Platelet-activating factor and solute transport processes in the kidney

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Platelet-activating factor (PAF) represents a group of 1-alkyl-2-acetyl-sn-glycero-3-phosphocholines that is a class of lipid mediators involved in many physiological (e.g., cell-cell signaling, regulation of blood pressure, and reproduction biology) and pathological (e.g., endotoxic/septic shock, immunological and inflammatory diseases, and ischemia and reperfusion injury) processes in the body (15, 28). Much of the research on PAF and the kidney has focused on the role of PAF in renal vascular and glomerular function. This is understandable given that PAF receptor antagonists can improve the outcome of many kidney diseases with a vascular or glomerular etiology (22, 31). Consequently, little is known about the possible actions of PAF on other aspects of kidney function. PAF is likely to act on tubular structures, because PAF receptor mRNA is present on all segments of the nephron, with particular abundance in the proximal tubule, comparable to the highest levels seen in the glomerulus (1). Although it is presently unclear whether tubule epithelial cells can synthesize PAF, it is well established that glomerular and renal medullary interstitial cells can generate PAF (36). Therefore, it is not an unreasonable expectation that the intrarenal generation of PAF may not only act locally on glomerular and renal medullary interstitial cells but also gain access to PAF receptors present on the tubular epithelium to influence cellular processes. In addition, circulating PAF may gain access to proximal tubular cells through glomerular filtration and/or be potentially synthesized and released from inflammatory blood cell types infiltrating into the kidney in pathological conditions.

Studies in anesthetized dogs and rats have generally shown that PAF administration is associated with a decrease in urinary water and electrolyte excretion that is secondary to PAF-induced falls in systemic blood pressure, extracellular fluid volume and cardiac output and/or PAF-induced falls in renal blood flow (RBF) and glomerular filtration rate (22, 35, 36, 40, 42). Furthermore, intravenous infusion of PAF at doses that had no effect on systemic and renal hemodynamics was associated with minimal changes in urinary

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excretion variables in anesthetized dogs (40). Similarly, studies employing isolated rat kidney perfused at constant pressure have shown that PAF infusion at doses that did not influence renal vascular resistance or glomerular filtration had no effect on urine flow rates (UVs) (32). Therefore, there are a number of reports from whole animal and isolated kidney studies suggesting that PAF has no measurable effect on tubular transport processes independent of changes in renal blood pressures and flows. Nevertheless, a direct action of PAF on renal tubules to modulate transcellular electrolyte transport has been suggested from measurements of transport function in microperfused isolated mouse thick ascending limbs, isolated rabbit cortical collecting tubules, and rat inner medullary collecting duct cell monolayers (2, 5, 19, 29).

Our initial purpose was to investigate whether PAF has a possible role to play in the regulation of rat kidney hemodynamic and urinary excretory function in vivo. The experimental strategy employed in these experiments was to infuse hypotensive doses of PAF into the renal artery of anesthetized rats and prevent blood pressure changes being transmitted to the kidney. This would allow us to 1) address the effect of PAF on rat renal vascular function, for which there is considerable confusion in the literature; and 2) determine whether PAF could influence urinary water and electrolyte excretion independent of blood pressure changes. The contribution of nitric oxide to the effect of PAF in the kidney was also examined, because nitric oxide can be a secondary mediator of PAF actions in the cardiovascular system (6, 7, 13, 15, 17). Our in vivo findings then led us to examine the direct actions of PAF on freshly isolated rat proximal tubules.

METHODS

**In vivo studies.** Adult male Wistar rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (60 mg/kg), and the trachea was cannulated to allow for a patent air passage. Catheters were then inserted into 1) the left jugular vein for the intravenous infusion of saline, pressor agents, or periodic injections of diluted anesthetic at a rate of 45 μl/min; 2) the right carotid artery for systemic blood pressure measurement; 3) the iliolumbar artery and maneuvered into the aorta and then into the left renal artery for the intrarenal administration of PAF and other agents at a rate of 55 μl/min; 4) the left femoral artery and advanced into the aorta just below the iliolumbar artery junction to estimate renal arterial blood pressure; and 5) the left ureter for the collection of urine. A silk thread was passed around the aorta rostral to the left kidney and attached to a screw device to allow constriction of the aorta and, thereby, regulation of renal arterial blood pressure. The left kidney was surgically and chemically (10% phenol in ethanol) denervated. RBF was recorded with a noncannulating flow probe placed around the left renal artery and connected to an electromagnetic flowmeter. A Valeo HPLC injection valve was interspersed into the intrarenal line, allowing the bolus administration (6 μl) of agents directly into the renal arterial circulation. Body temperature was monitored and maintained at ~37°C. At the end of all surgical procedures, 5 ml/kg of saline was administered intravenously over a 2-min period to replace surgical fluid losses. A minimum of 1 h was allowed for stabilization of cardiovascular and renal function parameters before the experimental protocol was begun.

