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Pharmacological Modulation of Platelet Activating Factor (PAF)-induced Bronchoconstriction and Hypertension in Anaesthetized Guinea-pigs

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Abstract—The effect of PAF has been examined in anaesthetized guinea-pigs. Intravenous (i.v.) administration of PAF (10 ng kg^{-1}) did not modify the respiratory response but decreased the arterial blood pressure. A high dose of PAF (200 ng kg^{-1}) caused marked bronchoconstriction and concomitant hypertension. The cyclooxygenase inhibitors aspirin (5 mg kg^{-1}) and indomethacin (5 mg kg^{-1}) and the thromboxane A_2 (TXA_2) receptor antagonist BM-13.177 (1 mg kg^{-1}) failed to inhibit the peak bronchoconstrictive response but significantly inhibited the prolonged response following peak response. These inhibitors also attenuated PAF-induced hypertension. On the other hand, the lipoxygenase inhibitors phenidone (10 mg kg^{-1}) and NDGA (5 mg kg^{-1}) and the leukotriene (LT) receptor antagonist FPL-55712 (2 mg kg^{-1}) affected neither bronchoconstriction nor hypertension induced by PAF. However, when aspirin was given in combination with NDGA, phenidone or FPL-55712, the peak and the following prolonged bronchoconstriction were significantly inhibited. The suppression of PAF-induced hypertension by aspirin was not further inhibited by the combination of these inhibitors. These results indicate that in anaesthetized guinea-pigs PAF-induced bronchoconstriction is composed of a dual response, a direct action (peak response) and an indirect action (prolonged response). The latter may be produced by the generation of TXA_2 and lipoxygenase products, while PAF-induced hypertension is indirectly mediated by the generation of TXA_2.

Platelet activating factor (PAF) is a phospholipid mediator generated in inflammatory and allergic reactions (Pincard et al 1982). In-vitro, PAF produces the activation of platelets or leucocytes (Voelkel et al 1986) and contraction of smooth muscles (Tokumura et al 1984; Touvay et al 1987). In-vivo, intravenous administration of PAF into guinea-pigs provokes bronchoconstriction (Vargaftig et al 1980). PAF-induced bronchoconstriction is not prevented by cyclo-oxygenase inhibitors, antihistamine or anti-5-hydroxytryptamine agents, but the combination of these agents suppresses it (Vargaftig et al 1982). Furthermore, high doses of lipoxygenase inhibitors suppress the bronchoconstriction (Bonnet et al 1983; Lewis et al 1984). Those studies suggest that cyclo-oxygenase and lipoxygenase products are involved in PAF-induced bronchoconstriction. On the other hand, PAF causes hypotension followed by hypertensive responses (Tanaka et al 1983). However, the relation between PAF-induced bronchoconstriction and change in systemic blood pressure is not well known.

In the present study, we have examined the role of cyclo-oxygenase and lipoxygenase products on PAF-induced bronchoconstriction and hypertension in anaesthetized guinea-pigs.

Materials and Methods

The sources of materials used were: PAF (L-α-phosphatidylcholine-β-acetyl-γ-*O*-hexadecyl), indomethacin, nordihydroguaiaretic acid (NDGA), 1-phenyl-3-pyrazolidone (phenidone) and fatty acid free-bovine serum albumin (BSA) were all purchased from Sigma Chemical Co. (St. Louis, USA); aspirin was obtained from Iwaki Pharmaceutical Co. (Tokyo Japan). BM-13.177 [4-(2-(benzene sulphamido)ethyl)phenoxyacetic acid], FPL-55712 [sodium 7-(3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy)-4-oxo-8-propyl-4H-chromene-2-carboxylate], and OKY-046 [(E)-3-(4-(1-imidazolylmethyl)-phenyl)-2-propenoic acid $HCl.H_2O$] were synthesized in our laboratory.

