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Effect of the ANF Analog A68828 in Cisplatin-Induced Acute Renal Failure

DAVID M. POLLOCK, MARK HOLST and TERRY J. OPGENORTH
Abbott Laboratories, Pharmaceutical Discovery, Abbott Park, Illinois
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ABSTRACT

Experiments were conducted to determine the effects of the reduced-size atrial natriuretic factor (ANF) analog, A68828, on renal function in rats with cisplatin (CP)-induced acute renal failure. CP was given as a single intraperitoneal injection (7.5 mg/kg) 3 days before experiments. In separate groups of rats, the renal response to intravenous infusion of A68828 at 3, 10 or 30 μg/kg/min or ANF[1–28] at 0.03, 0.1 or 0.3 μg/kg/min for 2 hr was evaluated. Another group of CP-treated rats were infused with the vehicle (0.1% bovine serum albumin in 0.9% NaCl). CP treatment resulted in a marked decline in glomerular filtration rate (GFR), arterial pressure, heart rate and reabsorption of water and electrolytes compared to untreated control animals. Infusion of A68828 produced a dose-dependent improvement in the glomerular filtration rate. The highest dose of A68828 produced a nearly 3-fold increase in the glomerular filtration rate, whereas arterial pressure was decreased; heart rate was unchanged. Despite producing a significant diuresis and natriuresis, net tubular reabsorption of water and sodium was also increased. Similar dose-dependent effects were observed with the native peptide, ANF[1–28]. These data indicate that infusion of the reduced-sized analog of ANF, A68828, can significantly improve glomerular and tubular function in rats with acute renal failure induced by CP.

CP is an antineoplastic agent used clinically to treat solid tumors (Einhorn and Williams, 1979). Although CP is a promising anticancer agent, its adverse effects, namely nephrotoxicity, has limited its usefulness. In rats, a single injection of CP can produce a severe reduction in GFR and inhibit tubular reabsorption of water and sodium (Chopra *et al.*, 1982; Winston and Safirstein, 1985). Efforts to prevent the nephrotoxic effects of CP have primarily focused on preventing damage to the tubular epithelium and not on reversing the glomerular effects. Such maneuvers include volume-induced diuresis (Cvitkovic *et al.*, 1977; Hayes *et al.*, 1977), inhibition of tubular CP secretion with probenecid (Ross and Gale, 1969), and treatment with oxygen free-radical scavengers (McGinness *et al.*, 1978).

ANF has the unique ability to increase GFR by dilation of the preglomerular vasculature and promote diuresis and natriuresis by a combination of glomerular and tubular effects (Goetz, 1988). Therefore, ANF or ANF analogs have been considered to have therapeutic potential for the treatment of acute renal failure (Conger *et al.*, 1989; Gianello, 1990; Nakamoto *et al.*, 1987; Shaw *et al.*, 1987). ANF has been shown to at least partially restore GFR in several models of acute renal failure, including CP nephrotoxicity (Capasso *et al.*, 1987), and may also have some cytoprotective properties (von Ruecker *et al.*, 1989). The purpose of the current study was to determine effects of A68828, a novel reduced-size analog of ANF (Pollock and Opgenorth, 1990), on CP-induced acute renal failure in the rat and compare this effect to the native peptide, ANF[1–28].

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Methods

All experiments were performed on male Sprague-Dawley rats (200–300 g; Charles River, Cambridge, MA) maintained on standard rodent chow (Purina, St. Louis, MO) with free access to tap water.