**Intrarenal PAF infusion in rats with raised systemic blood pressure and regulated renal arterial blood pressure.** The experimental protocol consisted of four 25-min periods. A baseline period was followed by a second period (control) in which AVP was infused intravenously at 15 ng·min⁻¹·kg⁻¹ to raise systemic blood pressure and continued at this rate throughout the entire experiment while renal arterial blood pressure was maintained at pre-AVP-infusion levels. PAF was then infused intrarenally at 2.5 ng·min⁻¹·kg⁻¹ during the third period (experimental) and then terminated during the fourth period (recovery). This infusion dose rate of PAF was chosen because it produces a small fall in systemic blood pressure without altering RBF (11, 13) and thus provides an online confirmation of the biological activity of PAF. About 10 min before the start of the second period, the rats were given a combined intravenous AVP infusion (15 ng·min⁻¹·kg⁻¹) and intrarenal Nω-nitro-l-arginine methyl ester (l-NAME) infusion (~0.4 mg/kg bolus + 0.5 ng·min⁻¹·kg⁻¹), and this was maintained throughout the entire experiment. Urine was not collected during the first 5 min of a 25-min period so that preformed urine would escape the dead space in the collection system. At the end of each experiment, an in vivo calibration of the flow probe was undertaken using the left renal artery or left femoral artery and collecting timed blood samples.

**In vitro studies.** Proximal tubules were isolated from the renal cortex of anesthetized male Wistar rats by in vivo and in vitro collagenase digestion and Percoll density centrifugation. Dissociated tubules were placed in a closed, thermoregulated chamber, and tissue oxygen consumption (QO₂) was measured by a Clarke oxygen electrode. All procedures have been previously described in detail (12). QO₂ can be used as an online integrated index of sodium transport activity because of the tight coupling between Na⁺-K⁺-ATPase activity and mitochondrial oxidative phosphorylation (23). In experiments using receptor antagonists or signaling pathway inhibitors, these were added to the proximal tubule suspension (preincubated for 10–30 min) and chamber. All other drugs were added as 25-μl boluses to the tubule-containing chamber via its injection port. To minimize the variability of QO₂ from different tubule preparations (basal pressure and nystatin-stimulated QO₂ averaged 34 and 57 nmol O₂·min⁻¹·mg protein⁻¹, respectively), the effects of drug treatments were expressed as a percent change from baseline values. All drug solutions were prepared fresh daily, and their molar concentrations indicate the final concentrations achieved in the chamber.

The following experiments were performed in rat proximal tubule suspensions. First, concentration-QO₂ response curves were generated for PAF in untreated and nystatin-treated proximal tubules to ascertain the sensitivity and membrane location of PAF actions. Second, whether the actions of PAF were mediated by a PAF receptor and/or related to a PAF metabolite was determined. Third, whether the actions of PAF were due to an effect on cell respiratory processes and/or active Na⁺-K⁺-ATPase activity was examined. Fourth, possible intracellular signaling pathways mediating the functional effects of PAF on proximal tubule QO₂ were identified.

**Drugs.** We received a gift of BN-52021 (Institut Henri Beaufour). l-α-phosphatidylcholine, β-acetyl-γ-O-hexadecyl(PAF), D-α-phosphatidylcholine, β-acetyl-γ-O-hexadecyl (d-PAF), l-ωlysophosphatidylcholine, β-acetyl-ω-O-hexadecyl (lyso-PAF), l-NAME, 17-octadecenoic acid, and all other drugs were purchased from Sigma.
Table 1. Renal effects of PAF (2.5 ng·min⁻¹·kg⁻¹) in AVP-treated rats with RPP held constant

