Abnormal Glomerular Response to Atrial Natriuretic Peptide in Rats with Aortocaval Fistulas

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ABSTRACT
Heart failure is characterized by a blunted natriuretic and diuretic response to atrial natriuretic peptide (ANP). To investigate this, a rat model of compensated high-output heart failure was used to determine whether glomerular response to ANP differs in animals with high cardiac output compared with control animals. An aortocaval (AC) fistula was made below the level of the renal arteries in male Sprague-Dawley rats. At 6 wk, one group of AC fistula (N = 6) and control rats (N = 6) was injected with radiolabeled microspheres for determination of hemodynamic parameters, including cardiac output, renal blood flow, and vascular resistance. Rats with AC fistulas had significant changes in cardiac output (21.8 ± 1.7 mL/min, P = 0.0001), renal blood flow (3.4 ± 0.7 versus 8.4 ± 1.9 mL/min, P ≤ 0.05; 3.0 ± 0.4 versus 7.2 ± 1.9 mL/min Right, P ≤ 0.05), and total vascular resistance (0.6 ± 0.1 versus 2.7 ± 0.4 mm Hg/mL per min, P < 0.001) compared with control animals, respectively. In another group of animals, after 6 wk, glomeruli were isolated from kidneys. Extracellular (EC) and intracellular (IC) cGMP was measured as an indicator of glomerular response to ANP. Early glomerular response to ANP (10^-8mol/L) showed a similar acute 13- to 18-fold rise in IC cGMP after 30 sec exposure to ANP (P ≤ 0.0001 versus no ANP; N = 4 AC fistula rats and N = 4 control rats). During 1-h incubations with ANP, glomerular response was characterized by a five- to sevenfold increase in EC cGMP. However, glomerular of AC fistula rats produced significantly less EC cGMP than did those of control animals (21.3 ± 2.5 versus 44 ± 4.9 fMol cGMP/2000 glomeruli, P ≤ 0.005; N = 5 AC fistula rats and N = 5 control rats, respectively). Probenecid-sensitive transport of EC cGMP between AC fistula and control rats (86% decrease versus 82% decrease) was similar. However, glomeruli from AC fistula animals had significantly less phosphodiesterase activity compared with control animals (3.6 ± 0.4 versus 5.4 ± 0.7 nMol cGMP/mg protein per min, P = 0.01; N = 4 AC fistula rats and N = 5 control rats, respectively). It is speculated that reduced glomerular generation of cGMP in response to ANP contributes to sodium retention in heart failure, but may be compensated for in part by decreased phosphodiesterase-mediated hydrolysis of cGMP.

Key Words: cGMP, phosphodiesterase, sodium retention, heart failure

Clinically compensated and uncompensated heart failure is often accompanied by sodium retention (1,2). This positive sodium balance is the result of multifactorial responses that act to maintain cardiovascular homeostasis, including increased sympathetic tone, activation of the renin-angiotensin system, and increased plasma vasopressin (3–5). In response to increased intravascular volume associated with heart failure, circulating atrial natriuretic peptide (ANP) concentration is greatly elevated in attempting to counterbalance sodium retention (1,5,6). Despite measurable increases in circulating ANP, the renal response of natriuresis is blunted in animals with heart failure (1,7). Furthermore, in animals with heart failure as a result of a variety of causes, the infusion of ANP does not result in a natriuretic response to the same magnitude as that in control animals (1,7–9). This has been accompanied by lower levels of urinary cGMP in some models (8). In other models, urinary cGMP is elevated to the same level as that of control animals, or higher (10).

We have previously described a decreased glomerular response to ANP in the neonatal rat, which is characterized by relative sodium retention (11,12). In this model, after prolonged incubation with ANP, the accumulation of extracellular cGMP was lower in glomeruli from preweaned rats than in adult rats (12). This finding was in agreement with the physiologic observation that there is a lower urinary cGMP response in preweaned rats and a lower amount of sodium excreted in response to ANP (11). Micropuncture studies indicate that ANP increases the glomerular production of cGMP in glomerular ultrafiltrate without causing significant increases in systemic or renal venous cGMP levels (13). Therefore, glomerular cells presumably produce the majority of cGMP found in glomerular ultrafiltrate.

We hypothesize that when ANP is infused in animals...
with abnormal cardiac function, the blunted natriuresis and diuresis may be related to an alteration in the glomerular response to this peptide. To determine if glomerular response mechanisms decrease when cardiac function is altered, we tested the glomerular response to ANP in rats with AC fistulas and with circulatory overload as a result of increased cardiac output. In addition, cellular mechanisms that could compensate for or explain this altered glomerular response to ANP were examined.

