Renal and Anti-Aldosterone Actions of Vasopressin-2 Receptor Antagonism and B-Type Natriuretic Peptide in Experimental Heart Failure

Lisa C. Costello-Boerrigter, MD, PhD; Guido Boerrigter, MD; Alessandro Cataliotti, MD, PhD; Gail J. Harty; John C. Burnett, Jr, MD

Background—Hemodynamic and neurohumoral function can affect the efficacy of diuretic therapy in congestive heart failure. Arginine vasopressin increases water reabsorption through the V2 receptor in the collecting duct, whereas B-type natriuretic peptide (BNP) decreases sodium reabsorption in the collecting duct. We hypothesized that combining BNP to the V2-receptor antagonist tolvaptan (TLV) would enhance renal excretory function by augmenting sodium excretion together with aquaresis without adversely affecting renal hemodynamics in experimental congestive heart failure.

Methods and Results—Congestive heart failure was induced in 3 groups (n = 6 per group) of dogs by tachypacing. A acute experiment was done after 10 days. After baseline measurements, study groups received a 0.1 mg/kg IV bolus of TLV alone (TLV), TLV in combination with BNP (TLV+BNP; 50 ng/kg/min), or BNP alone (BNP). Mean arterial pressure increased with TLV, remained unchanged with TLV+BNP, and decreased with BNP (+5±1 mm Hg versus −1±1 mm Hg versus −15±1 mm Hg; P <0.05). Renal blood flow and glomerular filtration rate were preserved with all regimens. Urine flow increased in all 3 groups but significantly more so with TLV+BNP (TLV: +0.4±0.1 mL/min versus TLV+BNP: +2.4±0.5 mL/min versus BNP: +0.8±0.3 mL/min; P <0.05). Only TLV+BNP and BNP were natriuretic (P <0.05), whereas only TLV and TLV+BNP increased electrolyte-free water excretion (P <0.05). Compared with TLV alone, TLV+BNP prevented an increase in aldosterone (P <0.05).

Conclusions—Coadministration of TLV and BNP in experimental HF resulted in a beneficial profile of renal, neurohumoral, and hemodynamic actions, specifically potent diuresis with natriuresis, neutral effect on mean arterial pressure, and lack of aldosterone activation. (Circ Heart Fail. 2010;3:412-419.)

Key Words: arginine vasopressin • heart failure • kidney • natriuretic peptides • pharmacology • V2 receptor antagonist • experimental model

Arginine vasopressin (AVP, also called antidiuretic hormone) is a 9-amino acid peptide secreted from the posterior pituitary in response to high plasma osmolality and hypotension. Its major actions are to reduce free water excretion and maintain blood pressure. The latter is mediated by V1A receptors in the vasculature, whereas the former is mediated by V2 receptors in the renal inner medullary collecting duct. Binding to the V2 receptor, adenylyl cyclase generates the second messenger cAMP, which promotes translocation of the water channel aquaporin 2 into the luminal membrane and thus increases water permeability and water reabsorption. In congestive heart failure (CHF), AVP secretion is increased, which can lead to hyponatremia and congestion.1 Both combined V1A/V2 as well as selective V2 receptor antagonists have been developed.

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Tolvaptan (TLV) is a V2-receptor antagonist and has recently been approved by the Food and Drug Administration for the treatment of euclidean and hypervolemic hyponatremia. Indeed, the large multicenter Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study with Tolvaptan (EVEREST) reported that chronic TLV was safe and induced an aquaresis as demonstrated by a reduction in body weight during the initial hospitalization for acute decompensated HF. Associated with this was an improvement in dyspnea, although TLV neither improved nor worsened the primary outcome variable, which was mortality.2,3 Although V2 receptor antagonism can aid in the management of congestion through aquaresis, it lacks natriuretic actions, and its cardiovascular actions remain incompletely defined.

