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Research Article

60% of chronic caval dogs with ascites did not respond to atrial natriuretic peptide (ANP) (75 ng.kg-1.min-1) with a natriuresis (TIVC-NR; delta UNaV = 2 +/- 0.8 mu eq/min) whereas the remaining 40% responded normally (TIVC-R; delta UNaV = 216 +/- 50 mu eq/min). Since proximal tubule neutral endopeptidase 24:11 (NEP) destroys most of intrarenal luminal ANP and kinins, we attempted to convert TIVC-NR into TIVC-R by providing NEP inhibition with SQ 28603 at 30 mg/kg. This potent and specific NEP inhibitor produced a natriuresis when administered alone to nine TIVC-NR dogs (delta UNaV = 67 +/- 2 mu eq/min) and permitted a natriuresis in the presence of ANP (delta UNaV = 97 +/- 18 mu eq/min). A natriuretic response to ANP could also be induced in TIVC-NR dogs by providing renal arterial bradykinin or intravenous captopril, a kininase inhibitor. Urodilatin, a natriuretic peptide not destroyed by intrarenal NEP was without effect in TIVC-NR dogs but increased UNaV when given to TIVC-R and normal dogs. Providing bradykinin to TIVC-NR now permitted an increment in delta UNaV (62 mu eq/min) when urodilatin was reinfused. TIVC-R dogs could be converted into TIVC-NR by pretreating with a specific bradykinin antagonist before infusing ANP. We conclude that TIVC-NR dogs are deficient in intrarenal kinins but are converted to responding dogs after NEP inhibition because […]

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Renal Tubular Responsiveness to Atrial Natriuretic Peptide in Sodium-retaining Chronic Caval Dogs

A Possible Role for Kinins and Luminal Actions of the Peptide

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Abstract

60% of chronic caval dogs with ascites did not respond to atrial natriuretic peptide (ANP) (75 ng · kg^{-1} · min^{-1}) with a natriuresis (TIVC-NR; $\Delta U_{Na}V$ = 2±0.8 μeq/min) whereas the remaining 40% responded normally (TIVC-R; $\Delta U_{Na}V$ = 216±50 μeq/min). Since proximal tubule neutral endopeptidase 24:11 (NEP) destroys most of intrarenal luminal ANP and kinins, we attempted to convert TIVC-NR into TIVC-R by providing NEP inhibition with SQ 28603 at 30 mg/kg. This potent and specific NEP inhibitor produced a natriuresis when administered alone to nine TIVC-NR dogs ($\Delta U_{Na}V$ = 67±2 μeq/min) and permitted a natriuresis in the presence of ANP ($\Delta U_{Na}V$ = 97±18 μeq/min). A natriuretic response to ANP could also be induced in TIVC-NR dogs by providing renal arterial bradykinin or intravenous captopril, a kininase inhibitor. Urodilatin, a natriuretic peptide not destroyed by intrarenal NEP was without effect in TIVC-NR dogs but increased $U_{Na}V$ when given to TIVC-R and normal dogs. Providing bradykinin to TIVC-NR now permitted an increment in $\Delta U_{Na}V$ (62 μeq/min) when urodilatin was reinfused. TIVC-R dogs could be converted into TIVC-NR by pretreating with a specific bradykinin antagonist before infusing ANP. We conclude that TIVC-NR dogs are deficient in intrarenal kinins but are converted to responding dogs after NEP inhibition because of increased kinin delivery to the inner medullary collecting duct. (*J. Clin. Invest.* 1992. 90:1425–1435.) Key words: neutral endopeptidase • ascites • sodium excretion • cGMP

Introduction

In our laboratory, all normal dogs uniformly respond with a brisk natriuresis to an infusion of atrial natriuretic peptide (ANP),[1] whereas edematous dogs usually show a heterogeneous response. About half of all sodium-retaining chronic caval (TIVC) or cirrhotic dogs with ascites will respond to an ANP infusion with a brisk natriuresis whereas the remaining half will show no natriuresis whatsoever (1, 2). This spectrum of heterogeneous response has also been reported for cirrhotic rats (3), cirrhotic men (4), and sodium-retaining bile duct–ligated dogs without ascites (5). Such a heterogeneous response to ANP appears to be unique to this peptide, since we have demonstrated that TIVC "nonresponders" will respond normally to various diuretics active in different nephron segments (6).

The lack of tubular sensitivity to ANP in ~ 50% of sodium-retaining TIVC dogs with ascites appears to be transient and therefore functional in nature, since when these nonresponders return to sodium balance (though maintaining their ascites), a normal natriuretic response to ANP reappears (1). Despite intensive investigation (1, 2, 7) we have so far been unable to detect physiological variables that discriminate between natriuretic responders and nonresponders among TIVC or cirrhotic dogs. All of these experimental animals, no matter their response to ANP, appear physiologically equivalent. Moreover, in a recent series of experiments (8), we deliberately attempted to convert TIVC-responding dogs into nonresponders and vice versa by manipulating the intrarenal environment (e.g., catecholamine and angiotensin infusions, angiotensin and adrenergic blockade, adenosine receptor antagonism, etc.) with various pharmacological infusions calculated to either attenuate or promote ANP effects. These attempts proved unsuccessful.

Neutral endopeptidase 24:11 (NEP 24:11) within the brush border of the proximal convoluted tubules serves as a major route for degradation of filtered ANP (9). Recently, evidence has been adduced that endopeptidase inhibition may promote a natriuresis in animal models of sodium retention showing an attenuated response to the natriuretic effects of ANP (10, 11). In the present study, we have investigated the possible role of excessive endopeptidase degradation of ANP as a possible cause for the tubular insensitivity to this peptide in a population of TIVC dogs with urinary sodium retention and ascites unresponsive to pharmacological infusions of this potent natriuretic agent. Because kinins may also be degraded by this en-

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1. *Abbreviations used in this paper:* ABP, arterial blood pressure; ANP, atrial natriuretic peptide; BKA, bradykinin receptor antagonist; CVP, central venous pressure; FE, fractional excretion; iANP, immunoreactive ANP; IMCD, inner medullary collecting duct; NEP, neutral endopeptidase; NEP 24:11, neutral endopeptidase 24:11; PAH, para-aminohippurate; TIVC, chronic caval dog; NR, not responding; R, responding; $U_{Na}V$, urinary sodium excretion.

zyme system (12), we examined the role of kinin availability as a possible modulator of the natriuretic effects of ANP.

Methods

A total of 47 chronic conditioned dogs of either sex survived the procedure of thoracotomy and partial constriction of the supradiaphragmatic vena cava. These dogs were studied both in the alert, unanesthetized state or anesthetized, depending on the experimental protocols. Studies were also performed on 20 acute, unconditioned dogs of either sex selected for good health. They were also studied in the anesthetized or unanesthetized state as required. The preparation of the TIVC dogs was carried out under sterile conditions, using sodium thiopentone intravenous anaesthetic (20 mg/kg) as previously described in great detail for this laboratory (13). The animals were fully recovered, mobile, and eating by the first postoperative day. The dogs received a standard chow diet containing 45 meq Na^+/d. All postoperative care was supervised by senior veterinarians from the McGill Animal Resources Centre, and all components of the study protocols received approval from the University Animal Utilization Ethics Committee. These dogs developed detectable ascites usually within 5–9 d and were studied shortly thereafter. When either TIVC or acute dogs were studied standing quietly in a Pavlov sling, the following procedures were used: All dogs were sedated with an atropine–xylazine mixture given intramuscularly (5). Urine was collected by a standard washout technique through a Foley catheter inserted into the bladder. All infusions were given intravenously through polyethylene catheters placed by direct venipuncture in the saphenous or antecubital veins. Inulin and para-aminohippurate (PAH) were given at 0.5 ml/min through a PE50 catheter placed in one antecubital vein to measure GFR and renal plasma flow, respectively. All infusions were given with constant-speed infusion pumps. Clearance periods were ≥ 10 min in duration and were performed by the constant-infusion technique (13). Blood was sampled at the midpoint of each period from a PE 190 or 205 catheter placed in the abdominal vena cava from a saphenous venipuncture. At least three clearance periods were taken in each experimental phase and averaged. Where arterial blood pressure (ABP) and central venous pressure (CVP) were to be measured, appropriate catheters were placed in the right carotid artery and jugular vein several days before the experiment under thiopentone anesthesia and protected with a felt collar after subcutaneous tunneling. ABP was measured by mercury manometry and CVP was measured by saline manometry, with the zero reference point in each case fixed at heart level.

