In Vitro and In Vivo Protective Effect of Atriopeptin III on Ischemic Acute Renal Failure

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Abstract

The effect of atriopeptin III (AP-III) on ameliorate ischemic acute renal failure was first examined in the isolated perfused kidney. Isolated rat kidneys were clamped for 1 h and reperfused for 30 min without therapy and then perfused with either 0 (control) or 100 μg/dl AP-III. In this system AP-III significantly improved renal plasma flow (39.6±2.4 vs. 32.2±2.1 ml/min per g; $P < 0.05$) inulin clearance (182.6±49.2 vs. 24.6±6.2 μl/min per g; $P < 0.05$), urine flow (52.9±12.1 vs. 7.1±0.8 μl/min per g, $P < 0.01$), and net tubular sodium reabsorption (21.2±6.6 vs. 2.9±0.9 μmol/min per g, $P < 0.05$) as compared with control. A second series of in vivo studies experiments were performed using 1 h of bilateral renal artery clamping followed by an intravenous infusion of either saline alone (control) or AP-III (0.20 μg/kg per min) for 60 min. The results demonstrated that inulin clearance (244.4±25.1 vs. 15.8±8.2 μl/min per 100 g; $P < 0.01$), urine flow (23.1±5.9 vs. 1.1±0.5 μl/min per 100 g; $P < 0.01$), and net tubular sodium reabsorption (38.9±4.7 vs. 4.3±1.6 μmol/min per 100 g; $P < 0.01$) were significantly higher in AP-III–treated rats than controls during the hour of AP-III infusion. In 1 h posttreatment study this significant protective effect of AP-III was documented to persist. In more chronic studies animals treated acutely with AP-III had lower serum creatinine concentration at 24 h (1.8±0.3 vs. 3.3±0.4 mg/dl; $P < 0.01$) and 48 h (1.0±0.2 vs. 2.4±0.4 mg/dl; $P < 0.01$) after the 60 min of ischemia than controls. Renal adenosine triphosphate regeneration as assessed by P-31 nuclear magnetic resonance during reflow was also significantly improved in AP-III–treated animals at 1 h (3.03±0.30 vs. 1.45±0.40 μmol/g dry wt; $P < 0.05$) and 2 h (3.98±0.46 vs. 1.80±0.05 μmol/g dry wt; $P < 0.01$) of reflow as compared with control rats. Thus, AP-III significantly ameliorates ischemic acute renal failure both in vitro and in vivo in the rat.

Introduction

The hallmark of ischemic acute renal failure is a profound diminution in glomerular filtration rate that is disproportionate to any decrease in renal blood flow (1). Efforts to prevent or attenuate renal ischemic injury should therefore result in maintenance of glomerular filtration rate. In this regard, the newly discovered hormone, atrial natriuretic factor (ANF),[1] has been shown to possess receptors on the glomerulus (2). Moreover, studies of glomerular dynamics using micropuncture techniques indicate a selective increase in glomerular filtration rate independent of glomerular plasma flow, because of vasodilation of the afferent arteriole combined with an increase in postglomerular resistance (3, 4). These characteristics suggested that ANF might provide unique protection against ischemic acute renal failure.

Methods

Studies were performed in the isolated perfused kidney and an in vivo model of ischemic acute renal failure.

Experiments in an isolated perfused kidney (group I). Male Sprague-Dawley rats weighing 320–420 g fed on rat chow (Ralston Purina Co., St. Louis, MO) and allowed free access to tap water were used. Rats were anesthetized with pentobarbital given intraperitoneally, and kidneys were dissected using the method described by Nishiitsutsuji-Uwo, Ross, and Krebs (5), as modified by Ross, Epstein, and Leaf (6). Experiments were performed using a recirculating perfusion circuit and perfusion media which has been described in a previous paper (7). Briefly, the perfusion media consisted of 6.7% albumin in Krebs-Henseleit saline supplemented with glucose 5 mM, 20 amino acids as described by Epstein et al. (8), and inulin 25 mg/dl. ^{14}C inulin (New England Nuclear, Boston, MA) was also added to the perfusion media. The perfusion media was adjusted to pH 7.4 and maintained at that level by continuous gassing with 95% O_2/5% CO_2. The temperature of the perfusion media was maintained at 37°C during each experiment. After cannulation of the right renal artery and brief flushing of the right kidney with perfusion media the arterial tubing was clamped, producing ischemia to that kidney for 1 h in situ. After this ischemic period, the clamp was released and the right kidney was removed from the animal and placed on the perfusion circuit where it was perfused with 100 mmHg perfusion pressure for 90 min. After the first 30 min of reperfusion either 0, 10, or 100 μg of atriopeptin III (AP-III) (Peninsula Laboratories, Inc., Belmont, CA) was added in a randomized blind fashion to 100 ml of perfusate. During reperfusion urine was collected during 15-min collection periods, and plasma samples were drawn at the midpoint of each collection period. Renal plasma flow (RPF) was measured with a Brooks flowmeter (Thomas Scientific, Philadelphia, PA). Urine flow (V), inulin clearance (C_{In}), fractional sodium reabsorption (FR_{Na}), net sodium reabsorption (T_{Na}), and net urinary sodium excretion ($U_{Na}V$) were calculated as described previously (7).

