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Conditional knockout of collecting duct bradykinin B_2 receptors exacerbates angiotensin II-induced hypertension during high salt intake

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Abstract

We elucidated the role of collecting duct kinin B_2 receptor (B_2R) in the development of salt-sensitivity and angiotensin II (ANG II)-induced hypertension. To this end, we used a Cre-Lox recombination strategy to generate mice lacking *Bdkrb2* gene for B_2R in the collecting duct (Hoxb7-Cre$^{tg/+}$:Bdkrb2$^{flox/flox}$). In 3 groups of control (Bdkrb2$^{flox/flox}$) and 3 groups of UB$^{Bdkrb2-/-}$ mice, systolic blood pressure (SBP) responses to high salt intake (4 or 8% NaCl; HS) were monitored by radiotelemetry in comparison with standard salt diet (0.4% NaCl) prior to and during subcutaneous ANG II infusion (1000 ng/min/kg) via osmotic minipumps. High salt intakes alone for 2 weeks did not alter SBP in either strain. ANG II significantly increased SBP equally in control (121 ± 2 to 156 ± 3 mmHg) and UB$^{Bdkrb2-/-}$ mice (120 ± 2 to 153 ± 2 mmHg). The development of ANG II-induced hypertension was exacerbated by 4%HS in both control (125 ± 3 to 164 ± 5 mmHg) and UB$^{Bdkrb2-/-}$ mice (124 ± 2 to 162 ± 3 mmHg) during 2 weeks. Interestingly 8%HS caused a more profound and earlier ANG II-induced hypertension in UB$^{Bdkrb2-/-}$ (129 ± 2 to 166 ± 3 mmHg) as compared to control (128 ± 2 to 158 ± 2 mmHg) and it was accompanied by body weight loss and increased mortality. In conclusion, targeted inactivation of B_2R in the renal collecting duct does not cause salt-sensitivity; however, collecting duct B_2R attenuates the hypertensive actions of ANG II action under conditions of very high salt intake.

Keywords

bradykinin receptor; kallikrein–kinin system; collecting duct; angiotensin II; hypertension; Cre recombinase; high salt diet

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Conflict of interest: none

Introduction

The kallikrein–kinin system (KKS) plays a physiological role in controlling vascular tone, renal hemodynamics and tubular function, and thus contributes to the regulation of blood pressure.[1-6] Bradykinin is the most biologically active peptide of KKS and acts mainly as a local hormone by activating specific G protein coupled receptors, known as B_1 and B_2 receptors (B_1R and B_2R), with most of the cardiovascular effects being mediated by the B_2R.[2-6] The B_2R protein is constitutively expressed in most tissues and over-expression of B_2R causes hypotension in transgenic mice.[7] Vascular endothelial cells express B_2R abundantly, where it is functionally linked to activation of endothelial nitric oxide (NO) synthase. Expression of B_1R is minimal under normal circumstances, but is induced by inflammation and organ damage.[3,6,8] Furthermore, the B_2R forms a complex with angiotensin converting enzyme, and this is thought to play a role in cross-talk between the renin-angiotensin system (RAS) and KKS.[3,6,9] The integrative role of KKS is further supported by involvement of B_2R activation in renin and NO release.[6,10-12] These interactions may significantly contribute to the regulation of kidney function. Indeed, several studies have suggested that inappropriate function of KKS can contribute in hypertension.[2-4,9-11] It is likely that the KKS selectively buffers the activity of vasoconstrictors such as angiotensin II (ANG II).

The enhanced susceptibility of mice lacking B_2R to high salt intake and also to ANG II - dependent hypertension and renal vasoconstriction clearly illustrates the important role of B_2R in the control of tubular electrolyte transport mechanisms, particularly in sodium handling.[1,4,10,11] Bradykinin exerts direct inhibitory effects on the epithelial sodium channel (ENaC) in vitro.[13-15] The cross-talk between the KKS, RAS and NO likely constitutes an important pathway in the regulation of sodium homeostasis and blood pressure. However, the relative contributions of endothelial vs. collecting duct B_2R to the regulation of salt sensitivity and angiotensin-mediated hypertension have been difficult to determine due to lack of tissue-specific targeted mice.

In order to delineate the integrative role of KKS, particularly B_2R - RAS interactions on the development of salt and ANGII-dependent forms of hypertension and potentially in progression of a variety of kidney diseases,[2-6,9] we utilized a gene targeting strategy to inactivate B_2R specifically in the collecting duct. We hypothesized that Bdkrb2 gene inactivation in the collecting duct favors enhanced tubular sodium retention during high salt intakes and causes salt-sensitive hypertension and hypertension-associated end-organ damage. We also tested the hypothesis that Bdkrb2 gene inactivation in the collecting duct worsens the development and severity of ANG II-induced hypertension.

