Distinct roles for renal particulate and soluble guanylyl cyclases in preserving renal function in experimental acute heart failure


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Acute heart failure (AHF) represents an important clinical challenge and poses a major healthcare problem as hospitalization for AHF continues to increase and outcomes remain poor (31, 41). Specially, the worsening of renal function in the setting of human AHF strongly predicts poor outcomes, such as rehospitalization and increased mortality (2, 19, 20, 35, 46). To preserve and/or enhance renal function in AHF, an understanding of intrarenal factors that may protect the kidney in this syndrome may provide a direction on optimal use of current therapies and also lead to newer therapeutic strategies (14).

From an integrated cardiorenal physiological perspective, AHF involves acute cardiac overload with release of the natriuretic peptides (NP) atrial and brain NP (ANP and BNP, respectively), which activate the particulate guanylyl cyclase (pGC) NP receptor-A (NPR-A), resulting in the generation of the second messenger cGMP and the effector PKG. Of note, the NPR-A is also the target of the intrarenal NP urodilatin that, like ANP and BNP, is being developed for the treatment of AHF (33). Infusion of these three NPs in animals and humans results in natriuresis and diuresis and, at certain doses, an increase in glomerular filtration rate (GFR; 9, 10, 23). It should be noted that in severe experimental or human heart failure, a renal hyporesponsiveness may occur to the NP system (NPS) with excessive hypotension linked to worsening renal function (29, 37, 42). The importance of the NPS and the NPR-A in renal regulation is underscored by studies of genetic and pharmacologic receptor disruption characterized by impaired renal sodium handling and often hypertension (5, 12, 24, 34).

A reduction of renal perfusion pressure as could occur with AHF has been reported to activate the intrarenal nitric oxide (NO) pathway in which NO stimulates soluble guanylate cyclase (sGC) localized to the cell cytosol. This is in contrast to pGC, which is membrane bound and activated by the NPS. sGC activation also results in the increase of cGMP and PKG. From a clinical perspective in AHF both sodium nitroprusside and nitroglycerin are widely used for the treatment of AHF. Both are sGC activators and potent vasodilators. Recently, we employed nitroglycerin and the novel direct activator of sGC, BAY 41-2272, in a model of AHF and observed potent renal vasodilation without natriuresis and diuresis, although both produced a significant reduction in arterial pressure together with cardiac unloading (4). Importantly, genetic or pharmacologic disruption of the NO/cGMP system also involves alteration of physiological control of cardiorenal function most often characterized by hypertension secondary to systemic and renal vasoconstriction (36, 38).

Recently, the concept of compartmentalization of cyclic nucleotide signaling has been advanced especially in the heart in which pGC and sGC have been demonstrated to have distinct roles in cardiomyocyte function (8, 18). Furthermore, Airhart et al. (1) have reported that the pGC agonist ANP but not the sGC agonist S-nitroso-N-acetyl-penicillamine stimulates the translocation of PKG to the plasma membrane of renal cells augmenting GC activity of the NPR-A receptor to which ANP, BNP, and urodilatin bind (1). This strongly supports in vitro distinct functional roles for pGC and sGC in the kidney.

Understanding the intrarenal roles of the NP/cGMP and NO/cGMP pathways in vivo in AHF in the control of renal function would advance our knowledge of renal adaptations in this syndrome. Therefore, the objective of our present study was to confirm and extend previous investigations and define the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
in vivo in a large animal model of AHF produced by rapid ventricular pacing, the physiological properties of the endogenous NP/cGMP, and NO/cGMP pathways in the control of renal hemodynamic and excretory function. We hypothesized that each pathway would play specific roles in the maintenance of renal function in AHF consistent with distinct GC enzymes for each system.

