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Platelet activating factor and the responses of rat isolated stomach strip to prostaglandin E_2

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Abstract—The effect of platelet activating factor (PAF) on contractions evoked by acetylcholine, 5-hydroxytryptamine (5-HT) and prostaglandin E_2 (PGE_2) was studied in-vitro on rat stomach strip. Addition of PAF to the organ bath increased PGE- but not 5-HT- or acetylcholine-evoked responses. The effect of PAF was unaffected by atropine, methysergide or indomethacin, but prevented by a specific PAF receptor antagonist BN 52021. The data support a specific interaction between PAF and PGE_2 on rat stomach strip.

Lyso-PAF and arachidonic acid are precursors of PAF (platelet activating factor) and prostaglandins, respectively. Both these precursors are released from membrane-bound phospholipids by the action of phospholipase A_2. Their common origin could, in part, explain some biological effects that both prostaglandin E_2 (PGE_2) and PAF share when released in response to different stimuli.

However, some of these biological activities, such as local vasodilatation (Chu et al 1988), hyperalgesia (Dallob et al 1987), vascular permeability (Oh-ishi et al 1986), pleural exudation (Martins et al 1989), are modulated by PAF inhibitors and by the products of the cyclo-oxygenase or lipoxygenase pathways, indicating an interaction between PAF and arachidonate metabolites. In addition PGE_2 potentiates the inflammatory response to PAF in animals (Morley et al 1983) and in man (McGivern & Basran 1984). Furthermore PAF and PGE_2 may regulate the biosynthesis of one another. We now demonstrate that under conditions where PAF is itself inactive, it potentiates the effect of PGE_2, but not of acetylcholine or 5-hydroxytryptamine (5-HT).

Materials and methods

Experiments were carried out in-vitro using stomach strips (prepared longitudinally) from male Wistar rats, 150–170 g, killed by cervical dislocation. Strips were mounted in 10 mL organ baths containing warmed (37°C) and oxygenated (95% O_2–5% CO_2) Krebs solution of the following composition (g L^{-1}); NaCl 6·9, KCl 0·35, $MgSO_4 \cdot 7H_2O$ 0·29, KH_2PO_4 0·16, $CaCl_2$ 0·28, $NaHCO_3$ 2·1, glucose 2·0) and connected to an isotonic transducer to record the longitudinal muscle activity. The resting tension was adjusted to 2 g and maintained throughout the experiment. The agonists studied were PGE_2 (0·3–2·4 ng mL^{-1}), acetylcholine (5–20 ng mL^{-1}), and 5-HT (2·5–10 ng mL^{-1}); they remained in contact with the tissue until the maximal effect occurred (90–120 s) and then washed out. After at least three control agonist contractions, PAF (12 pg mL^{-1}) was added to the bath 2 min before the next addition of agonist. PAF (Sigma, Italy) was dissolved in Tris buffer (pH 7·4) containing 0·1% bovine serum albumin. In some experiments the cholinergic antagonist atropine sulphate (0·2 μg mL^{-1}), the 5-HT antagonist methysergide chloride (0·2 μg mL^{-1}), the prostaglandin synthesis inhibitor indomethacin (2 μg mL^{-1}), or the PAF antagonist BN 52021 (2 μg mL^{-1}) (Braquet 1985), was added to the Krebs solution. The results are shown as percent increase of the respective controls ± s.e. and the raw data were analysed statistically by Student's *t*-test for paired or unpaired data (2-tailed tests). The latent period was defined as the time between the addition of PGE_2 and the start of the contraction.

