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Investigation of the inhibitory effects of PGE_2 and selective EP agonists on chemotaxis of human neutrophils

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1 The aims of this study were to investigate the inhibitory effects of prostaglandin E_2 (PGE_2) on chemotaxis of N-formyl-methionyl-leucine-phenylalanine (FMLP)-stimulated human neutrophils, and to test the hypothesis that cyclic AMP is the second messenger involved. For this purpose, the inhibitory effect of selective EP agonists, and the modulatory effects of the adenylate cyclase inhibitor, SQ 22536, the protein kinase A (PKA) inhibitors H-89 and Rp-cAMPS, and the type IV phosphodiesterase (PDE) inhibitors, rolipram and Ro20-1724 have been examined.

2 Chemotaxis has been measured using blindwell chambers. When human neutrophils were stimulated with FMLP (100 nM), PGE_2 inhibited chemotaxis in a concentration-dependent manner (0.01-10 μM), with an EC_{50} of 90 ± 24.5 nM, a maximum effect ranging from 45-75% and a mean inhibition of $64.5 \pm 2.4\%$.

3 The EP_2-receptor agonists, 11-deoxy PGE_1, butaprost and AH 13205 also inhibited chemotaxis. The order of potency of these agonists was PGE_2 > butaprost ($EC_{50} = 106.4 \pm 63$ nM) > 11-deoxy PGE_1 ($EC_{50} = 140.9 \pm 64.7$ nM) > AH 13205 ($EC_{50} = 1.58 \pm 0.73$ μM). Correlation of the ability of EP_2 agonists to increase cyclic AMP and to inhibit chemotaxis was poor ($r = 0.38$).

4 The IP agonist, cicaprost gave similar increases in cyclic AMP to those achieved with PGE_2, yet produced 50% of the maximum inhibition of chemotaxis observed with PGE_2.

5 Slight potentiation of the inhibitory effects of PGE_2 after type IV PDE block was observed with rolipram (EC_{50} for $PGE_2 = 57.2 \pm 5.9$; 35.2 ± 6.8 nM) but not Ro20-1724 (EC_{50} for $PGE_2 = 216.0 \pm 59.7$; 97.8 ± 50.6 nM). Type IV PDE inhibitors are themselves potent inhibitors of chemotaxis with EC_{50} values of 23.0 ± 2.3 and 73.6 ± 10.3 nM for rolipram and Ro20-1724, respectively.

6 Inhibition of cyclic AMP production with the adenylate cyclase inhibitor SQ 22,536 (0.1 mM) failed to antagonize inhibition of chemotaxis by PGE_2 (EC_{50}s for PGE_2 of 57.2 ± 5.9 and 56.8 ± 27.3 nM, in the absence and presence of SQ 22,536, respectively) despite a reduction in the increase in cyclic AMP induced by PGE_2.

7 Inhibition of PKA with either H-89 (10 μM) or Rp cyclic AMPS (10 μM) similarly failed to antagonize inhibition of chemotaxis by PGE_2; EC_{50} for PGE_2 of 90 ± 40 and PGE_2 + H-89 60 ± 17 nM; PGE_2 216.0 ± 58.7 and PGE_2 + Rp cyclic AMP 76.9 ± 14.7 nM.

8 Of the two PKA inhibitors tested, H-89 (10 μM) and Rp cyclic AMPS (10 μM), the more effective inhibitor of PGE_2-induced inhibition of neutrophil superoxide anion generation was H-89 (EC_{50}s for PGE_2 were 0.36 ± 0.1 and > 10 μM, respectively). We have previously shown this to be a cyclic AMP-dependent effect of PGE_2.

9 Confirmation of block of PKA by H-89 was suggested by the finding that H-89 blocked inhibition of superoxide anion generation observed with the type IV PDE inhibitors rolipram and Ro20-1724; EC_{50}s of 12.9 ± 8.9 nM for rolipram alone and rolipram + H-89 > 1 μM; Ro20-1724 alone 59.5 ± 28.1 nM and Ro20-1724 + H-89 > 1 μM.

10 The results suggest that inhibition of chemotaxis by PGE_2 and EP_2 agonists is not mediated by increased neutrophil cyclic AMP levels.

Keywords: Neutrophils; chemotaxis; PGE analogues; cyclic AMP

Introduction

Human neutrophil activation is inhibited by prostaglandin E_2 (PGE_2) (Wong & Freund, 1980; Hecker *et al.*, 1989) and PGD_2 (Ney & Schror, 1991) and this has been linked to the ability of these compounds to activate adenylate cyclase. It has previously been shown that inhibition of N-formyl-methionyl-leucine-phenylalanine (FMLP)-induced superoxide generation by PGE_2 is mediated by the occupation of EP_2 receptors and activation of adenylate cyclase, leading to the elevation of intracellular levels of adenosine 3':5'-cyclic monophosphate (cyclic AMP) (Talpain *et al.*, 1995). In this study, the ability of selective EP_2 agonists to inhibit chemotaxis has been examined to test the hypothesis that cyclic AMP is the second messenger mediating inhibition of chemotaxis in human neutrophils.