Dose-response studies. The relative potency and efficacy of A68828 and ANF[1–28] were compared using a step-up dose-response protocol. Rats were anesthetized with Inactin (100 mg/kg; Byk Gulden Konstanz, FRG) and placed on a thermoregulated heated blanket (Harvard Apparatus, Cambridge, MA), where rectal temperature was maintained at 37°C. A tracheotomy was performed (PE205) to allow free breathing. The femoral artery was cannulated (PE50) for blood sampling and measurement of arterial blood pressure using a Statham pressure transducer connected to a recorder (Modular Instruments, Inc., Southeastern, PA). The femoral vein was cannulated for infusion of peptides. The vehicle of 0.1% BSA in 0.9% NaCl (saline) was continuously administered at 0.6 ml/hr. The bladder was cannulated (PE160) for urine collection. After completion of surgical procedures, the animals were allowed to equilibrate for 90 min. Arterial pressure measurements and timed urine collections were made during a 60-min control period followed by sequential half-log incremental doses of peptide at 15-min intervals. Values during the control period were averaged and used to calculate the percent change in arterial pressure during peptide infusion. Changes in urinary sodium excretion were calculated as the log of the ratio between the value during peptide

ABBREVIATIONS: ANF, atrial natriuretic factor; CP, cisplatin; GFR, glomerular filtration rate; BSA, bovine serum albumin.

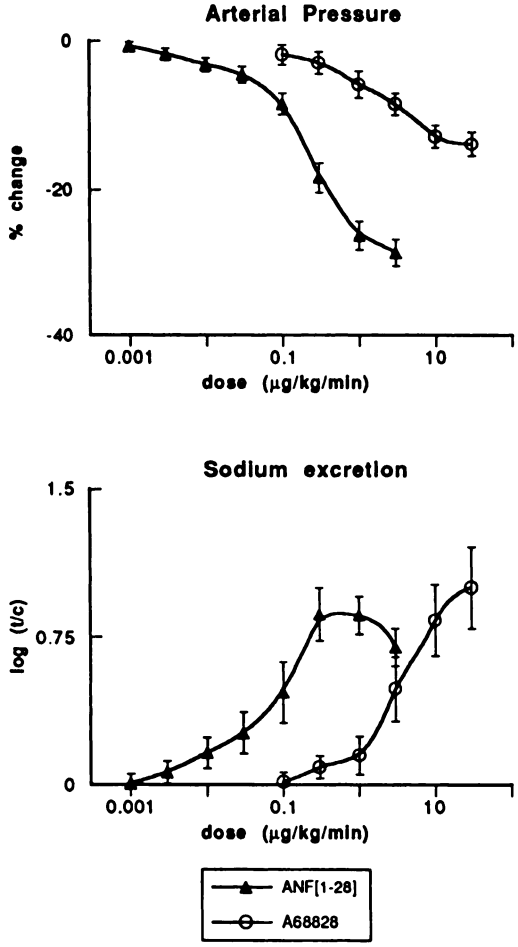

Fig. 1. Dose-related effects of A68828 (n = 11) and ANF[1–28] (n = 13) on mean arterial pressure and sodium excretion in normal anesthetized rats. The control reference values for mean arterial pressure and sodium excretion were 108 ± 2 mm Hg and 2.0 ± 0.4 μEq/min/kg b.wt., respectively. Values represent mean ± S.E.

TABLE 1

Effect of cisplatin on renal function and hemodynamics

Cisplatin was administered at a dose of 7.5 mg/kg i.p. 3 days before clearance measurements.

Variable	Cisplatin	Vehicle	P
GFR (ml/min/g kidney wt.)	0.08 ± 0.01	1.03 ± 0.05	<.0001
Urine flow rate (μl/min)	21.8 ± 2.0	22.9 ± 4.6	NS
Sodium excretion (μEq/min)	2.0 ± 0.2	2.9 ± 0.7	NS
Potassium excretion (μEq/min)	0.7 ± 0.1	2.5 ± 0.2	<.001
Absolute water reabsorption (μl/min)	186 ± 25	2512 ± 140	<.0001
Absolute sodium reabsorption (μEq/min)	29 ± 4	357 ± 18	<.0001
Absolute potassium reabsorption (μEq/min)	0.1 ± 0.1	6.7 ± 0.5	<.0001
Fractional excretion of water (%)	14 ± 1	0.9 ± 0.2	<.0001
Fractional excretion of sodium (%)	9.1 ± 0.9	0.8 ± 0.2	<.0001
Fractional excretion of potassium (%)	96 ± 6	27 ± 2	<.0001
Arterial pressure (mm Hg)	87 ± 2	97 ± 3	<.01
Heart rate (bpm)	360 ± 6	397 ± 12	<.005
Hematocrit (%)	46.4 ± 0.6	43.8 ± 0.5	<.05
Number of animals	42	11	

infusion and the average control value (*e.g.*, a 10× increase would be converted to a value of 1).

