Hepoxilin A₃ inhibits the rise in free intracellular calcium evoked by formyl-methionyl-leucyl-phenylalanine, platelet-activating factor and leukotriene B₄

Odette LANEUVILLE,*† Denis REYNAUD,* Sergio GRINSTEIN,*‡ Santosh NIGAM§ and Cecil R. PACE-ASCIAC*†||

*Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Canada M5G 1X8,

†Department of Pharmacology, University of Toronto, Toronto, Canada M5S 1A8, ‡Department of Biochemistry, University of Toronto, Toronto, Canada M5S 1A8,

and §Department of Gynaecology, Free University of Berlin, D-1000 Berlin, Germany

We have previously shown that the hepoxilins are capable of increasing the intracellular free concentration of calcium ($[Ca^{2+}]_i$) in human neutrophils through a pertussis toxin-sensitive, extracellular calcium-independent pathway involving the mobilization of calcium from internal stores. A subsequent hepoxilin-induced and extracellular calcium-dependent influx of calcium is observed. In an effort to investigate further the role of these compounds in the human neutrophil, we investigated their potential effects on the action of known agonists such as formyl-methionine-leucine-phenylalanine (fMLP), platelet-activating factor (PAF) and leukotriene B₄ (LTB₄) on the mobilization of calcium. Hepoxilins dose-dependently inhibited the increases in $[Ca^{2+}]_i$ induced by fMLP, PAF and LTB₄. The hepoxilin concentration required for inhibition was around 100 ng/ml (3×10^{-7} M). This concentration of hepoxilin did not cause any measurable change in $[Ca^{2+}]_i$. The extent of inhibition of the

agonist-evoked rise in $[Ca^{2+}]_i$ by hepoxilins was proportional to the increase in the calcium response evoked by hepoxilin beyond its threshold concentration. Additional experiments were carried out to investigate the mechanism for the hepoxilin effect. Using calcium-free medium and in the presence of sufficient amounts of thapsigargin (200 ng/ml) to maximally block the calcium pump (thereby achieving a constant rate of calcium leakage from stores), hepoxilin A₃ increased further this rate of calcium leakage, indicating that hepoxilin acts by rapidly draining calcium from stores. Its potential (additional) thapsigargin-like action in blocking the pump, however, cannot be ruled out by these experiments. These observations suggest that the hepoxilins may serve an important negative regulatory function in the agonist-induced mobilization of calcium in these cells by depleting calcium stores.

INTRODUCTION

Important functional properties of human neutrophils are known to be closely coupled to changes in the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$). In fact, an elevation of $[Ca^{2+}]_i$ in response to several agonists may be required for stimulation of NADPH oxidase, exocytosis of secretory granules, phagocytosis and chemotaxis, all of which play a significant role in host defence mechanisms against invading micro-organisms (Sha'afi and Molski, 1988). Chemotactic factors such as formyl-methionyl-leucyl-phenylalanine (fMLP) cause the release of arachidonic acid and stimulate its subsequent metabolism (Bokoch and Reed, 1980; Rubin et al., 1981). Arachidonic acid is metabolized into a variety of products, including prostaglandins, leukotrienes, lipoxins, hepoxilins, etc. Leukotriene B₄ (LTB₄) is the most potent chemotactic factor known (Ford-Hutchinson et al., 1980). We have previously shown that hepoxilins, metabolites of arachidonic acid formed via the 12-lipoxygenase pathway (Pace-Asciak et al., 1983; Pace-Asciak, 1984), evoke a rise in $[Ca^{2+}]_i$ (Dho et al., 1990) and they also cause the release of diacylglycerol and arachidonic acid through an $Ins(1,4,5)P_3$ -independent pathway (Nigam et al., 1990, 1993). We were therefore interested in investigating whether the hepoxilins also influence the actions of other known agonists in evoking the release of $[Ca^{2+}]_i$. The data presented herein invoke a possible physiological function of the hepoxilins and provide further insight into the mechanism of

action of hepoxilins as modulators of inflammation in the neutrophil.

