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References

BONTA, I.L. & PARNHAM, M.J. (1977). Prostaglandins and chronic inflammation. *Biochem. Pharmac.* (in press).

BONTA, I.L., PARNHAM, M.J. & ADOLFS, M.J.P. (1977). Reduced exudation and increased tissue proliferation during chronic inflammation in rats deprived of endogenous prostaglandin precursors. *Prostaglandins*, **14**, 295–308.

CHANG, W.-C. & TSURUFUJI, S. (1976). Differences in the mode of exudative reaction between early phase and late phase of carrageenin-induced inflammation in rats. *Eur. J. Pharmac.*, **36**, 7–14.

DiPASQUALE, G., RASSAERT, C., RICHTER, R., WELAJ, P. & TRIPP, L. (1973). Influence of prostaglandins (PG)E_2 and F_{2alpha} on the inflammatory process. *Prostaglandins*, **3**, 741–757.

HIGGS, G.A., HARVEY, E.A., FERREIRA, S.H. & VANE, J.R. (1976). The effects of antiinflammatory drugs on the production of prostaglandins *in vivo*, in *Advances in Prostaglandin and Thromboxane Research*, Vol. 1. Ed. Samuelsson, B. & Paoletti, R., pp. 105–110. Raven Press, New York.

Comparison of the effects of prostaglandin analogues on rabbit platelets, rabbit isolated vascular tissues and rabbit skin microvasculature

D.E. MacINTYRE, J. WESTWICK & T.J. WILLIAMS

Department of Pathology, Tennis Court Road, Cambridge and Department of Pharmacology, Royal College of Surgeons, Lincoln's Inn Fields, London WC2A 3PN

The unstable prostaglandin endoperoxides produce platelet aggregation (Hamberg & Samuelsson, 1974), contract rabbit aorta (Vargaftig & Zirinis, 1973, Willis, 1974), and produce a transient vasoconstriction in the microvasculature followed by a dilatation (Lewis, Westwick & Williams, 1977. Prostaglandin E_2 produces no platelet aggregation, has no activity on rabbit aorta, but it is a potent vasodilator. We have examined a range of stable prostaglandin analogues in order to evaluate their structure-activity relationships to the parent compounds. This has been investigated *in vitro* using rabbit platelet aggregation (Gordon & Drummond, 1974) and isolated vascular smooth muscle (Furchgott & Badrakom, 1953; Bunting, Moncada & Vane, 1976), and *in vitro* by measuring rabbit skin blood flow (Williams, 1976).

The bicyclic compounds resembled the endoperoxides in that they produced aggregation and rabbit aorta contracting activity (see Table 1). ICI 86841 showed less aggregating activity and less activity on blood flow (significant only at high doses). All the bicyclic compounds tested, with the notable exception of azo PGH_2 were active on the rabbit aorta. From the above results it might appear that reductions in blood flow were due to thrombus formation *in vivo*. However, Wy 40659 was a potent aggregatory substance (equipotent with Wy 19110), with no activity on blood flow. Thus, in general, an observed reduction in blood-flow probably represents vasoconstriction. These two compounds (Wy 40659, Wy 19110) had similar activity on rabbit aorta.

For the monocyclic compounds to be potent vasoconstrictors they appear to require both 11-deoxy and 16-methyl groups. Compounds having only 11-deoxy groups, or 15-methyl groups, or 16-methyl groups, were found to be vasodilators. These compounds resembled PGE_2 itself, having poor aggregatory activity, low activity on rabbit aorta, relaxant activity on rabbit mesenteric artery, but potent vasodilatator activity *in vivo*. The exception to this was Wy 18189, which showed some aggregatory activity.

The bicyclic compounds tested resemble the endoperoxides in their structure and aggregatory activity. The correlation with vasoconstrictor activity suggests that native endoperoxides are vasoconstrictors. This probably explains the transient vasoconstriction which we have previously observed with PGG_2.

