Vol. 152, No. 1, 1988
April 15, 1988

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 129-135

GLOMERULI FROM ISCHEMIC RAT KIDNEYS PRODUCE INCREASED AMOUNTS OF PLATELET ACTIVATING FACTOR

Antonio López-Farré, Miguel Torralbo and José M. López-Novoa

Laboratory of Renal Physiopathology, Fundación Jiménez Diaz-CSIC, Avda Reyes Católicos 2, 28040 Madrid, Spain

Received February 16, 1988

SUMMARY: The production of Platelet Activating Factor (PAF-acether) by glomeruli isolated from rats subjected to a 60 min renal ischemia has been studied. PAF-acether has been purified by Sep-Pak columns and measured by its ability to release serotonin from previously loaded rabbit platelets. Glomeruli from ischemic kidneys had higher amounts of PAF-acether activity than glomeruli from sham operated rats. These data suggest a role for PAF-acether in the renal functional alterations induced by renal ischemia.

Platelet activating factor (PAF-acether, 1-O-alkyl-2-**O**-acetyl-**sn**-glycero-3-phosphocholine) is an alkyl phosphoglyceride with numerous biological actions on renal function (1,2). The intrarenal infusion of PAF-acether causes important reduction in glomerular filtration rate (GFR) and renal plasma flow (RPF) (3,4). It has been reported that PAF-acether is produced by the kidney (2,5), and that it is synthesized and released by isolated glomeruli and glomerular mesangial cells (6,7). In addition, PAF-acether has been recovered in the effluent from ischemic kidneys (2). For these reasons, PAF-acether could be a mediator in the reduction of RPF and GFR observed after renal ischemia (8). Thus, the purpose of the present study was to analyze whether the glomeruli isolated from rats after temporal renal ischemia were able to produce increased amounts of PAF-acether.

METHODS

Renal ischemia.- Experiments were performed in 15 animals. In 9 of them, transitory renal ischemia was induced as previously described. In brief, under ether anesthesia, a small dorsal incision was made, the kidney dissected free from perirenal fat and the renal pedicle isolated. Then heparin (1000 U) was injected intravenously, and the renal artery was clamped with an

0006-291X/88 $1.50

atraumatic vascular clamp for 60 min. Clamp was then released and reflow allowed for 30 min. Six rats suffered a sham operation.

Incubation of isolated glomeruli and PAF-acether extraction.- Kidneys were removed and glomeruli isolated by a sieving technique, as previously described (9). This method provides isolated glomeruli, most of them without Bowman capsule, and with a tubular contamination lower than 5 %. Protein concentration of the glomerular suspension was determined using the method of Lowry. Freshly isolated glomeruli were placed in tubes containing 1.5 ml of a Tris-glucose buffer (Tris 20 mM, NaCl 130 mM, KCl 10 mM, $CaCl_2$ 2.5 mM, Sodium acetate 10 mM and glucose 5 mM, pH 7.45), containing phenylmethylsulfonyl fluoride (PMSF, 10^{-4} M, final concentration) to prevent rapid intracellular degradation of PAF-acether (10). Tubes were incubated at room temperature for 30 min and glomeruli were separated for centrifugation at 3000 rpm for 3 min. Supernatant was mixed with 5 ml of cold methanol acidified with acetic acid (49:1; v:v), and PAF-acether was extracted as described in a previous work (5). Glomeruli were mixed with 5 ml of cold acidified methanol and sonicated for 30 sec and slowly stirred for 30 min. Then tubes were centrifuged and the pellet was again mixed with 5 ml of cold methanol, stirred for 30 min and centrifuged. Both methanol phases were pooled. PAF-acether was extracted from the methanol phase as previously described. In brief, chloroform and water were added to the methanol to a proportion of 1:0.9:1 vol:vol and gently stirred. After phase formation by centrifugation, chloroform phase was removed and new chloroform added to the methanol:water in the same proportion as above reported. The process was repeated and both chloroform extracts were pooled and dried under N_2 atmosphere. PAF-acether extraction was performed with silica cartridges (Sep-Pak, Waters, Milford Ma, USA). Cartridges were rinsed with 5 ml of chloroform and then the sample resuspended in 5 ml of chloroform acidified to pH 3-4 with HCl was applied to the cartridge. The column was eluted sequentially with 5 ml of chloroform methanol (3:1), 5 ml of chloroform- methanol (3:2) 5 ml of chloroform-methanol (1:3), and 5 ml of methanol-water 3:1. The last fraction containing PAF-acether was collected and dried under nitrogen atmosphere. In separate studies [^{3}H]-PGE_2 and [^{3}H]-TXB_2 (Amersham) were added to the sample and this one processed as above described.

