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An EP receptor with a novel pharmacological profile in the T-cell line Jurkat

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1 Comparison of the rank order of potency of the natural prostanoids prostaglandin E_2 (PGE_2), PGD_2, $PGF_{2\alpha}$ and carbaprostacyclin in stimulating cyclic AMP in Jurkat cells is consistent with the presence of an EP receptor.

2 Lack of responsiveness to the EP_1/EP_3 selective agonist, sulprostone, and the EP_2 agonists, butaprost and AH 13205, indicates that this receptor is not of the EP_1, EP_2 or EP_3 subtypes.

3 Inhibition of PGE_2-stimulated cyclic AMP by the EP_4 antagonist, AH 23848 is non-competitive, unlike the competitive antagonism reported in the pig saphenous vein EP_4 preparation. Furthermore, 16,16-dimethyl PGE_2 is 100 fold less potent than PGE_2 in Jurkat cells, while these agonists are equipotent in the rabbit jugular vein purported EP_4 preparation. In addition, 1-OH PGE_1, which also is active in the rabbit jugular vein preparation, is inactive in Jurkat cells at concentrations up to 1×10^{-4} M. These data are not wholly consistent with any adenylate cyclase coupled EP receptor described to date.

4 It is postulated that an EP receptor, positively coupled to adenylate cyclase, with a unique pharmacological profile is present in Jurkat cells.

Keywords: Prostaglandins; EP-receptors; cyclic AMP; human lymphocytes; Jurkat cells; prostaglandin receptor agonists; prostaglandin receptor antagonists

Introduction

Prostaglandins of the E series are generally believed to downregulate the immune system (Goodwin & Ceuppens, 1983; Plaut, 1987). This is based, in part, on early *in vivo* studies of allograft rejection, autoimmune responses and anti-arthritic effects in animals (Zurier & Quagliata, 1971; Anderson *et al.*, 1977; Strom *et al.*, 1977; Whittum *et al.*, 1985). Consistent with this idea are a number of *in vitro* studies in which PGE_2 has been reported to inhibit T-cell mitogenesis and interleukin-2 (IL-2) production (Walker *et al.*, 1983; Mary *et al.*, 1987; Betz & Fox, 1991; Elliott *et al.*, 1992). Although its mechanism of immunosuppression is not entirely clear, PGE_2 has been shown to bind to cell surface receptors on lymphocytes (Goodwin *et al.*, 1979; Eriksen *et al.*, 1985) and, apparently, to mediate its effects through stimulation of the second messenger adenosine 3':5'-cyclic monophosphate (cyclic AMP) (Rincon *et al.*, 1988; Bastin *et al.*, 1990; Lingk *et al.*, 1990; Minakuchi *et al.*, 1990). This hypothesis is supported by the observations that suppression of T-cell responses is correlated with elevations of cyclic AMP brought about by non-receptor mechanisms (eg. treatment with dibutyryl cyclic AMP or forskolin) (Krause & Deutsch, 1991; Anastassiou *et al.*, 1992) and with activation of cyclic AMP-dependent protein kinase (Skålhegg *et al.*, 1994).

Prostanoid receptors have been classified into five major types based on their affinity for, and functional responsiveness to, the natural prostanoids PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2 and the stable thromboxane A_2 (TxA_2)-mimetics (Kennedy *et al.*, 1982). These receptors are designated EP, DP, FP, IP and TP, respectively. The EP receptor, in turn, has been divided into four subtypes: EP_1, EP_2, EP_3 and EP_4 (Coleman *et al.*, 1994b). A pharmacological characterization of these receptors has been based primarily on rank order potencies of the natural prostanoid agonists and selected synthetic analogues. We adopted this approach in the present study to define the PG receptor subtype positively coupled to adenylate cyclase in human T-cells. Specifically, we have examined the effects of natural prostanoids and synthetic analogues on endogenous cyclic AMP levels in the human leukaemic T-cell line, Jurkat. Initial comparisons with PGE_2 were made utilizing 16,16-dimethyl PGE_2 and 11-deoxy PGE_1. 16,16-dimethyl PGE_2 and 11-deoxy PGE_1 have both been found to exhibit little selectivity of action at the various EP-receptor subtypes, having been reported to have agonist activity on preparations containing EP_1, EP_2, EP_3 and EP_4-receptors (Coleman *et al.*, 1988; Reeves *et al.*, 1988; Lawrence & Jones, 1992; Lawrence *et al.*, 1992). The activities of more selective agonists are described below. The results of our studies indicate that an EP receptor linked to adenylate cyclase, but pharmacologically distinct from all EP receptors described to date, is present on these cells.

