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Renal Metabolism and Urinary Excretion of Platelet-activating Factor in the Rat*

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The origin of platelet-activating factor (PAF) in the urine remains ill defined. The present study documents that [^{3}H]PAF (3.5 μCi) injected into the renal artery of isolated control rat kidney preparations perfused at constant pressure with a cell-free medium containing 1% bovine serum albumin (BSA) was excreted in negligible amounts (0.034%) in the urine, whereas 6% was retained by the kidney. When kidneys were perfused with a BSA-free medium, 0.029 and 71% of the total radioactivity added to the perfusate was recovered in the urine and in the renal tissue, respectively. [^{3}H]PAF urine excretion in proteinuric kidneys from adriamycin-treated rats was still negligible (0.015%). Analysis of the renal tissue-retained radioactivity in control and proteinuric kidneys perfused with 1% BSA indicated metabolism into long chain acyl-*sn*-glycero-3-phosphorylcholine species, lyso-PAF, glycerols, and intact PAF. Thin layer chromatography analysis of [^{3}H]glycerol fraction in these renal extracts showed two major components comigrating with 1-*O*-alkylglycerol and 1-*O*-alkyl-2-fatty acylglycerol. Isolated proximal tubules, but not glomeruli from nephrotic rats exposed to increasing concentrations of BSA (0–4%), had a higher PAF uptake than control tubules for BSA concentrations ranging from 0 to 0.1%.

Our findings in the isolated perfused kidneys indicate that, in normal conditions, circulating PAF is excreted in the urine in negligible amounts and that the altered glomerular permeability to proteins does not affect this excretion rate. Moreover, analysis of renal tissue radioactivity documented that the renal metabolism of PAF is comparable in control and nephrotic kidneys.

Platelet-activating factor (PAF),[1] or 1-*O*-alkyl-2-acetyl-GPC, is an unique phospholipid with a broad range of biological activities that may be relevant in the development of inflammatory reactions (1, 2). In addition to its proaggregatory and proinflammatory activities, PAF has been suspected to play an important role in glomerulonephritis, favoring the process of immune complex formation in glomeruli (3) and possibly participating in the development of proteinuria (4, 5).

Urine collected from experimental animals and humans under physiological conditions contains a measurable amount of PAF (6, 7). Evidence is now available indicating that the urinary excretion of PAF is increased in experimental[2] and human lupus nephritis (6) during the acute phase of the disease concomitant with accumulation of platelets and polymorphonuclear cells in the glomerular capillary lumens.

However, the source of PAF detected in the urine remains ill defined. Theoretically, urinary PAF could originate from the systemic circulation after activation of circulating cells or from the resident kidney cells. In this context, it has been reported that isolated perfused rat kidneys challenged with ionophore A23187 release PAF in the venous effluent, suggesting that kidney possesses the enzymatic machinery to synthesize PAF (8). This possibility is further supported by the observation that isolated glomeruli, as well as mesangial cells in culture, generate PAF in response to appropriate stimuli (9–11).

Since PAF has a high affinity to plasma proteins, particularly albumin (12, 13), one would expect that, in renal diseases characterized by massive proteinuria, the increased urinary excretion of PAF could be the result of an elevated protein flux through the glomerular capillary wall. Alternatively, in this condition, the exaggerated PAF urinary excretion might reflect an increased renal synthesis of the phospholipid.

The present study was designed to investigate (*a*) whether exogenous PAF injected into the renal artery can be recovered in the urine, (*b*) whether increased protein flux through the glomerular capillary barrier is associated with enhanced excretion of exogenous PAF, and (*c*) whether the uptake and the metabolic fate of PAF are comparable in control and nephrotic kidneys.

These issues have been addressed in isolated perfused rat kidney preparation.

MATERIALS AND METHODS[3]

RESULTS

[^{3}H]Ether Lipid Distribution in Isolated Perfused Rat Kidney—Fig. 1 depicts the time course of radioactivity recovery in venous effluent following the addition of [^{3}H]PAF to the perfusion medium. In all three groups of isolated kidney

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[1] The abbreviations used are: PAF, platelet-activating factor; GPC, *sn*-glycero-3-phosphorylcholine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PS, phosphatidylserine; LPS, lysophosphatidylserine; PI, phosphatidylinositol; SPH, sphingomyelin; HPLC, high performance liquid chromatography; BSA, bovine serum albumin; ADR, adriamycin.

[2] M. Morigi, D. Macconi, E. Riccardi, P. Boccardo, P. Zilio, T. Bertani, and G. Remuzzi, *J. Pharmacol. Exp. Ther.*, submitted for publication.

[3] The "Materials and Methods" are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

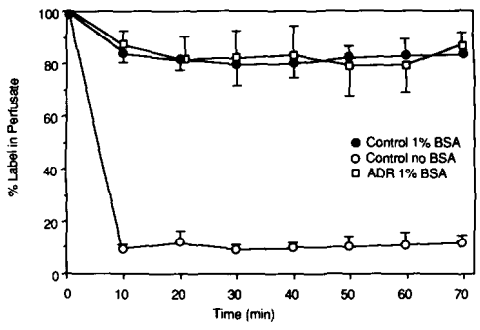

FIG. 1. **Time course for radioactivity recovery in venous effluent following [^{3}H]PAF injection into the renal artery of isolated control kidneys perfused with or without 1% BSA and proteinuric kidneys.** Values are means of three experiments ± S.D.

TABLE I

[^{3}H]ether lipid distribution (%) in isolated rat kidney perfused with [^{3}H]PAF

Values are means ± S.D. (n = 3). Entries represent the percentage of total injected radioactivity.

