Effects of PAF on excitatory neuro-effector transmission in dog airways

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1 Effects of PAF on excitatory neuro-effector transmission in smooth muscle cells of mucosa-free trachea and epithelium-intact bronchiole of the dog were investigated, by isometric tension recording, microelectrode and double sucrose-gap methods.

2 PAF (10^{-11} – 10^{-7} M) dose-dependently enhanced the amplitude of contraction evoked by repetitive field stimulations (10 stimuli at 20 Hz) in both tracheal and bronchiolar tissues. At higher concentrations PAF ($>10^{-8}$ M) increased the amplitude of contraction to a greater extent in the bronchiole than in the trachea.

3 In both muscle tissues, in parallel to the amplitude of contraction, PAF markedly enhanced the amplitude of excitatory junction potentials (e.j.ps) evoked by a single field stimulation in a dose-dependent manner, with no change in the resting membrane potential or input membrane resistance of the smooth muscle cells. PAF (5×10^{-7} M) enhanced the amplitude of e.j.p. to a greater extent in the bronchiole than in the trachealis. In contrast, lyso-PAF (10^{-10} – 10^{-7} M) showed no effect on e.j.p. amplitude in bronchiolar tissues. At a high concentration (10^{-7} M) lyso-PAF slightly enhanced the e.j.p. amplitude in tracheal tissue, however the lyso-PAF induced stimulation of e.j.p. amplitude in the trachea was small compared to that of PAF.

4 PAF (10^{-7} M) had no effect on the membrane depolarization induced by acetylcholine (ACh, 10^{-9} – 10^{-5} M) and carbachol (10^{-9} – 10^{-5} M) in tracheal smooth muscle cells.

5 The PAF-antagonists CV3988 (5×10^{-7} M) or WEB2086 (5×10^{-7} M) significantly enhanced the e.j.p. amplitude themselves, PAF (5×10^{-8} M) further enhanced the e.j.p. amplitude in the presence of WEB2086 (5×10^{-7} M) but not CV3988 (5×10^{-7} M). In contrast, the new PAF-antagonist, E 6123 (5×10^{-8} M), did not affect the e.j.p. amplitude itself, and completely inhibited the increase in e.j.p. amplitude caused by 5×10^{-8} M PAF. On the other hand, in the presence of the H₁-antagonist, mepyramine, PAF (5×10^{-8} M) further enhanced the e.j.p. amplitude.

6 The leukotriene synthesis inhibitor AA-861 (10^{-6} M) or leukotriene antagonist ONO1078 (10^{-7} M) inhibited the increase in e.j.p. amplitude caused by 5×10^{-8} M PAF, respectively.

7 In the presence of AA-861 (10^{-6} M), leukotriene B₄ (LTB₄, 10^{-8} M) or LTD₄ (10^{-8} M) slightly, and LTC₄ (10^{-8} M) markedly enhanced the e.j.p. amplitude. In contrast, LTE₄ (10^{-8} M) significantly suppressed the e.j.p. amplitude.

8 PAF (5×10^{-8} M) attenuated the depression phenomena of e.j.ps observed during double stimulus experiments at different time intervals (5–10 s), but had no effect on the summation of e.j.ps during repetitive field stimulation at a high frequency (20 Hz) in the trachealis.

9 These results indicate that PAF potentiates excitatory neuro-effector transmission mainly through stimulating the release of lipoxigenase products, mainly LTC₄ in the dog airway smooth muscle tissues.

Keywords: PAF; trachea, canine; bronchiole; excitatory neuro-effector transmission

Introduction

Platelet activating factor (PAF) is a phospholipid that was first derived from rabbit basophils upon IgE-mediated immunological challenge (Benveniste *et al.*, 1972). Later, it was shown that PAF can be synthesized and released by several immune and non-immune stimuli from a variety of cell types, including macrophages, platelets, monocytes and endothelial cells (Vargaftig *et al.*, 1981a,b; O'Flaherty & Wykle, 1983; Braquet *et al.*, 1987).

