
Prostaglandins and model aspects of thrombosis

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Summary

The possible role of prostaglandins (PGs) in thrombosis was determined by examining their effects in models of thrombosis which included platelet aggregation (in vitro), blood flow (in vivo) and thrombus formation (in vivo). It was found that blood flow and thrombosis can be effectively modulated by the various types of prostaglandins produced by the blood and the vascular tissue.

Introduction

Although platelets have been established to be of primary importance in arterial thrombosis (Genton, Weily and Steele, 1973), the change which initiates arterial thrombosis probably occurs in the wall of the blood vessel rather than in the platelet. Platelet interaction with damaged vessel walls, altered blood flow, and platelet aggregation, are contributory events to arterial thrombosis. Model systems in vivo and in vitro representing these events have been developed, and a number of naturally occurring prostaglandins (PGs) have been examined in each system to determine the possible role of PGs in thrombosis. The PGs examined included the relatively stable E₂, F₁₂₀, D₂, and E₁, plus some of their pulmonary metabolites. Also the labile PGG₂ which is the precursor of the '2 series PGs', thromboxanes (Hamberg, Svensson and Samuelsson, 1975) and prostacyclin (Moncada et al., 1976) have been examined.

Methods

Platelet aggregation

This was quantitated as an increase in light transmission in stirred platelet-rich plasma (PRP) (Born and Cross, 1963). The effect of PG on ADP-induced human platelet aggregation was performed as described by Westwick (1976). Briefly, following a 2-min pre-incubation period of the PRP at 37°C, 50 μl of vehicle or prostaglandin (final concentration 5 × 10⁻⁸ to 5 × 10⁻⁵ mol/l) was added followed 3 min later by ADP (1·3 × 10⁻⁶ mol/l). Each concentration of PG was tested 3–5 times with the same number of vehicle-only aggregations. Results were expressed as a percentage of the control height of primary aggregation. Dose-response curves were plotted and IC₅₀ values (μmol/l) were calculated.

Blood flow

The hamster cheek pouch preparation (HCP) as described by Lewis and Westwick (1975) was modified so that arterioles of 25–45 μm resting diameter could be monitored for up to 3 hr. Constriction and dilatation of the arterioles were measured by a photometric technique (Hutchings et al., 1976). The relatively stable prostaglandins, E₂, F₁₂₀, E₁ and their pulmonary metabolites plus PGD₂ were infused for 5-min intervals into the Krebs solution superfusing the cheek pouch in concentrations ranging from 0·6 to 400 ng/ml/min (Lewis and Westwick, 1976). Each concentration was assayed in a minimum of 4 hamsters. The unstable PGG₂ (half-life in saline at 38°C is 6 min) was stored in dry acetone at −20°C at 500 μg/ml. When required the stock solution was diluted either in saline or acetone at 0°C and applied immediately (0·5–5 μl) into the Krebs solution adjacent to the arteriole being monitored. PGG₂ was examined in doses of 5–500 ng.

Thrombus formation

Prostaglandin (G₂, D₂, E₂ and E₁) or vehicle was applied peri-vascularly to arterioles (40–70 μm diameter) of HCP followed 30 sec later by electrical micro-damage (Westwick and Lewis, unpublished data, 1977) and 1 min later by perivascular applications of ADP (10⁻⁶ mol/l final concentration). Thrombus formation was quantitated by measuring the total time during which thrombi were present within a period of 10 min. Each animal was its own control and results were expressed as percentage difference (mean ± s.e. mean) from control.

Results

Platelet aggregation

The 15-keto and 13,14-dihydro-15-keto-PGE₂, PGE₂ and PGF₁₂₀ were inactive at concentrations below 50 μmol/l. However, 13,14-dihydro derivatives
were very active with the following IC\textsubscript{50} (\textmu mol/l) values: PGE\textsubscript{1} (0.08), 13,14-dihydro-PGE\textsubscript{1} (0.037), PGE\textsubscript{2} (0.0), 13,14-dihydro-PGE\textsubscript{2} (0.0). Also PGE\textsubscript{2} and 13,14-dihydro-PGE\textsubscript{2} produced a significant potentiation of platelet aggregation at 0.08—0.26 \textmu mol/l (Fig. 1).

**Blood flow**

PGE\textsubscript{2} was the most potent vasodilator substance examined producing a 50% increase in diameter in a concentration of 1.2 ng/ml/min. The relative potencies were as follows PGE\textsubscript{2}>13,14-dihydro-PGE\textsubscript{2}>13,14-dihydro-PGE\textsubscript{1}>PGE\textsubscript{1}>bradykinin. The other pulmonary metabolites can be regarded an inactive, for example 15-keto-PGE\textsubscript{2} is 200 times less active than the parent PGE\textsubscript{2}. PGD\textsubscript{2} (>20 ng) produced a small vasoconstriction.

