Acute Phosphodiesterase 5 Inhibition Mimics Hemodynamic Effects of B-Type Natriuretic Peptide and Potentiates B-Type Natriuretic Peptide Effects in Failing But Not Normal Canine Heart

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Objectives
The aim of this work was to test whether acute phosphodiesterase 5 (PDE5) inhibition via sildenafil (SIL) mimics and/or potentiates cardiorenal effects of exogenous natriuretic peptide (NP) infusion.

Background
Heart failure (HF) is often accompanied by elevated NP secretion yet blunted responsiveness. Such NP resistance may, in part, relate to increased cyclic guanosine monophosphate (cGMP) catabolism by PDE5.

Methods
Dogs (n = 7) were studied before and after tachypacing-induced HF. Animals received 30-min infusion of B-type natriuretic peptide (BNP) (2 μg/kg bolus, 0.02 μg/kg/min), and on a separate day SIL (1 mg/kg, intravenous), followed by BNP (SIL + BNP). Phosphodiesterase 5 activity was measured in lung, vasculature, and kidney.

Results
At baseline (non-failing), BNP lowered central venous, pulmonary capillary wedge, diastolic, mean pulmonary artery, and mean arterial pressure. Sildenafil had no effects, and SIL + BNP was similar to BNP alone. In contrast, SIL lowered these pressures similarly to BNP in dogs with HF, and SIL + BNP was additive in further reducing pulmonary pressures over BNP alone. Plasma cGMP/plasma BNP ratio was markedly reduced with HF, indicating NP resistance. Sildenafil plus BNP increased this ratio in HF, but had no effect in non-failing animals. Sildenafil had no independent diuretic/natriuretic effects nor did it enhance BNP effects under baseline or HF conditions. In HF, PDE5 activity was significantly increased in the systemic and pulmonary vasculature and in the kidney.

Conclusions
The PDE5 activity in systemic and pulmonary vasculature increases in HF rendering hemodynamic responses to PDE5 inhibition identical to those from BNP infusion. Natriuretic peptide desensitization in HF relates, in part, to increased PDE5 activity, supporting a therapeutic role for PDE5 inhibition. (J Am Coll Cardiol 2007;49:1079–88) © 2007 by the American College of Cardiology Foundation

Heart failure (HF) is characterized by progressive cardiac dysfunction often accompanied by pulmonary and systemic vasoconstriction and renal salt and water retention. Activation of the endogenous natriuretic peptide (NP) system, and subsequent release of atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) is thought to play an important counter-balancing role against the cardiorenal sequelae of HF (1). However, the net response to endogenous NP is insufficient, and even pharmacologic levels achieved by exogenous infusion frequently fail to elicit adequate hemodynamic and/or renal effects, highlighting profound NP resistance (2–4).

Natriuretic peptides bind to the natriuretic peptide receptor type-A (NPR-A) coupled to particulate guanylate cyclase, eliciting their biologic action by increasing cyclic 3', 5'-guanosine monophosphate (cGMP) synthesis. Cyclic guanosine monophosphate is degraded by phosphodiesterases such as PDE5 (5,6). In HF, the ratios of plasma cGMP/plasma ANP, and urine cGMP/plasma ANP fall markedly, suggesting impaired cGMP generation despite high NP stimulation, consistent with NP resistance (7–9). Up-regulation of PDE5 has been shown to contribute to NP resistance in the
kidney in animal models of HF and other salt retaining states (10–12). Vascular PDE5 activity is increased in experimental models of pulmonary hypertension. Since pulmonary hypertension is often manifest in HF, a similar increase in PDE5 could occur and contribute to NP resistance in HF (13,14). If so, this would suggest that PDE5 inhibitors such as sildenafil (SIL) might provide equal, if not greater, hemodynamic benefits as NP agents.

Accordingly, the present study tested the hypothesis that PDE5 activity is augmented in both vasculature and kidney in experimental dilated HF, and that PDE5 inhibition mimics hemodynamic and renal effects of exogenous NP administration in this condition. We further determined whether PDE5 inhibition enhances cardiovascular-renal actions of exogenous NP.

