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# BENEFICIAL EFFECT OF ATRIAL NATRIURETIC FACTOR ON ISCHEMICALLY INJURED KIDNEYS IN THE RAT

## A NEW APPROACH TO IMPROVE EARLY RENAL FUNCTION[1]

PIERRE GIANELLO,[2] DIDIER POELAERT, ADRIEN RAMBOUX, JEAN-PAUL SQUIFFLET, ADRIEN BERBINSCHI, JULIAN DONCKIER, JEAN-MARIE KETELSLEGERS, LUC LAMBOTTE, AND GUY ALEXANDRE

*Departments of Transplantation, Anesthesiology, Endocrinology, and Experimental Surgery, University of Louvain Medical School, 1200 Brussels, Belgium*

**In a rat experimental study we investigated whether the atrial natriuretic peptide by itself is able to improve early renal function after an ischemic injury. Two groups of Wistar male rats underwent a right nephrectomy and a left renal artery occlusion for 30 min and were infused for 2 hr after ischemia with isotonic saline or rat atrial natriuretic peptides (αANF: 28 amino acids (AP 28) and atriopeptin III (AP 24): 24 amino acids). ANF infusion increased the urinary flow ($P<0.001$), the urinary sodium concentration ($P<0.001$), the sodium excretion rate ($P<0.0001$), and improved the glomerular filtration rate (GFR) recovery ($P<0.02$) determined at the end of the 2-hr infusion period. AP 24 exhibited higher natriuretics activities than AP 28. The effect of both peptides upon GFR recovery was equivalent. These effects of ANF observed after acute ischemia suggest that this peptide may be beneficial on the resumption of renal function in the early phases following transplantation.**

Since the discovery by Debold et al., that the atria of mammals, including humans, contain a natriuretic factor, major progress has been made in identifying and determining the effects of the atrial natriuretic factor (ANF)* (*1*, *2*). The natriuretic peptide is important in physiological conditions—not only as a powerful natriuretic substance but also as a potential modulator of GFR, renal blood flow, and renal vascular resistance (*3*).

Its diuretic and natriuretic effect, recently demonstrated at physiological and pharmacological plasma concentrations is, in part, mediated by an increase of glomerular filtration (*4*). These effects do not require an increase in renal blood flow but are attributed to the rise in glomerular capillary pressure resulting from afferent arteriolar vasodilation and concurrent efferent arteriolar vasoconstriction (3, 5–8). Moreover, its natriuretic effect may also be mediated by an inhibition of the renin-angiotensin-aldosterone system (*9*) and by a direct tubular effect (*10*).

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[2] Address correspondence to: P. Gianello, M.D., Department of Transplantation, Cliniques Universitaires St-Luc, Avenue Hippocrate 10, 1200 Brussels, Belgium.

* Abbreviations used: ANF, atrial natriuretic factor; DGF, delayed graft function; EDTA Cr51, Ethylene diamineotetraacetic acid labeled with 51 chrome; GFR, glomerular filtration rate.

If the natriuretic peptide appears to be important in physiological conditions (*11*, *12*) it may also preserve natriuresis effects in pathological situations with renal impairment. Indeed, the natriuretic effects of ANF have been demonstrated in the experimental chronic renal failure model of subtotally (5/6) nephrectomized rats (*13*).

The present experimental protocol has been conducted to study the ANF effects after acute renal failure, to define its possible interest for clinical kidney transplantation.

## MATERIALS AND METHODS

Wistar male rats weighing 200–250 g were used in this study.

All animals were fed a standard rat chow ad libitum and were housed at 24°C, with an alternating cycle of 12 h light/dark for at least three days before the experiment.

The day before the operation, all the animals were separately housed in metabolic cages for the first preoperative assessment: urea, creatinine and sodium were measured in serum and in 24-hr urinary collection; glomerular filtration rate (GFR) was assessed by ethylenediaminotetraacetate $^{Cr51}$ (Table 1) (*14*).

The next day, anesthesia was induced by an intraperitoneal injection of pentobarbital sodium (25 mg/kg) and a polyethylene catheter (PE-10; Clay Adams) placed in the left femoral vein. Through a midline incision, a right nephrectomy was performed and the left renal artery occluded with a smooth vascular clamp for 30 min. Thereafter, the bladder was drained with a silastic catheter for sampling the urinary output. During the ischemic period, the body temperature was controlled and maintained between 36°C and 37°C. After acute renal ischemia, the abdominal wall was sutured and the animals were placed in individual restraining cages. During the next 2 hr, the animals were infused with isotonic sodium chloride or ANF. The control group (n=22) received the isotonic sodium chloride solution (NaCl 0.9%) at a rate of 1 ml/h for 2 hr; in the ANF group (n=19), natriuretic peptides (Peninsula Laboratories Europe, Ltd.) dissolved in isotonic saline solution were infused at the same rate as in the controls, at a dose of 0.5 μg/kg/min.

