Cardiovascular and Renal Action of Platelet-Activating Factor in Anesthetized Dogs

SUBBARAO VEMULAPALLI, PETER J.S. CHIU, AND ALLEN BARNETT

SUMMARY Platelet-activating factor (PAF) has hypotensive effects similar to those of antihypertensive polar renomedullary lipid (APRL), a potent endogenous hypotensive lipid. In this study the cardiovascular and renal effects of PAF were characterized in anesthetized dogs. Intravenous infusion of PAF at 0.1 μg/kg/min for 1 hour caused marked reduction in arterial blood pressure and cardiac output and was accompanied by minimal changes in heart rate. Concomitantly, renal blood flow, glomerular filtration rate, urine flow, and fractional excretion of Na+ and K+ fell significantly. Plasma renin activity was greatly stimulated (11.9 ± 1.66 vs 3.26 ± 0.45 ng/angiotensin I/ml/hr for the placebo group). There were no significant alterations in any of these parameters following PAF at a lower dose (0.03 μg/kg/min for 1 hour). In a separate study, PAF at 0.1 μg/kg/min for 20 minutes produced a decrease in left ventricular myocardial contractile force, concomitant with bradycardia and hypotension, which indicated the presence of a negative inotropic activity. It is concluded that systemic administration of PAF has a deleterious effect on kidney function due to arterial hypotension and diminished cardiac output. (Hypertension 6: 489-493, 1984)

Key Words • platelet-activating factor • antihypertensive polar renomedullary lipid • blood pressure • cardiac output • myocardial contractility • renal blood flow • glomerular filtration rate

Platelet-activating factor (PAF, PAF-acether), a glycerophospholipid released from stimulated basophils, macrophages, and platelets, is considered an important mediator of inflammatory and allergic reactions.1-3 The chemical structure of PAF has been identified as 1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine (alkyl ether analogs of phosphatidylcholine, AEPC), which is similar in hypotensive effect to that of antihypertensive polar renomedullary lipid (APRL).4-6 APRL and antihypertensive neutral renomedullary lipid (ANRL) are potent endogenous hypotensive lipids isolated from renal medulla.

PAF, APRL, or ANRL, in microgram doses, lowered arterial pressure in guinea pigs, rats, and rabbits in the normal and hypertensive state following intravenous,7-10 intramuscular, or oral administrations.11-13 We first reported that PAF infusion significantly reduced myocardial performance and renal function in anesthetized dogs.14 Recently, it was demonstrated that intravenous administration of bolus doses (5-20 μg/kg) of PAF to anesthetized dogs caused marked reductions in blood pressure (BP), heart rate (HR), cardiac output (CO), left ventricle (LV) dp/dt, blood volume, and coronary perfusion pressure and led to acute circulatory collapse.15 Our present work is an extension of these studies. Effects of PAF on cardiac contractility and other cardiovascular measures were evaluated. In addition, concomitant changes in renal hemodynamics and electrolyte excretion were determined.

Materials and Methods

The methods used were described in detail previously.16 Briefly, mongrel dogs of either sex weighing 13 to 21 kg were anesthetized with sodium pentobarbital 30 mg/kg i.v. and artificially respired with a Harvard respirator. The right brachial artery, vein, and the left cephalic vein were cannulated for collection of blood samples, drug administration, and inulin infusion, respectively. The right femoral artery was cannulated and connected to a Statham pressure transducer to record BP. The left renal artery was exposed through a flank incision, and an electromagnetic flow probe (Micron Instruments, Los Angeles, California) was placed around the renal artery to monitor renal blood flow. Approximately 200 ml of physiological saline was infused i.v. over 30 to 45 minutes to replace fluid loss incurred during surgery and to ensure adequate urine flow. Both ureters were cannulated via a suprapubic incision for continuous urine collection.

