Effects of platelet activating factor on contractile force and ^{45}Ca fluxes in guinea-pig isolated atria

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- 1 The effects of platelet activating factor (PAF) were studied on the electromechanical properties and $^{45}\text{Ca}^{2+}$ fluxes of guinea-pig isolated atria.
- 2 Both in spontaneously beating and electrically driven atria, PAF (10^{-12} – 10^{-7} M) increased atrial rate but produced a biphasic effect on contractile force. At low concentrations (up to 10^{-10} M) it produced a positive inotropic effect, while at higher concentrations PAF exerted a negative inotropic effect. A similar biphasic effect was observed in the slow contractions elicited by isoprenaline in K^{+} -depolarized atrial fibres.
- 3 The positive inotropic effect of PAF was prevented by verapamil, whereas pretreatment of atria with propranolol, phentolamine, indomethacin or atropine did not modify its positive and negative inotropic actions. BN 52021, a specific PAF antagonist, abolished both the positive and negative inotropic effects.
- 4 PAF had no effect on the characteristics of the action potentials recorded in either normally polarized or K^{+} -depolarized (slow action potential) atrial fibres.
- 5 At concentrations at which it increased contractile force, PAF potentiated the contractile responses to Ca^{2+} (0.9–9 mM), whereas at negative inotropic concentrations it inhibited them. The negative inotropic effect of PAF was partially reversed in 70% Na^{+} medium.
- 6 At 10^{-11} M, PAF increased $^{45}\text{Ca}^{2+}$ uptake and reduced the rate coefficient (k_{cm}) for the $^{45}\text{Ca}^{2+}$ efflux. This increase in $^{45}\text{Ca}^{2+}$ uptake was abolished in atria pretreated with verapamil or BN 52021. However, 10^{-7} M PAF modified neither $^{45}\text{Ca}^{2+}$ uptake nor efflux in atrial muscle.
- 7 These results suggest that in guinea-pig atria the biphasic inotropic effects of PAF cannot be explained through modifications in the slow inward Ca^{2+} current or in Na^{+} – Ca^{2+} exchange, but may be related to changes in trans-sarcolemmal Ca^{2+} entry mediated by specific PAF receptors.

Introduction

Platelet-activating factor [PAF, 1-0-hexadecyl-2-0-acetyl-sn-glycyl-3-phosphorylcholine] is a polar phospholipid which exhibits a wide range of biological activities (Braquet *et al.*, 1987). In anaesthetized animals, PAF produced hypotension, transient apnoea and increased vascular permeability (Halonen *et al.*, 1980; Sánchez-Crespo *et al.*, 1982; Bessin *et al.*, 1983). In guinea-pig isolated perfused hearts, PAF reduced coronary blood flow and contractile force and produced conduction arrhythmias (Benveniste *et al.*, 1983; Levi *et al.*, 1984; González-Morales & Tamargo, 1988) and thus, it was proposed that PAF may play a major role in cardiac abnormalities occurring during cardiac anaphylaxis (Levi *et al.*, 1984). However, discordant results on contractile force have been reported. Thus, while some authors have described a concentration-dependent negative inotropic effect on guinea-pig isolated atria and papillary muscles (Camussi *et al.*, 1984; Levi *et al.*, 1984; Alloatti *et al.*, 1987; Robertson *et al.*, 1988) and human papillary muscles (Alloatti *et al.*, 1986), others have described a biphasic effect of PAF, a positive inotropic effect being observed at low concentrations, whereas high concentrations produced a negative one (Tamargo *et al.*, 1985; Nayaka & Tohse, 1986). The mechanism(s) of the inotropic effects of PAF are unknown at present, but it is unlikely that they are secondary to formed blood elements or changes in coronary blood flow, since they have also been observed in noncoronary-perfused isolated atria and papillary muscles. Previous experimental evidence attributed the negative inotropic effect to a decrease in the slow inward Ca^{2+} current (Camussi *et al.*, 1984; González-Morales & Tamargo, 1988) and/or to a decrease in intracellular Na^{+} activity mediated by the Na^{+} – Ca^{2+} exchanger (Robertson *et al.*, 1988), whereas the positive inotropic effect was attributed to an increase in Ca^{2+} influx via the slow inward Ca^{2+} current (Tamargo *et al.*, 1985). In fact, PAF has been found to increase $^{45}\text{Ca}^{2+}$ influx in mouse macrophages (Braquet *et al.*, 1987), human (Valone

& Johnson, 1985) and rabbit platelets (Lee *et al.*, 1981) and $^{45}\text{Ca}^{2+}$ uptake in guinea-pig papillary muscles (Tamargo *et al.*, 1988).