<table>
<thead>
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<th>Baseline</th>
<th>Control</th>
<th>Experimental</th>
<th>Recovery</th>
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<tr>
<td>MAP, mm/Hg</td>
<td>113.6 ± 2.3</td>
<td>141.4 ± 5.0b</td>
<td>125.8 ± 6.4d</td>
<td>136.2 ± 7.3</td>
</tr>
<tr>
<td>RPP, mm/Hg</td>
<td>109.9 ± 2.6</td>
<td>111.6 ± 2.0</td>
<td>109.8 ± 2.6</td>
<td>109.8 ± 2.8</td>
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<tr>
<td>RBF, ml·min⁻¹·kg⁻¹</td>
<td>25.2 ± 1.7</td>
<td>25.0 ± 1.8</td>
<td>29.2 ± 2.1f</td>
<td>26.1 ± 1.8</td>
</tr>
<tr>
<td>UV, μl·min⁻¹·kg⁻¹</td>
<td>13.4 ± 1.9</td>
<td>12.9 ± 1.8</td>
<td>20.8 ± 2.7f</td>
<td>17.0 ± 2.2f</td>
</tr>
<tr>
<td>UNaV, μeq·min⁻¹·kg⁻¹</td>
<td>2.0 ± 0.6</td>
<td>2.0 ± 0.7</td>
<td>5.5 ± 1.5f</td>
<td>4.8 ± 1.0f</td>
</tr>
<tr>
<td>UKV, μeq·min⁻¹·kg⁻¹</td>
<td>5.0 ± 0.6</td>
<td>4.8 ± 0.4</td>
<td>7.1 ± 1.0f</td>
<td>5.5 ± 0.5</td>
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Values are means ± SE (n = 7). PAF, platelet-activating factor; MAP, mean arterial blood pressure; RPP, renal arterial blood pressure; RBF, renal blood flow; UV, urinary flow rate; UNaV, urinary Na⁺ excretion rate; UKV, urinary K⁺ excretion rate; control, intravenous AVP infusion; experimental, intravenous AVP and intrarenal PAF infusion; recovery, intravenous AVP infusion. *P < 0.001 from baseline value using one-way ANOVA and Student-Newman-Keuls test. †P < 0.001 from control value using one-way ANOVA and Student-Newman-Keuls test. n = 7.

Statistics. Data are shown as means ± SE. Multiple groups were analyzed by one-way ANOVA with a paired Student’s t-test and between two groups using an unpaired Student’s t-test. Statistical significance was taken as a P value ≤0.05.

RESULTS

In vivo studies. Results obtained from AVP- and PAF-infused rats in the absence or presence of L-NAME (nitric oxide synthase inhibitor) are shown in Tables 1 and 2, respectively. As shown in Table 1, intravenous AVP infusions elevated mean arterial blood pressure (MAP) during which renal arterial blood pressure (RPP) was effectively regulated at pre-AVP/L-NAME infusion levels and RBF, UV, urinary sodium excretion (UNaV), and urinary potassium excretion (UKV) remained unchanged. Intrarenal PAF infusion at 2.5 ng·min⁻¹·kg⁻¹ resulted in a small fall in MAP, no change in RPP, and increased RBF (Fig. 1), UV, UNaV, and UKV by 17.0 ± 1.7, 62.1 ± 14.4, 188.2 ± 38.9, and 46.8 ± 13.4%, respectively. Complete recovery of MAP, RBF, and UKV were observed on cessation of the PAF infusion, whereas UV and UNaV remained elevated. Data in Table 2 show that MAP increased during simultaneous AVP and L-NAME infusion, which tended to be greater than that observed in rats infused with AVP alone. RPP was effectively regulated at pre-AVP/L-NAME infusion levels and RBF, UV, UNaV, and UKV fell by 29.7 ± 1.8, 41.7 ± 6.6, 32.3 ± 17.7, and 48.8 ± 6.5%, respectively. Intrarenal infusion of PAF at 2.5 ng·min⁻¹·kg⁻¹ did not alter MAP or RPP and was associated with a very small decrease in RBF (~5%) that continued to fall after termination of the PAF infusion and likely reflected a failure to achieve a plateau phase during nitric oxide synthase inhibition. The PAF infusion period was also associated with an increase in UNaV and UKV of 113.5 ± 41.1 and 36.7 ± 13.2%, respectively, as well as a trend toward an increased UV (P = 0.054, one-way ANOVA and Student’s t-test). Urinary water and solute excretion rates remained elevated on cessation of the PAF infusion.

