L-Platelet Activating Factor Induces Changes on Renal Vascular Resistance, Vascular Reactivity, and Renin Release in the Isolated Perfused Rat Kidney

Ullrich Schwertschlag, Harald Scherf, John G. Gerber, Melvin Mathias, and Alan S. Nies

Rat kidneys were isolated and perfused with a modified Krebs-Henseleit buffer containing 4% albumin. Perfsusate recirculated except during L-platelet activating factor (L-PAF), angiotensin II (ang II), and norepinephrine (NE) infusions. L-PAF caused a dose-dependent decrease in renovascular resistance (RVR): -6 ± 3% at 10^-9 M, -12 ± 6% at 10^-8 M, -18 ± 3% at 10^-7 M and -20 ± 7% at 10^-6 M. L-PAF increased immunoreactive PGE (iPGE) and thromboxane (iTXB) release into the venous effluent from 2.4 ± 0.2 to 3.9 ± 0.4 ng/min (p<0.05) and from 2.1 ± 0.4 to 3.5 ± 0.5 ng/min (p<0.05), respectively. Vasodilation by L-PAF (10^-7 M) in the presence of indomethacin (INDO) (5 μM) was enhanced compared to the non-INDO response (RVR change: L-PAF = -18 ± 3% vs. L-PAF + INDO = -26 ± 3%; p<0.05). As a control for specificity, the structural isomer D-PAF was infused at 10^-9 M, 10^-8 M, and 10^-7 M. None of these concentrations changed renal vascular resistance. To study the vascular receptor responsible for L-PAF-induced vasodilation, dose-response curves to NE and ang II were established with and without L-PAF (10^-7 M). The NE dose-response curve was unchanged by L-PAF, whereas the ang II dose-response curve was shifted to the right by one order of magnitude. In kidneys pretreated with INDO (5 μM), the L-PAF-induced shift of the ang II dose-response relation was increased to 2-3 orders of magnitude. Renin release was unchanged at 10^-9 M, 10^-8 M, and 10^-7 M L-PAF, but rose threefold at 10^-6 M concomitant with maximal vasodilation. We conclude that L-PAF is a potent vasodilator of the rat renal vasculature, specifically antagonizing ang-II-induced vasoconstriction. L-PAF also releases prostaglandins, which antagonize the vasodilation. Renin release is increased at a high dose of L-PAF and may be an indirect effect secondary to decreased perfusion pressure. (Circulation Research 1987;60:534-539)

Platelet-activating factor (L-PAF) is a biologically active phospholipid that has been studied most extensively as both a platelet-aggregating and a neutrophil-activating inflammatory agent. It has been found to be released from immunologically stimulated macrophages and neutrophils,1 from glomerular and renal medullary interstitial cells,2 and from the ionophore-stimulated isolated perfused kidney.3 In addition to its aggregating and proinflammatory actions, L-PAF possesses potent vascular actions, and its structural similarity to the renal medullary antihypertensive polar lipid has been proposed.4-6 Following unclipping of the renal artery of the one-kidney, one-clip hypertensive rat, L-PAF was released into the renal venous blood associated with the fall in blood pressure that was blunted by a specific platelet-activating factor antagonist.5,7 The detection of L-PAF in the normal circulation and its absence in anephric man and animals8 further supports the importance of the kidney as a source for this lipid. L-PAF in microgram doses lowers blood pressure in normotensive and hypertensive rats9-14 and in normotensive dogs.15-17 These effects were shown to be independent of sympathetic innervation7,10,11,13,14 or β- and α-adrenergic blockade.18 The action of this potent vasoactive lipid on renal hemodynamics is largely unknown. Thus, the present study was designed to examine the effect of L-PAF on renal vascular resistance, renin release, and vascular reactivity in the isolated perfused rat kidney, devoid of blood-borne elements, innervation, and systemic vascular responses.

Materials and Methods

Isolated Perfused Rat Kidney

Kidneys were isolated and perfused as previously described.18 Briefly, kidneys taken from male Sprague-Dawley rats (180–200 g body weight) were perfused at a constant flow to generate a perfusion pressure of 110–120 mm Hg in a recirculating system. The perfusion fluid consisted of a modified Krebs-Henseleit buffer containing 4% albumin and 5 mM glucose. The use of albumin instead of hydroxyethylstarch allows for basal vascular tone in the isolated...
perfused kidney preparation. The experiments were performed after an equilibration period of 30 minutes. During infusion of L-PAF, angiotensin II (ang II), and norepinephrine (NE), the perfusion fluid was not recirculated. Pressure and flow were measured continuously.