METHODS

Animals

Male adult Sprague-Dawley rats (150 to 200 gm) were anesthetized with 50 mg/kg pentobarbital ip and subjected to aortocaval shunting by the method of Garcia and Diebold (14). In brief, an 18-gauge needle was introduced into the abdominal aorta below the level of the renal arteries and then passed into the inferior vena cava to create an aortocaval fistula. Control animals had an 18-gauge needle introduced into the abdominal aorta only. The aortic entry point was sealed with a drop of cyanoacrylate glue and the animals were allowed to recover. They received a standard diet (Rat, Mouse, Hamster Feed 3000; Agway, NY) and water ad libitum. After shunt procedure, some animals were housed in metabolic cages for urine collections. Six wk after the shunting procedure, studies were done to assess physiologic changes and to assay glomerular response to ANP. These studies were done in accordance with institutional guidelines and committee-approved animal protocols at the University of Virginia.

Characterization of Physiologic and Hemodynamic Parameters

Physiologic parameters, including body weight, heart weight, and hematocrit value, were measured. For hemodynamic studies, rats were anesthetized with 60 mg/kg sodium pentobarbital ip, and a carotid artery was cannulated for measurement of mean blood pressure and heart rate. A jugular vein was cannulated for infusion of 0.9% NaCl at 3 mL/kg per h. A femoral artery was cannulated into a reference blood sample during microsphere infusion as described previously (15). Radiolabeled microspheres 12 to 15 microns in diameter and labeled with 85Sr (New England Nuclear, Boston, MA) (12) were injected into the aortic root. Cardiac output, renal blood flow, total vascular resistance, and renal vascular resistance were calculated as described previously (15). Urine sodium levels were measured by flame photometer (Model 435; Corning Glass Works, Medfield, MA).

Glomerular Response Studies

Glomeruli were isolated from rat kidneys by mechanical sieving in a glomerular buffer of modified Dulbecco's phosphate-buffered saline containing (in mmol/L) 130 NaCl, 2.5 KCl, 7.7 NaH2PO4, 5.5 glucose, 0.85 CaCl2, 0.5 MgCl2, 4.75 NaHCO3, 0.5 N-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pH 7.4, with NaOH and 0.2 g/dL BSA as previously described (12). Glomeruli were further purified from accompanying tubules by a digestion in collagenase (10 mg/50 mL glomerular buffer, CLS2 298 µg/mg; Worthington Biochemical Corp, Freehold, NJ) and DNase I (1.5 mg/50 mL glomerular buffer, Type IV Bovine Pancreas, D5025; Sigma Chemical Co., St. Louis, MO) followed by rinsing and collection on 75-µm and 45-µm sieves. The purity of the glomerular suspensions was >95%. The concentration of glomeruli in each assay tube was adjusted to 2000 glomeruli. Glomerular incubation with ANP was done as previously described (12,16). In brief, acute glomerular response to ANP was tested after a preincubation period of 5 min at 37°C. Glomeruli were then incubated with ANP (10−8 mol/L; Peninsula Laboratories, Belmont, CA) for intervals of 0 to 5 min at 37°C. Isobutylmethylxanthine (IBMX) (1 mmol/L) was present in these assays. To assay glomerular response to ANP over longer intervals, glomeruli were preincubated at 37°C for 15 min before addition of ANP (10−6 mol/L). After addition of ANP, incubations were continued for up to 4 h at 37°C. IBMX was omitted from the longer incubations because of concerns of maintaining the activity of this reagent over 4 h. Pilot studies showed no significant difference in glomerular response with or without IBMX in the 4-h incubations (data not shown). At the end of each incubation, glomeruli and media were separated by a 15-s centrifugation at 14,000 x g. Intracellular (glomerular) and extracellular (media) cGMP concentration were measured as the indicators of glomerular response to ANP as previously described by RIA (New England Nuclear, Boston, MA) (12).

Phosphodiesterase hydrolysis of cGMP was measured in glomerular homogenates of AC fistula and control animals by the method of Kincaid and Manganiello (17). Organic-acid-transporter activity, a transport system for extruding cGMP from glomerular cells to the extracellular space, was assessed by adding the pharmacologic blocker of the organic-acid transporter probenecid (1 mmol/L) to some incubations of glomerular response to ANP as previously described (12).

Statistical Analysis

Glomerular data are shown as the mean plus or minus standard error of the mean for N = 4 to 5 animals and eight to ten measurements of each time point or condition. Statistical comparisons of glomerular response to ANP between control and AC-fistula animals were made using multiple analysis of variance. Differences in phosphodiesterase activity were compared by t test for independent data. Physiologic and hemodynamic data were shown as the mean plus or minus standard error of the mean for N = 6 control and N = 6 AC-fistula animals. Statistical comparisons of physiologic and hemodynamic data were done using t test for independent data. Statistical significance was defined as P < 0.05.

