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LABORATORY INVESTIGATION

Platelet activating factor inhibits Cl and K transport in the medullary thick ascending limb

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Platelet activating factor inhibits Cl and K transport in the medullary thick ascending limb. Since the kidney medulla was reported to generate platelet activating factor (PAF), we investigated a possible effect of this agent on the reabsorptive function of in vitro microperfused medullary thick ascending limbs from mouse kidney (mTAL). PAF, 10^{-7} M in the bath, significantly decreased the net chloride flux (JCl) from 48.8 ± 7.1 to 27.4 ± 5.7 pmol/min. This effect was reversible, blocked by the antagonist BN 50730, and not reproduced by the inactive metabolite lyso-PAF. PAF inhibited the transepithelial potential difference with a threshold at 10^{-9} M. In the presence of isoproterenol, the PAF-induced decrease of JCl was not significantly different from that observed in basal conditions; moreover, PAF did not modify the adenylate cyclase activity in isolated mTALs, either in basal condition or under stimulation by isoproterenol. The effect of PAF on JCl was not prevented by mepacrine, NDGA associated with proadifen, or adenosine deaminase. When the apical Na-K-2Cl cotransport was blocked by furosemide or bumetanide, a net K secretion occurred (−1.1 ± 0.2 pmol/min), which was significantly decreased by PAF (−0.06 ± 0.3 pmol/min). Moreover, it was verified on isolated mTALs that PAF did not modify the Na,K-ATPase activity. It is concluded that PAF inhibits the reabsorptive function of the mTAL, as indicated by the decrease of Cl reabsorption and K secretion. This effect could not be accounted for by adenosine or arachidonic acid metabolite action, and was not mediated by an inhibition of the adenylate cyclase activity.

Platelet activating factor (PAF, 1-0-akyl-2-0-acetyl-SN-glycero-3 phosphocholine), a mediator of anaphylactic shock and inflammation, was shown to be produced by a wide variety of cells [reviewed in 1]. The generation of PAF by the mesangial cells of the glomeruli [2] and by the renal medulla cells [3] suggested a paracrine action of this agent. As far as the effect of PAF on renal function was concerned, numerous studies [reviewed in 1] reported that PAF induced a decline of the glomerular filtration rate, an effect that might partly result from mesangial cells contraction, thromboxane A_2 production, and/or decrease in blood pressure [4, 5]. Furthermore, PAF was proposed as a potential mediator in the pathogenesis of glomerulonephritis [6].

Only few data are available about a possible direct tubular effect of PAF [7, 8]. The studies, moreover, were performed in vivo, thus the decrease in urinary flow rate and sodium excretion [8] as well as of calcium and magnesium excretion [7] observed during PAF infusion could not be definitely dissociated from hemodynamic effects. Nevertheless, a direct action of PAF on the collecting tubule was recently suggested by the report of a PAF induced increase in cytosolic free calcium concentration in cultured inner medullary collecting duct cells [9].

The aim of the present work was to investigate a possible effect of PAF on the reabsorptive function of the medullary thick ascending limb of Henle's loop (mTAL), especially on net chloride reabsorption and net potassium secretion, by the in vitro microperfusion technique. The main results indicated that: i) PAF decreased chloride reabsorption, potassium secretion, and the transepithelial potential difference (PDt); ii) PAF did not act by inhibiting the adenylate cyclase activity; iii) these effects were not likely to be mediated by arachidonic acid metabolite or adenosine. In addition to prostaglandin E_2 (PGE_2) [10] PAF thus represents an inhibiting agent of the mTAL function.