In the lung, PAF has potent effects on various tissues and cells. For example it increases pulmonary vascular pressure, induces pulmonary oedema (Hamasaki *et al.*, 1984) and causes a long lasting, non-specific increase in bronchial responsiveness in experimental animals (Mazzoni *et al.*, 1985; Barnes *et al.*, 1987; Robertson & Page, 1987; Robertson *et al.*, 1988). Recently, it was shown that PAF increases the responsiveness of dog airway smooth muscle to parasympathetic stimuli *in vivo* (Leef *et al.*, 1987; Bethel *et al.*, 1989). The authors concluded that airway hyperresponsiveness elicited by PAF results from regional stimulation and/or release of mediators that augment contractility of airway

smooth muscle. To investigate further the mechanisms involved in increased responsiveness of airway smooth muscle, we performed comparative studies on the effect of PAF and lyso-PAF on the contraction and excitatory junction potential (e.j.p.) recorded from epithelium-intact bronchial and mucosa-free tracheal tissues, in response to electrical field stimulation (EFS) with isometric tension recording, double sucrose gap and microelectrode methods.

Methods

Adult mongrel dogs of either sex, weighing 10–13 kg were anaesthetized by i.v. administration of pentobarbitone (30 mg kg^{-1}). Segments of the cervical trachea were excised and whole pulmonary lobes were quickly resected from the main bronchus. A dorsal strip of transversely running tracheal smooth muscles was separated from the cartilage, and the mucosa and adventitial areolar tissue were carefully removed, leaving just smooth muscle tissue. The tracheal smooth muscle was cut to a width of 2.0–2.5 mm and a length of about 15 mm for use with the double sucrose gap

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method. Small airways (about 1 mm in diameter) were carefully excised from the lung tissue under microscopic observation, and lung parenchyma and pulmonary vessels running along a bronchiolar branch were removed. Histological investigations revealed that the tissues used for the present experiments had a diameter of 0.8–1.1 mm, and were composed of smooth muscle layers and mucous membrane but lacked cartilage, thereby indicating that the tissue comprised bronchioles (Cumming, 1972; Inoue & Ito, 1986; Ito & Inoue, 1989). A piece of bronchiole (1 mm in diameter, 4–5 mm in length), and ring preparations (0.8–1 mm in diameter, 1–2 mm wide) were used for the microelectrode and tension recording experiments, respectively. The preparation was bathed in a modified Krebs solution of the following ionic concentration (mM), Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134.0, H_2PO_4^- 1.2, HCO_3^- 15.5 and glucose 11.5. The solution was aerated with 97% O_2 and 3% CO_2 and the pH was 7.3–7.4. The double sucrose gap method was used to record simultaneously the membrane potential and tension development from the tracheal smooth muscles. The chamber used has been described elsewhere (Ito & Tajima, 1981). To produce neurogenic responses, EFS was applied between ring electrodes placed in the centre pool of the apparatus, using an electronic stimulator (Nihon Kohden SEN-7103). Single and repetitive stimulation was applied with current pulse of 50 μs in duration and 10–20 V in strength. The voltage of the current pulse was adjusted so that an e.j.p. of a defined amplitude was evoked by a single pulse. Drugs were dissolved in Krebs solution and applied to the tissue through the centre pool of the double sucrose gap apparatus, using a multi-way tap (dead-time approximately 30 s).

For intracellular recording of the membrane potential from a single cell, thin strips of tracheal tissue 10–15 mm in length 4–5 mm in width and 0.3–0.4 mm thick or a piece of intact bronchiole (1 mm in diameter, 4–5 mm in length) were used. The dog tracheal preparations used in these experiments were dissected entirely free from the overlying mucosal layer and its cartilagenous attachments. In the case of bronchioles, the airway tissues were dissected out from the lung tissue under microscopic observation, and smooth muscle cells were impaled with the microelectrode from the outer surface of the tissue so that the epithelial layer inside the lumen was intact.

