J. Pharm. Pharmacol. 1992, 44: 344–348
Received May 20, 1991

Effects of Platelet Activating Factor on Contractions and ^{45}Ca Influx Induced by Noradrenaline and Potassium in Rat Rubbed and Intact Aorta. Comparison with Its Hypotensive Effect in Anaesthetized Normotensive Rats

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Abstract—In order to clarify the mechanism of hypotensive activity of platelet activating factor (PAF), the effects of this drug on blood pressure in anaesthetized normotensive rats, on KCl- and noradrenaline-induced ^{45}Ca uptake and contractile responses in rat aorta rings with and without endothelium were studied. PAF (3 $\mu g\ kg^{-1}$, i.v.) showed long-lasting hypotensive effects in anaesthetized normotensive rats accompanied by a significant increase in heart rate. PAF (0·1–10 μM) did not relax the contractions induced by noradrenaline (10 μM) or K^+ (60 mM) in rubbed or intact rat aorta. PAF did not affect the basal uptake of $^{45}Ca^{2+}$ nor that induced by the two vasoconstrictor agents. In experiments in a calcium free medium, PAF (10 μM) had no effect on the noradrenaline- (10 μM) induced contractions. These results suggest that the hypotensive activity of PAF in normotensive anaesthetized rats is not due to a direct effect on rubbed and intact rat aorta rings (acting within the cell or blocking Ca^{2+} influx through L-type transmembrane calcium channels).

Hypotension is a characteristic result of the action of platelet activating factor (PAF) on the circulatory system (Muirhead et al 1981; Bessin et al 1983). However, the mechanism of this hypotensive activity is not fully clarified. Two main causes for hypotension have been proposed: vasodilation in various organs (Lai et al 1983; Kenzora et al 1984; Chu et al 1988) and reduction of cardiac output (Benveniste et al 1983; Kenzora et al 1984; Levi et al 1984; Sybertz et al 1985).

The effects of PAF on vascular smooth muscle has been reported to differ, depending on the preparation, from constriction (Benveniste et al 1983) to dilation (Lai et al 1983; Sybertz et al 1985; Chu et al 1988; Langente et al 1988).

Since the initial work of Furchgott & Zawadski (1980), the potential importance of the endothelial system in the modulation of the effects of several drugs on the vascular smooth muscle tone has been shown (Alosachie & Godfraind 1988; Vanhoutte 1989). However, the involvement of endothelial cells in the vasodilation produced by PAF is not clear. Kamitani et al (1984), Kasuya et al (1984) and Chiba et al (1990) reported that the integrity of the endothelial system is essential for the vasorelaxant action of PAF, whereas Lefer & Lefer (1986) demonstrated that endothelial cells are not implicated in this action.

In view of those conflicting reports, in the present work the effects of PAF on blood pressure of anaesthetized normotensive rats, on tension responses to KCl (K^+) and noradrenaline, and on ^{45}Ca uptake in rat aorta with and without endothelium have been investigated.

A preliminary account of this study has appeared elsewhere (Orallo et al 1991).

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Materials and Methods

Blood pressure measurements in anaesthetized normotensive rats

Normotensive male Sprague-Dawley rats, 250–300 g, were anaesthetized with urethane (1·26 g kg^{-1}, i.p.) and kept warm (36·5–37·5°C) with an overhead lamp. Cannules were inserted in the trachea to facilitate spontaneous respiration and in a common carotid artery for blood pressure measurement. Systolic and diastolic arterial pressure were monitored by means of a TRA 021 Letica pressure transducer on a Letica Unigraph 1000-506 device. Heart rate was obtained from the arterial pulse wave on a digital counter (Panlab 0602) connected to the polygraph output.

The preparation was allowed to equilibrate for at least 30 min before drug administration. After blood pressure and heart rate stabilization, 0·1 mL of PAF solution (3 $\mu g\ mL^{-1}$)/100 g body weight was injected intravenously via the right femoral vein, in order to observe the effects on blood pressure and heart rate.

Contraction studies

Male Sprague-Dawley rats, 250–300 g, were killed by a blow on the head. The thoracic aorta was rapidly removed, stripped of endothelium (in some experiments) by rubbing the intimal surface with a cotton bud, cut in cylindrical segments 4 mm in length and immediately transferred to an organ bath containing 20 mL of Krebs solution of the following composition (mM): NaCl 119, KCl 4·7, $CaCl_2 \cdot 2H_2O$ 2·5, KH_2PO_4 1·2, $MgSO_4 \cdot 7H_2O$ 1·2, $NaHCO_3$ 25, glucose 11, ascorbic acid 0·6 and disodium salt of ethylenediaminetetraacetic acid (EDTA) 0·03. Calcium-free solution was prepared by omission of calcium when required. The

solution was thermoregulated at 37°C and bubbled with 95% O_2–5% CO_2.