MATERIALS AND METHODS

Materials

Hepoxilins were kindly provided as their methyl esters by Dr. E. J. Corey, Harvard University, Cambridge, MA, U.S.A., and were prepared as described (Corey and Su, 1984, 1990). The two isomers of hepoxilin A₃ (HxA₃) and trioxilin A₃ (TrXA₃) were dissolved in ethanol and added to the neutrophils in a volume of 1–5 μ l, so that the final concentration of ethanol or DMSO did not exceed 0.5%. Platelet-activating factor (PAF) and fMLP were purchased from Sigma, and were dissolved in ethanol to a concentration of 1 μ M. LTB₄ was purchased from Cayman Laboratories and dissolved in ethanol to a concentration of 1 μ M. Staurosporine was purchased from Boehringer-Mannheim and thapsigargin was from Sigma.

Cell preparation

Neutrophils were isolated from heparinized (20 units/ml) venous blood collected from healthy volunteers according to Boyum (1968) and Dho et al. (1990). Erythrocytes were removed by 4.5% dextran sedimentation for 45 min at room temperature, and the supernatant was subjected to Ficoll (Pharmacia, Sweden)

Abbreviations used: $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; HxA₃, hepoxilin A₃ [8(R)- and 8(S)-hydroxy-11(S),12(S)-epoxyeicosa-5Z,9E,14Z-trienoic acid]; TrXA₃, trioxilin A₃ [8(R) and 8(S),11(R),12(S)-trihydroxyeicosa-5Z,9E,14Z-trienoic acid]; HxA₃-C, hepoxilin A₃-C [8(R) and 8(S),12(S)-dihydroxy-11(R)-glutathionyleicosa-5Z,9E,14Z-trienoic acid]; fMLP, formyl-methionyl-leucyl-phenylalanine; PAF, platelet-activating factor; LTB₄, leukotriene B₄; Indo-1-AM, 1-[2-amino-5-(6-carboxyindol-2-yl)-phenoxy]-2-(2'-amino-5'-methylphenoxy)ethane-*NNN'*-tetra-acetic acid penta-acetoxy methyl ester; DMSO, dimethyl sulphoxide.

|| To whom correspondence should be addressed.

density gradient centrifugation. Neutrophils were obtained from the pellet. Contaminating erythrocytes were eliminated by lysis in ammonium chloride (0.85%). After an additional washing step, the cells were counted and adjusted to a final concentration of 10^7 cells/ml in RPMI 1640 medium (Sigma).

Indo-1-AM loading

$[Ca^{2+}]_i$ was measured using the fluorescent indicator Indo-1-AM (Calbiochem). Freshly prepared neutrophil suspension (1 ml ; 1×10^7 cells) was loaded with 3 mM (final concentration $3 \mu\text{M}$) of the acetoxymethyl ester precursor of the calcium indicator for a period of 30 min at 37°C . Extracellular dye was removed by centrifugation and the cells were resuspended in fresh RPMI 1640. Dye-loaded cells were kept at room temperature on a rotator.

Measurement of $[Ca^{2+}]_i$ by spectrofluorometry

For each measurement, 2×10^6 cells were added to 1 ml of assay medium (composition in mM : NaCl 140, KCl 5, MgCl_2 1, CaCl_2 1, HEPES sodium-free 10 and glucose 10, pH 7.3) in a temperature-controlled plastic cuvette (Diamed Lab., Toronto, Canada) at 37°C . The cell suspension was continuously stirred. Fluorescence was continuously monitored with a Perkin-Elmer fluorescence spectrophotometer (model 650-40) and recorded on a chart recorder (LKB model 2210) set at 1 cm/min . The excitation wavelength was set at 331 nm , the emission wavelength was set at 410 nm , and slits of excitation and emission were set at 3 and 15 nm respectively. A calibration procedure was carried out for each sample. Maximal fluorescence was achieved by adding ionomycin (Sigma) at 1 mM (final concentration $1 \mu\text{M}$); minimal fluorescence was obtained by adding an excess of MnCl_2 (final concentration 3 mM) according to Grinstein and Furuya (1984).