The ANF group was divided into two subgroups according to types of synthetic natriuretic peptide at rat being used: the first subgroup (n=9) received αANF, a 28-amino-acid peptide (AP 28) and the second subgroup (n=10) was infused with atriopeptin III (AP 24), a 24-amino-acid peptide. At the end of the 2-hr infusion period, the following parameters were analyzed: urinary output, sodium concentration, sodium excretion rate, and GFR recovery (EDTA $^{Cr}$51).

Plasma ANF was obtained by radioimmunoassay as equal values of thawed serum samples, and a 25% solution of polyethylene glycol 6000 in phosphate buffer 0.1 M, pH 7.4, containing 0.1% Triton X-100 and 0.1% human albumin, were mixed and allowed to stand for 1 hr at 4°C. After centrifugation at 2800 rpm for 30 min, supernatants were serially diluted and immunoreactive ANP levels measured in RIA by competi-

tion with $^{125}$I-αANP (human). Tracer (specific activity of 74 TBQ/mmol, [±150,000 cpm/tube]) was from Amersham; rabbit antibodies against 1–28 α human ANP (100% crossreactive with 1–28 rat ANP and rat atriopeptin III) and synthetic human αANP standard were from Peninsula Laboratories Inc. (Belmont, CA). Serum levels of ANP were calculated in picograms per milliliter after correction for recovery of synthetic ANP added to control rat serum and processed like the samples. Recovery and IC 50 were 89% and 69 pg/ml, respectively.

All values are expressed as mean ± SEM. Statistical analysis included Student's unpaired or paired *t* test or Wilcoxon's rank sum test.

## RESULTS

As shown in Table 1, renal function of both groups of rats was comparable before the experiment with regard to the following data: urinary flow; serum urea, creatinine and sodium; urinary urea, creatinine, and sodium; urea and creatinine clearance and GFR and sodium excretion rate. After a 30-min ischemic period in the control group, there was a significant decrease in the urinary flow ($P<0.001$), sodium excretion rate, and GFR ($P<0.001$) but urinary sodium concentration remained unchanged (Table 2). Two hours of ANF infusion, initiated after the ischemic period, improved recovery of early renal function significantly. Indeed, the urinary flow (Fig. 1) was 5-fold higher in the ANF group than in the controls ($P<0.001$); ANF also caused a significant 1.4-fold increase in urinary sodium concentration (Fig. 2; $P<0.001$), such that the total sodium excretion rate was 6 times higher in the treated than in the control group (Fig. 3) ($P<0.0001$). We also found that ANF significantly improved the GFR recovery after ischemia (Fig. 4; $P<0.02$).

The comparison between the two types of rat natriuretic peptides demonstrated that AP 24 had a more pronounced natriuretic effect than AP 28 upon urinary flow (16.5±2.8 μl/min versus 6.6±1.7 μl/min) ($P<0.01$) and sodium excretion rate (2.6±0.38 μEq/min versus 1.09±0.31 μEq/min) ($P<0.01$), respectively. However, its effect upon GFR recovery was similar with the two types of peptides (48.4±1.16% versus 43.5±3.4%).

ANF plasma concentrations did not change 2 hours after ischemia in the control group, rose from 57±7.1 pg/ml to 555±60 pg/ml in the AP 28 subgroup, and increased to 337±36 pg/ml in the AP 24 subgroup ($P<0.01$).

## DISCUSSION

The present study clearly demonstrates that ANF infusion improved the early renal function of ischemically injured kidneys with regard to urinary flow, sodium excretion rate, and GFR recovery. Preglomerular vasoconstriction and tubular obstruction are assumed to play a major role in the genesis of acute renal failure caused by ischemia (*15*), whereas ANF appears as an important modulator of renal blood flow and can induce vasodilatation in vasoconstricted kidneys (*3*). In addition, the best GFR recovery after ischemia in the ANF group results from an afferent arteriolar vasodilatation and concurrent efferent arteriolar vasoconstriction, as previously reported in the intact kidney (*3, 6, 7, 16*).