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Methods

Male Hartley guinea-pigs (300–400 g) were anaesthetized with urethane (1·5 g kg^{-1} i.p.). Arterial blood pressure was recorded through a cannula in the left carotid artery by a pressure transducer (Nihon Kohden, MPU-0. 5A) coupled to a multipurpose polygraph (Nihon Kohden, PMP-6008).

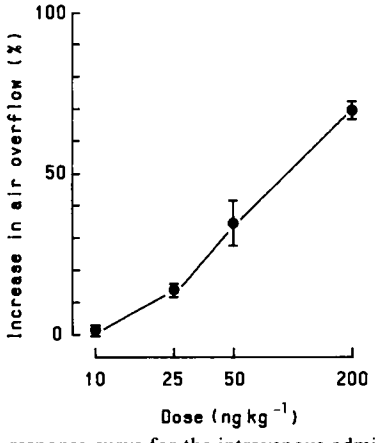

FIG. 1. Dose-response curve for the intravenous administration of PAF on pulmonary response in anaesthetized guinea-pigs. This response is shown by percent increase in air overflow volume compared with its maximum. Results are the mean ± s.e.m. of 6–8 guinea-pigs.

Basal mean arterial blood pressure (MAP) was 40±4 mmHg. The jugular vein was cannulated for the administration of drugs. The tracheal cannula was inserted to permit measurement of air overflow by the method of Konzett & Rössler (1940). Respiration was carried out in the following ways: (1) 15–20 mL kg^{-1} $stroke^{-1}$, (2) 40–50 strokes min^{-1}. The increase in respiratory overflow volume provoked by PAF was given as a percentage of the maximal overflow volume (100%) obtained by clamping off the trachea.

PAF was dissolved in 0·9% NaCl (saline) with 0·1% BSA; aspirin was dissolved in 8·4% $NaHCO_3$; indomethacin, phenidone and NDGA were dissolved in 100% polyethylene glycol 400; and FPL-55712 was dissolved in saline containing 0·3% Tween 80. Vehicles had no effect on the responses of PAF in anaesthetized guinea-pigs. Aspirin and indomethacin were administered 10 min before, phenidone, NDGA, and OKY-046 5 min before, and FPL-55712 and BM-13.177 1 min before challenge with PAF. All data were analysed using Student's *t*-test for unpaired data.

Results

In anaesthetized guinea-pigs, intravenous administration of PAF caused dose-dependent bronchoconstriction at doses of 10–200 ng kg^{-1} (Fig. 1). The response reached a peak 30s after the PAF injection and then was sustained at a lower value (Fig. 2, right upper panel). A low dose of PAF (10 ng kg^{-1} i.v.) caused marked hypotension (peak: 10–20 s), while a high dose of PAF (200 ng kg^{-1}) provoked transient hypotension followed by potent hypertension (peak: 60 s) (Fig. 2, lower panels). Arterial pressure changes induced by 20–100 ng kg^{-1} PAF were not uniform (data not shown).

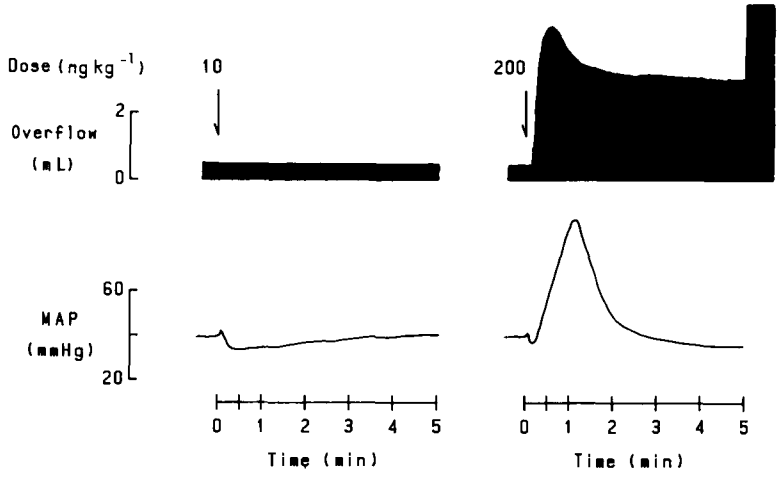

FIG. 2. Typical tracings of pulmonary response and MAP after intravenous administration of PAF to anaesthetized guinea-pigs. PAF was injected at the points indicated by arrows. The maximal overflow volume was achieved by clamping off the trachea.

