An Enhanced Effect of Arginine Vasopressin in Bradykinin B$_2$ Receptor Null Mutant Mice

Marcos E. Alfie, Shainda Alim, Dharmesh Mehta, Edward G. Shesely, Oscar A. Carretero

Abstract—Under water restriction, arginine vasopressin (AVP) is released and promotes water reabsorption in the distal nephron, mainly through AVP V$_2$-receptors. It has been proposed that renal kinins counteract the hydro-osmotic effect of AVP. We hypothesized that kinins acting through B$_2$ receptors antagonize the urinary concentrating effect of AVP. To test this, bradykinin B$_2$ receptor knockout mice (B$_2$-KO) and 129/SvEv mice (controls) were placed in metabolic cages and urine collected for 24 hours (water ad libitum). After that, urine was again collected from the same mice during 24 hours of water restriction. Urinary volume (UV), urinary osmolarity (UOsm), and urinary Na$^+$ (U$_{Na}$V) and K$^+$ (U$_{K}$V) excretion were determined. On water restriction, UV in controls decreased by $\approx 25\%$, whereas in B$_2$-KO mice there was almost a 60% drop in urinary output ($P=0.001$ versus controls). In the controls, water restriction increased UOsm by 347 mOsm/kg H$_2$O, $\approx 14\%$ above baseline (NS), whereas in knockout mice the increase was 3 times that seen in the controls: $>1000$ mOsm/kg H$_2$O ($P=0.001$ versus controls). Compared with normohydration, U$_{Na}$V and U$_{K}$V in the water-restricted state increased more in controls than in B$_2$-KO mice. This difference in electrolyte excretion could be explained by greater dehydration in the controls (dehydration natriuresis). In a second protocol, we tried to mimic the effect of endogenous AVP by exogenous administration of an AVP V$_2$-receptor agonist, desmopressin (DDAVP). To suppress endogenous AVP levels before DDAVP administration, mice were volume-overloaded with dextrose and alcohol. UOsm was 685$\pm$125 and 561$\pm$58 mOsm/kg H$_2$O in water-loaded controls and B$_2$-KO mice, respectively. After DDAVP was injected subcutaneously at a dose of 1 $\mu$g/kg, UOsm increased to 1175$\pm$86 mOsm/kg H$_2$O ($\Delta+490$ mOsm) in the controls and 2347$\pm$518 mOsm/kg H$_2$O ($\Delta+1786$ mOsm) in B$_2$-KO mice ($P<0.05$ versus controls). We concluded that water restriction or exogenous administration of an AVP V$_2$-receptor agonist has a greater urinary concentrating effect in B$_2$-KO mice than in controls, suggesting that endogenous kinins acting through B$_2$ receptors oppose the antidiuretic effect of AVP in vivo. (Hypertension. 1999;33:1436-1440.)

Key Words: mice, knockout $\blacktriangleright$ bradykinin $\blacktriangleleft$ argipressin $\blacktriangleright$ desmopressin $\blacktriangleleft$ urine

Kinin$\mathrm{s}$ are released from a protein precursor, kininogen, by plasma and tissue enzymes collectively termed kinin$\mathrm{ases}$; the best known are plasma and tissue kallikrein. Kinins act as local hormones by activating the release of endothelium-derived relaxing factor and prostaglandins. They act mainly through 2 different types of receptors, B$_1$ and B$_2$. Most of the known effects of kinins (vasodilatation, bronchoconstriction, diuresis, and natriuresis) are mediated by B$_2$ receptors, which belong to a family of peptide hormone receptors linked to G proteins. In the kidney, kallikrein and kininogen are found in the distal nephron; the interaction between renal kallikrein and kininogen results in kinin formation late in the collecting tubules.

Arginine vasopressin (AVP) is a nonapeptide synthesized in the paraventricular and supraoptic nuclei of the hypothalamus. From there, it is transported along the axons to the posterior pituitary gland, where it is stored until it is released into the peripheral circulation in response to an appropriate stimulus, such as increased plasma osmolarity, reduced cardiac output, or increased diastolic pulmonary blood volume, or decreased arterial blood pressure. AVP interacts with at least 2 types of receptors, V$_1$ and V$_2$. V$_1$ receptors activate phospholipase C which in turn increases cytosolic free Ca$^{2+}$, thereby mediating contraction of vascular smooth muscle. V$_2$ receptors activate adenylyl cyclase, increasing intracellular cAMP levels. The most important response to V$_2$ receptor-adenyl cyclase stimulation is increased water permeability of the luminal membrane of the cortical and medullary collecting tubules, exeriting a powerful antidiuretic effect. This makes AVP the major determinant of the rate of renal water excretion. 

