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Renal effects of platelet-activating factor in the rat

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Abstract

The renal glomerular and tubular effects of the platelet-activating factor (Paf-acether) were studied, by clearance techniques, in acutely thyroparathyroidectomized Brattleboro rats infused with Paf-acether at the rate of 1.25, 2.5 or 5 ng/min/100 g bw. Paf-acether infusion was accompanied by decreases of urinary flow rate, calcium, and magnesium urinary excretion, whereas decreases of mean arterial pressure and glomerular filtration rate did not exceed 20% of control values for the highest perfusion rate of Paf-acether. These changes in tubular function were partially (calcium excretion) or totally (urinary flow rate and magnesium excretion) reversed after Paf-acether infusion was discontinued. Sodium and potassium excretion did not vary significantly during Paf-acether infusion, but increased dramatically after discontinuation of Paf-acether infusion. Infusion of lyso-Paf-acether or ethoxy-Paf-acether, two biologically less active structural analogues of Paf-acether, did not elicit any change in the variables studied. These data suggest that Paf-acether, when locally released by the kidney in pathological conditions, might affect both glomerular and tubular functions, possibly through different mechanisms.

Introduction

Paf-acether (platelet-activating factor; 1-0-alkyl-2-0-acetyl-sn-glycero-3-phosphocholine) [1, 2] has a wide spectrum of cellular sources and biological actions. It was first shown to be released from basophils, and to participate in immediate hypersensitivity [3]. Then it was demonstrated that it is released from inflammatory [4, 5] and non-inflammatory [6] cell types as well as whole organs: isolated rat kidney [7] and guinea pig heart [8]. Further, Paf-acether has been suspected to play a role in the pathogenesis of glomerulonephritis [9], nephrotic syndrome [10], and rabbit acute serum sickness [11]. Finally, it was established that vascular permeability increased after intraarterial bolus injection of Paf-acether in the isolated perfused rat kidney, and that renal blood flow and glomerular filtration rate (GFR) decreased dramatically during intravenous Paf-acether infusion in the dog [12, 13].

The aim of the present study was to examine, in the rat, a possible effect of Paf-acether, infused at various rates, on the renal excretion of water and electrolytes, and to compare these effects with those of two chemical analogues with decreased biological activity. We show that Paf-acether, but not lyso-Paf-acether or ethoxy-Paf-acether, affect tubular functions and that the resulting changes are likely to be due to a direct effect of Paf-acether on tubular epithelium.

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Material and methods

Materials

Totally synthetic Paf-acether (1-0-octadecyl-2-0-acetyl-sn-glycero-3-phosphocholine) and 2-ethoxy-Paf-acether (1-0-octadecyl-2-0-ethyl-rac-glycero-3-phosphocholine) were gifts from Pr. J. J. Godfroid (Université Paris 7, France), 2-lyso-Paf-acether (2-lyso-hexadecyl-sn-glycero-3-phosphocholine) was purchased from Berchtold (Biochemisches Lab., Bern, Switzerland), bovine serum albumin (BSA) from Sigma (St. Louis, MO, USA), sodium-5-ethyl-5(1-methyl-propyl)-2-thio-barbiturate (Inactin®) from International Apotheke, (Hamburg, FRG), methoxy-^{3}H inulin (specific activity: 100–500 Ci/g) from New England Nuclear (Boston, MA, USA), and radiophosphate ^{32}P (^{32}P neutral sodium phosphate) from the Commissariat à l'Energie Atomique (Saclay, France).
Homozygous Brattleboro (DI) rats, bred in the Département de Biologie (CEN Saclay, France), by J. M. Juvanon, were fed with a commercial chow (UAR, Paris, France) of constant composition (mmol/kg: Na: 130, K: 177, Ca: 230, Mg: 64, P: 297; vit. D_3: 4030 U/kg; proteins: 200 g/kg; carbohydrates: 540 g/kg).