Methods

Mice

The experiments were performed on 10-16 weeks old HoxB7-Cre:Bdkrb2$^{flox/flox}$, designated UB$^{Bdkrb2-/-}$ and control Bdkrb2$^{flox/flox}$ mice (n=52). Conditional Bdkrb2$^{flox/flox}$ mice on C57Bl6 genetic background were generated by the El-Dahr laboratory at Tulane University Health Science Center, New Orleans, LA, USA. Briefly, the homologous recombination

strategy involved replacing the wild type Bdkrb2 allele with Floxed Bdkrb2 exon3 and a selectable marker Neomycin resistance gene. This construct was electroporated into C57Bl6 mouse ES, selected with neomycin and used to generate the transgenic mouse strain. Bdkrb2 floxed mice carrying the NEO cassette were bred to Flipase mice to remove the NEO cassette. Bdkrb2 floxed mice were then crossed to Hoxb7Cre-GFP transgenic mice which express cre recombinase in ureteric bud lineage and its derivatives to conditionally remove Bdkrb2 in the collecting duct lineage. Deletion of Bdkrb2 gene was confirmed by the absence of exon 3 DNA and mRNA in FACSorted collecting duct cells (taking advantage of the GFP cassette in the Hoxb7Cre-GFP transgene) from newborn Hoxb7-Cre:Bdkrb2 floxed mice. Loss of Bdkrb2 in the collecting duct was assessed by section immunofluorescence (Figure 1). All of the experiments were approved by the Institutional Animal Care and Use Committees and by the Ministry of Health of the Czech Republic.

Monitoring of blood pressure in conscious animals

According to the recommendation for cardiovascular studies in experimental animals,[16] radio-telemetry device (Data Science International) was used to measure pressure and other cardiovascular parameters as recommended.[17] Radiotransmitters (TA11PA-C10, DSI, St. Paul, MN, USA) were implanted in anesthetized mice as previously reported [18] and validated in our laboratory.[19,20] A midline skin incision 2 cm long from chin to manubrium was performed to isolate the common carotid artery. A blunt trocar was passed from the neck incision to abdominal region through the lateral aspect under the skin. The catheter of the implant was introduced via common carotid artery to the aortic arch. The transmitter body was placed under the skin in the abdominal region. The skin is sutured and topical antiseptic was applied. After 8-10 days of recovery, the monitoring was initiated continuously using the telemetry data acquisition system.

Only animals giving stable records were randomly divided into experimental groups receiving different diets (Harlan-Teklad, Madison, WI, USA): normal-salt (NS) 0.4% NaCl, high salt (4HS), and very high salt 8% NaCl (8HS). Based on salt intake, six experimental groups were monitored: 1. Wild-type Control ($Bdkrb2^{flox/flox}$) + NS (n=6); 2. Control + 4HS (n=6); 3. Control + 8HS (n=8); and 4. $UB^{Bdkrb2-/-}$ + NS (n=6); 5. $UB^{Bdkrb2-/-}$ + 4HS (n=6); 6. $UB^{Bdkrb2-/-}$ + 8HS (n=8). These animals were subjected to the following 31-day experimental protocol of cardiovascular parameters recording: after two days of basal cardiovascular monitoring and urine collection, two weeks various salt intakes were tested. Second urine collection was conducted at day 7 to determine excretory responses to high salt diets. At day 14, ANG II infusion is initiated for another two week period during various salt intakes. Third urine collection was conducted at day 20 to assess sodium excretion in ANG II - infused mice.

Urine collection in conscious mice

24-hour urine samples were collected in conscious mice using metabolic cages.[18-20] Animals were housed individually in metabolic cages and urine was collected for 24 hours into sterile tubes during radiotelemetry recording. Urine volumes were determined from each urine collection and samples were centrifuged (3,000 rpm / 3 min; 4°C) and preserved for analysis. Urinary concentrations of sodium and potassium are assessed by flame

photometry. Urinary NO metabolites (nitrate/nitrite; NOx) were analyzed by colorimetric assay (Caymen Chemical, Ann Arbor, MI, USA).[19]

ANG II-induced model of hypertension in mice

Under anesthesia, osmotic minipumps (1004 Alzet, Cupertino, CA) were implanted subcutaneously to deliver 1000 ng of ANG II per kg per min, as described previously.[10,18] After implantation, the animals were monitored for another two week period during various salt intakes.