When dogs were studied in the anaesthetized state, sodium pentobarbital (25 mg/kg, intravenously) was used for induction with small supplemental amounts given as required throughout the study. Urine was collected through a Foley catheter, except for those studies where the left renal artery was selectively perfused, in which case the ureters were cannulated directly through a retroperitoneal approach to avoid spilling ascites. In normal dogs without ascites, the ureters were cannulated through a lower abdominal midline incision. Blood for inulin and PAH levels were taken from an arterial cannula placed in one femoral artery. Anesthetized dogs were intubated and ventilated with a Harvard Dog Respirator (Harvard Apparatus, South Natick MA). When the left kidney was to be perfused a 26-g curved needle was inserted into the artery by techniques previously described and held in place with several drops of adhesive (Permabond 910; Permabond International, Englewood, NJ) (13).

Several protocols were used in these studies, as follows:

(*a*) Nine normal dogs were studied standing quietly in a Pavlov sling. After control clearance periods, ANP was infused at 75 ng/kg per min intravenous. After a 10-min waiting period, another set of clearance periods were collected. Plasma for inulin and PAH were collected at the midpoint of each urine collection whereas plasma for ANP levels was carefully collected as previously described (1, 2) at the midpoint of each experimental phase. After a 90-min recovery period, repeat clearance collections were again taken, and the NEP 24:11 inhibitor SQ 28603 was injected in a dose of 30 mg/kg intravenous in 20 ml of isotonic $NaHCO_3$ (provided by Bristol-Myers Squibb Research Institute [Princeton, NJ]; *N*-2-mercaptomethyl-1-oxo-3-phenylpropyl B alanine [SQ 28603] is a highly specific antagonist of NEP 24:11 and is thought to have only weak actions on other peptidases contained within the proximal convoluted tubule brush border). After a 10-min wait, clearance studies were repeated. ANP was then reinfused at the previous dose level and, after another 10-min waiting period, a final set of three urine collections were made.

(*b*) This protocol was also followed in nine TIVC dogs who were deemed nonresponders to an infusion of ANP ($\Delta U_{Na}V < 20$ μeq/min).

(*c*) The protocol was also followed for seven TIVC responders who showed a brisk natriuresis to ANP ($\Delta U_{Na}V > 20$ μeq/min).

(*d*) The following protocols were employed in additional groups of responding TIVC dogs: (*i*) After the initial ANP infusion and a 90-min recovery period, aprotinin was given intravenously as a bolus of 50,000 kallikrein inhibitor units (KIU) and then infused as a constant infusion of 10,000 KIU/min to blunt the generation of intrarenal kinins. After a 10-min wait, clearances were taken, ANP was then reinfused at previous dose levels and after another 10-min waiting period, another set of clearance periods were taken ($n = 5$). (*ii*) A similar protocol as in (*i*), but a specific antagonist of the bradykinin receptor (D-Arg^0, Hyp^3, thi^5, D-Phe^7, thi^8) bradykinin (BKA) (IAF Biochem International, Inc., Montreal, Canada) was administered into the left renal artery at 15 μg/kg per min after the initial ANP infusion. In separate pilot studies, it was determined that this dose administered to three normal dogs prevented the increased renal plasma flow and natriuresis of bradykinin (3 μg/kg per min) given into the left renal artery. The right kidney was used as a control. After a set of clearances, a second ANP infusion was given intravenously while the BKA was still being infused into the left renal artery ($n = 4$).

(*e*) In additional groups of nonresponding TIVC dogs, the following protocols were employed: (*i*) In four TIVC dogs unresponsive to an initial intravenous infusion of ANP, urodilatin was infused at 100 ng/kg per min i.v. after a 1-h recovery period. After a 10-min wait, three clearance collections were taken. In an additional three dogs, the intravenous urodilatin was administered along with bradykinin given into the left renal artery at 3 μg/kg per min. (*ii*) Four TIVC nonresponders studied in the anesthetized state were given the initial intravenous ANP infusion of 75 ng/kg per min while isotonic saline at 0.5 ml/min was infused through the left renal arterial catheter. After a 1-h recovery period, the renal arterial infusion was switched to bradykinin 3 μg/kg per min delivered at 0.5 ml/min. After obtaining three urine collections after a 10-min waiting period, the intravenous ANP infusion was readministered concurrent with the bradykinin. After a 10-min stabilization period, another set of urine collections were obtained. (*iii*) In four nonresponding TIVC dogs, bradykinin at 3 μg/kg per min was administered into the left renal artery after an intravenous infusion of 8-Br-cGMP (16 μg/kg per min) had failed to initiate a natriuretic response. Although the renal arterial bradykinin was being infused, this second messenger analogue for ANP was readministered intravenously in an identical dose. (*iv*) In four nonresponding TIVC dogs we administered captopril intravenously 20 μg/kg per min. This dose has been shown to prevent angiotensin II generation (14). After three clearance periods, ANP was readministered concurrent with the captopril and a second set of clearances taken. In these studies, the captopril was being used as an inhibitor of intrarenal kininase II.

In additional groups of normal dogs, serving as controls, we administered urodilatin 100 ng/kg per min ($n = 3$), captopril, and ANP as above ($n = 3$).

Inulin was measured by an anthrone technique and PAH was measured by an autoanalyzer technique. These methods have previously been described in detail for this laboratory (13). Sodium in the urine and plasma was measured by flame photometry (13). Plasma protein and hematocrit were measured by techniques previously described (13). Immunoreactive ANP was analyzed in plasma and urine by techniques previously described in great detail (1, 2). Extraction of samples was done on SepPAK C_{18} cartridges with 60% acetonitrile–0.1% tri-

fluoracetic acid elution. Recovery of synthetic human ANP (1-28) added to plasma was 75%. Intra-assay coefficient of variation was from 6 to 11%, depending on levels of hormone being assayed, and interassay coefficient of variation was between 10 and 11%.

Statistical significance was taken at the 5% probability level. Group means between experimental groups were analyzed by the unpaired t test, and within groups values were evaluated with a two-way analysis of variance for repeated measures or a paired t test as required. Data are presented as mean±SE.

Results

A total of 47 TIVC dogs were examined in this study. Of these, 28 were identified as natriuretic nonresponders to ANP ($\Delta U_{Na}V < 20$ μeq/min) and 19 were identified as natriuretic responders ($\Delta U_{Na}V \geq 20$ μeq/min). In addition, we studied 20 normal control animals fed a similar salt diet. Fig. 1 summarizes the spectrum of natriuretic response to ANP (75 ng/kg per min intravenously) in all three populations of dogs. The profile of $\Delta U_{Na}V$ was similar for both control and TIVC responding dogs whereas the TIVC nonresponders averaged an increment in urinary sodium excretion of only 2±0.8 μeq/min.

Table I summarizes some features of these TIVC dogs after they had developed urinary sodium retention and ascites some 5-9 d after surgery. All dogs were in good health with easily detectable volumes of ascites. The dogs in each TIVC group were quite similar, differing only in the natriuretic response to ANP and in baseline plasma iANP levels. The observation of a lower baseline level for plasma iANP in TIVC nonresponders has been an inconsistent finding in our laboratory (1, 2, 5-7), and will be commented upon further in the Discussion section.

