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5-HYDROXYTRYPTAMINE UPTAKE AND RELEASE IN RELATION TO AGGREGATION OF RABBIT PLATELETS

By H. R. BAUMGARTNER

From the Department of Experimental Medicine, F. Hoffmann-La Roche and Co. Ltd., Basle, Switzerland

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SUMMARY

1. The aggregation of blood platelets was measured *in vitro* at different time intervals after the addition of 5-hydroxytryptamine (5-HT) or of reserpine to platelet-rich plasma of untreated rabbits and of rabbits injected with reserpine and 5-HT, i.e. while the platelets were taking up 5-HT or releasing it under the influence of reserpine.

2. When 5-HT was added to stirred platelet-rich plasma the platelets aggregated reversibly within 1 min. The velocity of aggregation increased with 5-HT concentrations of 0·1–30 μM and decreased with higher concentrations.

3. (−)-Adrenaline, which alone did not produce aggregation, markedly accelerated the aggregation caused by 5-HT. The acceleration was greatest when 5-HT and adrenaline were added simultaneously.

4. 5-HT added to the platelet-rich plasma in amounts that exceeded the 5-HT capacity of the platelets progressively diminished the velocity of aggregation produced by 5-HT plus adrenaline until aggregation was completely inhibited. Smaller amounts of 5-HT produced a transient inhibition of aggregation.

5. The aggregation of platelets from reserpinized rabbits was inhibited by less 5-HT than the aggregation of platelets from normal rabbits.

6. (−)-Adrenaline aggregated platelets of untreated rabbits but not those of reserpinized rabbits or of rabbits injected with 5-HT when reserpine was added *in vitro* 1–30 min previously.

7. Platelets obtained from rabbits treated first with reserpine and subsequently injected with 5-HT were not aggregated by 5-HT plus adrenaline. During incubation *in vitro* these platelets progressively recovered their aggregability but this recovery was delayed by the monoamine oxidase inhibitor Pargyline.

8. Imipramine in concentrations which did not influence platelet aggre-

gation by adenosine diphosphate (ADP) abolished aggregation produced by 5-HT or by 5-HT plus adrenaline.

9. The inhibitory effect of adenosine on platelet aggregation was concentration-dependent and similar whether aggregation was produced by ADP, 5-HT or 5-HT plus adrenaline.

10. It is proposed that aggregation brought about by 5-HT is connected with its active uptake into the platelets and is caused by ADP formed from ATP during the active uptake of the amine. When 5-HT is no longer actively taken up, it also ceases to cause or to potentiate aggregation.

INTRODUCTION

The preceding paper has shown that rabbit platelets 'saturated' with 5-hydroxytryptamine (5-HT) are no longer aggregated by it, whether or not the 5-HT storage organelles are full or empty. Unlike human platelets, those of the rabbit are not aggregated by adrenaline but, as with human platelets, adrenaline increases the aggregation by 5-HT; conversely, 5-HT sensitizes rabbit platelets so that adrenaline causes aggregation in the presence of low concentrations of 5-HT (Baumgartner & Born, 1968, 1969).

Rabbit platelets, like those of other species, take up 5-HT from the plasma against high concentration gradients. The transfer of 5-HT into platelets occurs both by diffusion and by an active, i.e. energy-requiring mechanism (Born & Gillson, 1959; Born & Bricknell, 1959; for review see Pletscher, 1968). An active mechanism operates at the level of the plasma membrane (Pletscher, Burkhard, Tranzer & Gey, 1967; Pletscher & Tranzer, 1967); another mechanism is apparently responsible for the accumulation of 5-HT in the intracellular storage organelles (Tranzer, Da Prada & Pletscher, 1966; Da Prada, Pletscher, Tranzer & Knuchel, 1967).

Earlier work suggested a connexion between the ability of platelets to take up 5-HT and its aggregating effect (Baumgartner & Born, 1968, 1969). This paper describes experiments designed to clarify this connexion by measurements of the aggregation of rabbit platelets during the uptake and release of 5-HT.

METHODS

Sixty-five rabbits of either sex from commercial sources weighing 2·5–3·5 kg were used.

Platelet-rich plasma was prepared as described in the preceding paper (Baumgartner & Born, 1969).

Platelet counting. In each sample of plasma the concentration of platelets was determined with a Coulter Counter Model F (Coulter Electronics Ltd., Dunstable, Bedfordshire, England) before the aggregation experiments and again after approximately 1 and 2 hr. The Coulter Counter was equipped with a tube (aperture of orifice 50 μ) and a 0·5 ml. manometer. The instrument was calibrated using suspensions of polyvinyl toluene latex particles of 3·35 μ^3