	Control (1% BSA)	Control (no BSA)	ADR (1% BSA)
	%	%	%
Venous effluent	83.6 ± 1.42	11.85 ± 2.65[a]	85.33 ± 5.97
Urine	0.034 ± 0.01	0.029 ± 0.005	0.015 ± 0.005[b]
Kidney	5.62 ± 1.2	71.4 ± 7.92[a]	7.89 ± 0.5

[a] $p < 0.01$ *versus* control (1% BSA) and ADR (1% BSA).
[b] $p < 0.05$ *versus* control (1% BSA) and control (no BSA).

preparations, the amount of tritium in the recirculating perfusate decreased during the perfusion, with minimum values obtained 10–20 min after exposure of kidneys to [^{3}H]PAF. Thereafter, the percentage of radioactivity residual in the perfusate remained quite constant until the end of the experiment.

As shown in Table I, in the control isolated kidney perfused with 1% BSA, when [^{3}H]PAF was added to the perfusion medium, the extractable radiolabel could be recovered at the end of the perfusion with an 89% yield of administered dose from venous effluent, urine, and tissue extract. The remainder of the radioactivity appeared to remain absorbed to the tubing of the perfusion apparatus. After 70 min of perfusion, 84% of the radiolabel was recovered in the venous effluent, whereas a negligible amount of radioactivity was found in the urine. Another 6% of the added radioactivity was recovered in the extract of the kidney homogenate. When isolated kidneys were perfused with a BSA-free medium, a significantly ($p <$ 0.01) lower amount of extractable radioactivity was observed in the venous effluent (Table I). However, the amount of radiolabel that appeared in the urine during the 70-min time course averaged only 0.029% of the [^{3}H]PAF added to the perfusate, a value comparable to that reported for kidneys perfused with 1% BSA. The radiolabel component was greatest in the kidney homogenate with a value of 71% yield of the administered dose (Table I). Isolated kidneys from ADR-treated rats perfused with 1% BSA medium were markedly proteinuric. Values of urinary protein excretion were 103 ± 41 μg/min, significantly higher than in isolated kidneys from control rats perfused with 1% BSA (13.5 ± 0.7 μg/min, $p <$ 0.05). In this condition, the radioactivity recovered in the venous effluent after 70 min of perfusion with medium containing [^{3}H]PAF was, on average, 85% of the administered dose (Table I). The amount of radiolabel found in the urine was still negligible, but significantly ($p < 0.05$) lower than that reported in control kidneys perfused with or without 1% BSA (Table I). However, since in proteinuric kidneys the urine output was markedly reduced (ADR kidneys, 36.93 ± 11.6; control (1% BSA), 107.36 ± 15.43; control (no BSA), 148.1 ± 39.2 μl/min), the radioactivity recovered in the urine, when factored per milliliter of urine, was comparable to that estimated in control kidneys perfused with 1% BSA (ADR kidneys, 0.0058 ± 0.0003%; control (1% BSA), 0.0045 ± 0.001%), but significantly ($p < 0.01$) higher than in control kidneys perfused with BSA-free medium (0.0029 ± 0.0003%). The amount of radiolabel that appeared in the proteinuric kidney homogenate, by the end of the study, was numerically higher than that in control kidneys perfused with 1% BSA, but the difference did not reach statistical significance (Table I).

Characterization of Venous Effluent and Renal Tissue-retained [^{3}H]PAF Metabolites—Fig. 2 shows a representative fractionation of radiolabeled phospholipid extract from venous effluent obtained at the end of the experiment in control isolated kidneys perfused with 1% BSA. HPLC analysis yielded a radiochromatogram containing two peaks. The largest peak, approximately 85–90% of total radiolabel, had the same retention time as authentic [^{3}H]PAF. The minor peak corresponded to 5% of total recovered radioactivity and comigrated with [^{3}H]lyso-PAF. Virtually identical results were obtained for the distribution of venous effluent phospholipid radioactivity from proteinuric kidneys, with two peaks of [^{3}H]PAF (90%) and [^{3}H]lyso-PAF (4%), respectively. Despite being qualitatively similar, the radiochromatogram obtained from HPLC analysis of the venous effluent phospholipid extract from control kidneys perfused with BSA-free medium showed a higher percentage of [^{3}H]lyso-PAF (about 30%) than in control kidney perfused with 1% BSA and proteinuric kidneys. The remaining unmetabolized [^{3}H]PAF averaged 60%.

A comparable percentage of [^{3}H]glycerols was found in the venous effluent from control kidneys perfused with 1% BSA (5%) and from proteinuric kidneys (4%). The percentage of [^{3}H]glycerol was slightly higher in the venous effluent from kidneys perfused with BSA-free medium (about 6%), as compared with the other two groups of isolated kidneys; however, this difference did not reach statistical significance.

Fig. 3 shows a representative radiochromatogram of the semipurified kidney phospholipid extract from control isolated kidney perfused with 1% BSA. HPLC analysis revealed a major peak (55% of the tissue radiolabel), with the same retention time as authentic 1-*O*-alkyl-2-acyl-GPC and two additional peaks (15 and 12%) comigrating with authentic

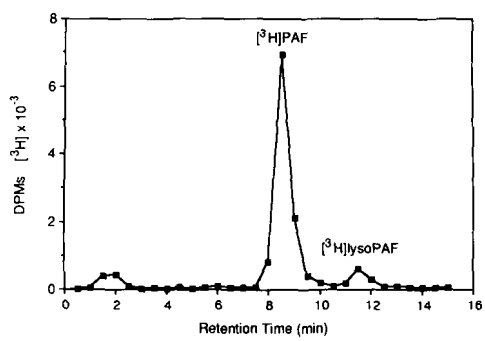

FIG. 2. **HPLC radiochromatogram of the semipurified renal venous effluent after recirculating perfusion with [^{3}H]PAF in control isolated rat kidney perfused with 1% BSA.** The perfusate was purified and analyzed by straight phase HPLC as described under "Materials and Methods." Solvent system, acetonitrile:methanol:phosphoric acid (130:5:1.5, v/v/v); flow rate, 1.5 ml/min. *DPM*, disintegrations/min.