Endopeptidase inhibition. Table II summarizes the effects of administering endopeptidase inhibitor to nine normal dogs. When ANP alone was infused, the subsequent natriuresis was

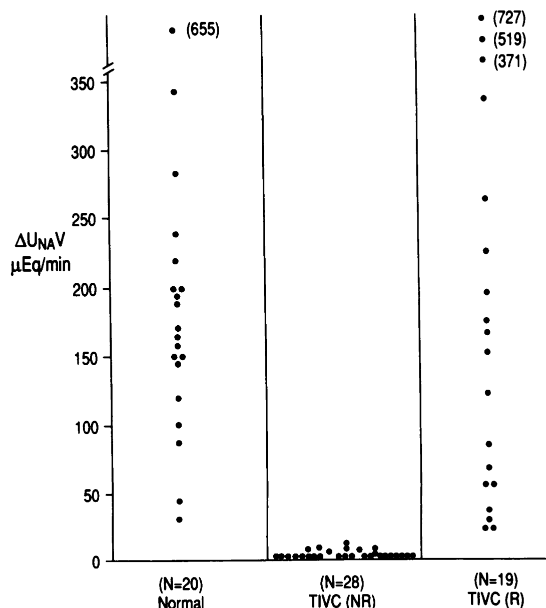

Figure 1. The change in urinary sodium excretion ($\Delta U_{Na}V$) from control levels after an intravenous infusion of ANP (75 ng/kg per min) in normal dogs, caval dogs unresponsive to ANP [*TIVC(NR)*], and caval dogs responsive to ANP [*TIVC(R)*]. Natriuretic responders were those who had a $\Delta U_{Na}V \geq 20$ μeq/min.

Table I. Baseline Data in TIVC Dogs Comparing Natriuretic Responders (n = 19) to Nonresponders (n = 28)

	Responders	Nonresponders
Body weight (kg)	16.7±1.3	16.7±1.1
ABP (mmHg)	106±8	104±7
CVP (cm H_2O)	2.1±0.9	2.9±0.6
GFR (ml/min)	45±2.9	47±2.7
C_{PAH} (ml/min)	124±8	119±6
V‡ (ml/min)	2.6±0.13	2.3±0.11
$U_{[Na]}^{+}$§ (meq/liter)	8±2	12±3
Baseline $U_{Na}V$‖ (μeq/min)	22±7	26±4
$\Delta U_{Na}V$ (post-ANP infusion) (μeq/min)	211±50	3±0.8*
Hematocrit (%)	41.7±2.4	45.6±1.8
Plasma iANP (pg/ml)	91±21	38±12*
Postinfusion plasma iANP (pg/ml)	1,458±123	1,853±312

* $P < 0.05$. ‡ V, urine flow rate. § U_{Na}, urinary sodium concentration. ‖ $U_{Na}V$, urinary sodium excretion.

associated with a small but significant increment in urinary ANP excretion, but a major reduction in the fractional excretion of the peptide. This was due to an enormous capacity to either degrade the filtered peptide or bind it to silent receptors. Indeed, if one assumes that the circulating peptide was freely filtered, only 30 pg/min of a filtered load of 138,780 pg/min escaped degradation within the kidney. (We will use the term "degrade" within the text as a single description for enzymatic degradation and binding to silent C receptors. Though data are lacking concerning ANP tubular reabsorption or secretion, the equation used in Table II [degraded ANP load = filtered load − excreted load] seems a reasonable assumption. To the extent there is tubular secretion or reabsorption of the peptide is the extent this equation is an approximation and would be similar to a net clearance value encompassing both these tubular processes.) The infusion of the NEP inhibitor was associated with a modest increment in $U_{Na}V$, unassociated with any change in ABP or GFR. Plasma levels of iANP increased slightly but significantly and the urinary excretion of ANP rose markedly. The fractional excretion of the peptide now rose dramatically from baseline levels as ANP degradation declined. When ANP was reinfused, the natriuretic response was magnified compared with the initial natriuresis. The Δ for $U_{Na}V$ initially was 234±63 μeq/min, whereas for the second ANP infusion the change (compared with recovery phase) was 352±39 μeq/min ($P < 0.05$). This magnification of the ANP-induced natriuresis was associated with an increment in GFR not different from the initial ΔGFR (6 vs. 10 ml/min, NS). Plasma iANP levels more than doubled (+129%) compared with the first infusion and the urinary excretion of ANP rose dramatically. Fractional excretion (FE) of ANP increased still further from the NEP inhibitor only phase by 0.7%. The degraded ANP load rose in absolute terms, but fell in fractional terms compared with the initial ANP infusion.

In five separate normal control dogs, studied as part of a separate protocol, the effect of vehicle for the NEP inhibitor, i.e., 20 ml of isotonic $NaHCO_3$, was given to test the effect on

Table II. Endopeptidase Inhibition in Nine Control Dogs

	Control	ANP_1	Recovery	SQ28603*	SQ28603 + ANP_2
V (ml/min)	2.5±0.11	4.0±0.42§	3.2±0.40	3.4±0.43	6.2±0.63¶
$U_{Na}V$ (μeq/min)	44±15	278±72§	45±17	94±32‖	397±50¶
GFR (ml/min)	54±5.8	60±6.9§	51±5	50±6	61±7¶
C_{PAH} (ml/min)	145±14	168±18§	128±11	117±6	145±13¶
ABP (mmHg)	141±5	124±6§	131±5	134±4.6	121±6¶
Hematocrit (%)	50±1.5	56±2§	52±2	52±1.2	55±1.5¶
Plasma iANP (pg/ml)	49±7	2,313±341§	75±9.7	126±19‖	5,300±839¶
Urine iANP (pg/ml)	2.7±0.6	7.4±1§	3±0.3	80±18‖	2,668±484¶
UV_{ANP} (pg/min)	6.6±3.0	30±10§	9.6±2	274±24‖	16,382±250¶
FE ANP (%)‡	0.25±0.09	0.02±0.009§	0.25±0.10	4.40±0.7‖	5.1±0.6¶
Degraded ANP load (pg/min)	2,640±420	138,750±18,000§	3,815±994	6,026±744‖	306,918±82,680¶

For abbreviations see text and Table I. * SQ 28603 = NEP 24:11 inhibitor. ‡ F.E. = fractional excretion. § $P < 0.05$ compared with control phase. ‖ $P < 0.05$ compared with recovery phase. ¶ $P < 0.05$ compared with SQ28603 phase. Degraded load = filtered load − excreted load.

$U_{Na}V$. This infusion was without effect (36±8 vs. 39±11 μeq/min, NS). A similar lack of effect was observed in three responding and nonresponding TIVC dogs, respectively.

Nonresponding TIVC dogs. The NEP inhibitor was administered to nine nonresponding TIVC dogs with sodium retention and ascites, and these data are summarized in Table III. The provision of the Squibb compound caused a significant natriuresis in all dogs that was unassociated with any increment in GFR, renal perfusion, ABP, or plasma levels of iANP. Though urinary excretion of ANP tended to rise, this change was not significant nor was the increment in FE of ANP. The rise in urinary excretion of ANP may be physiologically significant, however, since the rate of urinary excretion of ANP during the infusion of the NEP inhibitor just barely escaped statistical significance. When ANP was reinfused in the presence of NEP inhibition, $U_{Na}V$ increased still further so that $\Delta U_{Na}V$ of 96±18 μeq/min (compared with recovery phase) clearly represented a significant natriuretic effect when compared with the initial response ($\Delta U_{Na}V$ = 3±0.8 μeq/min, $P < 0.05$). Plasma levels of iANP increased by 201% compared with the initial infusion, and the fractional excretion of ANP increased dramatically to 17.7±7.9%.

Responding TIVC dogs. Table IV summarizes the data for the responding TIVC dogs. Provision of NEP inhibition also spontaneously increased $U_{Na}V$ by ~ 46±7 μeq/min ($P < 0.05$) and also magnified the ANP-induced natriuretic response ($\Delta U_{Na}V$ = 280±43 compared with 211±50 μeq/min initially, $P < 0.05$). The modest natriuretic effect of NEP inhibition alone occurred with a significant increment in plasma iANP and with significant increments in the urinary excretion of this peptide. When ANP was reinfused in the presence of NEP inhibition, plasma levels increased by 287% and FE of the peptide increased to 9±2.6%.

