Br. J. Pharmac. (1976), **56**, 209–218

SHAPE CHANGE AND AGGREGATION OF BLOOD PLATELETS: INTERACTION BETWEEN THE EFFECTS OF ADENOSINE DIPHOSPHATE, 5-HYDROXYTRYPTAMINE AND ADRENALINE

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1 The interaction of effects between 5-hydroxytryptamine (5-HT) and adenosine diphosphate (ADP) on human or rabbit platelets was investigated *in vitro*. The initial platelet shape change and their aggregation were measured in stirred, citrated platelet-rich plasma (PRP) at 37°C by recording the rate and extent of changes in light scattering and light transmission.

2 Both the velocity and extent of aggregation and the velocity and extent of the rapid morphological change caused by ADP were enhanced by simultaneous addition of 5-HT. Methysergide but not imipramine inhibited the 5-HT effects.

3 Platelets were made refractory to the aggregating and shape changing effect of either ADP or 5-HT by repeated aggregation with the particular agent; platelets made refractory to ADP retained their responsiveness to 5-HT and platelets made refractory to 5-HT responded to ADP. Platelets pre-incubated for 3–10 min with 5-HT without aggregation showed greatly reduced aggregation on subsequent addition of ADP. Methysergide inhibited all the effects of 5-HT whilst imipramine was inactive.

4 When the shape change or aggregation of platelets induced by ADP was submaximal, addition of 5-HT increased it further. Pre-incubation of PRP with 5-HT before the addition of ADP resulted in failure of the secondary induction of aggregation or shape change by 5-HT. The secondary induction by 5-HT also did not occur in the presence of methysergide; imipramine had no inhibitory effect. Similar secondary induction of aggregation was shown by adrenaline injected during aggregation by ADP; the adrenaline effect was removed by phentolamine but not by propranolol.

5 Our results show that the initial change in shape of platelets and their aggregation can be induced by ADP or 5-HT in specific manner. The interaction of the effect of these substances on platelets can result in either increase in platelet sensitivity or, under certain conditions, decrease in platelet responsiveness. The increase or depression of platelet reactivity appears to be a highly specific effect and is probably mediated at specific receptors involved with platelet activation.

Introduction

Mammalian blood platelets contain relatively high amounts of 5-hydroxytryptamine (Rand & Reid, 1951) which they are able to concentrate against concentration gradients by a membrane transport system that is coupled to sodium influx and which may be energy-dependent (Hardisty & Stacey, 1955; Born & Gillson, 1959; Sneddon, 1973). It was thought that platelets do not synthetize 5-hydroxytryptamine (Paasonen, 1965) but recently 5-hydroxytryptophan decarboxylase activity has been demonstrated *in vitro* in extracts of human and beef platelets (Marmaras & Mimikos, 1971). Another mechanism is thought to be responsible for the accumulation of 5-hydroxytryptamine (5-HT) in cytoplasmic organelles; specifically that 5-HT forms a stable complex with adenosine triphosphate (Born, Ingram & Stacey, 1958; Da Prada & Pletscher, 1968; Pletscher, Da Prada & Tranzer, 1969). Most of this stored 5-HT is released together with adenosine triphosphate (ATP), by aggregating agents such as thrombin (Grette, 1962), adenosine diphosphate (ADP) and collagen (Packham, Guccione, Chang & Mustard, 1973). It has been suggested that such a release reaction accompanies aggregation, at least *in vitro* (Mills & Roberts, 1967; Mustard & Packham, 1970).

In the platelets of some mammalian species 5-HT induces a rapid morphological change that is followed by reversible aggregation. In human platelet-rich

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plasma (PRP) aggregation velocity accelerates with 5-HT concentrations up to approximately 10 μM and decelerates again with concentrations above this level (Baumgartner & Born, 1968). In citrated rabbit PRP 5-HT does not itself cause aggregation but potentiates aggregation induced by ADP (Sinakos & Caen, 1967). Furthermore, incubation of platelets with 5-HT can, under certain conditions, diminish the aggregating effect of ADP as well as aggregation by 5-HT itself (Baumgartner & Born, 1969). Experiments with 5-HT antagonists have established that 5-HT initiates aggregation by reacting with a receptor similar to the D-receptor of smooth muscle (Michal, 1969). More recent evidence suggests the existence of two different receptors, one mediating shape change and aggregation by 5-HT and the other uptake of the amine (Born, Juengjaroen & Michal, 1972).

This paper provides additional observations on the shape change and aggregation of platelets induced by 5-HT, and on its interaction with ADP and adrenaline. The effects of the 5-HT antagonists methysergide and imipramine, which block shape change/aggregation by 5-HT and its uptake respectively, are also presented.