Methods

Microperfusion experiments

mTALs were microperfused in vitro following the technique first described by Burg et al [11] and detailed elsewhere [12]. Briefly, male Swiss White mice, 18 to 25 g body wt, were killed by cervical dislocation and exsanguinated. Coronal slices were then cut from both kidneys and were immediately immersed in a cold perfusing solution (see *Composition infra*) added to 0.4% bovine serum albumin (Sigma Chemical Co., St. Louis, Missouri, USA). mTALs (length = 563 ± 34 μm) were harvested from the inner stripe of the outer medulla. The segments were then transferred to a Lucite chamber (1.5 ml) in which the bath was continuously flowing at 5 ml/min. Bath temperature was thermostatically maintained at 37 ± 0.5°C (YSI probe, Yellow Springs Instruments Co., Yellow Springs, Ohio, USA). When the tubular fluid was collected (all series except IV; see *Infra*), the tubules were perfused at a rate of about 3 nl/min. When the transepithelial potential difference (PDt) was recorded and no tubular fluid collected (series IV), the tubular flow rate was more than 10 nl/min. In this condition, it was supposed that no sodium chloride gradient between the bath and the perfusate occurred and consequently that no dilution potential was generated.

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Each collection experiment lasted two hours. Unless otherwise mentioned, a 30-minute experimental period was preceded by a 30-minute control period and followed by a 30-minute recovery period. Ten minutes were allowed for equilibration between the control and the experimental periods as well as between the experimental and recovery periods. The luminal fluid was collected every 10 minutes so that three samples per period of 30 minutes were collected.

Nine series of experiments were performed:

Series I (N = 12). PAF (Eurobio, Paris, France) was added to the bath in the experimental period, at the final concentration of 10^{-7} M. The solvent (chloroform) was present in the bath throughout the experiment (1/10,000 vol/vol).

Series II (N = 5). The protocol was identical to series I. In addition, a specific antagonist of PAF, BN 50730 (gift from Beaufour, Les Ulis, France), was present in the bath at the final concentration of 2.10^{-6} M throughout the experiment.

Series III (N = 6). Lyso-PAF (Eurobio, Paris, France) was added to the bath in the experimental period at the final concentration of 10^{-7} M.

Series IV (N = 8). PAF at final concentrations ranging from 10^{-11} to 10^{-6} M was added to the bath. On each tubule, two to four PAF concentrations were tested. For each tested concentration, the control, experimental, and recovery periods lasted about 10 minutes, during which the PDt was continuously recorded.

Series V (N = 5). The protocol was the same as in series I. In addition, mepacrine (Sigma), a phospholipase A_2 inhibitor, was present in the bath at the final concentration of 6.10^{-6} M throughout the experiment.

Series VI (N = 5). Nordihydroguaiaretic acid (NDGA, Sigma) and proadifen (Eurobio), inhibitors of lipooxygenase and cytochrome P_{450} monooxygenase, respectively, were both added to the bath at the final concentration of 10^{-6} M throughout the experiment. Two tubules were used as the time control. In three tubules, PAF was added during the experimental period as in series I. No recovery period was performed in this series.

Series VII (N = 7). The protocol was identical to series I, however, in order to prevent a possible effect of endogenous adenosine, adenosine desaminase (ADA, Boehringer, Mannheim, Germany) was present in the bath at the final concentration of 2.5 mg/liter throughout the experiment. Moreover, in one tubule, adenosine desaminase was present in the perfusate at the same concentration as in the bath, and in two tubules, a specific A_1 adenosine antagonist, 1,3-Dipropyl-8-(2-amino-4-chlorophenyl) xanthine (PACPX, Research Biochemicals Inc., Natick, Massachusetts, USA), was present in the bath at the final concentration of 10^{-7} M, as well.

Series VIII (N = 5). The protocol was as in series I; in addition, isoproterenol (Isuprel©) was present in the bath at the final concentration of 10^{-7} M throughout the experiment.

Series IX (N = 6). As in series I. In addition, tubules were perfused with furosemide (Lasilix©, N = 2) at the final concentration of 10^{-4} M, or bumetanide (gift of Hoffman LaRoche Laboratory, Basel, Switzerland, N = 4) at the final concentration of 6.10^{-6} M, throughout the experiment.

