Renal and blood pressure phenotype in 18-mo-old bradykinin B$_2$R(−/−)CRD mice

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Bernard, Lisa M. Harrison, Susana Dipp, and Samir S. El-Dahr. Renal and blood pressure phenotype in 18-mo-old bradykinin B$_2$R(−/−)CRD mice. Am J Physiol Regul Integr Comp Physiol 285: R782–R790, 2003. First published June 12, 2003; 10.1152/ajpregu.00133.2003.—Aberrant gene-environment interactions are implicated in the pathogenesis of congenital renal dysgenesis (CRD), a leading cause of renal failure in infants and children. We have recently developed an animal model of CRD that is caused by gestational salt stress (5% NaCl diet; HS) of bradykinin B$_2$R null mice [B$_2$R(−/−)]CRD; El-Dahr SS, Harrison-Bernard LM, Dipp S, Yosipov IV, and Meleg-Smith S. Physiol Genomics 3: 121–131, 2000]. Developing B$_2$R(−/−)CRD mice exhibit tubular and glomerular cysts, stromal expansion, and loss of corticomedullary differentiation. In addition, B$_2$R(−/−)CRD mice exhibit transient hypertension from 2 to 4 mo of age. The present study was designed to determine the long-term consequences of CRD on renal morphology and salt sensitivity of blood pressure in B$_2$R(−/−)CRD mice. One-year- and 18-mo-old B$_2$R(−/−)CRD mice exhibited stunted renal growth, glomerular cystic abnormalities, and collecting duct ectasia. Moreover, tumors of mesenchymal cell origin emerged in the dysplastic kidneys of 90% of 1-yr-old and 100% of 18-mo-old B$_2$R(−/−)CRD mice but not in age-matched B$_2$R(−/−) or wild-type mice. When challenged with an HS diet, 18-mo-old B$_2$R(−/−)CRD exhibited a significant rise in systolic and diastolic blood pressures and more pronounced natriuresis and diuresis compared with salt-loaded 18-mo-old wild-type mice. Kidney aquaporin-2 expression was decreased by 50%, whereas renin, ANG type 1 receptor, and Na$^+$/K$^+$-ATPase levels were not different in B$_2$R(−/−)CRD mice compared with controls. In conclusion, this study demonstrates that B$_2$R(−/−)CRD mice exhibit permanent phenotypic and functional abnormalities in renal growth and differentiation. This novel model of human disease links gene-environment interactions with renal development and blood pressure homeostasis.

kidney development; salt sensitivity; kallikrein-kinin; aquaporin

CONGENITAL RENAL DYSGENESIS (CRD) accounts for ~30% of chronic renal failure cases in infants and children (12). We recently developed a model of CRD caused by gestational salt stress of mice with targeted disruption of the bradykinin B2 receptor gene (gene: Bdkrb2; protein: B2R; see Ref. 10). The developing kidney ex-presses all of the components of the tissue kallikrein-kinin system (8, 9). The kidney abnormality in B$_2$R(−/−) progeny is evident histologically on embryonic day 16 and consists of distorted renal architecture, foci of tubular dysgenesis, and cyst formation (10). Moreover, the abnormality is intrinsic to the embryo, because B$_2$R homozygous offspring from heterozygous parents exhibit the same phenotype as offspring from homozygous null parents (10). In contrast, B$_2$R mutant mice maintained on a normal salt intake or salt-loaded wild-type mice do not develop renal abnormalities (10). Thus this model of CRD depends on the cooperation of both a defined genetic defect and a specific environmental stressor heretofore termed B$_2$R(−/−)CRD.