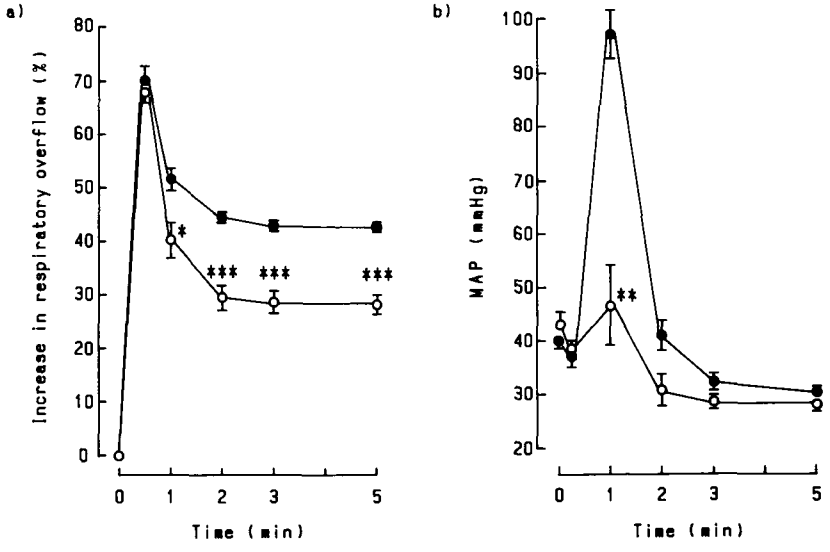

FIG. 3. Time course of the effect of aspirin on PAF (200 ng kg^{-1})-induced bronchoconstriction (a) and hypertension (b) in anaesthetized guinea-pigs. Aspirin (5 mg kg^{-1}) (○) was pretreated 5 min before the PAF injection. Results are the mean±s.e.m. of 6–7 separate experiments. $*P<0·05$, $**P<0·01$, $***P<0·001$, significantly different when compared with control response (●).

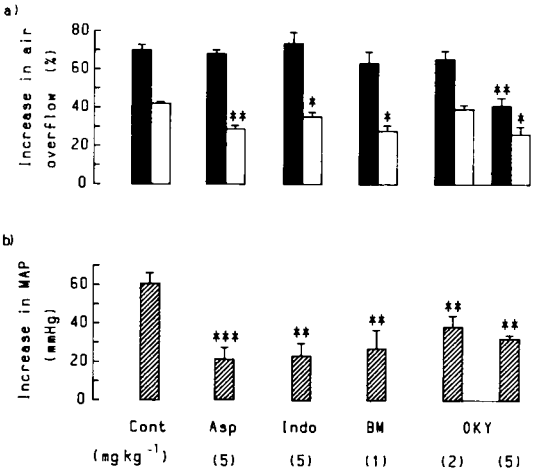

FIG. 4. Inhibitory effects of PAF-induced bronchoconstriction (a) and pressor response (b) by aspirin, indomethacin, BM-13.177 and OKY-046 in anaesthetized guinea-pigs. Aspirin (Asp) 5 mg kg^{-1}, indomethacin (Ind) 5 mg kg^{-1}, BM-13.177 (BM) 1 mg kg^{-1} and OKY-046 (OKY) 2 and 5 mg kg^{-1}, were pretreated 10, 10, 1 and 5 min before the PAF injection, respectively. Closed columns and open columns expressed the bronchoconstriction of the peak time and that of 5 min after the injection of PAF, respectively. Hatched columns indicate increase in MAP after PAF injection. Results are the mean ± s.e.m. of 5-7 separate experiments. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, significantly different when compared with control response (Cont).