METHODS

We conducted experiments in three groups of normal male mongrel dogs weighing 18–22 kg (6 dogs in each group). The study and all of the procedures were in accordance with the Animal Welfare Act and were approved by the Mayo Clinic Institutional Animal Care and Use Committee. All dogs underwent rapid ventricular pacing for 45 min to induce AHF, which, as in humans, is characterized by decreased cardiac output (CO), increases of cardiac filling pressures, plasma ANP, plasma cGMP, and urinary cGMP excretion (21, 25). Group 1 received saline vehicle and served as control; group 2 received HS-142-1, an NP receptor antagonist (0.5 mg/kg; intrarenal artery bolus) (39); and group 3 received L-NMMA (L-NMMA), an inhibitor of NO synthesis (50 μg·kg⁻¹·min⁻¹ intrarenal infusion) (36). The dosage of HS-142-1 and L-NMMA was based on studies that documented the effectiveness of the administration of these agents in attenuating the renal actions of the NP and NO in normal dogs (12, 36). All dogs were fasted the night before the experiment but were allowed free access to water until the time of the studies.

After induction of anesthesia with pentobarbital sodium (30 mg/kg iv, followed by supplemental doses when required to maintain the level of anesthesia), the animals were mechanically ventilated (Harvard Apparatus, Millis, MA) via an endotracheal tube with supplemental oxygen to ensure adequate oxygenation. The left femoral artery and vein were cannulated for arterial monitoring, intra-aortic blood sampling, and intravenous infusion. A balloon-tipped thermocatheter (American Edwards Laboratory, Santa Ana, CA) was placed in the left femoral artery and vein and advanced into the pulmonary artery and then connected to a CO computer (model 9502-A; American Edwards, Irvine, CA). The heart was exposed through a left thoracotomy. After a 1- to 2-cm pericardiotomy, an epicardial pacemaker lead for ventricular pacing was implanted into the right ventricle and connected to a pulse generator (model 4563A; Medtronic, Minneapolis, MN). The pericardium and the chest wall were then carefully closed. The left kidney was exposed and a polyethylene catheter was inserted into the ureter for timed urine collection, and a magnetic flow probe (Carolina Medical Electronics) was placed around the ipsilateral renal artery and connected to a flow meter (model FM501; Carolina Medical Electronics) to measure and record renal blood flow (RBF). A curved 22-gauge needle was inserted into the renal artery and connected via a polyethylene tube to a syringe pump (model 341-A; Sage Instruments). Patency of the intrarenal needle was maintained by saline infusion at a rate of 0.5 ml/min. A priming dose of inulin dissolved in isotonic saline solution was injected, followed by a constant infusion of 1 ml/min to achieve a steady plasma inulin level.

Following 60 min of equilibrium, a 15-min baseline clearance was performed. Hemodynamics were assessed and intra-aortic blood and urine samples were obtained at the midpoint of the clearance. Thereafter, rapid right ventricular (RV) pacing was initiated to acutely increase left ventricular filling pressure (PCWP) and decrease CO and mean arterial pressure (MAP) as previously reported (17, 26). Before pacing, heart rate in all groups was 128 ± 4 beats/min, and with pacing to increase PCWP threefold, it was significantly increased to 206 ± 5 beats/min. After 45 min of RV pacing, a second hemodynamic, blood and urine sample was collected for the baseline AHF clearance. Then, through the intrarenal arterial needle; group 1 received 3 ml of saline slowly infused over 1 min followed by a continuous infusion of 0.5 ml/min saline administered for the remaining two 15-min clearance periods. Group 2 was given HS-142-1 (0.5 mg/kg bolus) as previously reported to block the NPS followed by saline infusion as in group 1. Group 3 was given 3 ml of saline slowly infused over 1 min followed by a 30-min infusion of 50 μg·kg⁻¹·min⁻¹ of L-NMMA (0.5 ml/min). The methods of administration of the two antagonists were based upon previous studies that assessed the intrarenal role of the NP and/or NO systems in the physiological regulation of renal function (36, 40). All dogs were monitored for two more consecutive 15-min clearances in which RV pacing was maintained and hemodynamic data and blood, as well as urine collections, for clearance studies were obtained.