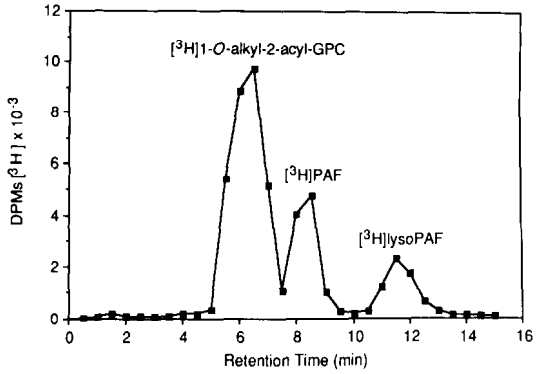

FIG. 3. **HPLC radiochromatogram of the semipurified extract from control rat kidneys after perfusion with [^{3}H]PAF in a medium containing 1% BSA.** The renal extract was purified and subjected to HPLC as described under "Materials and Methods." Solvent system, acetonitrile:methanol:phosphoric acid (130:5:1.5, v/v/v), flow rate, 1.5 ml/min. Fractions (0.75 ml) were collected and radioactivity was counted. *DPM*, disintegrations/min.

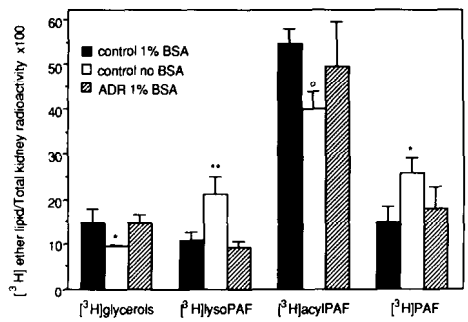

FIG. 4. **[^{3}H]PAF metabolism in isolated perfused rat kidneys after [^{3}H]PAF injection into the renal artery.** Tissues from control kidneys (perfused with medium with or without 1% BSA) and from proteinuric kidneys perfused with 1% BSA were purified and analyzed as described under "Materials and Methods." Results are expressed as the percentage of total tissue-retained radioactivity. Values are means ± S.D. ($n = 3$). **, $p < 0.01$ *versus* control with 1% BSA and ADR with 1% BSA. *, $p < 0.05$ *versus* control with 1% BSA and ADR with 1% BSA. °, $p < 0.05$ *versus* control with 1% BSA. *[^{3}H] acyl-PAF*, [^{3}H]alkyl-2-acyl-GPC.

PAF and lyso-PAF standards, respectively. Similarly, the metabolite profile of the phospholipid extract from proteinuric kidneys showed three peaks of radioactivity with retention times characteristic of 1-*O*-alkyl-2-acyl-GPC (50%), PAF (18%), and lyso-PAF (10%) (Fig. 4). By contrast, in control kidneys perfused with BSA-free medium, a significantly higher percentage of [^{3}H]lyso-PAF (22%, $p < 0.01$) and unmetabolized [^{3}H]PAF (37%, $p < 0.05$), together with a lower percentage of ^{3}H-labeled 1-*O*-alkyl-2-acyl-GPC (40%, $p < 0.05$) than in control kidneys perfused with 1% BSA, was recovered (Fig. 4).

A comparable percentage of [^{3}H]glycerols was found in control kidneys perfused with 1% BSA (15%) and in the proteinuric kidneys (15%), whereas the value was significantly lower in the control kidneys perfused with BSA-free medium, averaging 9% ($p < 0.05$).

Further analysis of the [^{3}H]glycerol fraction by thin layer chromatography showed one major component ($R_F = 0.29$) corresponding to 40–50% of the radiolabel and comigrating with authentic 1-*O*-hexadecyl-*rac*-glycerol. A second component, accounting for 40–50% of the radioactivity, migrated with an R_F (0.63) characteristic of synthetic 1-palmitoyl-2-arachidonylglycerol and corresponded to 1-*O*-alkyl-2-fatty acylglycerol. No metabolite corresponding to 1-*O*-hexadecyl-2-acetylglycerol was detected. A minor component, representing 8–9% of radioactivity, was also found close to the origin, possibly corresponding to the small aliquot of phospholipid metabolites coeluted with [^{3}H]glycerols in the chloroform fraction of the silicic acid column.

HPLC Analysis of Endogenous Renal Phospholipids—As shown in Fig. 5, fractionation of semipurified renal tissue extract by HPLC analysis yielded a chromatogram containing all the major phospholipid classes, namely phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, lysophosphatidylcholine, and sphingomyelin, either in control kidneys perfused with 1% BSA (*panel A*), without BSA (*panel B*), or in proteinuric kidneys (*panel C*). An unknown peak, whose retention time did not correspond to any of the phospholipid standards injected, was also seen, except in control kidneys perfused without BSA. A marked reduction of the peak comigrating with lysophosphatidylcholine standard was found in proteinuric but not control kidneys.

