

ASPIRIN AT THERAPEUTIC CONCENTRATIONS DOES NOT AFFECT 5-HYDROXYTRYPTAMINE UPTAKE BY PLATELETS

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Aspirin at therapeutic concentrations does not inhibit the uptake of 5-hydroxytryptamine (5-HT) by blood platelets nor induce release of 5-HT from platelets, although platelet aggregation responses to collagen and arachidonic acid (which are dependent on platelet prostaglandin synthesis) are abolished. This does not support an earlier claim that aspirin's effects on platelet function include inhibition of 5-HT transport.

Introduction Aspirin at plasma concentrations of 0.1–1 mM inhibits prostaglandin-dependent platelet aggregation and secretion (for review, see Smith & Silver, 1977). Rendu (1976) showed that 1.1 mM aspirin inhibited the uptake of 5-hydroxytryptamine (5-HT) by human platelets, and suggested that this action might contribute significantly to the spectrum of aspirin's inhibitory effects on platelets. A subsequent correction (Erratum: *Br. J. Pharmac.* (1976), 58 (1)) indicated that an aspirin concentration of 110 mM had in fact been used in these experiments, but this correction did not explain how a concentration of 110 mM aspirin (which greatly exceeds the drug's aqueous solubility) was achieved in a physiological medium, and therefore more methodological details of these experiments are needed to evaluate the results adequately. In addition, the question originally addressed by Rendu (1976) remained unanswered—does aspirin at therapeutic concentrations affect 5-HT uptake by platelets? We therefore measured the uptake of 5-HT by platelets in plasma samples with up to 1 mM aspirin added, and in samples from volunteers who had ingested aspirin. The potency of aspirin against platelet responses that are dependent on prostaglandin synthesis was tested by measuring aggregation induced by collagen and arachidonic acid in replicate samples.

Methods Human citrated platelet rich plasma (PRP) was prepared by differential centrifugation of blood from volunteers who had taken no medication for at least 10 days. Uptake of 5-HT was measured as

described by Drummond & Gordon (1976). Briefly, 0.1 ml samples of PRP were incubated with 5-hydroxy-[G-³H]-tryptamine creatinine sulphate (0.5 Ci/mmol; The Radiochemical Centre, Amersham), final concentration 1 μ M, at 37°C for 2 min except where otherwise stated. Uptake was terminated by addition of 0.5 ml ice cold iso-osmotic saline containing 0.4% (w/v) disodium edetate (EDTA) and centrifuging immediately (14,700 g; 30 seconds). The cell pellet was washed with 1 ml iso-osmotic saline and the uptake of 5-HT determined by liquid scintillation counting of the radioactivity in the pellet. A 5-HT concentration of 1 μ M was chosen because this is the apparent K_m value for 5-HT uptake by human platelets under these conditions (Gordon & Olverman, 1976).

Platelet aggregation was measured photometrically (Born, 1962) in 0.1 ml samples of PRP (Gordon & Drummond, 1974). Agents used to induce platelet aggregation were fibrillar collagen (10 μ g/ml) and arachidonic acid (1 mM). Both of these agents stimulate platelets by activating prostaglandin synthesis.

For studies *in vitro* aspirin (final concentrations 5–1000 μ M) was preincubated in PRP at 37°C for 10 minutes. For studies *ex vivo*, PRP was prepared from volunteers immediately before and 2 h after ingestion of 900 mg aspirin. The platelet count in each sample was adjusted to 3.5×10^8 cells/ml.

Results Uptake of 5-HT 2 h after ingestion of aspirin was unaltered (Figure 1a) although platelet aggregation responses to collagen and arachidonic acid were abolished (Figure 1b). When tested *in vitro*, aspirin in concentrations of 25–1000 μ M progressively inhibited aggregation induced by collagen and arachidonic acid, but did not affect 5-HT uptake (Figure 1c).

Since Rendu (1976) had shown that the effect of aspirin on 5-HT transport was associated with 5-HT being released from the platelets, we investigated the effects of 1.1 mM aspirin on platelets preloaded with [³H]-5-HT. Samples of PRP were incubated for 30 min at 37°C with 1 μ M [³H]-5-HT. The amount of

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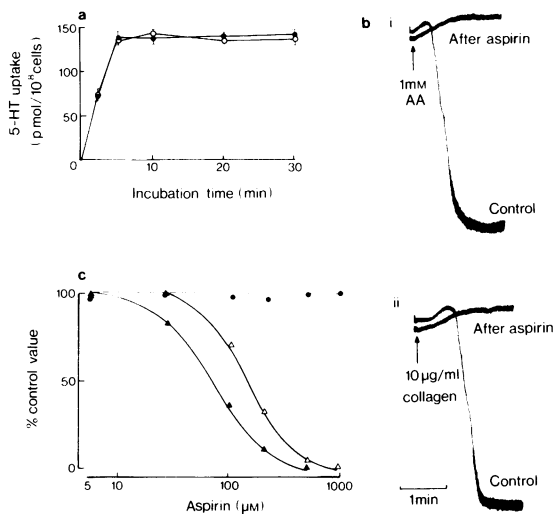


Figure 1 Effect of aspirin on the uptake of 5-hydroxytryptamine (5-HT) by human platelets and on platelet aggregation induced by collagen (10 μg/ml) and arachidonic acid (1 mM). (a) 5-HT uptake measured before (●) and 2 h after (○) ingestion of 900 mg aspirin. Points shown are means, vertical bars show s.e. mean, $n=6$. Samples of PRP were incubated at 37°C with 1 μM [³H]-5-HT, and uptake was determined as described in the text. (b) Platelet aggregation responses to (i) 1 mM arachidonic acid (AA) and (ii) to 10 μg/ml collagen, measured before and 2 h after ingestion of 900 mg aspirin. Aggregating agents were added at the arrows, and responses measured photometrically (see text). (c) 5-HT uptake and platelet aggregation responses measured after 10 min preincubation with aspirin (5–1000 μM). Replicate samples of PRP were taken for determination of 5-HT uptake (●) as described in the text, and for measurement of aggregation responses to 10 μg/ml collagen (▲) and to 1 mM arachidonic acid (Δ). All measurements are expressed as percentage of values obtained in control samples containing an equivalent volume of iso-osmotic saline. Points shown are means of triplicate determinations.

[³H]-5-HT transported was (mean ± s.e.) 143 ± 3 pmol/10⁸ cells ($n=6$). Addition of 1.1 mM aspirin did not release any [³H]-5-HT: 30 min after adding aspirin

the platelet content of [³H]-5-HT was 146 ± 4 pmol/10⁸ cells, and in matched control samples the platelet content of [³H]-5-HT, 30 min after adding an equivalent volume of iso-osmotic saline, was 144 ± 3 pmol/10⁸ cells.

Discussion Therapeutic doses of aspirin result in plasma levels of the drug between 0.1 and 0.5 mM, but concentrations above 1 mM are not achieved even after high doses. Results from many laboratories (including our own) have shown that aspirin at therapeutic concentrations can abolish platelet responses that are dependent on prostaglandin biosynthesis, but the results of our present study clearly demonstrate that biologically significant concentrations of aspirin do not inhibit 5-HT uptake or stimulate 5-HT secretion.

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