RESULTS

Physiologic and Hemodynamic Changes in AC-Fistula Animals

Physiologic characteristics of rats with AC fistulas compared with control rats are summarized in Table 1. There was a significant increase in heart weight and plasma ANP concentration in animals with AC fistulas. However, no changes were observed in mean blood pressure, body weight, or heart rate compared with control animals. The urinary sodium excretion rate was not different in AC-fistula rats compared with control rats (2.8 ± 0.1 mEq/24 h, N = 9 versus 3.3 ± 0.05 mEq/24 h, N = 2) respectively. Urinary cGMP concentration was compared in rats before and after placement of AC fistulas (3.8 ± 0.6 versus 2.6 ± 0.2 nmol cGMP/mL urine). There was a trend
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Figure 2. Effect of probenecid on glomerular response to ANP. Glomeruli from control (A) and AC-fistula (B) rats were incubated for 15 min without (solid bar) or with (hatched bar) probenecid (1 mmol/L), followed by a 1-h incubation with ANP (10⁻⁹ mol/L). Results show the mean ± SE of EC cGMP for N = 4 control and N = 4 AC-fistula rats, with eight determinations for each condition. * P < 0.0001 versus no probenecid; # P < 0.0003 versus control.

Figure 3. Phosphodiesterase activity in glomerular homogenate. Glomerular homogenate from control (solid) and AC-fistula (hatched) rats were assayed for hydrolysis of cGMP. Results show the mean ± SE phosphodiesterase activity expressed as nMol hydrolyzed cGMP/mg protein per min for glomerular homogenate of N = 5 control and N = 4 AC-fistula rats. * P < 0.01 versus control.

In humans and animals, there is a spectrum of physiologic and hemodynamic changes that occur with altered cardiac function, ranging from mild heart failure to severe decompensation of the cardiovascular system. It has been reported in both humans and animals that release of ANP and renal responsivity to ANP is also related to the severity of heart failure (18-21). In these studies, animals and humans with mild heart failure appear to have increased natriuresis and diuresis to endogenously elevated ANP (19,21). However, with severe heart failure, there is decreased renal response to endogenously elevated ANP, with less natriuresis, diuresis, and lower urinary or plasma cGMP (18,20,21). Under conditions of severe heart failure, there is also significantly less renal response to systemic infusion of ANP (18). In the study presented here, there is clear cellular evidence of a decrease in glomerular response to ANP, although the AC-fistula animals do not demonstrate uncompensated heart failure. The significant cardiomegaly and the changes in vascular resistance in these animals are consistent with the model of circulatory overload as a result of increased cardiac output.

Although the natriuretic and diuretic renal response to ANP has been demonstrated in humans and animals with heart failure, the glomerular response to ANP in the models of congestive heart failure and in AC-fistula rats has not been fully characterized. Studies of glomerular response to ANP in animal models of altered cardiac function have shown variable changes (8,22). Although it is possible that ANP receptors in the distal nephron may also be affected by changes in cardiac function, studies have not been done to confirm this. Glomerular response to ANP is determined by binding of ANP to a specific ANP receptor (23). The biologic receptor for ANP includes the catalytic subunit for a membrane-bound guanylyl cyclase (23). Thus, when ANP binds to the biologic receptor in the glomerulus, there is a measurable increase in IC and EC levels of cGMP (12,24,25). Evidence to support the role of EC cGMP as a regulator of sodium transport in the nephron comes from physiologic, micropuncture, and electrophysiologic studies (13,26). During ANP infusion, cGMP accumulates in plasma and urine. The increase in urine cGMP directly correlates with natriuresis and diuresis that occur as the result of the ANP infusion (11). Micropuncture studies have shown that in response to stimulation with ANP, the glomerulus generates large amounts of cGMP recovered in the glomerular ultrafiltrate (13). Because cGMP concentration does not increase along the length of the nephron, it is likely that the glomerulus is the major site of production for cGMP present in tubular fluid and in the urine (13). In intramedullary collecting duct vesicles, cGMP added to inside-out vesicles inhibits an amiloride-sensitive cation channel (26). In distal tubules, cGMP added to the luminal bath of the tubules is associated with a direct decrease in chloride ion conductance (27). Recent studies from our laboratory indicate that EC cGMP significantly reduces amiloride-inhibitable short-circuit current (sodium transport) in LLC-PK1 cells, a proximal-tubule cell line (28). Together, these data indicate a role for EC cGMP in the modulation of sodium and chloride transport in the lumen of the nephron.

