THE EFFECT OF ASPIRIN ON 5-HYDROXYTRYPTAMINE UPTAKE AND RELEASE BY HUMAN PLATELETS

F. RENDU

Department of Hemostasis and Experimental Thrombosis, E.R.A., 335 C.N.R.S., Research Unit 150 I.N.S.E.R.M., Hôpital Lariboisière, 2, rue Ambroise Paré, 75475 Paris Cedex 10, France

The influence of aspirin on 5-hydroxytryptamine (5-HT) uptake and storage by human blood platelets has been investigated. Uptake of 5-HT was strongly inhibited. In 30 min aspirin released 50% of the 5-HT that had been incorporated into the platelets prior to the addition of the aspirin. These results are discussed in terms of possible interference with a 5-HT membrane receptor and the impairment of 5-HT storage in the dense granules.

Introduction Acetyl salicylic acid (aspirin) has been shown to be an effective inhibitor of certain platelet functions. Platelets exposed *in vivo* or *in vitro* to this drug show a normal primary aggregation when the inducer is adenosine 5'-diphosphate (ADP) or adrenaline. Moreover the secondary wave of aggregation is abolished. Aggregation induced by collagen is markedly reduced. It has thus been reported that aspirin inhibits the release reaction responsible for the secondary aggregation as induced by ADP or adrenaline (Zucker & Peterson, 1968; Weiss, Aledort & Kochwa, 1968) and causes cell membrane damage (O'Brien, 1969).

The work reported here describes the action of aspirin on 5-HT uptake by blood platelets. 5-HT is accumulated in platelets from the surrounding medium against a high concentration gradient. It is well established that the amine is taken up by the platelet by two different mechanisms: passive diffusion which reflects the concentration gradient of 5-HT outside and inside the platelet, and active uptake which requires energy and which leads to a saturation level of intracellular 5-HT (Born, Juengjaroen & Michal, 1972; Da Prada & Pletscher, 1972). Several investigations from different laboratories, most of which are studies on the smooth muscle, indicate that the 5-HT receptor may be a proteolipid (Woolley & Gommi, 1965; Marcus, Safier & Ullman, 1972; Heyningen, 1974).

The results obtained in this study clearly show that aspirin diminishes 5-HT uptake. Discussion is open as to whether the mechanism of aspirin inhibition of the amine uptake through the cell membrane could be responsible for its inhibitory effect on release.

Methods Blood was collected from normal human volunteers in a hypercitrated medium (1 volume for 10 volumes final) and centrifuged for 15 min at 100 *g*. The concentration of platelets in the platelet rich plasma (PRP) was determined by counting using a phase contrast microscope and was adjusted to 300 000 per mm^3 by dilution with autologous plasma free of platelets.

[^{14}C]-5-HT was obtained from C.E.A. (Saclay) and its uptake was measured as previously described by Rendu & Caen (1973). The final concentration of [^{14}C]-5-HT added to the PRP was 3.7 mCi/mM. To assess the inhibition of [^{14}C]-5-HT uptake by aspirin, PRP was preheated 5 min at 37°C and aspirin (Theraplix) was added at a concentration of 1.1 mM without shaking for 5 min before the incubation with [^{14}C]-5-HT. The uptake was stopped at increasing time intervals by rapid centrifugation in an Eppendorf microcentrifuge. The radioactivity in the platelet pellet was measured in an Intertechnique Model SL 4000 liquid scintillation counter by the external standard method with an efficiency of 80%. To assess the effect of aspirin on the storage of 5-HT, aspirin was also added 30 min after the addition of [^{14}C]-5-HT to the PRP with the incubation prolonged for a further 30 min period. All results are expressed as the percentage of the total [^{14}C]-5-HT added to the PRP which was incorporated within the platelet pellet.

The basic fluorescent dye mepacrine (K and K Labs) at a concentration of 50 μM was incubated with PRP for 30 min at 37°C. This dye allows the visualization of the storage bodies (Lorez, Da Prada & Pletscher, 1975). The platelets were sedimented, resuspended in saline and ACD A and examined by fluorescence microscopy using a photomicroscope (Zeiss) equipped with a super pressure lamp (HBO 200 W/4) with vertical illumination.

