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Induction of prostacyclin receptor expression in human erythroleukemia cells

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We have identified both high-affinity ($K_D = 36 \pm 3$ nM) and low-affinity ($K_D = 2.1 \pm 0.8$ μM) prostacyclin (PGI_2)-receptor sites on human erythroleukemia (HEL) cells using the radiolabelled prostacyclin analogue, [^{3}H]iloprost. The addition of the phorbol ester, TPA, to the culture medium caused a 5–10-fold increase in the number of both the low- and the high-affinity sites, without any change in their affinity constants. Iloprost stimulated HEL cell membrane adenylate cyclase activity 5-fold. This stimulation was potentiated in the presence of GTP, indicating a conventional PGI_2 receptor-G_s-adenylate cyclase system. HEL cells represent a source of prostacyclin receptor mRNA which may be of value in expression cloning of this receptor.

Prostacyclin receptor; Phorbol ester; Adenylate cyclase

1. INTRODUCTION

Prostacyclin (PGI_2) is a potent platelet inhibitor and vasodilator. PGI_2 receptor sites have been identified in several tissues, including platelets [1,2], vascular smooth muscle [3] and the NCB-20 cell line [4]. Both the platelet and vascular effects of PGI_2 appear to be mediated, via a G-protein, by the adenylate cyclase enzyme.

Studies of the expression of platelet proteins have been difficult to perform due to the low capacity of the platelet for protein biosynthesis. Megakaryocytes, the platelet precursor cells, synthesize platelet proteins [5], but they are difficult to isolate in significant quantities. However, a human erythroleukemia (HEL) cell line has been established which expresses several megakaryocytic markers of differentiation. It has also been observed that the phorbol ester 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) induces the expression of various megakaryocyte/platelet markers in HEL cells [6].

In this study, we have examined the expression of PGI_2 receptors and the coupling of the receptor to adenylate cyclase in HEL cells.

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2. MATERIALS AND METHODS

2.1. *Cell culture*

Human erythroleukemia cells (HEL) were purchased from the American Type Culture Collection (ATCC) and cultured in suspension in RPMI 1640 medium supplemented with 10% fetal calf serum and antibiotics. TPA was dissolved in dimethyl sulfoxide (DMSO) and added to the culture medium at a final concentration of 160 nM for a period of 3 days.

2.2. *[^{3}H]Iloprost binding*

The HEL cell suspension (5×10^6 cells/ml) was incubated for 30 min at room temperature with 100 nM [^{3}H]iloprost (14.7 Ci/mmol) and 0–10 μM unlabelled iloprost in 50 mM Tris·HCl buffer, pH 7.4, containing 5 mM $MgCl_2$, in a final volume of 0.2 ml. The reaction was terminated by rapid filtration and washing on Whatman GF/C glass fibre filters. After addition of 10 ml of scintillation fluid, the filters were assayed for radioactivity.

Binding analyses were performed by computerized non-linear curve fitting using the LIGAND program, as described by Munson and Robard [7].

2.3. *Adenylate cyclase assays*

HEL cell membranes were prepared following lysis of the cells in hypotonic PBS containing 0.1 mM PMSF. The cells were then dounce homogenized and the nuclei and undisrupted cells

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removed by centrifugation at 800 × g for 10 min. The supernatant was centrifuged at 100000 × g for 1 h, and the resultant membrane pellet was resuspended in 50 mM Tris · HCl, pH 7.4, containing 5 mM $MgCl_2$ and 1 mM EDTA and stored at −70°C until use.

Adenylate cyclase activity was measured by the method of Salomon et al. [8]. ^{32}P-labelled cyclic AMP was separated by sequential chromatography on Dowex and alumina columns.

Results are expressed as means ± SE.

3. RESULTS

Binding of [^3H]iloprost to HEL cells yielded a curvilinear Scatchard plot, indicating that there were two PGI_2-binding sites present (fig.1). The high-affinity-binding site had a $K_D = 36 \pm 3$ nM and $B_{max} = 54 \pm 10$ fmol/10^6 cells. There were approximately five times as many low-affinity-binding sites, with $K_D = 2.1 \pm 0.8$ μM and $B_{max} = 293 \pm 56$ fmol/10^6 cells ($n = 8$).

We investigated the functional coupling of these PGI_2 receptors to the adenylate cyclase enzyme in HEL cell membranes. Iloprost (100 nM) maximally stimulated adenylate cyclase activity 5-fold. The EC_{50} for iloprost stimulation was 2 nM (fig.2). This is consistent with adenylate cyclase stimulation being mediated through the high-affinity iloprost-binding site.

