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Synergistic Inhibition of Human Polymorphonuclear Function by Prostaglandin E_1 and Linsidomine

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Abstract

Polymorphonuclear cells (PMN) are the dominating inflammatory cell population in acute tissue injury and contribute to host-defence mechanisms by formation and release of chemical mediators. The aim of the present study was to investigate whether chemoattractant-induced PMN stimulation can be synergistically antagonized by vasodilatory prostaglandins and nitric oxide (NO), both being formed by the vasculature in inflamed areas. PGE_1 (10 nM–10 μM) inhibited concentration-dependently formyl-methionyl-leucyl-phenylalanine (fMLP)-induced β-glucuronidase and oxygen radical ($O_2^{\cdot}$) release from human PMN. The NO donor linsidomine (100 μM) was ineffective, but significantly enhanced PGE_1 effects on oxygen radical generation and enzyme release. The non-selective phosphodiesterase inhibitor 3-isobutyl-l-methylxanthine (IBMX) (0·5 mM) potentiated PGE_1 effects on all parameters measured. The combination linsidomine (100 μM) plus IBMX (0·5 mM) did not additionally reduce β-glucuronidase release, but abolished fMLP-stimulated $O_2^{\cdot}$ generation. There was a stimulation of cAMP formation by PGE_1 but not by linsidomine, both in the absence and presence of IBMX.

It is concluded that the effects of linsidomine on PMN function and its synergism with PGE_1 are not tightly correlated with total cAMP accumulation. Alternatively, the inhibition of $O_2^{\cdot}$ generation by linsidomine may be related to its ability to modulate the activation of the NADPH oxidase system or to scavenge free oxygen radicals.

Human polymorphonuclear cells (PMN) are the dominant white cell population in inflammatory exudates. They accumulate at a site of tissue injury via the expression of adhesion molecules and modulate the local inflammatory response by the release of chemical mediators. These mediators, including PMN-derived adhesion molecules (L-selectin), oxygen-centred free radicals ($O_2^{\cdot}$), lysosomal enzymes and products of the arachidonic acid metabolism (leukotrienes) are vital factors in host defence (Cooke et al 1989; Jutila 1992). However, they may also become important for tissue injury, for example, if the noxious stimulus cannot be eliminated and PMN stimulation persists, resulting in generation of huge amounts of tissue-destructive products.

Another cause of non-selective tissue injury by activated PMN is the largely uncontrolled nature of mediator release once the activation cascade is turned on. Since it is generally appreciated that cAMP accumulation results in inhibition of neutrophil function (Cooke et al 1989), one may argue that locally formed mediators, that stimulate adenylate cyclase in PMN, may exert this control function. This includes β_2-adrenergic agonists and vasodilatory prostaglandins. Inhibitory effects of vasodilatory prostaglandins of the E- and D-series on several activation parameters of human PMN are well established and found to be related to cAMP accumulation (O'Flaherty et al 1979; Wong & Freund 1981; Fantone & Kinnes 1983; Gryglewski et al 1987; Hecker et al 1990; Wright et al 1990).

In contrast to cAMP, the role of cGMP in the regulation of PMN function is still poorly understood. Recent evidence suggests that nitric oxide (NO) may act as a scavenger of pro-adhesive superoxide anion, acting as an inhibitor of leukocyte adhesion (Reinhardt et al 1992). Linsidomine, the active metabolite of molsidomine, inhibits platelet aggregation (Reden 1990) and PAF-induced leukocyte-endothelium adhesion by releasing nitric oxide (Reinhardt et al 1992). Linsidomine might exert these inhibitory effects through increasing cGMP levels (Ney et al 1990; Schröder et al 1990). Because of enhanced local NO formation at inflammatory sites it appears to be possible that NO may synergize with vasodilatory prostaglandins to control the inflammatory process.

The purpose of this study was to investigate the possible interaction between two different inhibitory signals for PMN: NO and E-type prostaglandins. Oxygen-centred radical formation and lysosomal enzyme release were measured as parameters of cell activation and correlated with cAMP levels in formyl-methionyl-leucyl-phenylalanine (fMLP)-stimulated human PMN. The hypothesis was that a synergism between the two classes of mediators would occur (Schröder et al 1992) and that cAMP levels should be best correlated with inhibition of cell stimulation.