[^{3}H]PAF Uptake by Isolated Glomeruli and Proximal Tubules—Table II shows the effect of increasing concentrations of BSA on [^{3}H]PAF uptake by isolated glomeruli and proximal tubules obtained from control and ADR-treated rats. Incubation of isolated glomeruli from control rats for 30 min resulted in a progressive decline in [^{3}H]PAF uptake with increasing concentrations of BSA in the medium. Identical results were obtained in isolated glomeruli from ADR rats, whose uptake values were not significantly different as compared with controls. Similarly, [^{3}H]PAF uptake progressively

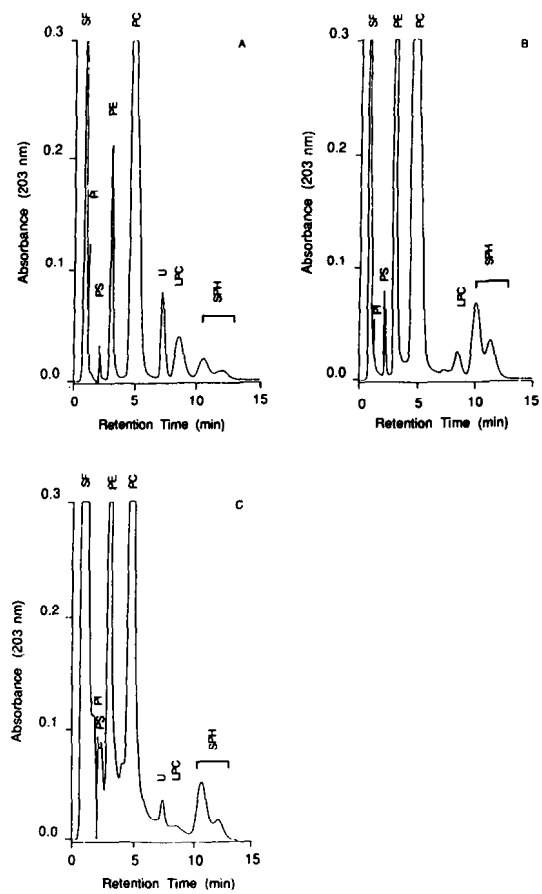

FIG. 5. **HPLC analysis of endogenous phospholipids extracted from renal tissue and chromatographed as described under "Materials and Methods."** *A*, control isolated rat kidney perfused with medium containing 1% BSA; *B*, control isolated rat kidney perfused with BSA-free medium; *C*, isolated perfused rat kidney from ADR-treated rat (1% BSA). *SF*, solvent front; *U*, unknown.

TABLE II

Effect of bovine serum albumin (BSA) on [^{3}H]PAF uptake by isolated glomeruli and proximal tubules

Values are means ± S.D. (n = 3).

BSA	Isolated glomeruli		Isolated proximal tubules	
	Control	ADR	Control	ADR
%	% uptake/mg protein		% uptake/mg protein	
0	47.26 ± 6.62	43.60 ± 2.75	55.35 ± 0.75	88.72 ± 4.64[a]
0.1	39.65 ± 1.88	39.12 ± 3.08	33.09 ± 4.69	44.93 ± 0.72[a]
1	14.61 ± 0.71	16.64 ± 2.43	9.31 ± 0.30	15.38 ± 0.21
4	6.81 ± 0.35	7.94 ± 0.96	5.64 ± 0.76	7.68 ± 2.05

[a] $p < 0.01$ *versus* control.

decreased in proximal tubular suspensions from both control and ADR-treated rats exposed to increasing concentrations of BSA. However, a significantly ($p < 0.01$) higher percentage of radiolabel uptake was found in isolated tubules from ADR-treated animals than in control rats at the final concentration of BSA in the incubation medium of 0 and 0.1% (Table II).

DISCUSSION

The present study documents that exogenous PAF added to the perfusion fluid of rat isolated perfused kidney was excreted in the urine only in negligible amounts. In these experiments, the amount of PAF remaining in the perfusion medium *versus* the amount taken up by the kidney was a function of the presence of albumin in the perfusate. Thus, in the presence of 1% BSA, 84% of the radiolabeled PAF was recovered from the venous effluent of the preparation, whereas 6% was retained by the kidney. When kidneys were perfused with an albumin-free medium, the amount of radiolabeled PAF recovered from the venous effluent decreased to 11%, whereas about 71% of exogenous PAF was retained by the kidney. These results indicate that albumin, which is a major transport plasma protein, plays a key role in regulating PAF renal uptake. It is known that PAF has a high affinity to albumin (12), which possesses the ability to solubilize PAF in aqueous solution (26). Hoppens *et al.* (27) demonstrated that albumin significantly reduces the biological activity of PAF by preventing the PAF in solution from interacting with target cells. Particularly, it has been documented that BSA inhibits specific and non-specific binding of PAF to platelets' plasma membranes (28). Thus, albumin would prevent PAF uptake to renal tissues by trapping it in the perfusion medium.

Despite a different percentage removal of PAF from the perfusate in control kidneys perfused with or without 1% of BSA, negligible and comparable amounts of [^{3}H]ether lipid were recovered in the urine. This result indicates that circulating PAF is unlikely to be excreted in urine by kidneys in which glomerular permselectivity to macromolecules is preserved.

One may wonder whether urinary excretion of PAF may change if protein flux through the glomerular capillary barrier increases as a consequence of changes in glomerular permselective properties. To test this possibility, [^{3}H]PAF infusion was repeated in isolated kidneys taken from ADR-treated animals that have profound changes in their glomerular permselective function (19) leading to severe proteinuria. The experiments showed that [^{3}H]PAF excretion was still negligible in urine collected from isolated kidneys of ADR-treated animals, so that no significant differences could be detected from control kidneys. One possible explanation is that PAF is filtered with albumin through the glomerulus of nephrotic kidneys but undergoes a subsequent reabsorption at tubular level.