Moreover, the pronounced natriuresis in the ANF group is probably also mediated by an inhibition of the renin-angiotensin-aldosterone system (*9*) and a decreased absorption in the collecting tubule (*10*) after ANF infusion, as reported in kidney without ischemic injury. In this study, the natriuresis was more pronounced, with the 24-amino-acid peptide (AP 24) than with the 28-amino-acids peptide (AP 28) but the GFR recovery in the two subgroups was similar. Comparable and opposite effects of AP 28 and AP 24 were reported on intact kidney in experimental biochemistry: the results were explained by a heterogenicity of AP receptors in vascular and renal tissues (*17, 18*). We can suggest that acute ischemia modifies these receptors, but it appears that the disulfide bridge between $^{129}$cystin and $^{145}$cystin is essential for the known biological activities of ANF (*5*). The plasma ANF levels reached in the ANF group are far higher than in physiological or pathological states (10 times baseline values). Further study will be required to determine the minimal dose-effect upon kidney submitted to acute ischemia. The present study has clearly demonstrated the beneficial effects of ANF infusion on the early renal function after acute

TABLE 1. Comparison of preoperative data in the two groups of rats[a]

| Group | Control (n=22) | Atrial natriuretic factor (n=19) | |
|---|---|---|---|
| Urinary volume (μl/min) | 10.6±1.34 | 9.2±1.93 | NS |
| Serum urea (mg%) | 37.3±2.6 | 46±2.8 | NS |
| Urinary urea (mg%) | 3492±722 | 3797±526 | NS |
| Urea clearance (ml/min) | 0.93±0.08 | 0.76±0.06 | NS |
| Urinary creatinine (mg%) | 68.1±6.5 | 93.6±13.3 | NS |
| Serum creatinine (mg%) | 0.58±0.02 | 0.6±0.02 | NS |
| Creatinine clearance (ml/min) | 1.01±0.04 | 1.1±0.2 | NS |
| EDTA $^{51}$Cr clearance (ml/min) | 1.98±0.05 | 1.92±0.02 | NS |
| [Na] Serum concentration (mEq/L) | 141.7±0.8 | 143.7±0.7 | NS |
| [Na] Urinary concentration (mEq/L) | 78±11 | 87±16 | NS |
| Urinary [Na] excretion rate (μEq/min) | 0.59±0.06 | 0.50±0.06 | NS |

[a] Values are expressed as mean ± SEM; statistical analysis by Student's *t* test.

TABLE 2. Effect of renal artery occlusion (30 min) on kidney function:control group (n=22)[a]

| | Before ischemia | After ischemia | *P* values |
|---|---|---|---|
| Urinary flow (μl/min) | 10.6±1.34 | 2.3±0.7 | <0.001 |
| Sodium concentration (mEq/L) | 78±11 | 106±10 | NS |
| Sodium excretion rate (μEq/min) | 0.59±0.06 | 0.31±0.11 | <0.001 |
| Glomerular filtration rate (ml/min) | 1.98±0.05 | 0.77±0.05 | <0.0001 |

Values are expressed as mean ± SEM; statistical analysis by Student's paired *t* test.

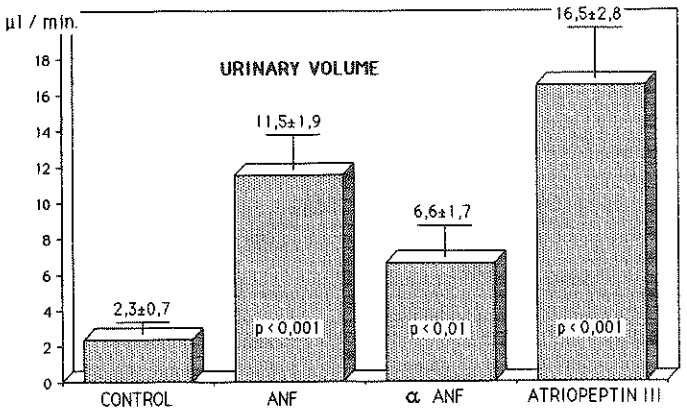


FIGURE 1. Urinary flow: results 2 hr after ANF or isotonic infusion in the postischemic period. Comparison of control group (n = 22), ANF total group (n = 19), AP 28 subgroup (n = 10) and AP 24 subgroup (n = 9) by Wilcoxon rank sum test (ANF, AP 24, and AP 28 versus control group).

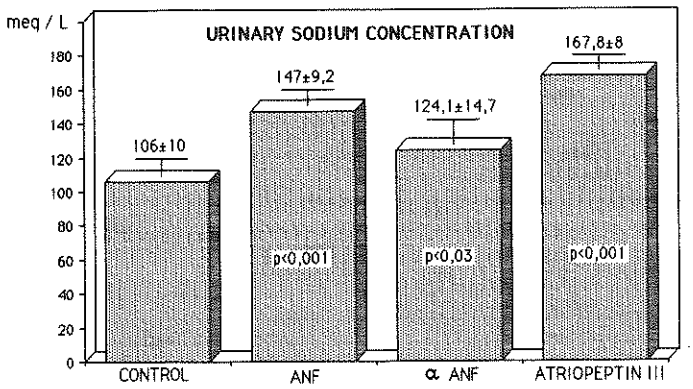


FIGURE 2. Sodium concentration: results 2 hr after ANF or isotonic infusion in the postischemic period. Comparison of control group (n = 22), ANF total group (n = 19), AP 28 subgroup (n = 10) and AP 24 subgroup (n = 9) by Wilcoxon rank sum test (ANF, AP 24, and AP 28 versus control group).