The lack of correlation between *in vitro* responses (eg to ICI 86841, Wy 40659, Wy 18189) indicates that responses of isolated vascular strips do not necessarily predict microvascular activity *in vivo*.

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Table 1 Effect of prostaglandin analogues on platelets *in vitro*, skin blood flow and isolated aorta of the rabbit

	Compound	*Platelet aggregation (threshold conc. μM)*	*Blood flow (% change)*	*Rabbit aorta (relative potency)*
Bicyclic analogues	15(S)-hydroxy-11α,9α-epoxy-methano-prostadienoic acid (U46619)	1	−68.8±1.8 (12)	0.37
	15(S)-hydroxy-9α,11α-epoxy-methano-prostadienoic acid (U44069)	2	−50.9±4.1 (12)	0.45
	15(S)-hydroxy-9α,11α-azo-prostadienoic acid (Azo PGH_2)	5	−65.5±7.7 (6)	0.06
	15(S)-hydroxy-9α,11α-ethenyl-prostadienoic acid (ICI 86841)	30	−14.1±12.1 (12)	0.52
Monocyclic analogues	11-deoxy-15(R)-hydroxy-16(RS)-methyl-PGE_2 (Wy 19068)	5	−54.5±4.9 (12)	0.76
	11-deoxy-15(S)-hydroxy-16(RS)-methyl-PGE_2 (Wy 19110)	6	−51.5±2.5 (12)	1
	11-deoxy-15(S)-hydroxy-15-methyl-PGE_2 (Wy 40659)	6	−5.7±7.5 (6)	0.50
	11-deoxy-15(RS)-hydroxy-15-methyl-PGE_2 (Wy 17186)	200	−0.1±5.1 (6)	0.08
	11-deoxy-PGE_2 (Wy 18189)	50	+41.2±13.2 (6)	0.21
	15(R)-hydroxy-16,16-dimethyl-PGE_2	150	+88.1±8.4 (6)	0
	15(S)-hydroxy-15-methyl-PGE_2	300	+108±15.4 (6)	0
	15(R)-hydroxy-15-methyl-PGE_2	>300	+53.8±9.2 (6)	0
	PGE_2	>300	+114.9±10.8 (6)	0
	Saline (control)	—	0.0±7.5 (12)	0

Platelet aggregation was quantified as the minimal active concentration of agonist that induced a detectable aggregation in citrated rabbit platelet-rich plasma. Results are the mean values of four determinations in separate animals.
Blood flow change (^{133}Xe washout) expressed as percentage increase (+) or decrease (−), compared with saline controls. Responses produced by intradermal injection of 100 ng of agent. Results are mean values ± s.e. mean of (n) determinations.
Rabbit aorta potency ratios were the means obtained from dose-response curves constructed using aortas from 5 animals. Each agent was tested at 3 to 5 doses per tissue.

References

BUNTING, S., MONCADA, S. & VANE, J.R. (1976). The effect of prostaglandin endoperoxides and thromboxane A_2 on strips of rabbit coeliac artery and certain other smooth muscle preparations. *Br. J. Pharmac.*, **57**, 422–423P.

FURCHGOTT, R.F. & BADRAKOM, S. (1953). Reactions of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. *J. Pharmac. exp. Ther.*, **108**, 128–142.

GORDON, J.L. & DRUMMOND, A.H. (1974). A simple fluorimetric micro-assay for adenine compounds in platelets and in plasma and its application to studies on the platelet release reaction. *Biochem. J.*, **138**, 165–169.

HAMBERG, M. & SAMUELSSON, B. (1974). Prostaglandin endoperoxides. Novel transformation of arachidonic acid in human platelets. *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3400–3404.

LEWIS, G.P., WESTWICK, J. & WILLIAMS, T.J. (1977). Microvascular responses produced by the prostaglandin endoperoxide PGG_2 *in vivo*. *Br. J. Pharmac.*, **59**, 442P.