In vivo experiments in ischemic acute renal failure (group II). Sprague-Dawley rats weighing 300–350 g fed as described previously were used for the in vivo studies. Anesthesia was accomplished with

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1. *Abbreviations used in this paper:* ANF, atrial natriuretic factor; AP-III, atriopeptin III; C_{In}, inulin clearance; FR_{Na}, fractional sodium reabsorption; MAP, mean arterial blood pressure; NMR, nuclear magnetic resonance; Pi, inorganic phosphate; RPF, renal plasma flow; T_{Na}, net sodium reabsorption; $U_{Na}V$, net urinary sodium excretion; V, urine flow.

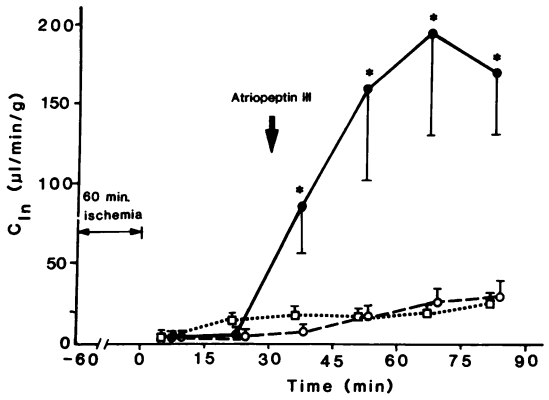

Figure 1. Effect of AP-III on C_{In} after ischemia in the isolated kidney. AP-III was infused at 100 µg/dl (*solid circles*) (n = 6) or 10 µg/dl (*open circles*) (n = 6). Control (*open squares*) (n = 5) received no AP-III. Results of experiments are mean±SEM. $^{*}P < 0.05$ compared with control.

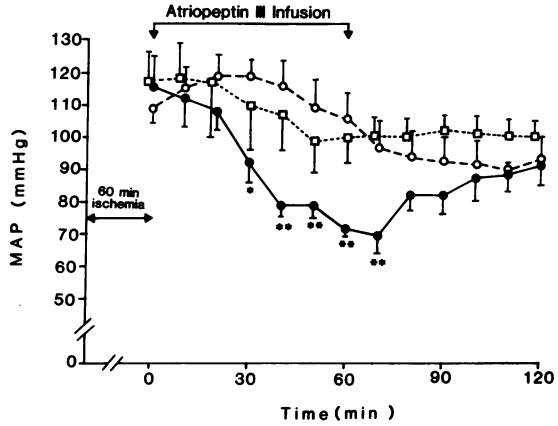

Figure 2. Effect of AP-III on mean arterial pressure (MAP) after ischemia in vivo. AP-III was infused at 0.2 µg/kg per min (*solid circles*) (n = 6) or 0.02 µg/kg per min (*open squares*) (n = 4) for 60 min. Control (*open circles*) (n = 6) received no AP-III. Results expressed as mean±SEM. $^{*}P < 0.05$; $^{**}P < 0.01$ compared with control.

pentobarbital as described above. A midline incision was performed and both renal arteries were clamped with vascular clamps for 1 h in situ. In the acute in vivo studies, an arterial catheter was placed in the carotid artery for monitoring blood pressure, and for infusion of AP-III a venous catheter was placed in the internal jugular vein. A bladder catheter was placed following a suprapubic incision to collect urine. In the subacute (24–48 h) studies, only the internal jugular catheter was placed. All catheters were made from PE-50 tubing.

In the acute studies, when the renal clamps were removed either 0, 0.02, or 0.2 µg/kg per min AP-III was infused for 1 h in a randomized, blinded fashion. The AP-III was dissolved in 100 µl of 0.2 M acetic acid and then added to the saline given to each animal during the first hour after clamp removal. All animals received 5 ml/kg per h of saline for 2 h after clamp removal. In the control experiments only the acetic acid was added to the saline. A bolus of ^{14}C inulin dissolved in 100 µl of saline was given 30 min before release of the vascular clamp, and ^{14}C inulin was added to the saline infusion so as to result in a constant plasma level. Animals also received a volume of normal saline each 30 min to replace urine output. During the 2 h after clamp removal, mean arterial blood pressure (MAP) was continuously recorded and V, C_{In}, FR_{Na}, and T_{Na} monitored every 30 min.