Therefore, the present study was undertaken to characterize further the mechanisms responsible for the inotropic effects of PAF, particularly as they may be mediated through changes (increase/decrease) of transmembrane Ca^{2+} movements. Thus, experiments were designed to (1) characterize the effects of PAF on contractile force and transmembrane action potentials in normal and depolarized media, and (2) determine its effect on $^{45}\text{Ca}^{2+}$ movement in guinea-pig atria, a cardiac preparation independent of coronary supply.

Methods

General procedure

Guinea-pigs of either sex weighing 350–450 g were stunned by a sharp blow on the head and their hearts were rapidly removed. Right and left atria were dissected and mounted vertically in 10 ml organ baths containing Tyrode solution of the following composition (mM): NaCl 137, KCl 5.4, CaCl_2 1.8, MgCl_2 1.05, NaHCO_3 11.9, NaH_2PO_4 0.42 and glucose 5.5. The solution was bubbled with 95% O_2 : 5% CO_2 and maintained at 34°C. In some experiments 30% of the Na^{+} concentration of the Tyrode solution was replaced by isosmotic sucrose. In others, Ca^{2+} concentration was varied from 0.9 to 9.0 mM. Left atria were electrically driven at a basal rate of 1 Hz through bipolar platinum electrodes with rectangular pulses (1 ms duration, twice threshold strength) delivered from a multipurpose programmable stimulator (Cibertec CS 220). Right atria were allowed to beat spontaneously. Rate and amplitude of contractions were measured isometrically by a force-displacement transducer and recorded on a Letica 2000 polygraph. Resting tension was adjusted to 1 g and a 30 min

equilibration period was allowed to elapse before control measurements were taken. The different parameters of isometric contractions and the sinus node recovery time (SNRT) were determined as previously described (Tamargo, 1980; Díez & Tamargo, 1987). After control values for each parameter were obtained, incremental concentrations of PAF were added to the bath to obtain a complete dose-response curve. The interval between doses of PAF was 10 min, since preliminary time-response studies indicated that its effect had stabilized in less than 10 min. The values for the different parameters obtained in the absence of PAF were used as a control and compared with those obtained after each increment in drug concentration. To study the interaction of different antagonists with the inotropic effects of PAF, dose-response curves to PAF were performed as above. The atria were washed and stabilized for 30 min. The antagonists were then added to the bath and after 15 min a new dose-response curve to PAF was obtained. Only one antagonist was used in each experiment.

For experiments on the interaction between PAF and the positive inotropic responses to CaCl_2 , the following procedure was used (Díez *et al.*, 1985). After control recordings were taken, increasing concentrations of CaCl_2 (0.9–9.0 mM) were added to the bath and a dose-response curve to Ca^{2+} was obtained. Once stable curves were obtained, the same procedure was repeated 10 min after the addition of PAF to the bathing media. Dose-response curves were expressed as percentages of the contractile response induced by 0.9 mM Ca^{2+} under control conditions.

Intracellular microelectrode recordings

Right and left atria were pinned to a Sylgard resin (Dow Corning) base of a chamber and perfused at a rate of 7 ml min^{-1} with Tyrode solution (34°C) bubbled with 95% O_2 :5% CO_2 . Preparations were stimulated as described earlier. Action potentials were recorded with glass microelectrodes ($10\text{--}20 \text{ M}\Omega$) filled with 3 M KCl as previously described (Díez *et al.*, 1985; Delpón *et al.*, 1989) and displayed on a Tektronix 5104 storage oscilloscope for photography. To study the effects of PAF on slow action potentials and contractions, atria were equilibrated in Tyrode solution and then rendered inexcitable by depolarizing with high K^+ (27 mM) Tyrode solution (Delpón *et al.*, 1989). Under these conditions atrial fibres became inexcitable despite intense electrical stimulation. Excitability was restored (i.e. slow action potentials and contractions) in atria driven at a basal rate of 0.4 Hz by adding isoprenaline (10^{-6} M) to the bathing media.

$^{45}\text{Ca}^{2+}$ uptake

To determine $^{45}\text{Ca}^{2+}$ uptake, left atria driven at a basal rate of 1 Hz were incubated in Tyrode solution for 30 min. In each experiment half of the atrium served as control and the other half as the experimental preparation. Following the equilibration period the experimental half was treated with PAF for 10 min and then both halves were exposed for various time intervals (2, 5 and 10 min) to $^{45}\text{Ca}^{2+}$ -labelled Tyrode solution (sp. act. 37 kBq ml^{-1} ; Amersham International, Buckinghamshire). At the desired time intervals, atria were removed, blotted on filter paper, dipped into Tyrode solution, reblotted and weighed. The atria were then placed in scintillation vials and 0.5 ml of Protosol 0.5 M (New England Nuclear) added and digested overnight at 50°C . Radioactivity was assayed on a liquid scintillation counter (LKB-Wallac 1211) as previously described (Barrigón *et al.*, 1982; Díez & Tamargo, 1987).