On arterioles which had a high resting tone, PGF\textsubscript{2} in low concentrations (5 ng) induced a vasodilatation only. Higher concentrations (25—100 ng) produced a short-lasting vasoconstriction (30 sec) followed by a protracted phase of vasodilatation (60—100% with 25—100 ng PGF\textsubscript{2}). On arterioles having a low vascular tone, a short-lasting vasoconstriction was induced, e.g. 25 ng produced 93 ± 2% constriction (mean ± s.e. mean, n = 6). Repeated doses of PGF\textsubscript{2} rapidly induced tachyphylaxis (Lewis, Westwick and Williams, 1977).

**Thrombus formation**

PGF\textsubscript{2} and PGE\textsubscript{1} produced a dose-related (12-5—1250 ng) inhibition (10 ± 8%—90 ± 15%) of thrombus formation. PGE\textsubscript{2} in low doses (12.5—125 ng) potentiated (49 ± 20%), while high doses (1250 ng) produced a weak inhibition (15—10%) of thrombus formation. PGD\textsubscript{2} did not produce a significant effect at concentrations up to 1250 ng.

**Discussion**

These results demonstrate that prostaglandins can exhibit both pro- and anti-thrombotic activity in model systems of thrombosis (Table 1). The E series PGs and the 13,14-dihydro-metabolites are very potent vasodilators in a number of species (for review see Messina, Weiner and Kaley, 1974). However, PGE\textsubscript{2} and PGE\textsubscript{4} (Bergstrom, Carlsson and Weeks, 1968) plus their 13,14-dihydro-metabolites have dramatically opposing effects on platelet aggregation (Westwick, 1976). PGE\textsubscript{1} is a very good inhibitor of thrombus formation in vivo in the hamster, as was also shown in the rabbit pial arteries by Emmons et al. (1967). Whereas it is clear that PGE\textsubscript{2} is pro-thrombotic, i.e. potentiates ADP-induced aggregation of human platelets and potentiates thrombus formation in HCP arterioles.

PGD\textsubscript{2} has been shown to be a potent inhibitor of human platelet aggregation but ineffective on rat platelets (Smith et al., 1974). In these experiments, PGD\textsubscript{2} did not inhibit thrombus formation in HCP arterioles, and it was a weak vaso-constrictor agent.

As might be expected from its key position in the metabolism of arachidonic acid (Johnson et al., 1976), the endoperoxide PGG\textsubscript{2} exhibited apparently opposing effects depending upon which model
TABLE 1. Summary of results, the number of + indicates potency. The vascular column refers to hamster cheek-pouch arterioles (HCPA); platelets (in vitro) column refers to human platelet aggregation; platelets (in vivo) column refers to thrombus formation in HCPA. For details, see method. n.t.=not tested. AGG = induces aggregation per se: ? indicates untested, but probable effect is demonstrated by its immediate precursor.

<table>
<thead>
<tr>
<th>Prostaglandin</th>
<th>Vascular dilatation</th>
<th>Vascular constriction</th>
<th>Platelets</th>
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<tr>
<td></td>
<td>in vitro</td>
<td>in vivo</td>
<td>in vitro</td>
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<tr>
<td>E1</td>
<td>+ + +</td>
<td>0</td>
<td>inhib. + +</td>
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<td>v. weak</td>
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<td>13,14-dh-15-k-E1</td>
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<td>13,14-dh-E1</td>
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<tr>
<td>E2</td>
<td>+ + +</td>
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<td>pot./inhib.</td>
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<td>v. weak</td>
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<td>13,14-dh-E2</td>
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<td>inhib. + +</td>
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system was used. PGG₂ was the only PG which produced a biphasic response on HCP arterioles, i.e. a transient vasoconstriction and a protracted vasodilatation. These responses soon became tachyphylactic, which did not happen when PGG₂ was assayed on isolated vascular tissues (Lewis et al., 1977). It seems unlikely because of its acute and tachyphylactic nature that PGG₂ could be responsible for protracted periods of vasoconstriction, although it could be responsible for acute periods of vasoconstriction as are often seen in response to injurious stimuli. The transient vasoconstriction was most likely produced by PGG₂ itself while the secondary protracted vasodilatation was most likely produced by one or many of its metabolites. A similar conclusion was reached by Hamberg et al. (1975) when examining the cardiovascular effects of PG endoperoxides in guinea-pigs. A clue to the identity of these metabolites was provided when PGG₂ was found to inhibit thrombus formation in vivo.

It appears likely that PGG₂ has been converted in vivo by HCP vascular tissue to prostacyclin, which produced the inhibition of thrombus formation. Prostacyclin can be generated by vessel wall microsomes in vitro and has been shown to inhibit platelet aggregation in vitro (Moncada et al., 1976).

From these experiments the metabolic fate of any PGG₂ produced in vivo is of utmost importance. For example, if PGG₂ is converted to PGE₂ then a pro-thrombotic situation will prevail, but if PGG₂ is converted to PGD₂ or prostacyclin, then an anti-thrombotic situation will exist. However, if PGG₂ comes in contact with platelets only, then a pro-thrombotic situation will arise owing either to the direct effect of PGG₂ or to thromboxane generation (Hamberg et al., 1975).

Thus blood flow and thrombosis can be locally modulated by the various types of prostaglandins produced by blood and vascular tissue.

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References


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