An Enhanced Effect of Arginine Vasopressin in Bradykinin B₂ 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 V₂-receptors. It has been proposed that renal kinins counteract the hydro-osmotic effect of AVP. We hypothesized that kinins acting through B₂ receptors antagonize the urinary concentrating effect of AVP. To test this, bradykinin B₂ receptor knockout mice (B₂-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 ≈25%, whereas in B₂-KO mice there was almost a 60% drop in urinary output (P<0.001 versus controls). In the controls, water restriction increased U Osm by 347 mOsm/kg H₂O, ≈14% above baseline (NS), whereas in knockout mice the increase was 3 times that seen in the controls: >1000 mOsm/kg H₂O (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 B₂-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 V₂-receptor agonist, desmopressin (DDAVP). To suppress endogenous AVP levels before DDAVP administration, mice were volume-overloaded with dextrose and alcohol. U Osm was 685 ± 125 and 561 ± 58 mOsm/kg H₂O in water-loaded controls and B₂-KO mice, respectively. After DDAVP was injected subcutaneously at a dose of 1 μg/kg, U Osm increased to 1175 ± 86 mOsm/kg H₂O (Δ+490 mOsm) in the controls and 2347 ± 518 mOsm/kg H₂O (Δ+1786 mOsm) in B₂-KO mice (P<0.05 versus controls). We concluded that water restriction or exogenous administration of an AVP V₂-receptor agonist has a greater urinary concentrating effect in B₂-KO mice than in controls, suggesting that endogenous kinins acting through B₂ receptors oppose the antidiuretic effect of AVP in vivo. (Hypertension. 1999;33:1436-1440.)

Key Words: mice, knockout ■ bradykinin ■ argipressin ■ desmopressin ■ urine

Kinin receptors belong to a family of peptide hormone receptors linked to G proteins.³ They act mainly through 2 different types of receptors, B₁ and B₂. Most of the known effects of kinins (vasodilatation, diuresis, and natriuresis) are mediated by B₂ receptors, which belong to a family of peptide hormone receptors linked to G proteins.³ In the kidney, kallikrein and kinogen are found in the distal nephron; the interaction between renal kallikrein and kinogen results in kinin formation late in the collecting tubules.¹–³,⁴–⁶

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 osmolality, reduced car-diopulmonary blood volume, or decreased arterial blood pressure.⁷,⁸ AVP interacts with at least 2 types of receptors, V₁ and V₂. V₁ receptors activate phospholipase C which in turn increases cytosolic free Ca²⁺, thereby mediating contraction of vascular smooth muscle. V₂ receptors activate adenyl cyclase, increasing intracellular cAMP levels. The most important response to V₂ receptor-adenyl cyclase stimulation is increased water permeability of the luminal membrane of the cortical and medullary collecting tubules, exerting a powerful antidiuretic effect. This makes AVP the major determinant of the rate of renal water excretion.⁸

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.⁹–¹¹ The nature of this relationship was suggested by reports that kinins promote free water excretion in dogs receiving AVP¹² and decrease AVP-stimulated water reabsorption in the amphibian urinary bladder,¹³ as well as in the rabbit cortical collecting duct perfused in vitro.¹⁴ Moreover, a
kallikrein inhibitor, aprotinin, augmented the renal response to AVP in Brattleboro rats. All of these studies suggested that kinins may attenuate AVP and induce antidiuresis.

Using homologous recombination, Borkowski et al recently developed bradykinin B2 receptor null mutant (knock-out) mice (B2-KO) in which the gene encoding for the bradykinin B2 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. This animal model provides the opportunity to investigate the interaction between the renal kallikrein-kinin system and AVP and to determine whether bradykinin B2 receptors play a role in such an interaction, thus avoiding confounding pharmacological approaches. Accordingly, we hypothesized that kinins acting through B2 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 B2-KO mice than in controls.

Methods

Animals
Homozgous B2-KO mice (−/−) 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.

Urine Parameters
Urinary volume (UV) was determined gravimetrically and expressed as μL/min. Urinary sodium and potassium concentrations were measured with a NOVA-1 ion electrolyte autoanalyzer (Nova Biomedical) and expressed as mOsm/kg H2O. Urinary osmolarity (UOsm) was determined with a freezing-point osmometer (Advanced Instruments) and expressed as mOsm/kg H2O.