$^{45}\text{Ca}^{2+}$ efflux

To determine $^{45}\text{Ca}^{2+}$ efflux following the equilibration period, left atria were incubated in $^{45}\text{Ca}^{2+}$ -labelled Tyrode solution (sp. act. 74 kBq ml^{-1}) for 2 h. Thereafter, the preparations were placed every 5 min for 60 min and every 10 min for

another period of 60 min in successive tubes containing 5 ml of Tyrode solution to complete a 120 min washout period. In each experiment one atrium served as control and another atrium was exposed to the action of the desired concentration of PAF (10^{-11} M and 10^{-7} M) from min 20 to the end of the washout period. After the efflux period of 120 min, radioactivity lost into the tubes and present at the end of the experiment in the atria was determined as described for the uptake experiments. From the final activity in muscles and from the amount of radioactivity lost from the atria at 5–10 min intervals the percentage of $^{45}\text{Ca}^{2+}$ remaining in the atria at each collecting time was obtained. Washout data were expressed as a percentage of radioactivity remaining in the atria at each time interval and were plotted semilogarithmically as desaturation curves. The rate coefficient for the $^{45}\text{Ca}^{2+}$ efflux, k_{cm} , was calculated as described by Holland *et al.* (1978).

Drugs

The following drugs were used: PAF (Bachem, Bubendorf, Switzerland), lyso-PAF (Bachem), BN 52021 (Institut Henri Beaufour, France), L 652,731 (Merck Sharp & Dohme), (–)-isoprenaline hydrochloride (Sigma), verapamil hydrochloride (Knoll), (–)-propranolol (ICI-Farma), atropine sulphate (Sigma), phentolamine hydrochloride (Ciba), indomethacin (Merck Sharp & Dohme) and bovine serum albumin (Sigma). Powdered PAF was dissolved in Tyrode solution containing bovine serum albumin (0.5%) to obtain a concentrated solution. Further dilutions were performed in Tyrode solution to obtain the desired final concentration between 10^{-12} M and 10^{-7} M . Other drugs were dissolved in distilled water. Ascorbic acid (10^{-4} M) was added to each solution of isoprenaline to prevent its oxidation. All concentrations refer to the salt.

Statistical analysis

Throughout the paper results are expressed as mean $\pm$ s.e.mean. Statistical analysis was performed by use of Student's *t* test based on paired or unpaired observations when appropriate, and differences were considered significant when $P < 0.05$. Regression analysis was used to study the relationship between k_{cm} and percentage of $^{45}\text{Ca}^{2+}$ remaining in the atria.

Results

Effects of PAF on right and left atria

The effects of PAF in a range of concentrations between 10^{-12} M and 10^{-7} M on rate and amplitude of spontaneous contractions were studied in 8 right atria. Control values for both parameters averaged $157.9 \pm 13.0 \text{ beats min}^{-1}$ and $440.1 \pm 67.3 \text{ mg}$, respectively. When added to the bathing medium, PAF produced a concentration-dependent increase in atrial rate which reached significant values ($P < 0.05$) at concentrations higher than 10^{-12} M (Figure 1a). Thus, at 10^{-8} M , PAF increased atrial rate by $11.3 \pm 4.0\%$ ($P < 0.01$). On contractile force PAF induced a biphasic effect, a positive followed by a negative inotropic effect (Figure 1a). At low concentrations (10^{-12} M – 10^{-10} M) PAF increased peak contractile force ($+14.3 \pm 5.1\%$ at 10^{-10} M , $P < 0.05$), while at higher concentrations it produced a concentration-dependent negative inotropic effect which reached significant values at 10^{-7} M ($-15.7 \pm 5.0\%$, $P < 0.05$). The onset of inotropic effects occurred within 2 min of its addition to the bath and reached steady-state values within 5–7 min. The effect of PAF on the SNRT was analyzed in another 8 spontaneously beating right atria. Control values for this parameter averaged $439.7 \pm 29.5 \text{ ms}$. Figure 1a shows that PAF produced a significant shortening of the SNRT at all concentrations tested and thus, 10^{-7} M PAF shortened the SNRT by $17.1 \pm 3.9\%$ ($P < 0.01$) below control values. At the same range of concen-

Figure 1 (a) Effect of PAF on peak contractile force (○) and rate (Δ) of spontaneous contractions and of the sinus node recovery time (▲) in isolated right atria. (b) Effect of PAF on peak contractile force (○), df/dt_{max} (●), time to peak tension (Δ) and time for total contraction (▲) in electrically driven left atria. Each point represents the mean of 8 experiments; vertical bars show the s.e.mean. * $P < 0.05$. C = control.

trations lyso-PAF did not modify atrial rate or contractile force.

