Potentiation of ADP-ase activity of aortic rings by prostaglandin synthetase inhibitors and PGE_1

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Lieberman & Lewis (1977) have demonstrated a Ca^{++}, Mg^{++} dependent ADP-ase located on the plasma membrane of rabbit aorta cell preparations which can inhibit ADP-induced platelet aggregation. We now report the presence of an ADP-ase activity in aortic rings, and that this activity is potentiated by two cyclo-oxygenase inhibitors, indomethacin and aspirin, and also by prostaglandin E_1.

Rabbit platelet-rich plasma (PRP) was prepared and the trimmed thoracic aorta was washed in saline and stored in 50 mM tris-HCl buffer at pH 7.4 at room temperature. Incubation mixtures (200 µl) containing either buffer alone (50 mM tris-HCl, pH 7.4 at 37°C) or buffer and 10^{-4} M ADP (0.12 µCi [8-^{14}C]-ADP per 200 µl) and either 0.1% v/v ethanol (drug vehicle) or indomethacin (6 µg/ml), or aspirin (100 µg/ml) or PGE_1 (100 ng/ml) were all pre-incubated at 37°C for 10 minutes. At time 0 min, a freshly cut and rewashed aortic ring (9.47 ± 0.52 to 7.25 ± 1.08 mg (mean ± s.e. mean)) was added to each incubate. At 0, 20 and 40 min a 45 µl aliquot of the incubate was removed.

The amount of platelet aggregation produced by 20 µl of this aliquot was calculated from ADP dose-response curves and expressed as percentage reduction of initial ADP concentration. The remaining 25 µl was acidified to pH 3.5 with 0.2 N perchloric acid and 20 µl was subjected to thin-layer chromatography on plastic backed silica gel plates with fluorescent indicator (Norman, Follett & Hector, 1974). Standard metabolites (ATP, ADP, AMP, adenosine, hypoxanthine, inosine, adenine) were co-chromatographed with the aliquots to permit u.v. visualization such that the area occupied by each could be marked, cut out and the amount of radioactivity indicating labelled metabolite determined for each area.

The concentration (mean ± s.e. mean, $n=5$) of ADP (nmoles [8-^{14}C]-ADP/ml) present in the incubation mixture at 0, 20 and 40 min are shown in Table 1. There is a time-dependent reduction in ADP concentration in the control experiments, which is potentiated by indomethacin, aspirin, and PGE_1, by 28, 40 and 86% respectively. In the control incubations, as the concentration of ADP decreased, that of AMP and inosine increased while the concentration of adenosine remained low, 3% of total radioactivity; in the aspirin, indomethacin and PGE_1 treated rings, the adenosine concentration was approx. 10% of total radioactivity at 20 minutes. When the PRP and rings were both treated with indomethacin (6 µg/ml), there was still inhibition (47.85 ± 1.5% (mean ± s.e. mean, $n=5$)) of ADP-induced platelet aggregation when the ring (≃15 mg) was added to PRP. This inhibitory activity disappeared (14.4 ± 5.28%, $n=5$) when the aortic rings were boiled.

Ho, Hermann, Towner & Walter (1977) and Bunting, Gryglewski, Moncada & Vane (1976) have demonstrated that aortic microsomes and arterial rings alone can inhibit platelet aggregation and have used this as evidence for prostacyclin generation. The existence of a plasma membrane ADP-ase may be another natural mechanism for limiting thrombotic events, and the mechanism is potentiated by three agents which also inhibit platelet aggregation.

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Table 1 Effect of drugs on the removal of ADP by aortic rings

Treatment	*nmoles [8-^{14}C]-ADP/ml			**% potentiation	P	Ring wts. mg
	0 min	20 min	40 min			
Control	93.06 ± 0.47	63.57 ± 0.98	31.17 ± 2.28			9.47 ± 0.52
Indomethacin (6 µg/ml)	94.41 ± 0.19	56.46 ± 3.31	26.44 ± 2.98	28.68 ± 11.2	> 0.05	7.25 ± 1.08
Aspirin (100 µg/ml)	93.82 ± 0.52	50.80 ± 2.3	16.13 ± 1.38	45.80 ± 5.3	< 0.001	7.69 ± 0.45
PGE_1 (100 ng/ml)	96.40 ± 0.34	41.52 ± 2.3	9.33 ± 1.86	86.0 ± 4.2	< 0.001	8.25 ± 0.50

Each value in the table is the mean (± s.e. mean) of 5 experiments.