Methods

Surgical preparation. The protocol was approved by the Johns Hopkins University Animal Care and Use Committee. Adult mongrel dogs (25 to 30 kg) were chronically instrumented for pressure-volume and hemodynamic monitoring in the conscious state, as described (15). This instrumentation included a micromanometer (P22; Königsburg Instruments, Pasadena, California) placed in the left ventricular (LV) apex to measure pressure, aortic and right atrial catheters for pressure measurements and blood sampling, and endocardial sonomicrometers (Sonometrics Corp., Ontario, Canada) placed to measure anteroposterior, septal-lateral, and apex-base dimensions from which LV volume was derived based on a prolate ellipsoid (16). Right ventricular apical epicardial pacing electrodes were placed to provide tachypacing. The dogs were provided 10 to 14 days to fully recover from surgery before study.

Experimental protocol. Animals were sedated with propofol (0.5 to 2 mg/kg bolus, 0.2 to 0.5 mg/kg every 5 to 10 min), and a balloon-tipped pulmonary artery catheter was inserted through a 7-F right external jugular venous sheath to measure pulmonary artery pressure and pulmonary capillary wedge pressure. A Foley catheter was inserted for urine collection. Animals recovered fully from sedation (~60 min), and were then allowed to stand freely in a minimally restricting sling for another 30 to 60 min before data collection.

Protocol I involved measurements of hemodynamics, blood, and urine collection (at 30-min intervals) at baseline and after infusion of canine BNP-32 (Phoenix Pharmaceuticals, Belmont, California; 2 μg/kg bolus, 0.02 μg/kg/min × 30 min into the right atrium). Protocol II utilized the same measurements, with animals first receiving a selective PDE5 inhibitor alone—SIL (SIL; 1 mg/kg × 30 min), followed by the same BNP-32 infusion as Protocol I. At least 48 to 72 h was provided between Protocol I and II, with right heart and urinary catheters removed at the end of each. After animals fully recovered, they underwent tachypacing at 210 beats/min × 4 weeks to induce dilated HF (15), after which the same 2 infusion protocols were repeated.

Data analysis. Hemodynamic recordings were digitized at 250 Hz. Steady state parameters were assessed at end expiration from an average of 10 to 15 sequential beats. Sonomicrometer-derived LV volumes were used to derive stroke volume (SV), cardiac output (SV × heart rate), and left ventricular ejection fraction (LVEF). In addition, systemic and pulmonary vascular resistance were calculated in the standard manner, and LV contractile function was measured based on pre-load-normalized maximal rate of pressure rise: (dP/dtmax/end-diastolic volume).

Blood/urine sample analysis. Blood was collected in tubes containing EDTA (BNP and cGMP) or heparin (SIL), and immediately placed on ice. Samples were centrifuged at 2K rpm for 30 min, plasma separated and stored at −20°C (cGMP and SIL assays) or −80°C (BNP assay). Plasma BNP was determined by immunoassay Bachem/Peninsula Laboratories, San Carlos, California) and reported as pg/ml. Plasma and urine cGMP were determined by immunoassay (Amersham Biosciences, Piscataway, New Jersey), and reported as pmol/ml. Plasma SIL was measured by HPLC (SFBC Analytical Laboratories, North Wales, Pennsylvania) reported as ng/ml.

Urine was collected in 50 ml polypropylene tubes and immediately placed on ice. Samples were centrifuged at 2K rpm for 30 min, urine separated from sediment and stored at −20°C until analyzed. Urine sodium concentration was measured by standard methods, and sodium excretion calculated as the product of urine flow rate and sodium concentration. Urine cGMP excretion was the product of cGMP concentration and urine flow rate. The ratio of plasma cGMP to plasma BNP (pcGMP/BNP), and urine cGMP to plasma BNP (ucGMP/BNP) were used as in vivo markers of NP signaling sensitivity.

Tissue PDE5 activity assays. The PDE5 activity was determined by fluorescence polarization assay (Molecular Devices, Sunnydale, California). Inferior vena cava, whole lung, pulmonary artery and vein, aorta, and whole kidney were obtained from 10 separate dogs: 5 normal dogs, and 5 dogs paced into HF using the identical pacing protocol as highlighted in the preceding text. None of these animals were subjected to either infusion protocol. Tissue homogenates were prepared from −80°C frozen tissue, with kidney further separated into renal glomeruli and inner medullary collecting ducts as described (17,18). Total cGMP esterase activity was measured, and the amount of total activity
inhibited by addition of either 0.5 μM SIL or 0.1 μM tadalafil used to determine activity attributable to PDE5.