Basal levels of calcium were measured over a period of 4 min or until stabilized for each sample. Cells were then treated with HxA_3 in a volume of $1 \mu\text{l}$ of ethanol. The calcium signal returned to a new steady state by 7 min or less after the addition of these agents. Then fMLP, PAF or LTB_4 was added and the $[Ca^{2+}]_i$ was recorded for a 10 min period. Experiments in the absence of extracellular Ca^{2+} were carried out by equilibrating the cells in assay medium lacking CaCl_2 and containing EGTA (1 mM). Calibration of the signal after testing with agonist required the addition of 1 mM CaCl_2 prior to the addition of ionomycin. Staurosporine, when used, was present at 100 nM , and was added 5 min prior to the addition of agonist. Thapsigargin was tested at three different concentrations (50 , 100 and 200 ng/ml) to determine the maximal rate of rise in $[Ca^{2+}]_i$, followed 5 min later by either HxA_3 or fMLP.

Statistics

Comparisons were made between controls (no HxA_3) and each individual dose of HxA_3 using Student's t test for unpaired data (an ANOVA test was first performed). The effects of HxA_3 on fMLP-, PAF- and LTB_4 -induced increases in $[Ca^{2+}]_i$ were evaluated by comparing the agonist response obtained in the absence of HxA_3 with that obtained in the presence of increasing doses of HxA_3 using Student's t test for unpaired data (an ANOVA was performed first and a search for significance using Student's t test was performed second). Statistics were performed on a MacIntosh computer using Statsview as statistics program.

RESULTS

Effects of hepxilins on $[Ca^{2+}]_i$

As demonstrated in an earlier study, HxA_3 caused an increase in $[Ca^{2+}]_i$ in human neutrophils which was made up of two components. The first is due to the mobilization of calcium from intracellular stores and is responsible for the bulk of the initial peak, while the second represents an influx of calcium from the extracellular medium and is responsible for a long-lasting plateau above the initial baseline. Both isomers of HxA_3 ($8R$ and $8S$) stimulated a rapid and transient increase in the $[Ca^{2+}]_i$. That the effects were due to the hepxilins themselves and not to their trihydroxy metabolites was shown by the lack of response to the latter products when added at a dose of $10 \mu\text{g}$ ($30 \mu\text{M}$) ($n = 2$) (results not shown). In control experiments the vehicle, ethanol, did not change significantly the basal levels of calcium over a period of 9 min (Figure 1a). The dose-dependence of both isomers of HxA_3 was also investigated. The increase in $[Ca^{2+}]_i$ induced by hepxilins was dose-related, with a threshold dose equal to

Figure 1 Effects of HxA_3 ($8R$ isomer) on the fMLP-induced increase in $[Ca^{2+}]_i$ in human neutrophils

Neutrophils, loaded with Indo-1-AM, were suspended in medium containing 1 mM Ca^{2+} and exposed to (a) ethanol vehicle ($5 \mu\text{l}$); (b) and (d) fMLP (2 nM); or (c) and (e) HxA_3 ($8R$ isomer) ($5 \mu\text{g}$). $[Ca^{2+}]_i$ was recorded for 7 min followed by addition of either fMLP (2 nM) (a, c and d) or HxA_3 ($8R$) ($5 \mu\text{g}$) (b and e). The traces are representative of three similar experiments with cells isolated from blood from different donors.

Figure 2 Concentration-dependent effects of the 8S (a) and 8R (b) isomers of HxA₃ in inducing an increase in $[\text{Ca}^{2+}]_i$ and in blocking the fMLP-induced increase in $[\text{Ca}^{2+}]_i$

Cells were exposed to ethanol or to different concentrations of HxA₃ in ethanol. $[\text{Ca}^{2+}]_i$ was recorded for 7 min after the addition of HxA₃, whereupon fMLP was then added. Data represent the maximal $[\text{Ca}^{2+}]_i$ calcium recorded after the addition of these compounds. Data are means $\pm$ S.E.M. ($n = 3$) and results are representative of experiments with cells derived from blood from different donors. Asterisks indicate significant differences from control ($P < 0.01$).