(Dow from SERVA, Entwicklungslabor, 69, Heidelberg, Germany) or of Puff Ball Spores of 21·5 μ^3 (Coulter Electronics) in the counting solution (see below). All solutions used for diluting the platelet-rich plasma or the suspension of reference particles were filtered through a 0·47 μ millipore filter (Millipore Filter Corporation, Bedford, Mass. 01730) and stored in closed semi-automatic pipettes (Mano Dispensor, Kirchner, Berne, Switzerland). For counting, 0·1 ml. of platelet-rich plasma was mixed with 1·9 ml. of a solution of trisodium citrate (mol. wt. 357·17) 3·8 g, formaldehyde (10 % solution neutralized with KOH) 10 ml., distilled water to 100 ml.). In this solution the concentration and the volume of platelets remained unchanged for at least 6 hr. Next, 0·1 ml. of the suspended platelets was transferred into 24·9 ml. of the counting solution (sodium chloride 0·9 g, potassium oxalate 0·05 g, formaldehyde (10 % solution neutralized with KOH) 5 ml., distilled water to 100 ml.) and mixed by inverting the tubes against Parafilm. In this suspension the platelets were counted. The concentration of platelets in the plasma (P) was calculated as follows: $P = 10^4 \times (X-(Y+B))$ platelets/ml. plasma; where X is the count with the counter set to include all particles down to a volume of 2 μ^3 (setting X for the instrument used was B 0·5, D 4, T 30); Y is the erythrocyte count (setting Y for the instrument used was B 0·5, D 32, T 30) and B is the blank count at setting X. If the blank count at setting X was over 1000 the solutions were refiltered. The blank count at setting Y should be under 50 and was therefore negligible.

The 5-HT concentration in platelets was measured spectrophotofluorimetrically (Bogdanski, Pletscher, Brodie & Udenfriend, 1956).

Platelet aggregation in stirred plasma was measured by the photometric method (Born, 1962) and expressed as aggregation velocity in mm/min; the recorder scale was calibrated as previously described (Baumgartner & Born, 1968, 1969).

Reagents. The aggregating agents were sodium adenosine diphosphate (ADP) and 5-hydroxytryptamine (5-HT) creatinine sulphate from the Sigma Chemical Company, St Louis 18, Missouri, U.S.A.; and (−)-adrenaline dihydrogen tartrate from Fluka AG, Buchs, Switzerland. They were made up in physiological saline and added to platelet-rich plasma as previously described (Baumgartner & Born, 1968, 1969). Adenosine was from the Sigma Chemical Company, reserpine (Serpasil) from Ciba AG, Basle and Imipramine (Tofranil, from Geigy S. A., Basle. Below, the final plasma concentrations are indicated.

RESULTS

5-Hydroxytryptamine caused swelling of the platelets followed by reversible aggregation (Baumgartner & Born, 1969) which depended on the 5-HT concentration (Fig. 1). The velocity of aggregation increased with concentrations up to 30 μM-5-HT; with higher concentrations the velocity decreased again. Indeed, in thirty-five experiments 1000 μM-5-HT caused only swelling and no aggregation. This was confirmed with the phase-contrast microscope and is similar to what was found with human platelets (Baumgartner & Born, 1968).

(−)-Adrenaline did not cause aggregation in fresh platelet-rich plasma. However, when the platelet concentration fell during storage by 1–10 %, adrenaline caused slight aggregation.

Interactions between 5-hydroxytryptamine and adrenaline. Adrenaline (5 μM) added to the platelet-rich plasma 1 min after 5-HT produced aggregation; the velocity depended on the concentration of 5-HT (Fig. 1). 5-HT (5 μM), added 1 min after adrenaline, also caused aggregation.

The synergism between 5-HT and adrenaline also depended on time. The shorter the interval of time between the addition of 5-HT and that of adrenaline, the greater the aggregation velocity (Fig. 2). When the time interval was plotted logarithmically against the velocity the result was a

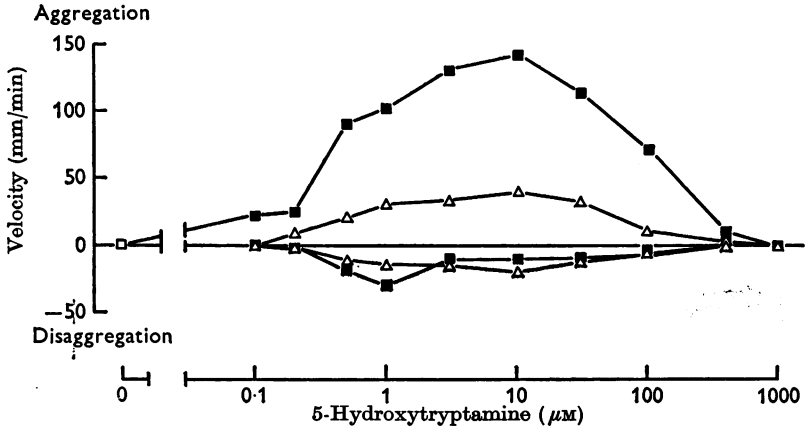

Fig. 1. Aggregation and disaggregation velocities produced by different concentrations of 5-HT, △, to which after 1 min (−)-adrenaline (5 μM) are added, ■. Velocity when adrenaline (5 μM) was added 1 min after saline (5 μl.), □. Typical of five experiments.