CP studies. In a separate group of rats, CP was administered as a single intraperitoneal injection, 7.5 mg/kg b.wt. (1.25 mg/ml 0.9% NaCl, Aldrich Chemical Co., Milwaukee, WI), 3 days before using the animals in clearance experiments. Control rats received an equivalent volume of the CP vehicle, 0.9% NaCl.

Anesthesia and surgical preparation were similar to that described in the dose-response series with some minor differences. The jugular vein was cannulated (PE50) for infusion of drug solutions; a maintenance infusion of 0.1% BSA in saline was administered at 0.6 ml/hr. The femoral vein catheter was then used for infusion of [^{3}H]inulin (Amersham Corp., Arlington Heights, IL) 2 μCi/100g b.wt./hr at a rate of 1.2 ml/hr and lactated Ringer's solution to replace urinary losses. Upon completion of surgery, 0.3 ml of 6.2% BSA in 0.9% NaCl was injected i.v. to replace minor losses of fluid during surgery and the [^{3}H] inulin infusion was initiated.

After a 60-min equilibration period, two 30-min control clearance periods were obtained. After the control periods, separate groups of rats were infused with A68828 at 3 (n = 5), 10 (n = 6) or 30 μg/kg/min (n = 5), ANF[1–28] at 0.03 (n = 6), 0.1 (n = 7) or 0.3 μg/kg/min (n = 7), or vehicle (0.1% BSA in saline) (n = 6) for a period of 2 hr. During the infusion periods, urine was collected at 15-min intervals and urinary losses above the maintained volume infusion rate were matched by an infusion of lactated Ringer's solution. In the CP-vehicle series, experiments were conducted in the same manner as the CP-treated group, except the concentration of the [^{3}H]inulin infusion rate was increased to 4 μCi/100g b.wt./hr. In all experiments, the duration of the infusion period was 2 hr. Blood samples (50–70 μl) were taken at the onset of the first clearance period and at 60-min intervals thereafter for determination of hematocrit and plasma [^{3}H]inulin and electrolyte concentrations.

ANF[1–28] and A68828 were both synthesized by the Abbott synthetic chemistry group. The amino acid sequence of A68828 is: ARG-CYS-CHA-GLY-GLY-ARG-ILE-ASP-ARG-ILE-PHE-ARG-CYS with a disulfide bond between the CYS residues. CHA represents cyclohexylamine.

Plasma and urinary sodium and potassium concentrations were analyzed by flame photometry (Instrumentation Laboratory, IL943, Lexington, MA). Plasma and urinary [^{3}H]inulin concentration were determined by scintillation counting (Beckman Instruments LS 5000TA, Fullerton, CA). Standard formulas were used to calculate GFR, fractional excretion rates, and absolute reabsorption of sodium and potassium. Data are expressed as means ± S.E. Student's t test for paired data was used to determine the statistical significance between the control clearance periods and drug infusion periods within groups of animals. Analysis of variance along with Fisher's PLSD *post hoc* comparison was used to determine the statistical significance between group means (Statview II, Abacus Concepts, Berkeley, CA). Data were considered significantly different at $P < .05$.

Results

The dose-related effects of A68828 and ANF[1–28] on mean arterial pressure and sodium excretion are compared in figure 1. Both peptides produced dose-related hypotension, although the maximum decrease in arterial pressure produced by the analog was roughly one-half the value for the native peptide. In contrast, both A68828 and ANF[1–28] displayed similar efficacy in natriuretic effects. For both of these variables the native peptide was roughly 50 times more potent.