In the study presented here, we have shown that

In humans and animals, there is a spectrum of physiologic and hemodynamic changes that occur with altered cardiac function, ranging from mild heart failure to severe decompensation of the cardiovascular system. It has been reported in both humans and animals that release of ANP and renal responsivity to
ANP-stimulated production of EC cGMP is significantly lower in glomeruli from AC-fistula rats. This finding corroborates the physiologic observations of others of a decrease in urinary cGMP as well as decreased natriuresis in response to ANP infusion in this model of high-output heart failure (6,7,18). There are models of sodium retention with decreased physiologic responsiveness to ANP that also are reported to have decreased glomerular response to ANP (1,11,29,30). In early postnatal development and in nephrotic syndrome, there is a similar decrease in the glomerular response to ANP. This may represent a general glomerular abnormality seen in states of positive sodium balance. EC cGMP represents an accumulation of cGMP that is continuously being transported out of glomerular cells. The concentration of EC cGMP may differ from that of IC cGMP if the organic-acid transporter for cyclic nucleotides is not working, if there is a change in the degradation of cGMP, if ANP receptor-linked guanylyl cyclase activity is altered, or if there is a decrease in substrate availability (GTP) for the guanylyl cyclase (25). The cellular mechanisms responsible for the decreased glomerular response do appear to differ. In the developing rat, glomeruli were found to have a decrease in organic-acid transporter activity (12). There was also less hydrolysis of cGMP in these glomeruli. In the developing rat kidney, the impaired transport of cGMP from the IC to the EC compartment by the organic-acid transporter may play a role in the blunted response to ANP, particularly if cGMP has a function as an EC second messenger for sodium transport in the nephron (26). In nephrotic rat glomeruli, hydrolysis of cGMP was significantly greater than in control glomeruli (29). The increased hydrolysis of second messenger, cGMP, in this model may contribute to the blunted physiologic response to ANP. In AC-fistula rats, there was no difference in organic-acid transporter activity, compared with control animals. However, there was significantly less hydrolysis of cGMP by glomerular homogenate of AC-fistula animals. This may be a compensatory response to maintain IC cGMP levels in the glomeruli of this model with relative resistance to ANP.

Because the biologic ANP receptor contains a guanylyl cyclase catalytic domain, alteration of biologic ANP receptors could account for the demonstrated difference in glomerular response to ANP (23). In models of heart failure, some studies have reported no change in kidney total ANP receptor binding and dissociation constant, and no change in receptor subtypes (10,22,30). Physiologic studies suggest there is a downregulation of ANP clearance receptors in the lungs and kidneys of animals with heart failure (31,32). Others have shown acutely in AC-fistula rats that there is a decrease in total ANP-binding sites in rats with AC fistulas, which is primarily the result of a decrease in the receptor density of the biologic receptor for ANP (33). Given these conflicting reports for renal ANP receptors in animal models of sodium retention, it is possible that a change in biologic receptor density could contribute to the observation of decreased glomerular response to ANP reported in the study presented here. Furthermore, there is evidence for an increase in degradation of ANP by neutral endopeptidase (32), which may also contribute to the decreased glomerular response to ANP in models of heart failure, including the AC-fistula rat.

It is possible that increased activity of the sympathetic nervous system and the renin-angiotensin system in heart failure could also modulate renal response to ANP. It has been reported that denervation of the kidneys of animals with ischemic heart failure restores renal responsiveness to ANP (34). Similarly, administration of drugs that block the production or action of angiotensin II are associated with decreased water retention in high-output heart failure (35). The interaction of these systems with ANP biologic-receptor activity may relate to decreased guanylyl cyclase activity or increased hydrolysis of cGMP (36).

Phosphodiesterase inhibition is part of the therapeutic management of patients with heart failure. In some cases, use of phosphodiesterase inhibitors has been associated with improvement in cardiac contractility (37). In other cases, there is only marginal improvement with the use of these agents (37). Our data suggest that there is a significant decrease in phosphodiesterase-mediated hydrolysis of cGMP in rat glomeruli. If this is true for other organs, such as the heart, it may help to explain the inconsistent clinical response to phosphodiesterase inhibitors in patients with heart failure.

In summary, we have shown that in rats with an AC shunt, glomerular generation of EC cGMP in response to ANP is significantly decreased compared with that of control-rat glomeruli. This is accompanied by decreased glomerular phosphodiesterase activity, which may serve to compensate for the decreased glomerular response by lessening hydrolysis of the cGMP. The decreased glomerular response to ANP may contribute to the sodium retention seen in models of heart failure, including high-output heart failure, and in other states of positive sodium balance.

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