Bioassay for PAF-acether activity. Bioassayable PAF-acether activity was determined by the release of [^{3}H]-serotonin from preloaded rabbit platelets in the presence of Indomethacin 10^{-5} M, as previously described (5). A standard curve of response to PAF-acether was made with concentrations between 3.8 x 10^{-11} M and 3.8 x 10^{-9} M. The amount of [^{3}H]-serotonin released for the lower standard was significantly higher that the non specific release. The amount of PAF acether activity was quantified by interpolating the values of [^{3}H]-serotonin release from platelets in presence of 100 µl of the sample by triplicate. Only the values corresponding to the linear portion of the curve (between 3.8 x 10^{-11} and 7.7 x 10^{-10} M) were used for quantitation. In some experiments, BN-52021 (Institut Henry Beaufour, Le Plessis Robinson, France), a PAF-acether receptor blocker, was added to the platelets before adding PAF-acether standars or the samples.

Characterization of PAF-acether activity.- According to previous publications, characterization of PAF-acether activity was performed with the following techniques:

a) One dimension thin layer chromatography (TLC). In some cases, samples were also purified by TLC. Briefly, samples were redissolved in chloroform :methanol (9/1, v/v) and developed using the solvent system propionic acid: propanol:chloroform:water (2:2:1:1, v:v). A zone of 0.5 cm above and under a Rf of 0.32 was scrapped and eluted in methanol and dried under N_2. This fraction was resuspended in a Hepes-buffered medium containing 0.25 % BSA, pH 7.4, to a final volume of 250 µl, and this suspension used to test for biological activity.

b) analysis by lipases. Fractions recovered from silica cartridges in the same fraction that synthetic PAF-acether were treated by phospholipase A_2 from Naja Naja (Sigma) and phospholipase C from B. Cereus (Sigma). Known amounts of synthetic PAF-acether were tested in the same way.

c) High Performance liquid chromatography. HPLC was performed using a dual pump Beckmann Model 324 system and an Ultrasphere-Si column (150 x 4.6 mm) using the conditions previously described (5). Under these conditions, the time of retention of synthetic [^{3}H]-PAF-acether was 24 min. PAF-acether was detected in HPLC eluates by the rabbit platelet assay described elsewhere.

Results are expressed as mean ± SEM. Comparisons between means were performed by unpaired Students T test.

RESULTS

Recovery of PAF-acether after methanolic extraction was 99.3 ± 0.12 % (n = 4). Table 1 shows that near 90% of the PAF-acether added to the column was recovered in the 4th fraction. Thus, PAF-acether recovery in the total purification procedure was 83.4 ± 1.4 % of the PAF-acether added (n = 4).

As expressed in table 1, near 90% of TXB_2 added to the sample was recovered with the first two solvents of the extraction procedure, and no significant amount of this compound appeared in the fraction in which PAF-acether appears.

Samples processed as above mentioned contained a substance which stimulated the release of serotonin from rabbit platelets in a dose-response manner similar to that of synthetic PAF acether. Variation coefficients of the total measurement procedure were

Table 1: Recovery of [^{3}H]-PAF-acether and [^{3}H]-TXB_2 during the extraction procedure (n = 4)

	Radioactivity recovered %	
	[^{3}H]-PAF	[^{3}H]-TXB_2
sample	5.46 ± 1.38	13.8 ± 0.12
C/ metOH 3:1	4.60 ± 0.98	61.1 ± 6.1
C/metOH 3:2	1.65 ± 0.99	26.5 ± 2.3
C/metOH 1:3	0.78 ± 0.53	5.38 ± 1.31
MetOH/H_2O 3:1	86.3 ± 6.9	1.32 ± 0.03
column	3.86 ± 1.20	2.52 ± 0.87
TOTAL	102.7	110.7

C = Chloroform. Data are mean ± SEM

Table 2: Inhibition by lipases of PAF-acether activity

	Total activity	Inhibition (%)	
		PA_{2}	PC
PAF	100	68 ± 12	81 ± 14
sample	100	70 ± 12	84 ± 5

PA_{2} : Phospholipase A_{2}. PC : Phospholipase C. Data are mean ± SEM.