To test such a possibility, we investigated the uptake of radiolabeled PAF by isolated glomeruli and proximal tubules from control and ADR-treated animals in the presence of increasing concentrations of BSA in the incubation medium. Incubation of isolated proximal tubules from nephrotic and control rats resulted in a significant [^{3}H]PAF uptake that declined with increasing concentrations of BSA in the incubation medium. However, isolated tubules from nephrotic rats had a higher uptake than control tubules, particularly for concentrations of BSA ranging from 0 to 0.1%. On the contrary, the uptake of [^{3}H]PAF by glomeruli from nephrotic or control animals did not differ significantly. These results may be interpreted as to indicate that proximal tubules, but not isolated glomeruli, from nephrotic kidneys possess a higher capacity to take up PAF than do control ones.

Another finding of the present study is that the percentage of PAF that is not retained in the kidney is only partly metabolized. Thus, analysis of residual radioactivity performed at the end of the infusion period revealed that, in the venous effluent, 85–90% of the total radioactivity was recovered as authentic PAF, whereas 5% of the total radioactivity corresponded to lyso-PAF and another 5% to glycerols. In contrast, lipid extracts from the kidney tissue show that PAF is rapidly and extensively metabolized by the isolated kidney. Actually, 55% of the tissue-retained PAF was found to be converted to long chain acyl-GPC species, 12% was converted to lyso-PAF, 15% to glycerols, and 15% was recovered as unchanged PAF. This metabolic pathway is qualitatively similar to what has been described previously in isolated cells (10) and in a previous study on the metabolism of intravenously injected PAF (29). Blank *et al.* (29) have reported that, after intravenous injection to rats, [^{3}H]PAF is rapidly uptaken and metabolized by a variety of tissues, mainly lung, liver, spleen, and kidney. In that study, a significant amount of the radiolabel within the kidney was associated with lyso-PAF and alkyl-2-acyl-GPC. Moreover, a previous study on isolated perfused lung demonstrated metabolism of PAF to alkyl-2-acyl-GPC (30). Studies on alveolar macrophages would suggest that these cells are involved in PAF metabolism but that the metabolites rapidly exit these cells so that most of the lyso-PAF formed after incubation of PAF with cultured alveolar macrophages is found in cell incubation medium (31). In the kidney, an intracellular acetylhydrolase for PAF has been documented in the cortex and partially characterized (32). It has been further documented that rat mesangial cells incorporate labeled lyso-PAF to alkyl-2-acyl-GPC species and rapidly convert exogenous labeled PAF to lyso-PAF and 1-*O*-alkyl-2-acyl-GPC (10). Given the qualitative similarities between the metabolism of PAF previously reported in mesangial cells and in the present results on the isolated perfused kidney, it is tempting to speculate that the mesangial cells' enzymatic machinery for PAF is responsible for the rapid metabolism of exogenous PAF we observed in isolated perfused kidney.

Even if alkyl-2-acyl-GPC represents the major metabolite recovered from renal tissue, a significant percentage of [^{3}H]PAF was seen to be converted to neutral lipids in our isolated perfused rat kidney model. Such a result is indicative of the existence of a second metabolic pathway for PAF in the kidney besides acetylhydrolase/acyltransferase. Phospholipase C activity with a high specificity for PAF has been previously described in various rabbit tissues including kidney (33). Moreover, isolated glomeruli from control and nephritic rabbits have been recently shown to convert lyso-PAF to alkylglycerol (34). In our isolated perfused rat kidney model, no metabolite corresponding to the 1-*O*-alkyl-2-acetylglycerol was recovered from tissue extracts; in fact, the glycerol frac-

tion consisted mainly of 1-*O*-alkylglycerol and 1-*O*-alkyl-2-acylglycerol. We would hypothesize that 1-*O*-alkylglycerol and 1-*O*-alkyl-2-acylglycerol are formed in the kidney by the action of phospholipase C on lyso-PAF and 1-*O*-alkyl-2-acyl-GPC, respectively.

The metabolism of PAF into alkyl-2-acyl-GPC and glycerols may reflect an inactivating mechanism to prevent local PAF concentration from reaching levels that can be associated with platelet activation and perturbations in glomerular capillary permeability (5, 35). However, whereas the kidney has been shown in this study to metabolize the majority of PAF, some intact PAF did survive. The metabolism of [^{3}H]PAF in isolated kidneys from nephrotic rats was similar to that observed in control animals. On the contrary, in kidneys perfused with BSA-free medium, the higher PAF uptake was associated with an increased amount of intact PAF in the kidney. The saturability of the PAF-inactivating pathway would be of significance in glomerular immune injury where an enhanced intraglomerular PAF synthesis would occur (36).

In conclusion, our present findings in the isolated perfused rat kidneys may be interpreted as indicating that (*a*) in normal conditions, circulating PAF is excreted in the urine in negligible amounts; (*b*) the kidney extensively metabolizes PAF to long chain acyl-GPC species, lyso-PAF, and glycerols; (*c*) changes in glomerular permeability to proteins do not affect the excretion rate of circulating PAF; and (*d*) the renal metabolism of PAF is comparable in control and nephrotic kidneys.