Blood was analyzed for electrolytes, lithium, inulin, and hormones (ANP, cGMP, and plasma renin activity). Blood for hormone analysis was placed in EDTA tubes on ice. After centrifugation at 2,500 rpm at 4°C for 10 min, plasma was separated and stored at −20°C until assay. Plasma levels of ANP, cGMP, and plasma renin activity were determined by radioimmunoassay (7, 29). Plasma and urine concentrations for electrolytes and lithium were determined by flame photometer (model IL943; Instrumentation Lab, Lexington, MA). Plasma and urine concentrations for inulin were measured by anthrone method for calculation of GFR (13). The corresponding hemodynamic and renal parameters were calculated: RVR = (MAP − RAP)/RBF, where RVR is renal vascular resistance and RAP is right atrial pressure; PFNaR% = (inulin clearance – lithium clearance)/inulin clearance × 100, where PFNaR is fractional sodium reabsorption; DFNaR (%) = (lithium clearance − sodium clearance)/lithium clearance × 100, where DFNaR is distal fractional sodium reabsorption; and UcGMP exc = UcGMP × urinary flow, where UcGMP exc is urinary cGMP excretion.

Statistical analysis. All data are expressed in means ± SE. The comparison between each measurement was performed by ANOVA for repeated measurement with Fishers protected least significant differences test. A significant difference was assumed at P < 0.05.

RESULTS

Model of AHF. The cardiorenal and humoral responses to experimental AHF are reported in Table 1. After 45 min of rapid RV pacing, MAP and CO were decreased, while PCWP and right atrial pressure were increased. RBF was decreased, while GFR, urine flow, and sodium excretion were preserved. Fractional sodium excretion decreased in association with an increase in proximal but not distal fractional reabsorption of sodium. Circulating ANP, plasma cGMP, and urinary cGMP excretion were decreased.

cGMP and ANP in AHF during HS-142-1 or L-NMMA administration. Figure 1 illustrates the comparison of absolute hormonal changes in response to saline control, HS-142-1, or L-NMMA in AHF. With regard to plasma cGMP (PcGMP), the second messenger for the NP and NO systems, there was a significant decrease with intrarenal HS-142-1 compared with either saline control or L-NMMA. This was also true for UcGMP excretion in which HS-142-1 decreased UcGMP significantly greater compared with either saline control or L-NMMA. With regard to plasma ANP, there was no significant difference, although there was a strong trend for an increase in ANP with L-NMMA, which may explain the trend for UcGMP to increase, as well, with L-NMMA.

Renal hemodynamic and excretory function in AHF during HS-142-1 or L-NMMA administration. Figure 2 illustrates the comparisons in absolute changes in renal hemodynamic and excretory function in response to saline, HS-142-1, or L-NMMA in AHF. With regard to RBF, a significant...
The goal of the present study was to define in a highly integrative large animal model of AHF the distinctive roles played by the endogenous nitric oxide-cGMP systems. Specifically, we sought to elucidate the roles of the endogenous NPS which functions via pGC and the endogenous NO that targets sGC. We defined the modulation of renal hemodynamic and excretory function in experimental AHF in the presence and absence of intrarenal inhibition of the intrarenal NPS by HS-142-1 to block activation of pGC and of the intrarenal NO system with intrarenal infusion of l-NMMA.

First, we found that renal function was acutely well preserved in this model of AHF. Second, we observed differential and complementary roles for these two endogenous cGMP-activating systems in AHF, whereby the endogenous NPS appears to play a greater role in the preservation of GFR and sodium excretion, while the endogenous NO system was more important in the control of RBF. Thus, the preservation of renal function in experimental AHF is mediated by dual cGMP systems which activate both pGC and sGC enzymes. Rapid ventricular pacing has been employed to produce AHF and chronic congestive heart failure (CHF) in which cardiovascular hemodynamics and renal adaptations closely mimic those occurring in humans with cardiac failure (21, 30).