The composition of the perfusing solution was as follows (in mM): NaCl 147, K_2HPO_4 2.4, NaH_2PO_4 0.6, $MgCl_2$ 1, $CaCl_2$ 1, urea 10, N-2-hydroxyethylpiperazine-N′-2-ethane sulfonic acid (HEPES) 5. The composition of the bathing solution was similar except for lower NaCl concentration, 142 mM, because of the addition of glucose 5 mM and Na acetate 5 mM. All solutions were adjusted to 300 mOsmol/kg H_2O with urea and to pH 7.38 to 7.42.

The PDt was recorded (Keithley Instruments, Cleveland, Ohio, USA) between two calomel electrodes connected to the bath and perfusate by 5% agar bridges in 0.9% NaCl. The volumes of the collected samples were measured with a calibrated constriction pipette, and the flow rate of perfusate was calculated assuming that water reabsorption was negligible in the mouse mTAL [13]. In the samples and perfusate, potassium and chloride concentrations were determined by ultramicron emission spectrophotometry [14] and microelectrometric titration [15], respectively. The results were expressed as net fluxes for the whole segment, calculated by multiplying the tubular flow rate by the concentration difference between the perfused and the collected fluid. Since it was reported that, in the mouse mTAL, chloride reabsorption decreases [16] as the luminal chloride concentration decreases, the net fluxes could not be considered as proportional to tubular length and therefore were not monitored by the tubular length.

The results from each period were pooled and considered as one point. The data were expressed as means ± SE. To calculate the inhibition of the net chloride flux (JCl) as the percentage of the control value, it was verified that a linear relationship existed between JCl in the experimental period and JCl in the nonexperimental periods (mean of control and recovery values). For series I, II, III, V, VII, and VIII, the correlation was significant ($0.01 < P < 0.001$). Statistical significance was evaluated in the same series by the paired Student's *t*-test, and between the series ($N = 7$) by the one-way analysis of variance followed by the least significant difference method.

Na,K-ATPase activity determination

The technique already reported for rat kidney [17] was modified as follows: male Swiss mice were anesthetized with pentobarbital sodium (0.5 mg/10 g body wt). The left kidney was perfused in situ via the abdominal aorta with 2 ml of incubation solution containing (in mM) NaCl 120, KCl 5, $CaCl_2$ 1, $MgSO_4$ 1, NaH_2PO_4 4, $NAHCO_3$ 4, glucose 5, lactate 10, pyruvate 1, essential and nonessential amino acids 4, vitamins (vitamins for Eagle's minimum essential medium, Eurobio) 30, and HEPES 20. Dextran 40,000 (0.3% wt/vol), collagenase (CLS II, 240 U/mg, Worthington) 0.3% wt/vol, and bovine serum albumin 0.2% wt/vol were added. The left kidney was removed and thin pyramids were then cut out along the corticopapillary axis of the kidney and were incubated for 15 minutes at 30°C in the incubation solution containing 0.1% collagenase. mTALs were microdissected from the inner stripe of the outer medulla in incubation solution maintained at 4°C. The length of each sample was determined by automatic image analysis.

ATPase activity was determined under V_{max} conditions by a radiochemical microassay, that is, measurement of ^{32}P released from ($^{32}P\alpha$) ATP, after permeabilization of cell membranes. ATPase activity was expressed as picomoles of inorganic phosphate liberated per millimeter of tubule length per hour. For each condition, ATPase was measured on four to six replicate samples. Na,K-ATPase activity was calculated as the

difference between the activities determined either in the presence or in the absence of Na. Results are presented as means ± SE of the values from several animals. Statistical significance was assessed by Student's *t*-test.