Nonresponding TIVC dogs: further studies. Because of the putative role that NEP 24:11 may play in kinin catabolism (12), we examined the possible role of kinins in the natriuretic response to ANP. Fig. 2 summarizes the data obtained from four nonresponding dogs when bradykinin was infused into the left renal artery before a reinfusion of ANP. Though bradykinin tended to increase urine flow and $U_{Na}V$ in the infused kidney, the changes were not significant. When ANP was reinfused in the presence of the kinin, the nonresponding TIVC dog was converted into a responder ($\Delta U_{Na}V$ = 114±1.9 μeq/min compared with recovery phase [$P < 0.05$]). The initial

Table III. Endopeptidase Inhibition in Nine Nonresponding TIVC Dogs

	Control	ANP_1	Recovery	SQ28603*	SQ28603 + ANP_2
V (ml/min)	2.28±0.11	2.39±0.14	2.20±0.04	2.37±0.11	2.47±0.11
$U_{Na}V$ (μeq/min)	2.6±0.37	4.4±0.08	3.3±0.08	70±17*	100±8§
GFR (ml/min)	47±2.7	51±4.7	40±5.7	42±6.3	39±4.9
C_{PAH} (ml/min)	119±6	141±20	120±13	123±11	113±9
ABP (mmHg)	104±7	92±8‡	95±6	92±9	88±7
Hematocrit (%)	45.6±1.8	48±2.1	45±3	45±5	46.6±4
Plasma iANP (pg/ml)	38±11.9	1,853±312‡	33±6.3	40±7.2	5,581±1,588§
Urine iANP (pg/ml)	6.5±1.9	13.5±4.2‡	8±3.3	25±10*	13,857±6,275§
UV_{ANP} (pg/min)	16.6±5	35.6±12‡	20±9	59±22	38,022±18,555§
FE ANP (%)*	2.3±0.6	0.04±0.01‡	2.1±0.8	7.9±4.3	17.7±7.9§
Filtered ANP load (pg/min)	1,284±458	103,545±31,180‡	1,323±269	1,469±200	217,000±86,323§
Degraded ANP (pg/min)	1,267±460	103,510±31,176‡	1,305±266	1,410±215	202,600±85,554§

For abbreviations see text and Table I. * $P < 0.05$ compared with recovery phase. ‡ $P < 0.05$ compared with control phase. § $P < 0.05$ compared with SQ28603 phase.

Table IV. Endopeptidase Inhibition in Seven Responding TIVC Dogs

	Control	ANP_1	Recovery	SQ28603*	SQ28603 + ANP_2
V (ml/min)	2.6±0.13	4.2±0.4*	3.5±0.3	3.4±0.5	4.8±0.4§
$U_{Na}V$ (μeq/min)	22±7	238±54*	21±7	68±19‡	305±116§
GFR (ml/min)	45±2.9	54±8.7*	44±6	49±7.2	57±9.5§
C_{PAH} (ml/min)	124±8	135±17	119±13	121±13	136±18
ABP (mmHg)	106±8	94±6*	103±7	104±5	94±8
Hematocrit (%)	41.7±2.4	48±2.1*	49.7±2.6	48.3±2.8	48.4±2.4
Plasma iANP (pg/ml)	91±20.7	1,458±123*	107±22	204±63‡	5,645±714§
Urine iANP (pg/ml)	20±10	15±6	7.5±2.5	190±87‡	5,261±1,788§
UV_{ANP} (pg/min)	30±16	109±49	23±8	474±277‡	35,299±14,000§
FE ANP (%)*	0.94±0.6	0.18±0.09	1.13±0.43	3.7±1‡	9±2.6§
Filtered ANP (pg/min)	5,352±948	83,725±17,680*	4,575±1,237	9,802±3,039	327,166±103,403§
Degraded ANP (pg/min)	5,322±957	83,616±17,675*	4,552±1,239	9,328±2,822	291,966±93,302§

For abbreviations see text and Table I. * $P < 0.05$ compared with control phase. ‡ $P < 0.05$ compared with recovery phase. § $P < 0.05$ compared with SQ28603 phase.

$\Delta U_{Na}V$ with ANP had been 3.4±3.1 μeq/min. For the right control kidney, the initial $\Delta U_{Na}V$ was 5±0.4 μeq/min and with the ANP reinfusion was 8±0.9 μeq/min. This value was not changed from the initial one and was significantly ($P < 0.05$) less than that observed in the experimental controlateral kidney.

In four nonresponding TIVC dogs, we administered captopril intravenously in an attempt to augment intrarenal kinin availability. Fig. 3 summarizes these data. Though captopril was without effect on $U_{Na}V$, GFR, or ABP (though there was a tendency for ABP to decrease), the reinfusion of ANP in the presence of captopril increased $U_{Na}V$ from 3.7±1.3 to 48±11 μeq/min ($P < 0.05$). The previous $\Delta U_{Na}V$ had been 9 μeq/min. This natriuresis occurred despite a blood pressure value that was significantly less in this final phase than recorded in the initial control phase. When administered to three normal dogs, captopril had no effect on $\Delta U_{Na}V$ (280 vs. 262 μeq/min, respectively) after an ANP infusion.

In four nonresponding TIVC dogs, the natriuretic peptide urodilatin was delivered intravenously at 100 ng/kg per min. Fig. 4 *A* summarizes these data. Urodilatin was unable to induce a natriuresis in these dogs, although when given to three normal dogs or to responding TIVC dogs, there was a prompt and significant natriuresis ($\Delta U_{Na}V$ = 230±8 and 206±11 μeq/min, respectively).

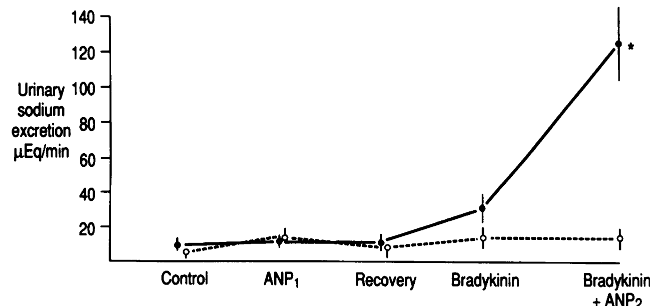

Figure 2. The natriuretic effect of ANP in four TIVC nonresponders when bradykinin is being infused into the left renal artery at 3 μg/kg per min. * $P < 0.05$ compared with previous phase. ●, Left kidney; ○, right kidney.

Fig. 4 *B* illustrates in a separate group of three dogs, the effect of prior administration of bradykinin to nonresponding TIVC dogs receiving urodilatin. The kinin now permits the urodilatin to induce a natriuresis in the left kidney ($\Delta U_{Na}V$ = 63 μeq/min), whereas the urodilatin reinfusion remains without effect on the control right kidney.

Finally, the effect of kinins on the renal response to the second messenger of ANP (cGMP) was examined in four nonresponding TIVC dogs with ascites. These data are given in Fig. 5. As previously demonstrated (8), in doses that cause marked natriuresis in both normal and responding TIVC dogs, 8-Br-cGMP is without effect in nonresponding TIVC dogs. The administration of bradykinin, however, to one kidney, now permits the induction of a natriuretic response with a second infusion of 8-Br-cGMP.

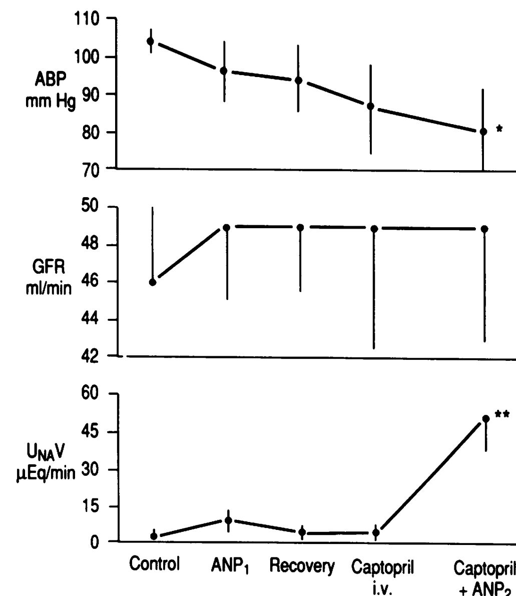

Figure 3. The effect of intravenous captopril on four TIVC nonresponders receiving an intravenous infusion of ANP. Note the rise in $U_{Na}V$ when ANP is administered in the presence of captopril despite the decline in ABP. * $P < 0.05$ compared with initial control phase; ** $P < 0.05$ compared with recovery phase.