Drugs
The AVP V2 receptor agonist desmopressin (DDAVP) ([deamino-cis1, D-Arg8]-vasopressin, Sigma) was used in protocol 2. 100 μL saline or a single dose of 1 μg/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 B2-KO Mice
Controls (129/SvEv) and B2-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 (normohydration state). UV, UOsm, UNaV, and UKV 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, UNaV, and UKV were again determined.

Protocol 2: Effect of DDAVP in Water-Loaded Controls and B2-KO Mice
Controls (129/SvEv) and B2-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 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 μL of saline (vehicle) SC, after which urine was collected for 15 hours. UV, UOsm, UNaV and UKV 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 μg/kg DDAVP SC instead of saline. Urine was collected for 15 hours. UV, UOsm, UNaV, and UKV 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 B2-KO) and a single repeated factor: experimental intervention (free access to water or 24-hour water deprivation 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, UNaV, and UKV) were subjected to the same analysis.
Figure 1. A, Absolute UV expressed as µL/min in controls (+/+) and B2-KO mice (−/−). White bars represent mice given water ad libitum; dark bars represent the same group subjected to 24-hour water restriction. ANOVA showed that the Δ for the decrease in UV was significantly greater in B2-KO mice compared with controls: P=0.001. B, Change in UV with 24-hour water restriction in controls (+/+) and B2-KO mice (−/−). ANOVA showed that the Δ for the decrease in UV was significantly greater in control mice vs B2-KO: P=0.003. C, Absolute UOsm expressed as mOsm/kg H2O in controls (+/+) and B2-KO mice (−/−). White bars represent mice given water ad libitum; dark bars represent the same group subjected to 24-hour water restriction. ANOVA showed that the Δ for the increase in UOsm was significantly greater in B2-KO mice compared with controls: P=0.001. D, Change in UOsm in response to 24-hour water restriction in controls (+/+) and B2-KO mice (−/−). (P=NS). Two-way ANOVA also showed a statistically significant difference in UNaV between controls and B2-KO mice: P=0.003.

Protocol 2: Effect of DDAVP in Water-Loaded Controls and B2-KO Mice

During water loading, UV in the controls was 3.45±0.84 µL/min, whereas B2-KO mice received the same amount of dextrose SC had UV 1.47±0.26 µL/min (P=0.049) (Figure 2). After receiving DDAVP, UV decreased to 0.95±0.37 µL/min in the controls (Δ−2.509 µL/min; P<0.01) and 0.37±0.13 µL/min in B2-KO mice (Δ−1.098 µL/min; P<0.02). Urine osmolality was 685±125 and 561±58 mOsm/kg H2O in water-loaded controls and B2-KO mice, respectively. When mice were injected with DDAVP SC at a dose of 1 µg/kg, urine osmolality increased to 1175±86 mOsm/kg H2O (Δ+490 mOsm) in the controls (P<0.025) and 2347±518 mOsm/kg H2O (Δ+1786 mOsm) in B2-KO mice (P<0.025). When analyzed by 2-way ANOVA, the Δ increase in UOsm was statistically greater in B2-KO mice versus controls: P=0.043.

Figure 2. A, Absolute UV expressed as µL/min in controls (+/+) and B2-KO mice (−/−). To inhibit endogenous AVP levels, all animals received 4.5 mL of 1% ethyl alcohol in 5% dextrose SC. White bars represent mice injected with saline as vehicle; dark bars represent the same group injected with DDAVP ~24 hours later at a dose of 1 µg/kg. ANOVA showed that the Δ for the decrease in UV was significantly greater in control mice vs B2-KO: P=0.036. B, Δ UV with DDAVP injection in controls (+/+) and B2-KO mice (−/−). C, Absolute UOsm expressed as mOsm/kg H2O in controls (+/+) and B2-KO mice (−/−). White bars represent mice injected with saline as vehicle; dark bars represent the same group injected with DDAVP ~24 hours later at a dose of 1 µg/kg. ANOVA showed that the Δ for the increase in UOsm was significantly greater in B2-KO mice vs controls: P=0.043. D, Δ UOsm with DDAVP injection in controls (+/+) and B2-KO mice (−/−). When mice were given vehicle, UNaV was 161.2±33.4 and 87.6±22.9 nmol/min in controls and B2-KO mice, respectively (P=0.09) (Table 2). When animals received DDAVP, UNaV was similar to vehicle in both groups: 149±64.9 nmol/min in controls and 68.8±17.87 nmol/min in B2-KO mice. UoV was statistically different in vehicle-injected controls and B2-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 B2-KO, respectively.