Several lines of evidence suggest that the aberrant renal phenotype of B$_2$R(−/−)CRD mice is the result of impaired terminal epithelial differentiation. First, the renal phenotype appears relatively late in fetal development in concert with morphological and functional tubular differentiation. Second, histomorphometric analysis indicates that the early inductive events of nephrogenesis proceed normally in the B$_2$R null mice (10). In addition to renal dysplasia, B$_2$R(−/−)CRD mice develop early onset hypertension (4). The elevated blood pressure (BP) can be measured as early as 6–8 wk of age and persists until ~12–14 wk of age, when it gradually declines toward normal values. The circulating renin-angiotensin system is not activated in B$_2$R(−/−)CRD mutants on the BL6 genetic background (4), yet these mice are highly sensitive to the chronic hypertensive effect of exogenous ANG II (5). The long-term relation of renal dysplasia with hypertension is not clear because tubular dysgenesis is usually associated with salt wasting (17a). Therefore, the objectives of the current study were to determine the long-term consequences of CRD on renal morphology and salt sensitivity of BP in B$_2$R(−/−)CRD mice.

METHODS

Animals and experimental protocols. Breeding pairs of B$_2$R(−/−) mice (C57/BL6) were placed on a high-salt diet of isocaloric chow (HS; 5% NaCl, TD no. 92102; Harlan Teklad, Madison, WI) 1 day before mating and for the duration of gestation to induce CRD in the progeny, as described previ-
ously (4, 10). The maternal diet was switched to normal salt (NS; 0.3% NaCl) 1 day postpartum. After weaning (days 21–25), the B2R(−/−)CRD progeny were maintained on NS. B2R(−/−) and B2R(+/+) mice were maintained on life-long NS and served as controls for the effects of age on renal structure and BP. To determine the long-term effects of CRD on BP, systolic BP (SBP) was measured in the same animals at 12 and 17 mo of age in B2R(−/−)CRD and B2R(+/+) mice on a life-long NS diet. To determine the long-term consequences of CRD on salt handling, 17-mo-old B2R(−/−)CRD and B2R(+/+) mice were placed on HS for 4 wk. BP was measured during the 4-wk HS period, and plasma electrolytes and urinary sodium excretion were analyzed at the end of the 4-wk HS diet, as outlined in Fig. 1 and described below.

**CNSconscious SBP measurements.** Serial measurements of conscious SBP were performed on male and female B2R(−/−)CRD (n = 18) and male B2R(+/+) (n = 4; originally 6 mice, 2 died between 12 and 18 mo of age) mice treated with an HS diet for 4 wk were administered 3 mg/100 g body wt bromodeoxyuridine (BrDU; Zymed Laboratories) intraperitoneally to measure the proliferative index in the kidneys. Animals were anesthetized with 50 mg/kg ip pentobarbital sodium and were placed on a heated platform and underwent 10 preliminary cycles. The average of these 10-cycle measurements, which each have a minimum of 6 out of 10 successful measurement cycles, was used for data analysis.

**Anesthetized BP measurements.** Before anesthesia, 18-mo-old male and female B2R(−/−)CRD (n = 20) and male B2R(+/+) (n = 6) mice at 12, 17, and 18 mo of age using a computer-automated tail-cuff system (Visitech BP-2000 Blood Pressure Analysis System; Visitech Systems; Apex, NC). Animals were placed on a heated platform and underwent 10 preliminary cycles. The average of these 10-cycle measurements, which each have a minimum of 6 out of 10 successful measurement cycles, was used for data analysis.

**Urine collections.** Male B2R(+/+) (n = 5) and B2R(−/−)CRD (n = 7) animals were placed in metabolic cages for a period of 24 h while on the NS diet at 17 mo of age and again at 18 mo of age after 4 wk on the HS diet. Urine was collected and analyzed for volume, sodium concentration, potassium concentration, and osmolality. Water intake was measured on the HS diet. Sodium and potassium were determined with a flame photometer (model 943; Instrumentation Laboratory, Lexington, MA). Osmolality was measured with a vapor pressure osmometer (model 5500; Wescor, Logan, UT).