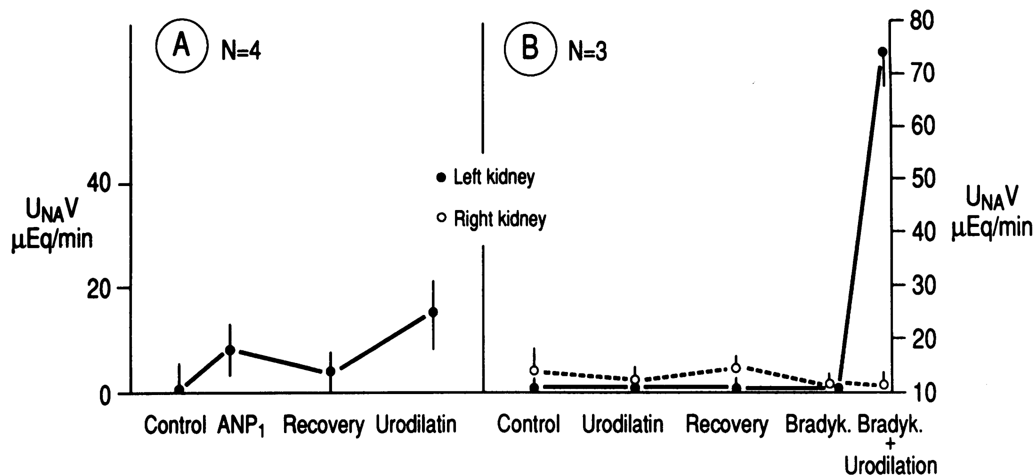

Figure 4. Urodilatin (100 ng/kg per min) administered to TIVC nonresponders without (*A*) and with bradykinin being infused into the left renal artery (*B*).

Responding TIVC dogs: further studies. To further test the hypothesis that kinins may be playing a role in the natriuretic response to ANP in TIVC dogs, we performed two sets of studies. In one, we administered aprotinin to five responders, and in another set, we administered a specific bradykinin antagonist to four responding dogs.

Fig. 6 summarizes the data with aprotinin. Although without effect on sodium excretion or urine flow, aprotinin caused significant blunting of the natriuretic response ($\Delta U_{Na}V$ 299±69 vs. 92±26 μeq/min, $P < 0.05$) when ANP was reinfused a second time. This agent by itself was without effect on GFR, ABP, or renal plasma flow.

When the BKA was administered into the left renal artery of four TIVC responders, it was able to dramatically attenuate the natriuretic response to ANP in the experimental kidney. The natriuresis of the contralateral right kidney reappeared with an ANP reinfusion and was unchanged to that observed when the dogs were initially exposed to intravenous ANP. These data are summarized in Fig. 7.

In three separate TIVC dogs responsive to the natriuretic effects of ANP, the BKA was administered into the left renal artery before intravenously infusing the NEP compound only. A second ANP infusion was not given. The initial natriuretic responses to ANP had been $\Delta U_{Na}V$ = 52±5 (left kidney) and 49±4.7 μeq/min (right kidney). When the NEP inhibitor was administered 90 min after cessation of the ANP infusion, $\Delta U_{Na}V$ for the right kidney was 31±2.6 μeq/min, but in the left kidney receiving the BKA, the $\Delta U_{Na}V$ declined to 5±0.9 μeq/min compared with a recovery period (NS). Thus, the BKA was able to attenuate the natriuresis induced by NEP inhibition alone in the absence of an exogenous ANP infusion.

Finally, we examined the possibility that bradykinin, captopril, aprotinin, or BKA caused increments in UV_{ANP} in the doses employed when infused into normal dogs. These data are

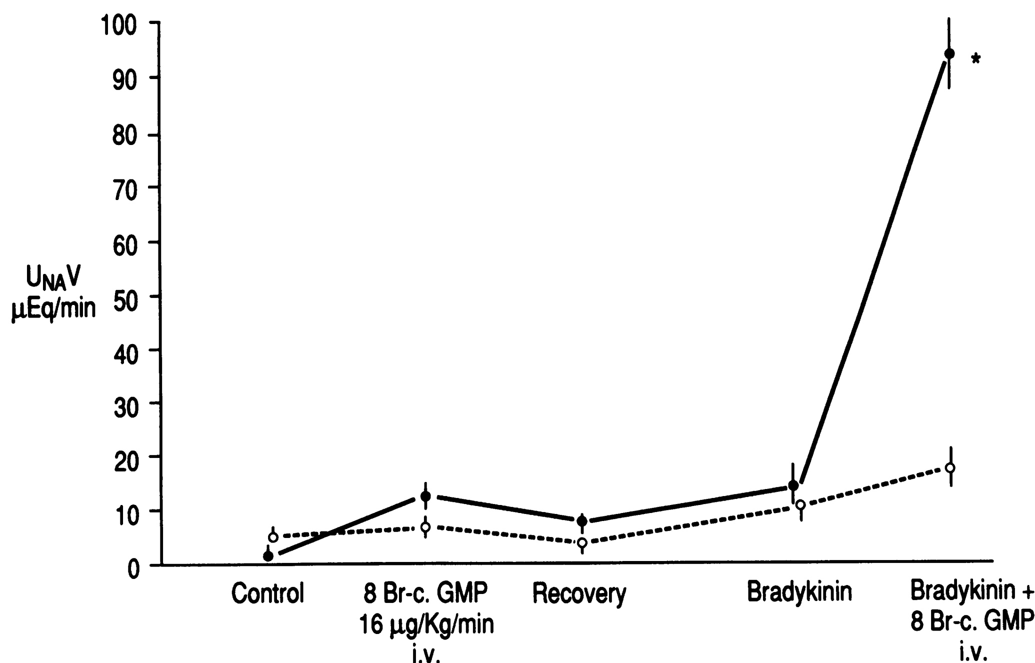

Figure 5. The effect of providing bradykinin into the left renal artery of four TIVC nonresponders receiving intravenous 8-Br-cGMP. **P* < 0.05 compared with previous phase. •, Left kidney; o, right kidney.

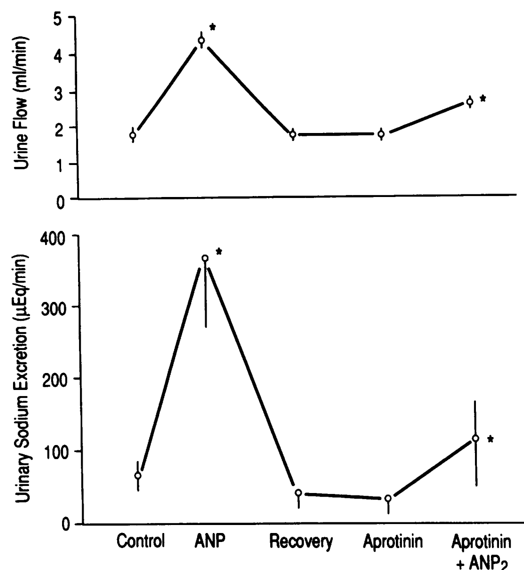

Figure 6. The effect of aprotinin on five TIVC dogs responsive to the natriuretic effects of ANP. Aprotinin attenuates the increment in urine flow and sodium excretion previously observed with ANP. *$P < 0.05$ compared with previous phase.

given in Table V. None of these agents is responsible for augmented ANP delivery to the inner medullary collecting ducts (IMCD).