Statistical analysis. Data are presented as mean ± SEM. Differences within and between groups were assessed by 2-way analysis of variance with repeated measures. The model included the physiologic parameter as the dependent variable, drug treatment (BNP alone, SIL alone, or SIL + BNP), the dog group (control animals vs. HF), and a dummy variable for each animal. An interaction term between dog group and drug treatment served to test for differential responsiveness due to the presence or absence of HF. A p value <0.05 was considered statistically significant. Differences between non-normally distributed data (cGMP and BNP) were determined using the Kruskal-Wallis test. Analysis was performed using Systat 10.2 (Systat Software, Inc., San Jose, California).

Results

Hemodynamic response to BNP, SIL, or both in control and HF dogs. Representative pressure-volume loop data at steady-state before and after infusion of BNP, SIL, and their combination in both a normal and HF animal are displayed in Figure 1. In healthy control animals, BNP shifted the loop downward to the left, reflecting principally venodilation (decline in pre-load) with no change in systemic resistance. Sildenafil alone had minimal effects, while combined SIL + BNP induced changes similar to BNP alone. In contrast with control animals, animals with HF showed very similar cardiac responses to BNP and SIL, each resulting in chamber unloading: primarily arterial dilation, along with reductions in LV end-diastolic pressure. The combination of both agents appeared to have a modest additive effect.

Summary results for both infusion protocols are provided in Table 1 and Figure 2. At baseline, HF animals had markedly reduced contractility (dP/dtmax/end-diastolic volume), LVEF, SV, and increased chamber volumes and LV end-diastolic pressure (all p < 0.01). There were no differences between baseline hemodynamic values in Protocols I and II; BNP (Protocol I) reduced end-systolic volume in both normal and HF animals, and decreased pre-load (end-diastolic volume and LV end-diastolic pressure). Stroke volume and LVEF rose in failure animals but not control animals. Contractility was unaltered in either group. Sildenafil alone (Protocol II) had minimal effects on cardiovascular hemodynamics in normal dogs. The only changes were a small decline in systemic vascular resistance and LV
Table 1

Hemodynamic Data at Baseline, and After BNP, SIL, or SIL + BNP in Dogs Before (Normal) and After Tachypacing-Induced HF

<table>
<thead>
<tr>
<th>Protocol I</th>
<th>Protocol II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>dP/dt/EDV (mm Hg/s/ml)</strong></td>
<td><strong>dP/dt/EDV (mm Hg/s/ml)</strong></td>
</tr>
<tr>
<td>Normal</td>
<td>30.3 ± 3.6</td>
</tr>
<tr>
<td>HF</td>
<td>12.3 ± 2.1*</td>
</tr>
<tr>
<td><strong>LVEF</strong></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0.37 ± 0.01</td>
</tr>
<tr>
<td>HF</td>
<td>0.20 ± 0.02*</td>
</tr>
<tr>
<td><strong>EDV (ml)</strong></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>91.0 ± 8.6</td>
</tr>
<tr>
<td>HF</td>
<td>133.5 ± 12.6</td>
</tr>
<tr>
<td><strong>ESV (ml)</strong></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>57.6 ± 5.7</td>
</tr>
<tr>
<td>HF</td>
<td>108.0 ± 11.7*</td>
</tr>
<tr>
<td><strong>HR (beats/min)</strong></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>100 ± 7</td>
</tr>
<tr>
<td>HF</td>
<td>127 ± 4*</td>
</tr>
<tr>
<td><strong>SV (ml)</strong></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>33.5 ± 3.2</td>
</tr>
<tr>
<td>HF</td>
<td>25.5 ± 1.9§</td>
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<tr>
<td><strong>CO (l/min)</strong></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>HF</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td><strong>LVSP (mm Hg)</strong></td>
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<tr>
<td>Normal</td>
<td>133.6 ± 4.4</td>
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<tr>
<td>HF</td>
<td>108.4 ± 3.8*</td>
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<tr>
<td><strong>PVR (dyne-sec-cm-5)</strong></td>
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<tr>
<td>Normal</td>
<td>221 ± 23</td>
</tr>
<tr>
<td>HF</td>
<td>142 ± 29</td>
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<tr>
<td><strong>SVR (dyne-sec-cm-5)</strong></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>2,703 ± 248</td>
</tr>
<tr>
<td>HF</td>
<td>2,286 ± 264</td>
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<tr>
<td><strong>PCWP (mm Hg)</strong></td>
<td></td>
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<tr>
<td>Normal</td>
<td>5.6 ± 0.9</td>
</tr>
<tr>
<td>HF</td>
<td>18.8 ± 1.9*</td>
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<tr>
<td><strong>LVEDP (mm Hg)</strong></td>
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</tr>
<tr>
<td>Normal</td>
<td>6.5 ± 0.9</td>
</tr>
<tr>
<td>HF</td>
<td>28.0 ± 2.6*</td>
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</table>