Results The rate of incorporation of [^{14}C]-5-HT by platelets in PRP is shown in Figure 1a. The concentration of [^{14}C]-5-HT added (5.4 μM) was such that the early part of the incorporation represented active uptake and the later part (after 30 min) passive diffusion (Da Prada & Pletscher, 1972). Pretreatment with aspirin resulted in a strong inhibition of the 5-HT incorporation. At a final concentration of 1.1 mM, aspirin inhibited both the rapid uptake and passive diffusion by 70%. The effect of aspirin on the storage of 5-HT that had already been incorporated in the platelets was also studied. PRP was incubated with

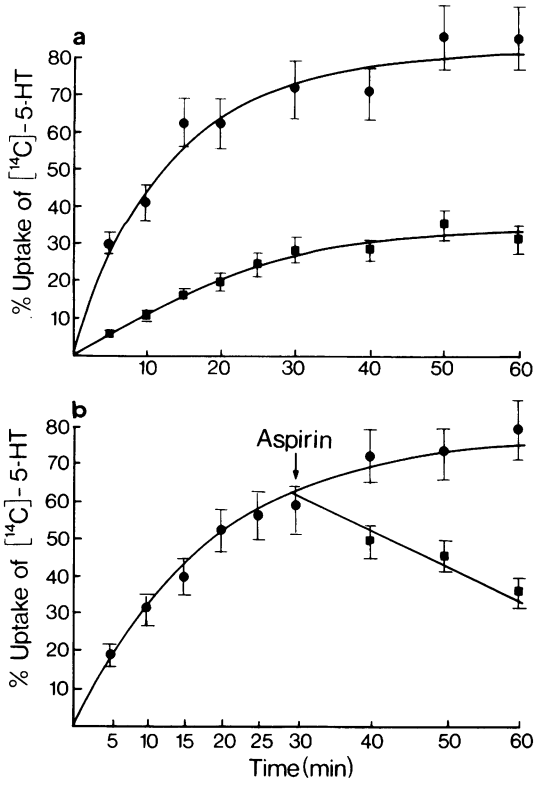

Figure 1 (a) The uptake of [^{14}C]-5-hydroxytryptamine (5-HT) by normal platelets (●) and by aspirin-(1.1 mM) treated platelets (■). (b) The uptake of [^{14}C]-5-HT by normal platelets (●) and the release of [^{14}C]-5-HT from platelets (■) after the addition of aspirin (1.1 mM).

[^{14}C]-5-HT for 30 minutes. Aspirin (1.1 mM) was added and the incubation prolonged for another 30 min period. Following the addition of the aspirin no further incorporation of [^{14}C]-5-HT was observed and there was a gradual decrease in the level of [^{14}C]-5-HT that had been incorporated; after 30 min incubation with aspirin 50% of the 5-HT that had been incorporated was liberated (Figure 1b).

Control platelets loaded *in vitro* with mepacrine (50 μM) and examined with the fluorescence microscope showed a green-yellow fluorescence which was concentrated in the dense granules. The drug thus enabled the visualization of the platelet 5-HT storage organelles. When the platelets were pretreated with aspirin before the addition of the mepacrine, the fluorescence observed in the dense granules was much weaker and was also diffused throughout the platelet.

Discussion Aspirin was observed to inhibit both the initial active and the later passive uptake of [^{14}C]-5-HT. This indicates that the action of the drug is complex, which is confirmed by the observations using mepacrine where in aspirin-treated platelets the fluorescence was diffused throughout the platelet cytoplasm and not only concentrated in or on the dense bodies. This suggests that aspirin not only has a surface effect and interferes with 5-HT uptake at the level of the receptor sites but also has a profound direct or indirect effect inside the platelet.

In addition, aspirin was unexpectedly observed to exert a 'releasing effect' which resulted in the liberation of at least 50% of the [^{14}C]-5-HT that had previously been incorporated. This aspirin-induced decrease in incorporated [^{14}C]-5-HT may be attributed to cell damage. It may, however, be a result of an impaired storage of the amine in the dense bodies, this also being compatible with the mepacrine fluorescence which is diffused throughout the platelet cytoplasm following aspirin pretreatment.

In many reports including those of Weiss *et al.* (1968); Zucker & Peterson (1968) and O'Brien (1969), the main action of aspirin on human platelets has been stated to be an inhibition of the release reaction. However, the results described here raise the question whether aspirin is really a release inhibitor. Al-Mondhiry, Marcus & Spaet (1970) have suggested that the action of aspirin may be at various sites in the cell. Recently it has been proposed that aspirin may impair the production of prostaglandins and their endoperoxide intermediates (Smith & Willis, 1971). Droller (1974) has also suggested that the inhibition of thrombin-induced cyclic adenosine 3′,5′-monophosphate formation in human platelets by aspirin is also related to an inhibition of prostaglandin synthesis. Thus the impairment of 5-HT uptake by platelets in the presence of aspirin may also be related to the inhibition of prostaglandin synthesis. Further investigations are needed to elucidate whether prostaglandin production has a role to play in 5-HT transport by blood platelets as has already been suggested by Sih, Takeguchi & Foss (1970).

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