In the subacute studies animals were acclimatized to metabolic cages, after which a baseline 24-h urine collection was performed. The rats were then anesthetized and a baseline plasma sample obtained from the tail artery. Then, as described above, bilateral renal ischemia was induced. Then either the vehicle or 0.2 µg/kg per min of AP-III was infused for 1 h. After 1 h of infusion, the venous catheter was removed, the vein ligated, and the midline incision closed with 3-0 chromic and 4-0 silk sutures. The animals were allowed to recover in their metabolic cages, and urine was collected for 2 d. Blood was sampled from the tail artery of anesthetized rats at 24 and 48 h after the renal artery clamping. 48 h after clamping, the right kidney of each rat was perfused with 2% glutaraldehyde for histologic studies.

Three control and three experimental rats were studied with ^{31}P nuclear magnetic resonance (NMR). These studies involved the iden-

Table I. Effect of AP-III on Renal Function after Ischemia in the Isolated Kidney

	Time	Control	AP-III	AP-III
	min	*0 µg/dl**	*10 µg/dl‡*	*100 µg/dl‡*
Renal plasma flow (*ml/min/g*)	0–30	27.6±3.1	23.8±2.2	25.6±2.7
	30–60	33.5±1.4	36.5±1.4	39.1±1.8§
	60–90	32.2±2.1	34.7±2.0	39.6±2.4§
C_{In} (*µl/min/g*)	0–30	11.6±4.2	3.9±1.6	4.3±0.8
	30–60	17.0±4.7	13.6±2.8	123.4±44.0§
	60–90	24.6±6.2	28.4±8.4	182.6±49.2§
V (*µl/min/g*)	0–30	3.3±0.4	2.6±1.2	1.3±0.4
	30–60	4.8±0.8	6.3±1.6	31.4±8.0§
	60–90	7.1±0.8	10.1±2.1	52.9±12.1‖
T_{Na} (*µmol/min/g*)	0–30	1.3±0.8	0.2±0.1	0.8±0.4
	30–60	2.3±0.8	1.4±0.3	14.8±6.0§
	60–90	2.9±0.9	3.0±1.2	21.2±6.6§
$U_{Na}V$ (*µmol/min/g*)	0–30	0.7±0.3	1.0±0.1	0.5±0.1
	30–60	0.7±0.1	0.9±0.2	3.6±0.8‖
	60–90	0.8±0.2	1.2±0.2	5.6±1.3‖

Results expressed as mean ± SEM. All data normalized per gram of left kidney weight.
* n = 5. ‡ n = 6. § $P < 0.05$. ‖ $P < 0.01$ vs. control.

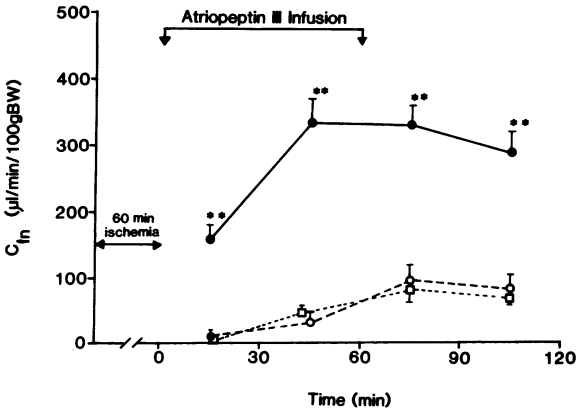

Figure 3. Effect of AP-III on C_{In} after ischemia in vivo. AP-III was infused at 0.2 µg/kg per min (*solid circles*) (n = 6) or 0.02 µg/kg per min (*open squares*) (n = 4) for 60 min. Control (*open circles*) (n = 6) received no AP-III. Results of experiments are mean±SEM. Results normalized per 100 g animal body weight. $^{**}P < 0.01$ compared with control.

Table II. Effect of AP-III Infusion on Recovery of Renal Function over 2 h after 60 min of Ischemia In Vivo

	Time	Control*	AP-III‡	AP-III*
	min		*0.02 μg/kg/min*	*0.20 μg/kg/min*
C_{In} (*μl/min/100 g*)	0–60	15.8±8.2	21.4±3.6	244.4±25.1§
	60–120	88.6±21.9	73.8±10.4	311.3±27.8§
V (*μl/min/100 g*)	0–60	1.1±0.5	1.1±0.4	23.1±5.9§
	60–120	2.6±0.4	4.3±0.6	12.8±3.8§
FR_{Na} (%)	0–60	94.1±1.0	92.6±1.0	94.4±2.1
	60–120	97.2±0.6	94.8±0.8‖	96.5±1.0
	0–60	4.3±1.6	5.9±1.7	38.9±4.7§
T_{Na} (*μmol/min/100 g*)	60–120	15.4±4.4	10.3±1.4	49.1±3.6§
$U_{Na}V$ (*μmol/min/100 g*)	0–60	0.2±0.1	0.2±0.1	3.3±0.8§
	60–120	0.3±0.1	0.5±0.1	2.0±0.6‖