Preparation of the animals

Nineteen DI rats (198 ± 4 g, mean weight ± SEM) were used. Chow was made available to all rats until 13–15 hours prior to the experiment. Free access to deionized water was allowed. After anesthesia was achieved (Inactin, 8 mg/100 g bw, IP), the animals were placed on a heated table and the rectal temperature was maintained between 37 and 38 °C. The mean arterial blood pressure (MAP) was monitored and blood samples were obtained through a catheter inserted in the right common carotid artery. A tracheotomy and a thyroparathyroidectomy were performed. Both ureters were catheterized. Infusions were performed as follows: NaCl 75 m*M* and ½ diluted Ringer's bicarbonate were infused via a tail vein and an external jugular vein, respectively, at a total rate of 100 µl/min/100 g bw. A priming dose of radioisotopes (^{3}H inulin and ^{32}P) was given at the end of the surgery via the tail vein, immediately followed by the sustaining dose.

Experimental protocol

After a 45-min equilibration period, urine was collected for ten 15-min periods (U_1 to U_{10}): three control periods, four periods during continuous infusion of Paf-acether or of its analogues, and three periods after discontinuation of Paf-acether infusion. A blood sample was obtained at mid time of U_1, U_3, U_5, U_7 and U_9.
Paf-acether or its analogues were dissolved in Ringer's bicarbonate containing 0.25% BSA (w:v). Paf-acether was infused, via the external jugular vein, at the rate of 1.25 ($n=4$), 2.5 ($n=5$) or 5 ng/min/100 g bw ($n=6$). Lyso-Paf-acether ($n=2$) and ethoxy-Paf-acether ($n=2$) were infused at the rate of 5 ng/min/100 g bw.

Analytical procedures

Blood samples were collected on dried lithium heparinate. Urine samples were weighed. The radioactivity of ^{32}P and ^{3}H was counted in a liquid scintillator (Intertechnique, SL 4000, Plaisir, France). Electrolyte concentrations in plasma and urine were determined as previously described in detail [14, 15]. Fractional excretions of Ca and Mg [14] were calculated taking into account previously measured ultrafilterability ratios which were 0.705 and 0.807 for Ca and Mg, respectively.
The left kidney was the experimental one. Results are expressed as means ± SEM. For statistical evaluation, mean values of control periods (C) (U_1 to U_3), of the last two experimental periods (E) (U_6–U_7) and of recovery periods (R) (U_8–U_{10}) were taken into account. Analysis of variance was applied to each parameter in each group and, when allowed by the F value, the modified t test was used for the different comparisons between periods [16].

Results

At 2.5 and 5 ng/min/100 g bw, Paf-acether induced a mild but significant decrease of MAP (Fig. 1). GFR decreased significantly during Paf-acether infusion only for the highest infusion rate. After the end of Paf-acether infusion, GFR increased significantly. Moreover, it reached a higher value than in control period in rats infused with 1.25 ng/min/100 g bw Paf-acether.

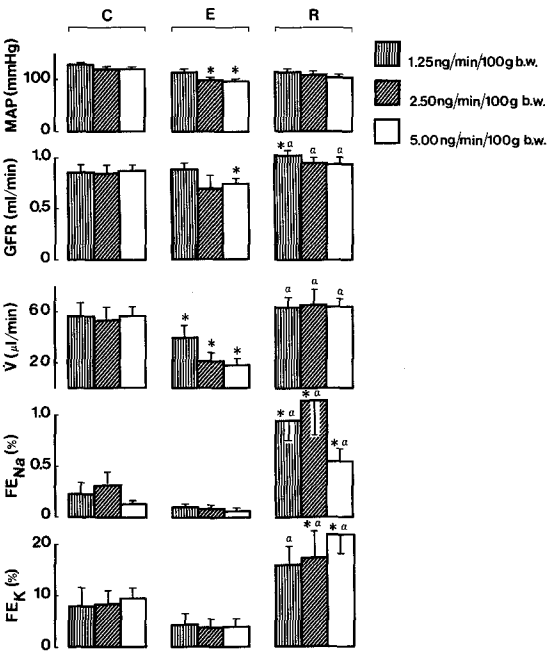

Figure 1
Effect of Paf-acether infusion on MAP, GFR, V̇ and Na and K excretions. C, E, R: control, experimental and recovery periods, respectively.
* significantly different from C, $p < 0.05$,
α significantly different from E, $p < 0.05$.

