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LOW CONCENTRATIONS OF PROSTAGLANDIN E_2 INHIBIT THE PROSTACYCLIN-INDUCED ELEVATION OF CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE IN ELICITED POPULATIONS OF RAT PERITONEAL MACROPHAGES

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1 Elevation of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) in elicited populations of rat peritoneal macrophages was used as a parameter to examine the influence of prostaglandin E_2 (PGE_2) on the effects of prostacyclin (PGI_2) and (±)-5E-13,14-didehydro-carbo-prostacyclin (DDH-carbo-PGI_2) *in vitro*.

2 PGE_2, within the range of 1.4×10^{-9} to 1.2×10^{-8} M, caused a concentration-dependent inhibition of the rise in cyclic AMP induced by 2.8×10^{-6} M PGI_2 or DDH-carbo-PGI_2.

3 With higher concentrations of PGE_2 the inhibition was either non-existent or masked by the effect of PGE_2 *per se* on cyclic AMP levels.

4 The present findings suggest that the earlier observed low responsiveness of granuloma macrophages to PGI_2, in terms of rise in cyclic AMP, is possibly due to permanent exposure of these cells to environmental endogenous PGE_2.

Introduction

Elevation of intracellular cyclic adenosine 3′,5′-monophosphate (cyclic AMP) levels in response to stimulation by prostaglandin E_2 (PGE_2) occurs in elicited populations of peritoneal macrophages and in macrophages isolated from carrageenin-induced granuloma (Bonney, Burger, Davies, Kuehl & Humes, 1980; Bonta, Adolfs & Parnham, 1981a). Prostacyclin (PGI_2) also stimulates the production of cyclic AMP in elicited peritoneal macrophages (Bonney *et al.*, 1980). In contrast, there is no elevation of cyclic AMP in macrophages from carrageenin granuloma when these cells are exposed to PGI_2 (Bonta, Adolfs & Parnham, 1981b). PGI_2 is a particularly well-known potent inhibitor of platelet aggregation and a stimulator of cyclic AMP elevation in platelets (Tateson, Moncada & Vane, 1977). However, exposure of platelets to PGE_2 renders these cells rather unresponsive to PGI_2, both in terms of anti-aggregation and elevation of cyclic AMP (Bonne, Martin, Watada & Regnault, 1981). We now show that PGE_2, in concentrations lower than those required to increase cyclic AMP in elicited peritoneal macrophages, inhibits the responsiveness of these cells to PGI_2 and to (±)-5E-13,14-didehydro-carbo-prostacyclin (DDH-carbo-PGI_2), a synthetic analogue not liable to undergo rapid decomposition.

Methods

Elicited macrophages were obtained by the method described by Bray & Gordon (1978). Male Wistar rats were given an intraperitoneal injection of 5 ml 1% solution of starch. After 24 h the peritoneal cavity was flushed with 2 × 20 ml of Gey's Balanced Salt Solution (GBBS). Subsequent handling of the cells was essentially carried out as described previously (Bonta *et al.*, 1981a). Briefly, separation by Ficoll-Isopaque centrifugation resulted in two cell layers, the upper one of which contained a 90% macrophage population. After washing the cells three times with GBBS, dilutions of 5×10^6 cells/ml GBBS were made for incubation. Viability (trypan blue exclusion) was usually found to be 98% or greater.

One ml samples of 5×10^6 cells were incubated for 5 min at 37°C with saline or the substances to be investigated. Thereafter 100 μl samples were taken for protein assay (Lowry, Rosebrough, Farr & Randall, 1951). The cell suspension was centrifuged at 12000 *g* for 0.5 min, the pellet resuspended in 150 μl Tris disodium edetate (EDTA) buffer (pH 7.4) and after 5 min in a boiling water-bath, again centrifuged at 12000 *g* for 5 min. The supernatant was stored at −70°C for subsequent cyclic AMP assay, which was

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carried out by the protein binding method (Gilman, 1970). Cyclic AMP values were expressed as a content in 10^6 macrophages.