Results expressed in mean±SEM. * $n = 6$. ‡ $n = 4$. § $P < 0.01$ vs. control. ‖ $P < 0.05$.

tical procedure described in the subacute in vivo protocol except that after renal arterial clamp removal, a left flank incision was made which exposed the kidney and a two-turn 1.5-cm-diameter surface coil was placed over the kidney. The kidney was also covered with a thin plastic covering to avoid tissue moisture losses. ^{31}P NMR spectroscopy was performed for 2 h after removal of the vascular clamps in a 1.89 Tesla 30-cm horizontal bore magnet (Oxford Research Systems) with a Bio-spec spectrometer (Bruker Instruments, Inc., Billerica, MA). At 2 h after clamping, the left kidney was freeze-clamped with Wollenberger clamps cooled in liquid nitrogen and subsequently extracted with perchloric acid for the determination of the tissue concentration of ATP (9). ^{31}P NMR spectra were baseline corrected and the relative areas under the beta-ATP peak and inorganic phosphate (Pi) peak calculated (10–14). Within each experiment, the different spectra were scaled to the same standard. The area under the beta ATP peak was calibrated to be equal to the ATP concentration determined by the destructive method described above. Tissue ATP and Pi concentrations for all time points within each experiment studied with ^{31}P NMR were then determined by peak areas relative to this beta-ATP peak (14). The intracellular pH was estimated by the chemical shift of Pi relative to internal proton resonance frequency using a pH titration curve determined for this instrument (10, 11).

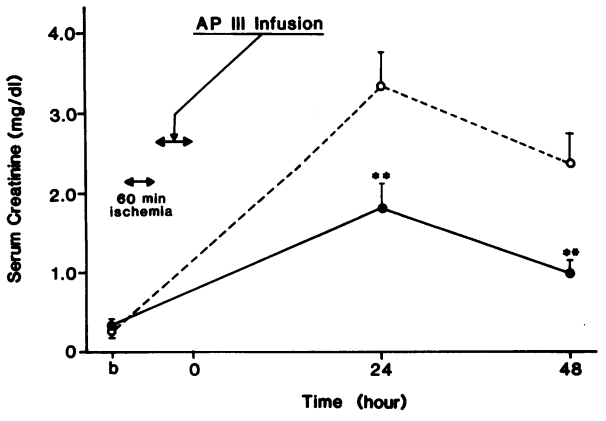

Figure 4. Effect of AP-III on serum creatinine after ischemia in vivo. AP-III was infused at 0.2 μg/kg per min (*solid circles*) ($n = 12$) for 60 min. Control (*open circles*) ($n = 12$) received no AP-III. *b*, blood taken before onset of ischemia. Results expressed as mean±SEM. *$P < 0.05$; **$P < 0.01$ compared with control.

The renal histology was examined in the following manner. At the end of the 48 h of reperfusion the right renal artery was cannulated across the aorta via the superior mesenteric artery and the kidney was fixed by perfusion for 7–10 min with 2% glutaraldehyde in 0.1 M phosphate buffer at pH 7.3. A 1–2-mm slice from the midportion of the kidney was removed and a 4 × 8-mm section containing cortex, outer medulla, and papilla was taken from this slice. This section was fixed for an additional 24 h in glutaraldehyde and then removed to 0.1 M phosphate buffer, pH 7.3. The tissue was dehydrated in graded ethanol, then infiltrated and embedded in glycol methacrylate (15). 1-μm-thick sections were stained with methylene blue. The sections were examined by light microscopy. For quantitation of injury in the S_3 segment of the proximal tubule the slides were placed on a mechanical stage and all proximal tubules encountered along a line through the mid-region of the outer stripe of the outer medulla were evaluated.

Table III. Effect of AP-III Infusion on Recovery of Renal Function over 48 h after 60 min of Ischemia In Vivo

	Time	Control	AP-III
	h	*n = 12*	*n = 12*
C_{Cr} (*μl/min/100 g*)	0–24	60±23	110±18*
	24–48	126±34	219±34*
FR_{Na} (%)	0–24	94.1±1.8	98.3±0.3*
	24–48	93.6±3.0	99.3±0.2*
T_{Na} (*μmol/min/100 g*)	0–24	7±3	15±3*
	24–48	12±3	38±6‡
$U_{Na}V$ (*μmol/min/100 g*)	0–24	0.127±0.013	0.145±0.021
	24–48	0.173±0.024	0.153±0.031
FE_K (%)	0–24	279±49	173±44*
	24–48	115±27	50±11*
	0–24	0.42±0.05	0.51±0.06
U_KV (*μmol/min/100 g*)	24–48	0.40±0.06	0.47±0.03

FE_K, fractional potassium excretion; C_{Cr}, creatinine clearance. Results expressed in mean±SEM.
* $P < 0.05$.
‡ $P < 0.01$ vs. control.