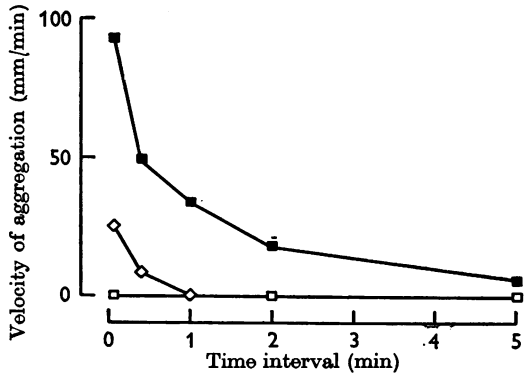

Fig. 2. Aggregation velocities produced by (−)-adrenaline (1 μM ■ and 0·2 μM ◇) added to platelet-rich plasma at different time intervals after the addition of 5-HT (5 μM). In the control experiment adrenaline (1 μM) was added at different time intervals after saline (5 μl.) □. Typical of seven experiments.

straight line; this is similar to what was found with human platelets (Baumgartner & Born, 1968). Aggregability disappeared more quickly with a lower concentration of adrenaline (Fig. 2) or with higher concentrations of previously added 5-HT. For example, adrenaline (5 μM) no longer produced aggregation when added 1 min after 5-HT (1000 μM) (Fig. 1).

Relation between 5-HT uptake and aggregation. Platelets of rabbits normally contain about 6 mμ-moles 5-HT/10^8 platelets. This can be increased *in vivo* or *in vitro* by about 50 % when the platelets are exposed to enough 5-HT.

Since aggregation by 5-HT alone was always small, use was made of the fact that the aggregation velocity was greatly increased in the presence of adrenaline. Therefore, in the subsequent experiments, the simultaneous

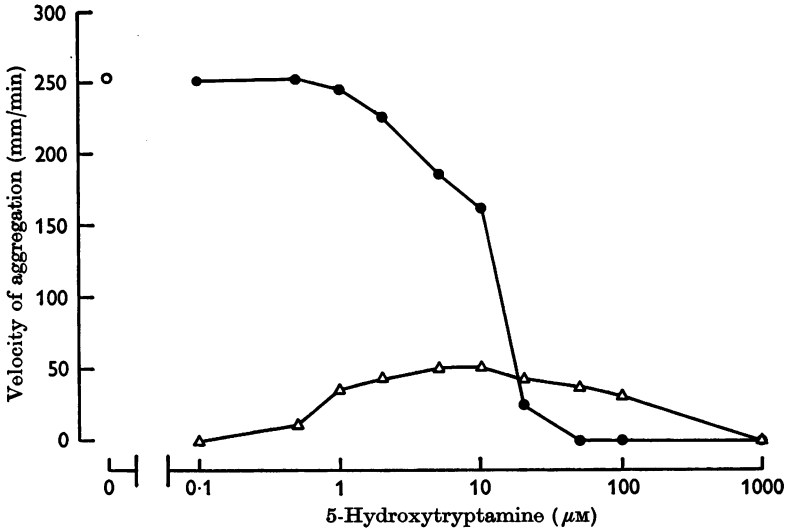

Fig. 3. Aggregation velocities produced by different concentrations of 5-hydroxytryptamine in platelet-rich plasma of an untreated rabbit △; and effect of these concentrations of 5-HT on aggregation velocities brought about by the indicator mixture [5-HT (5 μM) plus (−)-adrenaline (5 μM)] added to the same platelet-rich plasma after incubation for 1 hr at 37° C, ●. Control experiment; aggregation velocity produced by the indicator mixture 1 hr after the addition of saline (5 μl.) ○. Typical of six experiments.

addition of 5-HT and adrenaline was used as an indicator of the aggregating effectiveness of 5-HT itself. For example, in Fig. 3 the mixture of 5-HT (5 μM) and adrenaline (5 μM) produced a velocity of aggregation of 252 mm/min, whereas 5-HT (5 μM) alone gave a velocity of only 51 mm/min.

In the experiment represented in Fig. 3, 5-HT in various concentrations was added to normal platelet-rich rabbit plasma and the aggregation velocities were recorded. The dose–response curve was similar to that shown in Fig. 1. After disaggregation, which occurred within 1 min of adding 5-HT, the stirrer was removed and the plasma samples were incubated for 1 hr at 37° C. The samples were briefly stirred and the indicator mixture of 5-HT plus adrenaline was added and aggregation measured again. Figure 3

shows that incubation in concentrations of up to 1 μM-5-HT did not affect the aggregability of the platelets. Higher concentrations of 5-HT increasingly inhibited aggregation which was abolished by 50 μM or more. This result obtained *in vitro* is analogous to the abolition of aggregation by saturating platelets with 5-HT *in vivo* (Baumgartner & Born, 1969). The 5-HT concentration in plasma which produced a given degree of inhibition of aggregation depended on the concentration of the platelets. The lower the platelet concentration, the lower the 5-HT concentration required for a given inhibition.