Discussion

In recent studies, including the present investigation, conducted over an interval of several years (1, 2, 5–8), this laboratory has consistently demonstrated a heterogeneous natriuretic response to infusions of pharmacological doses of ANP (50–175 ng/kg per min) administered to sodium-retaining dogs with ascites. Approximately half of the animals respond with an increment in urinary sodium excretion not different from normal controls whereas the remaining half fail to demonstrate any natriuretic response. Because all of our studies, including the present one, have demonstrated no difference in postinfusion plasma levels of ANP (1, 2) and because recent studies in suspensions of IMCD cells isolated and prepared from TIVC responders and nonresponders have failed to demonstrate differences in ANP receptor density and affinity and in ANP-induced generation of cGMP (8), it seems reasonable to conclude that the absent natriuretic response to ANP in nonresponders cannot be due to deficiencies in ANP availability, binding, or second messenger generation at basolateral receptor sites.

Lack of response to ANP in TIVC nonresponders could therefore be due to (*i*) biologically inactive peptide, (*ii*) a reduction in sodium delivery to the IMCD, (*iii*) inhibitory effects of neurohumoral stimuli overriding the natriuretic effects of ANP, (*iv*) a problem with post-cGMP signal transduction, or (*v*) differences in the luminal delivery of ANP and/or other factors to the IMCD. In this regard, Gerbes et al. (15) have recently demonstrated in cirrhotic rats that clearance "C" receptors involved in degradation of ANP are markedly increased within the glomerulus compared with controls. They speculated that as a result diminished tubular delivery of ANP could be a factor producing insensitivity to the peptide in this experimental model.

Since identical lots of peptide were employed for both responding and unresponding TIVC dogs, the first possibility can be eliminated. Moreover, in the present study, ANP given to nonresponders could cause a hypotensive effect while not producing a natriuretic effect (Table III), and in other studies could raise GFR while not augmenting urinary sodium excretion (1, 2). We are thus dealing with a true dissociation of biological effects.

Though micropuncture studies have not been performed in any of our studies, we have demonstrated that filtered sodium load, 24-h sodium excretion, baseline sodium excretion in acute clearance studies, and baseline urinary sodium concentration have been equivalent for both canine populations (1, 2). Where we have measured $U_K^+/U_{Na} + U_K^+$ ratios, a measure for distal delivery and cation exchange (13, 16), we also have not detected differences between natriuretic responders and nonresponders. Thus, it seems unlikely that reduced distal de-

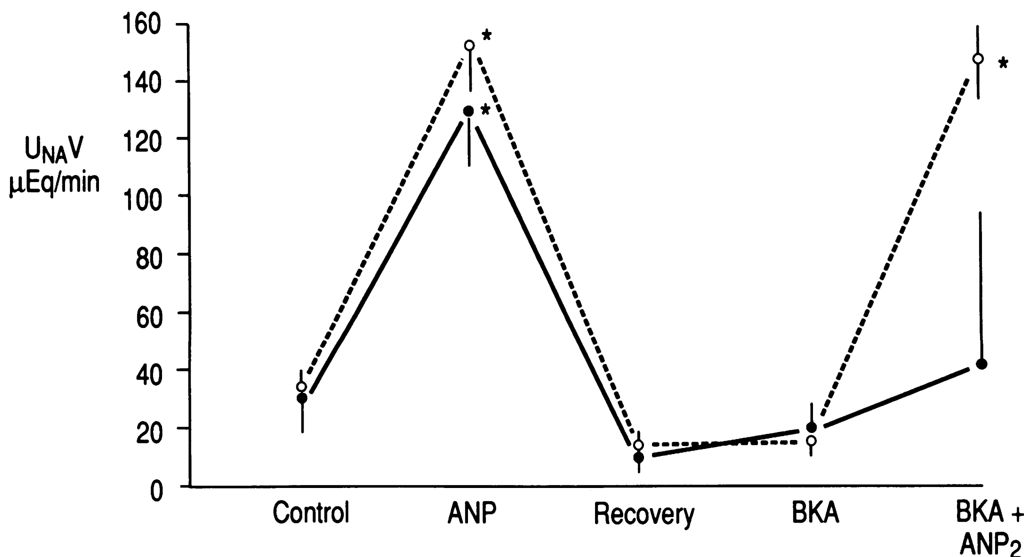

Figure 7. The effect of delivering a specific bradykinin antagonist into the left renal artery of four TIVC responders. *$P < 0.05$ compared with previous phase. ●, Left kidney; ○, right kidney.

Table 5. Influence of Various Agents on Urinary ANP Excretion (n = 6)

Agent	Control UV_{ANP}	Postagent UV_{ANP}
	pg/min	
Bradykinin (LRA)*	32±12.7	15.8±3
Captopril (i.v.)‡	17±8	24.6±4
Aprotinin (i.v.)	11.6±4	15.4±3
Bradykinin antagonist (LRA)	16±2.3	18.5±4.6

* LRA, agent delivered into left renal artery. ‡ i.v., agent delivered intravenously.

livery of sodium to the IMCD is a problem. Moreover, in a recent study, we have demonstrated that amiloride, a diuretic which inhibits Na^+ conductive pathways responsive to ANP, will induce a normal natriuresis in TIVC dogs unresponsive to ANP infusions (6). It thus seems unlikely that in our experimental model insufficient distal delivery of sodium could be a determinant for tubular unresponsiveness to ANP in TIVC nonresponders. It must be conceded, however, that Morali et al. (17) recently demonstrated in 10 cirrhotic patients with ascites unresponsive to an ANP infusion that mannitol induced modest natriuretic responses to this peptide in six of these patients. In the remaining four, however, neither mannitol nor mannitol plus ANP in combination was sufficient to increase urinary sodium excretion.

In an extensive series of studies in TIVC dogs, we have been unable to show differences between responders and nonresponders for plasma volume, GFR, renal perfusion, papillary plasma flow, aldosterone, endothelin, and vasopressin plasma levels (1, 2, 5–8) and have ruled out a role for catecholamines, angiotensin, low blood pressure, renal nerves, and adenosine in overriding and blunting an ANP-induced natriuresis in nonresponders. Although we have not yet excluded with certainty a problem with signal transduction distal to cGMP generation, the data from the present study suggest strongly that ANP and/or kinin delivery to the IMCD may be of great importance as a determinant in the natriuretic response to infused ANP, at least in TIVC nonresponders.

Since NEP 24:11 within the brush border of the proximal tubule prevents the largest part of filtered ANP from reaching the IMCD, the urinary excretion of ANP was minimal, varying from 6 to 30 pg/min in our experimental animals (Tables II–IV). Since ANP is known to exert a potent inhibitory effect on conductive sodium transport when present only on the luminal side of the IMCD (18), receptors must presumably exist at this site. Moreover, immunocytochemical evidence confirms that with a high degree of probability ANP receptors exist on the luminal aspect of both the cortical collecting duct and the IMCD (19). As Wilkins et al. (11) recently speculated, these receptors, receiving a small but fairly constant amount of natriuretic peptide and protected from "up or down" regulation by varying plasma ANP levels, may play an important role in determining sodium handling by the collecting duct. Indeed, in our studies, the administration of a NEP inhibitor to TIVC nonresponders was able to elicit a modest natriuresis in this group independent of any change to GFR, C_{PAH}, ABP, or plasma level of iANP (Table III). Although urinary concentrations of ANP increased markedly, UV_{ANP} or FE of ANP just barely escaped statistical significance while increasing. In the remaining groups of dogs, a natriuretic effect after administration of the NEP inhibitor was associated with increments in plasma iANP, as well as significant increments in UV_{ANP} and FE of ANP. Several groups of investigators (11, 20) have now suggested that intraluminal delivery of ANP to the IMCD (as opposed to the basolateral surface) may be critical in determining the magnitude of the natriuretic response.

The observation in our experiments that a natriuretic effect in TIVC dogs after administration of NEP inhibitor could occur in association with increased ANP urinary excretion, but also with a rise in plasma iANP levels (controls and TIVC responders), as well as the observation that the baseline ANP excretion was not less in TIVC nonresponders than other groups, or indeed was even higher during the initial ANP infusion than in control dogs, suggested that distal delivery of ANP was not necessarily correlated to sodium excretion in our TIVC dogs. The observation that administering NEP inhibitors to TIVC nonresponders was a potent method to induce tubular responsiveness to ANP in animals previously completely unresponsive to this peptide indeed suggested to us that excessive degradation of ANP by proximal tubular NEP 24:11 might be the cause of such unresponsiveness. Several laboratories (10, 11, 20, 21), working with dogs, humans, and rats, have now demonstrated that inhibition of NEP 24:11 with various compounds will indeed magnify the natriuretic response to ANP, even though changes in plasma ANP levels after such inhibition are variable.