Adenylate cyclase activity determination

Adenylate cyclase activity was determined in single mTAL tubules by use of the microassay previously described in the rat [18]. Tubule microdissection was carried out on mice kidney as reported above for Na,K-ATPase activity determination. mTALs were individually incubated at 0 to 4°C for 30 minutes in 0.5 μl of a hyposmotic solution containing 0.25 mM EDTA, 1 mM $MgCl_2$, 5×10^{-5} M GTP, 1 mg/ml bovine serum albumin, and 8 mM Tris (hydroxymethyl) aminomethane, Tris HCl, pH 7.6. When necessary, isoproterenol or PAF were added to this solution at a concentration of 10^{-7} M in the final incubate. Samples were then submitted to a brief freezing on dry ice before adenylate cyclase measurement was initiated by adding 2 μl of incubation medium to each of them. The composition of this medium was adjusted to obtain the following concentrations in the final incubate: cAMP 1 mM, EDTA 0.25 mM, $MgCl_2$ 4 mM, GTP 10^{-5} M, [$^{32}P\alpha$] ATP 0.30 to 0.35 mM (0.5 to 0.8 μCi/sample), phosphocreatine 20 mM, creatine kinase 1 mg/ml, and Tris HCl (pH 7.6) 100 mM. After a 30 minute incubation at 30°C, the reaction was stopped by adding 150 μl of a solution containing 3.3 mM ATP, 5 mM cAMP, 50 mM Tris HCl, and tracer amounts of [^{3}H]-cAMP for recovery calculations. The [^{32}P]-cAMP formed was separated from other labeled nucleotides by double filtration through Dowex and aluminium oxide columns, and the radioactivity present in the eluate was counted by liquid scintillation.

Adenylate cyclase activity was calculated as femtomoles of cAMP formed per 30 minutes of incubation period per millimeter tubular length. For each experimental condition, adenylate cyclase was determined on four to six replicate samples. Results were calculated as means ± SE of the value from several animals. Statistical analysis was performed by Student's *t*-test.

Results

Effect of PAF on the net chloride reabsorption and on PDt

The results obtained in series I (Fig. 1) showed that PAF significantly decreased the net chloride flux (JCl) from 48.8 ± 7.1 pmol/min to 27.4 ± 5.7 pmol/min ($P < 0.001$). This effect was partly reversible since JCl returned to 33.4 ± 7.9 pmol/min ($P < 0.05$) after removal of the PAF. This effect did not result from a decrease of the tubular flow rate since the flow rate was fairly constant throughout the experiment (V, nl · min: 3.08 ± 0.44, 2.94 ± 0.47, 2.73 ± 0.51, in control, experimental, and recovery periods, respectively, NS vs. the preceding period). The effect of PAF on JCl was completely blocked by BN 50730 (series II, Fig. 1). Moreover, it was not reproduced by lyso-PAF (series III), an inactive metabolite of PAF, since JCl was decreased by 6.4 ± 4.0% (nonsignificantly different from zero) versus 36.8 ± 3.8% in the presence of PAF ($P < 0.001$). However, the progressive decrease with time of JCl observed in the series III accounted partly for the incomplete reversibility of JCl after PAF removal (series I).

The effect of PAF at different concentrations was tested on PDt (series IV, Fig. 2). The mean control value of PDt for all the tested tubules was 6.9 ± 1.3 mV. PAF inhibited PDt with a threshold at 10^{-9} M (PDt: 90.4 ± 1.9% of the control value, $P < 0.01$). At 10^{-6} M, the inhibition of PDt was significantly higher ($P < 0.01$) than at 10^{-7} M, the concentration used in this study.

To investigate a possible mediation of the PAF effect by arachidonic acid metabolites or by adenosine, the inhibitory effect of PAF on JCl was evaluated in the presence of mepacrine, a phospholipase A_2 inhibitor (series V), and under conditions preventing a possible adenosine accumulation and/or action (ADA, series VII).