1.0 μg or 3 μM for HxA₃ (8R), and to 1.5 μg or 4.5 μM for HxA₃ (8S) (Figures 2a and 2b).

Effects of HxA₃ (8R and 8S isomers) on fMLP-, PAF- and LTB₄-induced increases in $[\text{Ca}^{2+}]_i$

The effects of HxA₃ on the fMLP-evoked rise in $[\text{Ca}^{2+}]_i$ are illustrated in Figures 1 and 2. Neutrophils were challenged first with 2 nM fMLP, followed 7 min later by the addition of the 8R isomer of HxA₃ at a concentration (5 μg ; 15 μM) known to increase significantly the $[\text{Ca}^{2+}]_i$ (Figures 1b and 2b). Results show that cells treated first with fMLP mobilized intracellular calcium, and this treatment did not affect the subsequent stimulation with HxA₃ or a second dose of fMLP (Figures 1b and 1d). Higher concentrations of fMLP, however, inhibited the

Figure 3 Concentration-dependent effects of HxA₃ (8R isomer) in inducing an increase in $[\text{Ca}^{2+}]_i$ and in inhibiting the PAF-induced increase in $[\text{Ca}^{2+}]_i$

Cells were exposed to ethanol or to different concentrations of HxA₃. $[\text{Ca}^{2+}]_i$ was recorded for 7 min after the addition of HxA₃, and PAF was then added. Data represent the maximal $[\text{Ca}^{2+}]_i$ recorded after the addition of these compounds. Each data point shows the mean $\pm$ S.E.M. ($n = 3$) and results are representative of experiments with cells derived from blood from different donors. Asterisks indicate significant differences from control ($P < 0.005$).

subsequent stimulation of hepxoxilins (results not shown). When cells were first challenged with either of the HxA₃ isomers, the subsequent challenge by fMLP or by a second addition of HxA₃ (Figures 1c and 1e) was inhibited. The threshold dose at which HxA₃ (8R isomer) began to block the fMLP-induced increase in $[\text{Ca}^{2+}]_i$ was 0.1 $\mu\text{g}/2 \times 10^6$ neutrophils, i.e. 0.3 μM (Figure 2). This effect of HxA₃ was dose-dependent: the higher the dose of hepxoxilins, the bigger the decrease in the fMLP response (Figure 2). With a dose of 2.0 μg (6 μM) of HxA₃ (either isomer), the response to fMLP was maximally inhibited (Figures 2a and 2b). Note that the doses of HxA₃ (8R and 8S isomers) required to initiate the blockade of the fMLP-induced increase in $[\text{Ca}^{2+}]_i$ (0.1 μg or 0.3 μM) are lower than the doses required to produce a significant increase of $[\text{Ca}^{2+}]_i$ (8R isomer, 1.0 μg or 3 μM ; 8S isomer, 1.5 μg or 4.5 μM). The fMLP-induced increase of $[\text{Ca}^{2+}]_i$ was unaffected when cells were pretreated with ethanol vehicle (Figure 1a). Staurosporine at 100 nM did not affect the rise in $[\text{Ca}^{2+}]_i$ brought about by either HxA₃ (6 $\mu\text{g}/\text{ml}$) or fMLP (2 nM) (results not shown), indicating that these effects are not mediated via activation of protein kinase C. Higher concentrations of staurosporine (1 μM) partially inhibited the hepxoxilin effect, suggesting that protein kinases other than protein kinase C are blocked at this concentration (Nigam et al., 1992), and these therefore may be involved in mediating the effects of hepxoxilin.