It has been argued that chemotaxis and chemokinesis are different magnitudes of the same phenomenom (Tranquillo *et al.*, 1988), and indeed that chemotaxis cannot occur without chemokinesis (Wilkinson, 1988). Here no distinction has been made between chemotaxis and chemokinesis, and cell locomotion has been measured by an adaptation of the Boyden chamber method (Boyden, 1962).

To test the hypothesis that cyclic AMP is the second messenger mediating inhibition of chemotaxis, the effects of the following agents have been studied: (1) the type IV PDE inhibitors, rolipram and Ro20-1724 (Schudt *et al.*, 1991); (2) the adenylate cyclase inhibitor, SQ 22,536 (Harris *et al.*, 1979) and (3) the PKA inhibitors, H-89 (Geilen *et al.*, 1992) and Rp-cyclic AMPS (Van Hasslez *et al.*, 1984). In addition the ability of EP_2 receptor agonists to inhibit chemotaxis has been compared with the ability of these compounds to increase neutrophil cyclic AMP levels (Armstrong & Talpain, 1994).

Methods

Isolation of human neutrophils

Human neutrophils were isolated as described previously by use of a discontinuous percoll gradient (Talpain *et al.*, 1995). With this method of separation, neutrophils represented 84.0±1.9% ($n=9$) of the polymorphonuclear leukocyte band, as determined by the % of cells magnetically tagged with CD 16 microbeads (Eurogenetics). The cells were >95% viable as determined by trypan blue exclusion, and were resuspended at a concentration of 1.5×10^6 cells ml^{-1}, in RPMI medium (containing 10 mM HEPES buffer (Gibco) with 0.4% human serum albumin added and filter sterilized) and kept at room temperature.

Chemotaxis procedure

Chemotaxis was measured in blindwell chambers where the chemoattractant (200 μl in RPMI medium) was placed in the lower well and separated from the cells (400 μl = 6×10^5 cells) in the upper well by a 3 μm nuclepore polycarbonate filter (Costar). FMLP (100 nM) was used as the chemoattractant. Cells were treated with inhibitor or RPMI medium immediately prior to addition to the upper well, and the chambers were then incubated for 45 min at 37°C in a moist, 5% CO_2 atmosphere.

Filters were washed and stained with Diff Quik (Baxter Diagnostics AG, fixative-fast green in methanol for 15 s, eosin G for 15 s, thiazine dye for 15 s and water for 15 s), and cells which had migrated through the filter were counted in 5 high power fields. Duplicate wells were prepared for every concentration of drug tested, and the number of cells which had migrated was averaged.

Superoxide anion production

Superoxide generation was assayed by spectrophotometric evaluation of the reduction of ferricytochrome C to ferrocytochrome C (A550nm). The method used was a modification of that published recently (Talpain *et al.*, 1995) to be analysed by a plate reader. Cells (1.5×10^6 ml^{-1}) were resuspended in PBS buffer (Sigma) containing 2.5 mg ml^{-1} cytochrome C and 5 μg ml^{-1} cytochalasin B. (PKA inhibitors were added at this stage when being tested.) Cells (450 μl) were incubated with PBS or inhibitory agent (50 μl) for 10 min at 37°C prior to the addition of FMLP (50 μl-final concentration = 100 nM). Ten min after the addition of FMLP, the reaction was terminated by immersing the tubes in ice for 5 min and the samples centrifuged at 300 *g*, 4°C, for 10 min, to sediment the cells. Samples (200 μl) from each tube were dispensed into a 96 well plate and the absorbance at 550 nm measured. Basal absorbance was taken as cells without FMLP. Each incubation was carried out in triplicate and the values averaged.

Measurement of cyclic AMP levels

Cells (5×10^6 ml^{-1}) were resuspended in Hank's buffer (Gibco) containing 0.25 mM isobutylmethyl xanthine (IBMX) and 1 ml aliquots of cells were incubated for 10 min at 37°C with buffer or prostaglandin. Cyclic AMP levels were measured by a protein binding assay after ethanolic extraction, as described previously (Armstrong & Talpain, 1994).

Data analysis

EC_{50} values were calculated (concentration of agonist required to produce 50% of the maximal effect of PGE_2) for all the EP-receptor agonists, in the presence and absence of antagonists using the Apple Macintosh programme 'Kaleidagraph'.

Statistical analysis

Data are expressed as the mean ± standard error of the mean (s.e.mean), of the averaged result taken from a minimum of three separate experiments. Data were analysed with Student's paired or unpaired 2 tailed *t* test as appropriate. In addition, data involving multiple comparisons were analysed by ANOVA (two factor with replication) using microsoft Excel. A value of $P<0.05$ has been taken as significant.