The effect of CP on renal function and hemodynamics before atrial peptide infusion are summarized for all animals in table 1. CP produced a highly significant decrease in GFR while sodium and water balance appeared to have been maintained; urine flow rate and sodium excretion were not different between CP and vehicle-treated rats. In contrast to sodium and water, potassium excretion was severely reduced. Net reabsorption calculated as the difference between the amount filtered and the amount reabsorbed was significantly reduced for water, sodium and potassium.

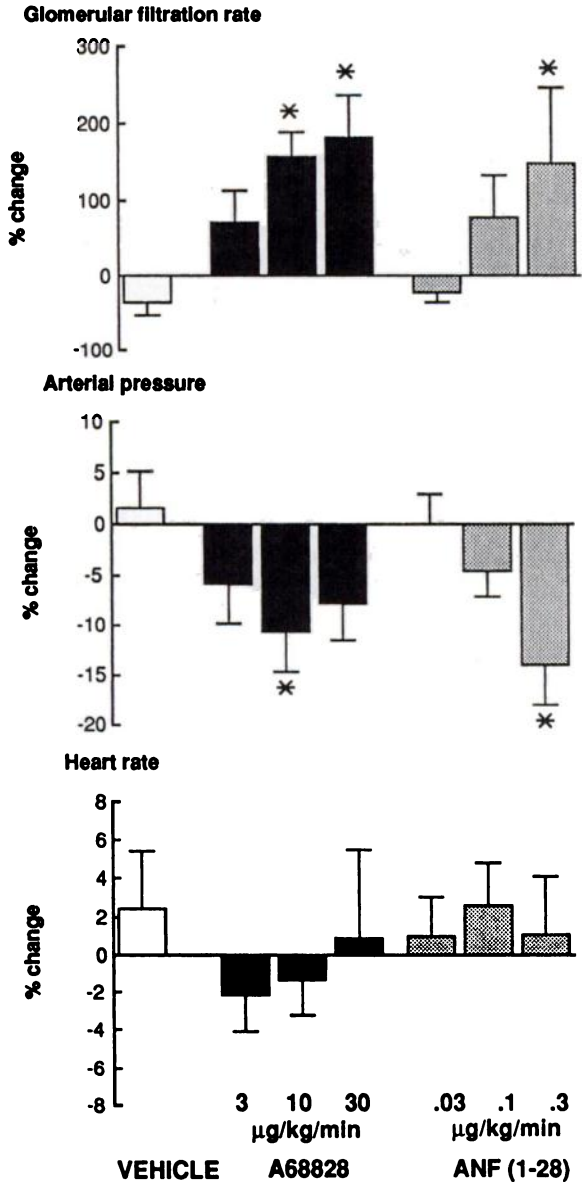

Fig. 2. Effect of intravenous infusion of A68828 and ANF[1–28] on glomerular filtration rate, arterial pressure, and heart rate in anesthetized rats treated with CP (7.5 mg/kg i.p.) 3 days before study. Values represent mean ± S.E. * represents $P < .05$ compared to vehicle controls.