The CO was measured with a Columbus CO computer (Columbus Instruments, Columbus, Ohio). After the surgical procedures had been completed, a priming
A dose of inulin 50 mg/kg was infused i.v. over 15 minutes and immediately followed by a sustaining infusion at the rate of 0.84 mg/kg/min throughout the experiments. One hour was allowed for equilibration before sample collection was started. Urine was collected every 20 minutes, and blood samples were taken at the beginning and end of each urine collection period. Following two control periods, PAF was administered by i.v. infusion at a rate of 0.03 or 0.1 μg/min/kg body wt for 1 hour. The experiments were continued for an additional hour after the high dose of PAF. PAF (Calbiochem-Behring, San Diego, California) used in this study is derived from egg yolk, with a biological activity similar to naturally occurring PAF (ED₅₀ 1.1 x 10⁻¹⁰ M in causing H3 serotonin release from rabbit platelets) and 99% pure (Dr. G. Kritchovsky, Calbiochem-Behring, personal communication). PAF was dissolved in 60% ethanol in prechilled plastic test tubes kept on ice, and 1 ml aliquots were frozen at -70°C. On the day of the experiment, a 1 ml aliquot was thawed on ice, diluted with saline, and immediately infused. Any remaining portions of PAF were discarded.

Urine and plasma samples were analyzed for inulin by the anthrone method¹⁷ and for sodium and potassium concentrations by flame photometry. Plasma renin activity was determined in blood samples collected 70 minutes before and shortly after the 1-hour infusion of PAF with use of a New England Nuclear assay kit, as described previously.¹⁶

Measurements of Myocardial Contractile Force

Myocardial contractile force (CF) was measured in anesthetized, artificially ventilated dogs weighing 15 to 19 kg. Through a left thoracotomy, the heart was exposed, and two small holes were made on the pericardium of the left ventricle. A precalibrated Walton-Brodie strain gauge arch was sutured onto the surface of the LV, and the strain gauge was stretched until maximum force was attained and then tightly fixed. The preparations were allowed to stabilize for 1 hour after the skin over the wound had been sutured. The right femoral artery and brachial vein were cannulated for monitoring blood pressure and PAF infusion, respectively. PAF was infused at the rate of 0.1 μg/kg/min for 20 minutes. All parameters were recorded on a four-channel Hewlett Packard recorder.

Statistics

The data were analyzed by analysis of variance, and significant differences (p < 0.05) from the baseline values were determined with Duncan's multiple range test. All values are presented as means ± se.

Results

Systemic Hemodynamic Effects

The effects of i.v. infusion of PAF at 0.03 and 0.1 μg/kg/min for 60 minutes on mean arterial BP, HR, CO, and total peripheral resistance (TPR) are summarized in Figure 1. The low dose of PAF produced no significant alterations. The high dose lowered BP as soon as the infusion was started. The peak effect occurred at 20 minutes into the infusion and persisted throughout. Concomitantly, CO fell and TPR was elevated. HR tended to decrease, but the changes were not statistically significant. A moderate recovery of BP, CO, and TPR appeared to occur 1 hour after the infusion was terminated. Infusion of 2-lyso PAF at 0.1/μg/kg/min for 60 minutes had no effect on any one of these measures (n = 3).

Measurements of Myocardial Contractile Force

Preliminary experiments indicated that infusion of PAF at 0.1 μg/kg/min for 20 minutes caused max-
maximum reduction in the CF of the LV. Therefore, this infusion rate was used in subsequent experiments. PAF decreased CF coincident with a fall in HR and BP (Figure 2). The peak effects occurred after 20 minutes of infusion. All measures tended to return to control values 60 minutes postinfusion, and they remained stable following the infusion of 2-lyso PAF at 0.2 μg/kg/min for 20 minutes (n = 3).