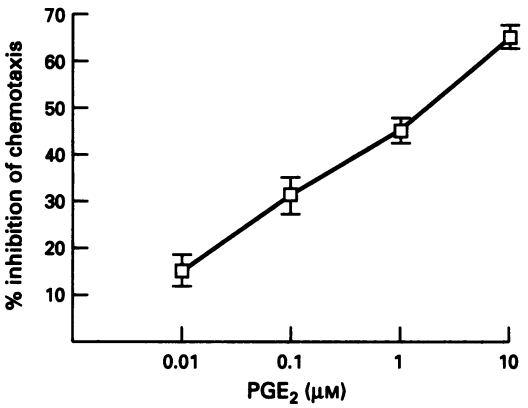

Figure 1 Log concentration-effect curve for PGE_2-inhibition of chemotaxis in human neutrophils induced by FMLP (100 nM). The values are the mean with s.e.mean of 16 different donors for PGE_2.

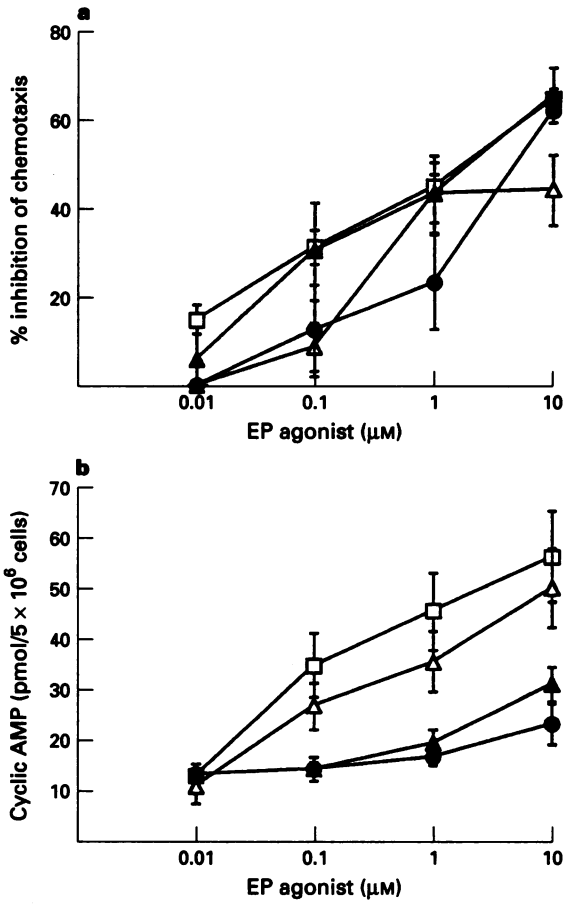

Figure 2 Log concentration-effect curves for (a) inhibition of FMLP-induced neutrophil chemotaxis and (b) increases in cyclic AMP levels in neutrophils induced by butaprost (▲), 11-deoxy-PGE_1 (△), and AH 13205 (●). Effect of PGE_2 (□) is given for comparison. The values are the mean with s.e.mean of at least 3–4 different donors.

Materials

The following compounds were gifts which are gratefully acknowledged: rolipram and cicaprost from Dr E. Schillinger, Schering AG, Berlin; Ro20-1724 (4-butoxy-4(methoxybenzyl)-2-imidazolidinone) from Roche; butaprost from Dr P. Gardiner, Bayer, U.K.; AH 13205 (*trans*-2[4-(1-hydroxyhexyl) pentylphenyl]-5-oxocyclo pentaneheptanoic acid) from Dr B. Bain, Glaxo, U.K.; 11-deoxy PGE_1 was purchased from Cayman Chemicals, U.S.A.; SQ 22,536 [(9-tetrahydro-2-furyl)adenine] was synthesized in the department by Dr N.H. Wilson.

PGE_2, FMLP and ferricytochrome C were purchased from Sigma; Cytochalasin B from Aldrich; Rp cyclic AMPS from R.B.I; H-89 (N-[2-bromocinnamyl(amino)ethyl]-5-isoquinoline sulphonamide) from Calbiochem.

Ethanolic stock solutions of the prostanoids (10–30 nM) were stored at −20°C.

Results

Inhibition of chemotaxis by PGE_2

FMLP (100 nM) induced a submaximal migration of cells (85.3±5.5% of the migration observed with 400 nM FMLP, $n=4$). PGE_2 (0.01–10 μM) caused a concentration-related inhibition of FMLP-induced chemotaxis (Figure 1), producing a maximal effect of 64.5±2.4% inhibition at a concentration of 10 μM ($EC_{50}=90\pm24.5$ nM, $n=16$). Maximal inhibition ranged from 45–75%.