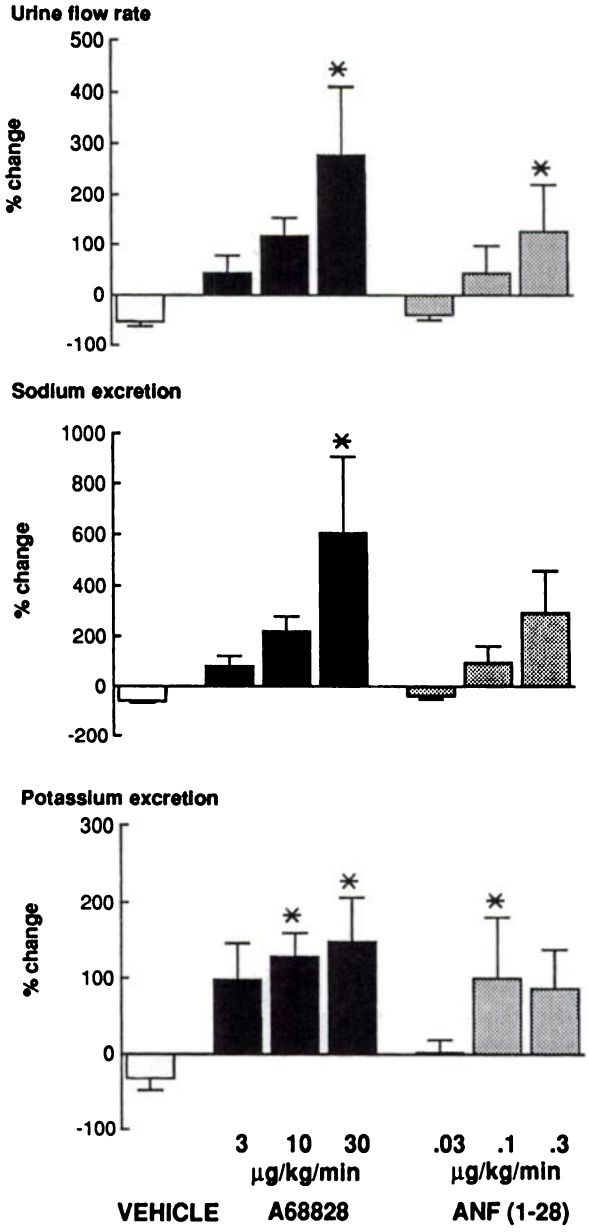

Fig. 3. Effect of intravenous infusion of A68828 and ANF[1–28] on urine flow rate and the excretion of sodium and potassium in anesthetized rats treated with cisplatin (7.5 mg/kg i.p.) 3 days before study. Values represent mean ± S.E. * represents $P < .05$ compared to vehicle controls.

Hemodynamic variables were also affected by CP. CP treatment resulted in a significant reduction in arterial pressure and heart rate along with an increase in hematocrit.

Changes in GFR, arterial pressure and heart rate produced by A68828, ANF[1–28] or the drug vehicle in CP-treated animals are presented in figure 2. As can be seen, both ANF and the analog produced a dose-dependent improvement in GFR during intravenous infusion. The highest dose of A68828 and ANF[1–28] produced a 150 to 200% increase in GFR as compared to the preinfusion control value. Nondrug infused control animals displayed a slight, but not statistically significant, tendency for GFR to decrease over the course of the experiment. This remarkable improvement in GFR occurred despite the hypotensive effects of ANF and the analog.

Changes in urine flow rate and sodium and potassium excretion produced by drug infusion are presented in figure 3. The increase in urine flow rate was significant only at the highest dose of each compound infused. The natriuretic response was significant only at 30 µg/kg/min with A68828. Potassium excretion was also increased by ANF[1–28] and A68828.

To assess further the effects of these compounds on renal tubular function, net reabsorption rates were calculated and presented in figure 4. A68828 and ANF[1–28] each produced a dose-dependent increase in the reabsorption of both water and sodium. The effects on potassium reabsorption were significantly increased only at the highest dose of ANF[1–28].

Comparison of the effects of A68828 and ANF[1–28] in nonrenal failure animals is presented in figure 5. These animals were injected with the CP vehicle (saline) 3 days before study and given an infusion of A68828 or ANF[1–28] at doses that were expected to produce comparable changes in renal function: 10 µg/kg/min and 0.3 µg/kg/min, respectively. As can be seen, both compounds had absolutely no effect on GFR while pro-