Materials and Methods

Preparation of PMN

Blood was taken by venipuncture from healthy donors who had not taken any medication within the last two weeks before the study. The blood was collected into acidic citrate/dextrose (Biostabil, 1/10; v/v). Platelet-rich plasma was separated by centrifugation at 300 *g* and room temperature for 10 min. The remaining sediment was diluted with phosphate-buffered saline (PBS). PMN were prepared following the procedure described by Böyum (1968) with slight modifications (Hecker et al 1990). Briefly, white cells were separated by dextran (6%) sedi-

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mentation. PMN were separated thereafter from other white cells by Ficoll–Paque-gradient centrifugation at 300 *g* for 20 min at 15°C. Any remaining red cells were destroyed by hypotonic lysis. The PMN were washed twice at 4°C in PBS and then resuspended in this solution, supplemented with 0·05% glucose. The PMN number was determined microscopically and adjusted to a final stock concentration of 5×10^7 cell mL^{-1}. This PMN preparation contained 95% PMN and their viability, tested by Trypan blue staining, was greater than 95%.

Measurement of oxygen-centred radical formation

PMN (5×10^6 cells) were incubated in Hanks balanced salt solution (HBSS, PBS, supplemented with 1 mM Ca^{2+} and 1 mM Mg^{2+}) at 37°C in a total volume of 1 mL. According to the protocol, PGE_1 (1 nM – 10 μM), linsidomine (1 μM – 1 mM) or a combination of both was added for 1·5 min. These incubations were performed in the presence or absence of IBMX (0·5 mM). The cells were stimulated with fMLP (30 nM) for another 1·5 min. Thus, the total incubation time was 3 min. The reaction was stopped at time 3 min by centrifugation at 12 000 *g* at 4°C. Superoxide anion (O_2^-) generation was measured spectrophotometrically (550 nm) in terms of superoxide dismutase (SOD, 300 int. units mL^{-1}) inhibitable reduction of ferricytochrome C (Babior et al 1976). The values were corrected for basal O_2^- generation by unstimulated cells.

Measurement of lysosomal enzyme release

PMN (5×10^6 cells) were incubated in HBSS containing cytochalasin B (5 μg mL^{-1}) at 37°C in a total volume of 1 mL. According to the protocol described above, the incubations (10 min) were performed in the presence or absence of IBMX. The cells were stimulated with fMLP (30 nM) for another 10 min. The reaction was stopped as indicated above and β-glucuronidase activity was measured in terms of release of phenolsulphthalein from phenolphthalein-β-glucuronide as previously described (Hecker et al 1990).

Cyclic nucleotide determinations

PMN ($2·5 \times 10^6$ cells) were incubated with the substances to be studied (PGE_1 and linsidomine alone or in combination) for 1·5 min at 37°C in HBSS supplemented with cytochalasin B (5 μg mL^{-1}) and with or without IBMX (0·5 nM). The cells were stimulated for another 1·5 min with fMLP (30 mM). In preliminary experiments, incubation times of 10 min for PGE_1 and linsidomine and 10 min stimulation with fMLP were also investigated and gave similar results (not shown). The total assay volume was 0·5 mL. The reaction was stopped by addition of perchloric acid (15%, 1/3 v/v). The cell membranes were disrupted by sonication, the pH was adjusted to 7·4 with ice-cold K_2CO_3 solution (2 M) and the cell debris removed by centrifugation. An aliquot (100 μL) from the supernatant was used for cAMP and cGMP determinations by specific radioimmunoassays, developed in our laboratory according to Steiner et al (1972).

Substances and solutions

Biostabil was from Biotest, Frankfurt, Germany; formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) from Calbiochem GmbH, Frankfurt, linsidomine from Cassella-Riedel, Frankfurt. [^{3}H]cAMP and [^{3}H]cGMP were from Du Pont de Nemours GmbH, Dreieich, Germany. Dextran T 500 and Ficoll Paque were from Pharmacia, Freiburg, Germany; PGE_1 was from Schwarz–Pharma, Monheim, Germany. Phenolphthalein-β-D-glucuronide was from Serva, Heidelberg, Germany; cAMP free acid, cGMP free acid, cytochalasin B, ferricytochrome C, 3-isobutyl-1-methylxanthine, and superoxide dismutase from bovine erythrocytes were from Sigma, Deisenhofen, Germany. All other substances and solvents were from E. Merck Darmstadt, Germany and of the highest purity available. Linsidomine and PGE_1 were kindly provided by the respective manufacturers.