Pretreatment with a cyclo-oxygenase inhibitor, aspirin (5 mg kg^{-1}), failed to suppress the peak response of bronchoconstriction induced by 200 ng kg^{-1} PAF, but significantly attenuated the subsequent prolonged response (Fig. 3a). Simultaneously, the PAF-induced hypertension was significantly reduced by aspirin (Fig. 3b). Similar inhibitory effects of PAF-induced bronchoconstriction and hypertension were observed with another cyclo-oxygenase inhibitor, indomethacin (5 mg kg^{-1}), and a TXA_2 antagonist, BM-13.177 (1 mg kg^{-1}) (Fig. 4). A TXA_2 synthetase inhibitor, OKY-046 (2 mg kg^{-1}) did not affect the peak and subsequent prolonged bronchoconstriction (5 min after the PAF injection), but did inhibit the concomitant hypertension. Moreover, a high dose of OKY-046 (5 mg kg^{-1}) inhibited both peak and prolonged bronchoconstriction as well as hypertension.

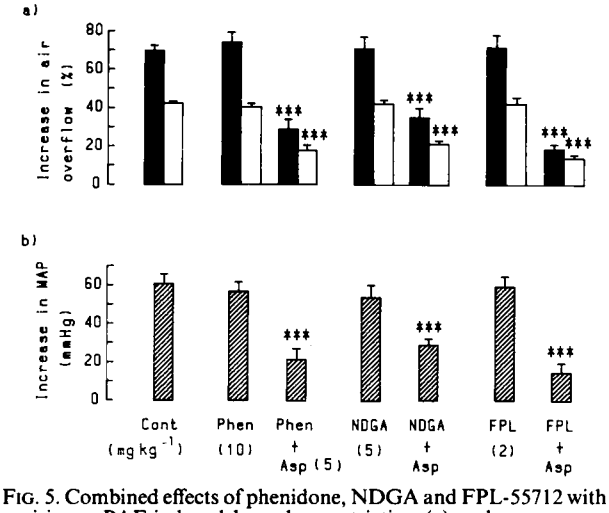

FIG. 5. Combined effects of phenidone, NDGA and FPL-55712 with aspirin on PAF-induced bronchoconstriction (a) and pressor responses (b) in anesthetized guinea-pigs. Phenidone (Phen) 10 mg kg^{-1}, NDGA 5 mg kg^{-1}, FPL-55712 (FPL) 2 mg kg^{-1} and aspirin (Asp) 5 mg kg^{-1} were pretreated 5, 5, 1, and 10 min before the PAF injection, respectively. Closed columns, open columns and hatched columns are explained in Fig. 4. Results are the mean ± s.e.m. of 5–7 separate experiments. $**P < 0.001$, $***P < 0.001$, significantly different when compared with control response (Cont).

On the one hand, the lipoxygenase inhibitors phenidone (10 mg kg^{-1}) and NDGA (5 mg kg^{-1}) and the leucotriene (LT) antagonist FPL-55712 (2 mg kg^{-1}) affected neither bronchoconstriction nor hypertension induced by PAF (Fig. 5). However, when aspirin (5 mg kg^{-1}) was combined with the above inhibitors, responses of peak and prolonged bronchoconstriction were significantly inhibited. But the inhibition of the PAF-induced hypertension by this combination was similar to that of aspirin (inhibition percent: aspirin 65%, aspirin + phenidone 64%, aspirin + NDGA 55%, aspirin + FPL-55712 74%). These results were also obtained with the combination of indomethacin (5 mg kg^{-1}) and phenidone (10 mg kg^{-1}) or FPL-55712 (2 mg kg^{-1}) and the combination of BM-13.177 (1 mg kg^{-1}) and FPL-55712 (2 mg kg^{-1}) (data not shown).

