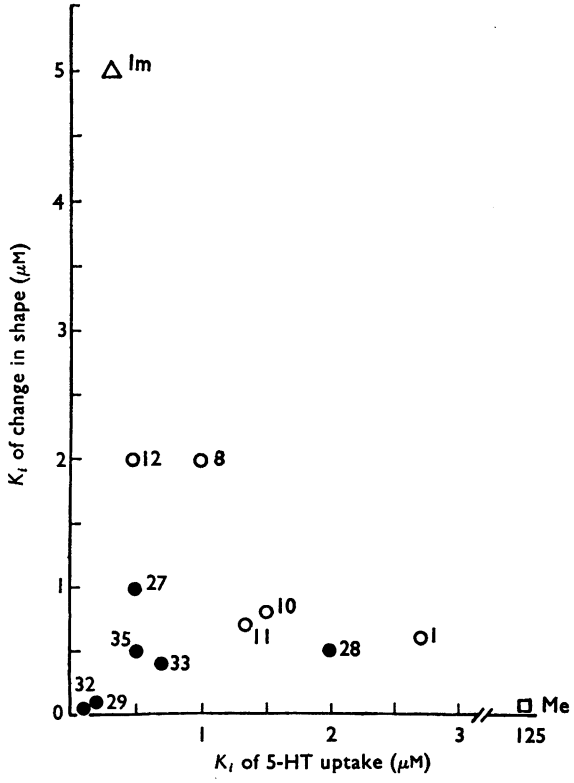


FIG. 12. Relationship between the inhibitory values (K_i) for the inhibition of change in shape (ordinate) induced by 5-HT and for the inhibition of 5-HT uptake (abscissa) in citrated human plasma rich in platelets. Analogues of 5-HT and tryptamine are numbered as in Table 1. ●, Analogues of 5-HT; ○, analogues of tryptamine. Im, Imipramine; Me, methysergide.

Probably the most interesting implications of the results emerge when the inhibitory potencies of the analogue towards shape change and aggregation on the one hand and towards uptake of 5-HT on the other hand are plotted against each other as have been done in Figs. 11 and 12. For 5-hydroxytryptamine and other analogues substituted on the indole ring, no evident correlation emerges. On the other hand, a remarkable feature emerges with the tryptamines: tryptamine itself is considerably more inhibitory towards shape change and aggregation than towards uptake. With increasing aliphatic substitution on the amino group the derivatives become increasingly potent inhibitors towards uptake and at the same time decreasingly potent inhibitors towards shape change or aggregation. The inverse relationship in this series becomes even more striking when the inhibitory potencies of methysergide and imipramine are plotted on the same scale (see Figs. 11 and 12): clearly methysergide falls at one extreme of the tryptamine curve whereas imipramine falls at the other. Such a difference has also been found by Haslam (personal communication).

The very great differences in the inhibitory potencies of methysergide and imipramine on the measured effect would, at first sight, be most straightforwardly explained by assuming two different types of 5-HT receptor on platelets, one mediating shape change and aggregation and the other mediating uptake. However, it is worthwhile to consider this in relation to the meaning of receptors and of specificities. Methysergide is a highly specific antagonist to 5-HT on smooth muscle as well as on platelets and its antagonism is, within certain limits, competitive. This suggests that methysergide does indeed compete with 5-HT for its active receptor, presumably because of the similarities in molecular structure. Imipramine, on the other hand, powerfully antagonizes not only the uptake of 5-HT by platelets but also that of noradrenaline, a very different molecule, by sympathetic nerve cells ; furthermore, these inhibitory effects of imipramine are non-competitive. It seems, therefore, that imipramine exerts its effect through something other than a direct competitive action on the 5-HT receptor. Our observation, that increasing aliphatic substitution of tryptamine makes for an increasingly imipramine-like inhibition of uptake of 5-HT by platelets and at the same time for a decreasing effect on change in shape and aggregation, suggests that these substances, like imipramine, do not compete with 5-HT at its active receptor but have a less specific reaction with the cell membrane which, secondarily, shows itself as the observed inhibition. These considerations bring to mind the difference between the receptor types for the catecholamines, because increasing aliphatic substitution on their amino group increases potency towards one type and decreases potency towards the other.

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