Renal Hemodynamic Effects

Changes in glomerular filtration rate (GFR), renal blood flow (RBF), and renal vascular resistance (RVR) following PAF infusion are summarized in Figure 3. The high dose of PAF caused a marked reduction in RBF in association with elevated RVR. Maximal changes occurred 20 minutes into the infusion and coincided with the maximal drop in BP and CO. In addition, GFR fell and reached a nadir after 40 minutes of infusion. The reduction in RBF and increase in RVR persisted after cessation of drug infusion, whereas GFR tended to return to baseline value more rapidly. The low dose of PAF did not significantly (p < 0.05) affect any of these measures. The high dose of PAF induced reductions in RBF and GFR that were accompanied by a significant fall in urine flow and fractional excretion of Na⁺ (FENa) and K⁺ (FEK) (data not shown). The low dose produced some modest effects, which are not statistically significant.

Measurements of Plasma Renin Activity

The effects of placebo (vehicle) and PAF on plasma renin activity (PRA) are shown in Table 1. The control PRA values in each of the three treatment groups were not significantly different. No significant increase in PRA over baseline controls occurred after placebo or after the low dose of PAF. However, the high dose of PAF markedly elevated PRA.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control (ng ANG I/ml/hr)</th>
<th>After treatment (ng ANG I/ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>5</td>
<td>1.75 ± 0.38</td>
</tr>
<tr>
<td>PAF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.03 μg/kg/min for 60 min</td>
<td>5</td>
<td>1.67 ± 0.35</td>
</tr>
<tr>
<td>0.1 μg/kg/min for 60 min</td>
<td>5</td>
<td>1.64 ± 0.25</td>
</tr>
</tbody>
</table>

The placebo group was tested under similar conditions but without measurements of cardiovascular and renal functions. The PAF-treated groups were the same as those in Figures 1–3.

*p < 0.01 vs placebo group; Duncan’s multiple range test.
Discussion

Alkyl ether analogs of AEPc, which have been studied as an APRL by Muirhead and associates,\textsuperscript{6, 18-19} have been proposed to be potent endogenous antihypertensive agents of renal origin. In comparison, AEPc, studied as a platelet-activating factor (PAF), has been implicated as a major mediator in anaphylactic shock.\textsuperscript{20}

That PAF is a potent hypotensive agent in normal and hypertensive animals is indisputable,\textsuperscript{7, 13, 18, 20-23} but the mechanism of this action remains unclear. In normotensive and hypertensive rats, PAF reversed the pressor responses to epinephrine and norepinephrine, which indicated that PAF is an alpha-adrenergic-receptor blocking agent.\textsuperscript{10, 11} It is therefore probable that the hypotensive effect of PAF is partly mediated by its alpha-adrenergic-receptor-blocking activity. However, a contradictory report by Sybertz et al.\textsuperscript{11} suggests that PAF attenuated the pressor responses to both phenylephrine and angiotensin and implies that it has a nonspecific inhibitory effect on vascular smooth muscle.

Intravenous administration of PAF caused marked reductions in BP and CO and significantly increased TPR. The marked increase in TPR seems to rule out a generalized vascular dilation as the cause of hypotension in our study on anesthetized dogs. Thus, our results are qualitatively similar to those of Bessin et al., who reported that PAF elevated TPR, attenuated all hemodynamic measures, and caused circulatory collapse in anesthetized dogs; our results differ quantitatively, however. This discrepancy could be due to the high doses of PAF employed by these workers (5-20 \textmu g/kg, i.v. bolus vs 0.03 and 0.1 \textmu g/kg/min infusion over 60 minutes in our study). PAF has been reported to reduce blood volume\textsuperscript{15} by increasing vascular permeability.\textsuperscript{24, 25} Therefore, the drastic increase in TPR may not be the result of the direct action of PAF on vasculature, but a compensatory reflex vasconstrictor mechanism for the drastically reduced plasma volume. The exact mechanism of action of PAF in lowering CO cannot be derived from our studies, although PAF-induced loss of plasma volume by extravasation\textsuperscript{15} or decreased venous return may contribute to the reduction of CO observed in our study. The reduction in myocardial CF induced by PAF may provide an alternate explanation for the diminished CO, as discussed below.