VARGAFTIG, B.B. & ZIRINIS, P. (1973). Platelet aggregation induced by arachidonic acid is accompanied by release of potential inflammatory mediators distinct from PGE_2 and $PGF_{2\alpha}$. *Nature, New Biol.*, **244**, 114–116.

WILLIS, A.L. (1974). Isolation of a chemical trigger for thrombosis. *Prostaglandins*, **5**, 1–25.

WILLIAMS, T.J. (1976). Simultaneous measurement of local plasma exudation and blood flow changes induced by intradermal injection of vasoactive substances, using [^{131}I] albumin and ^{133}Xe. *J. Physiol. (Lond.)*, **254**, 4–5P.

Changes in blood flow, histamine and prostaglandin E_2 in rabbit skin grafts

G.P. LEWIS & BEVERLEY A. MANGHAM

Department of Pharmacology, Royal College of Surgeons, London WC2A 3PN

When skin is transplanted either as autografts or homografts, distinct vascular changes occur during healing in and in the case of homografts also at the onset of rejection (Medawar, 1944). The question arises, do these vascular changes reflect the activity of pharmacological mediators? To investigate this possibility an attempt was made to correlate blood flow changes with the presence of mediators in the graft tissue.

Full thickness skin grafts (Jasani & Lewis, 1971) were used and blood flow changes were measured using a ^{133}Xenon clearance technique (Lewis, Peck, Williams & Young, 1976). Grafts were homogenised in 60% alcohol, dried and assayed for histamine and prostaglandin (PG) E_2. Histamine was measured fluorimetrically (Evans, Lewis & Thomson, 1973) and PGE_2 by radioimmunoassay (Hennam, Johnson, Newton & Collins, 1974).

Blood flow was first detected in the grafts on day 3 after grafting, becoming maximal by 12.00 h on day 4 in homografts and between days 4 and 6 in autografts. The blood flow continued in the autografts but could no longer be detected by day 5 in the homografts.

There is a striking similarity between the pattern of changes of histamine and of blood flow in both autografts and homografts. In both, the histamine content was low for the first 3 days. In autografts it rose on day 4, reaching a peak by day 5 (4.4 μg/g tissue) which was maintained until day 8, and fell sharply on day 9 to control level (2.3 μg/g tissue). In the homograft the histamine content reached two peaks. The first on day 4 at 12.00 h (12.5 μg/g tissue) corresponded with the maximum increase in blood flow. The second peak was after the onset of rejection (i.e. stoppage of blood flow) on day 5 (14.5 μg/g tissue). Between the two peaks, at a time which corresponded to the fall in blood flow, there was a significant fall in the level of histamine (5.8 μg/g tissue).

In both autografts and homografts the level of PGE_2 was raised above control immediately after grafting (87–125 ng/g tissue) but fell to control level by day 4 (15–28 ng/g tissue). It remained low in autografts throughout the experiment but rose sharply on day 4 at 12.00 h in homografts. This short-lived plateau of PGE_2 (90–95 ng/g tissue) occurred during the first peak of histamine and the peak of high blood flow. The level of PGE_2 increased further after the onset of rejection.

Histamine H_1-receptor antagonist mepyramine (but not H_2-receptor antagonist, metiamide), prolonged the increased blood flow in the homografts and increased their survival time from 5 days to 7–9 days. Indomethacin, on the other hand, did not prolong the increased blood flow and had no effect on rejection time but caused a decrease in the maximum blood flow at a time when there was an increase in PGE_2 content in the homografts. Neither antagonist affected the blood flow changes in autografts.

These findings suggest that: (a) histamine, via an H_1-receptor, is involved in the fall in blood flow associated with the rejection process; (b) although changes in histamine correspond with those in blood flow in autografts, it is present in a form which is not accessible to antagonists (see Kahlson, 1962; Schayer, 1962); (c) a prostaglandin, probably PGE_2, accounts