In the present study, after acute rapid ventricular pacing, CO decreased occurred with l-NMMA, which was greater than the changes observed with saline or HS-142-1. In contrast, GFR decreased more with HS-142-1 compared with control or l-NMMA. Thus, there was a greater decrease in filtration fraction that occurred with HS-142-1 as compared again to control saline or l-NMMA. Furthermore, only with HS-142-1 was there a significant decrease in sodium excretion compared with either saline control or l-NMMA.

**Renal tubular function in AHF during HS-142-1 or l-NMMA administration.** We utilized the lithium clearance technique to localize changes in the tubular handling of sodium at the level of the proximal and distal nephron during NP and NO blockade. Figure 3 illustrates comparison of the absolute changes in PFNaR and DFNaR in the three groups. With regard to PFNaR, there was no significant change in the absolute differences in all three groups. In contrast, in the distal nephron, abundant in NPR-A receptors, DFNaR significantly increased with HS-142-1 compared with saline control or l-NMMA.

**Cardiovascular hemodynamics in AHF during HS-142-1 or l-NMMA administration.** Table 2 reports the cardiovascular hemodynamics in AHF in the three groups before and after infusion of HS-142-1 or l-NMMA. MAP increased compared with baseline AHF in the HS-142-1 and l-NMMA groups. PCWP was increased only in the l-NMMA groups compared with baseline, while CO decreased in the control and l-NMMA groups.

**DISCUSSION**

The goal of the present study was to define in a highly integrative large animal model of AHF the distinctive roles in vivo of two endogenous cGMP activating neurohumoral systems.
significantly decreased and PCWP, ANP, and plasma and urinary excretion of cGMP, all increased. Although significant decreases in RBF and an increase in RVR were observed, GFR and renal sodium excretion were maintained corresponding to significant increases in filtration fraction and proximal fractional reabsorption of sodium. This compensated renal function occurred without significant changes in plasma renin activity.

A physiological role of the acute increase of endogenous ANP in AHF could be demonstrated by a pharmacological means employing a specific NP receptor antagonist, HS-142-1. This fungal-derived specific NP receptor antagonist has been widely used in the investigation of numerous physiological and pathophysiological studies related to the NPS (12, 15). Specifically, we observed that intrarenal HS-142-1 resulted in decreases in RBF and an increase in sodium reabsorption at the distal nephron without significant changes in RBF. HS-142-1 mediated decreases in GFR, indicating that the NPS may be important in maintaining glomerular hydrostatic pressure in AHF by dilating renal afferent arterioles, constricting renal efferent arterioles, or increasing glomerular ultrafiltration coefficient ($K_t$) or all of them. Our studies also suggest that in addition to a preserved GFR, endogenous ANP maintained sodium excretion also in part by reducing sodium reabsorption at the terminal nephron as demonstrated by the lithium clearance technique. The findings from our studies and those of others underscore the role of the NPs in AHF through their highly expressed pGC receptors in glomeruli and inner medullary collecting duct cells in the renal adaptation and maintenance of GFR and renal sodium excretion (6, 43).

Interestingly, there was an increase in MAP in the groups treated with either HS-142-1 or L-NMMA, which may reflect systemic overflow of these two inhibitors. In the L-NMMA group, there was a trend of ANP to increase. We believe that systemic spillover of L-NMMA may have increased arterial blood pressure and left atrial pressure, thus increasing plasma ANP, although insignificantly. The rise in urinary cGMP, we believe, reflects a more sensitive renal response to the mild increase in plasma ANP with stimulation of NPRA receptors in the kidney. We further advance the concept that a soluble cGMP activator would be associated with a decline in plasma ANP due to cardiac unloading, which we have observed recently employing the novel sGC activator, BAY 58-2667 (3). It should be noted that a decline in ANP would be viewed as an unfavorable occurrence, as it would withdraw pGC activation. This might argue for coadministration of an sGC activator with an NP, which would be underscored further by the now known compartmentalization of cGMP within cells (18).