Inhibition of chemotaxis by selective EP agonists

The selective EP_2-receptor agonists, 11-deoxy PGE_1 (Lawrence *et al.*, 1992), butaprost (Gardiner, 1986) and AH 13205 (Nials *et al.*, 1993) also inhibited chemotaxis, giving maximal inhibitions of 43.8±7.9% ($n=3$), 65.2±6.2% ($n=5$) and 61.9±2.7% ($n=3$), respectively at concentrations of 10 μM (Figure 2a). Taking the maximal effect of PGE_2 at 10 μM as 100% response, apparent EC_{50}s of 106.4±63 nM, 140.9±64.7 nM and 1.58±0.73 μM were determined for butaprost, 11-deoxy PGE_1 and AH 13205, respectively. For comparison, the ability of these analogues to increase neutrophil cyclic AMP levels is shown in Figure 2b.

Inhibition of chemotaxis by cicaprost

The selective IP agonist, cicaprost, was much weaker at inhibiting chemotaxis than PGE_2, giving a maximal effect of 28.5±8.7% at 10 μM, $n=5$, and an EC_{50} of >10 μM (Figure 3a). In contrast, cicaprost and PGE_2 were equiactive at increasing neutrophil cyclic AMP levels (Figure 3b).

Effect of Type IV PDE inhibitors

Inhibition of chemotaxis by PGE_2 after PDE block with rolipram (Figure 4a) and Ro20-1724 (Figure 4b) was investigated. A slight and significant ($P=0.002$ by ANOVA) additive effect was observed with rolipram but not Ro20-1724 (EC_{50}s for PGE_2 of 57.2±5.9; 35.2±6.8 nM, $n=5$ and 216.0±59.7; 97.8±50.6 nM, $n=4$, with rolipram (50 nM) and Ro20-1724

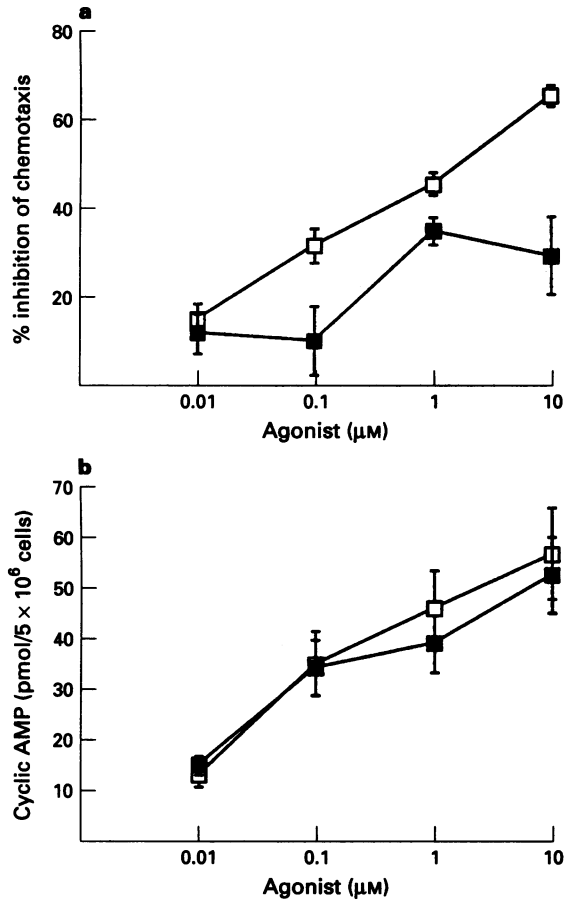

Figure 3 Log concentration-effect curves for (a) inhibition of FMLP-induced neutrophil chemotaxis and (b) increases in cyclic AMP levels in neutrophils induced by the IP receptor agonist, cicaprost (■). Effect of PGE_2 (□) is given for comparison. The values are the mean with s.e.mean of 5–10 different donors.

