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Malondialdehyde production and the release reaction in rat blood platelets: inhibition by aspirin and indomethacin *ex vivo*

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Intermediates in the biosynthesis of prostaglandins (PGs) play an important role in the secretion of platelet granule constituents, such as 5-hydroxytryptamine (5-HT), induced by collagen. Non-steroidal anti-inflammatory drugs (e.g. aspirin and indomethacin) are potent inhibitors of platelet PG synthesis and the platelet release reaction. The extent to which the collagen-induced platelet release reaction may depend on mechanisms independent of PG synthetase is not clear at present. Arachidonic acid, however (which can also induce the platelet release reaction), is the main precursor for platelet PG synthesis and therefore might be expected to act exclusively by a PG synthetase-dependent mechanism. One of the products of PG synthetase is malondialdehyde (MDA) which can be measured colorimetrically by its reaction with thiobarbituric acid (Flower, Cheung & Cushman, 1973). In the present study, we have compared the extent and duration of the effects of aspirin and indomethacin *ex vivo* on MDA production by rat platelets, and 5-HT release induced by collagen and arachidonic acid.

Rats were bled 90 min, 1, 2, 3 and 4 days after oral dosing and platelet-rich plasma. (PRP) was prepared as previously described (Gordon & Drummond, 1974). Platelet suspensions were prepared by centrifuging PRP at 850 *g* for 8 min in the presence of 7.5 mM disodium ethylenediamine tetraacetate (EDTA) and resuspending the cell pellets in phosphate buffered saline (pH 7.4). MDA production was assayed by the method of Stuart, Murphy & Oski (1975), after incubating 0.25 ml samples of platelet suspensions with 33 μM arachidonic acid (10 min; 37°C). 5-HT release induced by collagen (10 and 33 μg/ml) and arachidonic acid (1 mM) was measured in 0.1 ml of PRP prelabelled with 1 μM [^{3}H] 5-HT.

Aspirin (200 mg/kg p.o.) after 90 min abolished MDA production and 5-HT release induced by arachidonic acid and collagen (10 μg/ml). Release induced by collagen (33 μg/ml) was inhibited by about 50%. After 1 day all responses were still significantly inhibited. Release induced by both concentrations of collagen had returned to control values after 2 days, whereas arachidonic acid-induced release returned after 3 days. MDA production was still slightly inhibited after 4 days.

Indomethacin (8 mg/kg p.o.) after 90 min inhibited MDA production by 46%. Release induced by arachidonic acid, collagen (10 μg/ml), and collagen (33 μg/ml) was inhibited by 53%, 63% and 34% respectively (mean values from groups of 4 animals). After 1 day the release induced by collagen (10 μg/ml) was still inhibited by almost 70% but the other responses were inhibited by only ten to twenty per cent. All responses had returned to control values after 2 days.

The life span of rat platelets is about 4–5 days (Odell, Tausche & Gude, 1955), which correlates with the duration of aspirin's inhibitory effect on MDA production. A similar correlation has been found in man (Stuart *et al.*, 1975). Indomethacin's inhibitory effect on rat platelets does not persist throughout the life of the cell, which again is similar to its effect in man (Kocsis, Hernadovich, Silver, Smith & Ingerman, 1973).

Inhibition of MDA production by aspirin persisted after the platelet response to collagen had returned to normal, but this pattern was not observed with indomethacin: indeed, the response to the lower collagen concentration was still substantially inhibited by indomethacin when MDA production had almost returned to the control value. Release induced by arachidonic acid was more closely correlated with MDA production.

The relative contributions of PG synthetase dependent and independent mechanisms in the collagen-induced platelet release reaction remain to be evaluated but because PG synthetase inhibitors are at

present being evaluated as antithrombotic agents (Elwood, Cochrane, Burr, Sweetnam, Williams, Welsby, Hughes & Renten, 1974), this may be of considerable physiological importance.

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Transport of 5-hydroxytryptamine by rat and human platelets

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Blood platelets transport 5-hydroxytryptamine (5-HT) from the plasma and store it in granules as a macromolecular complex with ATP and calcium (Born & Gillson, 1959; Da Prada & Pletscher, 1968). This transport process is similar to that of serotonergic neurones, and for this reason platelets have been used with some success as models of aminergic neurones (Sneddon, 1973), particularly for investigating the effects of drugs on 5-HT uptake. Most investigations of 5-HT uptake by platelets have used human platelet-rich plasma (PRP), and as much of the experimental work on serotonergic neurones has been carried out in rats we wished to compare the characteristics of 5-HT transport by rat and human platelets.

Uptake of 5-HT by rat platelets was measured as described by Drummond & Gordon (1976), and the same technique was used for studies with human PRP. The initial rate of 5-HT was much faster in rat than in human PRP, and at submicromolar substrate concentrations the linear component of uptake in both species lasted for at most 10 seconds. In rat PRP, 5-HT uptake was so rapid that substrate depletion (with consequent limitation of uptake) occurred within 60 s, but this could be greatly reduced by diluting the PRP with cell-free plasma, to lower the platelet count from its normal value of 10^9 cells/ml to about 2×10^8 cells/ml (comparable with the platelet count in human PRP). Lineweaver-Burk analysis of uptake after 10 s incubation of PRP with 5-HT (0.3–2.5 μM) gave apparent K_m values of 1.0 μM for human platelets and 0.75 μM for rat. Values for V_{max} were 20 pmol 10^8 cells^{-1} 10 s^{-1} for human platelets and 60 pmol 10^8 cells^{-1} 10 s^{-1} for rat.

Uptake of 5-HT by both rat and human platelet was extremely temperature sensitive over the temperature range 17–37°C. At lower temperatures, human platelets transported very little 5-HT, whereas rat platelets apparently retained the ability to transport significant amounts at temperatures as low as 7°C. Since this transport by rat platelets at low temperatures was not inhibited by chlorimipramine, however, it seems likely that it represents facilitated diffusion rather than active uptake.

Tricyclic antidepressants, which are believed to exert their clinical effects partly by inhibiting 5-HT transport in serotonergic neurones, are also competitive inhibitors of platelet 5-HT uptake. In some studies, however, these compounds have been tested by incubating 5-HT in PRP for 1 min or more (Buczko, De Gaetano & Garattini, 1975; Horng & Wong, 1976), and because uptake then is much slower than the initial rate (especially in rat PRP) the potency of competitive inhibitors may be underestimated. For example, when we measured the uptake of 0.8 μM 5-HT by rat PRP after 10 s incubation at 37°C, the IC_{50} value for chlorimipramine was 0.1 μM, but when this experiment was repeated, incubating the 5-HT in PRP for 3 min, the IC_{50} value for chlorimipramine was 2.0 μM.

Uptake of 5-HT by rat platelets is inhibited by ADP (Drummond & Gordon, 1976). We found that ADP was a less potent inhibitor in human than in rat PRP, when uptake was measured after 10 s incubation of 5-