Methods

Preparation of platelet-rich plasma

Platelet-rich plasma (PRP) was prepared from human or rabbit citrated blood (trisodium citrate 0.38% final concentration) by centrifugation at 200 *g* for 10 or 15 min respectively. Decanted PRP was kept at room temperature and used as soon as possible after bleeding.

Platelet shape change and aggregation

Photometric records of platelet aggregation and the preceding shape change were obtained by the method of Michal & Born (1971) which allows simultaneous recordings of light transmission and scattering in the PRP at 37°C. When shape change only was required, 10 μl of 0.4M ethylene glycolbis-(2-aminoethyl) tetra-acetic acid was added to each ml of PRP before challenge with agonists. Platelet aggregation and shape change were quantitated by measuring the velocity and extent of the photometric records. When the effect produced by one agent was enhanced or inhibited in the presence of another agent, the responses obtained were expressed as percentage of the normal controls. When measuring the influence that pre-incubation with 5-HT had on the subsequent aggregation or shape change, a strict timing schedule was observed so that the total period during which the samples were warmed and stirred was identical in all cases and only the contact time with 5-HT was varied within this set period. Inhibitors were allowed 3 min contact before addition of the agonist, unless otherwise specified.

Drugs used were adenosine 5′-diphosphate (Sigma), 5-hydroxytryptamine creatinine sulphate (May & Baker), adrenaline acid tartrate (BDH), imipramine hydrochloride (ICI), phentolamine hydrochloride (Ciba-Geigy), (+)-propranolol hydrochloride (ICI) and methysergide bimaleate, gift from Sandoz, Ltd., Basle. All drugs were freshly dissolved in 0.9% w/v NaCl solution (saline) and added to PRP in volumes not exceeding 20 μl per 1 ml PRP.

Results

Enhancement of adenosine diphosphate-induced aggregation by 5-hydroxytryptamine

Both human and rabbit platelets aggregate in response to ADP in a concentration-dependent manner. Human platelets are aggregated by 5-HT but the aggregates are smaller and disaggregate more rapidly than aggregates produced by equal concentrations of ADP. When 5-HT and ADP are added simultaneously to stirred PRP, the resulting aggregation is faster than that caused by either of the aggregating agents alone. 5-HT alone does not aggregate platelets in rabbit citrated PRP, but as seen in human PRP, it increases the aggregating effect of ADP (Sinakos & Caen, 1967). Figure 1 shows the effect of four concentrations of 5-HT on the rate and extent of aggregation of rabbit platelets produced by different concentrations of ADP. The velocity as well as the extent of aggregation, i.e. reduction in number of single platelets (Born & Hume, 1967; Cronberg, 1970), which is measured by increase in light transmission are enhanced in the presence of 5-HT. The enhancement of aggregation is greatest with low concentrations of ADP, i.e. 0.2–1 μM when the rate and extent of aggregation are increased six to seven fold by the addition of 5-HT 1–5 μM. At higher ADP concentrations the enhancement by 5-HT is less. In rabbit PRP, ADP produces maximal velocity of aggregation at concentrations of 5–10 μM, and therefore no potentiating effect of 5-HT can be measured.

Enhancement of adenosine diphosphate-induced shape change by 5-hydroxytryptamine

The rapid morphological change of platelets, which precedes their aggregation, is induced by 5-HT or by ADP in both rabbit and human plasma. The rate as well as the extent of shape change, i.e. the number of platelets with changed shape, depends on the concentration of the aggregating agents and generally occurs with concentrations well below those needed to