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SUPPLEMENTAL MATERIAL TO:

Renal metabolism and urinary excretion of platelet-activating factor in the rat

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MATERIALS AND METHODS

Chemicals. [^{3}H]PAF ([1',2'-^{3}H]hexadecyl-2-acetyl-GPC; 56.7 Ci/mmol), [^{3}H]lysoPAF ([1',2'-^{3}H]alkyl-2-lyso-GPC); 52.0 Ci/mmol), [^{3}H]alkyl-2-acyl-GPC ([1',2'-^{3}H]alkyl-2-arachidonyl-GPC); 40 Ci/mmol) were purchased from New England Nuclear (Boston, MA). [^{3}H]lysoPAF and [^{3}H]alkyl-2-acyl-GPC were a mixture of isomers with hexadecyl, octadecyl and tetradecyl alkyl moiety. Unlabeled 1-*O* -hexadecyl-*rac* -glycerol (>99% pure by GC) was from Fluka Chemie (Buchs, Switzerland). The phospholipid standards phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine, lysophosphatidylethanolamine, phosphatidylserine, lysophosphatidylserine, phosphatidylinositol and sphingomyelin were a gift from Fidia Research Laboratories (Abano Terme, Italy). The purity and the ratio chemicals of these phospholipids has been described elsewhere (14,15). 1,3-dipalmitoyl-2-oleoyl-glycerol (approximately 99% pure by TLC) was obtained from Sigma Chemicals Co. (St. Louis, MO).

1-*O*- hexadecyl-2-acetyl-glycerol and 1-stearoyl-2-arachidonyl-glycerol were synthesized by hydrolysis with phospholipase C of 1-hexadecyl-2-acetyl-GPC (Bachem Feinchemikalen, Switzerland; >90% pure by TLC) or L-α-phosphatidylcholine-β-arachidonyl-γ-stearoyl (Sigma Chemicals Co.; approximately 99% pure by TLC) respectively. Briefly, about 1 mg of phospholipid was suspended in 4 ml of diethylether, then 2 µmol of NaCl, 22 µmol of $CaCl_2$ and 500 units of phospholipase C (from *Bacillus cereus* type II, Boheringer Mannheim , F.R.G.) in 1 ml of 0.08 M of borate buffer (pH=8) were added (16). The two-phase mixture was shaken at 37 °C for 90 min, the ether removed and the aqueous layer extracted twice with 2 ml of dichloromethane. The organic phases were joined and the purity of hydrolytic products were checked by thin layer chromatography carried out on 250-µm silica gel 60, (Merck, Darmstadt, Federal Republic of Germany) and developed in chloroform: methanol: acetic acid 96:4:1 (vol/vol/vol) (17). Developed TLC plates, sprayed with a solution of 50% sulforic acid and heated at 100 °C for 10 min, showed a single spot both for 1-*O* -hexadecyl-2-acetyl-glycerol and 1-stearoyl-2-arachidonyl-glycerol. R_F values were 0.51 for 1-*O* -hexadecyl-2-acetyl-glycerol and 0.63 for 1-stearoyl-2-arachidonyl-glycerol respectively.

HPLC and analytical grade solvent were purchased from Merck (Darmstadt, FRG). Bovine serum albumin (BSA Pentex, Fraction V) was obtained from Miles Laboratories (Elkhart, Indiana). Adriamycin was a gift from Farmitalia Carlo Erba (Milan, Italy).

Studies in isolated perfused rat kidney. Sprague-Dawley rats (Charles River Italia, Calco, Italy), 320-350 g body weight, housed in a constant-temperature room with a 12 h dark-12 h light cycle were used for these experiments. The animals were fasted overnight before surgery and had free access to tap water.

To assess the kidney capability to excrete and metabolize exogenous PAF, kidneys from normal rats (n=3) were isolated and perfused in a recirculating system at a constant pressure of 100 mmHg with an artificial cell-free medium containing 1% (wt/vol) BSA. Each kidney was allowed to equilibrate 15 to 20 min before any measurements were taken. After this period [^{3}H]PAF (3.5 µCi, diluted in 1 ml of perfusion medium) was injected into the renal artery as a bolus and the perfusion was continued for 70 min. The amount of PAF injected, corresponding to 60 pmol, was below the dose known to have hemodynamic and proteinuric effects in this preparation (5,18). Urine samples were collected over a 70 min period in preweighed tubes both for determination of urinary protein and [^{3}H]PAF excretion. Aliquots (1 ml) of the venous effluent were collected every 10 min after the injection of [^{3}H]PAF to evaluate the amount of [^{3}H]PAF residual and to analyze its metabolites. Perfusate and urine samples were kept in ice until analysis. At the end of the perfusion the kidney was removed and immediately frozen to assess [^{3}H]PAF renal uptake and metabolism. After each experiment the perfusion apparatus was washed with 300 ml of ethanol/water 1:1 (vol/vol) to recover the radioactivity adsorbed to the tubing.

To establish whether the urinary excretion of PAF and its renal metabolism is dependent on the presence of albumin in the perfusion fluid, additional experiments were performed in isolated kidneys (n=3) from normal rats perfused with albumin-free medium. For this purpose we duplicated the above described protocol except that no BSA was added to the perfusion medium. In this experimental condition [^{3}H]PAF was dissolved in less than 1 ml of perfusion solution added with 0.25% BSA before injection. Thus the final concentration of BSA in the total volume of perfusate was less than 0.001%.

