Early Onset Salt-Sensitive Hypertension in Bradykinin B2 Receptor Null Mice

Ludek Cervenka, Lisa M. Harrison-Bernard, Susana Dipp, Ginny Primrose, John D. Imig, Samir S. El-Dahr

Abstract—Kinins have been implicated in the hemodynamic adaptation to postnatal life. The present study examined the impact of bradykinin B2 receptor (B2R) gene disruption on the postnatal changes in blood pressure (BP) and the susceptibility to early onset salt-sensitive hypertension in mice. B2R null (−/−) and wild-type (+/+ ) mice were fed normal (NS, 1% NaCl) or high (HS, 5% NaCl) salt diets during pregnancy. After birth, the pups remained with their mothers until they were weaned and were subsequently continued on the respective maternal salt intake until 4 months of age. The age-related changes at 3 and 4 months in tail-cuff BP and anesthetized mean arterial pressure at 4 months were not different in NS/B2R−/− and NS/B2R+/+ mice. However, there was a mild increase in BP in NS/B2R−/− at 2 months versus NS/B2R+/+. In contrast, HS/B2R−/− mice manifested early onset and persistent elevations of tail-cuff BP (P<0.05) at 2, 3, and 4 months versus other groups. MAP was also higher in HS/B2R−/− than HS/B2R+/+, NS/B2R−/−, and NS/B2R+/+ (91±3 versus 75±5, 74±2, and 70±2 mm Hg, respectively; P<0.05). Kidney renin and angiotensin type 1 receptor mRNA levels were not different. Additional studies showed that a delay in the initiation of HS until after birth was accompanied by later development of hypertension, although postnatal discontinuation of HS resulted in a gradual return of BP to normal values by 4 months of age. The results demonstrate that (1) kinins protect the developing animal from salt-sensitive hypertension, (2) lack of B2R from early development does not alter the maturation of BP, and (3) exposure to a HS diet during fetal life is not sufficient in itself to induce long-term hypertension in either wild-type or B2R null mice. (Hypertension. 1999;34:176-180.)

Key Words: kallikrein-kinin system • renin-angiotensin system • receptors, bradykinin • blood pressure

The recent development of genetically engineered mice with targeted disruption of the bradykinin B2 receptor (B2R) gene1 has provided a unique opportunity to investigate the physiological relevance of the kallikrein-kinin system in the absence of pharmacological interventions. Alfie et al2 demonstrated that although B2R null mutant mice maintained on a normal salt (NS) diet are normotensive, chronic high salt (HS) intake provokes hypertension and decreases renal blood flow compared with wild-type controls. Studies by Madeddu et al3 and Emanueli et al4 have shown that B2R null mice have a slightly higher resting blood pressure (BP) than wild-type controls and that salt loading or doxycorticosterone treatment causes hypertension in these mice. Thus, B2R gene inactivation results in salt-sensitive hypertension in the adult animal.

Although the role of kinins in the regulation of renal and cardiovascular homeostasis in the adult animal has received much attention, the role of the kallikrein-kinin system in developmental physiology is largely unknown. Emerging evidence indicates that the developing distal nephron is endowed with a local kinin-generating system.5–8 In addition, there is a considerable amount of kallikrein-like activity and bradykinin (BK) B2R mRNA in the developing vascular wall and heart.5,8 Therefore, it is conceivable that endogenous kinins counterbalance the effects of vasoconstrictor and antinatriuretic systems during normal growth and development. Accordingly, the present study was designed to (1) compare the maturational changes in BP in wild-type and B2R null mice, and (2) examine the impact of B2R ablation on the susceptibility to early salt-sensitive hypertension. In addition, to assess the contribution of the renin-angiotensin system, we examined the effects of B2R inactivation and differing salt diets on renal renin and angiotensin type 1 (AT1) gene expression and angiotensin II (Ang II) levels.

Methods

Animal Groups

Targeted disruption of the B2R gene was accomplished by homologous recombination in embryonic stem cells as described by...
Borkowski et al.1 B2R null mice (−/−) of mixed 129Sv×C57BL/6J genetic background (F1) were generously provided by Drs Fred Hess and Howard Chen (Merck Research Laboratory, Rahway, NJ) and were bred on C57BL/6J background up to F2 at Tulane School of Medicine vivarium (New Orleans, La). This was performed to limit the possible effects of genetic background on the BP phenotype. Wild-type C57BL/6J [B2R+/+] mice were obtained from Charles Rivers Laboratories (Wilmington, Mass). Experiments were performed on male and female mice (5 to 7 pups per litter). The experimental protocol was approved by the Tulane School of Medicine Animal Care Committee and the procedures followed were in accordance with institutional guidelines.