**Dose-Response Curves**

A stock solution of L-PAF (1-O-hexadecyl-2-acetyl-sn-glycerol-3-phosphorylcholine) was prepared monthly in chloroform:methanol, (95:5, v:v) and stored at -20°C. Such stock solutions can be stored for up to 4 months without loss of bioactivity, as assessed by rabbit platelet aggregation. D-PAF (the unnatural isomer of PAF) stock solutions were prepared similarly. Immediately before the experiment, an aliquot was evaporated to dryness under nitrogen, resuspended in gassed perfusion fluid (pH 7.4), and infused for 3 minutes at a rate of 0.02–0.025 or 0.2–0.25 ml/min to give a concentration of L-PAF in the perfusate of 10⁻⁹ M, 10⁻⁸ M, 10⁻⁷ M, or 10⁻⁶ M. D-PAF was infused at 10⁻⁹ M–10⁻⁷ M. Stock solutions of ang II and NE, 1 mg/ml each, were prepared in 5 mM NaH₂PO₄ (pH = 5) and diluted to 10,000, 1,000, 500, 100, 1, and 0.1 ng/ml. One hundred microliters of these stock solutions were injected into the perfusion circuit immediately adjacent to the kidney. Dose-response curves were established in a cumulative manner with the vehicle (100 μl NaH₂PO₄) injected first, followed by each drug concentration, and the maximal response was recorded. In separate experiments, the effect of L-PAF on the NE and ang II dose-response relation was determined by infusing L-PAF (10⁻⁷ M) for 5 minutes before dose-response curves were started and continuing the L-PAF infusion throughout the dose-response studies.

**Assays**

Renin activity in renal venous samples was determined by incubation with plasma from nephrectomized rats for 30 minutes with the generated angiotensin II assayed by radioimmunoassay as described. Values are expressed as nanograms ang I/hr incubation/min perfusion. There was no interference with the renin activity assay by L-PAF at concentrations up to 10⁻⁶ M.

Immunoreactive prostaglandin E (iPGE) and thromboxane (iTXB) in the renal venous effluent (iTXB) in the renal venous effluent were assayed by a double antibody technique as described. Urinary PGE was extracted with dichloromethane and assayed by radioimmunoassay using an antibody purchased from Institut Pasteur, Paris. A concentration of L-PAF of 2 × 10⁻⁷ M did not interfere with the prostaglandin assays, but at 2 × 10⁻⁶ M L-PAF, the readings for both prostaglandins were falsely elevated. Therefore prostaglandin release was determined at the 10⁻⁷ M L-PAF concentration only.

The bioactivity of the L-PAF stock solution was determined by the platelet aggregation assay. Rabbit platelets were prepared by standard procedures and washed by passing platelet-rich plasma through a Sepharose 4B-200 column. Platelets were then equilibrated with Ca²⁺-containing tyrode in an aggregometer cuvette and L-PAF added. This assay is sensitive to 10⁻¹⁰ M final L-PAF concentration.

**Materials**

L-PAF, ang II amide, norepinephrine tartrate, and albumin fraction V were purchased from Sigma Chemical Co., St. Louis, Mo. D-PAF was kindly provided by Dr. R. Wykle, Winston-Salem, N.C. All other reagents were analytical grade. The PAF antagonist BN 52021 was kindly provided by Dr. P. Braquet, Le Plessis Robinson, France.

**Statistics**

Experiments with and without L-PAF were performed in a randomized manner. Mean renal vascular resistance (pressure/flow) between experimental groups was compared by Student’s t test for unpaired data. Means within a treatment group were compared by two-way analysis of variance and Dunnett’s t test. Differences were considered statistically different when p < 0.05.

**Results**

**Vasodilation by L-PAF**

Twenty to thirty seconds after onset of L-PAF infusion, renal vascular resistance (RVR) decreased in a dose-dependent manner and reached a stable lower level over a period of 30–60 seconds. L-PAF (10⁻⁷ M) increased iPGE and iTXB release into the renal venous effluent by about 70% as compared to preinfusion levels (Table 1). Preperfusion of the isolated perfused kidney with indomethacin (5 μM) significantly enhanced the fall of RVR produced by 10⁻⁷ M L-PAF (L-PAF alone: -18 ± 3%; L-PAF + INDO: -26 ± 37%; p < 0.05) (Figure 1). This dose of indomethacin reduced urinary iPGE excretion by 90% during the control period (data not shown). L-PAF at 10⁻⁹ M, 10⁻⁸ M, and 10⁻⁷ M did not cause a significant change in renin secretion, but at 10⁻⁶ M, renin secretion increased threefold concomitantly with maximal vasodilation (Figure 2).