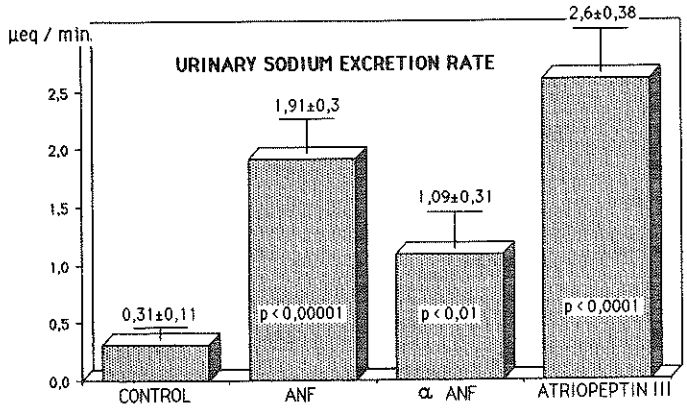


FIGURE 3. Sodium excretion rate: results 2 hr after ANF or isotonic infusion in the postischemic period. Comparison of control group (n = 22), ANF total group (n = 19), AP 28 subgroup (n = 10) and AP 24 subgroup (n = 9) by Wilcoxon rank sum test (ANF, AP 24, and AP 28 versus control group).

ischemia; in addition the increased GFR induced by the peptide could prevent late tubular obstruction by increasing tonicity of the medulla and subsequent tubular washout (*3*). Such an effect may perhaps decrease the rate of delayed graft function in

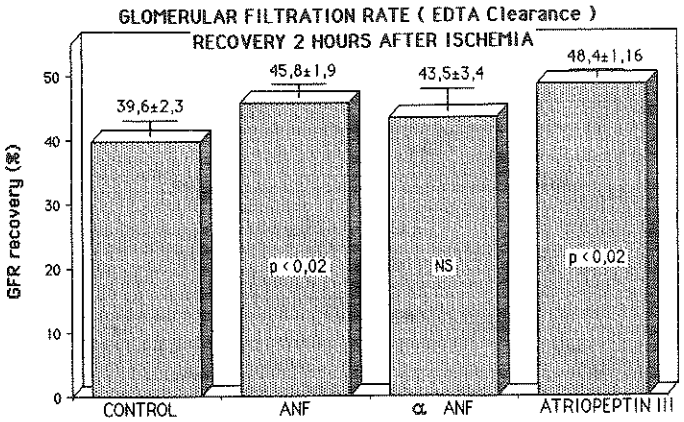


FIGURE 4. GFR recovery: results 2 hr after ANF or isotonic infusion in the postischemic period. Comparison of control group (n = 22), ANF total group (n = 19), AP 28 subgroup (n = 10) and AP 24 subgroup (n = 9) by Wilcoxon rank sum test (ANF, AP 24, and AP 28 versus control group).

human kidney transplantation. The relationship between an improved early renal function induced by ANF and the long-term results remains, nevertheless, to be established.

Up to now, various pharmacological agents have been used to improve the early graft function after warm and/or cold ischemia injuries. The beneficial effects of mannitol—and, more recently, the protective effects of calcium channel blockers have been described (*19–21*). Nevertheless, in experimental surgery, it is worth noticing that a perfusion of impermeant solute or an acute volume loading by simple saline infusion, by themselves, can protect against ischemic acute renal failure (*22, 23*); this protective effect was explained before the knowledge of ANF effects, at least in part, by better maintenance of the glomerular hydrostatic pressure and prevention of tubular obstruction. Now there is good evidence that ANF release occurs physiologically in response to volume expansion and is directly correlated with an increase in atrial pressure (24, 25, 26). Knowledge of the better GFR recovery after ischemic injury, and the correlation between volume loading and ANF release, permits the suggestion that this peptide participates in the mechanisms by which hyperhydration improves the recovery of renal function after kidney transplantation. Indeed, Carlier et al. have demonstrated that maximal hydration during anesthesia in patients receiving a kidney transplant improves the early graft function and decreases the incidence of delayed graft function (*27, 28*); in view of these results, Carlier et al. postulated that mediation of a humoral factor as well because in this clinical study the kidney was denervated (*27*). The results of experimental study and a better knowledge of the natriuretic peptide lead us to suspect that ANF is this hypothetical humoral factor.

In conclusion, we have demonstrated that ANF infusion can improve the GFR recovery and increase natriuresis after acute renal ischemia. It may be reasonable to suggest that ANF, by increasing of GFR and decreasing tubular obstruction through a washout of the kidney, can decrease the rate of delayed graft function in human kidney transplantation, and that the beneficial effect of hyperhydration was mediated by ANF.

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