Discussion

O'Donnell et al (1983) reported that PAF-induced bronchoconstriction was resistant to an LTD_4 antagonist FPL-55712 and the lipoxygenase inhibitors NDGA and 2-chloro-4-aminophenol. In the present study we showed that the cyclo-oxygenase inhibitors, aspirin and indomethacin, and the TXA_2 antagonist, BM-13.177 (Stegmeier et al 1984), inhibited PAF-induced bronchoconstriction and hypertension in anaesthetized guinea-pigs. These results suggest that PAF induces the generation of TXA_2, which contributes to the prolonged response following the peak response and the concomitant hypertension. The peak response of PAF-induced bronchoconstriction was not inhibited by these inhibitors. These results were similar to those of Lewis et al (1984), O'Donnell et al (1983) and Vargaftig et al (1982). The TXA_2 synthetase inhibitor OKY-046 (5 mg kg^{-1}) reduced peak bronchoconstriction and hypertension. Similar results have been observed with dazoxiben (Welton et al 1985) and FCE2218 (Giorgetti et al 1986). The inhibition of OKY-046 of peak bronchoconstriction might divert arachidonic acid metabolism toward prostacyclin (PGI_2) synthesis as well as dazoxiben (O'Keefe et al 1985).

In our results, the lipoxygenase inhibitors NDGA and phenidone were ineffective against the PAF-induced bronchoconstriction and hypertension. However, the combination of both inhibitors with aspirin dramatically inhibited the peak and prolonged bronchoconstriction, but the inhibitory activities on pressor response by these combinations were equal to those of aspirin alone. These synergistic effects by the combination of cyclo-oxygenase inhibitor with lipoxygenase inhibitor on PAF-induced bronchoconstriction suggest that lipooxygenase products, probably LTs, are involved in PAF-induced bronchoconstriction, because FPL-55712 did not affect PAF-induced bronchoconstriction but markedly inhibited it in combination with aspirin. The PAF-induced hypertensive response is not affected by the lipoxygenase inhibitor U-60257 in conscious guinea-pigs (Feuerstein et al 1985) and is abolished by propranolol in anaesthetized guinea-pigs (Lewis et al 1984). Förster et al (1980) reported that propranolol inhibited TXA_2 synthesis in

rat lungs and rabbit platelets, suggesting that in our data the hypertension induced by PAF probably resulted from the generation of TXA_2 in anaesthetized guinea-pigs. PAF has also been reported to stimulate LT release in rat lungs (Voelkel et al 1982) and guinea-pig trachea (Malo et al 1987) and TXA_2 release in guinea-pig lungs (Hamasaki et al 1984; Desquand et al 1986; Touvay et al 1986). Moreover, PAF enhances LTD_4-induced airway responses of rhesus monkeys (Patterson et al 1984), and LTD_4 and TXA_2 mimic U46619-induced contraction of guinea-pig trachea (Malo et al 1987). From these reports, we propose that PAF acts directly on peak bronchoconstriction but indirectly on the subsequent prolonged bronchoconstriction by generating TXA_2 and LTs, whereas PAF-induced hypertension may be due to the generation of TXA_2 in anaesthetized guinea-pigs.

Recently, several PAF antagonists have been reported. CV-3988 (Melden et al 1985), BN52021 (Desquand et al 1986), SRI-63-411 (Handley et al 1986) and FR-49175 (Okamoto et al 1986) can inhibit PAF-induced bronchoconstriction in guinea-pigs. Darius et al (1986) reported that a PAF antagonist, kadsurenone, prevented both bronchoconstriction and hypotension induced by PAF. Those reports indicate that, in our study, PAF was bound to a specific PAF receptor to provoke both bronchoconstriction and hypertension.

In conclusion, PAF-induced bronchoconstriction is constructed by a dual response; an initial direct action which is resistant to cyclo-oxygenase and lipoxygenase inhibitors and an indirect second action which is induced by generating TXA_2 and lipoxygenase products, whereas the pressor effect induced by PAF is mostly due to the generation of TXA_2.

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