Several observations in our laboratory suggest, however, that augmented availability of ANP may not be the entire cause for this increased natriuretic effect. Urodilatin is a peptide similar to ANP, first isolated from human urine by Schulz-Knappe et al. (22) and demonstrating potent vascular and natriuretic effects. Indeed, evidence has been presented that urodilatin may be that member of the ANP family primarily responsible for the regulation of urinary sodium excretion (23). Compared with ANP, it is NH_2 terminally extended by four amino acids and is thought to be produced within the kidney and to be resistant to the degradative effects of NEP 24:11 (23). If urodilatin was infused into TIVC nonresponders, and excessive degradation of ANP by NEP 24:11 was an important cause for the lack of natriuresis, then urodilatin escaping enzymatic breakdown should now be associated with a significant natriuresis. Although this occurred in normal dogs and TIVC responders, this peptide was without effect in TIVC nonresponders. Indeed, a natriuretic effect was not observed until the dogs were pretreated with a renal arterial infusion of bradykinin (Fig. 4). Given that NEP 24:11 can hydrolyze other peptides viz kinins, neurotensin, endothelin, etc. (12), the possibility exists that the delivery of kinins may be a determining factor for the tubular refractoriness to ANP in TIVC nonresponders. This idea receives support from the following observations. First, when captopril, an inhibitor of kininase and angiotensin-converting enzyme was infused into TIVC nonresponders, a natriuretic effect to a second infusion of ANP was observed (Fig. 3). No magnification of a natriuretic effect was observed when captopril was given to normal controls. We have recently demonstrated that angiotensin infused into TIVC responders will not convert them into nonresponders and similarly the provision of an angiotensin antagonist, saralasin, does not convert TIVC

nonresponders into responders after infusion of ANP (8). Captopril probably achieved its effect, therefore, by increasing the availability of intrarenal kinins in TIVC nonresponders. The absence of an effect in normal controls suggests that there exists an intrarenal availability of kinins beyond some critical level. The observation that $U_{Na}V$ did not increase following intravenous captopril alone despite an unchanged GFR (Fig. 3) suggests that this agent did not produce its permissive effect on ANP by augmented distal Na^+ delivery to the IMCD.

Second, though without effect on renal function or sodium excretion in the doses employed, bradykinin provided to TIVC nonresponders now permitted a natriuretic response to ANP where none previously could be elicited (Fig. 2). Even more compelling were the observations that aprotinin could blunt the natriuretic effect of ANP in TIVC responders (Fig. 6). This agent is a nonspecific polyvalent serine protease inhibitor capable of attenuating the effect of several proteases, including glandular kallikrein, but its physiological effects have generally been ascribed to its ability to inhibit kinin generation (12). Though without effect on kidney function in TIVC responders when administered alone, there was a dramatic decrement in $\Delta U_{Na}V$ after ANP administration (299±69 vs. 92±26 μeq/min, $P < 0.05$) when aprotinin was administered concurrently.

It has previously been reported that a specific BKA can blunt the many physiological functions attributable to kinins (24,25). Smits et al. (24) have demonstrated in anaesthetized rats that the potentiation of the natriuretic effects of ANP by NEP inhibitors was completely abolished by the identical BKA used in our present studies. These investigators concluded, particularly since NEP inhibition did not cause plasma ANP levels to rise (when given alone or concurrently with an ANP infusion), that potentiation of the natriuretic effects of ANP by NEP inhibition involved intrarenal accumulation of bradykinin.

In our studies we observed a similar phenomenon but had sufficient NEP inhibitor to study only three dogs in this way. When the BKA was given into one renal artery of TIVC responders, there was no effect on $U_{Na}V$. When the NEP inhibitor in usual doses was given intravenously, there was no increment in $\Delta U_{Na}V$ for the experimental kidney (5±0.9 μeq/min) whereas the $\Delta U_{Na}V$ for the control kidney was significant (31±2.6 μeq/min). We also demonstrated however that when the BKA was given to responding TIVC dogs receiving exogenous ANP, the natriuresis from the experimental kidney was severely blunted whereas that of the control kidney continued unabated (Fig. 7). Thus, in our hands, antagonism of bradykinin receptors was also capable of inhibiting a natriuretic effect when only exogenous ANP was infused, without simultaneous NEP inhibition.

This phenomenon has previously been reported by Sybertz et al. (24). These investigators administered to rats the same bradykinin antagonist used in our studies. They observed that bradykinin played a permissive role for the natriuretic effects of ANP since the BKA inhibited the natriuretic response to both NEP inhibitors and exogenous ANP itself. Unlike our present studies, these authors found that the BKA itself would decrease both urine flow and urinary sodium excretion, suggesting perhaps a more important role for kinins in regulating tubular handling of sodium in this species than in dogs. Of interest as well, was that the BKA did not abolish the antihypertensive effect of ANP with or without simultaneous NEP inhibitors.

The observations obtained in the present studies would indicate that the availability of intraluminal kinins may be a critical modulating factor for the natriuretic effects of ANP, and that both peptides must be present for a natriuretic response to be obtained after an exogenous ANP infusion or the delivery of "extra" ANP to the IMCD from the proximal tubule after NEP inhibition. Merely augmenting distal ANP delivery per se appears to be insufficient for a natriuresis to occur. Our studies with 8-Br-cGMP support this idea. Studies performed on cell suspensions prepared from the IMCD of TIVC and normal dogs indicate that the ability to generate cGMP is equivalent between these animals when exposed to ANP (8). Nevertheless, our present study as well as previous studies (8) indicate that TIVC nonresponders, but not normal dogs or TIVC responders, are refractory to the natriuretic effects of infused cGMP. Yet when bradykinin is supplied, TIVC nonresponders are now able to mount a natriuretic response to infused 8-Br-cGMP. These data suggest that the presence of kinins may be critical for ANP-induced signal transduction. Procedural difficulties in measuring urinary kinins precluded obtaining such data in the present study, but clearly some of these experiments must be repeated while assaying urinary and perhaps plasma kinin levels.

It must also be emphasized that despite whatever kinin–ANP interactions may be occurring within the collecting duct system, other factors may be operative in TIVC-NR dogs (e.g., limited distal Na^+ delivery, humoral factors antagonistic to ANP, etc.), limiting the natriuretic response to ANP. This is suggested by the observation that even in the presence of NEP inhibition, the peak $U_{Na}V$ after ANP administration or was 300–400 μeq/min in controls and TIVC-R dogs, but only 100 μeq/min in nonresponding TIVC dogs (Table III).

The schema illustrated in Fig. 8 summarize our ideas and explanations of our experimental findings. The left panel indicates normal events. ANP and kinins are freely filtered at the glomerular level (Fig. 8, site *1*). At site 2 in Fig. 8, NEP 24:11 along with kinases destroy virtually all of the filtered kinins so that none leaves the proximal tubule (27). In TIVC nonresponders, excess NEP activity at extrarenal sites could account for the diminished baseline levels of plasma iANP (Table I). Augmented reabsorption of this peptide after NEP inhibition seems unlikely given the extensive degradation by NEP 24:11 within the brush border of the proximal convoluted tubule. As well, previous studies in this model (1, 2, 16) have shown that differences in plasma volume cannot account for differences in baseline plasma iANP levels. Though plasma levels of iANP did not rise in TIVC-NR dogs after NEP inhibition (as might be anticipated if excessive nonrenal degradation were occurring in this group), it should be noted that fractional excretion of ANP (Table III) rose more in this group than the others. This rise in urinary excretion might have limited the rise in plasma levels. In this latter group as well, excess intrarenal NEP activity could lead to diminished distal delivery of ANP compared with TIVC responders. That NEP activity is related to kinin degradation seems clear. It is now believed that NEP 24:11 is at least as important as kininase 11 in degrading kinins within the nephron (12), and a recent report indicates that NEP 24:11 may account for 53–74% of intrarenal kinin degradation (28). In rats, urinary kininase activity appears to be largely provided by NEP 24:11 (27).