A relationship between the renal kallikrein-kinin system and AVP was inferred from the observation that excretion of renal kinins is increased by infusion of AVP in humans, dogs, and rats. The nature of this relationship was suggested by reports that kinins promote free water excretion in dogs receiving AVP and decrease AVP-stimulated water reabsorption in the amphibian urinary bladder, as well as in the rabbit cortical collecting duct perfused in vitro. Moreover, a
kallikrein inhibitor, aprotinin, augmented the renal response to AVP in Brattleboro rats.\textsuperscript{9} All of these studies suggested that kinins may attenuate AVP and induce diuresis.

Using homologous recombination, Borkowski et al\textsuperscript{15} recently developed bradykinin B\textsubscript{2} receptor null mutant (knockout) mice (B\textsubscript{2}-KO) in which the gene encoding for the bradykinin B\textsubscript{2} receptor protein was disrupted. We have shown that these mutant mice are completely nonresponsive to the acute vasodepressor effect of bradykinin, whereas the response to another endothelium-dependent vasodilator, acetylcholine, remains intact.\textsuperscript{16} This animal model provides the opportunity to investigate the interaction between the renal kallikrein-kinin system and AVP and to determine whether bradykinin B\textsubscript{2} receptors play a role in such an interaction, thus avoiding confounding pharmacological approaches. Accordingly, we hypothesized that kinins acting through B\textsubscript{2} receptors antagonize the urinary concentrating effect of AVP, and therefore increases in endogenous AVP or exogenous AVP administration would have a greater urinary concentrating effect in B\textsubscript{2}-KO mice than in controls.

## Methods

### Animals

Homozygous B\textsubscript{2}-KO mice (\textasciitilde \textasciitilde) were used in all experiments. These are a mixture of 2 different 129 substrains, with 129/SvEv being chosen as the wild-type controls because differences in simple sequence length polymorphisms were kept to a minimum and the strain is available commercially. Therefore, 129/SvEvTac purchased from Taconic Labs served as controls. All procedures were performed in accordance with institutional guidelines.

### Urine Collection

Metabolic cages with an inner diameter of 5 inches especially designed for mice were used to collect urine; food and water containers were placed outside the cage to avoid urine contamination. To decrease stress related to the new environment, mice were housed for at least 24 hours before starting the collection.

### Urinary Parameters

Urinary volume (UV) was determined gravimetrically and expressed as \( \mu \text{L/min} \). Urinary sodium and potassium concentrations were measured with a NOVA-1 ion electrolyte autoanalyzer (Nova Biochemical), and urinary sodium and potassium excretion \((U_{NaV}, U_{K V})\) were calculated and expressed as \( \text{mmol/min} \). Urinary osmolality (UOsm) was determined with a freezing-point osmometer (Advanced Instruments) and expressed as \( \text{mOsm/kg H}_2\text{O} \).

### Drugs

The AVP \( \text{V}_2 \) receptor agonist desmopressin (DDAVP) ([d-deamino
cis\textsuperscript{1}, D-Arg\textsuperscript{8}]-vasopressin, Sigma) was used in protocol 2. 100 \( \mu \text{L} \) saline or a single dose of 1 \( \mu \text{g/kg} \) DDAVP in saline was given subcutaneously (SC) to all mice.

Because DDAVP has very little effect on urine concentration in mice under normal conditions (possibly due to high levels of endogenous AVP), in protocol 2 we tried to suppress endogenous AVP levels. For this purpose, 1\% alcohol was added to the drinking water (because alcohol is known to inhibit AVP secretion), and, in addition, the mice were volume-loaded by 3 SC injections of 1.5 mL 1\% ethyl alcohol in 5\% dextrose 8 hours apart.

