Effect of prostaglandin E₂ on prostacyclin production by endothelial cells

IT has been suggested by Tomasi *et al.*¹ that prostaglandin (PG) E₂ inhibits the synthesis of PGI₂ (prostacyclin) by endothelial cells. This conclusion was based mainly on their observation that the inhibition of platelet aggregation produced by a suspension of isolated hepatic cells (described as "endothelial-rich") was reduced when the hepatic cell suspension was preincubated for 10 min with 1.5–3 µM PGE₂. An alternative explanation for this effect, apparently not considered by Tomasi *et al.* is that PGE₂ interferes with the action of PGI₂ rather than its synthesis. We therefore investigated the effects of PGE₂, first on PGI₂ synthesis by aortic endothelial cells, and second, on the inhibition of platelet aggregation caused by exogenous PGI₂.

Production of PGI₂ was measured by radioimmunoassay (RIA) of 6-oxo-PGF_{1α} (the stable metabolite of PGI₂) in the medium above monolayers of cultured endothelium. In this RIA, 2 µM PGE₂ showed appreciable cross reactivity, and all samples were therefore treated with 0.3 M KOH in methanol to convert PGE₂ to PGB₂, which showed very little cross reactivity. This treatment did not affect the immunoreactivity of 6-oxo-PGF_{1α}. The production of 6-oxo-PGF_{1α} was not reduced by 2 µM PGE₂ (Table 1); in fact, samples incubated with PGE₂ showed some increase in the amount of immunoreactive 6-oxo-PGF_{1α} present. Similar increases were, however, measured when 2 µM PGE₂ was added at the end of the incubation or when 2 µM PGE₂ was assayed in the same volume of medium alone. Figure 1 shows that as little as 0.1 µM PGE₂ abolished the inhibition of arachidonic acid-induced platelet aggregation produced by 20 nM PGI₂.

On the basis of these results we conclude that PGE₂, even at the relatively

Fig. 1 Effect of PGE₂ on the inhibition of platelet aggregation caused by PGI₂. Platelet aggregation was measured photometrically in 0.1-ml samples of human citrated platelet-rich plasma⁸. Aggregation was induced by 1 mM arachidonic acid alone (a) or 1 min after the addition of 20 nM PGI₂ (b). 0.1 µM PGE₂ was added 1 min before arachidonic acid either alone (c) or with 20 nM PGI₂ (d).

high concentration of 2 µM, does not block PGI₂ synthesis by aortic endothelial cells, but that a lower concentration of PGE₂ can overcome the inhibitory effect of PGI₂ on platelets. The action of PGE₂ may result from competition at the platelet receptor binding PGI₂, although this seems unlikely, as we have evidence that PGE₂ and PGE₁ act at different receptors on the platelet², and PGI₂ apparently acts at the same receptor as PGE₁ (ref. 3). Alternatively, this action could be a consequence of the ability of PGE₂ to facilitate platelet aggregation induced by several stimuli, including products of arachidonate metabolism⁴. Although the hypothesis advanced by Tomasi *et al.*¹ may be valid for the hepatic cell fraction they used (although this still remains to be unequivocally established), it does not apply generally to vascular endothelium.

RIA was from Drs F. Dray and H. Sors, and PGI₂ was from Dr P. L. Walton.

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TOMASI *ET AL.* REPLY—We had considered the possibility that PGE₂ interferes with the PGI₂ receptor, and experiments showing that PGE₂ at the concentrations used (1.5–3.0 µM) was unable to counteract PGI₂ (2–4 ng ml⁻¹) inhibition in platelet-rich plasma aggregated with sodium arachidonate, were carried out. We have now repeated these experiments with two different sources of PGI₂ with similar findings. However, we were surprised to observe that in the conditions described by Gordon *et al.* (0.1 µM PGE₂) the results were similar to those reported in their Fig. 1. We agree that this finding cannot be explained by an antagonism of PGE₂ on the PGI₂ receptor. The most likely explanation is that at low doses (0.1 µM) PGE₂ decreases cyclic AMP levels in platelets¹, counteracting the increase due to PGI₂ (ref. 2) and facilitating aggregation, whereas at higher doses, when PGE₂ has no effect or increases cyclic AMP¹, its action on PGI₂ inhibition is minimal or absent. Thus, in our conditions the effect of PGE₂ must be explained on the basis of a reduction of PGI₂ synthesis. This is supported by experiments showing that our preparations preincubated with PGE₂ and washed twice with buffer were much less effective than controls in inhibiting arachidonate-induced aggregation.

The failure of Gordon *et al.* to observe an effect of PGE₂ on 6-keto-PGF_{1α} formation may be accounted for: (1) by assuming that their endothelial cells from pig aorta do not contain a PGE₂-sensitive adenylate cyclase (which would be interesting to verify), or (2) by the possibility that PGI₂ is degraded mainly to 6,15-diketo-PGF_{1α} (ref. 3), which may not be detectable using a 6-keto-PGF_{1α} antiserum⁴.

We would be surprised if our hypothesis applies generally to vascular endothelium. We consider that the mechanism we have described may operate mainly inside an organ where PGE₂ produced by the major cell population would mediate a control on the endothelial cells lining the organ vasculature⁵.

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Table 1 The effect of PGE₂ on 6-oxo-PGF_{1α} production

Treatment	6-oxo-PGF _{1α} (pg)
a Endothelial cells alone	66 ± 2
b Endothelial cells + 2 µM PGE ₂	104 ± 13
c Endothelial cells alone; 2 µM PGE ₂ added after incubation	95 ± 6
d 2 µM PGE ₂ alone	22 ± 2

Figures are mean ± s.e.m. (six observations in each case) and represent pg per 10⁵ cells (or, in d, pg per sample). Porcine aortic endothelial cells⁵ were incubated in serum-free tissue culture medium for 10 min at 37°C either with (b) or without (a) PGE₂, and 6-oxo-PGF_{1α} was measured by RIA⁶. Controls (c, d) were incubated to estimate the interference of PGE₂ in the RIA.