The 32-amino-acid cardiac hormone B-type natriuretic peptide (BNP), like AVP, targets the renal inner medullary collecting duct, but rather than increasing water reabsorption it reduces sodium reabsorption contributing to natriuresis, especially if administered at nonhypotensive doses. Studies have established that mature BNP is a 32-amino-acid peptide...
and BNP, and BNP (TLV + BNP), and the third group received BNP alone (BNP). TLV was administered as an intravenous bolus (0.1 mg/kg), a gift from Otsuka America Pharmaceutical, Rockville, Md. Canine BNP was administered as an intravenous infusion (50 ng/kg · min); Phoenix Pharmaceuticals, Inc, Burlingame, Calif). Fifteen minutes after TLV administration or start of BNP infusion, a second 30-minute clearance was started.

**Assays**

Electrolytes were measured by flame photometry (IL943, Instrumentation Laboratory, Lexington, Mass). Plasma renin activity, aldosterone, atrial natriuretic peptide, and BNP were measured by radioimmunoassay as previously described.10 Glomerular filtration rate (GFR) was calculated by inulin clearance. Plasma and urine inulin were measured by the anthrone method.14 Osmolality was measured by using a vapor pressure osmometer (VAPRO 5520, Wescor, Inc, Logan, Utah). Electrolyte-free water clearance (E-C-H2O) was calculated as:

\[
E-C-H_2O = \text{urine flow} - \left[\text{urine flow} \times \frac{([Na]_U + [K]_U)}{[Na]_P}\right]
\]


**Statistical Analysis**

Data are provided as mean (SD) if normally distributed or as median (25th/75th percentile in tables, interquartile range in graphs) if not normally distributed. Parameters at baseline and with drug administration were compared within groups with paired t test or, for not normally distributed data, with Wilcoxon signed-rank test. Groups were compared by analyzing changes from baseline to drug administration clearance with 1-way ANOVA and post hoc Bonferroni test or, for not normally distributed data, with Kruskal-Wallis test and post hoc Dunn test. Statistical significance was noted at \(P<0.05\).

**Results**

Cardiorenal parameters at baseline (Table 1) were consistent with a CHF phenotype with increased systemic and renal vascular resistances, decreased CO, increased cardiac filling pressures, decreased urinary sodium excretion, and neurohumoral activation.11-13 There were some differences in baseline parameters among groups, mostly between the TLV and BNP groups. Changes from baseline within groups are shown in Table 2 and Figures 1 through 3.

**Cardiovascular Function**

Mean arterial pressure (MAP) increased with TLV, remained unchanged with TLV + BNP, and decreased with BNP, and this was significant among groups (Figure 1A). The same was true for renal perfusion pressure, which was calculated as difference between MAP and right atrial pressure. SVR, likewise, increased with TLV, remained unchanged with TLV + BNP, and decreased with BNP, but this was significant only between TLV and BNP (Figure 1B). CO and renal blood flow were unchanged in all groups. Right atrial pressure and renal vascular resistance decreased only with BNP, but this was not significant compared with the other groups. In contrast, pulmonary artery pressure and pulmonary capillary wedge pressure (PCWP) decreased with BNP, and this was significant compared with TLV or TLV + BNP.

**Renal Function**

Urine flow significantly increased in all 3 groups but significantly more so with TLV + BNP (Figure 2A). In contrast, urinary sodium excretion (Figure 2B) and potassium excre-
tion were increased with BNP alone and TLV+BNP, but not with TLV alone. Urine osmolality decreased with all 3 regimens. Electrolyte-free water clearance increased in both TLV and TLV+BNP but significantly more so with TLV+BNP (Figure 3C), and this was highly significant compared with TLV and BNP alone. GFR was unchanged with TLV, tended to increase with BNP (P=0.06), and significantly increased with TLV+BNP with no difference between groups (Figure 2D). Consistent with the administration of the GC-A agonist BNP, urinary excretion of the second messenger cGMP increased with BNP alone and with TLV+BNP, and this was significant compared with TLV alone.