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Results

PGE_2, 0·3–2·4 ng mL^{-1}, caused slow concentration-dependent contraction of the rat stomach strip with a latent period of 20–40 s. However, PAF 12 pg mL^{-1}, did not affect the muscle tone but increased the height of the contraction to PGE_2 (Table 1) and shortened the latent period to 5–10 s; these effects were totally removed by washing out the PAF. In contrast, lyso-PAF in concentrations 12–100 pg mL^{-1} did not affect the response to PGE_2 (0·3–2·4 ng mL^{-1}) (data not shown). Previous addition of atropine sulphate (0·2 μg mL^{-1}), methysergide chloride (0·2 μg mL^{-1}) or indomethacin (2 μg mL^{-1}), alone or in combination, in concentrations more than sufficient to suppress cholinergic and 5-HT-ergic activities and cyclo-oxygenase activity, did not affect the potentiating activity of PAF (Table 1). In contrast BN 52021 (2 μg mL^{-1}), a specific PAF receptor antagonist, prevented the potentiation. The blocking drugs had little or no effect (3–6% inhibition) on contractions but completely inhibited any spontaneous activity that was present. The effect of PAF on agonists other than PGE_2 was also examined; PAF 12 pg mL^{-1} did not affect the response to acetylcholine 5–20 ng mL^{-1} or 5-HT 2·5–10 μg mL^{-1}.

Table 1. Potentiating effect of PAF (12 pg mL^{-1}) on the contraction elicited by PGE_2 (0·3–2·4 μg mL^{-1}) in the presence of indomethacin, methysergide, atropine or BN 52021. PAF was added 2 min before adding PGE_2.

		Percent increase mean ± s.e. Krebs containing			
PGE_2 (ng mL^{-1})	Krebs solution	Indomethacin (2 μg mL^{-1})	Methysergide (0·2 μg mL^{-1})	Atropine (2 μg mL^{-1})	BN 52021 (2 μg mL^{-1})
0·3	54 ± 5[a] (9)	50 ± 3 (6)	52 ± 4 (6)	48 ± 3 (5)	9 ± 3[b] (7)
0·6	49 ± 6[a] (8)	48 ± 4 (8)	44 ± 4 (6)	47 ± 5 (7)	6 ± 2[b] (6)
1·2	48 ± 7[a] (10)	47 ± 4 (7)	47 ± 5 (6)	44 ± 5 (6)	4 ± 2[b] (5)
2·4	44 ± 6[a] (11)	41 ± 4 (7)	44 ± 5 (6)	44 ± 3 (6)	4 ± 2[b] (6)

Results are percent increase of response, mean ± s.e. The number of experiments is shown in parentheses. [a] $P < 0·01$ vs control; [b] $P < 0·01$ vs Krebs solution.

Discussion

The present experiments show that PAF (12 pg mL^{-1}) potentiates the response of rat stomach strips to PGE_2 but not to acetylcholine or 5-HT. In preliminary experiments we found that lower concentrations (1–6 pg mL^{-1}) of PAF were inactive or did not significantly potentiate (10–19%, $P < 0·1$) smooth muscle responses to PGE_2, while a higher concentration (24 pg mL^{-1}) alone induced a contraction. The concentration of PAF used in the present study (12 pg mL^{-1}) was itself inactive, but significantly (44–54%, $P < 0·01$) increased the amplitude of the contractions of smooth muscle to PGE_2. Several substances which potentiate smooth muscle responses to PGE_2 have been described (Capasso et al 1988) and it has been proposed that some of them act by liberating endogenous prostaglandins. Furthermore, it has been suggested that PAF stimulates the release of prostanoids by cells (Hirafuji & Ogura 1990) and there is also reason to believe that PAF infusion would enhance eicosanoid synthesis by the gastrointestinal tract. However, prostaglandin biosynthesis does not explain the synergism observed, since it was not inhibited by indomethacin. The experiments with atropine and methysergide indicate that acetylcholine and 5-HT are not involved in the potentiating effect of PAF.

Our results also exclude a sensitization of smooth muscle myofilaments as the potentiation is specific for PGE_2. Perhaps PAF accelerates the penetration or the exposure of PGE_2 to its receptors as indicated by the shortening of the latent period. Other mechanisms involved might be an increase in the number of available prostaglandin receptors, a sensitization of the prostaglandin receptors, a prevention of the binding of PGE_2 to silent receptors or a decrease of prostaglandin breakdown, so that more PGE_2 might be available at the active receptor sites. Regardless of the mechanism, an interaction between PAF and PGE_2 could play an important role in regulating gastrointestinal motility and other functions including clinical conditions in which both are released (e.g. diarrhoea and intestinal damage (Pinto et al 1989)).

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