Results

In preliminary trials, carried out within the concentration range of 2.8×10^{-8} to 2.8×10^{-6} M, DDH-carbo-PGI_2 was found to be marginally less potent than PGI_2 in stimulating cyclic AMP production in elicited peritoneal macrophages. In the present experiments, using a concentration of 2.8×10^{-6}, DDH-carbo-PGI_2 again proved slightly less effective than PGI_2 in causing a significant rise in cyclic AMP levels (Table 1). When either one of the two substances was added to the cells in association with low concentrations (1.4×10^{-9} to 1.2×10^{-8} M) of PGE_2, the stimulant effects of the two substances on cyclic AMP production were inhibited. The results in Table 1 clearly show that the progressive decline in elevation of cyclic AMP is strictly related to the concentration of PGE_2.

In a separate experiment we also examined the influence of higher concentrations of PGE_2 on the increase in cyclic AMP induced by DDH-carbo-PGI_2. The results in Table 2 show that in this experiment the levels of cyclic AMP in unstimulated cells were rather low, but the percentual rise induced by DDH-carbo-PGI_2 (2.8×10^{-6} M) proved of a similar magnitude to that observed in the first experiment (see Table 1). Furthermore, Table 2 demonstrates that, when concentrations of 2.8×10^{-8} and 5.6×10^{-8} M are used, PGE_2 caused a moderate, but significant and dose-dependent rise in cyclic AMP levels. When DDH-carbo-PGI_2 was added to the cells in association with 2.8×10^{-8} M PGE_2, the elevation of cyclic AMP proved smaller compared to that caused by DDH-carbo-PGI_2 alone. However, the addition of 5.6×10^{-8} M PGE_2 failed to interfere with the effect of DDH-carbo-PGI_2. Thus at this concentration of PGE_2 the inhibitory influence on the cyclic AMP elevating effect of DDH-carbo-PGI_2 is either non-existent or masked by the effect of PGE_2 *per se* on cyclic AMP levels.

Discussion

It has been reported that added PGE_2 causes a decrease in the responsiveness of platelets to PGE_1 and PGI_2 (Bonne *et al.*, 1981). The present observations show a similar interaction of PGE_2 with PGI_2 and with a synthetic stable analogue of PGI_2 in elicited peritoneal macrophages. There is evidence for a common prostaglandin receptor, including the same binding site for PGE_1, PGE_2 and PGI_2 (McDonald & Stuart, 1974; Shafer, Cooper, O'Hara & Handin, 1979; Siegl, Smith, Silver, Nicolaou & Ahern, 1979). PGE_2 proved potent in displacing

Table 1 Effects of low concentrations of prostaglandin E_2 (PGE_2) on elevation of cyclic AMP induced by either prostacyclin (PGI_2) or (±)-5E-13,14-didehydro-carbo-prostacyclin (DDH-carbo-PGI_2) in elicited populations of rat peritoneal macrophages

Substance added	*PGI_2 or DDH-carbo-PGI_2* (M)	*PGE_2* (M)	*Cyclic AMP*† (pmol/mg protein)	*% rise above saline control*
Saline			6.3±0.2	
PGI_2	2.8×10^{-6}		15.2±0.5§	141%
$PGI_2 + PGE_2$		1.4×10^{-9}	14.9±1.4^{NS}	136%
$PGI_2 + PGE_2$		2.8×10^{-9}	12.0±0.3*	90%
$PGI_2 + PGE_2$		5.6×10^{-9}	10.7±0.4*	70%
$PGI_2 + PGE_2$		1.2×10^{-8}	8.5±0.2**	33%
Saline			7.4±0.5	
DDH-carbo-PGI_2	2.8×10^{-6}		15.7±0.6§	112%
DDH + PGE_2		1.4×10^{-9}	14.4±1.1^{NS}	94%
DDH + PGE_2		2.8×10^{-9}	13.0±0.6*	76%
DDH + PGE_2		5.6×10^{-9}	9.8±0.5*	32%
DDH + PGE_2		1.2×10^{-8}	7.5±0.8**	1%

†Each value represents the mean ± s.e.mean of 6 observations, which were carried out as duplicate measurements on cell suspensions pooled from 5 rats.
§ Significance vs saline control, $P < 0.001$.
^{NS}Not significantly different from response to PGI_2 or DDH-carbo-PGI_2 alone.
*Significance vs PGI_2 or DDH-carbo-PGI_2 alone, $P < 0.05$.
**Significance vs PGI_2 or DDH-carbo-PGI_2 alone, $P < 0.01$.
Significance was determined by one-tailed Mann-Whitney U test. Up to the concentration of 1.2×10^{-8} M PGE_2 alone failed to cause any significant change in cyclic AMP levels of saline control cells.