* Concentration of ADP (nmoles [8-^{14}C]-ADP/ml) obtained after separation on silica gel plates of 20 µl of each acidified aliquot obtained as described in the text.

** % potentiation refers to the increase in ADP removed at 20 min by the drug-treated rings when compared with the amount of ADP removed by the control rings. Results were analysed by the student *t* test.

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A comparison of the effects of stable endoperoxide analogues with PGE_2 and $PGF_{2\alpha}$ on rat gastric secretion, *in vivo* and *in vitro*

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Arachidonic acid (AA), like PGE_2, reduces rat gastric secretion and this effect is antagonized by indomethacin (Frame, Main & Melarange, 1977), an inhibitor of cyclo-oxygenase. Since the cyclic endoperoxide intermediates may themselves be important physiologically and may account for at least some of the action of AA, we have investigated the effects of two stable analogues of endoperoxide, Upjohn 44069 and 46619 (Bundy, 1975) on gastric secretion *in vivo* and *in vitro*.

In vivo studies were conducted using the lumen-perfused rat stomach preparation. Acid secretion, mucosal blood flow (MBF) (aniline clearance, Main & Whittle, 1973) and blood pressure (BP) were recorded. Acid secretion was stimulated by pentagastrin (0.33 $\mu g\ kg^{-1}\ min^{-1}$ infused intravenously throughout the experiment) and drugs were given by i.v. infusion for 30 min periods. The results are the mean $\pm$ s.e. mean of four observations.

In doses of 5 and 12.5 $\mu g\ kg^{-1}\ min^{-1}$, U44069 caused a maximum inhibition of acid secretion of $22.6 \pm 5.9\%$ and $33.3 \pm 9.7\%$ respectively while U46619 caused inhibition of $33.0 \pm 7.8\%$ and $53.3 \pm 4.8\%$ respectively. PGE_2 in doses of 0.25 and 0.5 $\mu g\ kg^{-1}\ min^{-1}$ inhibited acid secretion by $26.4 \pm 8.5\%$ and $33.8 \pm 6.2\%$ respectively. $PGF_{2\alpha}$ (5 and 12.5 $\mu g\ kg^{-1}\ min^{-1}$) produced variable effects on secretion (increased in 5 experiments and decreased in 3 experiments). The results suggest that U44069 and U46619 are qualitatively similar to PGE_2 but are respectively about 25 and 10 times less potent. Inhibition of acid secretion was always associated with an increase in the MBF/acid ratio.

The effects of endoperoxides on B.P. were mixed at the lower dose-level. The higher dose, however, predominantly decreased BP, as did PGE_2. $PGF_{2\alpha}$ usually produced a small initial increase in BP followed by a fall.

In vitro studies were carried out on the isolated mucosa (Hearn & Main, 1975). Acid secretion was measured by pH stat and secretory responses to histamine (2.6 to 5.2×10^{-5} M present in the serosal solution for 30 min) were obtained at 75 min intervals. Both U44069 and U46619, in concentrations of 0.8 to 1.64×10^{-6}M, added to the serosal solution for 15 min prior to histamine, inhibited the responses to histamine and were approximately 10–20 times less potent than PGE_2.

The results show that endoperoxide analogues have qualitatively similar effects to PGE_2 on acid secretion but are considerably less active. If the activity of the analogues reflects that of the natural endoperoxides, the latter are unlikely to contribute significantly to the inhibitory effect of AA. The contribution of other products of cyclo-oxygenase to this inhibitory effect remains to be established since the effects of thromboxanes and PGI_2 on gastric secretion have not yet been reported.

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