J. Pharm. Pharmacol. 1996, 48: 955–958
Received December 11, 1995
Accepted February 27, 1996

Involvement of Prostaglandins and 5-Hydroxytryptamine in the Contractile Effect of Platelet-activating Factor in Rat Isolated Gastric Corpus

M. A. MARTÍNEZ-CUESTA, M. D. BARRACHINA, L. MORENO, S. CALATAYUD AND J. ESPLUGUES

Department of Pharmacology, Faculty of Medicine, University of Valencia, Valencia, Spain

Abstract

The present study characterizes the nature of the response to the platelet-activating factor (PAF) in isolated gastric corpus with and without mucosa.

PAF (10^{-8} M) induced contraction of rat isolated gastric corpus strips followed by desensitization of this tissue. Incubation of strips with the specific PAF-receptor antagonist WEB 2086 (5×10^{-8}–5×10^{-5} M), the prostaglandin blocker indomethacin (10^{-6} M) and the 5-hydroxytryptamine antagonist methysergide (10^{-5} M) reduced significantly the contraction induced by PAF. Neither of the histamine H_1/H_2 antagonists diphenhydramine (10^{-6} M) or cimetidine (10^{-5} M) affected the contraction induced by PAF. In contrast with the whole gastric corpus, in mucosa-free strips, the contractile effect of PAF was not modified by methysergide.

The present study supports the view that the effect of PAF is mediated by activation of specific PAF receptors and the release of prostaglandins and 5-hydroxytryptamine in isolated gastric corpus. Furthermore, our results suggest a role of the gastric mucosa via the release of 5-hydroxytryptamine which contributes to the contractile effect of PAF in the gastric smooth muscle.

Platelet-activating factor (PAF) (1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine) is an ether phospholipid released from a variety of inflammatory cells. This mediator which is a relevant activator of platelets and neutrophils, plays an important role in cardiac and systemic anaphylaxis as well as in the regulation of blood pressure.

In the gastrointestinal tract PAF exhibits a potent ulcerogenic activity and contracts a variety of gastrointestinal smooth muscle preparations. In-vivo studies have suggested that PAF increases gastric motility acutely by the release of histamine and 5-hydroxytryptamine (5-HT) (Esplugues & Whittle 1988, 1989).

In the present study the nature of the response to PAF was further characterized in rat isolated gastric corpus. The gastric mucosa contains a large number of cells which release mediators involved in gastrointestinal motility. Thus, the possible contribution of the gastric mucosa to the contractile effect of PAF was also evaluated.

Materials and Methods

Tissue preparations

Wistar rats of either sex, 250–300 g, were fasted overnight and killed by cervical dislocation and rapid exsanguination. The stomach was removed and opened at the small curvature. The tissue was then rinsed gently several times to remove any residual stomach contents, and circular muscle-mucosa strips (2×0.3 cm) were obtained from the gastric corpus. The strips were set up in 20-mL organ baths containing Krebs solution (composition in mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.18, $CaCl_2$ 2.5, KH_2PO_4 1.18, $NaHCO_3$ 25, glucose 11.1). The solution was gassed with 95% O_2/5% CO_2 and maintained at 37°C. Mechanical activity of the gastric corpus strips was recorded through a Ugo Basile 7006 isotonic transducer on a Gemini 70-70 recorder. Gastric corpus strips were equilibrated for 45 min under a basal tension of 2 g.

Correspondence: M. A. Martínez-Cuesta, Department of Pharmacology, Faculty of Medicine, Avda. Blasco Ibañez 15, 46010 Valencia, Spain.

Experimental protocols

Acetylcholine (10^{-5} M) was added to the strips to assess the contractile responsiveness of preparations. After a washout period, PAF (10^{-11}–10^{-5} M) was added to the organ bath. In preliminary experiments it was observed that PAF induces tachyphylaxis, thus the concentration effect-curve to PAF (10^{-11}–10^{-5} M) was constructed in a noncumulative manner using a fresh gastric strip for each measurement and the concentration 10^{-8} M of PAF was chosen for the pharmacological studies. In some experiments the specific PAF-receptor antagonist WEB 2086 (5×10^{-8}–5×10^{-5} M) was added to the organ baths 15 min before PAF 10^{-8} M exposure. Additional experiments were separately performed in the presence of different drugs: the non-specific neuronal blocker tetrodotoxin (5×10^{-8} M), capsaicin (10^{-6} M) which depletes neuropeptides from sensory neurons, the ganglionic antagonist hexamethonium (10^{-6} M), atropine (10^{-6} M), phentolamine (10^{-6} M), propranolol (10^{-6} M), indomethacin (10^{-6} M), methysergide (10^{-5} M), cimetidine (10^{-6} M) or diphenhydramine (10^{-6} M).