Comparison between the series (Fig. 3) indicated that the percentage of inhibition of JCl: 1) was not significantly different between the series PAF (series I), PAF plus mepacrine (series V), and PAF plus ADA (series VII); and 2) was significantly higher in the presence of PAF (series I, V, and VII) than in the presence of lyso-PAF (series III) and BN 50730 (series II). Moreover, in the presence of NDGA and proadifen (series VI),

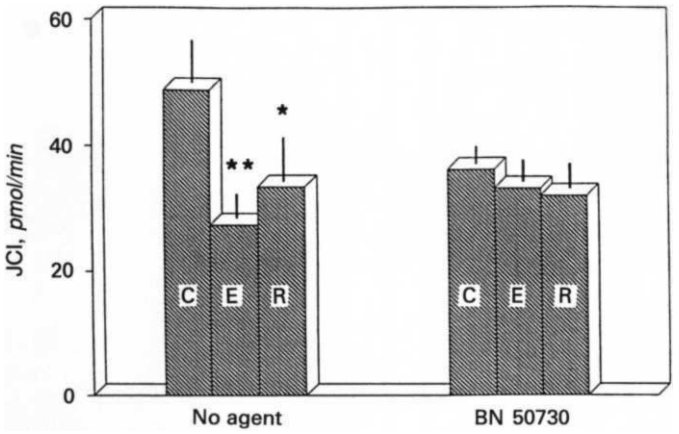

Fig. 1. *Effect of PAF on chloride reabsorption (JCl) in the absence and in the presence of BN 50730.* BN 50730, 2×10^{-6} M, was present during all the periods. C: control period; E: experimental period (PAF 10^{-7} M, series I and II); R: recovery period. *, ** Significantly different from the preceding period; * $P < 0.05$, ** $P < 0.001$. The control periods in the absence and in the presence of BN 50730 do not differ significantly.

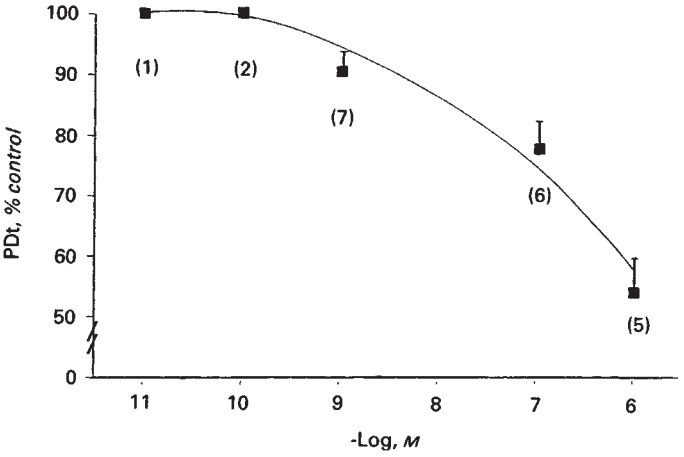

Fig. 2. *Inhibition of transepithelial potential difference (PDt) by different concentrations of PAF.* Abscissa: PAF concentration (log scale); ordinate: PDt, expressed as the percentage of the control value for each tubule. (*N*): number of determinations.

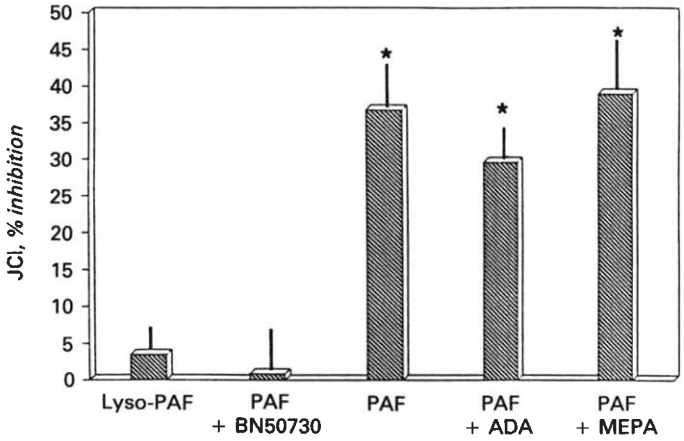

Fig. 3. *Inhibition of chloride reabsorption (JCl) in the presence of lyso-PAF 10^{-7} M (series III), PAF 10^{-7} M plus BN 50730 (2×10^{-6} M, series II), PAF 10^{-7} M (series I), PAF 10^{-7} M plus adenosine desaminase (ADA, 2.5 mg/liter, series VII), and PAF 10^{-7} M plus mepacrine (MEPA, 6×10^{-6} M, series V).* Ordinate: percentage of inhibition of JCl by comparison with the control value. For each tubule, the control value was the mean of the control and recovery periods. * Significantly different from lyso-PAF and BN 50730 ($P < 0.001$).

the decrease of JCl was 10.0 ± 1.3 pmol/min in the presence of PAF while it was only 4.3 ± 0.4 pmol/min in the absence of PAF.