As the inotropic effects of PAF were associated with an increase in atrial rate, the effects of cumulative concentrations of PAF on different parameters of isometric contractions were also studied in 13 left atria driven at a constant rate of 1 Hz. Control values of isometric contractions were: peak contractile force 592.6 ± 73.6 mg, df/dt_{max} 20.4 ± 2.8 mg ms⁻¹, time to peak tension 57.3 ± 3.0 ms and time for total contractions 200.3 ± 15.9 ms. Figure 1b shows that PAF also caused a biphasic effect on peak contractile force similar to that observed in right atria. In fact, at low concentrations (10^{-12} M– 10^{-10} M), PAF significantly increased peak contractile force as well as the df/dt_{max} , whereas at higher concentrations it produced a concentration-dependent negative inotropic action. The maximum positive inotropic effect was observed at 10^{-11} M PAF, which augmented peak contractile force by $18.8 \pm 4.9\%$ ($P < 0.01$) and df/dt_{max} by $25.4 \pm 4.7\%$ ($P < 0.001$). Both parameters were reduced by $15.5 \pm 4.4\%$ ($P < 0.01$) and $16.7 \pm 5.3\%$ ($P < 0.01$), respectively, at the highest concentration tested. At concentrations up to 10^{-10} M the positive inotropic effect was accompanied by a significant shortening of the time to peak tension ($9.0 \pm 2.4\%$ at 10^{-11} M, $P < 0.05$), but this effect was partly reversed at higher concentrations. PAF also prolonged the time for total contraction which reached significant values at 10^{-10} M ($11.4 \pm 3.8\%$, $P < 0.05$) and remained constant at higher concentrations.

In both right and left atria the positive and negative inotropic effects of PAF were completely reversed by washing the preparations with drug-free Tyrode solution and the negative effect by increasing the extracellular Ca^{2+} concentration to 3.6 mM or by adding isoprenaline (10^{-6} M) to the bathing medium.

Effect of different pretreatments on inotropic actions of PAF

In the presence of 10^{-6} M propranolol, PAF exerted similar positive ($+12.3 \pm 2.9\%$ as compared to $+18.6 \pm 8.6\%$ at 10^{-11} M PAF, $n = 6$) and negative inotropic effects ($-12.7 \pm 2.9\%$ as compared to $-16.3 \pm 10.5\%$ at 10^{-7} M PAF, $P > 0.05$). Similar results were obtained in atria pretreated with atropine (10^{-6} M), indomethacin (10^{-6} M) or phentolamine (10^{-6} M).

The effects of verapamil were studied on the inotropic responses to PAF (Figure 2). Verapamil, 10^{-6} M, by itself, decreased peak contractile force by $33.7 \pm 4.6\%$ ($n = 8$, $P < 0.001$), completely abolished the positive inotropic effect produced by low concentrations of PAF, and potentiated the

Figure 2 Effect of PAF on peak contractile force in electrically driven left atria in control conditions (○) and after pretreatment of the preparations with verapamil, 10^{-6} M (●). Each point represents the mean of 8 experiments; vertical bars show the s.e.mean. * $P < 0.05$; ** $P < 0.01$.

negative inotropic action induced by higher concentrations of PAF ($P < 0.05$). Thus, the dose-response curve to PAF was displaced downwards and to the left. Thus, the maximum negative inotropic effect of PAF in the absence and presence of verapamil was $-18.7 \pm 2.7\%$ and $-35.3 \pm 5.6\%$ ($P < 0.05$), respectively.

The PAF antagonist BN 52021 (10^{-7} M) (Braquet *et al.*, 1987) did not itself modify atrial contractile force, but suppressed both the positive inotropic effect of 10^{-11} M PAF ($112.6 \pm 2.0\%$ of control in the absence of BN 52021 vs. 98.6 ± 1.9 , $n = 7$, $P < 0.05$) and the negative inotropic effect of 10^{-7} M PAF ($83.0 \pm 2.2\%$ of control in the absence of drug vs. $97.6 \pm 2.2\%$ of control, $n = 7$, $P < 0.01$).

