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Cyclic-AMP level and eicosanoid release from alveolar macrophages are differentially affected by high and low dose of platelet activating factor

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Abstract—Antigen challenged alveolar macrophages (ac-AM) showed much higher basal prostaglandin E_2 (PGE_2) release (4.4-fold) and cAMP content (2.4-fold) than naive alveolar macrophages (AM). In naive AM 1 fM platelet activating factor (PAF) enhanced PGE_2 release from 115 to 157 ng/5 × 10^6 cells but was inactive at 1 nM or 1 μM. In ac-AC 1 fM PAF enhanced PGE_2 release from 510 to 670 ng/5 × 10^6 cells and inhibited leukotriene B_4 (LTB_4) release (from 6.0 to 4.8 ng/5 × 10^6 cells). At a 10^6-fold higher concentration PAF inhibited PGE_2 release (from 510 to 400 ng/5 × 10^6 cells) and stimulated LTB_4 release (from 6.0 to 8.2 ng/5 × 10^6 cells). PAF-induced increase or decrease in PGE_2 release was paralleled by changes in cellular cAMP (+35 and −17%, respectively). The specific PAF-antagonist BN 52021 completely reversed all PAF-induced effects while indomethacin inhibited only PAF-induced increase in PGE_2 release and cAMP leaving LTB_4 release unaffected. Similarly, the lipoxygenase inhibitor AA-861 inhibited PAF-induced rise in LTB_4 release leaving the enhancement in PGE_2 release and cAMP content unaffected. Present data show that PAF dose-dependently affects eicosanoid production and cAMP level in alveolar macrophages.

PAF* is released from a variety of cells known to be involved in pulmonary inflammatory reactions associated with asthma [Refs 1, 2 for review]. PAF has a wide spectrum of biological activity. It is a potent chemoattractant and releases various immuno-modulators like leukotrienes, prostaglandins [3–5] and cytokines [6–8] from different cells.

PAF induced responses are mediated via different pathways. PAF receptors are either coupled to phosphoinositide turnover, Ca^{2+} mobilization [1, 9] and stimulatory and inhibitory responses on cAMP production [10]. In AM enhancement of cAMP level inhibits phagocytosis and the release of oxygen radicals and lysosomal enzymes.

In AM, PAF induced a biphasic response on cAMP generation in antigen challenged, but not naive AM which was susceptible to inhibitors of arachidonic acid metabolism [11]. To ascertain whether these responses are mediated via modulation of eicosanoid production, we determined the release of PGE_2 and LTB_4 from naive and ac-AM in response to various concentrations of PAF.

Materials and Methods

Animals and sensitization. Male Hartley guinea pigs (300–500 g) were anaesthetized with sodium pentobarbitone (70 mg/kg, i.p.). Trachea were cannulated and bronchoalveolar lavage was performed by repeated lavages (8 mL volumes saline, total 150 mL) yielding naive cells. Ac-AM were obtained from animals previously (2 weeks) actively sensitized with ovalbumin (50 mg i.p. and i.v.).

Isolation and preparation of alveolar macrophages. Lavage fluids were filtered through gauze and centrifuged at 400 *g* for 10 min at 4°. Cells re-suspended in GBSS were centrifuged (400 *g*, 4°, 30 min) on Ficoll-Isopaque (Nycomed, Oslo, Norway). After three washings, cells were resuspended in GBSS (3 × 10^6/mL). Viability tested by Trypan Blue exclusion always exceeded 95%.

Incubation protocol. AM (3 × 10^6) were incubated for 15 min at 37° in the presence of 400 μM 3-isobutyl-1-methylxanthine (Janssen Chimica, Beerse, Belgium) and increasing doses of PAF (Sigma, St Louis, MO, U.S.A.). Preincubation time of BN 52021 (PAF-antagonist, gift from Dr P. Braquet), indomethacin or AA-861 (selective 5-lipoxygenase inhibitor, gift from Dr S. Terao) was 5 min. After incubation (15 min, 37°) cells were centrifuged, resuspended in 150 μL 50 mM Tris–HCl buffer (pH 7.4) and boiled for 3 min. LTB_4 was assayed in freeze-dried supernatant, resuspended in 250 μL methanol but PGE_2 was assayed directly in supernatant. Samples were stored at −80° until analysis.