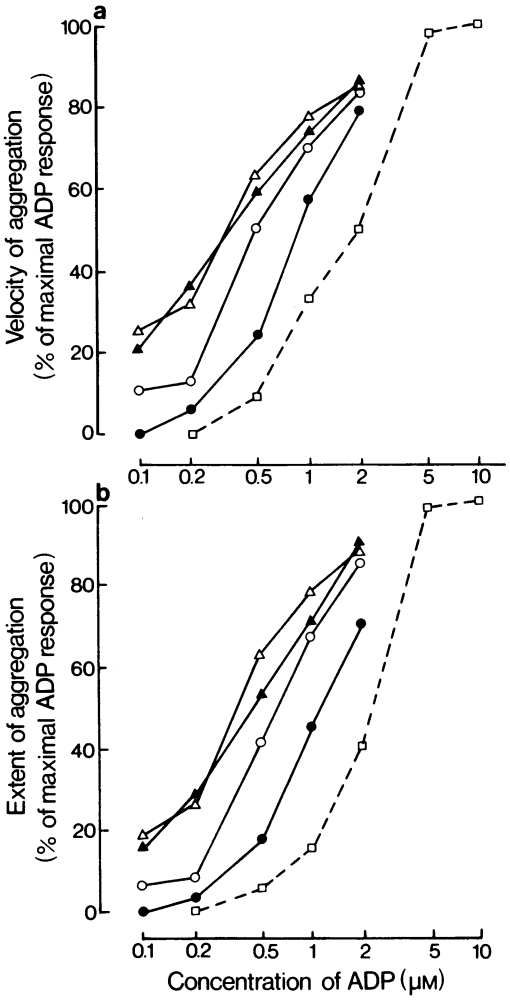

Figure 1 Potentiation by 5-hydroxytryptamine (5-HT) of platelet aggregation induced in rabbit platelet-rich plasma by adenosine diphosphate (ADP). Plots of (a) velocity of aggregation and (b) extent of aggregation. 5-HT was injected simultaneously with ADP in volumes of 10 μl per 1 ml plasma. (□) ADP alone; (●) ADP with 5-HT 0.5 μM; (○) ADP with 5-HT 1 μM; (▲) ADP with 5-HT 5 μM and (△) ADP with 5-HT 20 μM. Stirred platelet-rich plasma was maintained at 37°C and aggregation was measured by recording changes in light transmission.

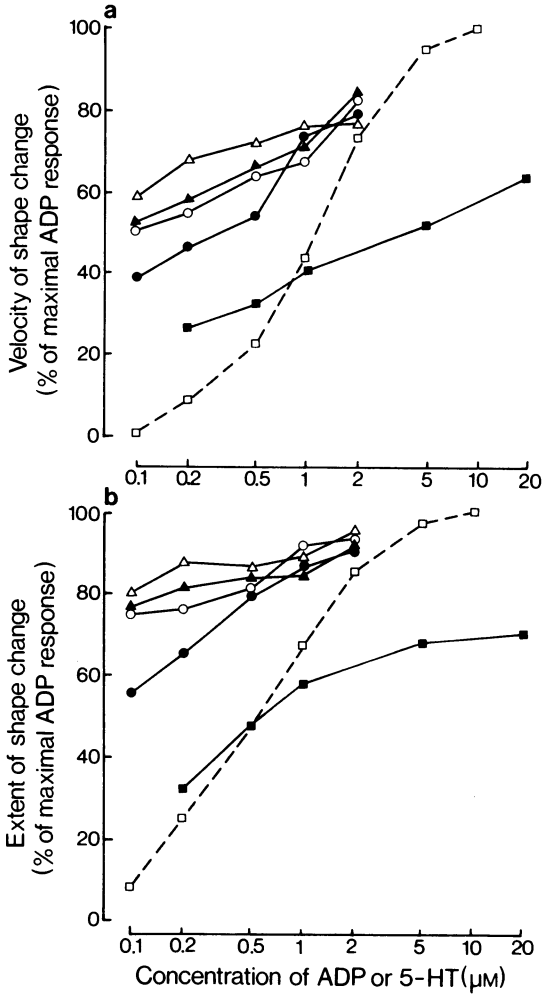

Figure 2 Potentiation of adenosine diphosphate (ADP)-induced shape change of rabbit platelets by 5-hydroxytryptamine (5-HT). Plots of (a) velocity of shape change and (b) extent of shape change. 5-HT was injected simultaneously with ADP. (□) ADP alone; (■) 5-HT alone; (●) ADP with 5-HT 0.5 μM; (○) ADP with 5-HT 1 μM; (▲) ADP with 5-HT 5 μM; (△) ADP with 5-HT 20 μM. Shape change was measured by recording changes in light scattering through stirred platelet-rich plasma at 37°C.

induce aggregation (Figure 2). The results were obtained in rabbit PRP by measuring 90° light scattering by platelets (Michal & Born, 1971). When 5-HT and ADP are injected together into PRP, the velocity and the extent of the morphological change is always greater than that produced by either agent alone. Concentrations of 5-HT which by themselves produce no detectable response, when added together with ADP bring about a rapid shape change of platelets. In rabbit PRP, the effect produced by low concentrations of ADP is markedly increased by very low concentrations of 5-HT (0.1–0.5 μM). Results of typical experiments are shown in Figure 3. As in the case of aggregation, 5-HT causes a greater enhancement of the effect of low concentrations of ADP (0.1–0.5 μM) than of concentrations of ADP which by themselves induce near maximal shape change (1–2 μM).