Effect of extracellular concentrations of calcium and sodium on the inotropic actions of PAF

It is known that as the Ca^{2+} concentration of the bathing medium is increased, or Na^{+} is decreased, Ca^{2+} influx and amplitude of contractions are augmented (Niedergerke, 1963). The influence of PAF on inotropic responses induced by increasing extracellular Ca^{2+} concentrations, $[Ca^{2+}]_o$, was evaluated on a paired basis in 8 left atria. A stepwise increase in $[Ca^{2+}]_o$ from 0.9 to 9.0 mM dose-dependently increased atrial contractile force. At 10^{-11} M PAF significantly increased the positive inotropic effect of each $[Ca^{2+}]_o$ and thus shifted the concentration-response curve of Ca^{2+} upwards (Figure 3). At 10^{-7} M PAF significantly decreased the positive inotropic effect of each $[Ca^{2+}]_o$ and shifted the curve downwards and to the right. Thus at concentrations which produced a positive inotropic effect, PAF was able to potentiate the inotropic responses to Ca^{2+} , whereas negative inotropic concentrations decreased these responses.

In another group of experiments the inotropic actions of PAF were studied in 6 left atria equilibrated for 30 min in Tyrode solution with Na^{+} concentration reduced to 70%. In 70% Na^{+} solution, peak contractile force was significantly increased over values obtained in normal Tyrode solution (759.4 ± 79.9 mg vs 561.4 ± 68.8 mg, $n = 6$, $P < 0.05$). Under these conditions PAF again exerted a biphasic effect on contractile force. However, when the effects observed in normal and 70% Na^{+} solution were compared (Figure 4) it was observed that the maximum positive inotropic effect of

Figure 3 Effect of PAF on the calcium-induced positive inotropic effect in electrically driven left atria. Each point represents the mean of 8 experiments; vertical bars show the s.e.mean. (○) Control; (●) PAF, 10^{-11} M; (▲) PAF, 10^{-7} M. * $P < 0.05$; ** $P < 0.01$.

10^{-11} M PAF was similar in both media ($+17.3 \pm 5.2\%$ change in contractile force as compared to $+12.8 \pm 6.1\%$), whereas at 10^{-7} M the negative inotropic effect observed in 70% Na^+ solution was significantly reduced when compared with that observed in normal Tyrode solution ($-5.7 \pm 25\%$ in contractile force as compared to $-14.8 \pm 4.1\%$, $P < 0.01$).

Effect of PAF on transmembrane action potentials

The electrophysiological effects of PAF in a range of concentrations between 10^{-11} M and 10^{-7} M, were studied on atrial transmembrane action potentials in 9 atria driven at a basal rate of 1 Hz. In contrast to ventricular muscle fibres, where PAF shortened the action potential duration (Camussi *et al.*,

Figure 4 Comparison of inotropic effects induced by PAF (10^{-11} M, 10^{-9} M and 10^{-7} M) in electrically driven left atria incubated in normal Tyrode solution (open columns) or in 70% Na^+ Tyrode solution (solid columns) expressed as percentage change from control contractile force. Values are the mean of 6 experiments; vertical bars show s.e.mean. * $P < 0.01$.

1984; Tamargo *et al.*, 1985), PAF had no effect on resting membrane potential, amplitude, maximum rate of depolarization (V_{max}) or action potential duration at any of the concentrations tested.

Effect of PAF on slow responses

In order to ascertain the effects of PAF on the slow inward current, the fast inward Na^+ current was voltage-inactivated by a partial depolarization by addition of 27 mM K^+ to the Tyrode solution and atria become inexcitable despite intense electrical stimulation. Addition of isoprenaline (10^{-6} M) to the bath restored electromechanical activity, i.e., slow action potentials and contractions, of the atria when they were stimulated at a basal rate of 0.2 Hz. In 8 right atria, peak contractile force of slow contractions induced by isoprenaline averaged 480.2 ± 48.9 mg. Addition of PAF produced an initial, non-significant, increase in peak contractile force ($6.1 \pm 3.9\%$ at 10^{-11} M), followed by a concentration-dependent decrease which reached significant values at concentrations higher than 10^{-9} M (Figure 5). This effect of PAF was rapidly reversed by increasing the Ca^{2+} concentration in the bathing medium. In contrast, under similar experimental conditions PAF (10^{-11} M, 10^{-10} M and 10^{-7} M) had no effect on amplitude, V_{max} and duration of the slow action potentials or on the resting membrane potential of atrial fibres depolarized to -46.3 ± 1.2 mV ($n = 6$).