**Western blot analysis of kidney protein.** Western blot analysis was performed on kidneys obtained from 18-mo-old B2R(−/−)CRD (n = 6) and B2R(+/+) (n = 4) mice on HS, as previously described (18). The following primary antibodies were used: anti-rat sheep polyclonal angiotensinogen antibody (1:6,000; see Ref. 7), anti-human rabbit polyclonal ANG type 1 receptor (AT1) antibody (1:200; N-10; sc-1173; Santa Cruz), anti-rat rabbit polyclonal aquaporin-2 (AQP2) antibody (1:200; AB3066; Chemicon), anti-rabbit mouse monocolonal Na+-K+-ATPase α1 (1:5,500; 35–369; Upstate Biotechnology), and anti-human rabbit polyclonal renin antibody (1:2,000; see Ref. 3). Membranes were reprobed with β-actin antibody (monoclonal anti-β-actin antibody, 1:4,000; A5491; Sigma). Signals were detected using enhanced chemiluminescence (Amersham), and protein expression was analyzed densitometrically using the Digital Imaging and Analysis Systems (Apha Innotech).

**Immunohistochemical analysis of kidney tissue sections.** Kidneys from 12- and 18-mo-old mice were fixed in 10% buffered formalin, dehydrated in graded solutions of alcohol, and embedded in paraffin blocks, and 5-μm sections were made and mounted on slides with Vectabond (Vector Laboratories, Burlingame, CA). Immunostaining was performed by the immunoperoxidase technique using the Vectastain Elite kit (Vector Laboratories, as previously described (10). Primary antibodies used include anti-human mouse monoclonal α-smooth muscle actin (1:100; NCL-SMA; Nova Castra Laboratories), AQP-2 (1:100; Chemicon International), anti-BrDU mouse monoclonal antibody (1:50; ZBU30; Zymed), anti-human rabbit polyclonal β-catenin (1:200; H-102, sc-7199; Santa Cruz Biotechnology), and E-cadherin (1:200; H-108, Santa Cruz Biotechnology). Controls consisted of tissue sections in which the primary antibodies were substituted with PBS or nonimmune serum.

**Lectin histochemistry.** Tissue sections were incubated with Dolichos biflorus agglutinin (0.0125 mg/ml; Sigma), as previously described (10).

**Data analysis.** Statistical analyses were performed using SigmaStat Statistical Software on the raw data by two-way ANOVA, followed by Tukey’s test or by unpaired t-test, as appropriate. A P value <0.05 was considered statistically significant. All data are presented as means ± SE.
RESULTS

Renal morphology. Histological studies were performed on kidney sections of B2R(+/+) and B2R(−/−)CRD mice (Fig. 2). Normal renal morphology is observed on postnatal day 2 of B2R(+/+) mice (Fig. 2A), 12 mo of age (Fig. 2D), and 18 mo of age (data not shown). Newborn B2R(−/−)CRD mice exhibit developmental renal abnormalities similar to what has previously been described (10), consisting of tubular cysts, a disorganized cortex, and poorly developed medullary rays (Fig. 2B). Dysgenic tubules in 18-mo-old B2R(−/−)CRD mouse kidneys stain positively for D. biflorus lectin, indicating collecting duct origin (Fig. 2E). The dysplastic tubules stained positively but faintly for AQP-2 Western blot below. Hyperplastic islands or tumorlets. An average of three to six tumorlets are observed per section and are located in the inner cortex closely associated with renal arteries, veins, and glomeruli (Fig. 2G).

Figure 3A and higher-magnification Fig. 3B show that smooth muscle α-actin positively stained blood vessels are seen at the center of a renal tumorlet, indicating high vascularization. The tumorlets are composed predominantly of mesenchymal, myofibroblastic cells (smooth muscle α-actin-positive; Fig. 3B) and, to a lesser extent, vimentin-positive cells (data not shown). Although the periphery of the tumorlet is positive for CD-45, a marker of hematopoietic cells, the tumorlet’s core is negative (Fig. 3C). Moreover, the tumorlets are positive for proliferating cell nuclear antigen (Fig. 3D) but negative for tubular epithelial markers, including β-catenin (Fig. 3, E and F), AT1 receptor (Fig. 4, A–C), angiotensinogen (Fig. 4, D and E), AQP-2, Na+K+-ATPase, and E-cadherin (data not shown). In the case of epithelial cell markers, no specific staining was observed in the tumorlets in spite of using intentionally high concentrations of antibodies. Importantly, 1-yr-old B2R(−/−) mice that were maintained on an NS diet during embryogenesis and postnata (n = 5) have normal renal architecture and no evidence of renal tumorigenesis (Fig. 3, G and H).