Methods

Cells

The human leukaemic T-cell line, Jurkat was obtained from ATCC (American Type Culture Collection, Rockville, MD, U.S.A.). The cells were cultured in RPMI 1640 (Gibco, Gaithersburg, MD, U.S.A.) supplemented with 10% foetal bovine serum (Hyclone, Logan, UT, U.S.A.), 50 u ml^{-1} penicillin, 50 μg ml^{-1} streptomycin, 2 mM L-glutamine, 50 μM β-mercaptoethanol, with 10 mM HEPES, pH 7.4. The cells were maintained at 37°C, in 5% CO_2, and split twice weekly to maintain a density of $5 \times 10^5 - 2 \times 10^6$ cells ml^{-1}. The cells were provided with fresh complete media 24 h before use.

Experimental procedure

Cells were washed once in serum-free RPMI/HEPES and then resuspended in RPMI/HEPES at 4×10^6 cells ml^{-1}; 0.5 ml aliquots were equilibrated for 1 h at 37°C in polypropylene tubes. Prostanoids were diluted in RPMI/HEPES from ethanol containing stock solutions just prior to use. The final ethanol concentration was 0.1%, which did not affect the measured responses in this test system. Jurkat cells were incubated with vehicle or drug at 37°C for the times indicated. The reaction was stopped by the addition of 0.3 N HCl.

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Cyclic AMP measurement

Acidified samples were heated at 80°C for 30 min and then centrifuged at 1300 *g* for 20 min. The supernatant fractions were neutralized and the cyclic AMP acetylated according to the method of Harper & Brooker (1975). Cyclic AMP content was determined by radioimmunoassay (RIA) using an automated RIA procedure (Attoflo Instruments). Data are presented as means ± s.e.mean. Within each experiment, samples were processed in duplicate.

Chemicals

PGE_2, PGD_2, $PGF_{2\alpha}$, carbaprostacyclin, 16,16-dimethyl PGE_2, 11-deoxy-PGE_1 and PGE_1-1-OH were obtained from Cayman (Ann Arbor, MI). 19(**R**)-OH PGE_2 was obtained from Biomol (Plymouth Meeting, PA). AH 13205 (rac-trans-2-[4-(1-hydroxyphexyl)phenyl]-5-oxocylopentane-heptanoic acid) and AH 23848 ([1α(z),2β5α]-7-[5-[[(1,1'-biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxo-cyclopentyl]-4-heptenoic acid) were gifts from Glaxo (Ware, U.K.). Sulprostone was obtained from Berlex (Cedar Knolls, NJ), while butaprost was prepared at Allergan (Irvine, CA). SQ29548([1S-(1α, 2β (5Z), 3β, 4α]-7[3-[[2-](phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo [2.21]hept-2-yl]-5-heptenoic acid) was purchased from Biomol (Plymouth Meeting, PA). All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

Results

Incubation of Jurkat cells in the presence of PGE_2 led to an increase in endogenous levels of cyclic AMP (Figure 1). The peak response (at 5 min) and subsequent plateau were PGE_2 concentration-dependent. The plateau phase was maintained for at least 40 min. These experiments were carried out without a phosphodiesterase (PDE) inhibitor. In the presence of the PDE inhibitor isobutylmethylxanthine (IBMX, 5×10^{-4} M), the peak cyclic AMP response was 4–8 fold higher (data not shown). For the remaining experiments, cyclic AMP levels were measured at 5 min after addition of the test agent, without a PDE inhibitor.

PGE_2 produced a concentration-dependent increase in cyclic AMP, with an EC_{50} of 6×10^{-8} M (Figure 2). PGD_2, $PGF_{2\alpha}$ and carbaprostacyclin had no effect on cyclic AMP levels at concentrations up to 1×10^{-5} M. Slight increases in cyclic AMP were observed when these drugs were tested at 1×10^{-4} M. PGE_2, therefore, is at least 5,000 times more potent than other natural prostanoids in this assay system.