To investigate the contribution of the gastric mucosa on the effect of PAF, additional experiments were performed with mucosa-free strips. These preparations were obtained by removing the mucosa from the gastric corpus with fine scissors. The tissues were set up in the organ baths as before.

Statistical analysis

The effect of PAF was expressed as a percentage of response to acetylcholine (10^{-5} M). Evaluation of results at different times for 30 min after PAF exposure did not show significant statistical differences; thus, results are quoted at 5 min. All results are expressed as means ± s.e.m. Comparisons between groups were made by Student's *t*-test and *P* values of 0·05 or less were considered as significant.

Drugs

Acetylcholine chloride, atropine sulphate, diphenhydramine hydrochloride, hexamethonium bromide, methysergide, propranolol hydrochloride, phentolamine hydrochloride and tetrodotoxin were purchased from Sigma Chemical Co. Cimetidine was obtained from SmithKline Beecham. All these drugs were dissolved in 0·9% saline immediately before use. PAF (Sigma Chemical Co.) was stored at −20°C as a stock solution in chloroform. A sample was dried under nitrogen when required, redissolved in vehicle (0·25% bovine serum albumin in saline), and kept on ice. Indomethacin (Sigma Chemical Co.) was dissolved in 1·25% sodium bicarbonate immediately before use. Capsaicin (Fluka Chemie AG.) was dissolved in absolute ethanol, Tween 80 and isotonic saline (10:10:80, v/v/v). Concentrated solutions of WEB 2086 (Boehringer Ingelheim) were prepared in dimethylsulphoxide (DMSO) daily and diluted in the tissue-bathing fluid so that the dimethylsulphoxide concentration did not exceed 0·1%. Solvent controls had no effect on the tissue responses studied.

Results and Discussion

PAF effect on gastric corpus strips

Esplugues & Whittle (1989) have described the increase in gastric motility induced by PAF in-vivo. As shown in Fig. 1, the present study confirms such an event since PAF (10^{-8} M) induced a rapid increase in resting tension of isolated rat gastric corpus which was maintained for at least the 30 min of the study. Additional PAF in the same tissue failed to produce a response even after repeated washing out with 10% BSA, indicating a tachyphylaxic effect. As shown in Fig. 2, the response to PAF was concentration-dependent (10^{-11}–10^{-6} M) with $E_{max} = 74{\cdot}4 \pm 8$ (% acetylcholine) and EC50 = $8{\cdot}9 \pm 2$ 10^{-8} M. The contraction induced by PAF 10^{-8} M, chosen for the pharmacological study, was 30·2 ± 3% acetylcholine (n = 18). A higher concentration (10^{-5} M) induced a biphasic response, characterized by a small contraction followed by relaxation. The contractile effect of PAF has also been described in other isolated gastrointestinal tissues such as rat stomach fundus (Levy 1987), guinea-pig ileum (Findlay et al 1981; Tokumura et al 1984) and rat colon (Tokumura et al 1988). Our results show that the incubation of tissues with the competitive PAF-receptor antagonists WEB 2086 (5×10^{-8}–5×10^{-5} M) induced a concentration-dependent inhibition of the response to PAF 10^{-8} M. Concentrations of WEB 2086 (5×10^{-7} M, 5×10^{-6} M and 5×10^{-5} M) significantly reduced the effect of PAF to 14 ± 7 ($P < 0{\cdot}05$, n = 4), 10 ± 4 ($P < 0.01$, n = 6) and 0·2 ± 0·1% acetylcholine ($P < 0.001$, n = 4), respectively. Thus, the effect of PAF was the consequence of a direct specific PAF-receptor activation in the rat gastric corpus, as has been described for other tissues (Hwang et al 1983, 1985; Nishihira et al 1985; Voelkel et al 1986), and associated with a subsequent tachyphylaxis of these tissues (Findlay et al 1981; Keraly et al 1983; Saijo et al 1985).