A conventional microelectrode filled with 3 M KCl (30–50 M Ω) was used throughout the experiments. Field stimulation was applied to the nerves through a pair of Ag-AgCl wires 3–5 mm apart and placed so that a current pulse would pass transversely across the tissue. Single and repetitive stimuli at 20 Hz were applied, with a pulse of 30–100 μs duration and 30–50 V from an electronic stimulator (Nihon Kohden SEN-7103). The chamber in which the strips were mounted had a volume of 2 ml, and was superfused at a rate of 3 ml min $^{-1}$ at a temperature of 35–36°C. To avoid recording artifacts due to twitch-like contraction of the muscle tissue, the preparation was pinned on a rubber plate in the chamber with insect pins 0.1 mm in diameter.

To measure the mechanical responses of the bronchiole, the ring preparations (0.8–1.0 mm in diameter, and 1 mm in length) were hooked over a pair of right-angled fine needles which were reduced in diameter by electrolysis to about 0.05 mm under microscopic observation. One of the needles was fixed at the bottom of the chamber and the other was connected to a manipulator. The other end of the needle was connected to an isometric tension transducer. The fixed needle was also used for electrical stimulation of the ring preparations.

Materials

The following drugs were used; indomethacin, acetylcholine hydrochloride, carbachol, physostigmine, leukotrienes B $_4$, C $_4$, D $_4$, E $_4$, PAF (Sigma, St. Louis, Mo, U.S.A.), guanethidine

(Tokyo Kasei, Tokyo), tetrodotoxin (Sankyo, Tokyo), lyso-PAF (Bachem, California, U.S.A.), leukotriene synthesis inhibitor AA-861 (2,3,5-trimethyl-6-(12-hydroxy-5,1 O-dodecadiynyl)-1,4-benzoquinone; Takeda, Tokyo), leukotriene antagonist ONO 1078 (4-oxo-8-[p-(4-phenylbutyloxy)benzoylamino]-2-(tetrazol-5-yl)-4H-1-benzopyran hemihydrate; ONO, Osaka), PAF antagonists WEB2086 (3-(4-(2-chlorophenyl)-9-methyl-6H-thieno-(3,2,f)(1,2,4)-triazolo) 4, 3-a) (1,4)-diazepine-2-yl)-1-(4-morpholinyl)-1-propanone) (Boehringer Ingelheim), CV3988 ((*RS*)-2-methoxy-3-(octadecylcarbamoyloxy)propyl-2-(3-thiazolio)ethyl phosphate; Takeda, Tokyo), and E 6123 ((*S*)-(+)-6-(2-chlorophenyl)-cyclopropanecarbonyl-8, 11-dimethyl-2,3,4,5-tetrahydro-8H-pyrido-[4',3':4,5] thieno[3,2f] [1,2,4] triazolo[4,3,-a][1,4]diazepine; Eisai, Ibaraki), mepyramine and atropine sulphate (Daiichi, Tokyo), FPL 55712 (7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy] -4-oxo-8-propyl-4H-1-benzopyran-2-carboxylic acid; Fisons, Ltd., Loughborough, U.K.).

Statistical evaluation

Results (membrane potential, amplitude of contractions, relaxations or e.j.ps) are expressed as mean $\pm$ s.d. and were analyzed by Student's *t* test for paired (amplitude of contractions, relaxations or e.j.ps) or unpaired data (membrane potential) to estimate the significance of differences between means ($P < 0.05$ was judged to be significant). For the measurement of resting membrane potential, 10–30 cells were impaled before and after drug application from the same preparations, and the experiments were repeated using 4–5 preparations, and the mean values were calculated from the collected data (unpaired data). Microelectrode or double-sucrose gap recordings of e.j.ps were obtained from the same cell or the same tissue throughout the experiments before and after the drug application, and data obtained from 5–8 cells or 4–8 preparations were used for the statistical analysis (paired data).

Results

Effects of PAF on contraction of dog trachea and bronchioles evoked by electrical field stimulation

The effects of PAF on the contractions evoked by electrical field stimulation (EFS) of cholinergic nerve fibres were studied in dog epithelium-free tracheal and epithelium-intact bronchiolar tissues, in the presence of indomethacin (10^{-5} M) and guanethidine (10^{-6} M) (see Methods).