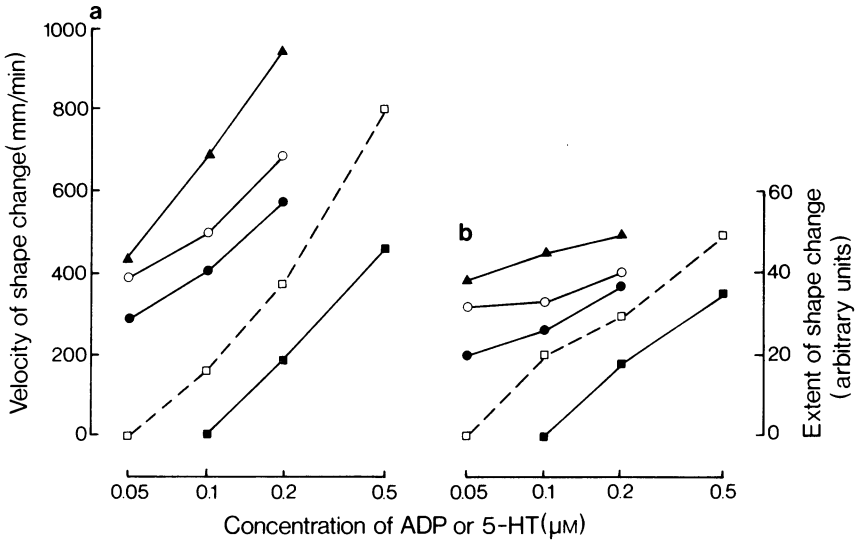

Figure 3 Potentiation by low concentrations of 5-hydroxytryptamine (5-HT) of platelet shape change caused by low concentrations of adenosine diphosphate (ADP) in rabbit platelet-rich plasma. Effect on (a) velocity of platelet shape change and (b) extent of shape change. (□) ADP alone; (■) 5-HT alone; (●) ADP with 5-HT 0.1 μM; (○) ADP with 5-HT 0.2 μM; (▲) ADP with 5-HT 0.5 μM. 5-HT was injected simultaneously with ADP.

Effect of inhibitors on aggregation and shape change

Incubation of PRP for 3 min at 37°C with methysergide (0.25 μM) before the addition of the aggregating agents (ADP and 5-HT) abolishes the enhancing effect of 5-HT on ADP (Figure 4). Methysergide at this concentration has no inhibitory effect on aggregation produced by ADP. Imipramine (0.5 μM) reduces the effect of 5-HT on ADP aggregation only slightly. A mixture of imipramine (0.5 μM) and methysergide (0.25 μM) has the same inhibitory effect as methysergide alone.

Similarly the increase by 5-HT of the velocity of shape change produced by ADP in human platelets is abolished by methysergide (0.25 μM); imipramine (0.5 μM) reduces the effect only slightly (Figure 5).

Table 1 The effect of pre-incubation of platelets with 5-hydroxytryptamine (5-HT) on their response to adenosine diphosphate (ADP) and potentiation by 5-HT of aggregation by ADP

Pre-incubation (min) with 5-HT (5 μM)	*ADP alone (0.5 μM) or ADP (0.5 μM) + 5-HT (10 μM)*	*Aggregation (% ADP control)**	
		Velocity	*Extent*
	Rabbit PRP		
No pre-incubation	ADP + 5-HT	145	152
3	ADP	120	122
	ADP + 5-HT	121	123
10	ADP	74	81
	ADP + 5-HT	86	92
	Human PRP		
No pre-incubation	ADP + 5-HT	191	272
3	ADP	74	59
	ADP + 5-HT	58	50
10	ADP	30	34
	ADP + 5-HT	32	32

* Aggregation by ADP alone at time zero is expressed as 100%.

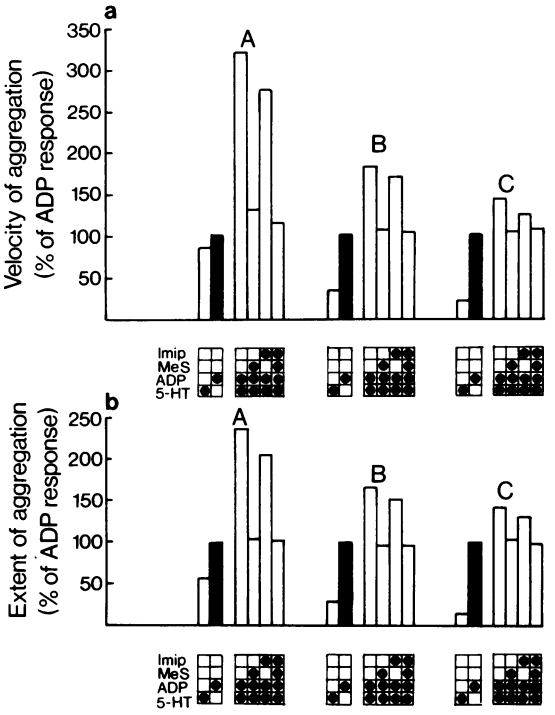

Figure 4 Effect of 0.5 μM imipramine (Imip) and 0.25 μM methysergide (MeS) on the potentiation by 5-hydroxytryptamine (5-HT, 0.5 μM) of (a) velocity and (b) extent of aggregation induced in human platelet-rich plasma by adenosine diphosphate (ADP). Concentrations of ADP: A=0.5 μM; B=1 μM; C=2 μM. 5-HT and ADP were injected simultaneously. Control responses to ADP alone (solid columns) were given a value of 100%. The inhibitiors were incubated in the platelet suspension for 3 min before addition of the ADP/5-HT mixture.