Stimulation of Jurkat cells with EP receptor-selective analogues produced the following results (Figure 3): incubation of cells in the presence of 11-deoxy PGE_1 produced a concentration-dependent stimulation of cyclic AMP synthesis. This prostaglandin analogue was just as efficacious as PGE_2 and only slightly less potent ($EC_{50} = 2 \times 10^{-7}$ M). Sulprostone, an EP_1/EP_3-selective analogue, and both AH 13205 and butaprost, EP_2-selective compounds, were inactive at concentrations up to 1×10^{-4} M. 1-OH PGE_1 also was inactive at concentrations up to 1×10^{-4} M. 16,16-dimethyl PGE_2 and 19(**R**)-OH PGE_2 were active only at higher concentrations tested, with EC_{50} values of 7.2×10^{-6} M and 8.0×10^{-5} M respectively. In summary, these data show that sulprostone, AH 13205, butaprost and 1-OH PGE_1 do not stimulate cyclic AMP production in Jurkat cells, while the remaining synthetic analogues tested were 40–1000 fold less potent than 11-deoxy PGE_1 or PGE_2 (Table 1). In addition, stimulation of cyclic AMP levels with 5×10^{-5} M forskolin was not inhibited by the prior addition (5 min) of sulprostone at concentrations up to 1×10^{-5} M (data not shown).

The effect of the EP_4 antagonist, AH 23848, on PGE_2-stimulated cyclic AMP in Jurkat cells was examined (Figure 4). Addition of AH 23848 (15 min prior to agonist) led to a concentration-dependent inhibition of cyclic AMP over a range of 1×10^{-6} M to 3×10^{-5} M. A shift to the right in the

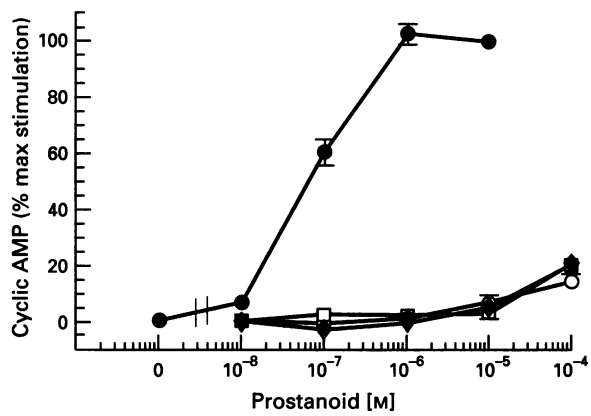

Figure 2 Log concentration-response curves for stimulation of cyclic AMP by prostanoids. Prostaglandin E_2 (PGE_2, ●); $PGF_{2\alpha}$ (□); PGD_2 (○); carbaprostacyclin (◆). Data presented here and in remaining figures as percentage of maximum stimulation, i.e. stimulation in the presence of 1×10^{-5} M PGE_2. Data represent the means ± s.e.mean of 3–14 experiments.

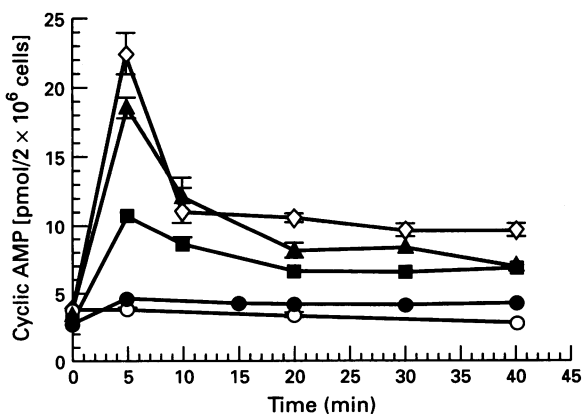

Figure 1 Endogenous levels of cyclic AMP over time after addition of increasing concentrations of prostaglandin E_2 (PGE_2). Vehicle control (○); PGE_2, 0.01 μM (●); PGE_2; 0.05 μM (■); PGE_2, 0.1 μM (▲); PGE_2, 1.0 μM (◇). Data represent the means ± s.e.mean of 3 experiments; in some cases the s.e.mean is smaller than the size of the symbol.