The effect of PAF on JCl was investigated when adenylate-cyclase activity was stimulated by isoproterenol (series VIII). As already shown in our laboratory [12], isoproterenol significantly increased JCl: In the control period (Table 1), JCl was significantly higher than in the absence of isoproterenol (74.4 ± 10.5 vs. 48.8 ± 7.1 pmol/min, series VIII vs. series I, $P < 0.01$). In the presence of isoproterenol, PAF induced a significant decrease of JCl (Table 1). The decrease of JCl was not significantly different from that obtained in the absence of isoproterenol (26.5 ± 4.0 vs. 21.4 ± 2.9 pmol/min, series VIII vs. series I, NS). Moreover, when adenylate cyclase activity was determined on isolated mTALs, the results indicated that PAF did not significantly modify the enzyme activity, either in basal condition or in the presence of isoproterenol (Table 2).

Effect of PAF on the net potassium secretion

Before any addition of a Na-K-2Cl cotransport blocker (series IX), the chloride concentration difference between the perfused and the collected fluid (ΔCl) was 17.8 ± 2.2 mM, a value not significantly different from that observed in the control period of series I (20.4 ± 4.2 mM) for similar tubular flow rates (3.08 ± 0.4 vs. 3.58 ± 0.3 nl/min, series I vs. series IX, NS). Administration of furosemide or bumetanide at the luminal side resulted in a fall of ΔCl to 1.0 ± 0.8 mM, as well as in an abolition of PDt from 5.8 ± 0.9 mV to 0.4 ± 0.2 mV (results from the first control sample). In this condition, a net significant K secretion (JK) occurred in the control period (−1.14 ± 0.21 pmol/min). In the presence of PAF (Fig. 4), JK was significantly decreased to a value not different from zero (−0.06 ± 0.28 pmol/min, $P < 0.01$ vs. control period). After removal of PAF (recovery period), a net potassium secretion resumed (−0.62 ± 0.16 pmol/min, significantly different from zero, $P < 0.01$).

Table 1. Effect of PAF on Cl reabsorption by mTALs in the presence of isoproterenol

Period	V *nl/min*	ΔCl *mM*	JCl *pmoles/min*
Control (isoproterenol)	3.1 ± 0.6	26.9 ± 5.3	74.4 ± 10.5
Experimental (isoproterenol + PAF)	3.1 ± 0.5	17.0 ± 4.3[b]	47.7 ± 9.5[a]
Recovery (isoproterenol)	3.0 ± 0.5	20.6 ± 4.2	56.6 ± 9.6[a,c]

Values are means ± SE. Abbreviations are: V̇, tubular flow rate; ΔCl, chloride concentration difference between the perfused and the collected fluid; JCl, net chloride flux. Difference in ΔCl between the recovery and the experimental periods was at the limit of significance (t = 2.4).

[a,b] Significantly different from the control period, [a] $P < 0.05$; [b] $P < 0.01$

[c] Significantly different from the experimental period, $P < 0.05$

Table 2. Effect of PAF on isoproterenol-sensitive adenylate cyclase activity in mTALs

	Adenylate cyclase *fmol · cAMP/30 min · mm*
Baseline	15.8 ± 0.2
PAF	13.6 ± 1.2
ISO	89.2 ± 10.9[a]
ISO + PAF	87.6 ± 5.6[a]

Values are means ± SE. For each condition, 5 tubule samples were obtained from 5 mice. Abbreviations are ISO, isoproterenol (10^{-7} M); ISO + PAF, isoproterenol (10^{-7} M) plus platelet-activating factor (10^{-7} M).