Effect of PAF on $^{45}\text{Ca}^{2+}$ uptake and $^{45}\text{Ca}^{2+}$ efflux

The effects of PAF (10^{-11} M to 10^{-7} M) on $^{45}\text{Ca}^{2+}$ uptake were studied in paired atria driven at a basal rate of 1 Hz and maintained in labelled solution for different time periods. As is shown in Figure 6, an increase in the incubation period in radioactive solution from 2 to 5 and 10 min augmented $^{45}\text{Ca}^{2+}$ uptake progressively from 0.526 ± 0.065 to 0.896 ± 0.137 and to 1.130 ± 0.105 mmol kg^{-1} . PAF, 10^{-11} M and 10^{-9} M, significantly increased $^{45}\text{Ca}^{2+}$ uptake by $51.8 \pm 5.4\%$ and $48.0 \pm 3.9\%$, respectively ($P < 0.01$) after incubation for 10 min but no significant modifications were observed with incubations of only 2 and 5 min. However, at 10^{-7} M, PAF did not modify $^{45}\text{Ca}^{2+}$ uptake at any time interval. Table 1 shows that the increase in $^{45}\text{Ca}^{2+}$ uptake after

Figure 5 Effect of PAF on slow contractions induced by isoprenaline (10^{-6} M) on K^+ (27 mM)-depolarized atria driven at 0.2 Hz. Each point represents the mean of 8 experiments; vertical bars show the s.e.mean. * $P < 0.05$.

Figure 6 Effect of PAF on $^{45}\text{Ca}^{2+}$ uptake in electrically driven left atria. Each point represents the mean of 6 to 18 experiments; vertical bars show the s.e.mean. (○) Control; (●) PAF, 10^{-11} M ; (△) PAF, 10^{-9} M ; (▲) PAF 10^{-7} M . * $P < 0.01$.

10 min incubation produced by 10^{-11} M PAF was inhibited in atria pretreated with verapamil (10^{-6} M) or BN 52021 (10^{-7} M). The effect of PAF (10^{-11} M and 10^{-7} M) on $^{45}\text{Ca}^{2+}$ efflux was evaluated in left atria driven at a basal rate of 1 Hz and incubated in $^{45}\text{Ca}^{2+}$ -labelled Tyrode solution for 2 h. In 4 atria PAF, 10^{-7} M , modified neither the percentage of $^{45}\text{Ca}^{2+}$ remaining in the atria nor the k_{cm} for $^{45}\text{Ca}^{2+}$ efflux from the tissue (data not shown). Figure 7 shows the effect of 10^{-11} M PAF on $^{45}\text{Ca}^{2+}$ remaining and k_{cm} when added from min 20 to the end of the washout period. In 6 atria the percentage of $^{45}\text{Ca}^{2+}$ remaining in the control atria after 120 min of washout was $3.59 \pm 0.58\%$, whereas in the experimental preparations it was 4.96 ± 1.05 . Moreover, PAF induced an upward shift of the washout curve, i.e., originated a decrease in $^{45}\text{Ca}^{2+}$ efflux from atrial cells. This effect was accompanied by a decrease of k_{cm} which reached significant values during the first 20 min following drug application (see inset in Figure 7). Therefore, the increase in uptake previously described can be interpreted as due to an increase of $^{45}\text{Ca}^{2+}$ influx.

Figure 7 Effect of PAF (10^{-11} M) on $^{45}\text{Ca}^{2+}$ efflux in left atria after 2 h incubation in radioactive Tyrode solution. The inset shows the effect of PAF on the rate coefficient for $^{45}\text{Ca}^{2+}$ efflux (k_{cm} , min^{-1}). Each point represents the mean of 6 experiments; vertical lines shown s.e.mean. (○) Control; (●) PAF, 10^{-11} M . * $P < 0.01$; ** $P < 0.001$.

Table 1 $^{45}\text{Ca}^{2+}$ uptake after 10 min incubation in radioactive solution in control left atria and in atria treated with PAF (10^{-11} M) and verapamil (10^{-6} M) or BN 52021 (10^{-7} M) plus PAF

Drug	n	$^{45}\text{Ca}^{2+}$ uptake
Control	6	1.233 ± 0.167
PAF	8	$1.811 \pm 0.152^*$
Verapamil + PAF	8	$1.274 \pm 0.123^{**}$
BN 52021 + PAF	6	$0.892 \pm 0.221^{**}$

* $P < 0.05$ vs. control; ** $P < 0.01$ vs. PAF.

n = number of experiments.