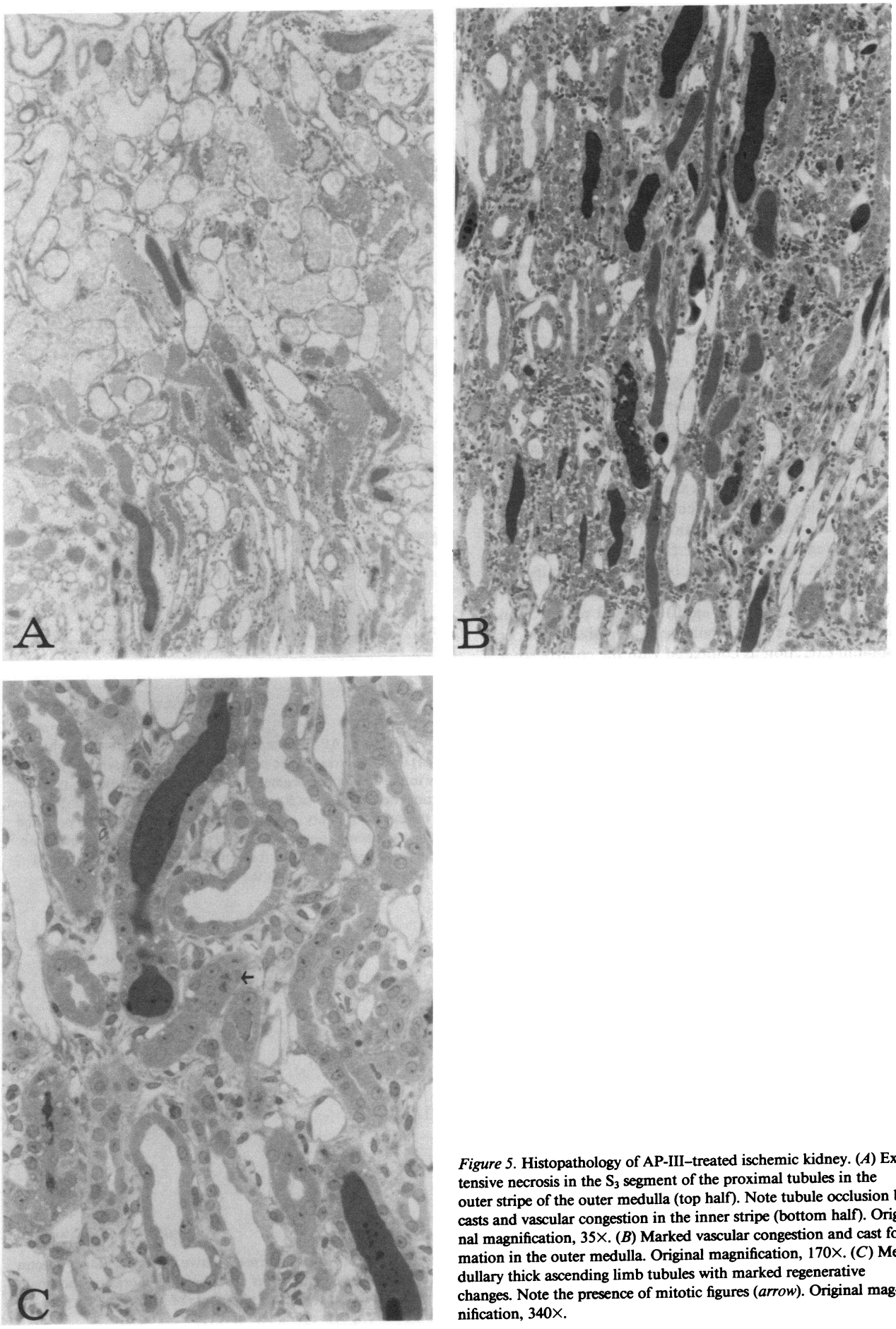

Figure 5. Histopathology of AP-III–treated ischemic kidney. (*A*) Extensive necrosis in the S_3 segment of the proximal tubules in the outer stripe of the outer medulla (top half). Note tubule occlusion by casts and vascular congestion in the inner stripe (bottom half). Original magnification, 35×. (*B*) Marked vascular congestion and cast formation in the outer medulla. Original magnification, 170×. (*C*) Medullary thick ascending limb tubules with marked regenerative changes. Note the presence of mitotic figures (*arrow*). Original magnification, 340×.

Tubular damage was defined as either overt coagulative necrosis of the lining epithelial cells or the presence of a flattened regenerative epithelium with necrotic cells in the lumen. The percentage of tubules with these findings was determined (16, 17). Similarly, the percent of tubules showing these changes in the S_1 and S_2 segments was determined by evaluating all proximal tubules with a 40× high-power field moved along a line in the superficial third of the cortex. Medullary congestion and cast formation were assessed semiquantitatively on a 0–4+ scale. In all the morphologic assessments the analysis was done by a renal pathologist without prior knowledge of the experimental conditions.