[a] Significantly different from basal value, $P < 0.001$

The basolateral entry of K was estimated by the determination of the Na,K-ATPase activity on isolated mTALs. PAF 10^{-7} M did not significantly modify the enzymatic activity (Table 3), this absence of effect persisted independent of the preincubation time.

Discussion

The present study represents the first demonstration of a direct tubular effect of PAF. The data showed that, in addition to PGE_2 [10], PAF could inhibit the reabsorptive function of the mTAL since it decreased chloride reabsorption, potassium secretion, and, consequently, PDt. Moreover, the results suggest that neither adenosine or arachidonic acid metabolite could account for the PAF effects and showed that, at variance with what was reported for PGE_2 [19, 20], the effect of PAF was not related to an inhibition of adenylate cyclase activity.

Direct effect of PAF

The present data showed that, in the mTAL, PAF decreased PDt as the consequence of the decrease of JCl and JK (see below). In the thick ascending limb, PDt could also be decreased either by adenosine, as recently shown in the cortical part [21], or by PGE_2 in the medullary part [10]. The present results indicated that adenosine could not entirely account for the inhibitory effect of PAF on JCl, since that effect persisted when adenosine accumulation and/or action on the mTAL was prevented by adenosine desaminase and an adenosine antagonist, the PACPX. As far as PGE_2 was concerned, the question of its possible role as a mediator of PAF was raised since PAF

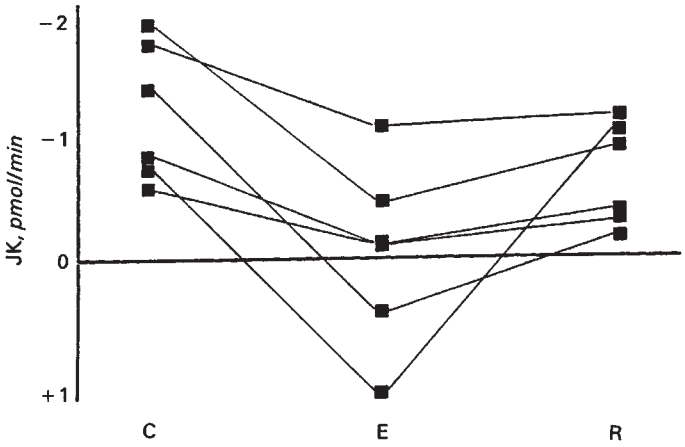

Fig. 4. *Effect of PAF on individual net fluxes of potassium (JK).* C: control period; E: experimental period (PAF 10^{-7} M, series IX); R: recovery period. Negative values indicate a net secretion.

Table 3. Effect of PAF on the Na,K-ATPase activity in isolated mTALs

Preincubation time *min*	Na,K-ATPase activity *pmol/hr · mm*	
	Control	PAF 10^{-7} M
0	5175 ± 310 (4)	5005 ± 322 (3)
10	4769 ± 1053 (2)	4843 ± 591 (3)
30	5180 ± 640 (3)	5016 ± 444 (3)
60	4848 (1)	4410 ± 443 (2)

Values are means ± SE. (*N*), five tubular samples were obtained from each mice, the number of mice is indicated in parenthesis for each condition. PAF 10^{-7} M was added either at the time of the enzymatic activity determination or 10 to 60 minutes before, in the incubation medium.

was reported to stimulate PGE_2 synthesis in the glomeruli [4]. Two lines of evidences allow us to reject this hypothesis: i) In the thick ascending limb of Henle's loop, undetectable levels of PGE_2 are produced [22, 23]; ii) the blockade of arachidonic acid production by mepacrine, a phospholipase A_2 inhibitor, failed to modify the effect of PAF on JCl. Moreover, provided that phospholipase A_2, lipooxygenase, and cytochrome P_{450} monooxygenase were completely blocked in the present conditions, the fact that the presence of NDGA plus proadifen did not prevent the effect of PAF suggests that no arachidonic acid metabolite was involved.