In patients with CHF, plasma nitrate, which is a stable end product of NO production, has been reported to be increased, although others have argued that in chronic CHF, NO may be deficient (45). The inhibition of NO by L-NMMA in the present study resulted in a further and significant decrease in RBF.
without significant decreases in GFR or renal sodium excretion. In physiological conditions, as well as in hypertensive patients, it has been shown that L-NMMA induces decreases in RBF without alterations in GFR or sodium excretion (22). Other groups observed similar results in normal conscious and anesthetized animals in RBF and GFR, however, Elsner et al. (16) and Majid et al. (27) reported decreases in sodium excretion after NO inhibition. Taken together, the maintenance of GFR and renal sodium excretion after NO inhibition in the present studies suggests that the role of the NO system may be more important in the maintenance of RBF.

The present study may have therapeutic implications worthy of further investigation. We demonstrated that endogenous ANP, which was activated shortly within 45 min after rapid ventricular pacing, may be important in the control of GFR and renal sodium excretion in experimental AHF. It is relevant to state that recent studies of chronic overexpression of BNP in a murine model of diabetic glomerulopathy resulted in a preservation ofglomerular structure and function (28). As a therapeutic strategy for AHF, ANP administration or agents that potentiate the effect of ANP may have renal therapeutic potential in AHF. Most recently, Cataliotti et al. (9) reported that coinfusion of BNP plus furosemide maximizes natriuresis and diuresis while preserving renal function in a canine model of chronic heart failure. Further, infusion of BNP without a bolus to minimize hypotension in patients undergoing cardiac surgery who have CHF resulted in improved GFR compared with standard therapy (32).

While the present studies advance a role for the endogenous NPS in experimental AHF, a renal hyperresponsiveness to the NP, such as BNP has been reported. Wang et al. (44) reported a blunted renal natriuretic and GFR response to acute BNP administration in patients hospitalized with heart failure and others (37) have reported worsening renal function. We speculate that the renal response to the NPs in CHF may be, in part, determined by the rate of degradation of cGMP by phosphodiesterase V (PDE V) within the kidney. Indeed, in a recent experimental study, acute BNP together with chronic use of the PDE V inhibitor sildenafil markedly potentiated GFR and the sodium excretion response in experimental CHF (11).

As discussed above, both nitroglycerin and sodium nitroprusside, which activate sGC, are used as potent vasodilators to unload the heart in AHF. One might conclude that such agents are renal vasodilators but would not necessarily enhance GFR or sodium excretion. Indeed, that is the case in experimental CHF using nitroglycerin or a direct sGC activator (4). Therefore, it is tempting to speculate that an optimal renal therapeutic strategy in AHF would be the use of nonhypotensive doses of a pGC agonist, such as ANP or BNP, together with a direct sGC activator, such as BAY 58-2667 which is effective even in the presence of oxidized sGC (3). Such a strategy would then target the glomeruli, the renal vasculature and sodium reabsorption. Potentiation of this dual cGMP system with PDE V inhibition would also maximize cGMP signaling and warrants further investigation.

While not studied in the present investigation, it is possible that blockade of the NPs could further activate endothelin (ET-1) which is inhibited by the NPs. Such an increase in ET-1 could conceivably activate ET-B receptors in the kidney and possibly explain lack of significant renal vasoconstriction. On the other hand, blockade of the NO system could theoretically attenuate the vasodilating actions of ET-B via NO, thus contributing to the greater renal vasoconstrictor response. Coblockade with L-NMMA and HS-142-1 would have given us another perspective, but due to the dependency of the kidney in AHF upon the cGMP system, we did not perform such experiments, since coblockade most likely would have resulted in a marked deterioration of renal function.

In summary, the present study demonstrates the differential roles of the NP and NO systems in the control of renal function in AHF. These two cGMP activating systems functioning through two distinct GC enzymes play a key role in maintaining renal function in experimental AHF that mimics human AHF. Specifically, the NPS plays the dominant role in maintaining sodium excretion and GFR, while the function of renal NO is the maintenance of RBF. These studies have both physiological and therapeutic implications warranting further research into cardiorenal interactions in this syndrome of AHF.

REFERENCES