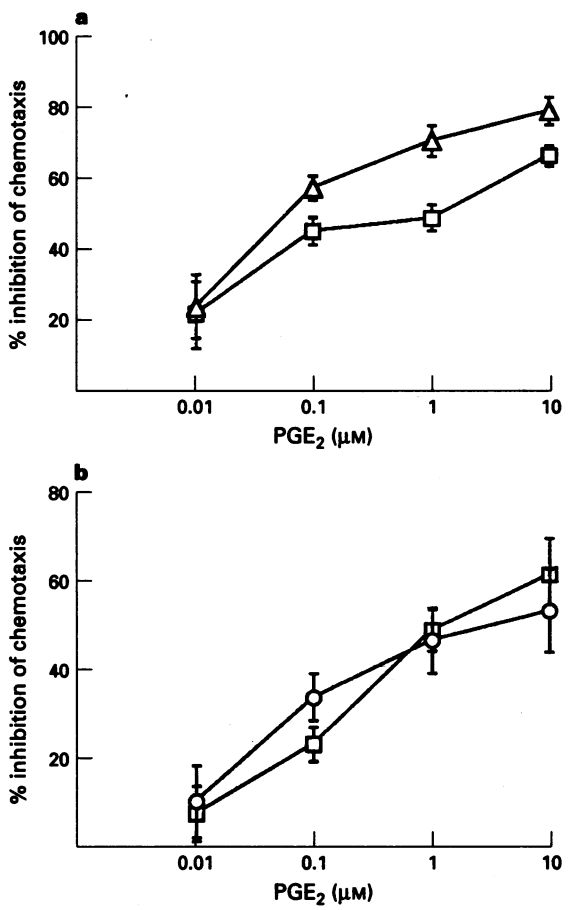

Figure 4 Log concentration-effect curves for inhibition of FMLP-stimulated neutrophil chemotaxis by PGE_2 in control cells (□) and cells treated with (a) rolipram (50 nM) (△) and (b) Ro20-1724 (50 nM) (○). The values are the mean with s.e.mean of 5 and 4 different donors respectively.

(50 nM) respectively). In these paired experiments, rolipram and Ro20-1724 at 50 nM inhibited the migration of neutrophils induced by FMLP (399.7±31.9 and 528±61.5 cells) by 51.4±9.5% and 33.4±4.8% respectively. Type IV PDE inhibitors are themselves potent inhibitors of neutrophil chemotaxis (Figure 5) giving EC_{50}s of 23.0±2.3 and 73.6±10.3 nM, maximum inhibition of 90.6±4.0 and 84.7±6.2% for rolipram and Ro20-1724 respectively, $n=3$.

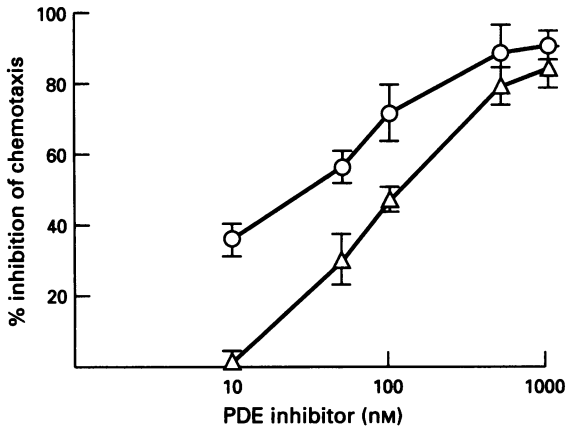

Figure 5 Log concentration-effect curves for inhibition by rolipram (○), and Ro20-1724 (△) of FMLP-stimulated neutrophil chemotaxis. The values are the mean with s.e.mean of 3 different donors.

Effect of inhibition of adenylate cyclase

Treating neutrophils with the adenylate cyclase inhibitor SQ 22,536 (0.1 mM) failed to antagonize inhibition of chemotaxis by PGE_2 (Figure 6a), giving EC_{50}s of 57.2±5.9 and 56.8±27.3 nM, $n=4$. Indeed a significant enhancement of the PGE_2 effect ($P=0.03$ determined by ANOVA) resulted from SQ 22,536-treatment. Control migration of cells (369.6±38.9 cells) was not affected by SQ 22,536 (348.2±53.5 cells, $P=0.67$). SQ 22,536 (0.1 mM) reduced the increase in neutrophil cyclic AMP observed with PGE_2 (Figure 6b).

Effect of inhibition of protein kinase A (PKA)

Inhibition of protein kinase A with either H-89 (10 μM) or Rp cyclic AMPS (10 μM) similarly failed to antagonize inhibition of chemotaxis (Figure 7a and b), giving EC_{50}s for PGE_2 of 90±40; 60±17 nM, $n=4$ and 216.0±58.7; 76.9±14.7 nM, $n=4$ respectively. No effect of H-89 or Rp-cyclic AMPS treatment was determined by ANOVA analysis. Control migration of cells (606±72.3 cells) was significantly reduced by H-89 treatment (497±102 cells, $P=0.02$), but not by Rp cyclic AMPS treatment (528±61.5 and 452.7±66.2 cells, respectively).

Effect of PKA inhibitors on inhibition of O_2^- generation by PGE_2

Of the two PKA inhibitors tested, H-89 (10 μM) and Rp cyclic AMPS (10 μM), the more effective inhibitor of PGE_2-induced inhibition of neutrophil superoxide anion generation was H-89, giving EC_{50}s for PGE_2 of 0.36±0.1 and >10 μM (Figure 8a