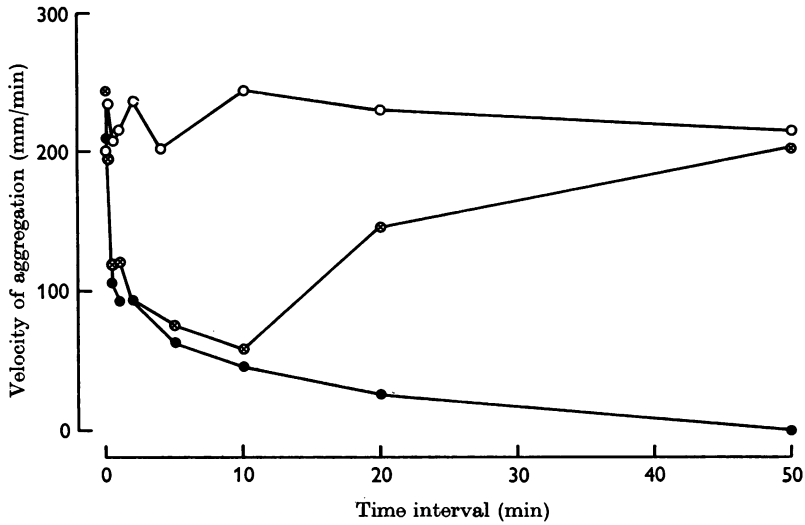

Fig. 4. Aggregation velocities produced by the indicator mixture [5-HT (5 μM) plus adrenaline (5 μM)] at different time intervals after the addition of saline (5 μl.) ○, 5-HT (5 μM) ⊗ and 5-HT (50 μM) ● at time 0. The concentration of platelets in the plasma was $5{\cdot}61 \times 10^8$/ml.; the initial concentration of 5-HT in the platelets 5·3 mμ-moles/10^8 platelets. Typical of three experiments.

In the next set of experiments (Fig. 4), aggregation velocities produced by 5-HT plus adrenaline were measured during the first 50 min after the addition of 5-HT, i.e. during the period in which it was taken up actively by the platelets. Three samples of platelet-rich plasma were incubated at 37° C; to one saline, to another 5-HT at 5 μM, and to the third 5-HT at 50 μM were added. These 5-HT concentrations were chosen because the lower one was below and the higher above the uptake capacity of the platelets (i.e. about 3 mμ-moles/10^8 platelets). In the saline controls the aggregation velocity remained about the same. With the high concentration of added 5-HT (50 μM) the velocity diminished progressively to zero after 50 min. With the lower 5-HT concentration there was a similar diminution

in the first 10 min but then the velocity increased again until, after 50 min, the platelets again aggregated as rapidly as the controls.

Aggregation by adrenaline during the release of 5-HT from platelets by reserpine. Reserpine causes the release of 5-HT from platelets *in vivo* and *in vitro* by an action on their 5-HT storage organelles (Pletscher *et al.* 1967). The released 5-HT is partially transformed into metabolites but most of it appears unchanged in the incubation medium (Bartholini & Pletscher, 1964). If the released 5-HT is taken up at the level of the plasma membrane again, in a similar way as in sympathetic nerve terminals (for review see von Euler, Rosell & Uvnäs, 1966), and if aggregation by 5-HT is indeed connected with its active uptake (Baumgartner & Born, 1969), the release of the platelets' own 5-HT might be expected to cause aggregation when, instead of the usual indicator mixture, only adrenaline is added. About 3 min after the addition of reserpine (1 μM) to platelet-rich plasma from normal rabbits, there was a slow decrease in light transmittance, indicating swelling of the platelets similar to that produced by added 5-HT.

In ten experiments adrenaline (5 μM) was added to the plasma at increasing time intervals after the reserpine (1 μM). When the interval was only a few seconds there was no aggregation of the platelets. With intervals of 1–8 min adrenaline produced aggregation with increasing velocities; with longer intervals the velocity decreased again (Fig. 5). With higher concentrations of reserpine (e.g. 10 μM) maximal velocities were brought about by adrenaline after shorter time intervals (e.g. 3 min). In control experiments adrenaline did not have such effects. Reserpine (1 μM) was also added to platelet-rich plasmas obtained from three reserpinized rabbits in which the concentration of 5-HT was less than 0·11 mμ-moles/10^8 platelets. Reserpine caused no swelling of the platelets and adrenaline (5 μM) added at different time intervals after reserpine caused no aggregation. Six other rabbits were injected intraperitoneally with 5-HT creatinine sulphate (20 mg/kg). When platelet-rich plasma was prepared 2 hr later, i.e. when the platelets were saturated with 5-HT (Baumgartner & Born, 1969) reserpine (1 μM) alone or with adrenaline (5 μM) added up to 30 min afterwards, again failed to produce aggregation.

Relation between aggregation by 5-HT and its metabolic inactivation in platelets. The evidence so far indicates that aggregation by 5-HT depends on its transport through the platelet membrane and that, under conditions in which the transport mechanism becomes saturated with 5-HT, its effectiveness as an aggregating agent disappears. It had already been shown (Baumgartner & Born, 1969) that platelets from reserpinized rabbits which were subsequently injected with 5-HT behaved like platelets saturated with 5-HT although their total content of the amine was

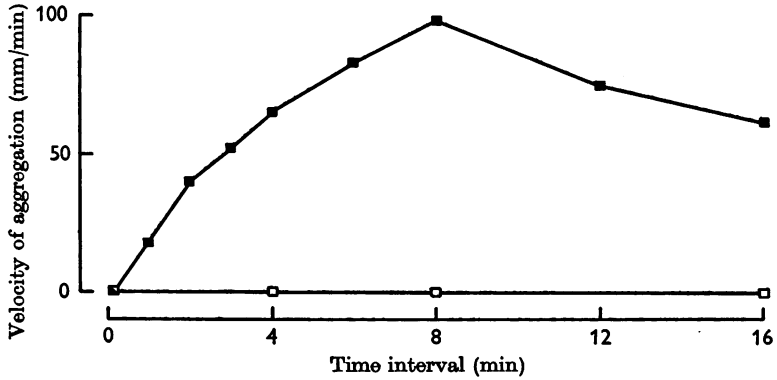

Fig. 5. Time dependent effect of reserpine on aggregation velocity produced by (−)-adrenaline. (−)-Adrenaline (5 μM) was added to platelet-rich plasma at different time intervals after HCl (0·001 N, 1 μl.) □ and after reserpine (1 μM) ■. Typical of ten experiments.