Excretery function of B2R(−/−)CRD and B2R(+ +) mice on NS and HS diets. A 24-h urine collection was obtained from 17-mo-old male B2R(+ +) (n = 5) and B2R(−/−)CRD (n = 7) mice while on the NS diet. Body weights were significantly higher in NS/B2R(−/−)CRD than NS/B2R(+ +) mice (Table 1). No significant weight change occurred in either group after 4 wk of HS diets (Table 1). Twenty-four-hour urine volume, urinary Na+ and K+ excretion, factored for body weight, and urine osmolality, are not different between 17-mo-old NS/B2R(−/−)CRD and NS/B2R(+ +) mice (Table 1). A second 24-h urine collection and 24-h water intake were measured after placing these two groups of mice on an HS diet for 4 wk. HS/B2R(+ +) mice showed a fivefold increase in urinary sodium excretion (P < 0.08) and a significant increase in urine volume compared with NS (P < 0.05; Table 1). We have previously reported that B2R(−/−)CRD mice exposed to gestational HS and switched to an NS diet postnatally exhibit significantly higher SBP at 2 and 3 mo of age with a gradual return of SBP to normal values by 4 mo of age (4). SBP of B2R(−/−)CRD mice measured at 12 and 17 mo of age averaged 116 ± 2 (n = 12) and 114 ± 2 (n = 20) mmHg, respectively. SBP of B2R(+ +) mice measured at 12 and 17 mo of age averaged 112 ± 2 (n = 8) and 115 ± 5 (n = 5) mmHg, respectively. There were no significant differences in SBP at 12 and 17 mo of age in B2R(−/−)CRD mice compared with B2R(+ +) mice maintained on the NS diet throughout postnatal life (Fig. 5).

Effect of HS diet on SBP profile in B2R(−/−)CRD and B2R(+ +) mice. In response to 4 wk of elevated dietary salt, 18-mo-old HS/B2R(−/−)CRD exhibited a significant rise in SBP, whereas SBP in HS/B2R(+ +) was not altered (Fig. 6). SBP of HS/B2R(−/−)CRD increased significantly from 114 ± 2 to 127 ± 3 mmHg after 18 days compared with the NS diet. SBP continued to increase over the following week in HS/B2R(−/−)CRD and was significantly higher on days 24 and 28 compared with HS/B2R(+ +) mice. SBP averaged 133 ± 3 in HS/B2R(−/−)CRD and 118 ± 8 mmHg in HS/B2R(+ +) (P < 0.05) at the end of 4 wk of the HS diet. The impact of salt loading on SBP is evidenced by the increase of 18 vs. 4 mmHg in HS/B2R(−/−)CRD and HS/B2R(+ +) mice, respectively (Fig. 6, inset).