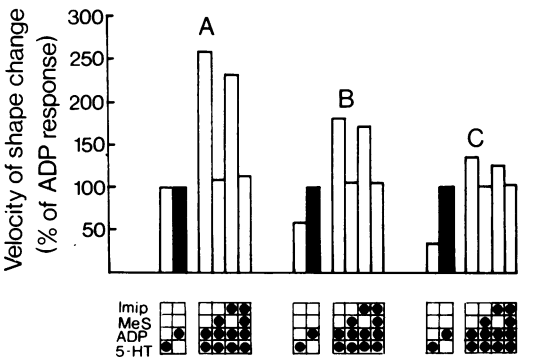

Figure 5 Effect of 0.5 μM imipramine (Imip) and 0.25 μM methysergide (MeS) on the potentiation by 5-hydroxytryptamine (5-HT, 0.5 μM) of the velocity of shape change induced in human platelet-rich plasma by adenosine diphosphate (ADP) (A=0.5 μM, B=1 μM and C=2 μM ADP). 5-HT and ADP were injected simultaneously. Control responses to ADP alone were given a value of 100% (solid columns). The inhibitors were incubated in the platelet-rich plasma for 3 min before the addition of the ADP/5-HT mixture.

Inhibition by 5-hydroxytryptamine itself

Enhancement of ADP-induced platelet aggregation by 5-HT is greatest when the two agents are injected into PRP simultaneously. When 5-HT is injected before ADP, the enhancing effect is smaller. By increasing the time of pre-incubation with 5-HT, not only the enhancing effect of 5-HT disappears but the effect of ADP is progressively reduced. Table 1 shows the effect of pre-incubating rabbit or human platelets with 5 μM 5-HT for 3 or 10 min before adding either ADP alone or a mixture of ADP and 5-HT. In rabbit PRP the velocity and extent of ADP aggregation after 3 min pre-incubation with 5-HT are still greater than aggregation caused by ADP alone; however, after 10 min, the velocity and extent are reduced. In human PRP pre-incubated with 5-HT for 3 min, aggregation by ADP or ADP plus 5-HT is inhibited, and after 10 min pre-incubation, velocity and extent are further reduced to approximately 30% of the control.

Platelet refractoriness to adenosine diphosphate and 5-hydroxytryptamine

Platelets are made refractory to ADP by repeated aggregation with ADP (Table 2). After the fourth injection of ADP (9 min), the extent of aggregation in human PRP is only 13–15% of the aggregation obtained with the first addition of ADP; injection of 5-HT into this PRP at time 12 min produces extensive aggregation which is twice as great as the 5-HT control. Methysergide (0.5 μM) inhibited the action of 5-HT only.

Platelets become rapidly refractory to repeated injections of 5-HT; a second injection of 5-HT given 3 min after the first fails to aggregate platelets (Table 2). However, sensitivity of these platelets to ADP remains unchanged, including the gradual development of refractoriness to repeated additions of this agent.

Rabbit platelets are also made refractory to ADP by repeated injections of this agent (Table 2). After three injections of ADP (1 μM) made at 3 min intervals, the platelets are no longer aggregated by ADP. However, when 5-HT is injected into the same PRP, aggregation is produced (29% of control) despite the inability of 5-HT to aggregate rabbit platelets that have not been exposed to ADP; subsequent additions of 5-HT produce no aggregation. The aggregating

Table 2 Platelet refractoriness to multiple injections of adenosine diphosphate (ADP, 1 μM) or 5-hydroxytryptamine (5-HT, 10 μM) in human or rabbit platelet-rich plasma (PRP)

Inhibitor	*Substance injected*	*Extent of aggregation (% ADP control)** *Time of injection (min)* 0	3	6	9	12	24
		Human PRP					
	ADP	100	47	19	13	5	6
	5-HT	67	0	0	0	0	
	ADP	100	68	29	15		3
	5-HT					200	
Methysergide (0.5 μM)	ADP	100	58	24	15		4
	5-HT					0	
	ADP			95	47	19	6
	5-HT	68	0				
		Rabbit PRP					
	ADP	100	7	0			0
	5-HT				29	0	
Methysergide (0.5 μM)	ADP	100	10	0			0
	5-HT				0	0	
Imipramine (0.5 μM)	ADP	100	9	0			0
	5-HT				28	0	
	ADP			96	26	0	
	5-HT	0	0				

* Aggregation by ADP alone at time zero is expressed as 100%. Inhibitors were pre-incubated in PRP for 3 minutes.

effect of 5-HT produced in platelets exposed to ADP is inhibited by methysergide (0.5 μM) but not by imipramine (0.5 μM). In rabbit platelets which do not aggregate after two injections of 5-HT given at 3 min intervals, addition of ADP results in normal aggregation and subsequent development of the refractory effect (Table 2).