Plasma and kidney ANG II and NOS activity analysis

In separate groups of mice treated with ANG II during NS and 8HS, we assessed ANG II levels in plasma and in the kidney (n = 6-8 in each group) by radioimmunoassay (EuroDiagnostica, Malmö, Sweden) as described in detail previously.[20,21] These mice were decapitated on day 4 after ANG II infusion or sham-operation. In the same tissue samples, NOS activity was determined in the renal cortex and medulla by measuring the rate of formation of L-[14C]citruline from L-[14C] arginine (Cayman Chem.Com. Ann Arbor, MI, USA) as described and validated previously.[20]

Histological evaluation of the kidney

At the end of experimental protocol, the animals were euthanized and the kidneys were collected for histological evaluation and compared to untreated control (n=6) and also $UB^{Bdkrb2-/-}$ mice (n=6). Glomerulosclerosis and tubular injury was assessed in the PAS stained kidney slices as we have described previously.[20,21]

Statistical analysis

Results are expressed as means ± SEM. Statistical analysis Using GraphPad Prism software (Graph Pad Software, San Diego, CA). The time course of the parameter within groups were conducted by the use of the repeated-measures ANOVA and Dunnett multiple comparisons test to analyze any change during the experimental protocol of salt or ANG II + salt administration as compared to basal data. Then we analyzed data during salt condition and during ANG II + salt separately to recognize the differences between control and $UB^{Bdkrb2-/-}$ stain in each time point of the protocol. Statistical comparisons between the groups were performed by two-way ANOVA, followed by Newman-Keuls test. $P < 0.05$ is considered as significant.

Results

The results of continuous monitoring of systolic blood pressure (SBP) by radiotelemetry are shown in Figure 2. Basal SBP was not different between mice lacking collecting duct B_2R ($UB^{Bdkrb2-/-}$) and $Bdkrb2^{flox/flox}$ (Control) mice. During the two weeks period of high salt intake, there were transient but non-significant SBP responses to 8HS diet only in both Control and $UB^{Bdkrb2-/-}$ mice (Fig. 2 A-C). As shown in Figure 2A, two week administration of ANG II caused similar SBP increases in both Control (121 ± 2 to 156 ± 3 mmHg) and $UB^{Bdkrb2-/-}$ mice (120 ± 2 to 153 ± 2 mmHg). High salt intake exacerbated the development of ANG II - induced hypertension, as expected. However, 4HS diet (Figure 2B)

enhanced the SBP rises during two week period to similar extent in both strains (Control, 125 ± 3 to 164 ± 5 mmHg and $UB^{Bdkrb2-/-}$ mice, 124 ± 2 to 162 ± 3 mmHg, respectively). In contrast, there was a significantly higher rise in SBP in ANG II - infused $UB^{Bdkrb2-/-}$ as compared to Control mice during the 1st week of 8HS diet (129 ± 2 to 166 ± 3 mmHg vs. 128 ± 2 to 158 ± 2 mmHg; $p<0.05$), (Figure 2C). Although this maximal responses to ANG II and 8HS were only transient in both $UB^{Bdkrb2-/-}$ and Control strains, it needs to be noted that SBP increases were significantly higher only in $UB^{Bdkrb2-/-}$ but not in Control when compared with those on ANG II and 4HS (Figure 2B).

The time course of heart rate (HR) during the experimental protocol is depicted in Figure 3. There were no significant differences between control and $UB^{Bdkrb2-/-}$ mice during various salt intakes or ANG II administration combined with salt diets. The observed transient drop in HR after the initial phase of ANG II infusion was more likely compensation to SBP rises. In groups on 8% HS diet, HR decreased during the second week of ANG II infusion possibly due to deteriorated health condition of the animals. Time course of body weight (BW) monitoring during the experimental protocol is presented in Figure 4. There were only slight alterations of BW due to ANG II infusion in comparison with basal data. In contrast, the combination of 8HS intake and ANG II infusion led to significant body weight loss in both strains (Figure 4C). This was associated with decreased SBP and followed by increasing mortality rate (up to 37 %) in both strains infused with chronic ANG II after the 24th day of experimental protocol (Figure 5). Therefore the experiment was ended 28th day to assess the renal tissue morphology.