PAF caused a decrease in myocardial CF with concomitant changes in BP and HR. This is the first time that the effects of PAF on contractility have been directly measured. Benveniste et al.\textsuperscript{26} and Levi\textsuperscript{27} recently reported that direct application of PAF to isolated hearts from normal or IgE-sensitized guinea pigs caused a negative inotropic effect attributable to coronary constriction. The plasma levels of thromboxane, (TXB\textsubscript{2}) increased after intraarterial or i.v. injection of PAF which led to coronary constriction.\textsuperscript{8, 13} In comparison, Prewitt et al.\textsuperscript{15} showed that APRL (0.01-4 \textmu g/ml) had no effect on the isolated spontaneously beating atria of normal guinea pigs. Cervoni et al.\textsuperscript{28} even reported that high concentrations of PAF (>10 \textmu M) had positive inotropic and chronotropic effects on spontaneously beating right atria and electrically driven left atria of rats.

The in vitro and in vivo cardiovascular and autonomic studies of PAF are fraught with discrepancies,\textsuperscript{22} which may be due to different lipid compositions of the materials used by individual groups. Yet, in most cases, the same material was used. It is probable that other unknown factors not present in vitro may work in conjunction with PAF in various tissues in vivo. Consequently, the exact mode of action with which PAF impairs cardiac contractility remains to be defined, although the reduction in coronary perfusion pressure or the metabolic acidosis induced by PAF\textsuperscript{15} may contribute to its negative inotropic effect. In the present study, CO and CF were determined in two separate groups of dogs. Since we did not measure LV pressure, we cannot compare LV stroke work to CF. Therefore, we cannot positively conclude whether the decrease in CO is predominant or the negative inotropic effect is predominant. However, Crandell et al.\textsuperscript{29} reported that PAF had no effect on CO in anesthetized SHR. Therefore, it is likely that PAF decreased CO by reducing CF in the anesthetized dogs.

In this study, PAF did not change HR in the dogs undergoing measurements of CO and kidney function, but caused bradycardia in the open-chest dogs in which myocardial CF was measured. The results resemble those obtained in anesthetized rats,\textsuperscript{12, 22} rabbits,\textsuperscript{20} and dogs,\textsuperscript{13} but differ from the data derived from conscious rat preparations in which PAF induced tachycardia instead.\textsuperscript{8, 11, 13} Inhibition of reflex compensatory adjustments in the face of arterial hypotension by anesthesia may account for the difference in results. In view of the variable HR responses to PAF, it is suggested that a decrease in myocardial contractility is more likely to play a significant role than bradycardia in diminished CO, with a subsequent drop in BP.

The great decrease in GFR and urine and electrolyte excretion was probably due to the fall in CO and BP. Similar renal changes can be produced after acute constriction of the thoracic inferior vena cava in dogs, which is associated with a decrease in CO and BP.\textsuperscript{30} The intense vasoconstriction, as reflected by elevated renal vascular resistance, may be due to a combination of direct and reflex action, aided further by increased circulating TXA\textsubscript{2}, and increased renin levels.\textsuperscript{8, 31} PAF significantly increased plasma norepinephrine in SHR.\textsuperscript{23} The increase in PRA might in part result from increased sympathetic nervous system activity and decreased renal perfusion caused by PAF.

Smith et al.\textsuperscript{31} have demonstrated that local application of APRL (0.5 \textmu g/ml) causes dilation of arterioles and venules in a cremaster muscle preparation from SHR and WKY rats. However, i.v. administration of APRL at doses that lowered BP resulted in cessation of blood flow in the cremaster preparation. Crandell et al.\textsuperscript{29} reported that PAF reduced blood flow to the heart by a direct effect on the coronary vasculature. Therefore, the preponderant effects of PAF appear to be
HEMODYNAMIC EFFECTS OF PLATELET-ACTIVATING FACTOR/Vemulapalli et al. 493

vasoconstriction in various vascular beds. Our present results suggest that PAF is an autacoid with multiple actions and that it is a potent vasoconstrictor when present in the systemic circulation, with deleterious cardiovascular and renal effects.

References

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