Eicosanoid and cAMP assay. PGE_2 and LTB_4 were assayed by ELISA (Cayman Chemical, Ann Arbor, MI, U.S.A.) with detection limits of 3 and 1 pg/mL, respectively. Intracellular cAMP was assayed by radioimmunoassay (RIA) using [^{3}H]cAMP (Amersham International, Amersham, U.K.) and an isolated binding protein [11].

Statistical analysis. Data are expressed as means ± SEM. Statistical significance was evaluated by the unpaired Student's *t*-test. A P-value < 0.05 was considered significant.

Results

Basal levels of cAMP and eicosanoid release. In ac-AM basal cAMP level was 2.4-fold higher than in naive macrophages (5.2 vs 2.2 pmol/5 × 10^6 AM). Basal PGE_2 release from naive AM and ac-AM was, respectively, 115 and 510 ng/5 × 10^6 AM. Basal release of LTB_4 from naive AM was below detection limit and amounted to 6.0 ng per 5 × 10^6 ac-AM. Neither BN 52021, indomethacin nor AA-861 affected these basal levels.

Effects of PAF on PGE_2 release from naive AM. Figure 1 shows that 1 fM PAF induced a 37% (42 ng/5 × 10^6 cells) increase in PGE_2 release which was inhibited by indomethacin (panel B) but not AA-861 (panel C). At higher concentrations (1 nM and 1 μM) PAF did not affect PGE_2 release.

Effects of PAF on eicosanoid release from ac-AM. Figure 2A shows that exposure of ac-AM to 1 fM PAF resulted in a 23% rise in PGE_2 release while 1 μM PAF induced PGE_2 release by 30%. The PAF-induced increase in PGE_2 release was reversed by indomethacin but not by AA-861. In contrast, AA-861 but not indomethacin inhibited the PAF-induced reduction in PGE_2 release.

Considering LTB_4 release from ac-AM, quite opposite results compared to PGE_2 release were obtained (Fig. 2B). At 1 fM, PAF decreased LTB_4 release from ac-AM by

* Abbreviations: AM, alveolar macrophages; PAF, platelet activating factor; ac-AM, antigen challenged alveolar macrophages; PG, prostaglandin; LT, leukotriene; GBSS, Gey's balanced salt solution.

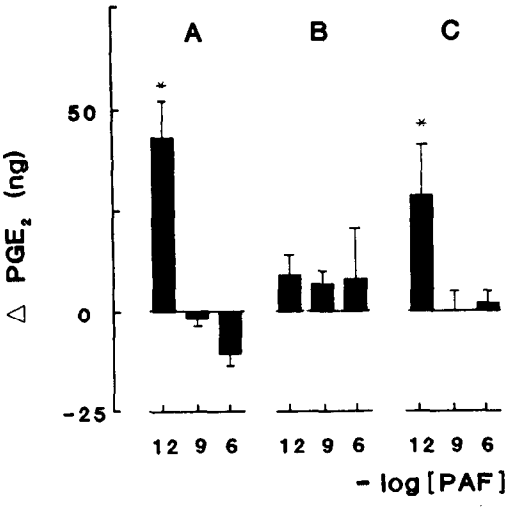

Fig. 1. Absolute change in PGE_2 release (ng/5 × 10^6 AM) from naive AM with respect to basal value (115 ng per 5 × 10^6 AM) by increasing doses of PAF (1 fM, 1 nM and 1 μM). (A) Vehicle; (B) in the presence of 3 μM indomethacin and (C) in the presence of 10 μM AA-861. Data are expressed as means ± SEM from three duplicate experiments. *P < 0.05 compared to basal value.