At Fig. 8, site 3, kinins enter the distal tubule, the source for

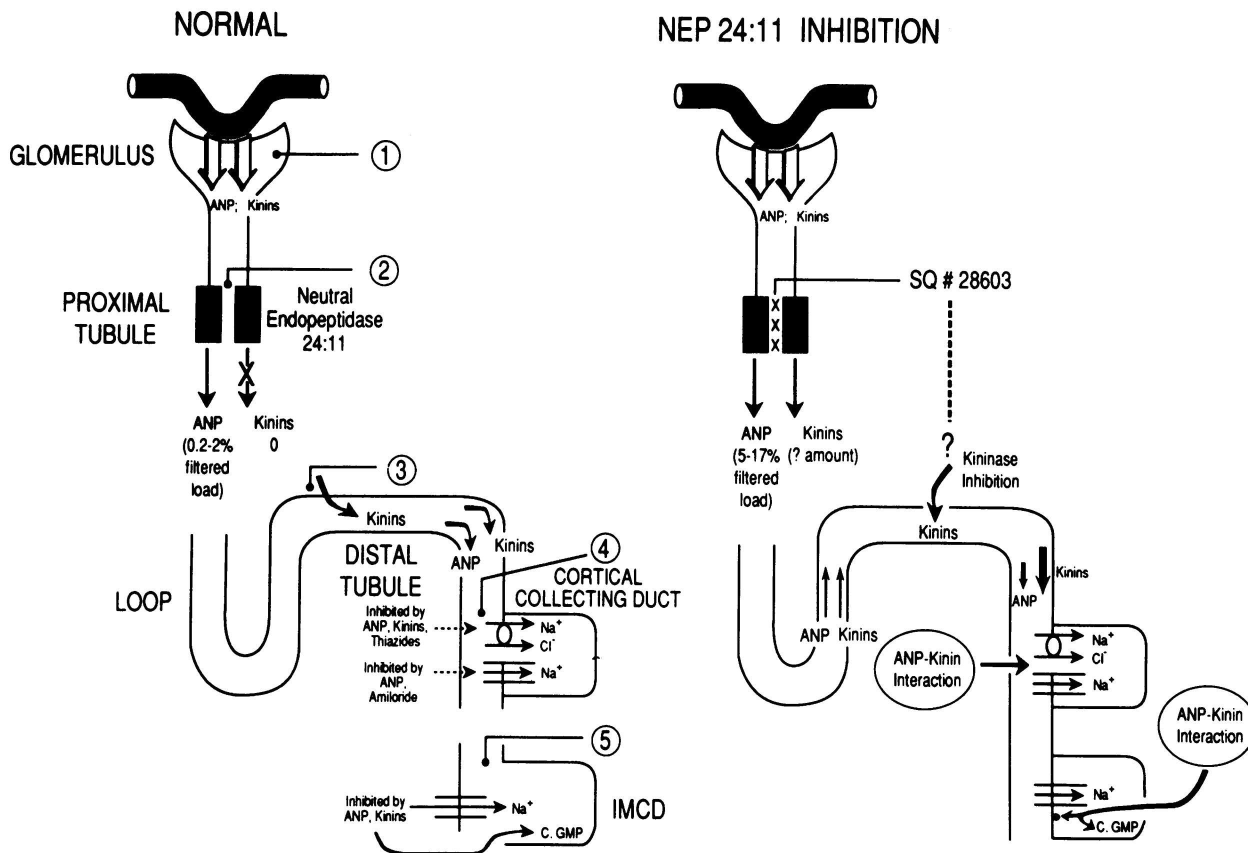

Figure 8. A summary of kinin–ANP interactions as they occur within the nephron normally (*left*) and after NEP 24:11 inhibition (*right*). See text for details.

kinin activity acting at more distal sites. A possible reduction in distal delivery of kinins in TIVC nonresponders suggest there may be reduced formation and/or excessive degradation at this site. Fig. 8, sites 4 and 5 indicate the major pathways for sodium reabsorption by conductive and electroneutral pathways in the cortical collecting duct and by conductive channels in the IMCD. Others have already supplied evidence that kinins may play an important permissive role at these transport sites. Zeidel et al. (29) recently demonstrated the ability of bradykinin to directly inhibit conductive Na^+ channels in rabbit IMCD cells. More recently, Stoos et al. (30) reported a synergistic effect for ANP and bradykinin on short circuit current, a measure of sodium transport in cultured cortical collecting duct cells (M-1 line). Each agent by itself was without effect on short circuit current but, when given together, reduced sodium transport by 15% whereas cGMP content of the cells increased. We have also shown at site 5 in Fig. 8 that kinins may play a permissive role for the actions of cGMP, at least in TIVC nonresponders.

The right panel of Fig. 8, summarizes possible effects after NEP 24:11 inhibition. The presence of SQ 28603 at Fig. 8, site 2 would augment the distal delivery of ANP and kinins. Since, however, kinins are thought not to reach the distal nephron from the proximal tubule normally, a lack of kinins from this source cannot be invoked as playing a role in the tubular insensitivity to ANP in TIVC nonresponders. As mentioned previously there may be a synthetic and/or degradative problem at the level of the distal nephron. This raises the possibility that the NEP inhibitor may be playing a beneficial role at Fig. 8, site 3, as illustrated in the diagram. It has been speculated that the kallikrein–kinin system may clear ANP from prohormone at this site (23), and this may be a determining factor.

In any event, after NEP inhibition, we postulate that increased delivery of kinins and ANP distally to Fig. 8, sites 4 and 5 may permit a magnified natriuresis in TIVC responders and induce a natriuresis in nonresponders as the delivery of kinins and ANP reach beyond some critical level. Since conductive and electroneutral pathways for sodium reabsorption are luminal, inhibition would presumably be at this site. Certainly bradykinin receptors (12) and ANP receptors (19) are thought to exist at this site. Our data also suggest that kinins are important for cGMP actions to occur, at least in TIVC nonresponders. That both substances viz ANP and kinins must be present together for an ANP-induced natriuresis to occur receives support from the observation that when antibodies to ANP are administered NEP inhibitors lose their natriuretic effects (31). Although other investigators have certainly suggested and provided evidence that kinins may be important for the natriuretic effects of ANP after NEP inhibition (20, 24, 26, 30), we believe that ours is the first such evidence collected in a model of sodium retention and ascites, where defects in kinin availability may serve as an explanation for the attenuated natriuretic effects of ANP.

In summary, we have uncovered two maneuvers that will convert TIVC dogs unresponsive to the natriuretic effects of ANP into responders. One is to inhibit NEP 24:11, the other is

to provide intrarenal kinins. Our observations in TIVC nonresponders that pretreatment with bradykinin is permissive for the natriuretic effects of ANP, urodilatin (a natriuretic peptide thought to escape intrarenal enzymatic cleavage), and cGMP, implies a final common pathway. Because urodilatin escapes NEP cleavage but is still not natriuretic in TIVC nonresponders until bradykinin is supplied, it is presumably not augmented distal delivery of natriuretic peptide that is critical after NEP inhibition, but rather the augmented delivery of kinins. This concept receives support from the observations that both aprotinin and a BKA will attenuate an ANP-induced natriuresis in TIVC responders. How kinins interact with ANP is not clear but they may modulate ligand (ANP) binding to IMCD luminal receptors or in some way be critical for full signal transduction. Why some 60% of our TIVC dogs in the present study appear to lack critical amounts of intrarenal kinins is not answered by our experiments but may be due to differences in the level of NEP (kininase) activity within the renal tubule. The observation by us that NEP-induced natriuresis occurs in TIVC nonresponders in the absence of raised plasma levels of ANP but seems to be associated with increased urinary excretion of ANP supports the view that the ANP-kinin interactions are probably occurring at the luminal as opposed to the basolateral surface of the IMCD.

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