Under basal condition of normal salt intake (0.4 % NaCl), daily sodium excretion was not different between the groups. During experimental period, sodium excretion was dependent on salt intake as expected; however, there were no significant differences between Control and $UB^{Bdkrb2-/-}$ mice on various salt diets and also during ANG II infusion combined with high salt intakes. Neither basal urinary NOx excretion nor NOx concentration during various salt intakes or ANG II administration combined with salt diets were significantly different between Control and $UB^{Bdkrb2-/-}$ mice. During ANG II infusion, we were able to collect urine only on day 20 due to worsening condition of ANG II - infused animals on 8HS. Excretory data are presented in Table 1.

Histological evaluation of glomerulosclerosis and tubulo-interstitial injury scores at the end of experiments revealed that excessive salt intakes did not significantly enhance the progression of renal damage induced by ANG II for this two week period in Control or mutant mice. Rare focal glomerulosclerosis was observed in all groups infused with ANG II and tubulo-interstitial changes such cellular infiltration and tubular atrophy. The results were classified as glomerulosclerosis index (GSI) and score of tubulo-interstitial injury (STI) are depicted in Table 2. Representative views of kidney sections are shown in the supplemental figure 1.

In the second series of experiments, ANG II concentrations in plasma (Figure 6A) and in the kidney (Figure 6B) were determined in Control and $UB^{Bdkrb2-/-}$ mice during NS and 8HS intakes or ANG II administration combined with various salt diets. This observation confirmed that the different in blood pressure responses to 8% high salt and ANG II

administration on day 4 did not reflect any alteration in ANG II levels in these mice. It can be assumed that ANG II levels remained similar in both control and $UB^{Bdkrb2-/-}$ during whole experimental protocol. Although there were expected decreases in ANG II levels during high salt intake and substantial increases after ANG II administration, we did not observe any significant differences in ANG II levels between Control and $UB^{Bdkrb2-/-}$ mice. In addition, NOS activity in the renal cortex (Figure 7A) and the renal medulla (Figure 7B) was also unaltered in $UB^{Bdkrb2-/-}$ mice during experimental conditions compared to Control strain. High salt intake led to the increases in renal NOS activity. On the other hand, ANG II treatment decreased NOS activity particularly in the renal medulla.

Discussion

To our knowledge, this is the first study that examined the blood pressure and renal phenotype in conditional B_2R knockout mice created by Cre-Lox recombination. In this model, exon 3 of the *Bdkrb2* gene was deleted by Cre-mediated recombination in the ureteric bud lineage, which gives rise to the mature collecting duct. Thus, B_2R was absent from the collecting duct from the earliest stages of kidney development. We surmised that collecting duct-specific deletion of Bdkrb2 predisposes to salt-sensitive hypertension due to inappropriate sodium retention. We tested two pathophysiological conditions: suppression of RAS by high salt intake and enhanced RAS activity induced by the continuous infusion of exogenous ANG II. Recent in vitro studies [13,14] have shown that bradykinin inhibits ENaC activity in the distal tubule predominately via B_2R. This mechanism also modulates the sodium transport by ENaC under high salt intake condition, suggesting that B_2R signaling protects against excessive salt retention and volume-expansion. On the other hand, ENaC activity is augmented in ANG II-induced hypertension.[22] Based on the described mechanisms, targeted inactivation of bradykinin receptors in the collecting duct could lead to an augmented ENaC activity contributing to the salt-sensitive responses during elevated sodium intake.

The major findings of this present study are as follows: 1) 4% or 8% high salt diets given for 2 weeks were not sufficient to induce hypertension in mice with collecting duct-specific deletion of Bdkrb2; 2) The development of ANG II-induced hypertension was not worsened by inactivation of B_2R in the collecting duct under normal salt conditions; 3) 4% salt intake exaggerated ANG II-induced hypertension in both strains to the similar extent; 4) 8% salt diet further accelerated the rise in blood pressure only in ANG II - infused mice lacking collecting duct B_2R. These results demonstrate that genetic inactivation of B_2R in the collecting duct does not contribute to the development of salt-sensitivity and ANG II-induced hypertension under normal or high salt condition. However, during very high salt conditions and inappropriate RAS activity, collecting duct B_2R counterbalance the action of the RAS in the regulation of sodium handling. It is important to point out two caveats of this study: a) our study does not rule out a role for collecting duct B_2R in salt-induced hypertension under conditions of more prolonged salt stress (longer than 2-4 weeks); and, b) our targeting strategy involved deletion of the B_2R since early development and in all cells types of the collecting duct. It is conceivable that selective deletion of B_2R from the principal cells later in development might reveal a role in salt sensitivity.

