The effect of pulmonary metabolites of prostaglandins E_1, E_2 and $F_{2\alpha}$ on ADP-induced aggregation of human and rabbit platelets

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Prostaglandins (PGs) have been extensively examined as regulators of platelet activity since Kloeze (1967, 1969) demonstrated that PGE_1 was a potent inhibitor of platelet aggregation. Recently the pulmonary metabolites of various prostaglandins have been shown to have considerable biological activity, e.g. on the bronchioles (Dawson, Lewis, McMahon & Sweatman, 1974), uterus (Crutchley & Piper, 1976) and blood pressure (Änggard, 1966).

In the present study, a comparison has been made of nine pulmonary metabolites on ADP-induced aggregation of human and rabbit platelets. Human and rabbit platelet-rich plasma (PRP) were prepared by the method of Holmsen, Storm & Day (1972). The platelet counts were adjusted to 300,000/μl and 400,000/μl in human and rabbit PRP respectively. Platelet aggregation was measured as an increase in light transmission as described by Born & Cross (1963). In order to test the normal reactivity of the platelets, with every batch of PRP a dose-response curve to ADP was obtained. A concentration of 1.3×10^{-6} ADP which produced about a 50% response, was selected for testing inhibition of aggregation. Following a 2 min pre-incubation period of the PRP to stabilize the temperature at 37°C, a constant volume (50 μl) of vehicle or prostaglandin (final concentrations 5×10^{-9} M to 5×10^{-5} M) was added, followed 3 min later by ADP. Each concentration of PG was tested 3–5 times and alternating control tests were made with vehicle alone.

These results were expressed as percentage of control response. Dose-response curves were plotted and the IC_{50} values (μM) are shown in Table 1.

These findings confirm that in man and rabbit, PGE_1 is a potent inhibitor of aggregation. However, the 13,14-dihydro-PGE_1 is also active in both species and is more than twice as active as the parent PGE_1 in man. While in high concentrations PGE_2 inhibits aggregation, in lower concentrations it causes potentiation of ADP-induced aggregation in man and rabbit. On the other hand, 13,14-dihydro-PGE_2 potentiates aggregation only in man. The extent to which human platelets metabolize prostaglandins and the effect of the metabolites on other platelet function tests are to be investigated.

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References

ÄNGGÅRD, E. (1966). The biological activities of three metabolites of prostaglandin E_1. *Acta physiol. scand.*, **66**, 509–510.

BORN, G.V.R. & CROSS, M.J. (1963). The aggregation of blood platelets. *J. Physiol. (Lond.)*, **168**, 178–185.

Table 1 Comparison of prostaglandin metabolites on ADP-induced platelet aggregation.

Prostaglandin	IC_{50} values (μM) Rabbit	Man
PGE_1	0.039	0.08
15-keto-PGE_1	>50.0	>50.0
13,14-dihydro-15-keto-PGE_1	>50.0	>50.0
13,14-dihydro-PGE_1	0.19	0.037
PGE_2	28.0*	13.0**
15-keto-PGE_2	>50.0	>50.0
13,14-dihydro-15-keto-PGE_2	>50.0	>50.0
13,14-dihydro-PGE_2	50.0	20.0**
$PGF_{2\alpha}$		10.0
15-keto-$PGF_{2\alpha}$	N.T.	>50.0
13,14-dihydro-15-keto-$PGF_{2\alpha}$		>50.0
13,14-dihydro-$PGF_{2\alpha}$		>50.0

* 0.25–2.7 μM and ** 0.08–0.26 μM produce potentiation ($P < 0.05$) of platelet aggregation. N.T. = not tested.

CRUTCHLEY, D.J. & PIPER, P.J. (1976). Bioassay and thin layer chromatography of prostaglandins and their pulmonary metabolites. *Br. J. Pharmac.* (in press).

DAWSON, W., LEWIS, R.L., McMAHON, R.E. & SWEATMAN, W.J.F. (1974). Potent bronchoconstrictor activity of 15-keto-prostaglandin $F_{2\alpha}$. *Nature (Lond.)*, **250**, 331–332.

HOLMSEN, H., STORM, E. & DAY, H.I. (1972). Determination of ATP and ADP in blood platelets. *Analyt. Biochem.*, **46**, 489–501.

KLOEZE, J. (1967). Influence of prostaglandins on platelet adhesiveness and platelet aggregation. Nobel Symposium 2, Stockholm, June 1966. In *Prostaglandins*, ed. S. Bergstrom & B. Samuelsson, pp. 241–252. Stockholm: Almqvist & Wiksell.

KLOEZE, J. (1969). Relationship between chemical structures and platelet aggregation activity of prostaglandins. *Biochim. Biophys. Acta*, **187**, 285–292.

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.