Urinary output decreased during Paf-acether infusion, and resumed basal values after Paf-acether was discontinued. The decrease of fractional excretion of Na (FE_{Na}) and K (FE_K) during Paf-acether infusion did not reach significance. However, excretions of Na and K increased during the recovery period to values significantly higher than control values.

The data about Ca, Mg and Pi are summarized in Table 1. As a consequence of parathyroidectomy, Pi urinary excretion was very low, and plasma calcium decreased throughout the experiment in the three groups [17]. During Paf-acether infusion, both absolute and fractional Ca excretions decreased significantly. At the end of Paf-acether infusion, Ca excretion increased but remained below the control level. Mg excretion decreased during Paf-acether infusion in a dose-dependent manner, and an almost complete reversion of this effect was observed after Paf-acether infusion was discontinued.

Table 1
Effects of Paf-acether on Ca and Mg urinary excretion.

		1.25[a]	2.50	5.00
P_{Ca}	C[b]	2.16±0.08	2.16±0.04	2.02±0.05
(m*M*)	E	2.07±0.09[c]	2.02±0.09[c]	1.91±0.05[c]
	R	2.02±0.09[c]	1.93±0.10[c]	1.80±0.07[c]
$U_{Ca}\times\dot{V}$	C	77.5±24.8	85.1±15.6	76.7±16.0
(nmol/min)	E	42.0±11.8[c]	15.8±4.9[c]	21.3±11.9[c]
	R	56.4±13.4	42.4±10.2	42.0±16.3
FE_{Ca}	C	5.54±1.25	6.51±0.70	6.11±1.14
(%)	E	2.97±0.69[c]	1.96±0.50[c]	1.94±1.01[c]
	R	3.62±0.71	3.70±0.91	3.39±1.27
P_{Mg}	C	0.54±0.02	0.54±0.01	0.57±0.03
(m*M*)	E	0.54±0.02	0.55±0.02	0.57±0.03
	R	0.55±0.02	0.56±0.02	0.58±0.03
$U_{Mg}\times\dot{V}$	C	71.9±17.4	74.7±10.2	74.8±13.6
(nmol/min)	E	48.2±13.3[c]	23.9±6.2[c]	29.2±10.8[c]
	R	66.6±17.1[d]	54.4±9.2[d]	65.4±9.8[d]
FE_{Mg}	C	18.15±3.05	20.80±2.80	19.09±2.92
(%)	E	11.85±2.91[c]	9.76±3.79[c]	8.36±2.69[c]
	R	14.06±3.07[d]	15.03±3.16[d]	15.08±2.22[d]
FE_{Pi}	C	0.13±0.03	0.48±0.35	0.57±0.32
(%)	E	0.10±0.01	0.29±0.13	0.50±0.29
	R	0.12±0.06	0.58±0.29	0.66±0.33

[a] Infusion rate of Paf-acether, in ng/min/100 g bw.
[b] C, E, R: control, experimental, recovery periods, respectively.
[c] Significantly different from the control value, $p < 0.05$.
[d] Significantly different from the experimental value, $p < 0.05$.

Table 2
Tubular effects of lyso-Paf-acether and ethoxy-Paf-acether[a].

		Lyso-Paf-acether	Ethoxy-Paf-acether
V̇	C	72.0±3.2	48.8±5.5
(µl/min)	E	78.1±1.2	56.7±19.2
GFR	C	0.879±0.106	0.965±0.002
(ml/min)	E	1.027±0.129	1.053±0.075
FE_{Pi}	C	0.20±0.05	0.10±0.01
(%)	E	0.23±0.12	0.10±0.01
$U_{Ca}\times\dot{V}$	C	109.5±2.2	83.1±0.8
(nmol/min)	E	105.8±9.8	75.1±29.7
FE_{Ca}	C	7.82±1.53	5.81±0.28
(%)	E	6.97±1.84	4.87±1.40
$U_{Mg}\times\dot{V}$	C	93.0±6.8	76.2±7.8
(nmol/min)	E	91.0±12.7	80.3±3.2
FE_{Mg}	C	25.61±2.70	18.72±2.56
(%)	E	22.27±3.65	16.89±0.23
FE_{Na}	C	0.49±0.45	0.11±0.08
(%)	E	1.17±0.96	0.31±0.03
FE_K	C	7.41±4.63	8.28±5.89
(%)	E	12.51±1.06	7.40±1.30

[a] C: control period; E: experimental period.