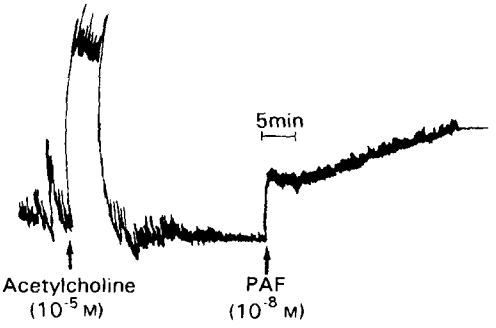

FIG. 1. Representative isotonic recording of the contraction induced by acetylcholine (10^{-5} M) and platelet-activating factor (PAF, 10^{-8} M) on a circular muscle-mucosa strip from the rat gastric corpus.

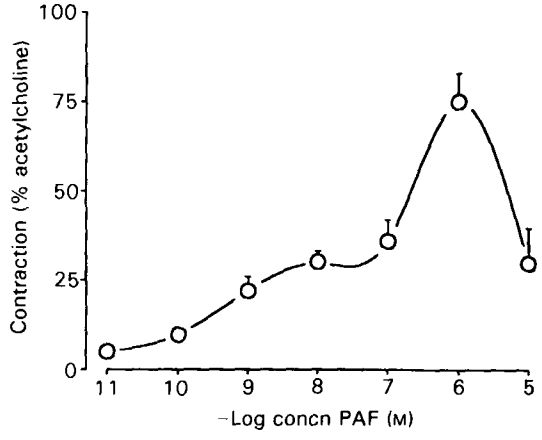

FIG. 2. Concentration ± response curve to PAF (10^{-11}–10^{-5} M) on rat isolated gastric corpus. Each tissue was exposed to a single PAF concentration and the effect was measured at 5 min. Each point represents the mean ± s.e.m. of the contraction induced by PAF as percentage of acetylcholine (10^{-5} M) contraction of at least 8 strips from different animals.

Table 1. Effect of various drugs on the contractile effect of PAF (10^{-8} M) in rat gastric corpus strips with mucosa.

	n	(% acetylcholine)
Control	18	30.2 ± 3
+ tetrodotoxin (5×10^{-7} M)	5	25.4 ± 3
+ atropine (10^{-6} M)	4	29.6 ± 2
+ phentolamine + propranolol (10^{-5} M)	6	23.1 ± 6
+ hexamethonium (10^{-6} M)	4	28.5 ± 8
+ capsaicin (10^{-6} M)	9	27.6 ± 5

Data show the mean ± s.e.m. of the contraction induced by PAF (10^{-8} M), expressed as a percentage of acetylcholine-induced contraction, in the presence or absence (control) of each blocker (n indicates number of animals).

However, the subsequent events to PAF-receptor activation are still poorly understood. A non-adrenergic, non-cholinergic neuronal activity has been described as the main mechanism involved in the increase of the gastric motility induced by PAF in-vivo (Esplugues & Whittle 1988, 1989). As shown in Table 1, such a neuronal mechanism was absent in isolated gastric tissue since incubation of the gastric strips with tetrodotoxin

Table 2. Effects of various drugs on the contractile effect of PAF (10^{-8} M) in rat gastric corpus strips with and without mucosa.

	PAF (% acetylcholine)			
	Gastric corpus with mucosa	n	Gastric corpus without mucosa	n
Control	30·2 ± 3	18	48·0 ± 4	26
+ indomethacin (10^{-6} M)	17·9 ± 2*	7	23·9 ± 3***	13
+ cimetidine (10^{-5} M)	36·1 ± 8	6	63·3 ± 7	9
+ diphenhydramine (10^{-6} M)	33·6 ± 5	5	40·9 ± 5	5
+ methysergide (10^{-5} M)	19·1 ± 4*	6	52·6 ± 5	9

Data show the mean ± s.e.m. of the contraction induced by PAF, expressed as a percentage of acetylcholine-induced contraction, in presence or absence (control) of each antagonist (n indicates number of animals). *$P < 0.05$, ***$P < 0.001$ compared with controls.