**Humoral Function**

There were no significant differences among groups with regard to changes in plasma sodium, potassium, and hematocrit. Plasma osmolality decreased with BNP compared with TLV and TLV+BNP. As expected, with the infusion of exogenous BNP, BNP levels increased in the TLV alone and TLV+BNP groups but were unchanged in the TLV group (Figure 3A). Plasma cGMP decreased with TLV and increased with BNP alone and with TLV+BNP (Figure 3B). Plasma renin activity was unchanged with TLV but decreased with TLV+BNP and with BNP, and this was significant between TLV and BNP (Figure 3C). Angiotensin II levels followed a similar pattern (levels tended to decrease with TLV+BNP; P=0.06), but this was not significant among groups. Aldosterone significantly increased from baseline in both the TLV alone and BNP alone groups but was unchanged in TLV+BNP, although this group had the largest diuresis (Figure 3D). A qualitative summary of the major changes in the 3 experimental groups is shown in Table 3.

**Discussion**

We report for the first time the cardiorenal actions of coadministration of the V2-antagonist TLV and the GC-A agonist BNP in experimental HF. TLV alone as expected was...
Coadministration of Tolvaptan (TLV) and BNP significantly reduced the mean arterial pressure (MAP) and systemic vascular resistance (SVR) compared to TLV alone or BNP alone. This effect was also consistent with changes in opposing directions produced by TLV and BNP, respectively. In the model of experimental heart failure, activated aldosterone increased potassium excretion and produced a natriuresis. Importantly, electrolyte-free water clearance was significantly increased with TLV alone, which can in part be due to increased delivery of sodium and water to the collecting duct through reductions in proximal sodium and water reabsorption previously reported with both atrial natriuretic peptide and BNP.

**Table 2. Changes in Cardiorenal and Neurohumoral Function From Baseline in the 3 Experimental Groups With TLV Alone, Tolvaptan and BNP, or BNP Alone**

<table>
<thead>
<tr>
<th>Hemodynamic function</th>
<th>Δ With TLV</th>
<th>Δ With TLV + BNP</th>
<th>Δ With BNP</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac output, L/min</td>
<td>−0.05 (0.07)</td>
<td>−0.08 (0.23)</td>
<td>+0.51 (1.13)</td>
<td>0.26</td>
</tr>
<tr>
<td>Right atrial pressure, mm Hg</td>
<td>−0.3 (0.8)</td>
<td>−0.07 (0.09)</td>
<td>−1.3 (0.6)*</td>
<td>0.15</td>
</tr>
<tr>
<td>Pulmonary capillary wedge pressure, mm Hg</td>
<td>−0.9 (2.0)</td>
<td>−1.2 (0.5)*</td>
<td>−4.0 (1.0)*</td>
<td>0.0029*</td>
</tr>
<tr>
<td>Renal perfusion pressure, mm Hg</td>
<td>+6 (3)</td>
<td>−1 (2)</td>
<td>−14 (3)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Renal blood flow, mL/min</td>
<td>+3 (9)</td>
<td>+14 (30)</td>
<td>+24 (34)</td>
<td>0.40</td>
</tr>
<tr>
<td>Renal vascular resistance, mm Hg · L−1 · min−1</td>
<td>+69 (210)</td>
<td>+43 (583)</td>
<td>−124 (85)*</td>
<td>0.56</td>
</tr>
</tbody>
</table>

**Renal function**

- Urinary potassium excretion, μEq/min: +5 (1/7) | +15 (8/21)* | +24 (15/39)* | 0.0027 |
- Filtration fraction: −0.04 (0.29) | +0.08 (0.32) | −0.03 (0.14) | 0.67 |
- Urinary cGMP excretion, pmol/min: +348 (−507/1120) | +5356 (2320/8142)* | +6369 (4196/7682)* | 0.0022 |
- Urine osmolality, mOsm/L: −708 (657)* | −1013 (468)* | −310 (92)* | 0.13 |