PAF and cyclic AMP

When adenylate cyclase was stimulated by isoproterenol, PAF exerted on JCl an effect that was similar to that observed in basal conditions. Moreover, the data indicated that PAF had no effect on adenylate cyclase activity neither in basal conditions or when stimulated by isoproterenol [12], thus the mechanism of PAF action could be different from that described for PGE_2. In the mTAL, indeed, PGE_2, which was shown to inhibit cyclic AMP synthesis [19, 20], displayed a pattern of action different from that reported here for PAF: PGE_2 failed to exert any effect on cyclic AMP content [19] as well as on PDt [10] in basal conditions, but decreased PDt and cAMP production when adenylate cyclase was hormonally stimulated.

Cellular mechanisms for the PAF effect on the mTAL function

The results presented here show that PAF decreased the net chloride reabsorption and the net potassium secretion in the mTAL. According to the model for sodium chloride reabsorption in this segment [24, 25] the transepithelial chloride reabsorption is active via an apical Na-K-2Cl cotransport, while the transepithelial sodium reabsorption is both active via the Na-K-2Cl cotransport and passive through the shunt pathway. The electrical driving force for the passive sodium reabsorption is a positive luminal PDt resulting from both potassium exit through an apical conductance and chloride exit through a basolateral conductance. The inhibition by PAF of JCl might thus result from either a decrease of the apical Na-K-2Cl cotransport activity and/or a decrease of the basolateral chloride conductance.

As far as potassium was concerned, PAF decreased its net secretion, likely in the absence of modification of the driving force since: i) a passive potassium transport was prevented in zero PDt conditions; ii) the apical entry of potassium was blocked by furosemide or bumetanide; iii) the basolateral entry of potassium was likely unchanged since the data indicated that the Na,K-ATPase activity was not modified in the presence of PAF. These results are compatible with a decreased potassium secretion resulting from a PAF-induced decrease of a potassium conductance. Since PAF was reported to increase cytosolic calcium concentration in many target cells [1], including the epithelial kidney cells [26], it is attractive to speculate, as a working hypothesis, that PAF induced the decrease in potassium secretion via an inhibition of the apical calcium-inhibited potassium channel [27].

Possible physiological relevance of the effect of PAF on the mTAL function

The present data indicated that PAF decreased PDt at a 10^{-9} M concentration. Several evidences suggest that this threshold of concentration obtained in vitro is compatible with an effect of endogenous PAF: i) In kidney homogenates, the medullary production of PAF was reported to be about 1.5 pmol/mg protein [3]. Assuming a concentration of 10 g protein per 100 g of wet tissue, one can estimate a PAF concentration of about 10^{-10} M in the medulla, thus only one order of magnitude below the concentration active in vitro. ii) The blockade by a PAF antagonist of the PAF-induced inhibition of JCl suggested, although it did not prove, that PAF exerted this effect via specific receptors. iii) Regarding the well-known effect of PAF on the contraction of the glomerular mesangial cells, Schlondorff et al [4] reported a value of Kd between 10^{-8} and 10^{-7} M.

PAF was reported to be a hypotensive agent that decreased renal blood flow, leading to a medullary hypoperfusion. In the mTAL, Brezis et al [28] showed a high sensitivity to hypoxia and that the resulting epithelium damages may be prevented by inhibition of the NaCl reabsorption. We thus suggest that the inhibitory effect of PAF on the mTAL reabsorptive function might result in a preservation of the cell integrity of this segment during renal injury.

In summary, the present work showed that PAF decreased chloride reabsorption and potassium secretion in the mTAL. This effect was not mediated by an inhibition of the adenylate-cyclase activity, and likely resulted from a direct tubular effect of PAF. The possible implication of this effect in the preservation of the tubular function during renal injury needs further investigation.

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