Shape change, like aggregation, diminishes after repeated injections of ADP and it is produced only by the first injection of 5-HT. Platelets made refractory to 5-HT change shape with ADP, and platelets made partially refractory to the shape changing action of ADP respond to addition of 5-HT.

Secondary induction of aggregation or shape change

When rabbit or human platelets are aggregated with ADP, the change in light transmission can be further increased by a second addition of ADP providing that the initial concentration of ADP causes a sub-maximal aggregating response and that the second addition is made at the peak of the initial aggregation

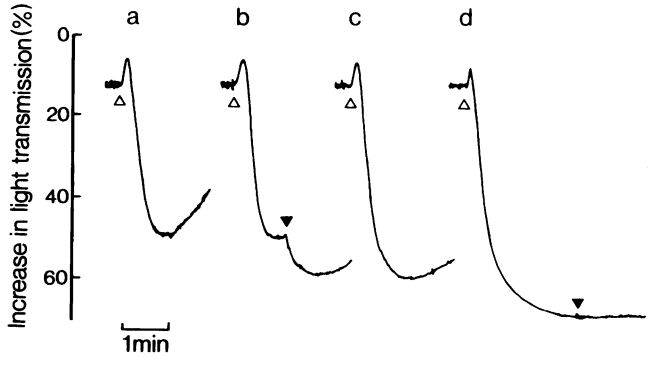

Figure 6 Change in light transmission in rabbit platelet-rich plasma caused by platelet aggregation induced by adenosine disphosphate (ADP, △): (a) 1 μM; (b) 1 μM; (c) 2 μM; (d) 10 μM; followed by 1 μM ADP (▲) in (b) and (d).

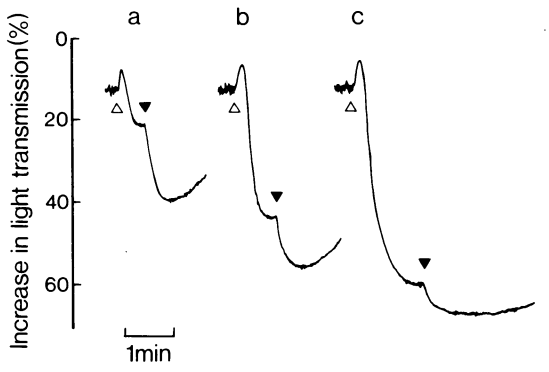

Figure 7 Secondary induction of aggregation by 10 μM 5-hydroxytryptamine (▲) of platelets in rabbit platelet-rich plasma aggregated initially by adenosine diphosphate (△): (a) 0.5 μM, (b) 1 μM and (c) 2 μM.

(Figure 6). The final extent of aggregation produced in this way is similar to aggregation caused by the combined amount of ADP added in one single injection. 5-HT injected as the second addition after ADP produces a similar increase in the aggregating effect (Figure 7). This additional effect is shown not only in human but also in rabbit PRP where 5-HT by itself does not aggregate platelets. Similarly, adrenaline which does not aggregate rabbit platelets nevertheless causes additional aggregation when added after ADP (Figure 8a).

The specificity of the additional aggregation was tested by incubating the PRP samples with antagonists of 5-HT and adrenaline for 3 min before aggregation. Pre-incubation with phentolamine (5 μM) removes the additional aggregation superimposed on ADP by adrenaline 10 μM (Figure 8f); propranolol (5 μM), imipramine (0.5 μM) and methysergide (0.25 μM) are inactive (Figure 8c, d and g). Incubation with methysergide abolishes the additional aggregating effect caused by 5-HT after the induction of aggregation by ADP (Figure 9d); imipramine, phentolamine and propranolol are inactive (Figure 9c, f and g).

Platelets aggregated by ADP do not show additional aggregation with 5-HT if they are pre-incubated for 3 min with 5-HT itself although the velocity and extent of initial aggregation by ADP are greater than without 5-HT pre-incubation (Figure 9e). This lack of additional aggregation in PRP samples pre-incubated with 5-HT resembles the self-inhibition by 5-HT of its potentiating effect on ADP already described (see Table 1). The self-inhibition is specific; pre-incubation with 5-HT does not inhibit the additional aggregation produced by either adrenaline or ADP (Figure 8e, Figure 10e).

Pre-incubation of PRP with adrenaline for 3 min before the addition of ADP increases the extent of platelet aggregation by ADP; such pre-incubation inhibits the effect of adrenaline added after ADP (Figure 8b) but not that of 5-HT (Figure 9b).