BP profile in anesthetized B2R(−/−)CRD and B2R(+ +) mice on the HS diet. Direct measurements of arterial BP were collected in anesthetized mice to confirm the observation of salt sensitivity in B2R(−/−)CRD mice made by tail-cuff SBP measurement. HS/B2R(−/−)CRD mice exhibited higher DBP compared with HS/B2R(+ +) mice when studied under pentobarbital sodium anesthesia [66 ± 3 and 51 ± 3 mmHg, respectively (P < 0.05); Fig. 7C]. MAP (Fig. 7A) and SBP (Fig. 7B) tended to be higher in HS/B2R(−/−)CRD than HS/B2R(+ +) mice, averaging 79 ± 4 and 62 ± 3 mmHg, respectively (P = 0.06). HR was not different.
Fig. 2. Renal morphology in B2R(+/+) (A and D) and B2R(−/−)CRD (B, C, and E–I) mice at birth and 12 and 18 mo of age. Normal renal morphology is seen in B2R(+/+) mice on postnatal day 2 (A) and at 1 yr (D). Nephrogenic zone (NZ) is demarcated in A, and normal glomerular morphology (arrow) is shown in D. B2R(−/−)CRD mice exposed to gestational HS demonstrate tubular cysts (*) at postnatal day 2 (B and at higher magnification in C) and at 18 mo of age (E). High-power magnification in E shows that the epithelial cells (arrows) lining the renal cysts are positive for the collecting duct marker Dolichos biflorus (DB; E). Aquaporin-2 (AQP-2) expression is downregulated in dysplastic collecting ducts as shown by the presence of a faint immunoreactivity (arrows in F). Renal tumors are found at 18 mo of age in B2R(−/−)CRD mice (G–I). These tumors are positive for bromodeoxyuridine (BrdU; G and H and higher magnification in I; arrows). PT, proximal convoluted tubule; CD, collecting duct; a, artery; v, vein. Original magnification ×20 (A, B, and H), ×40 (C, D, G, and I), and ×100 (E and F).
Fig. 3. Immunohistochemistry of tumorlets in 12-mo-old B2R(+/−)CRD dysplastic kidneys. Control section without primary antibody (A) and consecutive section immunostained for smooth muscle α-actin (B) showing the localization of a blood vessel at the center of the tumor (arrow). Tumorlets are negative for CD45, a marker of hematopoietic cell lineage (C). The dotted line outlines the core of the tumorlet, which is CD45 negative. The tumorlet is positive for proliferating cell nuclear antigen (PCNA; arrow), a proliferation marker (D). Anti-β-catenin antibody staining (E and F) shows positive immunoreactivity in the tubules (arrow), whereas the tumorlets are β-catenin negative (arrowhead). Kidney sections from 12-mo-old NS/B2R(−/−) mice (maintained on an NS diet since the time of conception) show normal morphology and an absence of dysplastic tubules and tumors (G and higher magnification in H). Original magnification ×20 (A, C, D, and G) and ×40 (B, E, F, and H).

between HS/B2R(−/−)CRD and HS/B2R(+/+) mice (449 ± 24 vs. 444 ± 61 beats/min; Fig. 7D).

Body weight was not different in male and female 18-mo-old HS/B2R(−/−)CRD and male HS/B2R(+/+) mice (Table 2). Plasma sodium and potassium concentrations were not different between the groups. Plasma protein concentration was significantly higher (P < 0.05); however, hematocrit values were not different in HS/B2R(−/−)CRD compared with HS/B2R(+/+) mice. Consistent with the postnatal phenotype of renal dysgenesis, total kidney weight and kidney-to-body weight ratios were reduced signifi-
cantly in adult HS/B2R(−/−)CRD compared with HS/B2R(+/+) mice (Table 2).

Kidney protein expression in B2R(−/−)CRD and B2R(+/+) mice on the HS diet. To assess whether the urinary excretory abnormalities in HS/B2R(−/−)CRD are associated with changes in tubular sodium and water transport mechanisms, the abundance of the renin-angiotensin system components, Na^+\-K^+\-ATPase, and AQP-2 protein expressions were determined in kidneys obtained from 18-mo-old HS/B2R(−/−)CRD and HS/B2R(+/+) mice by Western blot analysis. AQP-2 protein expression was significantly lower by 52% in kidneys of HS/B2R(−/−)CRD compared with HS/B2R(+/+) mice (P < 0.05; Fig. 8). Na^+\-K^+\-ATPase, renin, and AT1 receptor protein expressions were not different between the two groups, but there was a 50% elevation in angiotensinogen protein in HS/B2R(−/−)CRD (P < 0.05; data not shown).