- Hemodynamic data in normal and failing (HF) dogs at baseline and after acute administration of B-type natriuretic peptide (BNP), sildenafil (SIL), or the combination of SIL + BNP.
- *p < 0.05 and †p < 0.05 comparing baseline values of normal dogs to baseline values of HF dogs; ‡p < 0.01, §p < 0.001, and ¶p < 0.005 comparing treatment values to baseline values in normal and HF animals; †p < 0.05 for the interaction between drug treatment and HF.
- CO = cardiac output; EDV = end-diastolic volume; ESV = end-systolic volume; HR = heart rate; LVEDP = left ventricular end-diastolic pressure; LVEF = left ventricular ejection fraction; LVSP = left ventricular systolic pressure; PCWP = pulmonary capillary wedge pressure; PVR = pulmonary vascular resistance; SV = stroke volume; SVR = systemic vascular resistance.

end-diastolic pressure. However, in HF animals, the hemodynamic response to BNP and SIL was very similar. Figure 2 displays this comparison for central venous pressure, diastolic pulmonary arterial pressure (dPAP), mean pulmonary arterial pressure (mPAP), and mean arterial pressure. In control animals, all pressures declined with BNP but were unaltered by SIL. However, in failing dogs, pressure declines in response to BNP alone and SIL alone were nearly identical. Moreover, in HF animals, the combination of SIL + BNP reduced dPAP and mPAP (Fig. 2), as well as systolic pulmonary (p = 0.02) and aortic pressures (p = 0.03) to a greater degree than BNP alone, with a trend toward further reductions in mean arterial pressure (p = 0.08), and LV end-diastolic pressure (p = 0.08). The reductions in dPAP and mPAP, as well as heart rate, in HF animals treated with SIL were greater than those reported in normal dogs, indicating a differential hemodynamic responsiveness of the failing dog to acute PDE5 inhibition. B-type natriuretic peptide, however, did not demonstrate this differential effect, as it exerted similar hemodynamic actions in normal and failing dogs. Reductions in dPAP and mPAP in response to SIL + BNP were also greater in HF animals than in normal control animals (p < 0.003 and p < 0.06, respectively).

Pulmonary vascular resistance declined with SIL in HF animals, whereas BNP had no significant effect (Fig. 3).
Pulmonary vascular resistance declined similarly with SIL + BNP as with SIL alone, supporting the primary influence of SIL. Neither agent significantly changed pulmonary vascular resistance in non-failing controls.

**Vascular and renal PDE5 activity.** Figure 4A shows summary results for PDE5 activity from various vascular tissues in both control and HF animals. Enzyme activity rose significantly in whole lung, pulmonary artery, aorta, and inferior vena cava from HF animals versus control animals. This change is consistent with the amplified vasodilatory effects of PDE5 inhibition in these vascular beds in HF animals. Renal PDE5 activity (Fig. 4B) also increased as measured in whole kidney and inner medullary collecting ducts. The PDE5 activity in isolated glomeruli was not significantly altered, although total cGMP esterase activity was increased 31% (p < 0.03, not shown).