PGE_1 was dissolved in ethanol and fMLP in dimethylsulphoxide at 10 mM stock concentrations. Dilutions were made immediately before use with the appropriate buffer. Linsidomine was always freshly dissolved in buffer and used within 1 h. Neither of the solvents affected the assays at the final concentrations used.

Statistics

Data are mean ± s.e.m. of at least n = 5–6 PMN preparations from different donors, performed in duplicate. Data were analysed by analysis of variance (repeated measures) and checked for statistical significance by Student–Newman–Keul's test. $P < 0·05$ was considered significant.

Results

Oxygen radical formation

Effects of PGE_1 and linsidomine without IBMX. Basal O_2^- formation by unstimulated PMN amounted to 3·6 ± 0·7 nmol/5×10^6 cells. O_2^- formation was stimulated tenfold to 36·2 ± 2·7 nmol O_2^-/5×10^6 cells by 30 nM fMLP. This value was unchanged by 100 μM linsidomine (33·7 ± 2·4 nmol O_2^-/5×10^6 cells, n = 6, $P > 0·05$). However, a tenfold concentration of linsidomine caused a marked reduction in O_2^- formation (Table 1). From these data a concentration of 100 μM linsidomine was selected for further experiments.

Table 1. Effect of linsidomine on oxygen radical formation O_2^-, β-glucuronidase release and cAMP generation in human PMN.

Agonist	Linsidomine (μM)	Oxygen radical formation (nmol/5×10^6 PMN)	β-Glucuronidase (μg/5×10^6 PMN)	cAMP (pmol/5×10^6 PMN)
None	0	3·6 ± 0·7	3·1 ± 0·9	11·4 ± 1·0
30 nM fMLP	0	36·2 ± 2·7	25·3 ± 3·3	14·0 ± 2·2
	1	38·7 ± 9·0	23·2 ± 4·1	15·2 ± 2·1
	10	36·3 ± 3·0	24·2 ± 4·9	14·0 ± 1·0
	100	33·7 ± 2·4	23·4 ± 2·7	15·3 + 1·3
	1000	5·0 ± 2·2*	17·6 ± 2·8	15·0 ± 1·8

The data are mean ± s.e.m of 6 experiments performed in duplicate. *$P < 0·05$ compared with control (FMLP).

Table 2. Concentration–dependent inhibition of fMLP (30 nM)-induced O_2^- formation in human PMN in the absence and presence of IBMX (0·5 mM). Mean ± s.e.m. of 6 experiments. *$P < 0.05$ for PGE_1 alone compared with the same concentration plus linsidomine.

	PGE_1 (nM)	Inhibition (% control)	
		PGE_1 alone	PGE_1 plus linsidomine (100 μM)
Control	1	100 ± 4	81 ± 4*
	10	87·5 ± 6	71 ± 7*
	100	65·0 ± 7	44 ± 7*
	1000	42·0 ± 6	22 ± 6*
	10 000	25·0 ± 5	3 ± 5*
+ IBMX (0·5 mM)	1	92 ± 5	20 ± 10*
	10	62 ± 10	2 ± 3*
	100	22 ± 4	1 ± 1*
	1000	23 ± 10	2 ± 4*
	10 000	23 ± 2	3 ± 6*

Table 4. Concentration–dependent inhibition of fMLP (30 nM)-induced β-glucuronidase release from human PMN in the absence and presence of IBMX (0·5 mM). Mean ± s.e.m. of 6 experiments. *$P < 0.05$ for PGE_1 alone compared with the same concentration plus linsidomine.