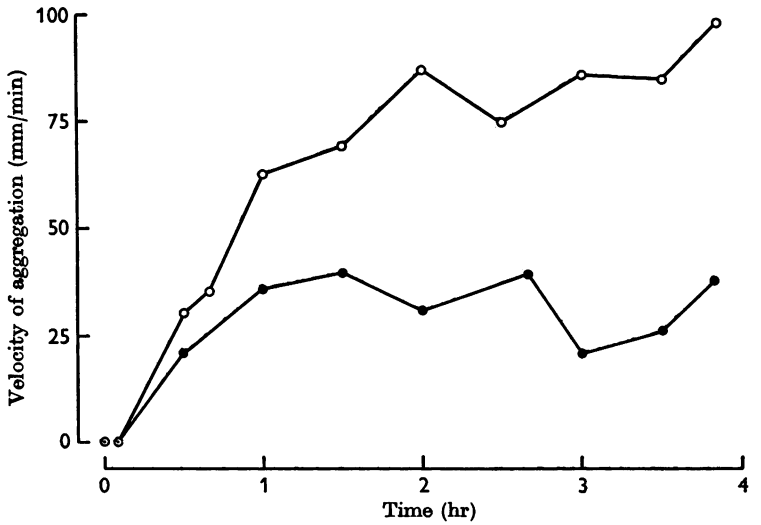

Fig. 6. Aggregation velocities produced by the indicator mixture [5-HT (5 μM) + adrenaline (5 μM)] at different times after the preparation of the platelet-rich plasma from a rabbit injected with reserpine (3 mg/kg, 21 hr before blood collection) and 5-hydroxytryptamine creatinine sulphate (20 mg/kg 2 hr before blood collection). ○ represents aggregation velocities produced with samples of platelet-rich plasma incubated at 37° C for different times after the addition of saline (2 μl.) at time 0. ● represents those after the addition of pargyline (20 μM in 2 μl. saline). In this experiment the 5-HT content at time 0 was 0·51 mμ-moles 5-HT/10^8 platelets; the concentration of platelets in the plasma was $4{\cdot}15 \times 10^8$ platelets/ml. plasma at the beginning (time 0) and $3{\cdot}88 \times 10^8$ platelets/ml. plasma at the end (time 3·8 hr) of the experiment. Typical of four experiments.

much lower. In such platelets, the metabolic inactivation of 5-HT is accelerated (Pletscher *et al.* 1967). This should result in a progressive return of aggregability by 5-HT with time as the amine is cleared from the transport mechanism in the membrane. This was tested in the following experiments.

Seven rabbits were injected intraperitoneally with reserpine (3/mg/kg) 20–25 hr before blood collection and with 5-hydroxytryptamine creatinine sulphate (20 mg/kg) 2 hr before. Platelet-rich plasma was prepared and the 5-HT content of the platelets was determined. Plasma samples were incubated in siliconized test tubes in a water bath at 37° C. The velocity of aggregation brought about by the indicator mixture was measured at intervals up to 4 hr. A result representative of seven experiments is shown in Fig. 6.

At the beginning, the platelets did not aggregate at all. With increasing duration of incubation, the platelets recovered their aggregability, after an interval that varied in different animals. In four rabbits the 5-HT content was 0·34–0·57 mμ-moles 5-HT/10^8 platelets; aggregation began to occur after 20–30 min. In an additional three rabbits the 5-HT content was 0·63–1·25 mμ-moles 5-HT/10^8 platelets; here the response only began after 2–3·5 hr. Thus, there was a rough correlation between 5-HT content and reappearance of aggregability.

The metabolism of 5-HT in platelets is inhibited by monoamine oxidase inhibitors (Bartholini, Pletscher & Bruderer, 1964; for review see Pletscher, 1968). The presence of such a drug should diminish the rate of recovery of aggregability. Figure 6 shows that this was indeed so when pargyline (20 μM) was added to samples of platelet-rich plasma at the start of incubation. These results show that the rate of 5-HT break-down in platelets can affect their aggregability by the amine.

Effect of imipramine. Imipramine inhibits the active uptake of 5-HT by platelets (Marshall, Stirling, Tait & Todrick, 1960; Fuks, Lanman & Schanker, 1964) by an action on their cell membrane (Pletscher *et al.* 1967; Pletscher & Tranzer, 1967). The effect of imipramine on aggregation velocity produced by ADP, by 5-HT alone or by 5-HT plus adrenaline is shown in Fig. 7. Concentrations of imipramine which did not influence the velocity of ADP aggregation abolished aggregation produced by 5-HT alone or by the indicator mixture.

