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Atrial Natriuretic Factor, Arachidonic Acid Metabolites and Acute Renal Ischemia: Experimental Protocol in the Rat

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Abstract. The effects of an atrial natriuretic factor (ANF) infusion upon the production of the arachidonic acid metabolites (thromboxane B_2, TxB_2; 6-keto-prostaglandin $F_{1\alpha}$, 6-keto-$PGF_{1\alpha}$, PGI_2, or prostaglandins E_2, PGE_2) were investigated after acute renal ischemia in the rat. This experimental protocol included a right nephrectomy and a 45-min left renal artery occlusion. Fifteen minutes after declamping, blood samples were collected from the left renal venous effluent for the assay of plasmatic prostanoid concentrations. Three experimental groups were studied: group I (n = 9) sham, no ischemia-group II (n = 9) control group, 45 min of left renal ischemia, followed by a 15-min revascularization, and group III (n = 10) ANF group, a similar ischemic protocol to that in group II was used but, after declamping, synthetic Atriopeptin III was infused (0.5 μg/kg/min) during the 15-min of vascular reflow. Fifteen minutes after declamping, TxB_2 secretion significantly increased after ischemia in the control and ANF groups: TxB_2: 210 ± 22.4 pg/ml (control group) and 234.8 ± 25.1 pg/ml (ANF group) versus 135.8 ± 17.8 pg/ml (sham group) ($p < 0.05$ and 0.01, respectively). On the other hand, the 6-keto-$PGF_{1\alpha}$ plasma levels were significantly higher after ischemia in the ANF group (221 ± 34 pg/ml) in comparison with the sham (129 ± 24.1 pg/ml) or with the control group (116.7 ± 12.5 pg/ml). The calculated TxB_2/6-keto-$PGF_{1\alpha}$ ratio was therefore higher in the control group, 1.93 ± 0.27, than in physiological conditions (sham group), 1.2 ± 0.17. In the ANF group, on the other hand, the TxB_2/PGI_2 ratio, 1.13 ± 0.08, was comparable to the basal conditions and therefore lower than in the control group ($p < 0.02$). The PGE_2 concentration increased significantly after ischemia but did not display any difference between the control and the ANF group. These experimental results suggests that the vascular properties of ANF, protecting the ischemically injured kidney, were mediated by an endogenous vasodilating substance production, i.e. PGI_2. Usefulness of ANF is therefore postulated in several clinical applications, i.e. kidney transplantation.

On the intact kidney, atrial natriuretic factor (ANF) seems to be not only a powerful natriuretic substance but also a regulator of the renal vascular resistance and renal blood flow [14]. Indeed, ANF produces a preglomerular artery vasodilatation with a concurrent efferent artery constriction increasing therefore the fraction filtration rate [15]. Likewise, after acute renal ischemia, ANF preserved these effects. Indeed, improved glomerular filtration rate (GFR) and increased natriuresis in uninephrectomized rats undergoing 30 or 60 min of renal artery occlusion [5, 16] were recently reported owing to a postischemic ANF infusion. Nevertheless, the relationship between the vascular effect of ANF upon the ischemically injured kidney and the arachidonic acid metabolites production remains unexplored. This experimental rat study investigated whether a synthetic ANF infusion affects the endogenous prostanoid production after an ischemic insult.

Material and Methods

Male Wistar rats weighing 250 g were used in this study. Anesthesia was induced by ether inhalation and maintained by intraperitoneal injections of pentobarbital sodium (2.5 mg/kg). Under microscope, a polyethylene catheter PE-20 (Clay-Adams, Parsippany, Becton Dickinson, N.J.) was introduced in the left femoral vein, and a right nephrectomy was performed through a midline laparotomy.

The left renal artery was dissected and occluded with a smooth vascular clamp for 45 min. During the whole ischemic period, the body temperature of all the animals was measured by a thermistor probe and maintained between 36 and 37 °C. After this ischemic insult, the clamp was removed. Fifteen minutes after declamping, a 4-ml blood sample was taken from the left renal venous effluent by direct puncture (through the vena cava) with a syringe containing ethylenediaminetetraacetic acid and indomethacin for the assay of the arachidonic acid metabolites: thromboxane B_2 (TxB_2), 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), and prostaglandin E_2 (PGE_2). The plasma was separated by centrifugation, and kept frozen at −20 °C until assayed.

Plasma Prostanoid Extraction

For prostanoid extraction, plasma samples were acidified at pH 3.5 with citric acid and passed through octadecyl (C18) columns (solid phase extraction system, J.T. Baker Chemicals, Phillipsburg, N.J.), previously rinsed with methanol and acidified water. These columns were sequentially washed with acidified water, 15% methanol and benzene, and, finally, the products were eluted with ethyl acetate. The ethyl acetate fraction was dried at 37 °C under nitrogen and redissolved in RIA buffer. Extraction efficiency was about 90% and this was taken into account in the calculation of the prostanoid concentration.