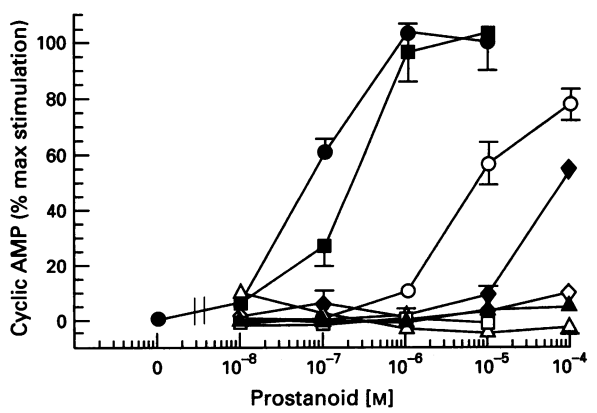

Figure 3 Log concentration-response curves for stimulation of cyclic AMP by prostaglandin E (PGE) analogues. PGE_2 (●); 11-deoxy PGE_1 (■); 16,16-dimethyl PGE_2 (○); 19(**R**)-OH PGE_2 (◆); sulprostone (◇); 1-OH PGE_1 (▲); butaprost (□); and AH 13205 (△). Data represent the means ± s.e.mean of 3–14 experiments.

Table 1 Potencies of prostanoids in stimulating cyclic AMP in Jurkat cells

Agonist	*Receptor selectivity**	EC_{50} (μM)	e.m.r.	n
PGE_2	EP	0.08 (0.05–0.12)	1	14
11-deoxy PGE_1	EP_1,EP_2,EP_3,EP_4	0.25 (0.09–0.42)	3 (1.7–4.3)	5
16,16-dimethyl PGE_2	EP_1,EP_2,EP_3,EP_4	14.0 (1.7–26.2)	150 (49–251)	6
Sulprostone	EP_1,EP_3	>100	>1500	3
AH 13205	EP_2	>100	>1500	3
Butaprost	EP_2	>100	>1500	3
19(R)-OH PGE_2	EP_2	79.7 (45.2–114.3)	1280 (310–2250)	3
1-OH PGE_1	EP_2,EP_4	>100	>1500	3

e.m.r. = equi-effective molar ratios. Data are expressed as means with 95% confidence limits in parentheses.
* Based on isolated tissue preparations as referenced in text.

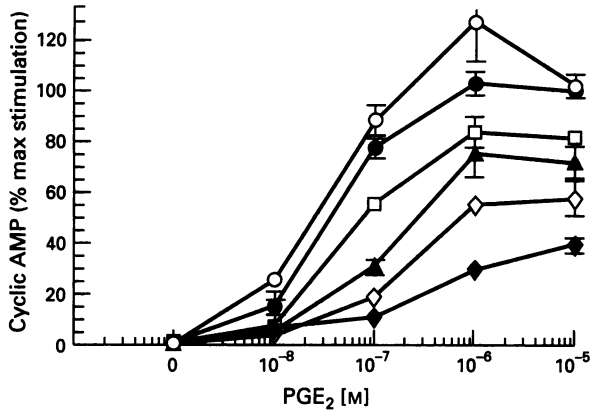

Figure 4 The effects of AH 23848 and SQ 29,548 on prostaglandin E_2 (PGE_2) stimulated cyclic AMP. PGE_2 alone (●); PGE_2 + AH 23848 1 μM (□); PGE_2 + AH 23848 3 μM (▲); PGE_2 + AH 23848 10 μM (◇); PGE_2 + AH 23848 30 μM (◆); PGE_2 + SQ 29,548 10 μM (○). Data represent the means ± s.e.mean of 3 experiments.

PGE_2 concentration-response curve and a depression of the maximum cellular response indicates a noncompetitive antagonism by AH 23848. The TP antagonist, SQ 29,548 (Ogletree *et al.*, 1985) did not affect PGE_2-stimulated cyclic AMP at the highest concentration tested (1×10^{-5} M). Since it could be argued that AH 23848 is altering the kinetics of PGE_2 stimulation of cyclic AMP rather than reducing the maximum transient elevation, we examined the time course of PGE_2-induced cyclic AMP both in the absence and presence of AH 23848. The antagonist caused a reduction in both the initial transient peak and the plateau with no alteration in the kinetics of the response (data not shown).

Discussion

Previous studies have shown that PGE_2 can stimulate cyclic AMP production in Jurkat cells (Mary *et al.*, 1987; Wacholtz *et al.*, 1991). This was reported to be a rapid response with a maximal increase observed within 5 min of stimulation. We also observed a transient, concentration-dependent elevation in cyclic AMP which rapidly fell to a concentration-dependent plateau, maintained over the 40 min observation period. The six to seven fold increase in cyclic AMP is consistent with earlier observations (Wacholtz *et al.*, 1991). The lack of activity seen with PGD_2, $PGF_{2\alpha}$ and carbaprostacyclin clearly indicate that this response is mediated by an EP-receptor.