Figure 1 shows the effects of PAF (10^{-11} – 10^{-7} M) on the amplitude of contractions evoked by repetitive field stimulation (10 stimuli at 20 Hz) in the tracheal and bronchiolar tissues. PAF (10^{-11} – 10^{-7} M) had no effect on the resting tension but enhanced the amplitude of contractions in the tracheal tissue dose-dependently to 1.3–1.9 times the control value. In the bronchioles, PAF (10^{-11} – 10^{-7} M) also enhanced the amplitude of contractions in a dose-dependent manner to 1.3–2.7 times the control and in some preparations it induced a phasic increase in muscle tone. As the PAF-induced phasic contraction did not occur consistently, no quantitative analysis was performed. However, the PAF-induced phasic contraction was partially suppressed by atropine (10^{-6} M) or FPL 55712 (10^{-6} M).

Effects of PAF and lyso-PAF on the amplitude of the excitatory junction potential of trachea and bronchioles

To assess the mechanisms involved in the enhancing effects of PAF on twitch contractions, we observed its effects on e.j.p. amplitude with microelectrode and double sucrose gap methods.

In the trachea, low concentrations of PAF (10^{-11} or 5×10^{-10} M) significantly enhanced the e.j.p. amplitude to

Figure 1 Effects of PAF (10^{-11} – 10^{-7} M) on the contractions of dog trachea (○) and bronchiole (●) evoked by repetitive field stimulation (10 stimuli at 20 Hz). The amplitude of contractions evoked by 10 stimuli at 20 Hz in normal Krebs solution was taken as a relative amplitude of 1.0. Each point is the mean value of 8–12 experiments carried out with 7–10 preparations from 5 dogs and vertical bars indicate $2 \times$ s.d.

1.12 ± 0.08 ($n = 4$, $P < 0.05$), and 1.20 ± 0.02 ($n = 4$, $P < 0.01$) times the control value with no change in the resting membrane potential. In the bronchiole, on the other hand, relatively higher concentrations of PAF (5×10^{-9} M) were required to enhance the e.j.p. amplitude significantly to 1.21 ± 0.01 ($n = 4$, $P < 0.01$). The double sucrose gap method was also used to record e.j.ps and the following contractions, and confirmed that PAF (10^{-7} M) similarly enhanced the e.j.p. amplitude to 1.30 ± 0.20 ($n = 3$, $P < 0.001$) in the trachea with no change in the resting membrane potential and the input membrane resistance (data not shown).

Figure 2 summarizes the effects of PAF on the relative amplitude of the e.j.p. and the resting membrane potential of the smooth muscle cells in dog tracheal and bronchiolar tissues.

We also observed the effects of lyso-PAF on the amplitude of e.j.p. and resting membrane potential of the smooth muscle cells in both muscle tissues under the same condition. As shown in Figure 3, lyso-PAF (10^{-10} – 10^{-7} M) showed no effect on e.j.p. amplitude and resting membrane potential in dog bronchiolar tissue. Similarly in the trachea, lyso-PAF (10^{-10} – 10^{-8} M) did not affect the e.j.p. amplitude. However, at a higher concentration (10^{-7} M), it significantly enhanced the e.j.p. amplitude to 1.13 ± 0.06 ($n = 4$, $P < 0.05$) in the trachea, although the lyso-PAF-induced stimulation of e.j.p. amplitude was small compared to that of PAF.

Effects of PAF on acetylcholine or carbachol-induced depolarization

As relatively low concentrations of PAF ($< 5 \times 10^{-9}$ M) enhanced the e.j.p. amplitude and contractions evoked by EFS with no change in the membrane potential or input membrane resistance, it was of interest to observe the effects

Figure 2 Effects of PAF (10^{-11} – 10^{-7} M) on the amplitude of e.j.p. and resting membrane potential (RP) of smooth muscle cells of dog trachea (○) and bronchiole (●). The amplitude of e.j.p. evoked by a single field stimulation in normal Krebs solution was taken as a relative value 1.0. Each point is the mean value derived from 4–20 experiments using 4–8 preparations, and vertical bars indicate $2 \times$ s.d. The e.j.p. and resting membrane potential were recorded by microelectrodes.