DISCUSSION

Genetic and environmental factors are implicated in the pathogenesis of CRD. The environmental component includes the fetal-maternal milieu. As such, we have developed a model of CRD resulting from the combination of an environmental stressor (gestational HS) combined with genetic deletion of the bradykinin B2 receptor. This animal model exhibits congenital collecting duct dysgenesis (Ref. 10 and this study). Long-term follow-up in the present study demonstrates that the collecting duct dysgenesis is permanent and irreversible. In addition, microscopic tumors of mesenchymal cells emerge in the dysplastic kidneys. Functional studies performed at 17–18 mo of age revealed that CRD mice show abnormalities in salt and water handling and a propensity to develop salt-induced hypertension. Importantly, 1-yr-old B2R(−/−) maintained on an NS diet since the time of conception revealed no abnormalities in renal development and had no renal tumors. Collectively, these findings present direct genetic evidence linking aberrant intrauterine gene-environment interactions with abnormal renal development and salt-sensitive hypertension.

A surprising finding of this study is the emergence of tumor growths in kidneys of 1-yr- and 18-mo-old B2R(−/−)CRD mice. Interestingly, studies in humans have described the emergence of nodular blastema tumors within dysplastic kidneys (16). Immunohistochemical staining of 1-yr-old and 18-mo-old B2R(−/−)CRD kidneys identified the cellular masses as proliferative (BrDU positive) and of mesenchymal cell origin (smooth muscle α-actin and vimentin positive). In com-

Fig. 4. Immunohistochemistry of tumorlets in 12-mo-old B2R(−/−)CRD dysplastic kidneys. A–C: ANG II type 1 (AT1) receptor immunoreactivity is present in renal tubules (arrowhead in B) and blood vessels (C) but not in the tumorlet or the dysplastic primitive duct (arrow in B). D and E: angiotensinogen (AGT) is expressed in tubules (arrows) but not in the tumorlet. The background nonspecific staining is the result of the use of intentionally high concentrations of AGT antibody. A glomerular cyst (glomer cyst) is seen in D. Original magnification ×20 (A) and ×40 (B–E).
Table 1. Urinary excretory parameters before (17 mo of age) and after (18 mo) salt loading of male B2R(+/+) and B2R(+/−)CRD mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NS/B2R(+/+) n=5–8</th>
<th>NS/B2R(+/−)CRD n=10–20</th>
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<tr>
<td>Body wt, g</td>
<td>33.7±1.3</td>
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<td>Water intake, ml·day⁻¹ 10 g</td>
<td>ND</td>
<td>2.9±0.4</td>
<td>4</td>
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<tr>
<td>Urine volume, ml·day⁻¹ 10 g body⁻¹</td>
<td>0.40±0.07</td>
<td>0.75±0.28†</td>
<td>4</td>
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<tr>
<td>Urine sodium excretion, mmol·day⁻¹·10 g body⁻¹</td>
<td>45±8</td>
<td>216±56‡</td>
<td>4</td>
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<tr>
<td>Urine potassium excretion, mmol·day⁻¹·10 g body⁻¹</td>
<td>100±24</td>
<td>52±13</td>
<td>7</td>
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<tr>
<td>Urine osmolality, mosmol·kgH₂O⁻¹</td>
<td>1,376±190</td>
<td>1,156±213</td>
<td>4</td>
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Values are means ± SE; n, no. of mice. NS, normal salt diet; HS, high-salt diet; B2R(+/+), B2 receptor (B2R) wild-type mice; B2R(+/−)CRD, congenital renal dysgenesis (CRD) B2 R-deficient mice. ND, not determined. HS diets were given at 17 mo of age for a period of 4 wk. †P < 0.05 vs. NS. *P < 0.05 vs. B2R(+/+) on a given diet. ‡P = 0.08 vs. NS.