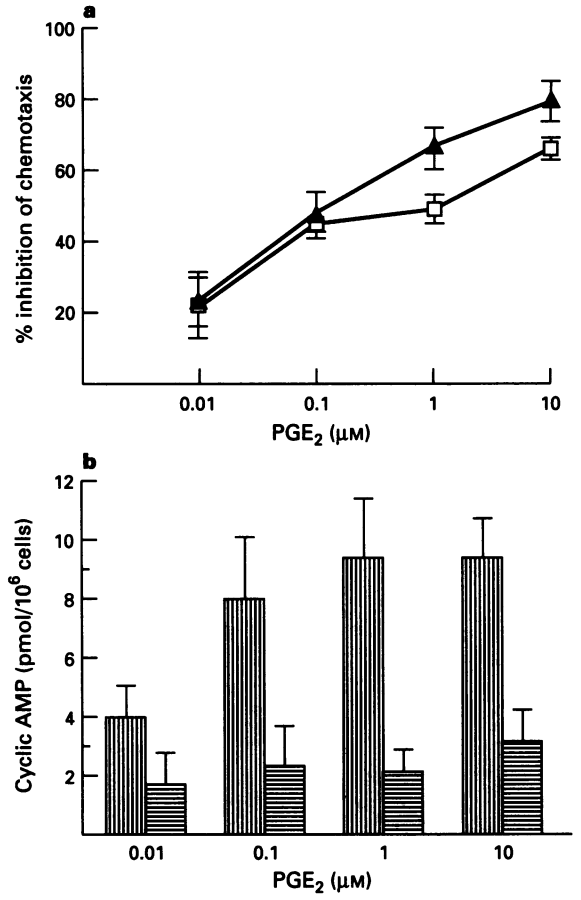

Figure 6 Effect of SQ 22,536 on PGE_2-induced inhibition of FMLP-stimulated neutrophil chemotaxis and increases in cyclic AMP levels. Inhibition by PGE_2 of chemotaxis of control cells (□) and cells treated with SQ 22,536 (▲) is shown in (a) and PGE_2-induced cyclic AMP levels in control cells (vertically lined columns) and cells treated with SQ 22,536 (horizontally lined columns) is shown in (b). The values are the mean with s.e.mean of 5 different donors.

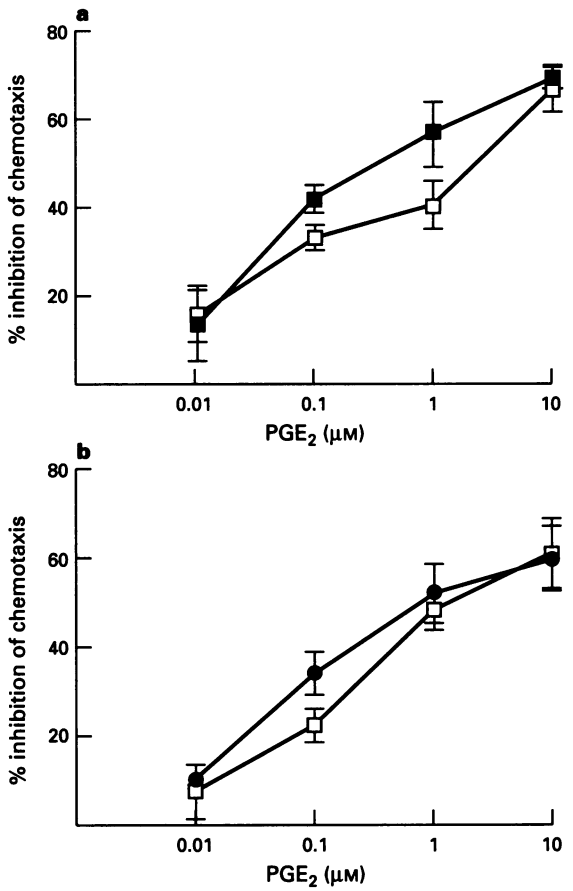

Figure 7 Log concentration-effect curves for inhibition of FMLP-stimulated neutrophil chemotaxis by PGE_2 in control cells (□) and cells treated with (a) H-89 (10 μM) (■) and (b) Rp-cyclic AMPS (10 μM) (●). The values are the mean with s.e.mean of 4 different donors.