Analysis of variance was used in all group comparisons. Comparison of individual means was done using an unpaired *t* test with Bonferroni's correction (18).

Results

Experiments in isolated perfused kidneys (group I). The administration of AP-III after 1 h of ischemia exhibited evidence of a dose response–mediated protection. The lower dose (10 μg/dl) of AP-III afforded no evidence of protection against the ischemic insult as assessed by inulin clearance. In contrast, 100 μg/dl of AP-III was associated with a significant preservation of inulin clearance. This protective effect of AP-III was observed within 15 min after the administration of the hormone and persisted for the remainder of the perfusion (Fig. 1). This improvement in inulin clearance was associated with a significant increase in renal plasma flow (Table I).

The low dose of AP-III (10 μg/dl) also had no effect on renal plasma flow, urine flow, tubular sodium reabsorption, or urinary sodium excretion as compared with the control studies. In contrast, the high dose of AP-III (100 μg/dl) was associated with both an increase in urine flow and urinary sodium excretion. The rise in urinary sodium excretion was observed within the first 15 min after the AP-III administration, thus correlating closely with the beneficial effect on inulin clearance. The rise in inulin clearance was also associated with a highly significant increase in net tubular sodium reabsorption.

In vivo experiments in ischemic acute renal failure (group II). The effects of the low (0.02 μg/kg per min) and high (0.2 μg/kg per min) doses of AP-III on MAP are shown in Fig. 2. The low dose of AP-III had essentially no effect on MAP, whereas the high dose resulted in a marked hypotensive effect that was demonstrable at 30 min and persisted until 10 min after the infusion of hormone was discontinued.

Similarly, whereas the low dose of AP-III did not significantly effect recovery of urine flow, inulin clearance, urinary sodium excretion, or net tubular sodium reabsorption, the high dose of AP-III significantly increased urine flow, inulin clearance (Fig. 3), urinary sodium excretion, and net tubular sodium reabsorption during both hours after removal of the clamps. These data are summarized in Table II.

In the subacute study, rats receiving the high dose of AP-III had significantly lower serum creatinines at 24 and 48 h than did control kidneys (Fig. 4). The AP-III–treated rats also had significantly higher creatinine clearances than the control rats despite comparable urine flow rates and urinary sodium excretions during the first and second day after ischemia. Fractional and net tubular sodium reabsorptions were higher during these times in the AP-III–treated rats. These data are summarized in Table III.

The histology was studied in 10 control and 8 AP-III–treated ischemic kidneys. The control ischemic kidneys

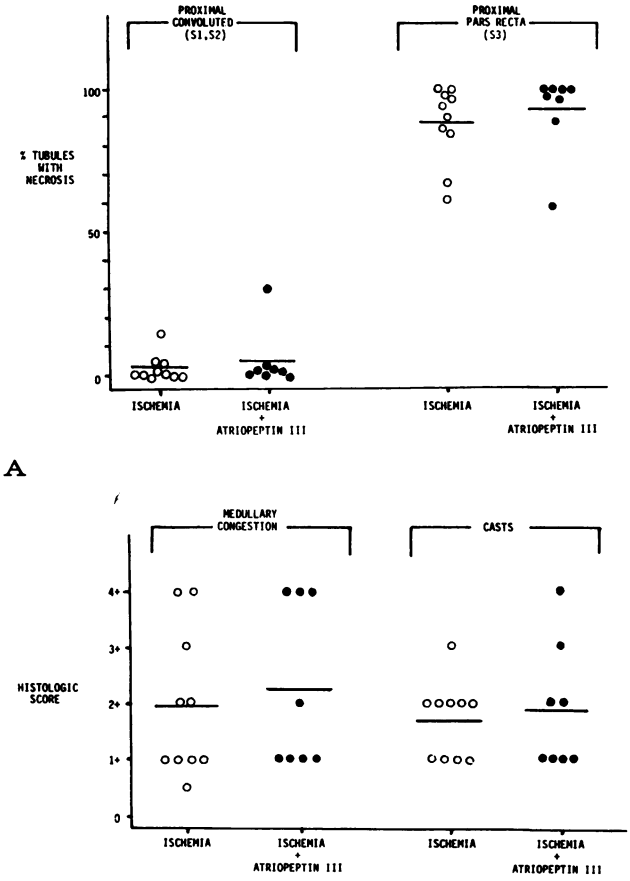

Figure 6. Effect of AP-III in renal ischemia: quantitative morphology. (*A*) Overt necrosis in the proximal tubule was quantitated separately in the convoluted segments (S_1, S_2) and in the pars recta (S_3). Necrosis was extensive in S_3 and only focal in S_1, S_2. AP-III did not alter the extent of injury. (*B*) Medullary congestion and the presence of casts was assessed semiquantitatively in a blinded protocol. Some degree of congestion and cast formation was consistently observed after ischemia with or without the AP-III.