Figure 3 Effects of lyso-PAF (10^{-10} – 10^{-7} M) on the amplitude of e.j.p. and resting membrane potential (RP) of smooth muscle cells of dog trachea (○) and bronchiole (●). The amplitude of e.j.p. evoked by a single field stimulation in normal Krebs solution was taken as a relative value 1.0. Each point is the mean value derived from 5–15 experiments using 5–8 preparations, and vertical bars indicate $2 \times$ s.d. The e.j.p. and resting membrane potential were recorded by microelectrode.

of PAF on the sensitivity of smooth muscle cells to ACh. For this purpose, the effect of PAF (10^{-7} M) on the membrane depolarization induced by various concentrations of carbachol or ACh in the presence or absence of physostigmine (10^{-6} M) was examined in dog trachea. The membrane potential of the tracheal smooth muscle ranged between -57 mV and -61 mV, with a mean value of -59.2 ± 1.4 mV ($n = 20$). ACh ($>10^{-9}$ M) depolarized the membrane dose-dependently, and the depolarization was enhanced in the presence of physostigmine (10^{-6} M), which produced a shift in the relationship between the dose of ACh and membrane potential to depolarized level (Figure 4). However, application of PAF did not affect the relationship between the membrane depolarization and dose of ACh in the presence or absence of physostigmine (data not shown). Similarly, carbachol ($>10^{-9}$ M) dose-dependently depolarized the membrane of the tracheal smooth muscle cells, and PAF showed no effect on the carbachol-induced membrane depolarization (Figure 4).

Effects of PAF antagonists and mepyramine on the amplitude of the e.j.p.

To study further the mechanisms involved in the enhancing effects of PAF on e.j.p. amplitude, we used the PAF antagonists, WEB 2086, CV3988 and E6123. At a concentration of 5×10^{-8} M, WEB 2086 did not affect the e.j.p. amplitude and resting membrane potential of the dog trachea, but at an increased concentration (5×10^{-7} M) this agent significantly enhanced the e.j.p. amplitude to 1.20 ± 0.10 ($n = 4$, $P < 0.01$) times the control with no change in the membrane potential. In the presence of WEB 2086 (5×10^{-7} M), PAF (5×10^{-8} M) further enhanced the e.j.p. amp-

litude to 1.51 ± 0.06 ($n = 4$, $P < 0.01$), indicating that WEB 2086 did not antagonize the action of PAF. PAF-antagonist CV3988 ($>5 \times 10^{-8}$ M) also enhanced the e.j.p. amplitude dose-dependently. After the pretreatment of the tissue with CV3988 (5×10^{-8} M), PAF (5×10^{-8} M) showed additional potentiating effects on e.j.p. amplitude. However, in the presence of an increased concentration of CV3988 (5×10^{-7} M), PAF (5×10^{-8} M) did not enhance the e.j.p. amplitude (Figure 5). However, it may be that the failure of PAF to enhance the e.j.p. amplitude in the presence of 5×10^{-7} M CV3988 is due to the drug having elicited the maximum possible stimulation of the e.j.p. itself. Therefore we used a new PAF antagonist, E6123, a member of the benzodiazepine class of PAF antagonists (Tsunoda *et al.*, 1990). E6123 (5×10^{-8} M) did not affect the e.j.p. amplitude and resting membrane potential of dog trachea, but completely suppressed the PAF (5×10^{-8} M)-induced stimulation of e.j.p. amplitude (Figure 5).

Similar experiments were repeated with mepyramine, since it is known that PAF stimulates the release of histamine from mast cells in the airway (see for example, Townley *et al.*, 1989) and it is known that released histamine stimulates the ACh release from the vagus nerve terminal through H_1 -receptors (Inoue & Ito, 1986). However, in the presence of the H_1 -receptor antagonist, mepyramine, PAF significantly enhanced the e.j.p. amplitude (Figure 5).