**Humoral function**

| Hematocrit, % | +1 (3) | 0 (2) | 0 (1) | 0.83 |
| Plasma Na+, mmol/L | +5 (8) | −1 (7) | −3 (6) | 0.12 |
| Plasma K+, mmol/L | −0.1 (0.2) | −0.1 (0.3) | −0.3 (0.2)* | 0.26 |
| Plasma osmolality, mOsm/L | +3 (10) | +2 (8) | −19 (17) | 0.015 |
| Angiotensin II, pg/mL | +5 (−20/18) | −15 (−79/−5) | −29 (−39/−21)* | 0.39 |

Values are mean (SD) for normally distributed data or median (25th/75th percentile) for not normally distributed data. P < 0.05 in post-hoc tests is indicated as follows: *TLV vs TLV + BNP, †TLV vs BNP, ‡TLV + BNP vs BNP. Δ, change from baseline level.

*P < 0.05 vs respective baseline level.

†P value for comparison of changes from baseline induced by TLV vs TLV + BNP vs BNP.

With regard to renal function, both TLV alone and BNP alone significantly increased urine flow, but TLV + BNP increased urine flow substantially more so. In contrast, TLV did not increase urinary sodium excretion, whereas BNP alone and TLV + BNP did. These findings are in keeping with TLV acting as a pure aquaretic through V2-receptor antagonism, whereas the natriuresis with TLV + BNP can be explained by the known natriuretic actions of BNP. Interestingly, electrolyte-free water clearance was significantly higher with TLV + BNP compared with TLV alone or BNP alone, which can in part be due to increased delivery of sodium and water to the collecting duct through reductions in proximal sodium and water reabsorption previously reported with both atrial natriuretic peptide and BNP. Also, although GFR remained unchanged with TLV alone, it increased with

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**Figure 1. Change in MAP (A) and SVR (B) with TLV, TLV + BNP, and BNP. P value in box is from ANOVA for comparison of groups. †P < 0.05 between groups. #P < 0.05 versus respective baseline. Data are mean (SD).**
TLV-BNP and tended to increase with BNP; however, this was not significant among groups. The GFR-enhancing action of BNP could be due to an increase in the coefficient of ultrafiltration or a differential effect on the afferent and efferent glomerular arteriole so as to increase glomerular hydrostatic pressure. Also, in cultured inner medullary collecting duct cells, atrial natriuretic peptide, which like BNP activates GC-A, has been reported to promote retrieval of AQP2 from the cell membrane to the cytosol, which may be able to enhance the aquaretic actions of TLV.

Diuretic therapy is frequently associated with the activation of sodium retaining neurohumoral systems, which can counteract the diuretic and natriuretic actions and reduce GFR. Diuretics may potently activate the RAAS with deleterious actions. In this study, TLV alone did not change plasma renin activity or angiotensin II but significantly increased aldosterone. The mechanism of the increase may be further stimulation of the unblocked V1A receptor in the adrenals. Indeed, it has been reported in mice that AVP induces aldosterone release from adrenal gland cells through the V1A receptor and that AVP activates the RAAS through the V1A receptor in macula densa cells. In contrast, addition of BNP to TLV significantly reduced plasma renin activity and prevented the increase in aldosterone. Again, these differential changes occurred despite the fact that TLV+BNP induced a greater diuresis and natriuresis. These findings are in keeping with the direct suppressing actions of GC-A activation on renin and aldosterone secretion. However, in this study, BNP alone also caused an increase in plasma aldosterone, presumably secondary to hypotension because when the hypotension was negated by combining TLV with BNP, this increase in aldosterone was not seen even though the diuretic response was greater. These results emphasize that aldosterone secretion is determined by a complex interplay of factors that include V1A receptor activation, GC-A activation, and blood pressure.