Fig. 5. SBP at 2, 3, 4, 12, and 17 mo of age in NS/B2R(+/−)CRD (solid bars; n = 10–20) and NS/B2R(+/+) (hatched bars; n = 5–8) mice. Data at 2, 3, and 4 mo adapted from Ref. 4. *P < 0.05 vs. B2R(+/+) mice at a given time point.

Fig. 6. SBP profile of B2R(+/−)CRD (●; n = 13–20) and B2R(+/+) (○; n = 4–5) mice beginning at 17 mo of age. Bars indicate time points for NS and HS diets. SBP was not different between the groups on NS, remained constant in B2R(+/+) on the HS diet, but increased significantly in B2R(+/−)CRD mice on the HS diet. The change in SBP from the last day of NS to the last day of HS is shown in inset (solid bar, B2R(+/−)CRD; hatched bar, B2R(+/+)). *P < 0.05 vs. NS. †P < 0.05 vs. B2R(+/+) mice.

parison, the tumorlets were negative for tubular epithelial cell markers such as angiotensinogen, Na⁺-K⁺-ATPase, AQP-2, E-cadherin, and β-catenin. Given the proximity of the tumorlets to glomeruli, the possibility that these cells originated from the extraglomerular mesangium was considered. Unfortunately, there are no specific markers for mesangial cells in the mouse. Finally, the core of the tumorlet was CD45 negative, indicating that the cells are not of hematopoietic origin. We hypothesize that the tumorlets originated from a population of renal stromal stem cells that maintained uncontrolled proliferation in the aberrant microenvironment of the dysplastic kidney. Additional studies are necessary to elucidate the cell biology of abnormal growth regulation in this model, particularly in light of the fact that hypertensive patients are predisposed to renal cancer (6, 13).

Alifie et al. (1) reported that SBP and MAP were higher in adult B2R(+/−) mice maintained on the HS diet for 8 wk compared with B2R(−/−) on the NS diet. Madeddu et al. (15) have shown that SBP and MAP were higher in B2R(+/+) than B2R(+/−) mice on the NS diet. It is possible that the difference between the two studies is related to the genetic background of the mice under study (129Sv vs. mixed 129/BL6). We have previously shown that B2R null mice exposed to a “postnatal” HS diet for 4 mo develop hypertension yet do not show signs of renal dysplasia (4). Only the combination of gestational HS diet and lack of B2R receptors leads to renal dysplasia. We have capitalized on this unique model of renal dysplasia to investigate...
the long-term effects of salt and water handling on BP, and renal morphology. The results of the present study show that, under conditions of normal salt intake, B2R(+/+)(HS/B2R(+/+))CRD mice remain normotensive up to 17 mo of age. However, B2R(+/+)CRD mice have a propensity to develop hypertension when challenged with a chronic dietary salt load. Loss of bradykinin’s natriuretic actions cannot be the sole reason for salt sensitivity in HS/B2R(+/+)(HS/B2R(+/+))CRD, since bradykinin’s actions via the B2R are natriuresis and diuresis, and these mice exhibited substantial increases in both salt and water excretion in response to chronic salt loading. Therefore, additional studies are warranted to elucidate the mechanisms of hypertension in 18-mo-old HS/B2R(+/+)CRD mice.

In the present study, we observed decreased kidney/collection duct AQP-2 expression in HS/B2R(−−)CRD

<table>
<thead>
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<th>Characteristic</th>
<th>HS/B2R(+/+) n</th>
<th>HS/B2R(−−)CRD n</th>
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<tbody>
<tr>
<td>Body wt, g</td>
<td>31.2 ± 2.1</td>
<td>33.2 ± 0.9</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>37.6 ± 3.1</td>
<td>42.1 ± 0.8</td>
</tr>
<tr>
<td>Plasma protein concentration, g/dl</td>
<td>4.2 ± 0.2</td>
<td>4.8 ± 0.1*</td>
</tr>
<tr>
<td>Plasma sodium concentration, meq/l</td>
<td>148 ± 1.3</td>
<td>148 ± 0.7</td>
</tr>
<tr>
<td>Plasma potassium concentration, meq/l</td>
<td>5.3 ± 0.7</td>
<td>5.0 ± 2.0</td>
</tr>
<tr>
<td>Kidney wt, mg</td>
<td>460 ± 35</td>
<td>421 ± 16*</td>
</tr>
<tr>
<td>Kidney wt/body wt, mg/g</td>
<td>14.5 ± 0.5</td>
<td>12.7 ± 0.3*</td>
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Values are means ± SE; n, no. of mice. *P < 0.05 vs. B2R(+/+).