The stimulation of adenylate cyclase by iloprost was potentiated in the presence of GTP, indicating that the stimulation is mediated by G_s, the adenylate cyclase stimulatory G-protein. The non-hydrolysable GTP analogs, Gpp(NH)p and GTPγS both stimulated adenylate cyclase activity,

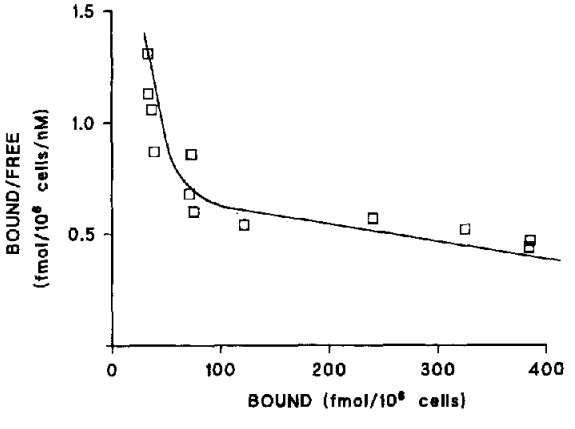

Fig.1. Scatchard plot of [^3H]iloprost binding to HEL cells (a representative experiment).

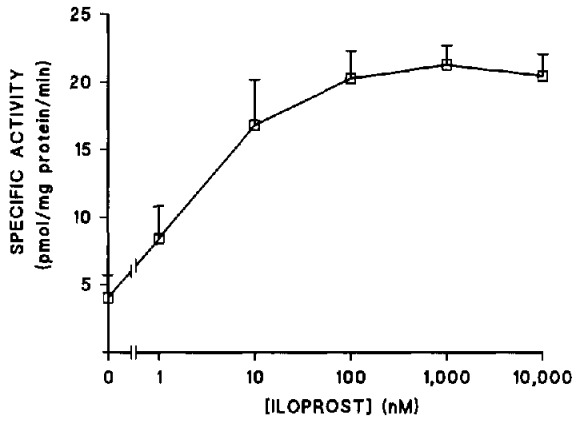

Fig.2. Stimulation of HEL cell membrane adenylate cyclase activity by iloprost ($n = 4$).

as did sodium fluoride, which also directly stimulates G_s (table 1).

We investigated the effect of TPA on the expression of the PGI_2 receptor in the HEL cells. In cells treated with 160 nM TPA, concomitant with the well characterized change in morphology of the cells, there was a significant ($p < 0.01$) increase in the density of both the high- and the low-affinity-binding sites (table 2). There was no change in the affinity of iloprost for these sites.

4. DISCUSSION

The present study demonstrates the presence of PGI_2 receptors in the HEL cell line. We have identified a high- and a low-affinity site for [^3H]iloprost. Two affinity states of the PGI_2 receptor have been identified in several tissues, e.g. platelets

Table 1

Stimulation of HEL cell membrane adenylate cyclase activity ($n = 4$)

Stimulant	Specific activity (pmol/mg per min)
Basal	4.0 ± 1.7
GTP (10 μM)	6.0 ± 1.2
Iloprost (1 μM)	21.3 ± 1.4
Iloprost (1 μM) + GTP (10 μM)	35.1 ± 3.7
Gpp(NH)p (10 μM)	57.0 ± 6.4
GTPγS (1 μM)	75.6 ± 4.0
NaF (10 mM)	56.0 ± 3.0

Table 2

Effect of the phorbol ester, TPA, on PGI_2 receptor number in HEL cells ($n = 7$)

	B_{max} (receptor sites/cell)	
	High-affinity site	Low-affinity site
Control	$0.4 \pm 0.05 \times 10^5$	$1.7 \pm 0.3 \times 10^5$
TPA-treated	$2.1 \pm 0.2 \times 10^5$	$14.0 \pm 4.0 \times 10^5$

[2], lung homogenate [9] and membranes from coronary arteries [3]. The high-affinity state of the receptor in HEL cells is the site which is coupled to stimulation of the adenylate cyclase enzyme by iloprost, as has previously been reported in platelet membranes [1,2]. This stimulation is mediated by a G-protein, probably G_s, as shown by the potentiation of iloprost stimulation of adenylate cyclase in the presence of 10 μM GTP. Stable GTP analogs, Gpp(NH)p and GTPγS also stimulated cyclase activity in these membranes, even in the absence of iloprost, suggesting a strong PGI_2 receptor-G_s coupling in these cells [10].

It has previously been reported that treatment of HEL cells with phorbol esters induces a dramatic macrophage-like shift in the phenotype of these cells [11]. This shift has been shown to be associated with the increased expression of several platelet proteins, for example glycoproteins IIb and IIIa, platelet factor 4 [6] and more recently the thromboxane A_2 receptor [12]. We investigated the regulation of expression of the PGI_2 receptor by the phorbol ester, TPA. The number of both high- and low-affinity sites increased 5–10-fold following TPA treatment. The affinity of these sites for [^{3}H]-iloprost did not change, however, indicating that the number but not the nature of the sites has been altered by TPA.

The PGI_2 receptor identified on HEL cells is similar to that present in platelets. The treatment of these cells with TPA represents a useful means of amplifying the expression of this receptor. These studies identify a source of mRNA for expression cloning of the PGI_2 receptor.

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