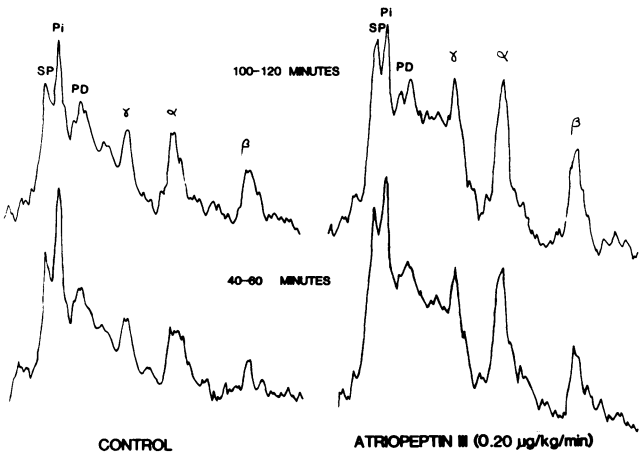

Figure 7. Effect of AP-III on representative ^{31}P NMR kidney spectra after ischemia in vivo. *SP,* sugar phosphate; *Pi,* inorganic phosphate; *PD,* phosphodiester; γ, gamma phosphate of ATP; α, alpha phosphate of ATP; β, beta phosphate of ATP.

showed morphology typical of the clamp-reflow model frequently described in the literature (16, 17). Extensive necrosis was present in the S_3 segment of the proximal tubule. The proximal convoluted tubule showed only focal damage, usually confined to the initial portion of the S_1 segment as has been observed previously (16). The medullary thick ascending limbs of the loop of Henle were generally cytologically intact, often collapsed, and often showed regenerative changes (enlarged nuclei with multiple prominent nucleoli and sometimes mitoses). Vascular congestion in the outer medulla was a consistent feature as was the presence of casts in collecting ducts. There were no significant differences in the morphology in the AP-III–treated and untreated ischemic kidneys, as is shown in Figs. 5 and 6.

^{31}P NMR studies demonstrated that rats treated with the high dose of AP-III had a statistically significant more rapid recovery of renal ATP concentration than control kidneys from 40 through 120 min postischemia. Representative spectra are shown in Fig. 7. The high dose AP-III kidneys exhibited trends toward more rapid lowering of intracellular Pi concentration and correction of intracellular acidosis as compared with the control kidneys, but these trends did not attain statistical significance (Table IV).

Discussion

Progressive renal injury has been demonstrated in the rat after renal ischemia has been induced by pedicle or arterial clamping. A reversible model of ischemic acute renal failure has been shown to occur after 45–60 min of clamp ischemia in this species, whereas longer periods of ischemia have been shown to induce irreversible injury (19, 20). 60 min of total ischemia was used in the present studies in an effort to maximize the reversible ischemic insult to renal function. Moreover, because maneuvers that attenuate renal ischemic injury have been shown in general to be more effective when administered before ischemic insult (21, 22), the postischemic infusion protocols employed were chosen to severely test any ameliorative effects of AP-III. Moreover, any potential clinical application of a protective agent is greater if shown to be effective after the ischemic insult.

AP-III was used in the present study, because it has been found in our laboratory to be more potent than other atrial natriuretic peptides in the isolated perfused rat kidney (23). The results indicate that AP-III used at a high dose of 100 μg/dl in the isolated kidney protocol significantly improved renal functional recovery as assessed by inulin clearance and net tubular sodium reabsorption. Because of the natriuretic properties of this hormone, fractional sodium reabsorption cannot be used to assess tubular function while the hormone is present. The lower dose of AP-III (10 μg/dl) employed in this protocol did not have any significant effects on renal functional recovery from the acute ischemic insult.

To further investigate the effects of this agent on ischemic acute renal failure, in vivo studies were performed. The lower dose of AP-III (0.02 μg/kg per min × 60 min) did not exhibit any significant effect on blood pressure or renal functional recovery. However, the higher dose of AP-III (0.2 μg/kg per min × 60 min) had a marked ameliorative effect on renal functional recovery despite significant hypotension. In the acute studies this ameliorative effect on renal function, as assessed by recovery of inulin clearance and net tubular sodium reabsorption, was shown to persist for at least 1 h after cessation of the AP-III infusion. This finding suggested that AP-III may exert a longer-term protective effect against ischemic acute renal failure. This possibility was examined by performing in vivo studies with a 48-h study period. In these studies a milder course of acute renal failure as assessed by serum creatinine and creatinine clearance occurred in the AP-III–treated rats as compared with control rats. Moreover, a higher net sodium reabsorption was also noted in the AP-III–treated animals, suggesting amelioration of tubular damage by this agent.