	PGE_1 (nM)	Inhibition (% control)	
		PGE_1 alone	PGE_1 plus linsidomine (100 μM)
Control	1	100 ± 1	82 ± 3*
	10	100 ± 2	77 ± 3*
	100	90 ± 3	72 ± 7*
	1000	78 ± 3	60 ± 4*
	10 000	58 ± 4	47 ± 4*
+ IBMX (0·5 mM)	1	92 ± 4	72 ± 10
	10	47 ± 6	38 ± 8
	100	25 ± 6	20 ± 10
	1000	18 ± 4	12 ± 6
	10 000	15 ± 4	10 ± 8

As expected, PGE_1 at 10 nM—10 μM caused a significant, concentration-dependent decrease in O_2^- generation. The inhibitory effect of PGE_1 on O_2^- was significantly enhanced in the presence of linsidomine at all concentrations of PGE_1 studied. Interestingly, the synergistic effect by linsidomine on PGE_1-induced decrease in O_2^- formation remained the same, i.e. about 20%, throughout the concentration–response curve of PGE_1 (Table 2).

Effects of PGE_1 and linsidomine in the presence of IBMX. In the presence of IBMX (0·5 mM), both basal and fMLP-stimulated O_2^- formation were significantly lower than in the absence of IBMX. Resting cells generated 1·0 ± 0·1 nmol O_2^-/5 × 10^6 cells, fMLP-stimulated cells 8·3 ± 0·6 nmol O_2^-/5 × 10^6 cells ($n = 6$; $P < 0.05$). There was a marked reduction of O_2^- formation by PGE_1 at 10 and 100 nM (40 ± 10% and 77 ± 4%, respectively). Further increase in PGE_1 concentrations did not elicit stronger suppressions of O_2^- formation and there was always a significant though reduced O_2^- generation even at 10 μM PGE_1 (Table 2).

A similar, although somewhat less pronounced inhibition of O_2^- formation was also obtained after linsidomine in the presence of IBMX. As shown in Table 3, linsidomine caused a concentration–dependent reduction of O_2^- formation at concentrations of 10 μM and higher. The combination PGE_1 plus linsidomine (100 μM) abolished O_2^- generation at 10 nM (Table 2).

β-Glucuronidase release

Effects of PGE_1 and linsidomine without IBMX. Basal enzyme release from unstimulated PMN was equivalent to 3·1 ± 0·9 μg phenolphthalein per 5 × 10^6 cells. This basal release was stimulated to 25·3 ± 3·3 μg phenolphthalein per 5 × 10^6 cells by 30 nM fMLP ($n = 6$). PGE_1 inhibited enzyme release by 40 ± 8% at 10 μM while linsidomine was ineffective up to 100 μM.

PGE_1 alone had a minor effect on β-glucuronidase release. Only at the highest concentration of 10 μM was there a significant suppression of enzyme release. Linsidomine synergized with PGE_1 at all concentrations of PGE_1. As already seen with O_2^- formation, this synergistic effect of linsidomine caused an additional 20% inhibition and was independent of the concentration of PGE_1 applied (Table 4).

Effects of PGE_1 and linsidomine in the presence of IBMX. IBMX alone affected neither basal or fMLP-stimulated β-glucuronidase release, which amounted to 2·0 ± 0·6 and 22·0 ± 2·9 μg phenolphthalein/5 × 10^6 cells, respectively. However, PGE_1 in the presence of IBMX caused a concentration–dependent reduction in β-glucuronidase release which became significant at 10 nM and reached more than 80% inhibition at 10 μM PGE_1 (Table 4).

The enzyme release in the presence of IBMX was unaffected by linsidomine up to 100 μM. Linsidomine (100 μM) also did not enhance the inhibitory effect of PGE_1 on β-glucuronidase release above the inhibition seen by IBMX alone (Table 4).

Table 3. Effect of linsidomine on oxygen radical formation O_2^-, β-glucuronidase release and cAMP generation in human PMN in the presence of IBMX (0·5 mM).

Agonist	Linsidomine (μM)	Oxygen radical formation (nmol/5 × 10^6 PMN)	β-Glucuronidase (μg/5 × 10^6 PMN)	cAMP (pmol/5 × 10^6 PMN)
None	0	1·0 + 0·1	2·0 ± 0·5	18·5 ± 2·0
30 nM fMLP	0	8·3 ± 0·5	22·0 + 2·9	29·8 + 4·6
	1	6·9 ± 1·0	21·3 + 3·2	30·5 ± 4·2
	10	5·5 + 1·4*	24·4 + 2·5	34·9 ± 7·1
	100	3·4 + 1·1*	20·3 ± 1·9	33·3 ± 4·6*
	1000	0·9 ± 0·6*	14·7 ± 1·6*	40·2 + 6·4*

The data are mean ± s.e.m. of 5–6 experiments performed in duplicate. *$P < 0.05$ compared with control.