The absence of acetylcholine vasorelaxant action in precontracted rings and a simple haematoxylin-eosin staining technique were used to verify the removal of endothelial cells and the integrity of underlying smooth muscle.

Two stainless steel pins were introduced through the lumen of each arterial segment: one pin was fixed to the organ baths and the other was connected to a CPOL force-displacement transducer for isometric tension recording by a computerized Celaster IOS 1 system.

After an equilibration period of at least 1 h under 2 g resting tension, isometric contractions induced by noradrenaline (10 μM) or K^+ (60 mM) (without keeping the osmolarity constant) were recorded for 15 min. Cumulative doses of PAF were then added, and the effect of each one observed for 10 min.

To obtain contractions in a calcium-free medium, artery preparations were equilibrated for 60 min in normal Krebs solution and then washed three times for 20 min with a calcium-free solution (containing 0·5 mM EGTA) before a noradrenaline (10 μM) contraction was elicited. To study the effects of PAF, the preparations were further washed in Krebs solution for 60 min (to fill the Ca^{2+} stores depleted by the first contraction). There was a further 20 min preincubation in calcium-free solution before a suitable concentration of PAF was added, followed 10 min later by noradrenaline (10 μM). Other tissues were subjected to the same procedure simultaneously, but with the omission of PAF.

^{45}Ca influx

Aorta rings weighing 5–9 mg were equilibrated for at least 60 min in physiological solution (composition (mM): NaCl 139, KCl 5, $MgCl_2$ 1, $CaCl_2 \cdot 2H_2O$ 1·5, Hepes, 5, glucose 10) maintained at 37°C and aerated with 100% O_2. Afterwards, the tissues were incubated for 5 min in a ^{45}Ca- (New England Nuclear, sp. act. 35 mCi mg^{-1}) containing medium (0·6 μCi mL^{-1}) with or without noradrenaline (10 μM) or K^+ (60 mM) to analyse the effect of these vasoconstrictor agents on ^{45}Ca uptake. To investigate the actions of PAF on this uptake, PAF was added to the bath 20 min before and during the incubation period with ^{45}Ca. Thereafter, the preparations were washed for 5 min in 500 mL of La^{3+} solution, composition (mM): NaCl 118, KCl 5·9, Tris-hydroxymethylaminomethane 5·4, $MgSO_4 \cdot 7\ H_2O$ 1·2, $LaCl_3 \cdot 7\ H_2O$ 50, glucose 11; pH = 6.8.

Afterwards the arteries were blotted, weighed and digested in 1 mL H_2O_2 (110 volumes) at 115°C for 90 min. After cooling, 5 mL of Ready-Safe Beckman were added and the radioactivity of the samples counted in a liquid scintillation counter (Beckman LS 3801).

Data presentation and statistical analysis

Unless otherwise specified, the results presented are mean ± s.e.m. The statistical significance of differences between two means ($P < 0.05$) was estimated by Student's two-tailed t-test for paired or unpaired data.

The ^{45}Ca tissue uptake was calculated as follows:

^{45}Ca uptake (nmol ^{45}Ca (kg wet tissue)$^{-1}$) = counts min^{-1} in tissue/wet tissue (kg) × nmol ^{45}Ca in 1 L solution/counts min^{-1} in 1 L solution.

Note that the numerator of the second factor in this expression is the concentration of ^{45}Ca, not the total Ca^{2+} concentration.

Drugs and chemicals

The following drugs were used: PAF (Sigma), (−)-noradrenaline bitartrate (Sigma) and urethane (Merck).

PAF was dissolved in 95% methanol to make a stock solution of 2 mg mL^{-1} and aliquots of this solution were then diluted with 0·9% NaCl (saline) (for i.v. bolus administration) or deionized water.

Noradrenaline was prepared daily with deionized water from a 100 mM stock solution kept at −20°C and containing 0·2% sodium bisulphite to prevent oxidation.

Urethane was dissolved in saline to make a solution of 25 g in 100 mL. This solution was kept at 4°C.

The chemicals used for the preparation of the physiological solutions were of analytical grade.

Results

Hypotensive effects of PAF in anaesthetized normotensive rats

PAF (3 μg kg^{-1}, i.v.) produced a pronounced and prolonged fall in mean blood pressure, accompanied by a significant increase in heart rate (Fig. 1). The maximal percentage of mean arterial pressure reduction was 57·62 ± 5·01% (n = 5). The hypotensive action was very rapid and the maximum effect was reached approximately after 8 min (Fig. 1).

