Modulation of thrombus formation *in vivo* by prostaglandins

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We have previously demonstrated the effects of prostaglandins (PG, E_2, E_1 and metabolites) (Lewis & Westwick, 1976) and PGG_2 (Lewis, Westwick & Williams, 1977) on the diameter of normal arterioles *in vivo*. We have now examined the possibility that arteriolar thrombosis can be locally modulated by prostaglandins produced by blood and vascular tissue. An animal model was developed which allowed quantitation of arteriolar thrombus formation during application of test substances to arterioles.

The hamster cheek pouch (HCP) preparation was set up as described by Lewis & Westwick (1975). Following a 45 min equilibration period, the flow of Krebs solution was switched off for 5 min and an arteriole (40–70 μm diameter) was selected. PG (G_2, D_2, E_2 and E_1) or vehicle was dropped onto the Krebs solution immediately adjacent to the selected arteriole, followed 30 s later by electrical micro-damage (+ve square wave DC pulses via 3 μm glass monopipette (filled 1 M KCl) at 10 Hz, 20 ms and current 25 μA for 2 s) and 1 min later by a similar application of ADP (10^{-6} M final bath concentration). The arteriole was continually observed and thrombus formation was quantitated by measuring the total time during which thrombi were present within a period of 10 minutes. The design of the experiment was such that each vessel received three applications of vehicle, five of test solution and two more of vehicle. Results were expressed as percentage difference from control and are shown in Table 1.

Table 1 Effect of prostaglandin (as a percentage difference from control) on thrombus formation in hamster cheek pouch arterioles

Prostaglandin	*Conc.* (ng/ml)*	*Percentage (mean ± s.e. mean)*	*P*	*Prostaglandin*	*Conc.* (ng/ml)*	*Percentage (mean ± s.e. mean)*	*P*
PGE_1	12.5	−11±8	NS	PGE_2	12.5	+11±35	NS
	125	−39±11	<0.05		125	+39±32	NS
	625	−57± 9	<0.05		1250	−14±11	NS
	1250	−97± 3	<0.05				
				PGG_2	12.5	−6±27	NS
PGD_2	125	−6± 1	NS		125	−13± 7	NS
	1250	−16±20	NS		625	−55±14	<0.05

NS = $P > 0.05$.
Conc.* = final bath concentration.

PGE_1 appears to be a potent inhibitor of thrombus formation *in vivo* as first described by Emmons, Hampton, Harrison, Honour & Mitchell (1967). PGD_2, which has been shown to be a potent inhibitor of human platelet aggregation, but ineffective on rat platelets (Smith, Silver, Ingerman & Kocsis, 1974) is also ineffective in preventing thrombus formation in the hamster.

These results indicate that the PGG_2 was converted by the vascular tissue *in vivo* to prostacyclin which produced the inhibition of thrombus formation. Prostacyclin formation might also explain in part the prolonged vasodilator activity observed in a previous study with this preparation (Lewis *et al.*, 1977). Prostacyclin was shown to be generated by vessel wall microsomes *in vitro* and to inhibit platelet aggregation *in vitro* (Moncada, Gryglewski, Bunting & Vane, 1976).

Thus, the effect of PGG_2 on thrombus formation appears to depend on the route of its metabolism. If it is metabolized by the enzyme system of the blood vessels as in the present experiments, it is converted to prostacyclin and inhibits thrombus formation, whereas if it came into contact with platelets it would be converted to thromboxane TxA_2 as shown by Hamberg, Svensson & Samuelsson (1975) and thrombus formation would be potentiated or even initiated.

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Inhibition of bile salt-induced gastric mucosal erosions by 16,16 dimethyl prostaglandin E_2 in the rat

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Exposure of the rat gastric mucosa to bile salt (sodium taurocholate, ST) and concurrent administration of indomethacin may lead to erosion formation. Such erosions can be inhibited by prostaglandins even in the presence of exogenous acid (Whittle, 1976) suggesting that prostaglandins have a protective effect on the mucosa, in addition to their anti-secretory actions.

We have investigated the effects of 16,16 dimethyl PGE_2 (MePG) on erosions induced by ST alone using the rat gastric chamber preparation (Mersereau & Hinchey, 1973). The mucosal solution (10–100 mM HCl made iso-osmotic with mannitol) was replaced every 0.5 h and H^+ loss, Na^+ gain and mucosal blood flow (MBF) (aniline clearance, Main & Whittle, 1973) were measured.

Erosion formation was related to the concentration of ST (present for one 0.5 h period) and acid (present from 2 h before until 2 h after ST). The erosion indices 1.5 h after exposure to 20 mM ST in 10 mM HCl (3 ± 2.5, $n=3$) or 2 mM ST in 100 mM HCl (0, 12, $n=2$) were not significantly different from controls (0 and 5.5 ± 5, for 10 and 100 mM HCl respectively, $n=4$).

With 5 mM ST (100 mM HCl) erosion formation began during the exposure period, rose rapidly in the next period and continued to rise for the remaining 1.5 h of the experiment. H^+ loss (per 0.5 h period) increased from 39 ± 11 to 99 ± 7 µmol ($P < 0.05$, $n=4$) during ST then fell steadily. Na^+ gain increased from 26.6 ± 4 to 39.8 ± 4 µmol ($P < 0.01$) during ST, reached a maximum (65.5 ± 5 µmol, $P < 0.01$) in the next period then declined. MBF was unchanged during ST but rose rapidly in the next period (237 ± 9% of basal, $P < 0.001$) and remained significantly elevated.

MePG (15 µg ml) in contact with the mucosa from 0.5 h before until 1 h after ST (5 mM, 100 mM HCl) inhibited erosion formation (index reduced from 44 ± 9 to 6 ± 3, at 1.5 h $P < 0.01$, $n=4$) while a lower concentration (5 µg ml) had no significant effect (32 ± 11, $n=4$).

Although no H^+ loss was noted with the low concentration of MePG prior to ST there was an increased loss (35 ± 4 to 59.5 ± 9 µmol, $P < 0.05$) with the high concentration. However, this latter concentration reduced the increased loss due to ST (from 99 ± 7 to 63 ± 15 µmol, $P < 0.05$). Na^+ gain increased from 17 ± 4 to 43 ± 5 µmol ($P < 0.01$) and 17.2 ± 2 to 36.7 ± 5 µmol ($P < 0.01$) for high and low concentrations respectively during the first period of MePG application but did not increase further during ST. MePG raised MBF to 142 ± 9% ($P < 0.01$) and 130 ± 4% ($P < 0.001$) for high and low concentrations respectively but failed to affect the further rise following ST.

These results show that MePG applied topically can protect the mucosa against ST. This protective action was associated with a reduction in the increased mucosal permeability to H^+ caused by ST. Protection is unlikely to result from the marked effects on Na^+ transport or MBF since both concentrations of MePG studied had similar effects on these parameters though the lower concentration did not prevent erosions.

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