Table 5. Effect of PGE_1 and linsidomine on cAMP generation in human PMN.

Agonist	PGE_1 (nM)	Linsidomine (μM)	cAMP (pmol/5 × 10^6 PMN)
None	0	0	11·5 ± 1·0
30 nM fMLP	0	0	14·0 ± 2·2
	1	0	13·2 ± 0·9
	10	0	16·5 ± 0·4
	100	0	13·8 ± 1·6
	1000	0	22·4 ± 3·2
	10000	0	53·6 ± 9·2*
	1	100	15·7 + 1·2
	10	100	15·8 ± 1·7
	100	100	17·6 ± 2·0
	1000	100	27·6 ± 6·1
	10000	100	43·0 ± 9·8*

The data are mean ± s.e.m. of 6 experiments performed in duplicate. *$P < 0·05$ compared with corresponding control.

Cyclic GMP

The basal level of cGMP in non-stimulated PMN amounted to 17·0 ± 3·0 pmol/5 × 10^6 cells (n = 6) and was not influenced by fMLP stimulation or IBMX pretreatment. The values at 10 μM PGE_1, 100 μM linsidomine and after combined administration of 10 μM PGE_1 plus 100 μM linsidomine were 18·9 ± 4·2, 22·9 ± 4·7 and 20·4 ± 4·4 pmol/5 × 10^6 cells, respectively. Thus, neither of these treatments affected cGMP levels ($P > 0·05$).

Cyclic AMP

Effects of PGE_1 and linsidomine without IBMX. Unstimulated PMN generated 11·5 ± 1·0 pmol cAMP/5 × 10^6 cells. This basal level of cAMP was slightly but not significantly elevated by fMLP, amounting to 14·0 ± 2·2 pmol/5 × 10^6 cells (n = 6; $P > 0·05$).

In the absence of IBMX, PGE_1 increased cAMP formation concentration–dependently up to 53·6 ± 9·2 pmol/5 × 10^6 cells at 10 μM. Linsidomine did not change cAMP formation, either alone (Table 1) or in the presence of PGE_1 (Table 5).

Effects of PGE_1 and linsidomine in the presence of IBMX. In the presence of IBMX the basal level of cAMP of unstimulated PMN amounted to 18·5 ± 2·0 pmol/5 × 10^6 cells. This was a slight but significant increase above the level in the absence of IBMX (n = 6; $P < 0·05$). Stimulation with fMLP enhanced cAMP formation to 30·4 ± 3·8 pmol cAMP/5 × 10^6 cells. This was equivalent to a 64% increase above basal level ($P < 0·05$; n = 6).

Table 6. Effect of PGE_1 and linsidomine on cAMP formation in human PMN in the presence of 0·5 mM IBMX.

Agonist	PGE_1 (nM)	Linsidomine (μM)	cAMP (pmol/5 × 10^6 PMN)
None	0	0	18·5 ± 2·0
30 nM fMLP	0	0	30·4 ± 3·8
	1	0	32·1 ± 5·1
	10	0	38·0 ± 3·5*
	100	0	71·2 ± 7·9*
	1000	0	244·8 ± 37·9*
	10000	0	549·2 ± 131·0*
	1	100	30·9 + 3·4
	10	100	43·9 ± 4·0
	100	100	83·1 ± 12·2*
	1000	100	227·4 ± 21·2*
	10000	100	689·0 ± 149·7*†

The data are mean ± s.e.m. of 6 experiments performed in duplicate. *$P < 0·05$ compared with corresponding control, †$P < 0·05$ compared with PGE_1 alone.

PGE_1 enhanced cAMP formation of fMLP-stimulated cells concentration–dependently at 10 nM–10 μM. Linsidomine at 100 μM slightly stimulated cAMP generation above the value seen after fMLP alone ($P < 0·05$; n = 5) (Table 3). Again, linsidomine did not affect PGE_1-induced cAMP accumulation above the level seen after PGE_1 alone. Only at the highest concentration (10 μM) of PGE_1 was there a minor though significant elevation ($P < 0·05$ vs PGE_1 alone, Table 6).