### Experimental Protocols

#### Protocol 1: Effect of 24-Hour Water Deprivation in Controls and B\textsubscript{2}-KO Mice

Controls (129/SvEv) and B\textsubscript{2}-KO mice \((n=6\) for each group) were placed in metabolic cages and were given food and water ad libitum.

Mice were kept in the cages for 72 hours. The first 48 hours were for adaptation; urine was collected during the last 24 hours (normohydrated state). UV, UOsm, \( U_{NaV} \), and \( U_{K V} \) were determined as indicated. Next, the same mice were subjected to 24 hours of water deprivation while urine was collected (water-restricted state). UV, UOsm, \( U_{NaV} \), and \( U_{K V} \) were again determined.

#### Protocol 2: Effect of DDAVP in Water-Loaded Controls and B\textsubscript{2}-KO Mice

Controls (129/SvEv) and B\textsubscript{2}-KO mice \((n=6\) for each group) were placed in metabolic cages and were given food and 1\% alcohol in 4\% dextrose for drinking water ad libitum to suppress endogenous levels of AVP. For the same purpose, mice were volume-loaded by giving them a 24-hour water restriction. 1.5 mL SC injection of 1\% alcohol in 5\% dextrose every 8 hours 3 times for a total volume of 4.5 mL. Along with the last injection, mice received 100 \( \mu \text{L} \) of saline (vehicle) SC, after which urine was collected for 15 hours. UV, UOsm, \( U_{NaV} \) and \( U_{K V} \) were determined as indicated. Approximately 24 hours later, the same mice were subjected to water loading, but this time, along with the last injection of dextrose and alcohol, the mice were injected with 1 \( \mu \text{g/kg} \) DDAVP SC instead of saline. Urine was collected for 15 hours, UV, UOsm, \( U_{NaV} \), and \( U_{K V} \) were again determined.

### Statistical Analysis

Values are expressed as mean±SEM. To evaluate the data from both protocols, we used ANOVA for repeated measures. The design had a single between factor: mouse type (controls and B\textsubscript{2}-KO) and a single repeated factor: experimental intervention (free access to water or 24-hour water restriction for protocol 1 and injection of vehicle or DDAVP for protocol 2). The analysis tests these 2 main effects and also examines the 2-way interaction. We consider an interaction significant if \( P \) is <0.05. All variables (UV, UOsm, \( U_{NaV} \), and \( U_{K V} \)) were subjected to the same analysis.

### Results

#### Protocol 1: Effect of 24-Hour Water Deprivation in Controls and B\textsubscript{2}-KO Mice

During water restriction, UV in the controls decreased from \( 0.88±0.052 \) to \( 0.66±0.025 \) \( \text{µL/min} \) (\( \Delta = -0.22 \) \( \text{µL/min}; P<0.01 \)), whereas in B\textsubscript{2}-KO mice it decreased from 1.08±0.169 to 0.45±0.072 \( \text{µL/min} \) (\( \Delta = -0.63 \) \( \text{µL/min}; P<0.001 \)) (Figure 1). Two-way ANOVA showed that the \( \Delta \) for the decrease in UV was significantly greater in B\textsubscript{2}-KO mice versus controls: \( P=0.001 \). Conversely, 24-hour water restriction increased urinary osmolality in the controls from 2386±169 to 2733±106 mOsm/kg H\textsubscript{2}O (\( \Delta = 347 \) mOsm; \( P=\text{NS} \)), whereas in B\textsubscript{2}-KO mice it increased from 2430±225 to 3467±211 mOsm/kg H\textsubscript{2}O (\( \Delta = 1037 \) mOsm; \( P<0.01 \)). ANOVA showed that the \( \Delta \) for the increase in UOsm was significantly greater in B\textsubscript{2}-KO mice versus controls: \( P=0.001 \).