The semilogarithmic plot of percentage of $^{45}\text{Ca}^{2+}$ remaining against time did not become linear even after 120 min of washout. Therefore, and to study further the effect of PAF on $^{45}\text{Ca}^{2+}$ efflux, the empirical approach of Reuter & Seitz (1968) was used. They found that the rate efflux of $^{45}\text{Ca}^{2+}$ from guinea-pig atria in Ca^{2+} -free washout medium was a function of the square of the amount of Ca^{2+} remaining in the tissue. Such a relationship would produce a straight line when the k_{cm} of the $^{45}\text{Ca}^{2+}$ efflux is plotted against the percentage of $^{45}\text{Ca}^{2+}$ remaining in atria, which expresses the rate of efflux as a function of the amount of isotope remaining in the tissue. As shown in Figure 8, this relationship fitted well to a straight line both in the absence ($r = 0.9634$, $P < 0.001$) and in the presence of PAF, 10^{-11} M ($r = 0.8696$, $P < 0.001$) but PAF produced a parallel shift to the right of the straight line. Because this shift is independent of the percentage of $^{45}\text{Ca}^{2+}$ remaining in the tissue, it may be concluded that the rate coefficient for $^{45}\text{Ca}^{2+}$ efflux in the presence of PAF is a constant. The shift was determined at each of the 14 plotted points on the control curve with a linear interpolation to obtain corresponding values in the presence of PAF. The value obtained for the PAF-induced shifts was $0.0101 \pm 0.0013\text{ min}^{-1}$ ($P < 0.001$), which means that the drug decreased the rate coefficient to this extent independently of the percentage of $^{45}\text{Ca}^{2+}$ remaining in the atria.

Discussion

The results of the present paper demonstrate that both in spontaneously beating and in electrically driven guinea-pig

Figure 8 Relationship between the rate coefficient of $^{45}\text{Ca}^{2+}$ efflux (k_{cm} , min^{-1} , ordinate scale) and the percentage of $^{45}\text{Ca}^{2+}$ remaining in the guinea-pig atria in the absence of (O, $r = 0.9634$, $P < 0.001$) and in the presence of 10^{-11} M PAF (●, $r = 0.8696$, $P < 0.001$). Data were taken from experiments of Figure 7.

left atria, PAF exhibited a biphasic inotropic effect. Thus, low concentrations of PAF (10^{-10} M) produced a positive inotropic effect whereas at higher concentrations it produced a negative inotropic effect. Changes in contractility were accompanied by a moderate increase in atrial rate and a shortening of the SNRT, which suggests that PAF may exert a stimulatory effect on cardiac pacemakers (Tamargo *et al.*, 1985; Alloatti *et al.*, 1988). These inotropic responses are independent of coronary supply, the presence of formed blood elements and frequency variations since they were almost similar both in noncoronary-perfused right and left atria. Pretreatment with phentolamine, propranolol, atropine or indomethacin did not modify these responses, thus indicating that they were not mediated through cardiac α - or β_1 -adrenoceptors, by muscarinic mechanisms or through prostaglandin synthesis. However, they were suppressed by the specific PAF antagonist BN 52021 (Braquet *et al.*, 1987), which suggests that PAF exerts direct and specific effects on atrial muscle fibres.

Even though the mechanism(s) responsible for the cardiac effects of PAF are unknown, they can be partly explained through modifications of trans-sarcolemmal Ca^{2+} fluxes. Thus, pretreatment with verapamil suppressed the positive inotropic effect of PAF. Previous evidence has demonstrated that verapamil prevented different effects of PAF both in isolated cardiac preparations and in anaesthetized animals (Tamargo *et al.*, 1985; Alloatti *et al.*, 1987). Furthermore, as previously described for platelets (Lee *et al.*, 1981; Valone & Johnson, 1985), macrophages (Braquet *et al.*, 1987) and ventricular papillary muscles (Tamargo *et al.*, 1988), low concentrations of PAF also increased $^{45}\text{Ca}^{2+}$ uptake in atrial fibres, this effect being suppressed in atria pretreated with verapamil or BN 52021. Since verapamil inhibits Ca^{2+} entry through voltage-dependent Ca^{2+} channels (Reuter, 1983) but had no effect on the Na^+ - Ca^{2+} exchange mechanism (Coraboeuf *et al.*, 1981), our data would suggest that the increase in $^{45}\text{Ca}^{2+}$ uptake induced by PAF may be related to an enhanced Ca^{2+} influx via slow channels. Nevertheless because verapamil also exhibits PAF receptor antagonist properties (Tuffin & Wade, 1985) it is difficult to ascertain whether its inhibitory effects on contractility and $^{45}\text{Ca}^{2+}$ uptake are due to a specific Ca^{2+} antagonism or to a competition at the cardiac receptor for PAF. In this regard the finding that in contrast to PAF antagonists, verapamil abolished the positive but potentiated the negative inotropic effect of PAF, may suggest that it acts more as a Ca^{2+} antagonist than as an antagonist of atrial