**Plasma BNP, cGMP, and pcGMP/BNP ratios.** Figures 5A to 5C display results for plasma BNP and cGMP levels and their ratio in both normal and HF dogs. In normal dogs, BNP infusion raised plasma BNP nearly 4-fold over baseline, and increased plasma cGMP by +36.2 ± 12.7 pmol/ml (p < 0.05). Sildenafil plus BNP did not alter the pcGMP/BNP over baseline or BNP alone, indicating proportional increases in plasma cGMP in relation to BNP in the non-failing circulation. However, in HF, basal plasma BNP was markedly elevated whereas plasma cGMP was only modestly increased.
over non-failure controls. As a result, the pcGMP/BNP ratio declined substantially (p < 0.0001), consistent with NP de-
sensitization. B-type natriuretic peptide infusion alone did not
further elevate plasma BNP or cGMP. However, plasma
cGMP rose substantially with SIL alone, and more so with
SIL + BNP; both responses were significantly above BNP
alone (Fig 5B). The paired disparity between cGMP change
with SIL versus BNP was far greater in HF than non-failing
animals (p = 0.025). Combined SIL + BNP raised plasma
cGMP 6- to 7-fold despite a trend toward declining BNP
levels by the end of the infusion (p = 0.15), increasing the
pcGMP/BNP ratio.

Influence of BNP, SIL, and both combined on renal
function. B-type natriuretic peptide increased urine flow
rate (0.75 ± 0.18 ml/min to 2.2 ± 0.52 ml/min; p < 0.05)
and sodium excretion (104.2 ± 39.3 μEq/min to 245.6 ±
57.3 μEq/min; p < 0.05) in normal dogs (Fig. 6). In
contrast, SIL had neither diuretic nor natriuretic effects,
while SIL + BNP induced changes similar to BNP alone.
In HF dogs, the renal response to BNP was blunted
compared with controls, with both urine flow and sodium
excretion change reaching borderline significance (p =
0.09). Sildenafil did not alter either parameter, and
combined SIL + BNP resulted in similar responses to
BNP alone. Renal PDE5 activity was increased in whole
kidney and inner medullary collecting ducts, but did not
reach significance in isolated glomeruli (Fig. 4, lower
panels).

Basal urine cGMP concentration and urine cGMP pro-
duction (data not shown) were not elevated in HF, and did
not change with BNP, although they trended to rise with
SIL + BNP (p = 0.06). The ucGMP/BNP ratio was
reduced in HF animals at baseline, was not significantly
altered by BNP alone, but did rise modestly with SIL +
BNP (p < 0.01 vs. baseline).

Discussion

This study demonstrates that acute administration of the
selective PDE5 inhibitor SIL is as effective in unloading the
failing heart as a moderate dose of exogenous BNP, whereas
in contrast with BNP, SIL has little hemodynamic impact on
normal animals. Moreover, SIL enhanced the hemodynamic
effects of BNP, particularly within the pulmonary circulation of
failing animals. Importantly, PDE5 activity significantly increased in the vasculature of HF relative to non-failing animals, suggesting a mechanism underlying enhanced hemodynamic responsiveness to SIL in HF. Renal PDE5 activity also increased with HF; however, SIL alone produced no diuretic or natriuretic effects, and the renal effects of SIL plus BNP were not additive to BNP alone. The data further support a role of PDE5 up-regulation as a contributor to NP desensitization, and suggest potential utility of combined treatment to maximize the hemodynamic benefits of endogenous or exogenous BNP.

In light of the vasodilatory and natriuretic actions of ANP and BNP, it was initially hypothesized that HF represented a state of NP deficiency. However, investigators observed that patients with elevated cardiac filling pressures and clinical HF had markedly elevated ANP and BNP levels, instead implying that HF is a state of NP resistance (19,20). This was supported by depressed pcGMP/BNP ratios (7,8), particularly as plasma and urine cGMP levels relate primarily to NP-derived, particulate guanylate cyclase-generated cGMP, rather than nitric oxide-derived, soluble guanylate cyclase-generated cGMP (2,21). Others found marked blunting of ANP- and BNP-mediated forearm vasodilation and impaired vascular cGMP production in HF patients (2,3), and marked increases in the molar ratio of ANP extraction to cGMP production in pulmonary and systemic vasculature of HF patients versus control patients (8,22). Diuretic and natriuretic responses to endogenous and exogenous NP and renal cGMP generation are also blunted in experimental and clinical HF (4,9,23).