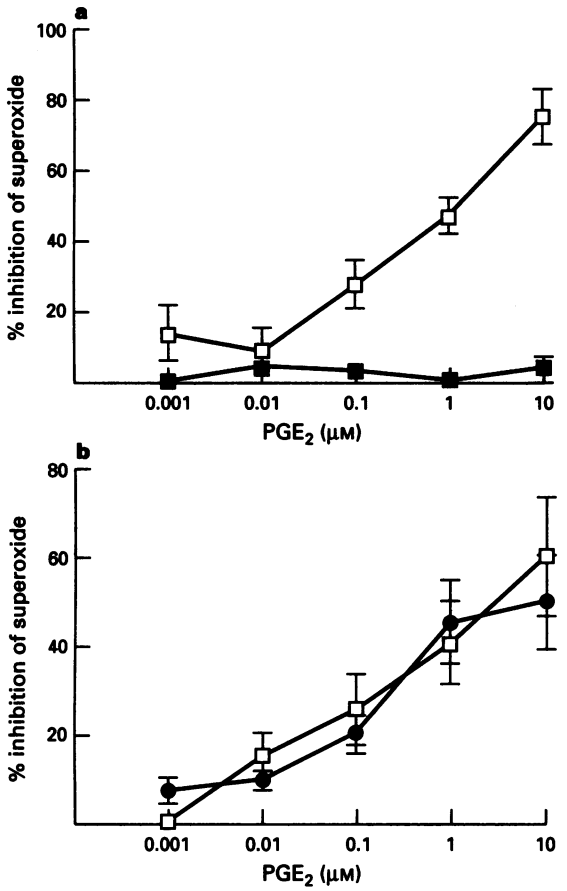

Figure 8 Log concentration-effect curves for inhibition of superoxide anion generation in FMLP-stimulated neutrophils by PGE_2 in control cells (□) and cells treated with (a) H-89 (10 μM) (■) and (b) Rp-cyclic AMPS (10 μM) (●). The values are the mean with s.e.mean of 4 and 3 different donors.

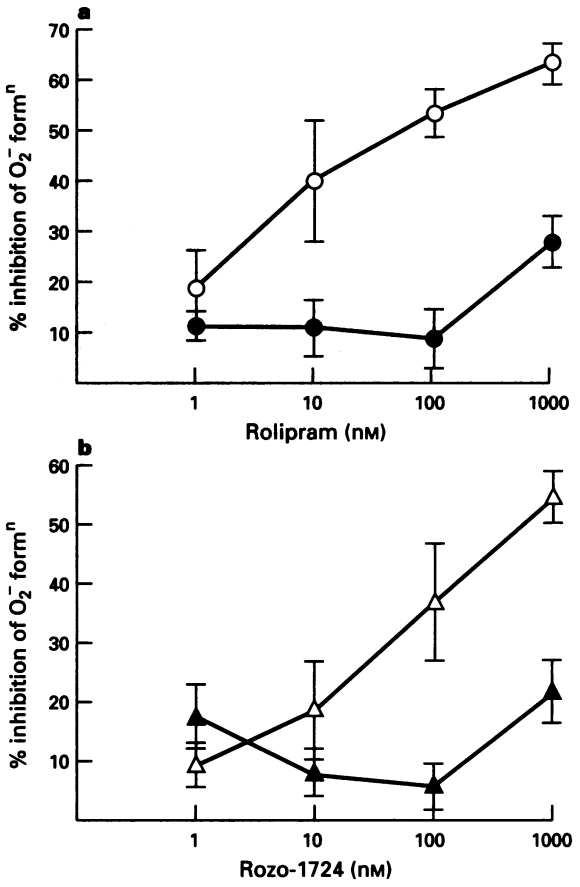

Figure 9 Log concentration-effect curves for inhibition of superoxide anion generation in FMLP-stimulated neutrophils by (a) rolipram in control cells (○) and cells treated with H-89 (10 μM) (●) and (b) Ro20-1724 in control cells (△) and cells treated with H-89 (10 μM) (▲). The values are the mean with s.e.mean of 4 different donors.

and b) which was highly significant by ANOVA analysis. H-89 treatment did not significantly affect the control response to FMLP (113.5±11.3%, $n=4$). Rp cyclic AMP did not significantly affect the PGE_2 response (EC_{50}s of 0.05±0.02 and 1.69±1.5 μM, $n=3$) or the control response to FMLP (100.2±5.5%). H-89 (10 μM) similarly blocked inhibition of O_2^- generation by rolipram and Ro20-1724 (Figure 9a and b), given EC_{50}s of 12.9±8.9 nM and >1.0 μM; 59.5±28.1 nM and >1.0 μM, $n=4$.

Discussion

Chemotaxis has been measured here as the total number of cells (counted in 5 high power fields) which have migrated from the upper well of the blindwell chamber into the filter in the 45 min incubation period. PGE_2 inhibited chemotaxis induced by a submaximal concentration of FMLP (100 nM), with an EC_{50} of 90 nM (Figure 1). Agonists with activity at EP_2 receptors also inhibited neutrophil chemotaxis (Figure 2a). The rank order of potency was PGE_2 > butaprost (EC_{50} = 106 nM) > 11-deoxy PGE_1 (EC_{50} = 141 nM) > AH 13205 (EC_{50} = 1.6 μM). The inhibitory effects of these EP_2 agonists did not correlate well with their ability to increase neutrophil cyclic AMP levels (Figure 2b), giving a correlation coefficient $r=0.38$ when the increase in cyclic AMP induced by 0.1, 1 and 10 μM of EP_2 agonist was compared with the % inhibition of chemotaxis observed at each concentration. Butaprost and AH 13205 are very weak agents at increasing cyclic AMP levels (Armstrong & Talpain, 1994), yet 11-deoxy PGE_1 which is slightly more effective and achieves the same maximal increase in cyclic AMP levels as PGE_2 consistently failed to achieve the maximal inhibition of chemotaxis observed with PGE_2, butaprost and AH 13205. As such, butaprost and AH 13205 are very poor inhibitors of neutrophil superoxide anion production (Talpain *et al.*, 1995), which we have suggested to be mediated by increased cyclic AMP levels. Furthermore, the IP agonist, cicaprost, gave similar increases in neutrophil cyclic AMP levels to those observed with PGE_2 (Figure 3b) yet only gave 50% of the maximum inhibition of chemotaxis observed with PGE_2 (Figure 3a). The fact that both 11-deoxy PGE_1 and cicaprost gave larger increases in cyclic AMP than those observed with butaprost and AH 13205, but were less effective inhibitors of chemotaxis, argues against the possibility that chemotaxis is particularly sensitive to inhibition by cyclic AMP such that agents which only weakly increase cyclic AMP levels could still be effective inhibitors of chemotaxis. If this were the case, 11-deoxy PGE_1 and cicaprost should still be more potent inhibitors of chemotaxis than butaprost and AH 13205.