Fig. 7. Hemodynamic profile of anesthetized B2R(−−)CRD (solid bars; n = 14) and B2R(+/+) (hatched bars; n = 4) mice on the HS diet. Mean arterial (A) and systolic blood (B) pressures of B2R(−−)CRD mice tended to be higher than B2R(+/+) mice. Diastolic BP (C) was significantly elevated in B2R(−−)CRD compared with B2R(+/+) mice. Heart rate (D) was not different between the two groups. *P < 0.05 vs. B2R(+/+) mice. bpm, beats/min.

Fig. 8. Kidney AQP-2 protein expression in B2R(−−)CRD (solid bar; n = 6) and B2R(+/+) (hatched bar; n = 4) mice, analyzed by Western blot analysis. Protein extracts were prepared from kidneys of 18-mo-old mice after 4 wk of the HS diet (20 μg total protein/lane). The membrane was reprobed with β-actin antibody. Data are expressed as the ratio of densitometric units to β-actin. AQP-2 protein expression was 50% lower in HS/B2R(−−)CRD mouse kidneys compared with HS/B2R(+/+). *P < 0.05 vs. B2R(+/+) mice. MW, mol wt.
comparing with age- and sex-matched wild-type mice, which may contribute, at least partly, to the diuresis. Interpretation of the exaggerated natriuresis in HS/B2R(−/−)CRD mice is more complex, because estimates of dietary salt intake are not available. It is important to note that higher body weights were observed in B2R(−/−)CRD compared with B2R(+/+) mice in both the mice on the NS and HS diets (Table 1), yet exaggerated natriuresis was only observed in the HS/B2R(−/−)CRD group. Also, differences in appetite cannot account for the natriuresis because a primary increase in salt appetite, resulting in a threefold increase in steady-state urinary sodium excretion, would be expected to cause a significant weight gain as a result of higher caloric intake. However, this did not occur because NS/B2R(−/−)CRD and HS/B2R(−/−)CRD had similar body weights on NS and HS diets (Table 1). The precise mechanisms of hypertension in HS/B2R(−/−)CRD mice remain to be defined. From a “clinical” standpoint, patients born with CRD initially present with complex electrolyte disturbances secondary to renal tubular salt wasting and/or concentrating defects, renal tubular acidosis, and varying degrees of renal functional impairment. Treatment involves careful replacement of fluid and electrolyte losses to maintain euvolesmia (12). It is unknown whether euvoletic CRD patients are susceptible to salt-induced hypertension, similar to the B2R(−/−)CRD mice.

In summary, 1-yr-old and 18-mo-old B2R(−/−)CRD mice with CRD exhibit persistent structural abnormalities such as renal tubular ectasia and glomerular cysts. An unexpected new finding was the development of mesenchymal-type tumor growth in the kidneys of B2R(−/−)CRD mice. BP, urine flow, and sodium excretion are normal at 17 mo of age in B2R(−/−)CRD mice maintained on an NS diet throughout postnatal life. However, when challenged with the HS diet for 4 wk, 18-mo-old B2R(−/−)CRD mice exhibit a rise in SBP and greater natriuretic and diuretic responses than salt-loaded B2R(+/+) mice. The diuresis is consistent with the downregulation of kidney AQP-2 expression (13). It is unknown whether euvoletic CRD patients are susceptible to salt-induced hypertension, similar to the B2R(−/−)CRD mice.

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DISCLOSURES

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