Several groups have previously demonstrated that global disruption of the B_2R gene in mice confers susceptibility to salt-sensitive hypertension.[1,2,23] In these studies it was not possible to determine whether the protective effects were mediated by epithelial (i.e., collecting duct) or vascular (i.e., endothelial) B_2R. The results of the present study indicate that the role of collecting duct B_2R seems to be restricted to conditions of high ANGII and very high salt intake. Under other conditions, collecting duct B_2R function appears to be fully compensated by other systems. One of such system that contributes to the regulation of local blood flow as well as the control of sodium transport is the crosstalk with NO release in the kidney.[24,25] It is generally accepted that NO produced in the kidney significantly influences both renal vascular and tubular function. Moreover, high salt stimulates NO production particularly in the kidney; NO promotes natriuresis and thus normalization of blood volume and BP.[26] Indeed, NO deficiency leads to the development of salt-sensitivity supporting the important role of NO in the regulation of tubular sodium handling.[19,27-29] In the present study, unaltered NOS activity in the kidney and urinary NOx excretions in the conditional mutant mice compared to Control mice reflect intact renal NO system under normal conditions and various salt intake and ANG II administration. These data support our notion that intact renal endothelial and tubular NO system could substitute the interrupted function of KKS in the collecting duct at least in this model. The exact role of NO in the regulation of kidney function in this model need to be further evaluated in the future study by blocking the NO system.

The physiological interactions of KKS with RAS are well recognized. In the conventional B_2R null mouse model, the hypertension induced by both the endogenous activation of RAS in 2K1C Goldblatt model and exogenous administration of subpressor doses of ANG II, is exacerbated.[10,11,30] In these animals, augmented ANG II levels result in decreased renal sodium excretion most likely due to increased sodium reabsorption in the distal nephron.[31,32] Thus we surmised that inactivation of B_2R in the distal nephron may worsen the development of ANG II-induced hypertension. However, our findings indicate that pressor responses to chronic infusion of ANG II are not augmented by inactivation of collecting duct B_2R. In contrast to the observation in B_2R null mice, suggesting that bradykinin mediated tubular effect may not be sufficient to oppose the prominent vascular and tubular effects of exogenous ANG II. In addition, our data did not reveal a difference in plasma or kidney angiotensin II levels between the groups under normal or very high salt condition and ANG II administration.

The present study also revealed the salt-sensitive component of ANG II -induced hypertension. As expected, high salt intake accelerated the development of hypertension in ANG II - infused mice. Although the 4% HS intake caused similar SBP responses in both mouse strains, 8% HS further accelerated the progression of hypertension only in the conditional B_2R mutant mice. This finding suggests that collecting duct B_2R oppose the RAS in the regulation of sodium handling under very high salt and ANG II conditions. It should be noted that this observation cannot be attributed to the changes in B_1R expression in our model. Although BdkrB1 receptor mRNA levels have been reported to be elevated in $BdkrB2^{-/-}$ kidneys (global KO),[33,34] we did not observe a difference in BdkrB1 mRNA in FACsorted collecting duct cells isolated from conditional $UB^{BdrB2f/fl}$ and $UB^{BdkrB2-/-}$ mice (data are not included). Histological evaluation of kidney tissue revealed no significant

chronic kidney injury induced by ANG II and salt stress the most likely due to the short-period protocol that limits this present study.[35] In the present study, we were limited by the deteriorated status of mice treated with ANG II during very high salt conditions. The longer protocol would require lower salt content and also infusions of lower ANG II concentrations. We cannot rule out that infusion of lower ANG II concentrations and for longer period of time may potentially unmask the interactions between high salt, ANG II and B_2R in this model. While important, these studies are beyond the scope of the present study and thus the possible protective effect of bradykinin mediated via B_2R particularly in the tubules against renal interstitial injury and fibrosis that has been described previously [3,36,37] remains to be tested in the future studies.