Natriuretic peptide desensitization and decreased cGMP generation in the vasculature and kidney in HF can have several causes including NPR-A receptor down-regulation, up-regulation of NP clearance receptors, neurohormonal counterregulation, and increased cGMP catabolism— principally by up-regulation of PDE5 (24). The activity of PDE5 has been found to increase in isolated glomeruli from failing canines, inner medullary collecting ducts of pregnant rats, and rats with a bile duct ligation model of cirrhosis, suggesting a role for PDE5 up-regulation in renal NP resistance for HF and other salt-retaining states (11,12,17). In addition, pulmonary vascular PDE5 activity increases in experimental pulmonary hypertension (13,14). Alternatively, a recent study showed that despite markedly increased plasma BNP levels determined by immuno-fluorescence assay in patients with HF, mass spectrometry did not reveal any BNP-32, the biologically active form of BNP (25). This raises the question as to whether altered forms of BNP may contribute to NP resistance in clinical HF. It remains unclear whether this paradigm applies to canine HF. However, our finding that SIL induced cardiac unloading along with substantial increases in plasma cGMP suggests that circulating BNP in our failing dogs was biologically active.

We observed increased pulmonary and systemic vascular PDE5 activity in experimental HF, compared with control animals, associated with biventricular unloading and marked
elevation of plasma cGMP with selective PDE5 inhibition in this setting. The PDE5 up-regulation was not isolated to the pulmonary vascular bed, suggesting that the stimulus, such as elevated circulating NP or counterregulatory neurohormones, was diffuse. Despite this, BNP and SIL exerted similar hemodynamic actions in HF animals, with the exception of pulmonary vascular resistance, that only declined with SIL. This indicates that NP resistance was more marked in the pulmonary vasculature, requiring PDE5 inhibition to yield vasodilator effects from BNP. This was further supported by the finding that SIL + BNP produced additive effects in the pulmonary circulation. Importantly, these changes did not occur with untoward reductions in systemic arterial pressure. Thus, the hemodynamic actions of acute PDE5 inhibition in the current study, either alone or combined with BNP, principally targeted sites with abundant PDE5 activity, such as the lung (26).

The physiologic significance of an interaction between PDE5 inhibition and circulating BNP was revealed by differential hemodynamic effects of SIL in HF versus normal dogs. In line with these observations, the relative increase in plasma cGMP in response to SIL was far greater in failing than non-failing animals, indicating that PDE5 inhibition enhanced endogenous NP signaling preferentially in HF. Moreover, the hemodynamic responses to exogenous BNP were enhanced through acute PDE5 inhibition, with a marked rise in the pcGMP/BNP ratio when SIL and BNP were combined. In contrast, SIL + BNP did not produce additive hemodynamic effects, elevate plasma cGMP levels, or alter the pcGMP/BNP ratio in non-failing animals despite increased circulating BNP levels after BNP infusion. This is consistent with the relatively low levels of vascular PDE5 activity in normal control animals. These results imply that a significant degree of the differential vascular responsiveness between HF and control animals occurs via post-NPR-A receptor modulation of NP signaling (i.e., at the level of PDE5).

Circulating BNP levels significantly rose with exogenous BNP in normal but not in failing dogs. This contrasts to prior animal and human studies have reported increased circulating BNP after its infusion in HF (27–30). One potential explanation is that the infused BNP lowered cardiac load reducing endogenous release and offsetting the change due to the infusion itself. Heart failure animals had very high basal BNP, so withdrawal could potentially have such an effect. This was supported by the SIL + BNP data, where unloading in HF dogs was even greater, and net BNP changes trended to a decline. In a prior canine study, basal ventricular dilatation and BNP were far below that in the current experiments, and less decline in endogenous release might explain a greater rise with exogenous infusion (27).

Another potential explanation for this difference lies in the half-life of BNP, which is only ~1.5 min in the dog (31). We administered BNP as a bolus followed by intravenous infusion; thus, 80% of the dose was delivered in the first minute of the 30-min period. Yet, biological effects of
BNP are thought to outlast its plasma half-life due to a longer $t_{1/2}$ of cGMP (~30 min in the dog) (31,32). The $t_{1/2}$ for BNP is 22 min in humans, which could also explain some differences to clinical studies. A phase-lag between BNP and cGMP levels could have artificially increased the pcGMP/BNP ratio with SIL + BNP in HF dogs. However, it could not explain baseline changes, the relative impairment of endogenous NP signaling in HF, nor marked disparity in cGMP response to BNP versus BNP + SIL. Though BNP was not assessed when SIL was administered alone, the latter also raised cGMP substantially, while likely lowering BNP due to hemodynamic unloading (in HF), so the pcGMP/BNP ratio would have increased.