Figure 2 depicts data showing that the PAF-induced rise in urinary water excretion rate and UNaV was significantly reduced in L-NAME-treated rats, with a similar trend observed for UKV (P = 0.082 using an unpaired Student’s t-test). Similar RBF and urinary excretory responses were observed in the absence or presence of L-NAME during an intrarenal infusion of PAF at the higher dose rate of 10 ng·min⁻¹·kg⁻¹ (not shown).

In vitro studies. Addition of PAF to nontreated fresh suspensions of rat proximal tubules resulted in a concentration-dependent inhibition of basal QO2 with a threshold concentration between 0.1 and 1 nM (Fig. 3). To examine whether PAF interfered with sodium exit from the proximal tubule cell, we treated cells with nystatin (sodium ionophore), which allows sodium to freely enter the cell and results in ~70% increase in QO2 due to enhanced basolateral Na⁺-K⁺-ATPase activity. Under nystatin-stimulated conditions, the proximal tubules were more sensitive to the inhibitory actions of PAF, with a threshold concentration of be...

Table 2. Renal effects of PAF (2.5 ng·min⁻¹·kg⁻¹) in AVP- and L-NAME-treated rats with RPP held constant

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<th>Baseline</th>
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<th>Experimental</th>
<th>Recovery</th>
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<tr>
<td>MAP, mm/Hg</td>
<td>117.3 ± 3.2</td>
<td>160.6 ± 4.5a</td>
<td>156.3 ± 4.1</td>
<td>157.1 ± 2.5</td>
</tr>
<tr>
<td>RPP, mm/Hg</td>
<td>114.8 ± 3.6</td>
<td>115.6 ± 3.6</td>
<td>115.1 ± 3.4</td>
<td>115.8 ± 3.5</td>
</tr>
<tr>
<td>RBF, ml·min⁻¹·kg⁻¹</td>
<td>25.3 ± 0.8</td>
<td>17.8 ± 0.8a</td>
<td>16.8 ± 0.8a</td>
<td>15.7 ± 0.6a</td>
</tr>
<tr>
<td>UV, μl·min⁻¹·kg⁻¹</td>
<td>12.5 ± 1.0</td>
<td>7.1 ± 0.6a</td>
<td>9.2 ± 1.0</td>
<td>9.2 ± 0.9a</td>
</tr>
<tr>
<td>UNaV, μeq·min⁻¹·kg⁻¹</td>
<td>1.4 ± 0.3</td>
<td>0.7 ± 0.1a</td>
<td>1.3 ± 0.1a</td>
<td>1.4 ± 0.2a</td>
</tr>
<tr>
<td>UKV, μeq·min⁻¹·kg⁻¹</td>
<td>5.3 ± 0.5</td>
<td>2.7 ± 0.4a</td>
<td>3.4 ± 0.3a</td>
<td>3.7 ± 0.4a</td>
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Values are means ± SE; n = 7 (blood pressure and flow data) or 6 (urinary excretion data). L-NAME, N⁷-nitro-L-arginine methyl ester. control, intravenous AVP and intrarenal L-NAME; experimental, intravenous AVP and intrarenal L-NAME and PAF infusion; recovery, intravenous AVP and intrarenal L-NAME infusion. *P < 0.001 from baseline value using one-way ANOVA and Student-Newman-Keuls test. †P < 0.05 from baseline value using one-way ANOVA and Student-Newman-Keuls test. ±P < 0.05 from control value using one-way ANOVA and Student-Newman-Keuls test. §P < 0.001 from control value using one-way ANOVA and Student-Newman-Keuls test.
between 1 and 10 pM (Fig. 3). These results suggested that one action of PAF could be to inhibit basolateral Na⁺-K⁺-ATPase activity. Subsequent studies employed 1 nM PAF because this concentration produced a near maximal inhibition of proximal tubule QO₂.

Figure 4 shows that only the levorotatory isomer of PAF was capable of inhibiting nystatin-stimulated proximal tubule QO₂ whereas vehicle and the dextrorotatory isomer of PAF (D-PAF) had no effect. This suggested that PAF acts through a stereospecific receptor site to exert its inhibitory effect on QO₂. We also found that the major product of PAF metabolism, lyso-PAF, did not alter nystatin-stimulated QO₂, implying that PAF itself was responsible for the observed biological response (Fig. 4).