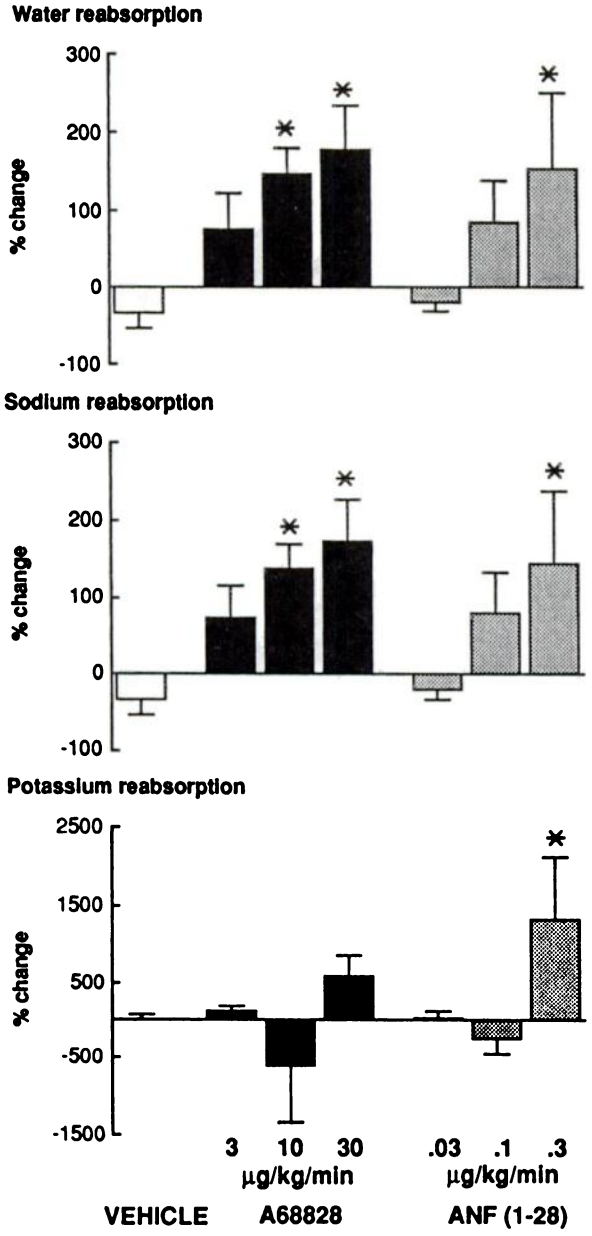

Fig. 4. Effect of intravenous infusion of A68828 and ANF[1–28] on the net reabsorption of water, sodium and potassium in anesthetized rats treated with CP (7.5 mg/kg i.p.) 3 days before study. Values represent mean ± S.E. * represents $P < .05$ compared to vehicle controls.

ducing only slight decreases in arterial pressure. Equivalent increases in urine flow rate and sodium excretion were observed for the two compounds at the doses used.

Discussion

We observed a severe reduction in GFR 72 hr after a single injection of CP compared to vehicle-treated animals. This impairment of renal function is typical of CP nephrotoxicity in Sprague-Dawley rats (Chopra *et al.*, 1982; Winston and Safirstein, 1985) Intravenous infusion of the ANF analog, A68828, produced a dose-dependent improvement in renal function in rats treated with CP. GFR was significantly increased despite reductions in mean arterial pressure. The analog also produced an enhancement of the net reabsorption of water and sodium concurrently with a significant diuresis and natriuresis. The