D-PAF was infused in concentrations of 10⁻⁹ M, 10⁻⁸ M, and 10⁻⁷ M; none of these concentrations had an effect on renal vascular resistance (Figure 1, lower panel).

**Norepinephrine Dose-Response**

Bolus injections of 100 μl NaH₂PO₄ 5 mM (i.e., vehicle) caused a transient increase of RVR by about 4% (given as 0 value in Figures 3–5). A dose-response curve to NE was established with and without L-PAF, and both curves were identical.

<table>
<thead>
<tr>
<th>Table 1. Renal Venous iPGE and iTXB</th>
<th>iPGE (ng/min)</th>
<th>iTXB (ng/min)</th>
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<tbody>
<tr>
<td>Control</td>
<td>2.4 ± 0.2</td>
<td>2.1 ± 0.4</td>
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<tr>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
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<tr>
<td>L-PAF (10⁻⁷ M)</td>
<td>3.9 ± 0.4</td>
<td>3.5 ± 0.5</td>
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**Figure 1.** Effects of L-PAF (upper panel) and D-PAF (lower panel) on renal vascular resistance (RVR) (mm Hg/ml perfusate flow x min⁻¹/lg kidney). Effect of L-PAF (10⁻⁷ M) plus indomethacin (5 x 10⁻⁶ M) is also shown. Data are means of 6 experiments ± SEM. Statistical comparisons were made to preinfusion RVR (C). *p<0.05; **p<0.01; #p<0.05 compared to L-PAF (10⁻⁷ M) without indomethacin.

**Angiotensin II Dose-Response**

With increasing doses of ang II, RVR rose in a linear fashion (r² = 0.98) (Figure 4). L-PAF shifted the ang II dose-response curve to the right and decreased the potency of ang II by about tenfold. INDO (5 μM) pretreatment of the isolated kidney significantly (p<0.05) increased the slope of the ang II curve (Figure 5). However, point by point the increases in RVR were not different from those without INDO (Figure 4, upper curve). After INDO pretreatment, L-PAF reduced the potency of ang II by 2–3 orders of magnitude.

**Discussion**

In the present study, L-PAF decreased perfusion pressure in the constant flow perfused isolated rat kidney. This fall in renal vascular resistance was enhanced by pretreatment with INDO. L-PAF has been shown to induce formation of arachidonate products in cultured rat mesangial cells,22 and we found L-PAF to increase iPGE and iTXB release from the isolated perfused rat kidney. Since PGE₂ and TXA₂ (thromboxane) were vasoconstrictors in our preparation,23 the enhanced fall of RVR in the presence of INDO was probably due to inhibition of the L-PAF-induced formation of these vasoconstrictive prostaglandins.

L-PAF may also have effects on vascular resistance by stimulating the formation of other arachidonate metabolites. For example, L-PAF has been found to increase leukotriene synthesis in the isolated rat lung,24 and the pulmonary vasoconstrictive responses of L-PAF have been shown to be antagonized by pretreat-

**Figure 2.** Renin release as a function of vasodilation by different concentrations of L-PAF. Vasodilation was significantly different from control at all L-PAF concentrations. Renin release was different from control only at 10⁻⁶ M. Data are means of 7–13 experiments ± SEM. Statistical comparisons were made to preinfusion levels. *p<0.05.

**Figure 3.** Norepinephrine dose-response curves in the presence (□—□) and absence (●—●) of L-PAF. Data are means of 6 experiments ± SEM. Optimal curve fitting was accomplished with an exponential function, r² = 0.98 and r² = 0.95, respectively.
ment with leukotriene antagonists such as diethylcarbamazine and FPL 55712. Since leukotrienes are also vasoconstrictors in the isolated perfused rat kidney, we cannot exclude the possibility that leukotrienes synthesized in response to L-PAF might limit the effects of L-PAF on RVR. However, we have been unable to detect any leukotriene-like activity in the renal venous effluent during L-PAF infusion using the isolated guinea pig ileum as bioassay (U. Schwertschlag, K. Stenmark, and R.C. Murphy, unpublished observations).