Effect of adenosine. Adenosine inhibits the aggregation brought about by ADP (Born, 1962; Born & Cross, 1963). Figure 8 shows that adenosine inhibited the velocity of aggregation by 5-HT alone or by 5-HT plus adrenaline in similar proportions.

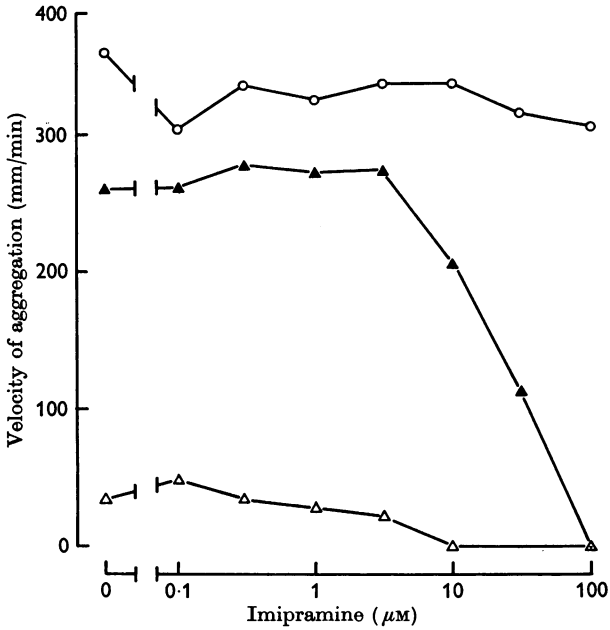

Fig. 7. Aggregation velocities produced by ADP (1 μM) ○, by 5-HT (5 μM) △ and by the indicator mixture [5-HT (5 μM) plus adrenaline (5 μM)] ▲ added 1 min after different concentrations of imipramine. Typical of five experiments.

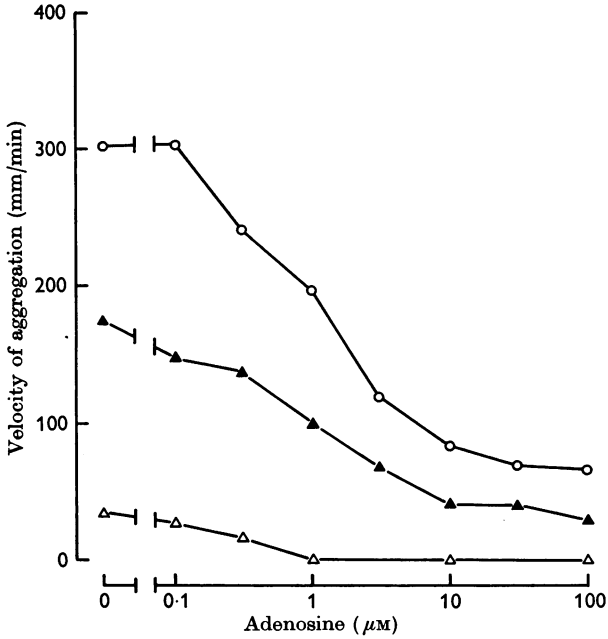

Fig. 8. Aggregation velocities produced by ADP (1 μM) ○, by 5-HT (5 μM) △ and by the indicator mixture ▲ added 1 min after different concentrations of adenosine.

DISCUSSION

This paper shows that the effects of 5-HT on aggregation of human platelets can also be demonstrated with rabbit platelets, the only difference being that adrenaline alone has no aggregating effect on the latter. Rabbit platelets are aggregated by adrenaline only when they are sensitized by 5-HT. Aggregation of human platelets by 5-HT is probably produced by the release or formation of ADP (Haslam, 1967). The abolition of aggregation by 5-HT with low concentrations of adenosine (Fig. 8) points to a similar mechanism for rabbit platelets. Thus the acceleration of 5-HT aggregation by adrenaline could well be due to the latter's potentiating effect on aggregation by ADP. Whether adrenaline augments the amount of ADP formed and/or released by 5-HT or whether it has a direct effect on the platelet membrane in the presence of 5-HT is not clear. Whatever the mechanism, the use of a mixture of 5-HT with adrenaline as an indicator for 5-HT aggregation is justified because, in the experiments presented, aggregation by the mixture was qualitatively always similar to that by 5-HT alone. Aggregation by adrenaline was brought about whether the 5-HT was added to the platelets from outside (Figs. 1 and 2) or whether it was liberated from the platelets, for example, by reserpine (Fig. 5). This finding is a further indication that reserpine does not interfere with the 5-HT uptake mechanism at the platelet surface but acts only on the 5-HT storage organelles (Pletscher *et al.* 1967; Pletscher & Tranzer, 1967).

When blood was collected from normal or reserpinized rabbits 2 hr after they had had an injection of 5-HT, their platelets were no longer aggregated by 5-HT or by 5-HT plus adrenaline; this inhibitory effect of 5-HT was independent of the total 5-HT content of the platelets (Baumgartner & Born, 1969). These findings are confirmed by the results obtained *in vitro* which are presented here. In order to reduce the aggregation velocity produced by the indicator mixture by 50% the platelets of reserpinized rabbits required a lower concentration of 5-HT in the plasma than did those of normal rabbits, i.e. $0{\cdot}79 \pm 0{\cdot}28$ (reserpinized) as opposed to $1{\cdot}82 \pm 0{\cdot}17$ mμ-moles 5-HT/10^8 platelets (normal). Thus, *in vitro* as well as *in vivo*, the uptake capacity for 5-HT of platelets from reserpinized rabbits is smaller than that of untreated rabbits.