The shape change of platelets produced by ADP is increased when 5-HT and ADP are injected together or by injecting 5-HT at the peak of the ADP shape change in the presence of ethylene glycol bis-(2-amino-ethyl) tetra-acetic acid (4 mM) to prevent aggregation. Methysergide, but not imipramine, removes the effect of 5-HT leaving the ADP shape change undiminished. Pre-incubation of platelets with 5-HT for 3 min prevents the effect of the second addition of 5-HT but the shape change produced by ADP is not inhibited. In this respect, aggregation and shape change resemble each other.

Adrenaline failed to induce an additional effect when injected at the peak of ADP-induced shape

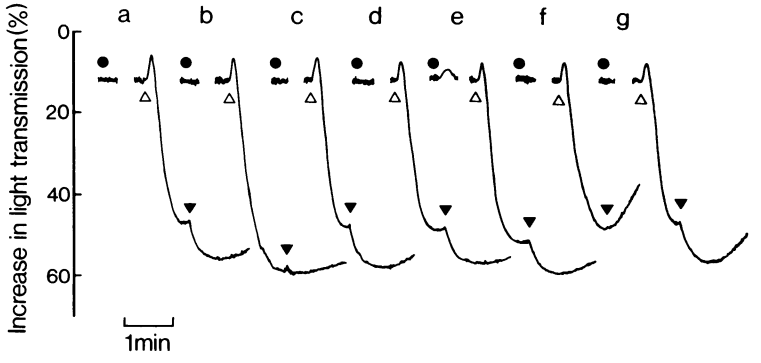

Figure 8 Effect of inhibitors on the secondary induction of aggregation of platelets in rabbit platelet-rich plasma caused by 10 μM adrenaline (▲) added after 1 μM adenosine diphosphate (△). Platelets were pre-incubated for 3 min (●) with: (a) saline control; (b) adrenaline 10 μM; (c) imipramine 0.5 μM; (d) methysergide 0.25 μM; (e) 5-hydroxytryptamine 10 μM; (f) phentolamine 5 μM; and (g) propranolol 5 μM.

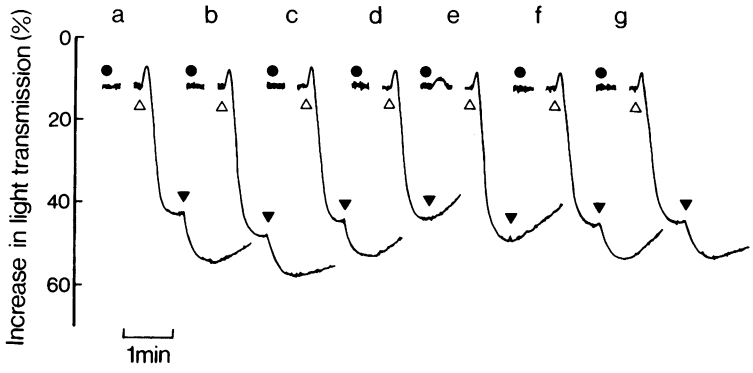

Figure 9 Effect of inhibitors on the secondary induction of aggregation by 5-hydroxytryptamine (5-HT) 5 μM (▲) of rabbit platelets aggregated by adenosine diphosphate 1 μM (△). Plasma was pre-incubated (●) for 3 min with: (a) saline control; (b) adrenaline 1 μM; (c) imipramine 0.5 μM; (d) methysergide 0.25 μM; (e) 5-HT 5 μM; (f) phentolamine 5 μM and (g) propranolol 5 μM.

change although adrenaline injected together with ADP did increase the shape change slightly. This lack of response to adrenaline is probably due to its inability to produce a rapid shape change; the shape change produced by adrenaline had a much slower time course than that produced by ADP or 5-HT.

Discussion

In some species including man, cat, dog and sheep, 5-HT causes platelets to aggregate more rapidly and completely than in other species, such as the guinea-pig and rat (Mills, 1970). The mechanism by which 5-HT causes aggregation is not yet clearly understood except that it is probably mediated via receptors similar to the D-receptors for 5-HT on smooth muscle (Michal, 1969). At one time the receptors responsible for aggregation seemed identical to those mediating 5-HT transport (Baumgartner & Born, 1969). More recent evidence indicates that the uptake and aggregating receptors are different (Born *et al.*, 1972).

The initial and immediate response of platelets to 5-HT is a rapid change in their shape, even when aggregation is not produced. This shape change can be caused by analogues of 5-HT which are not taken up by the platelets (Born *et al.*, 1972). Antagonists of 5-HT which inhibit the morphological change also inhibit aggregation by 5-HT, so that these effects are presumably mediated through the same receptor.