Addition of 5 μM FCCP (mitochondrial oxidative phosphorylation uncoupler) to control proximal tubules increased basal QO₂ by 217 ± 42% (P < 0.05, n = 4). In a separate group of experiments, FCCP caused a similar increase in basal QO₂ of 198 ± 22% (P < 0.001, n = 8), and the subsequent addition of 1 nM PAF was...
without effect (−2 ± 2%, n = 8). Conversely, control proximal tubules treated with 1 nM PAF decreased basal QO2 by 27 ± 7% (P < 0.05, n = 5) and did not impair the increase in QO2 on subsequent addition of FCCP (237 ± 27%, P < 0.001, n = 5). These findings suggest that the inhibitory effect of PAF on proximal tubule function was not due to an inhibition of mitochondrial respiratory activity. Treating proximal tubules with 5 mM ouabain (Na+/K+-ATPase inhibitor) reduced basal QO2 by 33 ± 5% (P < 0.001, n = 10) and abolished the stimulatory action of nystatin, confirming that the nystatin effect on QO2 was by increasing Na+/K+-ATPase activity. Conversely, ouabain reduced nystatin-stimulated proximal tubule QO2 by 58 ± 2% (P < 0.001, n = 9) and abolished the inhibitory action of PAF. Together, these findings indicate that PAF had direct actions on proximal tubule epithelium and that at least one effect was to suppress an ouabain-inhibitable component of transcellular sodium transport, namely, basolateral Na+/K+-ATPase pump activity.

To pharmacologically identify whether a receptor mediated the tubular action of PAF, we examined the ability of the phospholipid to inhibit nystatin-stimulated cellular transport in tubules preincubated with the PAF receptor antagonist BN-52021. As depicted in Fig. 4, we found that the inhibitory action of PAF on proximal tubule QO2 was abolished by 1 μM BN-52021. This effect of BN-52021 appeared to be specifically related to blocking the PAF receptor, because it did not affect the inhibitory action of 1 μM ANG IV on nystatin-stimulated proximal tubule QO2 (ANG IV, 19.5 ± 1.9%, vs. ANG IV + BN-52021, 18.2 ± 0.6%, n = 2 each), an ANG IV response known to be mediated by the angiotensin AT1 receptor (14).

We then examined possible intracellular signaling mechanisms involved in the inhibitory action of PAF on tubule QO2. The contribution of the nitric oxide-guanylate cyclase pathway was assessed by preincubating proximal tubules with either 10 μM methylene blue (guanylate cyclase inhibitor) or 100 μM L-NAME (nitric oxide synthase inhibitor). Curiously, we observed that the increase in QO2 on nystatin administration was quickly dissipated in tubules pretreated with methylene blue only. However, both methylene blue and L-NAME treatments did not interfere with the inhibitory action of PAF on nystatin-stimulated proximal tubule QO2 (Fig. 5). In contrast, phospholipase A2 (PLA2) inhibition with quinacrine (data for 0.1 and 1 mM were similar and combined), cytochrome-P-450 monooxygenase inhibition with SKF-525A (50 μM proadifen) or 17-octadecynoic acid (specific suicide inhibitor of cytochrome-P-450 fatty acid ω-hydroxylase, data for 1 and 10 μM were similar and combined) resulted in a 40–60% reduction in the effect of PAF on nystatin-stimulated proximal tubule QO2 (Fig. 5). Basal and nystatin-stimulated QO2 were similar in all drug-incubated tissues (the only exception was a depressed nystatin response in methylene blue-treated tissues) compared with untreated control tissue.

DISCUSSION

Early reports from our laboratory demonstrated that PAF administered as a bolus (10) or infusion (11) into the renal artery of anesthetized rats resulted in a decrease in renal vascular resistance and reactivity, and we had speculated on the involvement of endothelium-derived relaxing factor (nitric oxide) in these PAF-mediated processes. Later, we demonstrated an important role for nitric oxide in the PAF-induced attenuation of ANG II-mediated vasoconstriction in the rat renal vascular bed (13). The present study also suggests that nitric oxide contributes to the renal vasodilatory and systemic hypotensive responses to intrarenal PAF infusion in the rat.