Potentiating effects between SIL and BNP are concordant with recent data obtained in rats with hypoxic pulmonary hypertension, which found that SIL pre-treatment augmented pulmonary vasodilation and increased plasma cGMP levels with ANP infusion over ANP alone (33). Moreover, NPR-A receptor deficiency impairs pulmonary vasodilating effects of SIL or SIL + ANP in murine models of hypoxic pulmonary hypertension (34,35). We also found that SIL influenced hemodynamics only in the setting of high intrinsic NP, suggesting such activity may be important to enhancing the hemodynamic efficacy of PDE5 inhibitors.

Similar to the pcGMP/BNP ratio, the ucGMP/BNP ratio also declined in HF versus normal dogs, supporting impairment of in vivo renal NP signaling. While SIL + BNP also elevated this ratio, it was due less to increased urinary cGMP than to reduced plasma BNP. Thus, PDE5 sensitization in the kidney was not as robust as in the vasculature in this model, a conclusion further supported by the lack of significant diuretic-natriuretic effects with SIL in the HF animals. This result is somewhat different to prior studies employing zaprinast to block PDE5, which induced a rapid natriuresis in a rat arterio-venous fistula model of HF, or natriuresis and greater urinary cGMP production when infused directly in the renal artery in canine HF (9,23). Differences between these prior data and the current study may relate to the model and/or mode of drug administration (intravenous, which also lowered blood pressure, versus intrarenal), and less specificity of zaprinast for PDE5 (17,36). Neurohormonal counterregulation may also play a role, as acute angiotensin-converting enzyme inhibition and angiotensin II blockade both enhance renal responsiveness to ANP (37,38).

The plasma SIL level achieved in our protocol was 413 ng/ml, comparable to that measured in humans after a single 50-mg dose (39). This raises an intriguing possibility that acute PDE5 inhibition might provide an alternative approach to treat acutely decompensated HF, potentially avoiding the expense and inconvenience of intravenous NP. The inhibition of PDE5 alone or combined with BNP may be particularly efficacious in patients with the combination of advanced HF and pulmonary hypertension, and in particular those with reduced pcGMP/NP ratios, as this would suggest PDE5 up-regulation with greater NP desensitization (26). Chronic background PDE5 inhibition may also enhance endogenous NP responsiveness, but this remains to be demonstrated. Lastly, in pulmonary arterial hypertension, where NP levels are often normal or only modestly elevated, administration of BNP has recently shown to amplify the pulmonary vasodilatory effects of SIL therapy, supporting our findings (40-42).

The present study has several limitations. A relatively small sample size may have led to underestimation of more subtle hemodynamic effects of BNP, SIL, or their combination. Moreover, a greater sample size might have revealed effects on urine flow rate and sodium excretion, although the data did not even suggest a trend in this regard, whereas the hemodynamic effects were quite significant. We used a single dose of both SIL and BNP, and it remains possible that varying dosage combinations might better optimize (or worsen) their pharmacologic interaction. This may again be relevant to the renal data, where perhaps lower doses of SIL and/or BNP without any blood pressure changes may have had more effect on diuresis and natriuresis. In addition, we did not perform the experimental protocols in random order, which could potentially introduce bias. However, the 2 baselines were nearly identical suggesting such bias was unlikely. Lastly, we examined acute effects of SIL, more by way of a comparison with current clinical uses of BNP. Future studies are needed to determine if chronic PDE5 inhibition has additional benefit by reducing NP resistance and restoring intrinsic NP responsiveness.

Since its clinical introduction in 2001, administration of BNP has become integrated into the treatment of acutely decompensated HF (43). While originally tested for its capacity to benefit hemodynamics, current use is often aimed at assisting in diuresis of diuretic refractory patients. However, the treatment is costly, requires intravenous infusion, and recent meta-analysis has questioned the overall safety/efficacy profile and, in particular, potential involvement of BNP in renal complications (44,45). Our finding that an oral alternative such as SIL yields identical or better hemodynamic effects compared with clinically relevant BNP dosing suggests an intriguing alternative approach. Use of the pcGMP/BNP ratio may help identify individuals with a high degree of NP desensitization most likely to benefit from this approach or from combined PDE5 inhibition and low-dose BNP. Human studies are needed, but the current data help pave the way for such investigations.

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