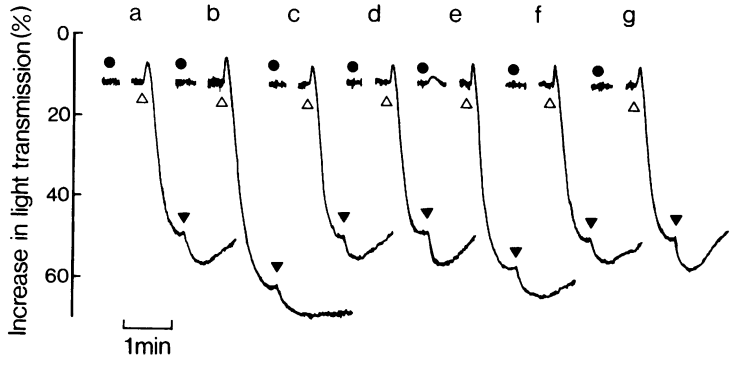

Figure 10 Effect of inhibitors on aggregation of rabbit platelets by adenosine diphosphate (ADP) 1 μM (△) followed by a second addition of ADP 1 μM (▲). Platelet-rich plasma was pre-incubated for 3 min (●) with: (a) saline control; (b) adrenaline 1 μM; (c) imipramine 0.5 μM; (d) methysergide 0.25 μM; (e) 5-HT 10 μM; (f) phentolamine 5 μM and (g) propranolol 5 μM.

Potentiation of one naturally occurring aggregating agent by another can be clearly demonstrated *in vitro*. Such interaction could also occur under *in vivo* conditions. We have demonstrated that the aggregating effect of ADP can be greatly potentiated *in vitro* by small concentrations of 5-HT. This may be of physiological importance because ADP is thought to be one of the substances causing platelet adhesion and aggregation *in vivo*. Concentrations of 5-HT and ADP which have no effect by themselves, are able to aggregate platelets when present in combination. In still smaller concentrations, these agents injected together can induce platelet shape change alone. The significance of the platelet shape change is not entirely clear, although platelets which do not change shape are defective in haemostatic function (Caen & Michel, 1972).

5-HT stored in platelets is liberated *in vitro* from the storage sites by collagen or thrombin and also by high concentrations of ADP. This release, which is an energy consuming process, is a characteristic platelet reaction and could be important in platelet behaviour *in vivo*. It has been demonstrated, for example, that 5-HT released from platelets during aggregation can accelerate and consolidate a platelet plug and formation of thrombus (Michal & Penglis, 1969).

The enhancing effect of 5-HT on shape change and aggregation induced by ADP seems to be caused by the action of 5-HT at plasma membrane receptor. Methysergide, but not imipramine, inhibits the potentiating action of 5-HT just as it blocks the direct action of the amine itself. These results indicate that 5-HT produces these effects via receptors different from those responsible for 5-HT uptake.

Platelets made refractory to ADP can still aggregate and change shape when exposed to 5-HT. In a similar manner platelets refractory to 5-HT aggregate and change shape in the presence of ADP. The effect of 5-HT is prevented by methysergide but not by imipramine. The additional aggregating and shape changing effect cannot be explained by simple synergism between the effects of the residual amounts of one agonist and the addition of another because under these conditions platelets are not sensitive to additions of high concentrations of the same agonist. These observations suggest that the effects of ADP and 5-HT are mediated by different mechanisms and that the refractory state induced through paralysis of one of these mechanisms need not prevent the effect of a different agonist. These results also support the suggestion by Evans & Gordon (1974) that refractoriness, at least as far as aggregation is concerned, does not represent a non-specific depression of platelet reactivity.

5-HT increased the extent of aggregation which had already been initiated by ADP provided that the initial effect of ADP was submaximal. Here too, methysergide specifically prevented the action of 5-HT and not that of ADP or adrenaline. Similarly, adrenaline potentiated the aggregation caused by ADP and this potentiating effect was antagonized by phentolamine; propranolol, methysergide and imipramine did not inhibit it. The additional aggregating effect caused by either adrenaline or 5-HT is therefore a specific and characteristic action of each of these substances.

These results show that platelet aggregation may be produced through stimulation of different receptors (by ADP, 5-HT or adrenaline) and that the effects can combine to give the overall response observed.

Under certain conditions, prior contact of platelets with 5-HT can render them resistant to the effect of further additions of 5-HT or other aggregating agents (Baumgartner & Born, 1969). Our results confirm these earlier observations. This inhibitory effect of 5-HT follows a time course similar to that of the active uptake process although uptake inhibitors, such as imipramine, do not interfere with the interaction of ADP and 5-HT effects on platelet aggregation and shape change. The situation is a complex one and it is not yet clear why the observed effect of 5-HT on platelets can be sometimes that of an agonist and at other times that of an antagonist or a 'stabilizing agent'. Nevertheless, this increase or depression of platelet reactivity appears to be a highly specific effect and is probably mediated at specific receptors involved with platelet activation.

F.M. is a Senior Research Fellow of the British Heart Foundation and M.M. is in receipt of a fellowship from the Wellcome Trust.

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(Received October 14, 1975.)