Effects of PAF on contractions induced by noradrenaline and high potassium

Noradrenaline (10 μM) produced a sustained contraction in rat aorta rings with and without endothelium, reaching

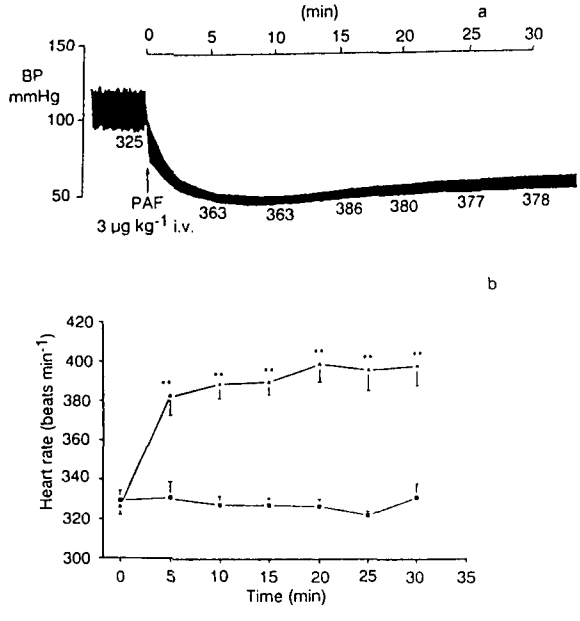

FIG. 1. Modifications of blood pressure and heart rate in anaesthetized normotensive rats after treatment with PAF (3 μg kg^{-1}, i.v.). a: The cardiovascular effects of PAF. Numbers along the x-axis represents heart rate values at single points. b: Effects of PAF (▲) on the heart rate. Each point is the mean of five experiments. Vertical bars indicate s.e.m. $^{**}P < 0.01$ compared with control values (■).

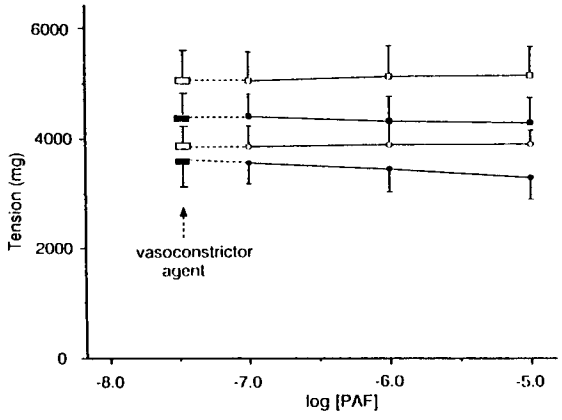

FIG. 2. Effects of PAF on noradrenaline- (10 μM; □, ■) and potassium-induced (60 mM; ○, ●) contractions in rat aorta rings with (■, ●) and without (□, ○) endothelium. Each point represents the mean ± s.e.m. from five experiments.

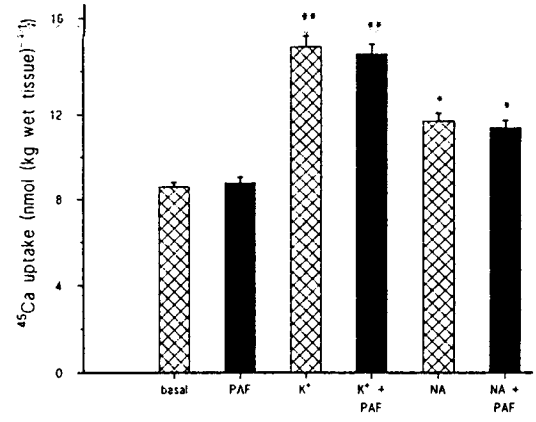

FIG. 4. Effect of PAF on ^{45}Ca uptake induced by noradrenaline (NA, 10 μM) and potassium (K^+, 60 mM) in rat aorta rings with endothelium. Error bars on the columns show s.e.m. of five experiments. $*P<0.05$, $**P<0.01$ with respect to the basal uptake. Similar results were obtained on rat rubbed aorta rings.

4397 ± 409 and 5059 ± 518 mg, respectively (n = 5). High potassium (60 mM) caused a tonic contraction in denuded and intact preparations, reaching 3670 ± 372 and 3586 ± 381 mg, respectively (n = 5). Mechanical removal of endothelium did not modify significantly the maximal tension induced by both vasoconstrictor agents.