The effect of HxA₃ (8R isomer) on the PAF-induced increase in $[\text{Ca}^{2+}]_i$ was also investigated. The dose of PAF used, 0.1 nM final concentration, was found to give a significant rise in the $[\text{Ca}^{2+}]_i$. HxA₃ (8R isomer) blocked the PAF response in a dose-related fashion with a threshold dose at 0.1 μg or 0.3 μM , as also observed with fMLP (Figure 3). A similar effect of HxA₃ (8R) on the LTB₄-induced rise in $[\text{Ca}^{2+}]_i$ was also observed with a hepxoxilin threshold of 0.1 μg (0.3 μM) (Figure 4).

Effects of HxA₃ (8S isomer) and fMLP on the rate of thapsigargin-induced calcium leakage in calcium-free medium

Thapsigargin, through blockade of the calcium pump, causes a slow leakage from calcium stores. This is more clearly shown

Figure 4 Concentration-dependent effects of HxA_3 (8R isomer) in inducing an increase in $[\text{Ca}^{2+}]_i$ and in inhibiting the LTB_4 -induced increase in $[\text{Ca}^{2+}]_i$.

Cells were exposed to ethanol or to different concentrations of HxA_3 . $[\text{Ca}^{2+}]_i$ was recorded for 7 min after the addition of HxA_3 , and LTB_4 was then added. Data represent the maximal $[\text{Ca}^{2+}]_i$ recorded after the addition of these compounds. Each data point shows the mean $\pm$ range ($n = 2$) and results are representative of experiments with cells derived from blood from different donors. Asterisks indicate significant differences from control ($P < 0.01$).

when cells are incubated in calcium-free medium, thereby eliminating calcium influx from extracellular medium. As shown in Figure 5(b), thapsigargin, at three concentrations (50, 100 and 200 ng/ml), caused an increase in the maximum $[\text{Ca}^{2+}]_i$ observed. The rate of increase was constant, indicating that the calcium pump is maximally inhibited at these concentrations of thap-

sigargin and that the rise in $[\text{Ca}^{2+}]_i$ occurs through drainage of calcium from intracellular stores. When a constant and maximal rate of drainage had been established (15 s), HxA_3 was added (Figure 5c). An immediate change in the rate of rise of $[\text{Ca}^{2+}]_i$ was observed, indicating that, since the pump had already been maximally inhibited by thapsigargin, HxA_3 must have affected the drainage of calcium from the stores. Similar effects were observed with fMLP (Figure 5e). These experiments indicate that HxA_3 acts on calcium stores by increasing the release of calcium from them. Whether it also acts at the level of the calcium pump cannot be established from these experiments.

DISCUSSION

HxA_3 stimulated a rapid and transient rise in $[\text{Ca}^{2+}]_i$ which was followed by a decrease to a level which was sustained above the resting $[\text{Ca}^{2+}]_i$ value. The initial rapid phase was ascribed to mobilization of calcium from intracellular stores, since it occurred in a calcium-free medium; this was followed by a slower influx of extracellular calcium, which was dependent on the presence of calcium in the extracellular medium (Dho et al., 1990). These effects of HxA_3 were not observed with the glutathione conjugate, $\text{HxA}_3\text{-C}$, or with the trihydroxy derivative, TrXA_3 , which were inactive within the range of concentrations used for HxA_3 (i.e. 0.1–5 μg , or 0.3–15 μM) (results not shown).

Previous studies have demonstrated that the heptoxilin-evoked rise in $[\text{Ca}^{2+}]_i$ is blocked by pertussis toxin, suggesting an involvement of receptors coupled to GTP-binding proteins (Dho et al., 1990). HxA_3 also induces the release of diacylglycerol and arachidonic acid from human neutrophils through a pertussis toxin-sensitive pathway (Nigam et al., 1990). No significant increase of $\text{Ins}(1,4,5)\text{P}_3$ could be detected in a similar study (Nigam et al., 1993). This suggests that heptoxilins release calcium by a mechanism independent of $\text{Ins}(1,4,5)\text{P}_3$. Inhibition of the agonist-induced release of intracellular Ca^{2+} by HxA_3 may be