Male and female B2R+/+ and B2R−/− mice were placed on either NS 1% (TD#00229) or HS 5% (TD#092102) isocaloric diets (Harlan Laboratories) 1 day before mating, and the diets were continued for the duration of pregnancy. After delivery, the pups remained with their mothers on the respective diets. Four groups of mice were designated as follows: NS/B2R+/+ (N=6), HS/B2R+/+ (N=6), NS/ B2R−/− (N=14), and HS/B2R−/− (N=12). Additional B2R−/− mice received (1) NS during gestation and the diet was switched to HS on day 1 or 10 after birth (end of nephrogenesis in mice); these groups were designated NS→HS, groups 1 (N=16) and 2 (N=18), respectively; or (2) HS during gestation and switched to NS on day 10 after birth (HS→NS, N=10). We used the term NS to indicate a lower salt content than HS (5-fold difference). The NS diet used in this study contained a slightly higher NaCl content than the standard laboratory chow (0.7%).

**BP Measurement**

Tail-cuff BP was measured in trained prewarmed conscious mice at 2, 3, and 4 months of age with a tail-cuff apparatus (IITC model 29 pulse amplifier, IITC Inc). Arterial pulsations detected by the tail-cuff photocell were displayed with output from the cuff pressure transducer on a digital storage oscilloscope (Tektronix model 2211, Tektronix, Inc) and measured with a built-in cursor function. Tail-cuff pressure values were derived from an average of 8 to 10 measurements per animal at each time point. The reproducibility of this method was tested periodically by repeating the BP measurements on the same animals on different days.

To measure intra-arterial mean arterial pressure (MAP), 4-month-old mice were anesthetized with a combination of thiobutabarbital (Inactin; 100 mg/kg) and ketamine (Ketaset; 10 mg/kg) IP and placed on a servo-controlled surgical table to maintain body temperature at 37°C. A tracheostomy was performed and a short polyethylene tracheal cannula inside a small plastic chamber into which humidified 95% O2/5% CO2 is continuously passed. This procedure occurred in 5-minute intervals. At the end of the experiment, the animals were euthanized with excess IV anesthesia.

**Northern Blot Analysis**

RNA extraction, gel electrophoresis, RNA transfer to membrane, and hybridization procedures were performed as previously described.5,7 The Northern blots were hybridized with random-primed 32P-labeled cDNAs for rat renin or mouse AT1 that were stripped and reprobed with human GAPDH. Signals were detected by autoradiography and quantified by scanning densitometry (Ultroscan, Pharmacia LKB).

**Measurement of Plasma and Kidney Ang II**

Plasma and kidney Ang II levels were measured in anesthetized mice by a radioimmunoassay with rabbit anti–Ang II antibody (Jean Sealy, Cornell School of Medicine, New York),8 monoiodinated 125I-labeled Ang II (Amersham), and Ang II standard as previously described.10 Results are reported in femtomoles per gram kidney weight or mL of plasma. The sensitivity of the Ang II assay is <5 fmol/L. The percentage of specific binding for Ang II averaged 39%, with nonspecific binding of 2.7%.

**Data Analysis and Statistics**

Comparisons among the groups were performed by Student t test or ANOVA followed by Tukey test. P<0.05 was considered statistically significant. All data are reported as mean±SEM.

**Results**

**Effect of B2R Ablation on BP Under NS or HS Conditions**

Body weights and kidney weight–to–body weight ratios at 2, 3, and 4 months of age are not different among the experimental groups, which indicates that the differences in BP cannot be related to differences in body size (Figure 1). Systolic BP (SBP) measured by the tail-cuff method in NS/B2R−/− mice is significantly higher than NS/B2R+/+ at 2 months but not at 3 or 4 months of age, which indicates that lack of kinin activity from early development does not have a long-lasting effect on the maturation of BP under conditions of NS intake (Figure 2A). SBP in HS/B2R−/− is not different from that of NS/B2R−/− mice at any time point, thus indicat-
Figure 2. A, SBP at 2, 3, and 4 months in wild-type B2R+/+ and null B2R−/− mice on NS and HS diet measured by conscious tail-cuff method. The number of animals is the same as in Figure 1. B, Comparison of age-related changes in SBP in B2R−/− mice on lifelong or postnatal HS diet (NS→HS B2R+/+ group 1, n=16; group 2, n=18). a indicates P<0.05 vs NS/B2R+/+ (n=6); b, P<0.05 vs HS/B2R+/+ (n=6); c, P<0.05 vs NS/B2R−/− (n=14); d, P<0.05 vs HS/B2R−/− (n=12); and e, P<0.05 vs HS→NS/B2R−/− (n=10).