In controls and B\textsubscript{2}-KO mice that received water ad libitum, \( U_{NaV} \) was similar between groups: 112±9.6 and 132.4±6.6 nmmol/min, respectively. However, when animals were subjected to 24-hour water deprivation, \( U_{NaV} \) increased to 200.7±28.4 nmmol/min in controls (\( \Delta = 88 \) nmmol/min; \( P<0.01 \)) versus only 156.6±24.2 nmmol/min in B\textsubscript{2}-KO mice (\( \Delta = 24 \) nmmol/min; \( P=\text{NS} \)) (Table 1). Two-way ANOVA for \( U_{NaV} \) revealed that the \( \Delta \) increase was borderline statistically greater in controls than in B\textsubscript{2}-KO mice: \( P=0.087 \). Finally, during water ad libitum, UV\textsubscript{3} was 213.1±9.1 and 278.3±12.4 nmmol/min for controls and B\textsubscript{2}-KO mice, respectively. During 24-hour water restriction, UV\textsubscript{3} increased by 93 nmmol/min to 305±30.9 nmmol/min in controls (\( P<0.01 \)) and decreased by 62 nmmol/min to 216.6±26.1 nmmol/min in B\textsubscript{2}-KO mice.
When mice were given vehicle, $U_{\text{NaV}}$ was 161.2±33.4 and 87.6±22.9 nmol/min in controls and B$_2$-KO mice, respectively (P=0.09) (Table 2). When animals received DDAVP, $U_{\text{NaV}}$ was similar to vehicle in both groups: 149±64.9 nmol/min in controls and 68.8±17.7 nmol/min in B$_2$-KO mice. $U_{\text{NaV}}$ was statistically different in vehicle-injected controls and B$_2$-KO mice: 184.3±31.5 versus 85.1±17.3 nmol/min, respectively (P<0.05), and it was not statistically different from vehicle after DDAVP injection in either group: 112.1±50.18 and 63.1±13.77 nmol/min for controls and B$_2$-KO mice, respectively.

Discussion

In this study we tested the hypothesis that kinins acting through the B$_2$ receptor antagonize the effect of AVP in vivo. For this purpose, we determined the effect of 24-hour water restriction and the effect of DDAVP administration on urinary concentrating capacity of controls and B$_2$-KO mice. The similarities in UV and UOsm between controls and B$_2$-KO mice given water ad libitum suggest either that kinins play little role in antagonizing AVP actions under normal

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**Table 1.** $U_{\text{NaV}}$ and $U_{\text{KV}}$ in Controls and B$_2$-KO During Normohydration and 24-Hour Water Restriction

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>129/SvEv (n=6)</th>
<th>B$_2$-KO (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$U_{\text{NaV}}$, nmol/min</td>
<td>Water Ad Libitum</td>
<td>24-hr Water Restriction</td>
</tr>
<tr>
<td>$U_{\text{NaV}}$, nmol/min</td>
<td>112.4±9.6</td>
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</tr>
<tr>
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<td>305±30.9$^*$</td>
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*P<0.01 vs 129/SvEv with water ad libitum.
that B2-KO mice have a greater urinary concentrating capacity. These changes in UOsm and UV seem to indicate that B2 receptors (and therefore a bradykinin B2-mediated response) the effect of the elevated AVP is unopposed and therefore these mice display greater urinary concentrating capacity than controls. We propose that in B2-KO animals that lack bradykinin B2 receptors and (therefore a bradykinin B2-mediated response) the effect of the elevated AVP is unopposed and therefore these mice display greater urinary concentrating capacity than controls. The data in the literature supporting this hypothesis come from in vitro microperfusion and cell culture studies. In 1985, Nasjletti's group showed that protein kinase C activators as well as bradykinin induce dose-dependent inhibition of AVP-stimulated cAMP synthesis but not generation of glucagon-, PGE2-, or forskolin-stimulated cAMP. Also in 1987, Friedlander demonstrated that protein kinase C activators as well as bradykinin induce dose-dependent inhibition of AVP-stimulated cAMP synthesis but not generation of glucagon-, PGE2-, or forskolin-stimulated cAMP. Also in 1987, Nasjletti's group showed that in Brattleboro rats AVP had a greater effect in vivo when animals were pretreated with the kallikrein inhibitor aprotinin. Because kinin generation was suppressed by blocking kallikrein, that study did not indicate which bradykinin receptor is responsible for opposing the effect of AVP in vivo. To the best of our knowledge, the present study using a mutant mouse model is the first to show that bradykinin blockade of the antidiuretic effect of AVP in vivo is mediated through B2 receptors. In our study we did not measure plasma AVP levels; therefore, one possibility is that the greater urine concentration observed in B2-KO mice during water restriction was due to a greater increase in plasma AVP levels versus controls. However, there seems to be a positive correlation between bradykinin and AVP in vivo: central administration of bradykinin stimulates AVP release. Therefore, in mice with a dysfunctional kallikrein-kinin system, one would not expect plasma AVP to be elevated. Furthermore, we observed a greater increase in UOsm in mutant mice versus controls when the AVP-V2 receptor agonist DDAVP was injected at equal doses in both groups. Thus, together these studies support the hypothesis that kinins acting through B2 receptors antagonize the urinary concentrating effect of AVP.