Radioimmunoassay

TxB_2, 6-keto-$PGF_{1\alpha}$ (the stable hydrolysis products of thromboxane A_2, TxA_2, and prostaglandin I_2, PGI2, respectively) and PGE_2 were assayed in extracted plasma samples and in unextracted tissue incubation medium by competitive binding RIA. All assays were performed with New England Nuclear RIA kits (Dreieich, FRG). Diluted samples were incubated with the appropriate ^{125}I-labeled tracer and specific rabbit antiserum for 16–24 h at 4 °C. Free and antibody-bound tracers were separated by the addition of polyethylene glycol. After centrifugation, the radioactivity in the pellets was measured with a γ-counter. Duplicate values were averaged and interpolated from a standard curve. Results are expressed as picograms per milliliter for plasma.

After the right nephrectomy, three experimental groups were assessed.

Sham group (n = 9). These animals underwent *no* ischemia. After 45 min of 'Sham ischemia', 1 ml of isotonic saline solution (NaCl 0.9%) was infused intravenously during 15 min through the left femoral catheter. The blood sample from the left renal venous effluent was taken 1 h after the beginning of the surgical procedure. The measurement of the plasmatic prostanoid concentrations in this group served as the basal plasmatic values without acute ischemic injury.

Control group (n = 9). Acute renal ischemia (45 min) was carried out by a selective left renal

artery clamping. After declamping, 1 ml of isotonic saline solution (NaCl 0.9%) was infused through the left femoral catheter during the 15-min period of revascularization. At the end of this period, the blood sample (4 ml) from the renal venous effluent was taken out as described above.

ANF group (n = 10). The same ischemic protocol as in the control group was performed. During the 15 min after declamping, synthetic rat Atriopeptin III (Peninsula Laboratories, UK) dissolved in 1 ml of isotonic saline solution was infused at a rate of 0.5 μg/kg/min.

All the parameters are expressed as means ± SEM; a one-way analysis of variance (ANOVA) was used for the comparison of each parameter between the three experimental groups and a Scheffé test for the comparison, two by two, in the case of a global statistical difference.

Results

All the results of the assay of the various prostanoid metabolites (TxB_2; 6-keto-$PGF_{1\alpha}$ and PGE_2) in the plasma samples are reported in table 1.

As expected, in the control group (45 min of ischemia), the TxB_2 concentration increases significantly after ischemia in comparison with the levels obtained in the sham group (without ischemia). Indeed, the TxB_2 plasmatic concentration reached 135.8 ± 17.8 pg/ml in the sham group versus 210 ± 22.4 pg/ml in the ischemically injured kidneys ($p < 0.05$). In the ANF group, the TxB_2 concentrations followed a similar increase, 234.8 ± 25.1 pg/ml, to that in the control group ($p < 0.05$; ANF vs. sham group).

In contrast, the PGI_2 metabolites (6-keto-$PGF_{1\alpha}$) plasma levels vary in a different manner in the sham or control group and in the ANF group. Indeed, 15 min after declamping the 6-keto-$PGF_{1\alpha}$ concentrations were comparable in the sham group (129 ± 24.1 pg/ml) and in the control group (116.7 ± 12.6 pg/ml, NS) whereas in the ANF group these $PGF_{1\alpha}$ plasma concentrations reached significantly higher plasmatic levels than in these two former groups: 221 ± 34 pg/ml ($p < 0.05$, vs. sham or control group).

Thus, the calculated TxB_2/6-keto-$PGF_{1\alpha}$ ratio reached 1.2 ± 0.17 in the sham group (physiological basal values) and was significantly higher in the control group, 1.93 ±

Table 1. Results of the measurements of the arachidonic acid metabolites in each experimental group

	Group I: sham (n = 9)	Group I vs. II	Group II: control (n = 9)	Group II vs. III	Group III: ANF (n = 10)	Group I vs. III	F[a]
TxB_2, pg/ml	135.8 ± 17.8	$p < 0.06$[b]	210 ± 22.4	NS	234.8 ± 25.1	0.01[b]	0.02
6-Keto-$PGF_{1\alpha}$, pg/ml	129 ± 24.1	NS	116.7 ± 12.6	$p < 0.02$[b]	221 ± 34	0.05[b]	0.02
PGE_2, pg/ml	15.4 ± 2.9	$p < 0.001$[b]	52.7 ± 8.9	NS	43.9 ± 4.7	0.001[b]	0.001
TxB_2/PGI_2	1.2 ± 0.17	$p < 0.05$[b]	1.93 ± 0.27	$p < 0.02$[b]	1.13 ± 0.08	NS	0.02

Values expressed in mean ± SEM.

[a] One-way analysis of variance (ANOVA).

[b] Scheffé test two by two when global statistical difference is demonstrated by ANOVA.