An intravenous infusion of AVP was used to raise systemic blood pressure and had minimal impact on RBF, highlighting the known insensitivity of the rat renal vasculature to the vasoconstrictor influences of intravenous AVP infusions (33, 41). Recently, it was proposed that renal sympathoinhibition accounts for the inability of intravenous pressor doses of AVP to decrease RBF in conscious rats, because renal denervation unmasked a vasopressin V1 receptor-mediated fall in RBF (33). Our findings in anesthetized rats would not support this proposal, because we found no evidence of an AVP-mediated decrease in RBF under conditions in which the associated increase in blood pressure was prevented from being transmitted to the denervated kidney. Therefore, we conclude that it is unlikely that the renal vasodilation induced by PAF...
was simply due to antagonism of a renal vasoconstrictor effect of AVP. In addition, PAF is a relatively poor inhibitor of the renal vasoconstriction induced by the intrarenal injection of high pharmacological doses of AVP (11). In previous studies, we could only show weak and transient renal vasodilatory responses to nonhypotensive intrarenal infusion rates of PAF (11). However, the experimental setup employed in the present study (preventing the PAF-induced systemic hypotension and associated increase in sympathetic outflow from being transmitted to the denervated kidney) allowed us to reveal a strong and sustained kidney vasodilatory response to intrarenal PAF infusions in normal rats in vivo.

To our knowledge, the present results are the first to demonstrate that PAF infusion into the rat renal artery can be associated with a significant diuresis, natriuresis, and kaliuresis, the magnitude of which was attenuated by nitric oxide synthase inhibition. However, opposing our in vivo findings is the report that the intrarenal infusion of PAF into anesthetized rats resulted in a decrease in urinary water excretion rate and UNaV that was largely due to PAF-induced falls in RBF and glomerular filtration rate (42). Although we cannot readily explain these hemodynamic discrepancies, it should be noted that a number of investigators have demonstrated that PAF can possess vasodilatory properties in the kidney (10, 17; see also citations in Ref. 11) and inhibit solute reabsorptive processes by acting at the level of the nephron (2, 5, 19, 29). In the present study, we regulated renal arterial blood pressure such that the AVP-induced rise in systemic blood pressure was not transmitted to the denervated kidney and found that urinary water and solute excretion remained unchanged. The actions of AVP in the kidney have been reported to range from a decrease to an increase in urinary water excretion rate and UNaV, as well as any conceivable combination of urinary excretory responses between these two extremes (3, 8, 9, 18, 20). This highlights the complex and multifactorial nature of AVP actions in the kidney to regulate urinary water and solute excretion, including integrated effects on blood pressure, sympathoinhibition, renal hemodynamics, and tubular function (3, 8, 9, 18, 20). Although one would expect the direct stimulatory actions of AVP on tubular transport processes to result in a decrease in urinary water excretion rate and UNaV (3), investigators have also reported that nonpressor doses of AVP infused into the kidney can be associated with no change in both renal hemodynamics and urinary water excretion rate and/or UNaV (8, 20). Consequently, we conclude that the subsequent intrarenal infusion of PAF was likely responsible for the observed increase in urinary water and solute excretion in the anesthetized rat. In addition to a significant role for nitric oxide in the glomerular and vascular actions of PAF in the kidney (13, 17, 24), the renal nitric oxide pathway is also known to be natriuretic by influencing several kidney systems, including the cortical and medullary microcirculation, interstitial hydrostatic pressure, and tubular transport processes (21, 25, 37). In the present study, blockade of nitric oxide synthesis was found to dramatically attenuate the ability of PAF to elicit a diuresis, natriuresis, and kaliuresis and suggests either a direct or indirect role of nitric oxide in these urinary water and electrolyte responses.