Figure 3. A, Effect of lifelong HS intake on MAP in 4 month-old anesthetized B2R+/+ and B2R−/− mice. B, Effect of IV BK (50 ng) or (C) Ang II (50 ng) on MAP. Statistical notations are the same as in Figure 2. The number of mice in each group is indicated in brackets.

The major finding of this study was that disruption of the B2R gene in mice confers a susceptibility to early onset salt-sensitive hypertension. The timing of the initiation of HS
intake during development does not seem to be crucial because HS intakes begun during embryogenesis or after birth are equally capable of inducing the hypertension. Lifelong HS intake in B2R null mutant mice is not associated with alterations of kidney renin or AT1 gene expression. This study also demonstrates that permanent elimination of kinin activity on the B2 receptor does not modify the maturational changes in BP under conditions of normal sodium intake.

Borkowski et al developed a mouse strain with a targeted disruption of the B2R gene in which the open reading frame of the B2R gene was replaced by a neomycin cassette. These mice lack all physiological responses to exogenous BK. With the use of adult B2R−/− mice (weight 26 to 30 g) derived from an inbred strain on a 129Sv genetic background, Alfie et al2 examined the effects of permanent loss of endogenous kinin activity on BP and renal hemodynamics. In B2R−/− mice maintained on a HS diet (3% in food and 1% saline in drinking water) for 8 weeks, tail-cuff BP and anesthetized MAP were 15 and 35 mm Hg higher than in null mice on NS, respectively. In contrast, there was no difference in tail-cuff BP or MAP in control mice fed either a NS or HS diet. In addition, renal blood flow was reduced by 20% and renal vascular resistance was doubled in B2R−/− on HS compared with controls. Subsequent studies by Madeddu et al3 in adult B2R null mice (weight 23 g) bred onto 129Sv genetic background reported that SBP and MAP were 15 mm Hg higher in B2R−/− than B2R+/+ mice on NS. B2R−/− mice showed exaggerated vasopressor responses to Ang II, and chronic administration of an AT1 receptor antagonist reduced the BP of B2R−/− to the levels of B2R+/+ mice.3 However, renin and AT1 gene expression were not different between the groups. In addition, chronic salt loading (0.84 mmol/g chow for 15 days) increased tail-cuff BP of B2R−/− mice by 35 mm Hg, whereas it was ineffective in B2R+/+ mice.3 Additional studies by the same group of investigators found that B2R null mice are highly susceptible to mineralocorticoid-induced hypertension.4 Together, these studies indicate that kinins play an important role in the prevention of salt-sensitive hypertension and that this may be achieved by maintaining renal blood flow under conditions of HS intake.

To our knowledge, the BP phenotype in B2R mutants of younger age groups has not been reported. Likewise, the effect of HS intake on the maturation of BP in B2R null mice is unknown. Madeddu et al11 administered the B2R antagonist icatibant (formerly known as Hoe 140) to pregnant Wistar rats and subsequently to their offspring maintained on NS diet. At 9 weeks of age, rats that were administered icatibant during prenatal and postnatal phases of life showed a modest BP elevation (≈8 mm Hg) versus vehicle-treated controls. In the present study, we found that permanent inactivation of B2R does not cause adult hypertension nor does it alter the maturation of BP under conditions of NS intake. We observed a small but statistically significant rise in tail-cuff SBP of NS/B2R−/− compared with NS/B2R+/+ at 2 months of age. However, this difference in BP was not sustained and could no longer be observed at 3 and 4 months of age, which indicated that the B2R null mutation did not have a long-lasting effect on the maturation of BP. In this regard, our findings are in agreement with those of Alfie et al2 who found that the BP of adult B2R null mice maintained on normal sodium intake is not different from wild-type controls. A technical limitation of investigations with mice is that measurement of BP is not feasible in young animals (≤1 month of age). Therefore, the earlier changes in BP during the evolution of hypertension in HS/B2R−/− mice were not determined. The BL6 strain used in this study is known to have a reduced BP when compared with other mouse strains, which suggests that protective genes might overcome the lack of B2R signaling and mask hypertension during early development.