Figure 5 Effects of HxA_3 (a and c) and fMLP (d and e) on the rate of rise of $[\text{Ca}^{2+}]_i$ induced by thapsigargin (b, c, and e) in neutrophils in calcium-free medium

In panels (c) and (e), HxA_3 and fMLP respectively were added to cells 15 s after thapsigargin treatment, a time point by which the steady rate of increase of $[\text{Ca}^{2+}]_i$ has been established. As shown, both HxA_3 and fMLP increased greatly the thapsigargin-induced rate of rise of $[\text{Ca}^{2+}]_i$, indicating an action on calcium stores.

due to the release of diacylglycerol (Nigam et al., 1990) followed by activation of protein kinase C. Indeed, Nigam et al. (1992) have shown that elevation of diacylglycerol independently of $\text{Ins}(1,4,5)\text{P}_3$ caused a reduction in the fMLP-induced rise in $[\text{Ca}^{2+}]_i$. In that study, diacylglycerol accumulation was shown to occur via a phospholipase D pathway. Recently we have also shown that HxA_3 stimulated the release of arachidonic acid and diacylglycerol via the formation of phosphatidic acid through activation of a phospholipase D pathway (Nigam et al., 1993). It is interesting to note that phosphatidic acid has been shown to stimulate the release of Ca^{2+} from intracellular stores (Moolenaar et al., 1986). Whether the hepoxilin-mediated effect on increase in $[\text{Ca}^{2+}]_i$ shown in our studies is dependent on the formation of phosphatidic acid or its further metabolites remains to be determined.

Preincubation of neutrophils with HxA_3 inhibited the fMLP-, PAF- and LTB_4 -induced increases in $[\text{Ca}^{2+}]_i$ in a dose-dependent fashion. The inhibitory effects of HxA_3 began to be observed at concentrations ($0.1 \mu\text{g}$ or $0.3 \mu\text{M}$) that, on their own, did not affect $[\text{Ca}^{2+}]_i$. HxA_3 therefore might have a significant effect on neutrophil activation in that it may modulate the stimulation of these cells by physiological stimuli.

In neutrophils, fMLP releases Ca^{2+} from internal stores and thereby further increases Ca^{2+} entry. An arachidonic acid metabolite derived through the cytochrome *P*-450 pathway has been suggested to be involved in the regulation of plasma membrane Ca^{2+} permeability controlled by the degree of filling of the intracellular calcium stores. It was reported that cytochrome *P*-450 inhibitors (econazole, miconazole and others) lower the store-dependent plasma membrane permeability of calcium in rat thymocytes (Alvarez et al., 1991). Recently it was shown that 11,12-epoxyeicosa-5Z,8Z,14Z-trienoic acid inhibits the influx of calcium into human platelets brought about by thapsigargin as well as thrombin, but it acted at concentrations much higher ($60 \mu\text{M}$) (Malcolm and Fitzpatrick, 1992) than that of HxA_3 ($0.1 \mu\text{M}$) used in our study. Whether HxA_3 interferes with the signal from intracellular stores that activates calcium channels in the plasma membrane remains to be determined.

Preliminary studies from our laboratory have shown that the effect of HxA_3 on $[\text{Ca}^{2+}]_i$ is not inhibited by cyclo-oxygenase inhibition (e.g. with indomethacin) or by the 5-lipoxygenase inhibitor MK886, indicating that the hepoxilin effect is not mediated through the formation of prostaglandins or LTB_4 (O. Laneuville, D. Reynaud, S. Grinstein, S. Nigam and C. R. Pace-Asciak, unpublished work). Another possibility to explain the action of HxA_3 may relate to the refilling of intracellular calcium stores. Refilling of intracellular stores depleted of calcium depends on extracellular calcium that largely or completely

bypasses the cytoplasm (Tsien and Tsien, 1990). Thapsigargin seems to act in a receptor-independent fashion by blocking the calcium pump of the internal stores without affecting the plasma membrane ATPase or generating inositol phosphates (Thastrup et al., 1989). Our experiments with calcium-free medium in the presence of thapsigargin to maximally block the calcium pump, thereby causing a slow drainage from calcium stores, indicated that HxA_3 further stimulates the release of intracellular $[\text{Ca}^{2+}]_i$. These experiments clearly demonstrate an action of hepoxilins on the intracellular calcium stores. Whether HxA_3 acts also as an endogenous blocker of the calcium pump involved in the refilling of calcium stores remains to be determined.