Effects of lipoxigenase inhibitor AA-861, leukotriene antagonist ONO1078 and leukotrienes on the amplitude of e.j.p. of the dog trachea

It is also known that PAF stimulates the lipoxigenase pathway to produce leukotrienes in the perfused lung (Jancar *et al.*, 1989). Therefore we also observed the effects of lipoxigenase inhibitor AA-861 and leukotriene antagonist ONO-1078 (Yamai *et al.*, 1989) on the action of PAF on e.j.p. amplitude. AA-861 (10^{-6} M) or ONO1078 (10^{-7} M) did not affect the e.j.p. amplitude and resting membrane potential of dog trachea themselves, but completely inhibited the PAF-induced stimulation of e.j.p. amplitude (Figure 5).

It was of interest to observe the effects of exogenously applied leukotrienes on the amplitude of e.j.p. of the dog trachea, since AA-861 and ONO1078 suppressed the enhancing effects of PAF on e.j.p. amplitude.

In the presence of AA-861 (10^{-6} M), LTB_4 (10^{-8} M) or LTD_4 (10^{-8} M) slightly, and LTC_4 (10^{-8} M) markedly enhanced the e.j.p. amplitude. In contrast, LTE_4 (10^{-8} M) significantly suppressed the e.j.p. amplitude. Figure 6 summarizes the effects of leukotrienes on the e.j.p. amplitude of dog trachea in the presence of AA-861 (10^{-6} M).

Effects of PAF on the depression phenomenon of e.j.p.

When conditioning and test EFS were applied in tandem at intervals of 5–60 s, the amplitude of the test e.j.p. was always smaller than the conditioning e.j.p., showing a marked depression phenomenon of e.j.ps (Ito & Tajima, 1981). Figure 7 shows the mean amplitude of the second e.j.p. relative to the first e.j.p. PAF (5×10^{-8} M) reduced the depression phenomenon within the time intervals of 5–10 s.

Effects of PAF on the e.j.p. evoked by repetitive field stimulation

When repetitive field stimulation at high frequency (20 Hz) was applied, e.j.ps showed summation in dog trachea (Ito & Tajima, 1981; Ito & Yoshitomi, 1988; Hakoda & Ito, 1990).

Figure 8 shows the effects of PAF on the relationship between the relative amplitude of e.j.p. and number of stimuli. PAF had no effect on the relationship.

Figure 4 Effects of PAF (10^{-7} M) on the membrane depolarization induced by ACh (a) and carbachol (b) in the dog tracheal smooth muscle cells. In (a): ($\blacktriangle$) ACh + physostigmine (10^{-6} M) + PAF (10^{-7} M); ($\bullet$) ACh + physostigmine (10^{-6} M); ($\circ$) control. In (b): ($\bullet$) carbachol + PAF (10^{-7} M); ($\circ$) control. Each point is the mean value of 15–30 experiments from 4–6 preparations. Bars indicate $2 \times$ s.d.

Figure 5 Effects of PAF (5×10^{-8} M), PAF-antagonists (WEB 2086, CV 3988 and E 6123), mepyramine, leukotriene synthesis inhibitor AA-861 (10^{-6} M) and leukotriene antagonist ONO 1078 (10^{-7} M) on the e.j.p. amplitude of dog trachea. The amplitude of e.j.p. evoked by a single field stimulation before application of drugs is defined as a relative amplitude of 1.0. Each value indicates mean of 4–8 experiments with 4–6 preparations and bars are s.d. All concentrations are molar.

Figure 6 Effects of AA-861 (10^{-6} M) and various leukotrienes (LTB₄, C₄, D₄ and E₄, 10^{-8} M each) on the amplitude of e.j.p. recorded with microelectrodes in the dog trachea. Each column is the mean value of 4–7 experiments with 4–5 preparations and horizontal bars indicate $2 \times$ s.d. All concentrations are molar.

Figure 7 The effects of PAF (5×10^{-8} M) on relative changes in the amplitude of the test e.j.p. (the second e.j.p.) following the conditioning e.j.p. (the first e.j.p.) measured at various time intervals (5–60 s): (●) PAF 5×10^{-8} M; (○) control. Each point is the mean value obtained from 5–7 experiments with 5–7 preparations and vertical bars indicate s.d. or $2 \times$ s.d.