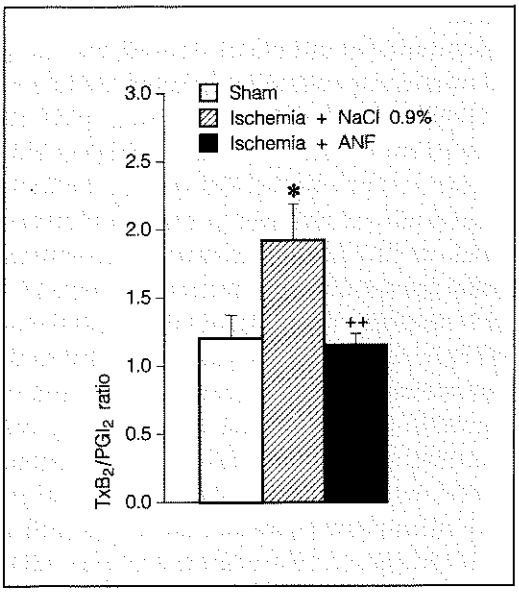

Fig. 1. TxA_2/PGI_2 ratio after 45 min of acute renal ischemia and 15 min of reperfusion in the sham group (□: without ischemia), in the control group (▨: 45 min of ischemia) and in the ANF group (■: 45 min of renal ischemia and 15 min of ANF infusion during the reperfusion period). * $p \leq 0.05$, control vs. sham; ++ $p \leq 0.02$, ANF vs. control.

0.27, in which the TxB_2 levels increased significantly whereas the PGI_2 production remained unchanged. An intravenous synthetic ANF infusion after declamping gave a similar calculated TxB_2/6-keto-$PGF_{1\alpha}$ ratio, 1.13 ± 0.08, to that in the sham group, and therefore significantly lower than in the control group ($p < 0.02$). In the ANF group, indeed, both TxB_2 and 6-keto-$PGF_{1\alpha}$ levels increased similarly so that the TxB_2/6-keto-$PGF_{1\alpha}$ ratio remained comparable to the ratio obtained in basal conditions (fig. 1).

On the other hand, acute renal ischemia produces an important rise of the PGE_2 concentrations (control/ANF groups vs. sham group) whereas the PGE_2 plasma levels did not display any difference between the control and the ANF group (table 1).

Discussion

As previously reported, acute renal ischemia significantly increases the plasmatic TxB_2 release whereas it affects the 6-keto-$PGF_{1\alpha}$ production less [2, 12]. This higher TxB_2/6-keto-$PGF_{1\alpha}$ ratio probably contributes to the renal cortical vasoconstriction and to the decrease in glomerular filtration rate usually described in ischemically injured kidneys [8]. This inference seems to be plausible since it has clearly been demonstrated that a synthetic PGI_2 or analogue infusion decreases the severity of the post-ischemic acute renal failure in both dogs and rats [3, 7]. In the same way, a thromboxane synthetase inhibitor protects the ischemically injured kidney through a TxA_2 release inhibition with a concurrent relative PGI_2 production [13].

Recently, atrial natriuretic peptide has been described as a protective substance against acute renal ischemia but the mechanisms of action involved remain unidentified [5, 16]. Indeed, in uninephrectomized rats, ANF provides after a renal artery occlusion (30 or 60 min) a significant improvement of the GFR and an increased natriuresis. The assumed mechanisms of these ANF effects include *vascular properties*, i.e. an elective preglomerular vasodilatation with a concurrent efferent artery vasconstriction, and other *endocrine* properties, i.e. an inhibition of the renin-angiotensin-aldosterone axis [6, 14, 15].

The relationship between the vascular ANF effects upon the ischemically injured

kidney and the possible involvement of the prostanoids remains nevertheless unexplored [10]. Our experimental results make it possible to demonstrate that after ischemia ANF is able to selectively stimulate the production of a vasodilating substance (2-fold) such as PGI_2. In contrast, the TxA_2 production seems to be unaffected by this peptide. Therefore, after a renal ischemic insult, the main important effect induced by an ANF infusion appears to be the recovery of a TxA_2/PGI_2 ratio comparable to the basal level without ischemia. On the contrary, an acute ischemic insult usually induces a higher TxA_2/PGI_2 ratio than in basal conditions without ischemia. The finding that ANF affects PGI_2 release rather than that of PGE_2 seems to demonstrate that the natriuretic peptide is a selective prostacyclin synthetase stimulator. The increase in PGI_2 activity induced by ANF after ischemia probably produces, as on intact kidney, a redistribution of the renal cortical blood flow to the deep cortex. Moreover, this higher prostacyclin activity might also lead to the improved GFR through an afferent artery vasodilatation. In contrast the natriuretic ANF effect seems to be independent of these prostanoids since on intact kidney, Salazar et al. [17] clearly demonstrated that a cyclooxygenase inhibitor does not influence the natriuresis due to ANF whereas the PGI_2 activity is inhibited.

All these results lead to suggest that the preglomerular artery vasodilatation obtained with an ANF infusion is probably mediated by these PGI_2 [17].

These results agree, moreover, with the fact that ANF prevents the ciclosporin nephrotoxic side effects since this drug on the contrary produces an afferent artery vasoconstriction through a higher TxA_2 production and therefore a higher TxB_2/PGI_2 ratio [1, 4, 9, 11].

In summary, this study demonstrates that the natriuretic peptide stimulates the PGI_2 production after acute renal ischemia. These results shed some light upon the vascular mechanisms of action induced by ANF after ischemia and further suggest interesting usefulness in kidney transplantation since ANF also appears to be a powerful natriuretic substance.

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