In platelets of reserpinized rabbits incubated with 5-HT the reappearance of 5-HT storage organelles containing osmiophilic material increases in proportion to the 5-HT content estimated fluorimetrically. To demonstrate this, high concentrations of 5-HT (5·7 mM) were necessary (Tranzer *et al.* 1966; Tranzer, Da Prada & Pletscher, 1968; J. P. Tranzer, personal communication). Even with such high concentrations there was much less osmiophilic material in the storage organelles of platelets from reserpinized

rabbits than in platelets from untreated rabbits, indicating that 5-HT must also be localized elsewhere. Whether this is in the cytoplasm of the platelets or in their plasma membrane or in both has still to be demonstrated. To abolish aggregation produced by 5-HT or by the indicator mixture much lower concentrations of 5-HT were sufficient, i.e. about 20 μM-5-HT for platelets of reserpinized rabbits and 50 μM for those of untreated rabbits. Most probably the 5-HT taken up by these platelets is at least partly localized in the cytoplasm and/or in the plasma membrane.

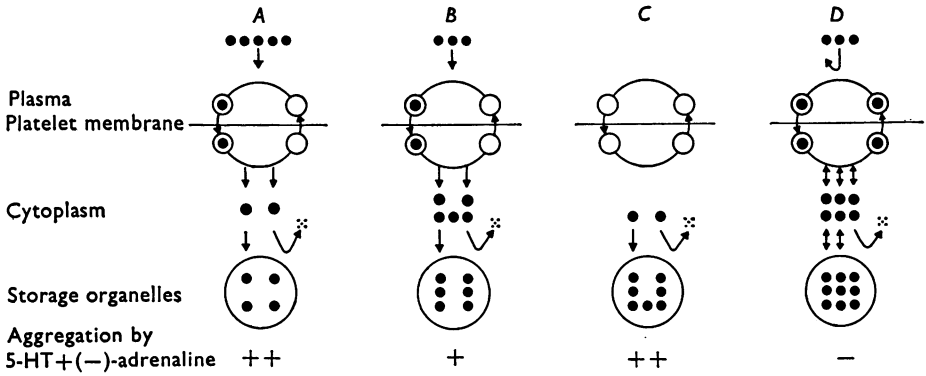

Fig. 9. Hypothesis to explain the inhibitory effects of 5-HT (5 and 50 μM) on platelet aggregation produced by the indicator mixture in four stages of the experiment represented in Fig. 5.

The symbols have the following meaning: molecule of 5-HT ● and of its metabolites :·:, receptor of the carrier system at the plasma membrane ○; carrier mechanism for active 5-HT uptake→. No aggregation −, low + and high aggregation velocity + +.

A represents the stage 0·1 min, *B* that of 10 min and *C* that of 50 min after the addition of 5-HT (5 μM); *D* that of 50 min after the addition of 5-HT (50 μM) to the platelet-rich plasma.

Baumgartner & Born (1968, 1969) have proposed the following hypothesis: 5-HT is actively taken up into platelets. Aggregation is probably caused by ADP formed from ATP during the energy-consuming uptake of 5-HT. Aggregation by 5-HT or by 5-HT plus adrenaline is abolished when the carrier mechanism for 5-HT at the level of the plasma membrane is saturated with 5-HT molecules. Whether the 5-HT is picked up from the carrier by the storage organelles or whether it is first liberated from the carrier into the cytoplasm is not yet clear. The latter is assumed in Figs. 9 and 10.

Uptake of 5-HT in vitro. Figure 9 shows how the hypothesis accounts for four stages of the experiment represented in Fig. 4. (*A*) Immediately after the addition of 5 μM-5-HT to platelet-rich plasma the carrier mechanism functions fully. The receptors return empty and bind other 5-HT mole-

cules. The addition of the indicator mixture causes high aggregation velocity. (*B*) 10 min after the addition of 5 μM-5-HT its concentration in the plasma has been reduced but uptake continues. Although the uptake capacity of the 5-HT storage organelles is not saturated, in the later stages uptake through the plasma membrane is partially saturated with 5-HT and fewer empty receptors return per unit time. The velocity of aggregation is therefore much diminished. (*C*) 50 min after the addition of 5 μM-5-HT all the amine has either been taken up by the storage organelles or decomposed in the cytoplasm of the platelets. The carrier mechanism is once again fully functional and all receptors return empty. Consequently the platelets aggregate again at the same rate as the controls. (*D*) 50 min after the addition of 50 μM-5-HT the platelets are saturated with amine. When the storage organelles are full of 5-HT its metabolic degradation is too slow to cope with the influx across the membrane. Consequently the carriers in the membrane remain full so that they do not return free, and the indicator mixture no longer causes the platelets to aggregate. These platelets correspond to those produced *in vivo* by the injection of 5-HT (Baumgartner & Born, 1969).