Figure 8 Effects of PAF (5×10^{-8} M) on the relationship between e.j.p. amplitude and the number of stimuli at 20 Hz: (○) control. (●) PAF (5×10^{-8} M). Each point is the mean value of 4–6 experiments with 4–6 preparations, vertical bars show s.d.

Discussion

The present study clearly shows an enhancing effect of PAF on the excitatory neuro-effector transmission in the dog trachea and bronchioles *in vitro*, presumably by increasing ACh release from the vagus nerve, since low concentrations of PAF (10^{-11} – 10^{-9} M) significantly increased the amplitude of e.j.p. and contractions evoked by nerve stimulation with no change in resting membrane potential, input membrane resistance or ACh-sensitivity of the smooth muscle cells. The enhancing action of PAF on neuro-effector transmission in the airway seems due to specific action of PAF, since lyso-PAF showed practically no effect on e.j.p. amplitude in both tissues and PAF antagonist E6123 completely suppressed the PAF-induced stimulation of e.j.p. amplitude. Furthermore, it seems that effects of PAF on release of ACh from the vagus

nerve terminal are not due to a direct action of PAF itself but through stimulation of lipoxygenase to produce leukotrienes in the tracheal and bronchiolar tissues. This view is mainly dependent on the observation that in the presence of the lipoxygenase inhibitor AA-861 or the leukotriene antagonist, ONO1078, PAF showed no effect on the e.j.p. amplitude.

Intravenous injections of PAF into the systemic circulation of living animals induces effects similar to an 'anaphylactic reaction' such as strong bronchoconstriction, acute inflammation and oedema, platelet aggregation and degranulation, and chemotaxis of neutrophils and eosinophils (Vargaftig *et al.*, 1981a,b; Page *et al.*, 1984). PAF-induced bronchoconstriction observed *in vivo* does not seem to be induced by a direct action of PAF on the bronchus, since the effects of PAF were less potent when the isolated lungs were perfused with cell-free solutions, or when isolated airway smooth muscle tissues were used in the experiments (Townley *et al.*, 1989). Thus the effects of PAF in lungs appear to depend on the method of application, which may reflect activation of different cell types.

Initially it was thought that platelets are essential for PAF to induce bronchoconstriction in various animal species, and in the dog it is largely dependent on secretion of 5-hydroxytryptamine released from platelets (Popovich *et al.*, 1988). However, it was also shown that in guinea-pig isolated lungs perfused with cell-free solutions, the injection of PAF induced a bronchoconstriction which was platelet-independent and blocked by cyclo-oxygenase inhibitors (Lefort *et al.*, 1984). Confirming this observation, there is a report indicating that the airway hyperresponsiveness induced by PAF may be dependent on thromboxane generation, since OKY-046, a thromboxane synthase inhibitor, suppressed PAF-induced bronchoconstriction and hyperresponsiveness in the dog (Chung *et al.*, 1986). PAF actually induces release of thromboxane A_2 (TXA $_2$) which preceded the increase in airway and pulmonary pressures (Lefort *et al.*, 1984), and inhalation of PAF increased the amount of a major urinary TXA $_2$ metabolite, 2,3-dinor-TXB $_2$ (Taylor *et al.*, 1991).

On the other hand, recent investigations indicate that when PAF was injected into isolated, perfused lungs, it stimulated the release of substance(s) causing contraction of bronchus, trachea and parenchymal strips which is unrelated to cyclo-oxygenase products (Jancar *et al.*, 1987; 1989). The authors concluded that a lipoxygenase product, possibly LTB $_4$ may be responsible for spasmogenic activity released by lungs following PAF stimulation, and that cyclo-oxygenase products subsequently released appear to result from the initial generation of LTB $_4$.

PAF causes vasoconstriction and oedema in non-sensitized rat lungs, possibly through the production of LTC $_4$ and D $_4$ (Voelkel *et al.*, 1982). As the present experiments were performed in the presence of indomethacin, it is possible to exclude the role of cyclo-oxygenase products on the enhancing actions of PAF on the excitatory neuro-effector transmission. Thus it is reasonable to assume that PAF stimulates 5-lipoxygenase to produce leukotrienes in the airway smooth muscle tissues, and in turn these leukotrienes enhance the excitatory neuro-effector transmission.