PAF (0·1–10 μM) did not relax the contractions induced by noradrenaline (10 μM) or high K^+ (60 mM) in rat rubbed or intact aorta rings (Fig. 2).

Effects of PAF on contractions induced by noradrenaline in calcium-free medium

In rat rubbed or intact aorta rings, noradrenaline (10 μM) produced a characteristic contraction with two distinct components: an initial transient contraction (fast component) (tension = 1067 ± 53 mg with endothelium and 1005 ± 74 mg without endothelium, n = 5) that relaxed to sustained tensions of 244 ± 23 and 316 ± 36 mg, respectively (slow component). These contractions were unaffected by the addition of PAF (10 μM) (fast component tension = 913 ± 74 and 918 ± 145 mg; slow component tension = 181 ± 18 and 280 ± 48 mg, n = 5, $P>0.05$) (Fig. 3).

The differences between contractions obtained in rubbed and intact aorta rings were not significant ($P>0.05$).

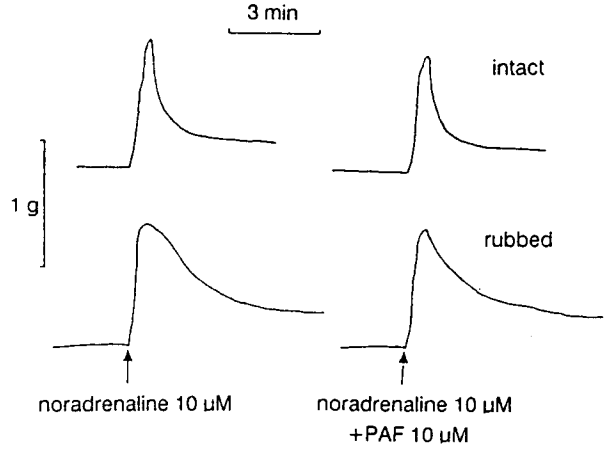

FIG. 3. Effect of PAF (10 μM) on noradrenaline-induced contractions in calcium-free medium in rat rubbed and intact aorta rings. Data are plotted as the mean from five experiments.

Effect of PAF on ^{45}Ca uptake

The calcium uptake by the segments of rat aorta in the absence of other agents (basal uptake) was 8·6 ± 0·22 nmol kg^{-1} (n = 5) in preparations with endothelium and 9·01 ± 0·32 nmol kg^{-1} (n = 5) in preparations without endothelium ($P>0.05$). The addition of PAF (1 μM) did not affect these values significantly (^{45}Ca tissue content: 8·8 ± 0·26 and 8·9 ± 0·37 nmol kg^{-1}, respectively, n = 5, $P>0.05$).

The vasoconstrictor agents, noradrenaline and high K^+, significantly increased the basal ^{45}Ca uptake in denuded and intact aorta rings (^{45}Ca tissue content: 11·7 ± 0·37 nmol kg^{-1} with endothelium and 12·6 ± 0·41 nmol kg^{-1} without endothelium (noradrenaline), n = 5, $P<0.05$; 15·1 ± 0·48 nmol kg^{-1} with endothelium and 16·5 ± 0·58 nmol kg^{-1} without endothelium (K^+), n = 5, $P<0.01$). The absence of the endothelial system did not affect significantly the ^{45}Ca influx elicited by noradrenaline or K^+.

PAF (1 μM) had no significant inhibiting action on noradrenaline or high K^+-induced ^{45}Ca uptake (tissue content of ^{45}Ca: 11·4 ± 0·33 (intact) and 12·8 ± 0·29 nmol kg^{-1} (rubbed) (noradrenaline); 14·8 ± 0·43 (intact) and 16·1 ± 0·47 nmol kg^{-1} (rubbed) (K^+), n = 5, $P>0.05$) (Fig. 4).

Discussion

In the present study, PAF showed long-lasting hypotensive activity in anaesthetized normotensive rats, which is in accordance with the results obtained by Prop et al (1981), Sánchez-Crespo et al (1982), Lai et al (1983) and Tanaka et al (1983).

It has been reported that high K^+ concentrations cause marked contractions in rat aorta tissue by depolarizing smooth muscle cells and increasing the influx of calcium

through L voltage-dependent channels (Spedding 1987; Bolton et al 1988; Karaki & Weiss 1988; Godfraind & Govoni 1989). It has also been shown that activation of α_1-adrenergic receptors by noradrenaline in rat aorta induces a two-phase contraction: an initial transient contraction, caused by the inositol tri-phosphate-mediated release of calcium from the intracellular stores, and a slow, sustained contraction, due to Ca^{2+} influx through the receptor-operated Ca^{2+} channels (Putney 1986; Karaki & Weiss 1988; Van Breemen & Saida 1989; Zelis & Moore 1989).