^{31}P NMR studies also demonstrated acceleration of cellular ATP regeneration as well as trends toward more rapid reduction of intracellular Pi concentrations and correction of intracellular acidosis in rats treated with AP-III. Although the histologic analysis did not reveal any significant differences between kidneys of rats treated with AP-III and saline at 48 h, the larger net tubular sodium reabsorption in AP-III–treated kidneys indicated a direct or secondary protective effect on tubular function. This observation is quite consistent with previous work by Hanley, who demonstrated a dissociation between morphologic evidence of injury and physiologic function in several nephron segments after ischemia (24).

Because the pathophysiology of ischemic acute renal failure involves both vascular and tubular factors (1), the protec-

Table IV. Effect of AP-III Infusion on Intracellular ATP, Inorganic Phosphorus, and pH

Treatment	Time	ATP	Pi	pH
	min	*μmol/g dw*	*μmol/g dw*	
Control *(n = 3)*	20–40	1.15±0.11	4.60±0.21	6.96±0.06
	40–60	1.45±0.40	3.47±0.40	7.02±0.02
	60–80	1.68±0.08	3.52±0.39	7.04±0.03
	80–100	1.81±0.29	2.61±0.20	7.08±0.05
	100–120	1.80±0.05	2.44±0.28	7.08±0.05
AP-III *(0.20 μg/kg/min × 60 min; n = 3)*	20–40	1.40±0.16	4.28±0.54	6.97±0.04
	40–60	3.03±0.30*	3.52±0.36	6.96±0.04
	60–80	3.22±0.36‡	3.27±0.07	7.09±0.07
	80–100	3.92±0.53‡	2.26±0.30	7.13±0.04
	100–120	3.98±0.46‡	1.87±0.24	7.15±0.03

dw, dry weight. Results expressed as mean±SEM. * $P < 0.05$; ‡ $P < 0.01$ vs. control.

tive effect of AP-III may involve effects both on glomerular hemodynamics (3, 4) and tubular function (25). The renal vasodilatory effect of AP-III, as demonstrated in the isolated kidney protocol, may improve the nutritional supply to cells recovering from ischemia and could explain the accelerated regeneration of ATP seen in the in vivo ^{31}P NMR studies. However, as nonspecific vasodilators have not been found to accelerate functional recovery in ischemic acute renal failure (26, 27), this vascular effect of AP-III is probably not the only factor involved in the hormone's protective effect. Furthermore, AP-III is more protective in the isolated kidney perfusion than verapamil (7).

Tubular obstruction secondary to cellular debris and diminished glomerular filtration pressure has been documented in several studies to be an important maintenance factor in ischemic acute renal failure (21, 28). Mannitol and polyethylene glycol have been shown to relieve this intratubular obstruction, presumably by washing out the cellular debris (21, 28). AP-III, with its ability to increase single nephron filtration as well as distal delivery of solutes and fluid, probably exerts similar protective effects (3, 4, 23, 29).

The clinical implications of these studies may extend beyond prevention against ischemic acute renal failure. It has been known for some time that a diminution in renal perfusion pressure secondary to cardiac dysfunction is associated with less renal vasoconstriction than that observed with a comparable fall in renal perfusion pressure induced by hypovolemia (30). Thus, the less severe renal consequences of hypotension due to cardiac dysfunction may be explained by the associated rise in atrial pressures causing increased circulating levels of human ANF (31), a hormone known to exert profound vascular and tubular effects on the kidney. It is also possible that the potential protection of saline expansion against ischemic acute renal failure might be related to an increase in endogenous AP-III. However, the use of exogenous AP-III is more effective because of much higher blood levels achieved by infusion. However, clinical studies are now finding significant hypotension to occur in normal volunteers. Therefore, any potential deleterious effects of the hypotension associated with systemic ANF might be avoided in clinical studies by the intrarenal infusion of lower doses which would avoid systemic effects but expose the kidney to concentrations comparable with those during the intravenous infusion. Alternatively, the simultaneous infusion of a pressor such as dopamine might also be used to avoid systemic hypotension during infusion of ANF.

In summary, the present results demonstrate that AP-III ameliorates ischemic acute renal failure in the isolated perfused kidney as well as in vivo in the rat. Effects of AP-III on the glomerular microcirculation and tubular function seem to be important factors in mediating this unique protective effect of AP-III. These observations merit further studies of the role of endogenous ANF in modifying renal vasoconstrictor stimuli as well as clinical investigations examining the effect of ANF in preventing or ameliorating acute ischemic renal injury.

Note Added in Proof

Since submission of our paper, ANF has been shown to ameliorate two other rat models of acute renal failure (32, 33).

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