9.54 % for a concentration of 0.08 ng/ml and 6.73% for a concentration of 0.4 ng/ml (n = 6). It can also be stated that the platelet [^{3}H]serotonin release induced by the sample or by synthetic PAF-acether was completely blocked by pretreatment of the platelets with BN-52021, a PAF-acether receptor blocker (1).

The identity of the product recovered in the extraction procedure with ability to aggregate rabbit platelet with PAF-acether was deduced from the following results. a) In TLC, it shows a Rf of 0.32, which is analogous to that of synthetic PAF-acether. b) treatment with phospholipases A_{2} and C induced a loss of biological activity similar to that of the synthetic compound (Table 2). c) HPLC analysis of the platelet activating activity showed a single peak with a time of retention of 22 ± 2 min, similar to that of synthetic compound.

Glomeruli from rats that have suffered one hour of renal ischemia contained greater amounts of PAF-acether both in the supernatant and in the glomeruli themselves, as shown in table 3.

DISCUSSION

The present results demonstrate that glomeruli from rats that have suffered one hour of renal ischemia produce greater amounts of PAF-acether than control glomeruli. PAF-acether has been determined as a substance able to stimulate serotonin

Table 3: PAF-acether activity in isolated renal glomeruli (ng/ml/mg glomerular protein)

		Supernatant	Glomeruli	Total
Sham	(6)	0.038 ± 0.014	0.11 ± 0.028	0.15 ± 0.032
Ischemic	(9)	0.211 ± 0.028*	0.25 ± 0.039*	0.46 ± 0.056*

* $p < 0.01$ vs Sham operated rats.

release by preloaded rabbit platelets. The possibility that this aggregation was caused by thromboxane A_2, which is also produced by ischemic glomeruli is discarded by the extraction procedure, which allows a complete separation of both products. In addition, PAF acether recovered from glomeruli shows the same physicochemical and biologic properties than synthetic PAF-acether. It must be noted that its activity is destroyed by phospholipases A_2 and C, and that its retention time on HPLC is identical to that of synthetic PAF-acether, thus suggesting that the product recovered and measured is PAF-acether. As a further test of the specificity of the method for quantifying PAF-acether, platelet pretreatment with BN-52021, a PAF-acether receptor blocker, completely prevented serotonin release induced by either synthetic PAF-acether or by the sample.

Production of PAF-acether from renal tissue has been previously documented (for review see ref 2). In addition, both Pirotzky et al. (7) and Schlondorff et al (6) have demonstrated that isolated rat glomeruli are able to produce PAF-acether when challenged with calcium ionophore A23187 or incubated at pH 9.5. The possibility that PAF-acether could be produced by platelets, polymorphonuclear neutrophils or monocytes contaminating the glomerulus is unlikely, because the kidneys were extensively perfused before removing them from the rats, and electronic microscopy examination of the isolated glomeruli reveals the lack of platelets and leukocytes entrapped in the capillary lumen. However the possibility remains that PAF-acether obtained from glomeruli may originate from resident mononuclear phagocytes, which have been demonstrated to exist in normal conditions in the glomeruli (11). Both endothelial and mesangial cells have been shown to be able to produce PAF-acether. In contrast, cultured glomerular epithelial cells were unable to produce PAF-acether, probably due to the lack of relevant activity of acetyl transferase in these cells (7). The glomerular PAF-acether formation seem to correlate with acetyl transferase activity of the microsomal fraction, which increases after the stimulation with ionophore A23187 (7). Similar data have been obtained by Schlondorff et al in cultured mesangial cells (6). Thus the mechanism of ischemia-induced- glomerular PAF-acether production seems to be based on the increased glomerular mesangial cell permeability to calcium induced by ischemia, as has been demonstrated for other renal cells (12).