The results presented in this study show that PAF does not relax the contractions induced by noradrenaline or K^+ in rat rubbed aorta (Kasuya et al 1984), which indicate that PAF does not act intracellularly or by blocking calcium influx through voltage-dependent and receptor-operated channels and that its in-vivo hypotensive action is not due to an in-vitro relaxant effect on aorta tissue. However, it should be emphasized that this hypotensive effect may be correlated with the potential vasorelaxant action of PAF on resistance vessels since this drug dilates mesenteric vascular bed of the rat (Langente et al 1988; Chiba et al 1990) and dog (Chu et al 1988).

The presence of endothelium does not modify the contractile effect induced by noradrenaline and high potassium, in agreement with previous studies (noradrenaline in rabbit aorta (Laher et al 1986); K^+ and noradrenaline in basilar arteries and rat aorta (Lai et al 1989)), and contrary to results obtained for phenylephrine by Malta et al (1986) and for noradrenaline by Martin et al (1986) in rat aorta. Possibly, the different Ca^{2+} concentrations of the physiological solutions (López-Jaramillo et al 1990) and the different techniques used for endothelium removal explain the different results reported in the same vascular tissue. Our results are supported by the following. The release of relaxant factors (e.g. EDRF) from endothelial cells is a calcium-dependent process but it is not modulated by calcium influx induced by high K^+, since voltage-dependent calcium channels are not present in those cells (for review see Angus & Cocks 1989; Ignarro 1989). In contrast to results obtained by Eglème et al (1984) and Carrier & White (1985), the release of EDRF does not seem to be regulated by noradrenaline, since to date no clear evidence for the presence of α_2-adrenoceptors on the rat vascular endothelium has been presented and several workers have concluded that such receptors do not exist (Dashwood & Jacobs 1985; Godfraind et al 1985; Martin et al 1986).

However, our data are not supported by the fact that the spontaneous release of EDRF can inhibit the contractions induced by several agonists in rat aorta (Bullock et al 1986).

On the other hand, in agreement with the results obtained by Lefer & Lefer (1986) in rabbit aorta, our results demonstrate that the presence of the endothelial system does not contribute to the PAF vasorelaxant action, suggesting that the hypotensive activity of PAF in anaesthetized normotensive rats is not due to a direct effect on the endothelial system, increasing endogenous relaxant factors release from endothelial cells. However, our data are in contrast to the results reported by Kamitani et al (1984) and Kasuya et al (1984) in rat aorta and Chiba et al (1990) in rat mesenteric artery, possibly due to the fact that these investigators used experimental conditions different from ours.

The lack of effect of PAF on calcium channels is shown by the experiments involving ^{45}Ca. In correlation with the contractility experiments, in the present work the basal and ^{45}Ca uptake induced by noradrenaline and K^+ was similar in rat rubbed and intact aorta rings, in agreement with previous studies in rabbit and rat aorta (Collins et al 1988) and in contrast to the results obtained for basal ^{45}Ca influx by Malta et al (1986) in rat aorta.

Basal, noradrenaline- and K^+-induced ^{45}Ca uptake is unchanged by the addition of PAF, which suggests that PAF does not block the transmembrane calcium movements through leakage, voltage-dependent and receptor-operated calcium channels in rat denuded or intact aorta.

The lack of effect to PAF within the cell is confirmed from the results with calcium-free medium. Addition of noradrenaline in the absence of external Ca^{2+} induces a fast and transient contraction, attributed to release of intracellular Ca^{2+} stores followed by a smaller slow and sustained contraction, that is thought possibly to involve the breakdown of phosphoinositide to diacylglycerol, activation of protein kinase C by the latter and induction of contraction in the presence of a low concentration of Ca^{2+} (Nishizuka 1984). In this work, the results in a calcium-free medium show two main findings consistent with the results obtained in physiological solution; mechanical removal of endothelium does not modify the contractile effect induced by noradrenaline in rat aorta, possibly due to the absence of α_2-adrenoceptors on the rat vascular endothelium (see above) and the spontaneous release of EDRF is a calcium-dependent process (Angus & Cocks 1989; Ignarro 1989). PAF does not act within the cell since it does not inhibit noradrenaline-induced contractions in a calcium-free medium in preparations with or without endothelium.

In conclusion, our results confirm the hypotensive activity of PAF in anaesthetized normotensive rats. This activity is not due to a direct vasorelaxant effect of the drug on rat rubbed and intact aorta rings.

Acknowledgements

This work was supported by grants from the Xunta de Galicia, Consellería de Educación (Spain).

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