Although this present study demonstrates that tissue specific conditional inactivation of B_2R in the collecting duct does not cause appreciable salt-sensitive pressor responses to high salt intake, tubular B_2R can partially counterbalance the action of the RAS in the regulation of sodium handling and blood pressure under very high salt condition, at least in the model of ANG II-induced hypertension. These observations imply that the absence of tubular B_2R signaling is compensated by other systems. Furthermore, in the renal medulla, Bdkrb2 is not only expressed in the collecting duct cells but also expressed in a great abundance in renomedullary interstitial cells (RMICs), as are ANG II/AT1 receptors.[38-40] It is theoretically conceivable that the Bdkrb2/NO/cGMP system in RMICs would act to compensate the loss of Bdkrb2 in the collecting ducts. Therefore, it is still important to assess possible interactions between KKS and other vasoactive systems that can modulate renal function and that are involved in the regulation of blood pressure. Furthermore, our findings prompt the need to distinguish the specific role of tubular and endothelial B_2R in pathophysiology of hypertension under normal and high salt conditions or exaggerated RAS activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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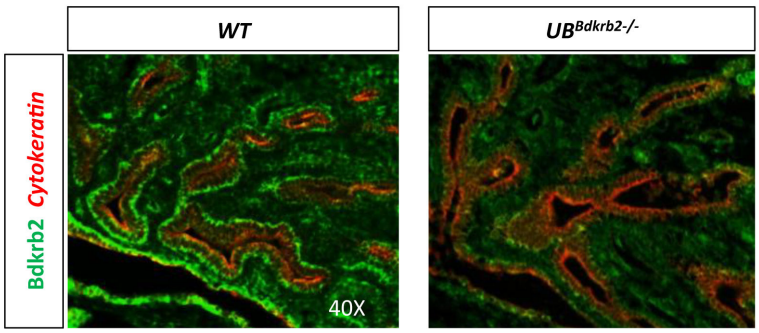

Figure 1.

Immunofluorescence for BdkrB2 receptor protein and cytokeratin (a marker of collecting duct) in newborn wild type (WT Control [**A**]) and mutant ($UB^{Bdkrb2-/-}$ [**B**]) kidneys.

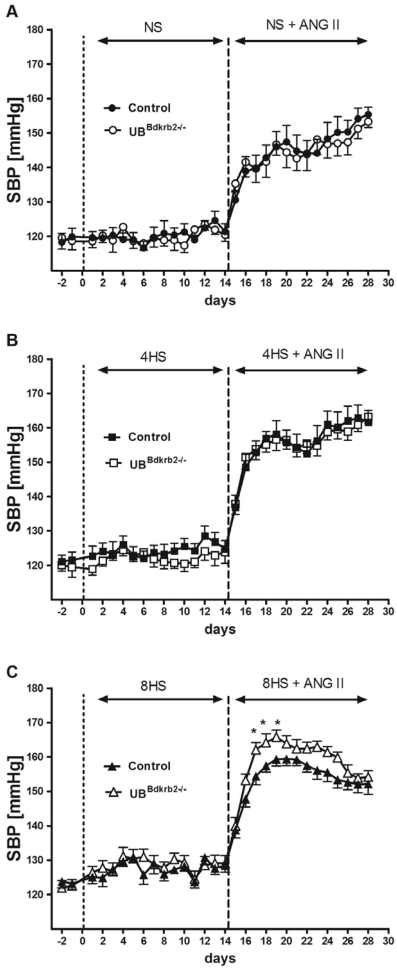

Figure 2.

Time course of systolic blood pressure (SBP) recorded by radiotelemetry during two weeks of various salt intakes (**A**, 0.4% normal salt diet - NS; **B**, 4% high salt - 4HS and **C**, 8% high salt - 8HS) and during angiotensin II (ANG II) infusion for another two weeks in Control and $UB^{Bdkrb2-/-}$ mice. *P<0.05 vs. Control mice.

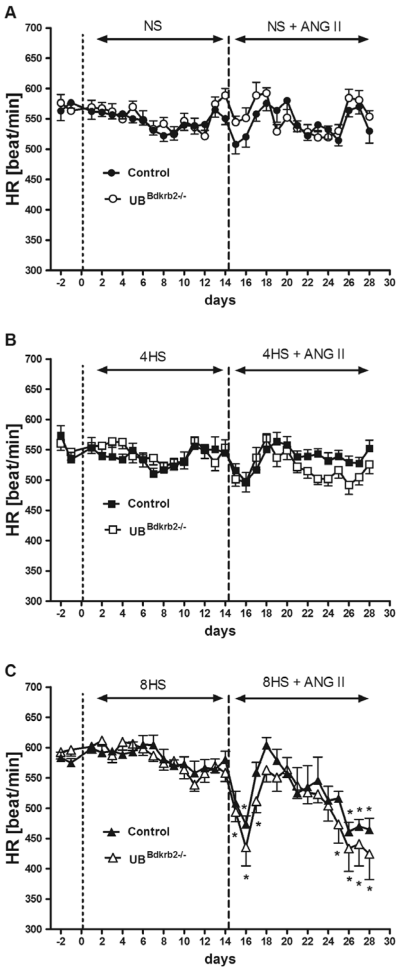

Figure 3.