These data support the hypothesis that an $\text{Ins}(1,4,5)\text{P}_3$ -independent calcium pool exists in human neutrophils which is stimulated by HxA_3 . HxA_3 may provide a useful tool for further studies on the nature of calcium pools in the human neutrophil and the communication between the $[\text{Ca}^{2+}]_i$ and agonist-evoked neutrophil activation.

This study was supported by grants from the MRC (S.G.), the Association for Cancer Research, U.K. (S.N.) and the MRC of Canada (C.R.P.-A). O.L. is a recipient of a graduate scholarship from the MRC and FCAR of Quebec.

REFERENCES

- Alvarez, J., Montero, M. and Garcia-Sancho, J. (1991) *Biochem. J.* **274**, 193–197
- Bokoch, G. and Reed, P. W. (1980) *J. Biol. Chem.* **255**, 10233–10238
- Boyum, A. (1968) *J. Clin. Invest.* **21**, 77–98
- Corey, E. J. and Su, W.-G. (1984) *Tetrahedron Lett.* **25**, 5119–5122
- Corey, E. J. and Su, W.-G. (1990) *Tetrahedron Lett.* **31**, 2113–2116
- Dho, S., Grinstein, S., Corey, E. J., Su, W.-G. and Pace-Asciak, C. R. (1990) *Biochem. J.* **266**, 63–68
- Ford-Hutchinson, A. W., Bray, M. A., Doig, M. V., Shipley, M. E. and Smith, M. J. H. (1980) *Nature (London)* **286**, 264–265
- Grinstein, S. and Furuya, W. (1984) *Biochem. Biophys. Res. Commun.* **122**, 755–762
- Malcolm, K. C. and Fitzpatrick, F. A. (1992) *J. Biol. Chem.* **267**, 19854–19858
- Moolenaar, W. H., Kruijer, W., Tilly, B. C., Verlaan, I., Bierman, A. J. and de Laat, S. W. (1986) *Nature (London)* **323**, 171–173
- Nigam, S., Nides, S., Cichon, G., Corey, E. J. and Pace-Asciak, C. R. (1990) *Biochem. Biophys. Res. Commun.* **171**, 944–948
- Nigam, S., Muller, S. and Walzog, B. (1992) *Biochim. Biophys. Acta* **1135**, 301–308
- Nigam, S., Muller, S. and Pace-Asciak, C. R. (1993) In *Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation and Radiation Injury* (Nigam, S., Marnett, L. J., Honn, K. V. and Walden, T. L., Jr., eds), pp 249–252. Kluwer Academic Publisher, Boston
- Pace-Asciak, C. R. (1984) *J. Biol. Chem.* **259**, 8332–8337
- Pace-Asciak, C. R., Granstrom, E. and Samuelsson, B. (1983) *J. Biol. Chem.* **258**, 6835–6840
- Rubin, R. P., Sink, L. E. and Freer, R. J. (1981) *Mol. Pharmacol.* **19**, 31–37
- Sha'afi, R. I. and Molski, T. F. P. (1988) *Prog. Allergy* **42**, 1–64
- Thastrup, O., Dawson, A. P., Scharff, O., Foder, B., Cullen, P. J., Drobak, B. K., Bjerrum, P. J., Christensen, S. B. and Hanley, M. R. (1989) *Agents Actions* **27**, 17–23
- Tsien, R. W. and Tsien, R. Y. (1990) *Annu. Rev. Cell Biol.* **6**, 715–760