Moreover, to investigate whether increased protein flux through the glomerular capillary barrier affects PAF urinary excretion and renal metabolism, a separate group of kidneys (n=3) were isolated from rats made nephrotic by a single intravenous injection of ADR (5 mg/kg, Farmitalia Carlo Erba Milan, Italy), as previously described (19). The development of the disease was assessed by the appearance of proteinuria in 24 hours urine samples collected in metabolic cages. Two weeks after ADR injection, when rats developed heavy proteinuria, kidneys were isolated and perfused with medium containing 1% (wt/vol) BSA. [^{3}H]PAF injection and sample collection during the perfusion period were performed in a manner identical to that described for normal rat kidney preparation.

Isolated perfused rat kidney: procedure and apparatus. Kidneys were isolated from rats following intraperitoneal thiopental sodium anesthesia (50 mg/kg), as previously described (5). Briefly, the rats were placed on a heated surgical table, the abdominal cavity was exposed and the right ureter cannulated with PE-10 polyethylene tubing (Clay-Adams, Parsippany, New Jersey). The open tip of a venous cannula (PE-240 polyethylene catheter) with its distal end closed was introduced into the vena cava below the right renal vein and secured in place. The right renal artery was cannulated with a short, blunted 19-gauge needle, via the mesenteric artery without interruption of flow to the kidney. The distal end of the venous cannula was opened and kidney washed with oxygenized perfusion solution. The kidney was perfused 'in situ' in a recirculating system. The perfusate, maintained at 37°C by a constant Haake D1 temperature circulator (Haake, Berlin, FRG) and gassed with a mixture of 95% O_2/5% CO_2 in a slowly rotating reservoir flask, was recirculated by a two-headed peristaltic pump (Gilson 2, Viller Le Bel, France). Through an in-line 8 µm filter (Sartorius, Gottingen, FRG) and a glass bubble trap, the perfusate was delivered to renal artery cannula. The effective pressure (pressure at the tip of the arterial cannula) was maintained constant at 100 mmHg throughout the experiments. The perfusate consisted of Krebs-Henseleit bicarbonate buffer containing 3.5 g/100 ml Ficoll 70 (Farmacia Fine Chemicals, Uppsala, Sweden), 1 g/100 ml bovine albumin (Pentex BSA Fraction V, Miles Laboratories, Elkhart, Indiana), 200 mg/100 ml glucose, 36 mg/100 ml urea, 50 mg/100 ml creatinine, and a mixture of amino acids that included methionine (0.5 mM), alanine (2.0 mM), glycine (2.0 mM), serine (2.0 mM), arginine (1.0 mM), isoleucine (1.0 mM), aspartic acid (3.0 mM) and cysteine (0.5 mM). The perfusate was filtered through a 0.45 µm membrane filter (Sartorius) before use and, when equilibrated with the gas mixture at 37°C, its pH was approximately 7.4. The total volume of the perfusate in the system was 250 ml.

Urine flow rate was determined gravimetrically. The perfusion pressure was continuously measured with a Statham transducer (Gould, Dusseldorf, FRG) connected by a polyethylene tubing to the perfusion line proximally to the arterial cannula and recorded on a Battaglia Rangoni KV 135 recorder (Battaglia Rangoni, Casalecchio di Reno, Italy). The effective perfusion pressure (cannula tip pressure) was derived by subtracting from the measured pressure the pressure drop known to occur across the arterial cannula at a given flow. Urine protein concentration was measured in duplicate samples by the Coomassie Brilliant blue dye-binding assay (20).

[^{3}H] ether-lipid distribution in venous effluent, urine, and renal tissue. To eliminate non lipid radioactive impurities perfusate and urine samples and renal tissue were extracted before analysis. Perfusate and urine collections were added with methanol (4:1 and 1.5:1 vol/vol, respectively). After gentle mixing for 30 min, samples were centrifuged at 2000 g for 15 min to precipitate denaturated proteins. Supernatants were extracted by the method of Bligh and Dyer (21). As far as kidneys, they were thawed to allow mincing, suspended in 20 ml of acidified methanol (pH=3 with HCl 1N) and homogenized three times for 30 sec with an Ultra-Turrax homogenizer (Jankle and Kunkel GMBH and Co., KG, IKA-WERK Staufen, FRG). Renal homogenate was centrifuged at 18000 g for 20 min, and the pellet was resuspended in 20 ml of acidified methanol, vortexed and centrifuged once again. Supernatants were pooled and subjected to extraction according to the Bligh and Dyer's procedure (21). Radiolabeled lipids recovered in the lower phase of the extracted samples were resuspended in chloroform/methanol 9:1 (vol/vol) and aliquots transferred to plastic vials to which 10 ml of scintillation mixture (Instagel II, Packard Instruments, Downers Grove, IL) was added. Radioactivity of the samples was determined by liquid scintillation counter (model LS 1701, Beckmann Instruments, Irvine, CA). Recovery of radioactivity after the extraction, as determined by counting aliquots of perfusate, urine, and renal homogenate before the analytical procedure, was about 98% both for perfusate and urine and 95% for kidney. The yield of [^{3}H]ether lipid recovered from the perfusate, urine and renal tissue, was expressed as extractable radioactivity divided by the total administered dose. Since PAF can adsorb to surfaces, the administered dose of [^{3}H]PAF was the difference between the tritium content in the bolus solution and that which was not injected but recovered from syringe.