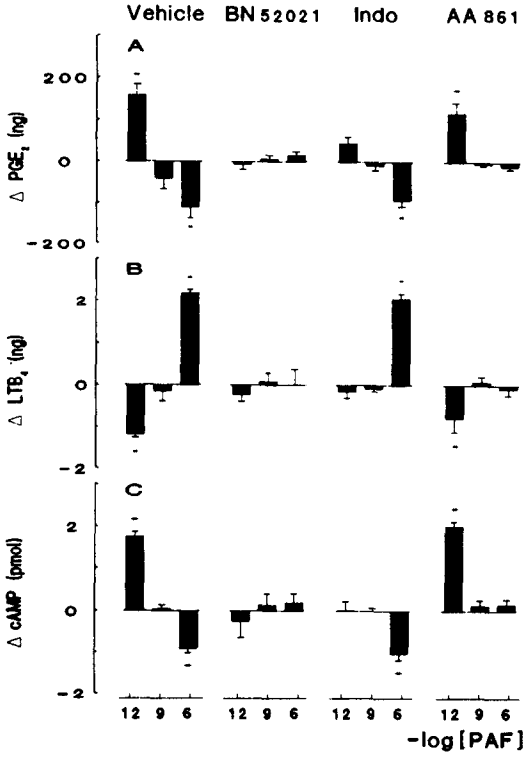

Fig. 2. Absolute change in cAMP level and PGE_2 and LTB_4 release from ac-AM with respect to basal values by increasing doses of PAF (1 fM, 1 nM and 1 μM). Values are expressed per 5 × 10^6 AM. Drug concentrations: BN 52021 at 10 μM; indomethacin (Indo) at 3 μM and AA-861 at 10 μM. Data are expressed as means ± SEM from nine duplicate experiments. *P < 0.05 compared to basal value.

27%, whereas at a 10^6-fold higher concentration LTB_4 release was increased 45%. PAF-induced decrease in LTB_4 release was inhibited by pretreatment of ac-AM with indomethacin but not AA-861. This is in contrast with PAF-induced increase in LTB_4 release which was not affected by indomethacin and could be fully blocked with AA-861. BN 52021 fully inhibited all PAF-induced changes in eicosanoid release.

Using different PAF concentrations a striking analogy (Fig. 2C) between cAMP level and PGE_2 release (but not LTB_4 release) was observed. PAF (1 fM) enhanced cAMP level (reversed by indomethacin) and 1 μM PAF reduced cAMP level (reversed by AA-861).

Discussion

PAF-induced changes in cAMP production in AM are related and probably even result from alterations in arachidonic acid metabolism [11]. PGE_2 known to stimulate adenylyl cyclase may be responsible for the increased cAMP level via enhanced basal secretion of PGE_2 from ac-AM.

Exposure of antigen challenged AM to 1 fM of PAF enhances both release of PGE_2 and the content of cAMP, but diminishes LTB_4 release. PAF at 1 μM reduces both PGE_2 release and cAMP production but stimulates LTB_4-secretion. Apparently at low concentration of PAF cyclooxygenase is activated considering enhanced PGE_2 release and its inhibition by indomethacin but not AA-861 suggesting that prostanoids like PGE_2 or PGI_2 are responsible for PAF-induced cAMP production.

The reduction in LTB_4 release from ac-AM by 1 fM PAF might be due to preferential metabolism of the limited pool of free arachidonic acid into prostanoids. At high concentration of PAF the reverse is observed which can be explained as follows. Phospholipase A_2 and lipoxygenase but not cyclooxygenase are Ca^{2+}-dependent enzymes. Thus PAF at micromolar concentration may mobilize Ca^{2+} [1, 9] and shunt arachidonic acid metabolism towards the lipoxygenase pathway promoting leukotriene generation (like LTB_4). Consequently, the limited pool of free arachidonic acid will then be less available for prostanoid production.

In a similar way the change in LTB_4 and PGE_2 release by 1 fM PAF after pretreatment with indomethacin can be explained: indomethacin inhibits PGE_2 production thus promoting LTB_4 production. A comparable mechanism is observed in ac-AM exposed to 1 μM PAF after pretreatment with AA-861.

PAF modulates intracellular cAMP level in ac-AM but not in naive macrophages. We showed previously that adenylyl cyclase responsiveness to salbutamol and PGE_2 is enhanced in ac-AM, an effect probably due to an increase in number of G_s-subunits [12]. Therefore, despite the modest PAF-induced increase in PGE_2 secretion from naive macrophages, the amount of PGE_2 is insufficient to substantially stimulate cAMP production. Ac-AM, however, respond to 1 fM PAF with a 4-fold higher PGE_2-production (an increase of 160 vs 42 ng/5 × 10^6 cells). Together with improved coupling between prostanoid-receptors and adenylyl cyclase, this results in higher cellular cAMP content.

Similar biphasic responses of PAF have been observed [6, 7]. Release of TNF, IL-6 and LTB_4 release from rat AM was differentially stimulated by 1 fM to 1 μM PAF [7, 8]: the dose–response curve being bell-shaped with a peak effect at 0.1 fM. Others [6] showed enhanced IL-1 release by PAF 0.1 to 1 fM and inhibition at higher concentration from rat spleen adherent monocytes.

In conclusion, PAF differentially affects AM arachidonic acid metabolism: low and high concentrations promoting, respectively, PGE_2 and LTB_4 production and released prostanoids are probably responsible for subsequent cAMP production.

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