Discussion

In this study we tested the hypothesis that kinins acting through the B1 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 B2-KO mice. The similarities in UV and UOsm between controls and B2-KO mice given water ad libitum suggest either that kinins play little role in antagonizing AVP actions under normal

<table>
<thead>
<tr>
<th>TABLE 1. UNaV and UoV in Controls and B2-KO During Normohydration and 24-Hour Water Restriction</th>
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<tr>
<td>Electrolyte Excretion</td>
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<tr>
<td>UNaV, nmol/min</td>
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<td>UoV, nmol/min</td>
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*P<0.01 vs 129/SvEv with water ad libitum.
TABLE 2. $U_{NaV}$ and $U_KV$ in Water-Loaded Controls and B$_2$-KO After SC Injection of Either Vehicle or 1 $\mu$g/kg DDAVP

<table>
<thead>
<tr>
<th>Electrolyte Excretion</th>
<th>129/SvEv (n=5)</th>
<th>B$_2$-KO (n=6)</th>
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<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>Desmopressin</td>
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<tr>
<td>$U_{NaV}$, nmol/min</td>
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</tr>
<tr>
<td>$U_KV$, nmol/min</td>
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<td>112.1±50.18</td>
</tr>
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$^*$P<0.05 vs 129/SvEv injected with vehicle.

conditions, or that compensatory mechanisms may have developed in the knockout animals that will only be uncovered under stress situations. Therefore, we decided to increase AVP levels in different ways. First we tested the effect of endogenous increases in AVP by restricting water access for 24 hours. Although we did not measure plasma AVP levels, it is well documented that water deprivation and the subsequent increase in plasma osmolarity comprise one of the main stimuli for AVP release. In the controls, water restriction increased UOsm by 347 mOsm/kg H$_2$O, ≈14% above baseline (NS), whereas in knockout mice the increase was 3 times that seen in the controls: more than 1000 mOsm/kg H$_2$O or ≈42% above baseline (Figure 1). In terms of UV, there was also a differential response between groups. During water restriction, UV in controls decreased by ≈25%, whereas in B$_2$-KO mice there was almost a 60% drop in urinary output (Figure 1). These changes in UOsm and UV seem to indicate that B$_2$-KO mice have a greater urinary concentrating capacity than controls. We propose that in B$_2$-KO animals that lack bradykinin B$_2$ receptors (and therefore a bradykinin B$_2$-mediated response) the effect of the elevated AVP is unopposed and therefore these mice display greater urinary concentration. This is in agreement with the hypothesis that bradykinin opposes the antidiuretic effect of AVP. Most of the data in the literature supporting this hypothesis come from in vitro microperfusion and cell culture studies. In 1985, Schuster showed that bradykinin in the bath but not in the lumen blunted the hydro-osmotic effect of AVP, which was overcome by exogenous cAMP. 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-, PGE$_2$-, or forskolin-stimulated cAMP. Also in 1987, Nasjletti’s group showed that in Brattleboro rats AVP had a greater effect in water-restricted animals. We speculate that because the effect of AVP is unopposed in B$_2$-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 B$_2$-KO mice versus controls. During water restriction, $U_{NaV}$ increased by 44% in controls but decreased by 22% in B$_2$-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 $U_{NaV}$ observed in this group. Furthermore, because kinins inhibit Na$^+$ reabsorption, promoting natriuresis, and because the effect of bradykinin is absent in B$_2$-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-V$_2$ receptor agonist. Based on the literature, we decided to use DDAVP at a dose of 1 $\mu$g/kg. In preliminary experiments, we found that urine collected for 15 hours after SC injection of 1 $\mu$g/kg DDAVP did not display increased osmolarity in either controls or B$_2$-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 B$_2$-KO mice. However, UOsm responded differently in the 2 groups. Whereas there was an ≈-3-fold increase in UOsm in B$_2$-KO mice, controls displayed only a 70% increase (Figure 2). Contrary to the increase in electrolyte excretion we had observed in mutant mice versus controls when the AVP-V$_2$ receptor agonist DDAVP was injected at equal doses in both groups. Thus, together these studies support the hypothesis that kinins acting through B$_2$ receptors antagonize the urinary concentrating effect of AVP.
observed in the first protocol (dehydration-induced natriuresis), exogenous administration of DDAVP did not alter UNaV and UNaV in either group.

An interesting observation was that during volume loading, UV in control mice was $3.45 \pm 0.84 \text{ mL/min}$, only 1.47 times higher than 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.

**Acknowledgment**

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**References**

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