These results suggested that unlike inhibition of superoxide anion generation, inhibition of chemotaxis by PGE_2 and EP_2 agonists is not mediated by increased cyclic AMP levels. This is supported by the finding that inhibition of the breakdown of neutrophil cyclic AMP with the type IV PDE inhibitors rolipram or Ro20-1724 (Figure 4a and b) did not potentiate inhibition of chemotaxis observed with PGE_2. The slight additive effect observed with rolipram may relate to the 50% inhibition of FMLP-induced cell migration by rolipram at 50 nM. Indeed rolipram and Ro20-1724 were potent inhibitors of neutrophil chemotaxis (Figure 5) giving EC_{50}s of 23 and 74 nM and maximal inhibitions of 91 and 85%. In contrast, rolipram markedly potentiated inhibition of superoxide anion generation by PGE_2 at concentrations as low as 2 nM (Talpain *et al.*, 1995). Furthermore, the adenylate cyclase inhibitor, SQ 22,536

(0.1 mM) did not antagonize inhibition of chemotaxis by PGE_2 (Figure 6a) but antagonized inhibition of superoxide anion generation (Talpain *et al.*, 1995) and reduced the increase in cyclic AMP observed with PGE_2 (Figure 6b). Inhibition of PKA with either H-89 (10 μM) or Rp cyclic AMPS (10 μM) similarly failed to antagonize inhibition of chemotaxis by PGE_2 (Figure 7a and b). Of these two inhibitors, results with H-89 are the more convincing, as a dramatic block of inhibition of superoxide anion generation by PGE_2 was observed (Figure 8a) giving an EC_{50} for PGE_2 of 0.36 μM and >10 μM respectively. This was not observed with Rp cyclic AMPS (Figure 8b) suggesting that at a concentration of 10 μM, Rp cyclic AMPS did not give a significant block of PKA in the human neutrophil. Indeed this may relate to the report that Rp cyclic AMPS can contain biologically significant amounts of adenosine (Musgrave *et al.*, 1993) and can itself inhibit neutrophil superoxide anion generations, albeit at higher concentrations (100 μM). Confirmation that H-89 was blocking PKA was obtained from the results with rolipram and Ro20-1724, where H-89 markedly blocked inhibition of superoxide anion generation by these PDE inhibitors, giving EC_{50}s of 13 nM and >1 μM; 60 nM and >1 μM (Figure 9a and b).

Since rolipram and Ro20-1724 were potent inhibitors of chemotaxis (Figure 5) it suggests that chemotaxis is sensitive to inhibition by cyclic AMP, although increases in cyclic AMP (from 30 s to 10 min, at 30 s intervals) with these compounds could not be detected.

Since PGE_2 appears to inhibit superoxide anion generation by a mechanism which is cyclic AMP-dependent, this suggests that these two processes, chemotaxis and superoxide anion generation, can be inhibited differentially. These two events have also been dissociated from each other in terms of signal transduction mechanisms. Recent evidence suggests that increased neutrophil cyclic GMP levels are associated with chemotaxis (Wyatt *et al.*, 1991; Belenky *et al.*, 1993) whereas activation of phospholipase D has been implicated in superoxide anion generation (Thelen *et al.*, 1993). It is suggested that NO production is responsible for the observed increases in cyclic GMP, and inhibitors of NO production such as L-NMMA inhibit chemotaxis. In addition, chemotaxis and superoxide anion generation can be dissociated by use of botulinum C2 toxin (Norgauer *et al.*, 1988) which enhances O_2^- release but inhibits neutrophil migration. The mechanism by which PGE_2 inhibits chemotaxis is under investigation.

In conclusion, these results suggest that PGE_2 and EP_2 agonists inhibit chemotaxis of human neutrophils by a cyclic AMP-independent mechanism. Since inhibition of superoxide anion generation by these EP agonists is dependent on cyclic AMP (Talpain *et al.*, 1995) it suggests that chemotaxis and superoxide anion generation in human neutrophils can be regulated differentially.

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