Table 2 Influence of higher concentrations of prostaglandin E_2 (PGE_2) on elevation of cyclic AMP induced by (±)-5E-13,14-didehydro-carbo-prostacyclin (DDH-carbo-PGI_2) in elicited populations of rat peritoneal macrophages

Substances added	*DDH-carbo-PGI_2* (M)	*PGE_2* (M)	*cyclic AMP*† (pmol/mg protein)	*% rise above saline control*
Saline			1.7 ± 0.1	
DDH-carbo-PGI_2	2.8×10^{-6}		3.5 ± 0.4**	105%
PGE_2		2.8×10^{-8}	2.4 ± 0.2*	41%
PGE_2		5.6×10^{-8}	2.8 ± 0.3*	65%
DDH-carbo-PGI_2 + PGE_2	2.8×10^{-6}	2.8×10^{-8}	2.7 ± 0.3*	59%
DDH-carbo-PGI_2 + PGE_2	2.8×10^{-6}	5.6×10^{-8}	3.4 ± 0.4**	100%

†Each value represents the mean ± s.e.mean of 6 observations, which were carried out as duplicate measurements on cell suspensions pooled from 5 rats.
*Significance vs saline control, $P < 0.05$.
**Significance vs saline control, $P < 0.01$.
Significance was determined by one-tailed Mann Whitney U test.

PGE_1 from its specific binding sites in platelets (Bonne *et al.*, 1981). These authors also presented results which suggested that direct inhibition of adenylate cyclase, rather than occupancy of the receptor, might be the mechanism underlying the prostaglandin-antagonistic activity of PGE_2. However, the effect of PGE_2 was biphasic and the authors concluded that the inhibition of adenylate cyclase is partially hidden, since PGE_2 stimulated the enzyme, through binding to the specific PGE receptor after all. The experiments described in the present paper do not enable us to decide whether either of the proposed mechanisms with platelets was the prevailing component in the prostacyclin-antagonistic effect of PGE_2 with macrophages. To our knowledge no specific receptor binding studies with prostaglandins on macrophages have been published. The two-fold mechanisms as suggested by Bonne *et al.* (1981) could just as well be valid for macrophages, because we also find that at a concentration of PGE_2 above that which causes apparently maximal interference with DDH-carbo-PGI_2, the inhibition is not observed. Since at concentrations above 10^{-8} M, PGE_2 itself elevates the cyclic AMP levels in elicited peritoneal macrophages (Bonta *et al.*, 1981b), this effect of PGE_2 might have masked the prostacyclin-antagonistic activity, as measured by the same parameter (see: Table 2).

Irrespective of the details of the mechanism by which low concentrations of PGE_2 exert the prostacyclin-antagonistic activity on macrophages, this finding possibly helps to explain the observation, which showed that granuloma-derived macrophages, in terms of cyclic AMP rise, are far less responsive to PGI_2 than are elicited peritoneal macrophages (Bonta *et al.*, 1981b). Resident peritoneal macrophages are less responsive to either PGE_2 or PGI_2 than are elicited macrophages from the peritoneal cavity (Bonney *et al.*, 1980). The authors speculated that, since endogenous formation of PGE_2 and/or PGI_2 might modulate the ability of the cell to respond to added prostaglandins, the higher capacity of resident macrophages to secrete PGE_2 and PGI_2 renders these cells rather unresponsive when exposed to exogenously added prostaglandins. Macrophages embedded in granuloma tissue are, almost certainly, permanently exposed to endogenous arachidonate conversion products (e.g. their own prostaglandins and those from surrounding fibroblasts). In this context, it was shown that PGE_2 is the major cyclooxygenase-generated product of arachidonate conversion by carrageenin-induced granuloma tissue *in vivo* (Bragt & Bonta, 1979). The low concentrations of PGE_2, at which the prostacyclin-antagonistic effects are exerted, are of a similar magnitude to the levels of PGE released locally during carrageenin-induced granulomatous inflammation (Bragt, Bonta & Adolfs, 1980). Therefore, the present results seem to warrant the proposal that the endogenous PGE_2 in granuloma tissue is responsible for the low responsiveness of granuloma-derived macrophages to exogenously added PGI_2 in terms of cyclic AMP level. This might, at least partially, account for the distinction between PGE_2 and PGI_2 as inhibitors of granulomatous inflammation (Parnham, Bonta & Adolfs, 1979).

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