Time course of heart rate (HR) recorded by radiotelemetry during two weeks of various salt intakes (**A**, 0,4% normal salt diet - NS; **B**, 4% high salt - 4HS and **C**, 8% high salt - 8HS) and during angiotensin II (ANG II) infusion for another two weeks in Control and $UB^{Bdkrb2-/-}$ mice. *P<0.05 vs. 8HS alone. There were no differences between groups.

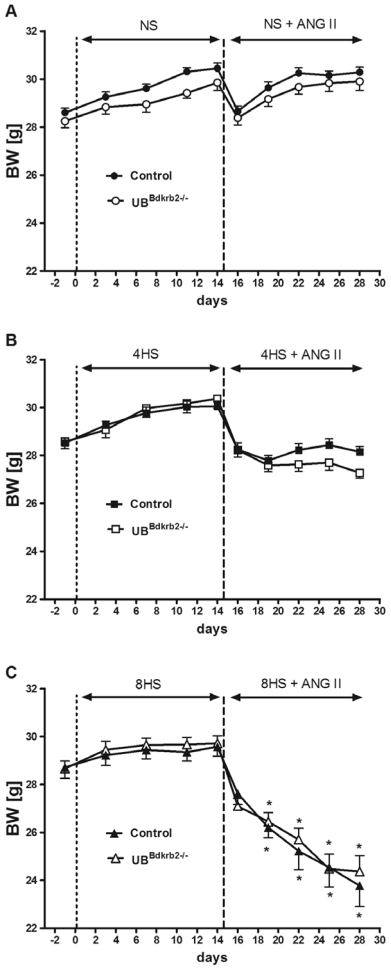

Figure 4.

Time course of body weight (BW) during two weeks of various salt intakes (**A**, 0,4% normal salt diet - NS; **B**, 4% high salt - 4HS and **C**, 8% high salt - 8HS) and during angiotensin II (ANG II) infusion for another two weeks in Control and $UB^{Bdkrb2-/-}$ mice. *P<0.05 vs. 8HS alone. No significant differences in BW between mouse strains on the same diet.

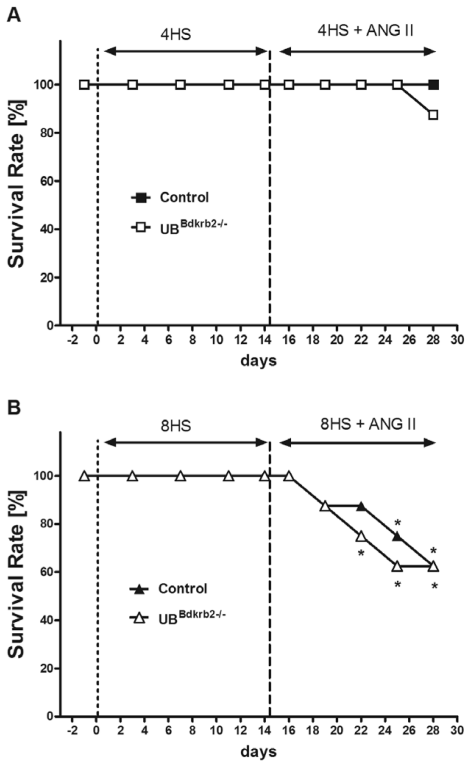

Figure 5.

Time course of survival rate during high salt intakes (**A**, 4% high salt - 4HS and **B**, 8% high salt - 8HS) and during angiotensin II (ANG II) infusion for another two weeks in Control and $UB^{Bdkrb2-/-}$ mice. *P<0.05 vs. 8HS alone. No significant differences in survival rate between mouse strains on the same diet.

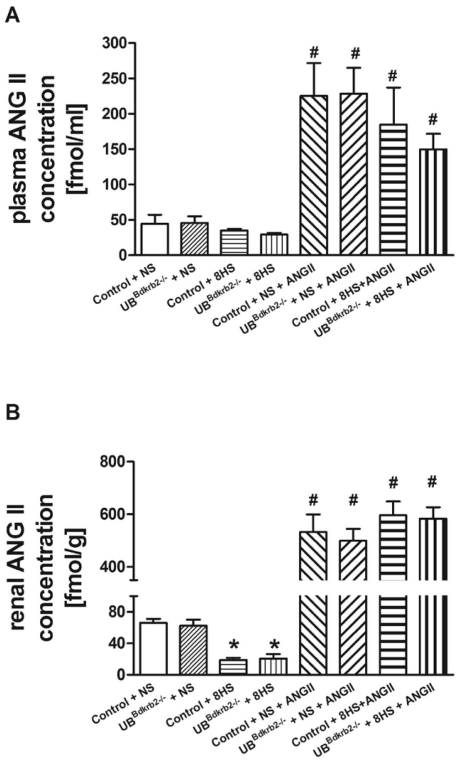

Figure 6.