With respect to hemodynamic actions, TLV increased MAP and SVR, although CO remained unchanged. As previously noted, TLV acts by blocking V2 receptors in the collecting duct cells, but it leaves V1A receptors in the vasculature readily accessible to endogenous AVP. Therefore, the increase in MAP and SVR may be explained by increased binding to the V1A receptor by AVP blocked from the V2 receptor. To our knowledge, this is the first invasive hemodynamic study of acute V2-receptor antagonism with TLV in a model of CHF that documents an acute vasoconstriction and an increase in blood pressure. Addition of BNP with its vasodilating actions prevented this increase in MAP and SVR, thus resulting in a neutral effect. We also observed that cardiac filling pressures did not acutely decrease with TLV despite the aquaretic actions of TLV. It is possible that the increase in SVR and afterload offset an unloading action of TLV. Also, a more prolonged period of observation and diuresis may have demonstrated a decrease in PCWP. Indeed, Udelson et al reported that in the ECLIPSE [Effect of Tolvaptan on Hemodynamic Parameters in Subjects with Heart Failure] trial TLV dose-dependently increased urine output and also decreased PCWP compared with placebo. No significant differences were reported for the secondary end points of systolic blood pressure, systemic or pulmonary vascular resistance, cardiac index, or heart rate. Of note, most of these patients were on concomitant medication, and it is unclear to
what extent the volume loss could have offset a V$_{1A}$-mediated increase in blood pressure. Nonetheless, in our study, renal hemodynamics were preserved despite the increase in SVR as GFR, renal blood flow, and renal vascular resistance remained unchanged. Addition of BNP to TLV also did not decrease right atrial pressure, whereas PCWP decreased with TLV+BNP, but this was not significant compared with TLV. Similar to TLV alone, TLV+BNP did not change renal blood flow and renal vascular resistance. BNP alone reduced MAP compared with TLV and TLV+BNP. The same was true for PCWP, which was probably due to the larger decrease in afterload rather than a diuretic effect, which was higher in TLV+BNP. Importantly, corresponding to the reduction in MAP, BNP alone decreased renal perfusion pressure, which may offset some of the direct renal enhancing actions of BNP.

A major therapeutic aim in patients with CHF is to induce renal excretion of water and sodium to reduce congestion without impairing renal function. Conventional diuretics such as loop diuretics and thiazides act primarily as saluretics by blocking sodium channels in the luminal membrane of tubular cells, thus increasing intraluminal electrolyte concentration and ultimately, for osmotic reasons, water excretion is then increased. Disadvantages are that increased sodium in the tubule can reduce GFR through tubuloglomerular feedback, promote hypertrophy of tubular cells in more distant nephron segments, and increase the potential for electrolyte imbalance such as hyponatremia if the electrolyte loss is high relative to the water excretion. Indeed, renal dysfunction in terms of decreased GFR as well as hyponatremia is an important complication in the treatment of CHF and is associated with increased morbidity and mortality. Therefore, the development of strategies to enhance water and sodium excretion without inducing renal dysfunction and electrolyte imbalance is a high priority.

TLV represents the first selective and orally available V$_{2}$-receptor antagonist, and as mentioned earlier, it has been evaluated recently in patients hospitalized with acute decompensated CHF in the EVEREST trial. A recombinant form of human BNP, nesiritide, was approved for the treatment of acute decompensated HF in the United States in 2001 and is significant for...