Renin release was increased by L-PAF only at the dose that produced maximal vasodilation. This might indicate that L-PAF has no direct effect on renin release and that the observed increase in renin release was secondary to the fall in perfusion pressure. This finding is similar to results by Finke et al who demonstrated that a critical threshold perfusion pressure was required to produce an increase of renin secretion in vivo. Although plasma renin activity was found to be increased by L-PAF in vivo in the dog and in the rat, these increases in renin activity may have been caused by a reflex increase in sympathetic tone secondary to hypotension caused by L-PAF. Thus, there is no evidence available that PAF can directly release renin.

Although the mechanism of the vasodilatory action of L-PAF is unknown, vascular relaxation by L-PAF has been reported to be independent of sympathetic tone and cholinergic, histaminergic H₁, or β-adrenergic receptors. Since there is no sympathetic innervation to the isolated perfused rat kidney, an interaction of L-PAF with this system would not be likely. Additionally, if L-PAF was an α-adrenergic antagonist, then the NE dose-response curve should have been shifted by L-PAF, which was not the case. This observation is in contrast to those by Smith et al and Caillard et al, who reported that L-PAF counteracted the NE-induced increase in peripheral resistance in the rat but had no effect on the response to ang II. These investigators concluded from their results that L-PAF was an α-adrenergic antagonist. In contrast to these findings, Kamitani et al, Masugi et al, and Faber et al found that in the rat in vivo, blood pressure changes produced by ang II, NE, and vasopressin were similarly shifted by L-PAF and concluded that L-PAF was a nonspecific vasodilator. Kamitani et al also showed no effect of L-PAF on the NE dose-response curve in the isolated rat aorta, and Cervoni et al found no evidence for α-adrenergic blockade using ligand binding techniques. Thus, the bulk of data, including our own, do not indicate that L-PAF is an α-adrenergic blocker.

In contrast to the lack of effect of L-PAF on NE-induced vasoconstriction, we found that the effect of ang II was inhibited by L-PAF. This is similar to the antagonism by L-PAF of ang-II-induced vasoconstriction in isolated perfused rat lung recently reported by Gillespie and Bowdy. It is of interest that Kamitani et al found that L-PAF relaxed KC1-induced contraction in the rat aorta while having no effect on NE-induced contraction. KC1-induced contraction is dependent on extracellular calcium, as is ang-II-induced vasoconstriction, whereas NE can induce vasoconstriction in the absence of extracellular calcium by mobilizing intracellular calcium stores. This may suggest that L-PAF interferes with calcium entry into the cells. The specific effect on ang-II-induced vasoconstriction in the isolated kidney contrasts with the nonspecific vasodilation seen in the rat in vivo. The reason for this difference is unknown.

The inhibition of ang-II–mediated contraction by L-PAF was further enhanced by pretreatment of the isolated perfused rat kidney with INDO. Thus, it appears that prostaglandin synthesis may attenuate not only the vasodilator effect of L-PAF alone but also the ability of
L-PAF to inhibit ang-II-mediated vasoconstriction. In this regard, the isolated rat kidney may not be representative of other species since in this model, PGE$_2$ was a vasoconstrictor and prostaglandin I$_2$ was either inactive or was a weak vasodilator. Although L-PAF had an action to reduce ang-II-induced vasoconstriction in this model, the L-PAF-mediated vasodilation observed in the absence of ang II is not likely related to inhibition of endogenous ang II since renin substrate was not present in the preparation. Thus L-PAF has a direct vasodilator effect in addition to its ability to inhibit ang-II-induced vasoconstriction. The specificity of L-PAF-induced vasodilation was addressed: the unnatural isomer D-PAF had no activity in all preparations. In addition, the L-PAF receptor antagonist BN 52021 at 10 $\mu$M completely abolished L-PAF–induced vasodilation (data not shown).

In conclusion, our data indicate that L-PAF in the isolated perfused rat kidney is a vasodilator and releases vasconstrictor cyclooxygenase products. Further, L-PAF has a selective effect to inhibit the vasocostriction produced by ang II without influencing NE-induced vasoconstriction. The mechanism of this selective effect is unknown. Finally, L-PAF stimulation of renin release in our model may be secondary to the vasodilation and consequent drop in perfusion pressure produced by L-PAF.

References


**KEY WORDS** • Platelet activating factor • vasodilation • renal circulation
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