**Figure 4.** Northern blot analysis. A, Kidney renin mRNA levels factored for GAPDH in 4 month-old B2R−/− and B2R+/+ mice on lifelong NS or HS a indicates P < 0.05 vs NS/B2R+/+. B, Ang II AT1 receptor mRNA levels factored for GAPDH in 4 month-old B2R−/− and B2R+/+ mice on lifelong HS. Each lane contained 20 μg of total kidney RNA.

**Figure 5.** Plasma and kidney Ang II peptide levels. NS→HS and HS→NS refer to switching of salt diets at 10 days of postnatal life. *P < 0.05 vs kidney levels in the corresponding group.
The development of salt-sensitive hypertension in young B₂R null mice suggests that the kallikrein-kinin system plays an important role in the hemodynamic adaptation to postnatal life under conditions of HS intake. We have demonstrated previously that the renal and cardiovascular systems express local, developmentally regulated, kinin-generating systems. Vascular and renal kallikrein-like activity increases 10– to 20-fold from birth to adulthood in the rat. In addition, B₂R and kininogen gene expression in the kidney, aorta, and heart is 10– to 30-fold higher in developing animals versus adult animals. Accordingly, we speculate that lack of kinin’s natriuretic and vasodilator activities contribute to the pathogenesis of the salt-sensitive hypertension in B₂R-deficient mice. In addition, we tested whether the renin-angiotensin system contributes to the hypertension in B₂R null mice on lifelong HS. No significant differences were detected in renal renin or AT₁ receptor mRNA levels in the mutant animals on HS, and kidney Ang II levels were appropriately suppressed in mutant mice that received HS from early postnatal life. We also found that the BP responses to intravenous Ang II were similar in B₂R null mutants and wild-type mice. However, the latter data should be interpreted with caution because of the small number of animals in the NS/B₂R−/− group, the possible influence of anesthesia on Ang II activity, and that a full dose-response curve was not performed. As suggested previously, unopposed activity of Ang II in the absence of kinin activity may contribute to hypertension in B₂R null mice on HS. The role of the renin-angiotensin system in mediating salt sensitivity in B₂R mutants deserves additional study.

Barker has proposed that adult arterial hypertension has its roots during early development. Developmental insults or stressors may alter the maturation of blood pressure and/or predispose to salt sensitivity. For example, intrauterine protein undernutrition is associated with later development of hypertension in the offspring. In the present study, we found that exposure to a HS environment only during fetal life is not sufficient in itself to cause long-term hypertension in B₂R null mice. Altered renal development is less likely to be a factor in the development of hypertension because delaying salt loading until the end of nephrogenesis in mice (day 10) was associated with the same magnitude of hypertension as in null mice on lifelong HS.

On the basis of these and other results, we conclude that the kallikrein-kinin system protects both developing and mature animals from salt-sensitive hypertension. The B₂R-deficient mouse is a monogenetic model that can be used to study the developmental aspects of salt-sensitive hypertension.

Acknowledgments
This study was supported by National Institutes of Health grant DK-53595, a Grant-in-Aid from the American Heart Association, National Center, and a National Kidney Foundation Scientific Award (S.S.E.-D.). L.M.H.-B. was supported by a Grant-in-Aid from the Louisiana Affiliate of the American Heart Association. J.D.I. was supported by National Institutes of Health grant HL-59699. L.C. is a postdoctoral fellow supported by a training award from the International Society of Nephrology. We are grateful to Fred Hess and Howard Chen (Merck Research Laboratories) for providing the B₂R null mice and to Geoffrey Schoffield (Tulane School of Medicine, New Orleans, La) for use of the tail-cuff BP apparatus.

References
Early Onset Salt-Sensitive Hypertension in Bradykinin B_2 Receptor Null Mice
Ludek Cervenka, Lisa M. Harrison-Bernard, Susana Dipp, Ginny Primrose, John D. Imig and Samir S. El-Dahr

Hypertension. 1999;34:176-180
doi: 10.1161/01.HYP.34.2.176

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/34/2/176