The rank order of potency of selected synthetic EP agonists suggests that the receptor linked to adenylate cyclase in Jurkat cells is not of the EP_1, EP_2 or EP_3 subtypes. The EP_1/EP_3-selective agonist, sulprostone (Coleman *et al.*, 1994b) was inactive at concentrations up to 1×10^{-4} M. Although, in general, EP_1 and EP_3 receptors have not been shown to be positively coupled to adenylate cyclase, variants of the EP_3 subtype have recently been reported to be linked to stimulation of cyclic AMP production (Namba *et al.*, 1993). The presence of such variants in Jurkat cells would not be consistent with our data.

Two highly selective prostanoid EP_2 agonists have been described: butaprost (Gardiner, 1986) and AH 13205 (Nials *et al.*, 1993). Although both drugs are reported to be 10–100 fold less potent than PGE_2 at EP_2 receptors, in the present study no stimulation of cyclic AMP was observed at concentrations up to 1×10^{-5} M and 1×10^{-4} M, respectively. Furthermore, the EP_2 agonist, 19(R)-OH PGE_2 was 400 times less potent in Jurkat cells than reported both in the cat trachea EP_2 preparation (Woodward *et al.*, 1993) and in cat ciliary muscle (Chen & Woodward, 1992). These data argue against the presence of an EP_2 receptor in Jurkat cells.

Recently, Lawrence & Jones (1992) postulated the existence of more than one EP_2 receptor based on studies of the relaxant effect of PGE_2 and selected prostanoids on the rabbit isolated jugular vein. This hypothesis was based primarily on the modest activity of butaprost compared to 16,16-dimethyl PGE_2 and 11-deoxy PGE_1 in this preparation. All three compounds are of similar potency in the EP_2 cat trachea preparation. Coleman *et al.* (1994a) made similar observations in the pig saphenous vein, where the potent relaxant effect of PGE_2 was not mimicked by sulprostone (EP_1/EP_3) or AH 13205 (EP_2). Furthermore, the TP-receptor antagonists AH 22921 and AH 23848 demonstrated a weak competitive inhibition of PGE_2 in this preparation, although they had no activity in the guinea-pig fundus (EP_1), rabbit ear artery (EP_2) or guinea-pig vas deferens (EP_3). It also was argued that the high absolute sensitivity (EC_{50} < 1.0 nM) of the EP receptors to PGE_2 in the pig saphenous and rabbit jugular veins is qualitatively different from that observed with EP_2 receptor-mediated effects. Coleman *et al.* (1994a) postulated, therefore, that the EP receptor found in the pig saphenous vein and perhaps in the rabbit jugular vein, represents a distinct subtype, termed EP_4.

In Jurkat cells, PGE_2 stimulates cyclic AMP production, but the overall activity profile does not parallel the EP receptor pharmacology reported for the rabbit jugular or pig saphenous veins. First, 16,16-dimethyl PGE_2 was much less potent than PGE_2 (e.m.r. 150). This is different from that observed in the rabbit jugular vein where 16,16-dimethyl PGE_2 and PGE_2 were essentially equipotent (Lawrence & Jones, 1992). Second, 1-OH PGE_1, which is active in the rabbit jugular vein preparation (EC_{50} 2.5×10^{-7} M; e.m.r. 291) (Chen & Woodward, unpublished observation), was inactive in Jurkat cells at concentrations up to 1×10^{-4} M. Furthermore, AH 23848, which has been shown to be a competitive antagonist in both the pig saphenous vein (Coleman *et al.*, 1994a) and rabbit ductus arteriosus (Smith *et al.*, 1994), clearly acts as a non-competitive inhibitor of PGE_2 in Jurkat cells. Taken together, we suggest that the pharmacological profile exhibited by prostanoids in Jurkat cells is not consistent with those previously reported for EP receptors found in rabbit jugular or pig saphenous vein preparations, nor for the EP_1, EP_2 or EP_3 re-

ceptor subtypes. Whether this profile represents a unique subtype or a variant of a previously described receptor will require further study.

How this adenylate cyclase-linked EP receptor is involved in regulation of T-cell activation is not known. The correlation between increases in cyclic AMP and inhibition of IL-2 production and cell proliferation has been well documented. We have observed similar responses in our own laboratory (unpublished observations). The presence of a novel receptor on T-cells presents the possibility of regulating immune function without significant effects on smooth muscle or gastric epithelium.

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