Angiotensin II (ANG II) concentrations in plasma (A) and in the kidney (B) on day 4 after ANG II infusion or sham-operation during 0.4% normal salt diet - NS and 8% high salt - 8HS in Control and $UB^{Bdkrb2-/-}$ mice. *P<0.05 vs. NS groups, #P<0.05 vs. corresponding groups without ANG II. No significant differences in ANG II levels between mouse strains on the same diet.

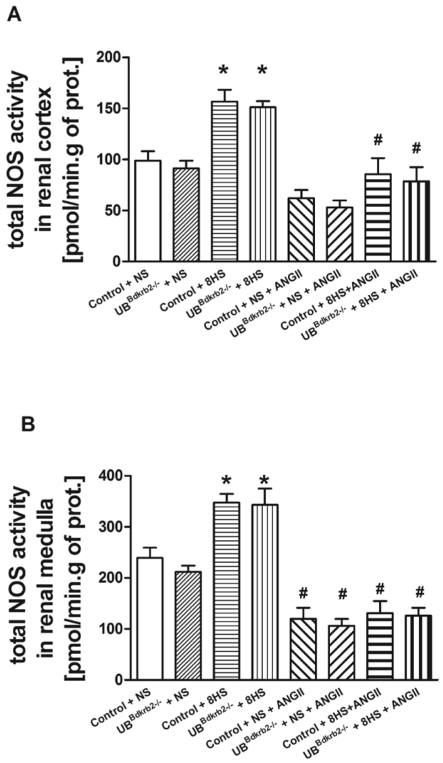

Figure 7.

Total nitric oxide synthase (NOS) activity in renal cortex (A) and in renal medulla (B) on day 4 after ANG II infusion or sham-operation during 0.4% normal salt diet - NS and 8% high salt - 8HS in Control and $UB^{Bdkrb2-/-}$ mice. *P<0.05 vs. NS groups, #P<0.05 vs. corresponding groups without ANG II. No significant differences in NOS activity between mouse strains on the same diet.

Table 1

Daily urinary excretion of sodium (UNaV) and nitrate/nitrite (UNOxV) on day 20 during ANG II administration and various salt intakes (NS, 4HS and 8HS) in Control and $UB^{Bdkrb2-/-}$ mice.

Group	UNaV [mmol/day]	UNOxV [μmol/day]
Control + NS + ANGII (n=6)	0.11 ± 0.02	0.43 ± 0.04
$UB^{Bdkrb2-/-}$ + NS + ANGII (n=6)	0.12 ± 0.03	0.39 ± 0.03
Control + 4HS + ANGII (n=6)	1.41 ± 0.16 *	0.31 ± 0.02 *
$UB^{Bdkrb2-/-}$ + 4HS + ANGII (n=6)	1.32 ± 0.17 *	0.30 ± 0.01 *
Control + 8HS + ANGII (n=8)	2.41 ± 0.22 *#	0.28 ± 0.01 *
$UB^{Bdkrb2-/-}$+ 8HS + ANGII (n= 8)	2.39 ± 0.19 *#	0.29 ± 0.02 *

* P<0.05 vs. NS groups,

# P<0.05 vs. 4HS groups.

No significant differences in UNaV and UNOxV between mouse strains on the same diet.

Table 2

Histological evaluation of glomerulosclerosis index (GSI) and score of tubulo-interstitial injury (STI) at the end of experimental protocol after two weeks of various salt intakes and ANG II infusion for another two weeks in Control and $UB^{Bdkrb2-/-}$ mice. No significant differences in renal damage between groups.

Group	GSI	STI
Control + NS + ANGII (n=6)	0.113 ± 0.028	0.051 ± 0.010
$UB^{Bdkrb2-/-}$ + NS + ANGII (n=6)	0.125 ± 0.032	0.069 ± 0.017
Control + 4HS + ANGII (n=6)	0.128 ± 0.031	0.071 ± 0.016
$UB^{Bdkrb2-/-}$ + 4HS + ANGII (n=6)	0.131 ± 0.038	0.067 ± 0.018
Control + 8HS + ANGII (n=8)	0.147 ± 0.048	0.074 ± 0.016
$UB^{Bdkrb2-/-}$+ 8HS + ANGII (n= 8)	0.153 ± 0.052	0.073 ± 0.015