PAF receptors. However, despite all this evidence, since PAF had no effect on the V_{max} of the slow action potentials elicited in K^+ -depolarized atria, a direct index of cardiac Ca^{2+} current (Malecot & Trautwein, 1987), it seems unlikely that an increase in Ca^{2+} entry via the slow channels can be responsible for its positive inotropic effect. At higher concentrations ($> 10^{-9}$ M) PAF decreased atrial contractility as previously reported (Levi *et al.*, 1984; Alloatti *et al.*, 1987; Robertson *et al.*, 1987). In ventricular muscle fibres where PAF shortened the action potential duration (Camussi *et al.*, 1984; Tamargo *et al.*, 1985; Robertson *et al.*, 1988) this negative inotropic effect has been attributed to the reduction in the time available for Ca^{2+} entry through the slow channels (Camussi *et al.*, 1984; Tamargo *et al.*, 1985) or to a reduced Na^+ influx (Robertson *et al.*, 1988). However, both explanations seem unlikely in atrial fibres where PAF had no effect on duration and V_{max} , an indirect index of the fast inward Na^+ current of the atrial action potential. Moreover, some evidence suggests that the negative inotropic effect of PAF may be due to a decrease in Ca^{2+} entry through the slow channels. Thus, verapamil potentiated this negative inotropic effect and at the same range of concentrations at which it exerted its negative inotropic effect, PAF also decreased the amplitude of the slow contractions. Furthermore, the negative inotropic effect was reversed by adding isoprenaline, which increased the slow inward Ca^{2+} current in atrial fibres (Reuter, 1983). However, because at this range of concentrations PAF did not decrease the V_{max} of the slow action potentials it appears unlikely that inhibition of atrial contractility can be related to a decrease in Ca^{2+} entry through the slow channels.

If changes in Ca^{2+} entry through slow channels are not responsible for the inotropic effects of PAF, entry of Ca^{2+} by passive perfusion down its concentration gradient or in exchange for Na^+ are two alternative and viable possibilities. The contractile force of guinea-pig isolated atria is a function of $[\text{Ca}^{2+}]_o$ (Scholz, 1969). Low concentrations of PAF shifted to the left the curve relating tension to $[\text{Ca}^{2+}]_o$, so that less Ca^{2+} is required to produce the same amount of tension. Thus, it is possible that PAF could enhance atrial contractility acting on the cardiac sarcolemma increasing its conductance for Ca^{2+} . The inwardly directed transmembrane electro-mechanical gradient for Ca^{2+} ions would result in an increased net influx of Ca^{2+} into the cell which could then potentiate the contractile effect of Ca^{2+} entering during the cardiac action potential. Such an effect may explain an increased net extra influx of Ca^{2+} independent of the slow channels as well as the PAF-induced increase of $^{45}\text{Ca}^{2+}$ uptake reported in platelets (Valone & Johnson, 1985) where there is no evidence of voltage-dependent Ca^{2+} channels (Doyle & Ruegg, 1985). This increase in Ca^{2+} entry together with the decrease in $^{45}\text{Ca}^{2+}$ efflux would lead to an increase in $[\text{Ca}^{2+}]_i$ and contractile force. The opposite results were found at concentrations of PAF producing a negative inotropic effect, thus suggesting that it may reduce Ca^{2+} entry into atrial fibres. The negative inotropic effect was reduced when the Ca^{2+} concentration was increased or Na^+ concentration in the bathing media was decreased, i.e. when Ca^{2+} entry into atrial fibres was increased (Niedergerke, 1963; Langer, 1973). These results suggest not only that Ca^{2+} antagonized the negative inotropic effect of PAF but also that this effect can be related to a decrease in Ca^{2+} entry into atria.

In conclusion, this study suggests that in guinea-pig atria the inotropic effects of PAF may be related to changes in trans-sarcolemmal Ca^{2+} entry. Since both the inotropic effects and the increase of $^{45}\text{Ca}^{2+}$ uptake induced by PAF were abolished by BN 52021, a specific antagonist of PAF receptors, these results suggest that in atrial fibres, PAF exerts direct effects which seem to be mediated by specific receptors possibly similar to those described in platelets (Hwang & Lam, 1986).

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