Discussion

Nitric oxide (NO) and vasodilator prostaglandins, such as PGE_1, PGE_2 and prostacyclin have synergistic effects on a number of cells involved in the inflammatory process. Both NO and PGE_1, inhibit platelet activation and smooth muscle tone. NO donors and prostacyclin have also been shown to inhibit enzyme release from aged human neutrophils (Korbut et al 1989). However, Palapies et al (1992) did not detect any synergism between linsidomine and PGE_2 in rat neutrophils, although they confirmed a synergistic inhibitory effect of both compounds in human platelets.

The present study shows that fMLP-induced activation of human PMN can be synergistically antagonized by both PGE_1 and linsidomine. The combined action of linsidomine and PGE_1 in the absence of PDE inhibition appeared to be additive and there was no evidence for any potentiation which would have been expected if both compounds synergize via identical pathways of cellular signal transduction. In this respect, a regulatory function of cAMP in modulating signal transduction and mediator release is generally accepted. Increased intracellular cAMP levels in neutrophils resulted in reduced chemotaxis, lysosomal enzyme release and respiratory burst (Bergman et al 1978; Stephens & Snyderman 1982). Cyclic AMP is also a secondmessenger of prostaglandin-induced inhibition of stimulation of human neutrophils (O'Flaherty et al 1979; Wong & Freund 1981; Fantone & Kinnes 1983; Hecker et al 1990; Wright et al 1990).

In this study, significant stimulation of cAMP levels and inhibition of β-glucuronidase release by PGE_1 in the absence of PDE inhibition was only obtained at the high concentration of 10 μM. However, a concentration-dependent, significant inhibition of O_2^- formation was already detected at one-thousandth of these concentrations. This suggests that differences exist between these two parameters.

The non-selective phosphodiesterase inhibitor IBMX had a profound inhibitory effect on O_2^- generation. IBMX also enhanced the inhibitory effect of PGE_1 on fMLP-induced O_2^- formation and β-glucuronidase release and potentiated the cAMP stimulation by PGE_1. Interestingly, there appeared not to be a linear correlation between cAMP accumulation and the inhibitory effect of PGE_1 on PMN function; despite the marked increase in cAMP levels, PGE_1 up to 100 nM did not further inhibit O_2^- formation and β-glucuronidase release. Lad et al (1985) had also noted a significant inhibition of O_2^- formation

and lysozyme release in human PMN by PGE_1 at unchanged cAMP levels. Moreover, there was a supra-additive synergism between PGE_1 and IBMX with respect to superoxide anion generation (Simchovitz et al 1980). This agrees well with our data and is consistent with the hypothesis that probably only a small fraction of cAMP is involved in the inhibitory effect of PGE_1 on these neutrophil functions.

Linsidomine at a concentration that affected neither biological parameters nor cyclic nucleotide levels, significantly enhanced the inhibitory effects of PGE_1 on O_2^- generation and β-glucuronidase release: O_2^- generation was inhibited at 1 nM and β-glucuronidase release at 1 μM. Interestingly, there was no increase in either cAMP or cGMP levels and the action of linsidomine appeared to be essentially identical at all concentrations of PGE_1 used.

The inhibitory action of linsidomine on lysosomal enzyme release and leukotriene B_4 formation has been linked to an increased formation of cGMP via stimulation of the soluble guanylate cyclase in PMN (Ney et al 1990; Schröder et al 1990). The failure to detect any significant increase of cGMP levels by linsidomine in this study was surprising and appeared to be at variance with these previous data. However, it should be noted that two different techniques for cGMP measurement have been used, resulting in markedly different cGMP levels. Determination of cGMP by the method of Harper & Brooker (1975) includes an acetylation step and results in basal cGMP levels of about 0·1 pmol/10^6 cells (range 0·03–0·3) in 5 different studies, including our own previous investigations (Zurier et al 1974; Smolien et al 1980; Salvemini et al 1989; Ney et al 1990; Schröder et al 1990). In contrast, cGMP determination by the method of Steiner et al (1972) which was used here, yielded 1·54 pmol/10^6 cells (range: 0·48–3·40) in 5 other studies (Ignarro & George 1974a, b; Smith & Ignarro 1975; Anderson & Van Rensburg 1979; Strobach & Schröder, unpublished). These data demonstrate resting levels of cGMP about 1/15 of those in assays using the acetylation procedure. We have, therefore, directly compared both techniques in additional experiments using cells from the same PMN preparation and found $0{\cdot}05 \pm 0{\cdot}03$ pmol/10^6 cells in the acetylation assay compared with $1{\cdot}11 \pm 0{\cdot}14$ pmol/10^6 cells without acetylation (Strobach & Schröder, unpublished). Thus, data obtained using these different assay procedures may not be directly comparable.