In contrast, neither lyso-Paf-acether nor ethoxy-Paf-acether elicited any significant alteration of the different variables, as shown in Table 2.

Discussion

The present study demonstrates that infusion of Paf-acether, but not of lyso-Paf-acether or ethoxy-Paf-acether, deeply affected the renal function in the rat.

Paf-acether was demonstrated to be a potent hypotensive agent in both normal and hypertensive rats [18–20]. Therefore, we chose infusion rates so that no or only mild decreases of MAP were observed in order to remain in the glomerular filtration rate autoregulation range [21], and to allow a valuable interpretation of tubular changes. Indeed, GFR decreased only for the highest rate of Paf-acether infusion. The enhanced GFR value observed during the recovery period in the first group might be the consequence of an increased, Paf-acether-induced glomerular synthesis of vasodilatory prostaglandins [22]. Such a mechanism might also partly account for the high Na excretion observed during the last period in the three groups. A decreased Na and K excretion during Paf-acether infusion was previously reported in the dog [13]. However, in the latter study, the infused amounts of Paf-acether were large enough to cause a marked decrease of renal blood flow and GFR [12, 13].

The Paf-acether-induced decrease of urinary flow rate might be due to an increased proximal reabsorption of fluid. Such an increase, while GFR was preserved, might be accounted for by i) a decrease of renal blood flow [23], ii) its redistribution towards juxtamedullary nephrons [24], which possess longer proximal tubules [25], and iii) by an increase in the water permeability of the collecting duct, which, however, seems unlikely in the absence of vasopressin.

Previous demonstrations that tubular reabsorption of divalent cations Ca and Mg was enhanced by several polypeptidic hormones (i.e. vasopressin, parathyroid hormone and calcitonin) [14, 26, 27] has led us to examine the effect of Paf-acether on the tubular handling of Ca and Mg in the absence of the above mentioned hormones, that is in acutely thyroparathyroidectomized Brattleboro rats. In these conditions the decrease not only of absolute excretion, but also of fractional excretion of Ca and Mg suggests and enhanced tubular reabsorptive capacity during Paf-acether infusion. A possible link between Ca and Mg excretion on the one hand, and urinary output on the other hand, has been evaluated by others [27], who concluded that Ca, but not Mg excretion, was positively correlated to urinary flow rate.

As regards Mg, its proximal reabsorption usually accounts for less than 5% of the filtered load, whereas the bulk of reabsorption occurs in the thick ascending limb of Henle's loop (TAL) [14, 28]. Therefore, the dramatic decrease of Mg excretion during Paf-acether infusion is most likely to be the consequence of an increased Mg reabsorption in this segment, and might reflect a direct action of Paf-acether on Mg transport by TAL. The mechanism of such an effect might involve an increase of the passive, voltage-dependent, intercellular Mg reabsorption in the medullary ascending limb [29], as a consequence of a possible increase in the lumen-positive transepithelial potential. Indeed, Paf-acether was shown to increase phosphatidylinositol breakdown in several tissues [22, 30], thereby increasing cytosolic calcium concentration [31, 32]. Paf-acether could therefore stimulate calcium-sensitive potassium channels which have been located in the apical membrane of medullary thick ascending limb cells [33], and then enhance the potassium blackflux to the lumen which was shown to generate the lumen-positive transepithelial potential [29].

Finally, the renal effects of Paf-acether were not shared by its two analogues lyso-Paf-acether and ethoxy-Paf-acether, supporting the need of a chemical integrity for the biological effect, as reported in other systems [34], and suggesting the presence of receptors for Paf-acether in the kidney.

In conclusion, these results show that Paf-acether, even when infused at rates which do not decrease GFR, induces an increase of Ca, and Mg reabsorption. Such events might occur in those circumstances where renal Paf-acether production is increased, and might be accounted for, at least partly, by a direct tubular action, apart from general or renal hemodynamic changes.

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