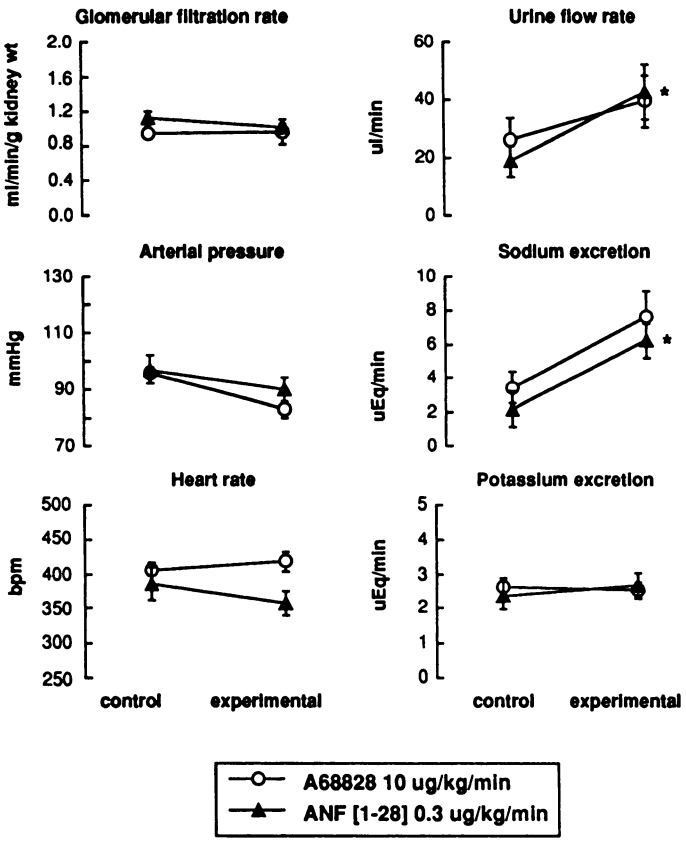

Fig. 5. Effect of intravenous infusion of A68828 and ANF[1–28] on selected variables in normal, anesthetized Sprague-Dawley rats. Peptides were infused intravenously during the experimental period. Values represent mean ± S.E. * represents $P < .05$ compared to the control period.

native peptide, ANF[1–28], produced similar effects but at a lower dose range.

Our results with the ANF analog and ANF[1–28] agree with the study of Capasso *et al.* (1987), who examined the effect of the native peptide in rats with CP-induced acute renal failure. These investigators observed that a high dose of ANF (12 µg/kg bolus plus 1 µg/kg/min) produced a 2-fold increase in GFR in the CP-treated groups similar to the maximum effect we observed using lower doses. Thus, it would appear as though the increases reported in the current study are at or near the maximum effect that can be obtained in an acute setting.

In CP-treated rats, the efficacy of A68828 was similar to that of the native peptide, ANF[1–28]. The only difference that we observed between A68828 and ANF[1–28] in nephrotoxic animals was potency. Although our results are consistent with the idea that the analog is producing its biological effect by activating ANF receptors, there were some interesting pharmacological differences in normal rats. Although A68828 is equally efficacious in producing natriuresis as ANF[1–28] in normal rats, it was not capable of producing the same degree of hypotension, as is the case with the native peptide. This may prove to be extremely beneficial because the blood pressure lowering effects of atrial peptides may limit the potential therapeutic usefulness of these compounds (Conger *et al.*, 1989).

Acute renal failure, including that induced by CP, is characterized by a significant decline in GFR produced by an increase in preglomerular resistance and increased proximal tubular pressure as a result of tubular necrosis and cellular debris

obstructing the tubules (Finn, 1980). Given that ANF increases net ultrafiltration pressure in normal animals, primarily *via* dilation of the preglomerular vasculature (Mendez *et al.*, 1986), it seems reasonable to suggest that this is how ANF analogs increase GFR in CP-induced acute renal failure. Thus, the increases in whole kidney GFR that we observed in the current study could be due to increases in single nephron GFR and/or to an increased number of filtering nephrons by relieving obstruction. The latter possibility has yet to be experimentally tested, but could occur as a result of the increase in flow and pressure produced by the glomerular effects of the analog followed by relief of the obstructed nephron.

ANF analogs have been shown to be capable of improving renal function in at least one other model of acute renal failure (Pollock and Opgenorth, 1990). As in CP-induced renal failure, A68828 increased GFR and enhanced water and sodium reabsorption in the postischemic kidney similar to ANF[1-28]. Several other laboratories have also characterized the beneficial effects of ANF in the postischemic kidney (Shaw *et al.*, 1987; Nakamoto *et al.*, 1987; Conger *et al.*, 1989). An improvement in renal functional parameters produced by larger ANF peptides has also been reported for norepinephrine- and gentamicin-induced acute renal failure (Schafferhans *et al.*, 1986; Schafferhans *et al.*, 1988).

In summary, we observed that A68828 significantly increases GFR and tubular reabsorption of water and sodium during CP-induced acute renal failure. Tubular function also appears to have been improved because excretion and net reabsorption of water and electrolytes was increased. These results support the possibility that ANF analogs could be useful in restoring renal function following acute nephrotoxicity.

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Send reprint requests to: David M. Pollock, Ph.D., Abbott Laboratories, D-47V, AP9/2, Abbott Park, Il 60064.