The increased glomerular PAF-acether production by ischemic glomeruli could explain some physiopathological mechanisms of postischemic acute renal failure. Thus intrarenal infusion of PAF-acether induced marked decrease of renal blood flow and glomerular filtration rate even in the absence of significant changes of systemic hemodynamics and extracellular fluid volume (3,4). In addition, it has been demonstrated that PAF-acether induces a reduction in isolated glomeruli planar area (13,14) and cultured mesangial cell cross-sectional area (15), These changes have been related to a decrease in Kf, and subsequently in GFR. The participation of increased glomerular PAF-acether production in the physiopathology of ischemic acute renal failure is also supported by recent results form our laboratory demonstrating that BN-52021, a specific PAF-acether-receptor blocking agent, prevents against ischemic-induced acute renal failure in rats (Lopez-Farré, Torralbo, Braquet, López-Novoa, unpublished results). In addition, blockade of PAF-acether production by Verapamil could also explain the beneficial effect of this drug in the evolution of ischemic acute renal failure (15).

ACKNOWLEDGMENTS: The authors acknowledge the advice of Dr. A. Barat with electronic microscopy. We also acknowledge Dr. P. Braquet. Institut Henri Beaufour, for giving us BN-52021. This work has been supported in part by grants from FIS (884/87) and CAICYT (594/84). M. Torralbo is a Fellow of FIS. A. López-Farré is a Fellow of the Conchita Rabago de Jiménez Díaz Foundation.

REFERENCES

1. Braquet, P., Touqui, L., Shen, T.Y., and Vargaftig B.B. (1987) Pharmacol. Rev. 39,97-145.
2. Camussi, G. (1986) Kidney Int. 29,469-477.
3. Hebert, R.L., Sirois, P., Braquet, P. and Plante, G.E. (1987) Prostag. Leukot. Med. 26,189-202.
4. Santos, J.C., Sanz, E., Caramelo, C., Hernando, L., and López-Novoa, J.M. (1985) Kidney Int 28,587.
5. Caramelo, C., Fernandez-Gallardo, S., Iñarrea, P., Santos, J.C., López-Novoa, J.M., and Sanchez Crespo, M. (1984) Biochem. Biophys. Res. Commun. 120,789-796.
6. Schlondorff, D., Goldwasser, P., Neuwirth, R., Satriano, J.A., and Clay, K.L. (1986) Am. J. Physiol. 250,F1123-F1127.
7. Pirotzky, E., Ninio, E., Bidault, J., Pfister, A., Benveniste, J. (1984) Lab Invest. 51,567-572.
8. Arendshorst, W.J., Finn, W.F., and Gottschalk, C.W. (1975) Circ. Res. 37,558-567.
9. Rodriguez-Puyol, D., Arriba, G., Blanchart, A., Santos, J.C., Caramelo, C., Fernandez-Cruz, A., Hernando, L., López-Novoa, J.M. (1986) Biochem. Biophys. Res. Commun. 138,496-501.

10. Touqui, L., Matsui, M., and Vargaftig, B.B. (1985) Biochem. J. 229,811-816.
11. Schreiner, G.F., Kiely, J.M., Cotran, R.S., and Unanue, E.R. (1981) J. Clin. Invest. 68,290-298.
12. Schrier, R.W., Arnold, P.E., VanPutten, V.J., and Burke T.J. (1987) Kidney Int. 32,313-321.
13. Arriba, G., Barrio, V. Hernando, L., López-Novoa, J.M. and Rodriguez-Puyol, D. (1987) Nephrol. Dialysis, Transplant 2,224-227.
14. Barrio, V, Arriba, G., López-Novoa, J.M. and Rodriguez-Puyol, D. (1987) Eur. J. Pharmacol. 135,93-96.
15. Schlondorff, D., Satriano, J.A., Hagege, J., Perez, J. and Baud, L. (1984) J. Clin. Invest. 73,1227-1231.
16. Burke, T.J., Arnold, P.E., Gordon, J.A., Bulger, R.E., Dobyan, D.C., and Schrier, R.W. (1984) J. Clin. Invest 74, 1830-1841, 1984.