(5×10^{-7} M) did not affect the contraction induced by PAF. Furthermore, local sensory afferent neurons have been associated with gastric microcirculatory response to PAF (Piqué et al 1990). Incubation of gastric strips with capsaicin (10^{-6} M), which damages the afferent nerve endings, did not modify the response to PAF (10^{-8} M) (Table 1). Likewise, hexamethonium (10^{-6} M), phentolamine and propranolol (10^{-6} M) had no effect (Table 1). The absence of the contribution of nerve endings in the effect of PAF have also been observed in isolated strips from guinea-pig ileum (Findlay et al 1981; Tokumura et al 1984), rat colon (Tokumura et al 1988) and rat stomach fundus (Levy 1987). Our findings suggest that the effect induced by PAF in the gastric corpus is not modulated by a local nervous mechanism. However, we can not reject the possible contribution of the integrity of the enteric nervous system or even the CNS for the effect of PAF in-vivo.

PAF is associated with inflammatory responses characterized by the release of mediators such as prostaglandins, histamine and 5-HT. As shown in Table 2, the incubation of the gastric tissues with methysergide (10^{-5} M) and indomethacin (10^{-6} M) induced a significant inhibition of PAF effect, suggesting that local releases of 5-HT and prostaglandins could be involved in the contractile effect of PAF on gastric corpus strips. However, the contractile effect of PAF was not modified by diphenhydramine (10^{-6} M) or cimetidine (10^{-5} M) (Table 2), indicating that other mediators such as histamine did not participate in the effect of PAF. Although a non-neuronal residual increase of gastric motility induced by PAF in-vivo was attributed to the acute release of histamine (Esplugues & Whittle 1988, 1989), it could be relevant to note the possible contribution of humoral circulation in the effect of PAF in-vivo.

PAF effect on mucosa-free strips

In mucosa-free strips, the profile of contractile effect of PAF (10^{-8} M) was similar to the gastric strips with mucosa, but the magnitude of response was greatly increased (48 ± 4·3% acetylcholine, $P < 0.05$, $n = 26$). As shown in Table 2, the incubation of mucosa-free strips with histamine H_1/H_2-receptor antagonists or methysergide did not modify the contractile effect of PAF. Incubation of tissues with indomethacin (10^{-6} M) significantly inhibited the effect of PAF (49 ± 6%), similar to the effect on strips with mucosa (42 ± 6%).

The role of these mediators on PAF response in the smooth muscle is still a matter of discussion. Several reports on guinea-pig ileum and rat colon suggest that the effect of PAF is not due to activation of histaminergic or serotonergic receptors (Findlay et al 1981; Tokumura et al 1984, 1988). Moreover, in isolated rat stomach fundus, neither these mediators nor the products from arachidonic acid by the cyclo-oxygenase pathway affected the contraction induced by PAF (Levy 1987). The gastric mucosa clearly shows an important difference between gastric fundus and corpus which could explain the differences in the stomach preparations. In gastric mucosa-free strips the higher contraction induced by PAF could be the consequence of a higher accessibility of PAF to smooth muscle. In addition, a loss of mediators released from the gastric mucosa could affect the response of mucosa-free strips to PAF; the contractile effect of PAF in gastric mucosa-free strips was mediated by prostaglandins, as well as in the whole tissue, but not by 5-HT. In-vivo studies on rat gastric motility confirm that spasmogenic amines such as 5-HT can be released from mast cells (Esplugues & Whittle 1988, 1989), so the contractile effect of PAF may be partly due to the action of 5-HT release from the mucosal mast cells.

Acknowledgement

M. A. Martínez-Cuesta and M. D. Barrachina are the recipients of fellowships sponsored respectively by Formación de Profesorado Universitario (Ministerio de Educación y Ciencia) and Conselleria de Educació i Ciència (Generalitat Valenciana, Spain). Lucrecia Moreno and Sara Calatayud are the recipients of fellowships sponsored by Shering Plough Laboratories.

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