Release of 5-hydroxytryptamine in vitro. Figure 10*A* shows the normal condition. All 5-HT receptors are empty, the carrier mechanism is at rest and the storage organelles are not saturated. As long as no 5-HT is transported by the carrier through the platelet membrane thereby forming or releasing ADP, adrenaline alone does not cause aggregation (Fig. 1). Aggregation by adrenaline added to stirred platelet-rich plasma after reserpine (Fig. 5) is explained by the hypothesis on the additional assumption that the 5-HT released from the storage organelles by reserpine is taken up again through the plasma membrane; this is similar to the reuptake of noradrenaline in sympathetic nerve terminals of reserpinized animals (for evidence see von Euler *et al.* 1966). The finding that platelets from reserpinized animals metabolize *exogenous* 5-HT (Bartholini *et al.* 1964) supports this assumption. Therefore, it is suggested that the first 5-HT molecules to be released are bound by the receptors of the carrier system and taken up again through the plasma membrane of the platelets (Fig. 10*B*). This sensitizes the platelets to adrenaline just as if the 5-HT had been added to the plasma. Consequently the velocity of aggregation produced by adrenaline added after reserpine increases at first to an extent which depends on the amount of 5-HT liberated from the platelets (compare Figs. 5 and 1). Several minutes after adding reserpine the reabsorbed 5-HT accumulates in the cytoplasm, its uptake into the storage organelles being blocked by reserpine which, moreover, continues to release more amine. Fewer receptors return empty to bind the released 5-HT so that the velocity of aggregation by adrenaline decreases (Fig. 5). This

effect is similar to that produced by higher 5-HT concentrations (Fig. 1) or by longer time intervals after the addition of exogenous 5-HT (Fig. 2).

After the addition of reserpine *in vitro* to platelet-rich plasma of reserpinized rabbits with less than 0·11 mμ-moles 5-HT/10^8 platelets, adrenaline does not produce aggregation. According to the hypothesis there is no 5-HT present in the storage organelles that could be released (Fig. 10*C*). (*D*) After adding reserpine *in vitro* to the platelet-rich plasma of rabbits

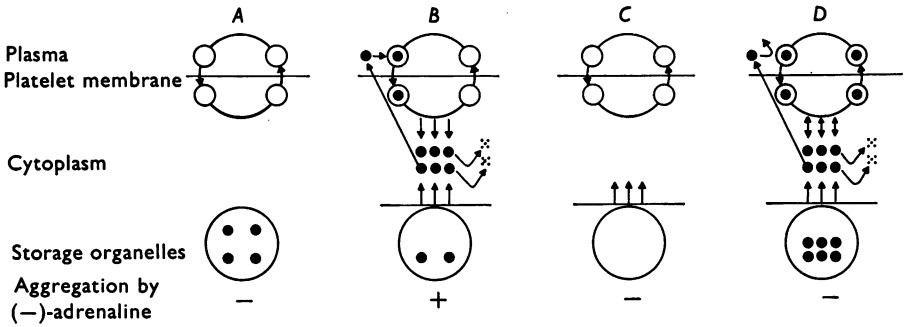

Fig. 10. Hypothesis to explain the effects of reserpine (1 μM) on platelet aggregation produced by (−)-adrenaline shown in Fig. 6.

The meaning of the symbols is stated in Fig. 9.

A platelets of a normal rabbit; *B* reserpine added *in vitro* to platelets of a normal rabbit some minutes before; *C* reserpine added *in vitro* to platelets of a reserpinized rabbit 1–30 min before and *D* reserpine added *in vitro* to platelets of a 5-HT injected rabbit 1–30 min before.

treated with 5-HT, adrenaline produces no aggregation for at least 30 min. This is because 5-HT occupies the carrier system so that what is released by reserpine finds the receptors occupied.

When reserpinized rabbits are injected with 5-HT the aggregability of their platelets returns progressively *in vitro* (Fig. 6). The 5-HT content of these platelets is low (about 0·6 mμ-moles 5-HT/10^8 platelets) and the break-down of 5-HT is accelerated (Pletscher *et al.* 1967). It would seem that 5-HT is progressively removed from the carrier system so that its capacity for transferring 5-HT through the platelet membrane increases. Consequently the velocity of aggregation increases with time (Fig. 6). Furthermore, the velocity of aggregation is decreased when the break-down of 5-HT is decreased by a monoamine oxidase inhibitor.

Imipramine and related compounds have an inhibitory effect on platelet aggregation (Mills & Roberts, 1967), which is probably due to the competitive inhibition of the active uptake of the amine at the level of the platelet membrane (Fuks *et al.* 1964; Pletscher *et al.* 1967; Pletscher & Tranzer, 1967). In terms of the hypothesis this means that the receptors

of the carrier system are occupied by imipramine (Baumgartner & Born, 1968).

The results presented in this paper are all in agreement with the hypothesis proposed in the preceding paper (Baumgartner & Born, 1969). Nevertheless, it remains a working hypothesis. For example, it has still to be demonstrated that the uptake of 5-HT into platelets is associated with the formation or release of ADP, and also that 5-HT is taken up again at the level of the platelet membrane after its release by reserpine.

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