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Stimulation of platelets by bis-enoic prostaglandins

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Prostaglandins (PGs) can stimulate or inhibit platelets: PGE_1 and PGD_2 are inhibitory (Kloeze, 1967; Smith, Silver, Ingerman & Kocsis, 1974), 11-deoxy-15-methyl-15RS-PGE_2 (Wy-17,186) stimulates aggregation directly (Fenichel, Stokes & Alburn, 1975), and PGE_2 stimulates or inhibits depending on the conditions (MacIntyre & Gordon, 1975).

We investigated the effects of $PGF_{2\alpha}$, PGE_2, Wy-17,186, 16,16-dimethyl-PGE_2, 15(R)-15-methyl-PGE_2 and 15(S)-15-methyl-PGE_2 methyl ester in human and pig platelet-rich plasma (PRP) as described previously (Gordon & Drummond, 1974; MacIntyre & Gordon, 1975). All these compounds except 15(R)-15-methyl PGE_2 induced platelet aggregation in pig heparinized PRP; minimum active concentrations (μg/ml) were respectively 2.0, 0.8, 0.6, 0.2, >100, 3.0.

In human citrated PRP, only 16,16-dimethyl-PGE_2 and Wy-17,186 induced substantial aggregation; 15(S)-15-methyl-PGE_2 methyl ester had a slight effect. At 3 μg/ml, 16,16-dimethyl-PGE_2 and Wy-17,186 released 40–70% of platelet granule constituents (measured by prelabelling with [^{14}C]-serotonin) and less than 10% of cytoplasmic constituents (measured by prelabelling with [^{3}H]-adenine). Aggregation and release induced by the methylated prostaglandins were inhibited by PGD_2, PGE_1, PGE_2, $PGF_{2\alpha}$ and 15(R)-15-methyl-PGE_2: against aggregation induced by ADP, PGD_2 and PGE_1 were similarly effective, but the other inhibitory PGs were much less active (Table 1).

These findings support the concept that blood platelets can provide a valuable model for studying the characteristics of cellular receptors for PGs. Methylation at positions 15 and 16, and the stereospecificity of this substitution, clearly affect the stimulatory or inhibitory potency of prostaglandins. Furthermore, the differential potency of inhibitory PGs against platelet aggregation induced by PGE_2 derivatives or ADP suggests that the site of action may be at the stimulatory receptor in the former case, but at a separate site (e.g. adenylate cyclase) in the latter.

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Table 1 Inhibition of human platelet aggregation by prostaglandins

	IC_{50} values (μg/ml) Aggregating Agent		
Prostaglandin	*16,16-diMe-PGE_2 (3 μg/ml)*	*Wy-17,186 (3 μg/ml)*	*ADP (1 μM)*
PGD_2	0.06	0.07	0.01
PGE_1	0.02	0.06	0.01
PGE_2	0.20	0.80	10
15-Me-15(R)-PGE_2	50	45	>100
$PGF_{2\alpha}$	2.5	4.0	⩾100

Results are mean values of triplicate determinations.

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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