Table 3. Qualitative Summary of Major Changes in the 3 Experimental Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TLV</th>
<th>TLV+BNP</th>
<th>BNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemodynamic function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>↑</td>
<td>↔</td>
<td>↓</td>
</tr>
<tr>
<td>Systemic vascular resistance</td>
<td>↑</td>
<td>↔</td>
<td>↓</td>
</tr>
<tr>
<td>Renal perfusion pressure</td>
<td>↑</td>
<td>↔</td>
<td>↓</td>
</tr>
<tr>
<td>Renal function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine flow</td>
<td>↑</td>
<td>↑↑</td>
<td>↑</td>
</tr>
<tr>
<td>Urinary sodium excretion</td>
<td>↔</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Electrolyte-free water excretion</td>
<td>↑ ↑</td>
<td>↔</td>
<td></td>
</tr>
<tr>
<td>Humoral function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma renin activity</td>
<td>↔</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>↑</td>
<td>↔</td>
<td>↑</td>
</tr>
</tbody>
</table>

Changes are within-group changes. Differences between groups were mostly but not always significant (see Tables 1 and 2 and Figures for details). ↑ indicates increase; ↓, decrease; ↔, no change; ↑↑, increase greater than ↑.
also being evaluated for other indications. There is still controversy regarding the value of BNP, particularly its renal actions, which may in part be secondary to a dosing issue, because some studies used regimens with doses high enough to induce significant hypotension.\textsuperscript{4,7,32,33} In contrast, BNP in lower doses was associated with improved renal function.\textsuperscript{7,32} Although a small number of patients in the EVEREST trial received both TLV and BNP, to the best of our knowledge, our study is the first to formally assess coadministration of TLV and BNP, which again cotarget the inner medullary collecting duct cells in the control of sodium and water homeostasis. Coadministration of TLV and BNP may be a beneficial strategy to mobilize congestion in patients with CHF. The tendency of TLV to increase MAP and SVR may prevent some of the hypotension reported with BNP.

There are several limitations to this study. The neurohumoral and renal alterations observed in this pacing model may not perfectly correspond to the alterations in CHF, for example, longer duration, atherosclerotic disease, and preexisting renal disease. The baseline differences, primarily between the TLV alone and BNP alone groups, could have affected the responses to the drugs. Also, we investigated only the short-term effects of the drugs.

In summary, addition of BNP augmented the diuretic actions of TLV and attenuated some of the adverse hemodynamic and neurohumoral effects seen with either TLV or BNP alone. Coadministration of TLV and BNP may represent a beneficial strategy to induce enhanced water and sodium excretion while maintaining renal perfusion pressure in CHF and suppressing the RAAS, thus representing a more physiological therapy for sodium and water retention in CHF. Further studies are required to assess whether these short-term findings in experimental CHF translate into improved outcomes in human CHF patients.

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Disclosures
None.

References


CLINICAL PERSPECTIVE

Hemodynamic and neurohumoral function can affect the efficacy of diuretic therapy in congestive heart failure, and conversely, it may be adversely affected by diuretic use. Arginine vasopressin increases water reabsorption through the V2 receptor in the collecting duct and increases blood pressure through the V1A receptor. B-type natriuretic peptide (BNP) decreases sodium reabsorption in the collecting duct and acts as a vasodilator. We hypothesized that combining BNP to the V2-receptor antagonist tolvaptan (TLV) would enhance renal excretory function by augmenting sodium excretion together with aquaresis without adversely affecting renal hemodynamics in experimental congestive heart failure. Congestive heart failure was induced in 3 groups of dogs by tachypacing for 10 days. In a short-term experiment, groups that were studied received TLV alone, TLV plus BNP, or BNP alone. Mean arterial pressure increased with TLV, remained unchanged with TLV + BNP, and decreased with BNP. Renal blood flow and glomerular filtration rate were preserved with all regimens. Urine flow increased in all 3 groups but significantly more so with TLV + BNP. Only TLV + BNP and BNP were natriuretic, whereas only TLV and TLV + BNP increased electrolyte-free water excretion. Compared to TLV alone, TLV + BNP prevented an increase in aldosterone. We conclude that coadministration of TLV and BNP in an animal pacing model of HF resulted in a beneficial profile of renal, neurohumoral, and hemodynamic actions, specifically potent diuresis with natriuresis, neutral effect on mean arterial pressure with preservation of renal perfusion pressure, and lack of aldosterone activation. These findings provide a rationale for future studies in human HF.
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