During water restriction, electrolyte excretion also behaved differently between groups. UNaV increased by 24 nmol/min in B2-KO, an 18% increase from baseline, whereas in controls it increased by 88 nmol/min, a 78% increase. Even though we did not conduct further studies to evaluate this increase in natriuresis in control mice, we believe it is the result of a phenomenon called dehydration-induced natriuresis. Although this phenomenon is not clearly understood, it seems to be a centrally mediated process by which many mammals increase sodium excretion when they become dehydrated, thereby contributing to restoration of body fluid osmolarity in water-restricted animals. We speculate that because the effect of AVP is unopposed in B2-KO mice, they can retain more fluid and therefore are more resistant to dehydration with 24-hour water restriction. This would explain the blunted dehydration-induced natriuresis observed in B2-KO mice versus controls. During water restriction, UNaV increased by 44% in controls but decreased by 22% in B2-KO mice. Increased Na+ delivery to the distal nephron promotes K+ secretion; therefore, the elevated dehydration-induced natriuresis in the controls could account for the increase in UNaV observed in this group. Furthermore, because kinins inhibit Na+ reabsorption, promoting natriuresis, and because the effect of bradykinin is absent in B2-KO mice, the blunted dehydration-induced natriuresis observed in these mice was expected.

We also tried to mimic the effect of endogenous AVP by exogenous administration of an AVP-V2 receptor agonist. Based on the literature, we decided to use DDAVP at a dose of 1 μg/kg. In preliminary experiments, we found that urine collected for 15 hours after SC injection of 1 μg/kg DDAVP did not display increased osmolarity in either controls or B2-KO mice. Therefore, to see an effect, we decided to suppress endogenous AVP levels before DDAVP administration by volume-overloading the animals with a solution of dextrose and alcohol, both of which are known to inhibit release of AVP. After injection of DDAVP, there was a 70% decrease in UV in both controls and B2-KO mice. However, UOsm responded differently in the 2 groups. Whereas there was an 2-fold increase in UOsm in B2-KO mice, controls displayed only a 70% increase (Figure 2). Contrary to the increase in electrolyte excretion we had

<table>
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*P<0.05 vs 129/SvEv injected with vehicle.
observed in the first protocol (dehydration-induced natriuresis), exogenous administration of DDAVP did not alter UNaV and UkV in either group.

An interesting observation was that during volume loading, UV in control mice was 3.45±0.84 μL/min, 3- to 4-fold higher versus non–volume-loaded controls. Conversely, B2–KO mice subjected to volume loading showed a UV of only 1.47±0.26 μL/min, only 36% higher than non–volume-loaded knockout mice. This observation agrees with our hypothesis, because in all probability AVP was not completely suppressed by volume loading, and therefore these hypothetical small amounts of AVP may have had an exaggerated effect in B2–KO mice, causing greater water reabsorption and hence decreased UV. Alternatively, we and others have shown that the renal kallikrein-kinin system regulates water and sodium excretion, promoting diuresis and natriuresis.6,22 Therefore, the observed difference in UV between controls and B2–KO mice subjected to volume loading suggests that kinins acting through B2 receptors may be essential for increased diuresis secondary to volume overload. Moreover, UNaV tended to be lower and UkV was statistically lower in B2–KO mice subjected to volume loading versus controls, suggesting an impaired natriuretic response to the infused volume.

In summary, our data indicate that endogenous increases in AVP or exogenous administration of an AVP-V2 receptor agonist have a greater urinary concentrating effect in B2–KO mice than in controls. This suggests that endogenous kinins acting through B2 receptors oppose the antidiuretic effect of AVP in vivo.

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References
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