Because our in vivo results do not allow us to dissociate the urinary excretory responses from the PAF-mediated renal hemodynamic effects, we examined the direct actions of PAF on isolated rat proximal tubules, because this nephron segment is a major site for the reabsorption of sodium and water and contains abundant PAF receptor mRNA (1). No information is available on the direct actions of PAF on this segment of the nephron. We found that PAF inhibited proximal tubule QO2 without influencing mitochondrial uncoupled QO2 rates, suggesting a reduction in energy-dependent transcellular solute transport. Proximal tubules were treated with nystatin (sodium ionophore that functionally bypasses the rate-limiting step of sodium entry into the cell), allowing an indirect assessment of sodium efflux from the cell via the basolateral Na+/K+-ATPase pump (23). Nystatin treatment increased the sensitivity of the proximal tubules to the inhibitory actions of PAF, suggesting that the lipid may act to reduce basolateral Na+/K+-ATPase pump activity. This conclusion is supported by the observation that ouabain, an Na+/K+-ATPase inhibitor, prevented the inhibitory effect of PAF on nystatin-stimulated proximal tubule QO2, as well as several reports that PAF can inhibit membrane Na+/K+-ATPase activity in a number of cell types (4, 16). In addition, endogenous PAF was found to contribute to the decrease in renal cortex Na+/K+-ATPase activity after reperfusion of the ischemic rat small intestine, an effect that could be blocked by the PAF receptor antagonist BN-52021 (39). Our results obtained with i-PAF, lyso-PAF, and PAF in the presence of BN-52021 would also be consistent with a PAF receptor mediating the biological response of PAF in isolated rat proximal tubules.

The dilatory action of PAF on the renal vasculature is largely dependent on the nitric oxide pathway (present study and Ref. 17), and others have shown that activation of guanylate cyclase and the subsequent rise in cGMP mediate many actions of nitric oxide in the body (26). We found that nitric oxide blockade with l-NAME attenuated the natriuretic and diuretic response to intrarenal PAF infusion in the rat in vivo. This reduced urinary excretory response could be due to l-NAME preventing PAF-induced renal vasodilation and/or reducing a natriuretic/diuretic action of PAF at the level of the nephron. Both nitric oxide and cGMP can be produced in the proximal tubule, with both capable of inhibiting proximal tubule Na+/K+-ATPase activity (21). However, we found that methylene blue and l-NAME at concentrations known to inhibit tubular guanylate cyclase and nitric oxide synthase activity, respectively, did not interfere with the inhibitory actions of PAF on rat proximal transport processes. This would seem to rule out nitric oxide as a candidate for mediating the direct inhibitory effect of PAF on proximal tubule solute reabsorptive function.
Although it is presently unknown whether PAF can stimulate nitric oxide biosynthesis and/or guanylate cyclase activity in the proximal tubule, the phospholipid can stimulate cGMP in glomerular cells when coincubated with endothelial cells (24) and inhibit solute reabsorptive function of the medullary thick ascending limb (mTAL) via a cGMP-dependent pathway (29). Our results do not rule out the possibility that PAF-stimulated nitric oxide/cGMP synthesis from the renal microcirculation and other nonproximal tubule sources may inhibit proximal tubule solute reabsorptive processes in vivo (21). Further downstream of the proximal tubule, investigators have demonstrated that PAF can reduce the ability of vasopressin to increase transepithelial resistance (a measure of active solute reabsorption) in cultured rat inner medullary collecting duct cells (19) or microperfused rabbit cortical collecting ducts (5). This would likely result in a natriuresis and diuresis in vivo, because this terminal segment of the nephron is important in regulating the final amount of sodium and water that is excreted in the urine.

The arachidonic acid pathway appeared to be of major importance in the direct actions of PAF in the rat proximal tubule. Inhibitors of PLA2 and cytochrome-P-450 monooxygenase were able to significantly attenuate the inhibitory actions of PAF on proximal tubule Na+/K+-ATPase activity through a PKC-dependent phosphorylation of the γ-subunit of ATPase, whereas nonproximal tubule sources may inhibit proximal tubule sodium reabsorption, perhaps PAF-stimulated nitric oxide synthesis from a nonproximal tubule source leading to a reduction in proximal tubule sodium reabsorption, 3) PAF activation of proximal tubule PLA2 and cytochrome-P-450 monooxygenase pathways to attenuate sodium reabsorption processes, and 4) PAF-mediated inhibition of solute reabsorption in the mTAL and collecting ducts.

In conclusion, a number of factors may potentially contribute to the PAF-induced increase in urinary water and electrolyte excretion observed in vivo and include 1) PAF-induced increase in kidney blood flow that was secondarily mediated by nitric oxide, 2) perhaps PAF-stimulated nitric oxide synthesis from a nonproximal tubule source leading to a reduction in proximal tubule sodium reabsorption, 3) PAF activation of proximal tubule PLA2 and cytochrome-P-450 monooxygenase pathways to attenuate sodium reabsorption processes, and 4) PAF-mediated inhibition of solute reabsorption in the mTAL and collecting ducts.

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PAF AND RENAL SOLUTE TRANSPORT