According to Harper & Brooker (1975), a too long-lasting acetylation step may imply the destruction of a more or less large cGMP fraction, eventually resulting in too low levels. Alternatively, it is possible that only a fraction of total cGMP is acetylated and may react different from the cGMP pool. Thus, we can neither exclude nor confirm a contribution of cGMP (below the detection limit in this assay) to the overall biological responses. However, we do not think that any change comparable with that of cAMP occurred with cGMP in this assay. In any case, the reasons for these marked assay-related differences in cGMP levels require clarification.

In rabbit platelets, cAMP is rapidly degraded by a zaprinast-sensitive phosphodiesterase which is also blocked by cGMP. Maurice & Haslam (1990) have put forward the concept of synergistic inhibitory interactions between NO and PGE_1, resulting as a consequence of inhibition of the low K_m value for cAMP-phosphodiesterase by cGMP. However, this enzyme appears not to be present in human PMN. In these cells, the vast majority of PDE belongs to the cAMP-specific, cGMP-insensitive type IV and there is no evidence for any significant amounts of cGMP-stimulated or -antagonized PDEs (Nielson et al 1990; Schudt et al 1991). Wright et al (1990) have proposed a response-specific regulatory role for the cAMP-specific, cGMP-insensitive PDE on human neutrophils and have attributed the effects of PDE inhibitors on oxygen radical generation to the modulatory effects of cAMP on the activation of the NADPH oxidase.

Our results demonstrate a closer relationship between cAMP accumulation and the inhibition of lysosomal enzyme release than to the inhibition of oxygen radical generation. Inhibition of oxygen radical formation probably implicates a more complex interaction. Different mechanisms of inhibition of oxygen-centred radical production are under discussion. These involve cAMP accumulation (Nielson et al 1990), inhibition of the NADPH oxidase (Cross & Jones 1989; Wright et al 1990) and enhanced degradation of already formed oxygen radicals (Gryglewski et al 1987).

Nakagawara & Minikami (1975) have reported inhibition of an isolated NADPH oxidase by cAMP. The potentiation of the effects of PGE_1 by IBMX on oxygen radical formation might be related to a cAMP-induced NADPH-oxidase inactivation. Nevertheless, the influence of cAMP on lysosomal enzyme release could be direct, as seen in other secretory processes. Linsidomine is thought to act in platelets and smooth muscle cells through formation of NO and activation of the soluble guanylate cyclase. Linsidomine is able to release NO spontaneously and to generate biologically relevant concentrations of superoxide anions in aqueous solution (Böger et al 1993). Oxygen radicals and haeme-containing proteins are known to inactivate NO (Gryglewski et al 1986; Ignarro 1990; Rubanyi & Vanhoutte 1986; Waldman & Murad 1987).

Trapping of NO by the NADPH oxidase or by oxygen radicals might contribute to the inhibitory effect of linsidomine and at the same time might prevent any detectable increase of cGMP levels. Furthermore, the inhibition of oxygen radical production might be attributed to the capacity of NO to scavenge free radicals rather than to inhibition of O_2^- formation (Cantor et al 1990; Lefer & Aoki 1990; Rubanyi et al 1991). Alternatively, released NO may be quenched by haeme-containing proteins, such as the NADPH oxidase (Ignarro 1990; Waldman & Murad 1987).

We conclude that linsidomine synergizes with PGE_1-induced inhibition of PMN function and that this action is additive and not directly related to the cAMP levels. This action may be related to changes in cGMP although this has not been directly confirmed in the present study. The effect of IBMX and linsidomine on oxygen radical generation might be related to their ability to inhibit the NADPH oxidase system. Linsidomine might in addition directly scavenge O_2^- radicals once released into the extracellular space.

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