PAF and arachidonic acid may be released from a common precursor, 1-alkyl-2-acyl-glycero-3-phosphocholine, in various cells (Snyder, 1985). Thus it seems probable that PAF might act as a potential regulator of arachidonic acid metabolism and *vice versa*. Although the PAF-antagonist CV 3988 possesses weak agonistic activity at high concentrations, it is likely to stimulate the endogenous generation of leukotrienes (Chung *et al.*, 1986; Braquet *et al.*, 1987; Taylor *et al.*, 1991). These findings could be explained by effects of CV 3988 on the e.j.p. amplitude in the absence and presence of PAF. In addition, in the case of PAF, inhibition of leukotriene synthesis by AA-861 produced similar results on the e.j.p. amplitude as with the PAF-antagonist, CV 3988, in our experiments, i.e. suppressing the PAF actions on the e.j.p.

amplitude. Furthermore, in the presence of leukotriene antagonist, ONO1078, which is a specific LTC₄ and LTD₄ antagonist (Yamai *et al.*, 1989) PAF did not show any effect on e.j.p. amplitude. These data further reinforce the explanation for the role of leukotrienes in the actions of PAF on excitatory neuro-effector transmission.

WEB 2086 did not antagonize the stimulating action of PAF on excitatory neuro-effector transmission, whereas CV 3988 in relatively high concentrations only partially suppressed the action of PAF. However, PAF-induced stimulation of e.j.p. amplitude was completely inhibited by E 6123, a new member of the benzodiazepine class of PAF antagonists (Tsunoda *et al.*, 1990). Recently, it was reported that among various PAF antagonists E 6123 potently suppresses PAF inhalation-induced bronchoconstriction in guinea-pigs with an ED₅₀ value of 1.3 µg kg⁻¹ which was lower than that of other PAF-antagonists such as WEB 2347 (ED₅₀ = 26 µg kg⁻¹) and Y-24180 (ED₅₀ = 12 µg kg⁻¹) (Sakuma *et al.*, 1991). Thus, the stimulating action of PAF on excitatory neuro-effector transmission is due to a specific action of PAF through PAF-receptors.

In the present experiments, there was a tendency for the enhancing effects of PAF on the amplitude of e.j.p. and contractions evoked by EFS to be more pronounced in the bronchioles. As far as tracheal muscle was concerned, the mucosa was removed entirely to leave just smooth muscle tissue. In contrast, bronchioles were carefully excised from the lung tissue under microscopic observation and smooth muscle cells were impaled with a microelectrode from the

adventitial surface of the tissue leaving the inner lumen intact. Thus, the experiments were performed with epithelium-free tracheal tissue and epithelium-intact bronchiolar tissues. It has been reported that the airway epithelium may contain surface receptors for PAF (Adler & Henke, 1991), and there is evidence that PAF stimulates the airway epithelium to produce cyclo-oxygenase products. Recent investigations also indicate that factor(s) released from epithelial cells in the airway control excitatory neuro-effector transmission possibly through lipoxygenase products of arachidonic acid (Xie *et al.*, 1992). Furthermore, LTC₄ applied exogenously in relatively low concentration enhanced the amplitude of e.j.p. and contractions evoked by EFS in the dog bronchiole. The present experiments indicate that PAF stimulates lipoxygenase in the airway smooth muscle cells and epithelial cells to produce leukotrienes and these modify excitatory neuro-effector transmission in the tracheal and bronchiolar tissues. PAF causes both acute bronchoconstriction (Leef *et al.*, 1987; Smith *et al.*, 1988) and persistent airway hyperresponsiveness of the airway (Chung *et al.*, 1986; Christman *et al.*, 1987). Thus, it is reasonable to assume that leukotrienes released from airway smooth muscle or epithelial cells in response to application of PAF contribute to both acute bronchoconstriction and hyperresponsiveness of the airway.

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