Analysis of [^{3}H]PAF and metabolites in the venous effluent and renal tissue. The extracts from perfusate samples, collected at the end of perfusion, and from renal tissue were purified according to the method of Pinckard *et al.* (1) with the following modifications: samples were resuspended in 1 ml chloroform/methanol 9:1 (vol/vol) and loaded on a column packed with 0.5 g of silicic acid (Bio-Rad Laboratories, Richmond, CA; 100-200 mesh) activated overnight at 100 °C. The column was eluted sequentially with 15 ml of chloroform, 15 ml of acetone, 15 ml of acetone/methanol (1:1,vol/vol), 30 ml of chloroform/methanol (1:4,vol/vol). [^{3}H]glycerols, [^{3}H]alkyl-2-acyl-GPC were recovered in the chloroform and in the acetone/methanol fraction respectively, whereas [^{3}H]PAF and [^{3}H]lysoPAF were eluted together in the fourth fraction. [^{3}H]alkyl-2-acyl-GPC, [^{3}H]PAF and [^{3}H]lysoPAF were analyzed by HPLC. The third and fourth fractions of the silicic acid column were combined and dried under a nitrogen stream. An aliquot of each sample was dissolved in 200 µl of chloroform/methanol 2:1 (vol/vol) and brought to 500 µl with the elution mixture. Samples were chromatographed by isocratic elution on a Perkin Elmer Silica A Column (2.6 x 250 mm), (Perkin-Elmer, CO, USA) connected to a Beckman model 342 Gradient Liquid Chromatograph (Beckman Instruments Inc., Berkeley,CA). The eluent was acetonitrile/methanol/85% phosphoric acid 130:5:1.5 (vol/vol/vol) as described by Chen and Kou (22). The flow rate was 1.5 ml/min and 0.75 ml fractions were collected and assayed for radioactivity by scintillation counting. [^{3}H]alkyl-2-acyl-GPC, [^{3}H]PAF, [^{3}H]lysoPAF were separated and identified on the basis of the retention time of the corresponding [^{3}H] standards.

To further analyze [^{3}H]glycerol metabolites, the chloroform fraction was dried under a nitrogen stream. The residue was dissolved in chloroform/methanol 9:1 (vol/vol) and spotted on a silica gel thin-layer plate (Silica gel 60 250 µm). 10 µg of unlabeled 1-*O* -hexadecyl-glycerol, 1-*O* -hexadecyl-2-acetyl-glycerol, 1-stearoyl-2-arachidonyl-glycerol and the triglyceride 1,3-dipalmitoyl-2-oleyl-glycerol was applied close to the samples. The thin-layer plates were developed in a solvent system of chloroform/methanol/acetic acid 96:4:1 (vol/vol/vol). Unlabeled lipid standard lanes were sprayed with a solution of 0.025% Rhodamine 6G (Merck Darmstad, FRG) in ethanol and visualized under ultraviolet light. The plates were divided into 1 cm segments, then the silica gel from each segment was scraped and radioactivity detected by liquid scintillation counting. The recovery after TLC was about 80%.

To visualize endogenous phospholipid profile the HPLC column effluent was continuously monitored for UV absorption at 203 nm by a 163 Variable Wavelength detector (Beckman Instruments Inc. Berkeley CA) coupled with a Shimadzu C-R3A Chromatopac Recorder-Integrator (Kjoto-Japan). To allow identification of major phospholipid peaks, the standards PC, LPC, PE, LPE, PS, LPS, PI and SPH (about 10 µg each) were injected and retention time determined.

Studies in isolated glomeruli and proximal tubules. To compare [^{3}H]PAF uptake by glomeruli and proximal tubules from control and nephrotic animals in the presence of increasing albumin concentrations in the incubation medium, two additional groups of control (n=8) and ADR treated rats (n=8) were used. To isolate glomeruli and proximal tubules, animals were anesthetized by intraperitoneal injection of thiopental sodium (50 mg/kg). The abdominal aorta was exposed via a midline incision, clamped above the renal arteries, cannulated with a 21-gauge needle and kidneys perfused with 60 to 80 ml of Krebs-Ringer phosphate buffer after incision of inferior vena cava to allow drainage of the perfusate.

Glomeruli from six control and nephrotic rats were isolated by differential sieving using a technique previously described (23) which provides about 95% pure glomerular preparation. The final pellet, consisting of decapsulated glomeruli, was resuspended in the same medium used to perfuse isolated kidneys but free of BSA.

Proximal tubules suspensions were prepared from kidneys of two control and nephrotic rats by Percoll gradient technique after collagenase digestion as described by Vinay *et al.* (24). Freshly prepared isolated tubules were pooled and resuspended as described for glomeruli.

Aliquots of glomeruli and proximal tubules suspensions (about 1 mg of protein each), were transferred to polypropylene tubes containing [^{3}H]PAF (0.2 µCi). The volume of each sample was brought to 2 ml by the addition of perfusate medium without BSA or containing increasing amounts of BSA to a final concentration of 0.1%, 1% and 4% (wt/vol), respectively. Incubations were done in triplicate, for 30 min at 37 °C under constant shaking. Then samples were transferred to clean tubes and immediately centrifuged at 2000 g for 15 min at 4 °C. Pellet and supernatant were separated; the pellet was washed twice with 1 ml of BSA free perfusate and finally resuspended in NaOH 1 N. Aliquots of the suspension were used for radioactivity counting and protein content determination. Protein concentration was determined according to the method of Lowry *et al.* (25). Supernatant and washing from each sample were combined and radioactivity counted. The amount of [^{3}H]PAF adsorbed to the wall of incubation tubes, as determined by counting the radioactivity residual in the tube itself, was less than 3% for all samples. [^{3}H]PAF uptake to proximal tubules and glomeruli was expressed as radioactivity linked to the pellet divided by the total administered label per mg of protein.

Statistical analysis. All results are expressed as mean ± SD. Data were analyzed by one-way or two-way analysis of variance, as appropriate. Significant level of differences between groups was established by using Duncan's multiple-range test or Tukey-Cicchetti test and defined as $P < 0.05$.