Normal Blood Pressure and Renal Function in Mice Lacking the Bradykinin B$_2$ Receptor

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Abstract—Telemetric blood pressure determinations, heart rate measurements, and pressure-natriuresis-diuresis experiments were used to characterize cardiovascular and renal function in bradykinin B$_2$ receptor knockout mice fed mouse chow containing 0.25% NaCl or mouse chow containing 4% NaCl. In B$_2$ receptor knockout mice fed usual mouse chow, the mean arterial blood pressure leveled between 108±1 and 110±3 mm Hg, and the heart rate leveled between 520±26 and 525±29 bpm, values that were not different from those measured in B$_1$ receptor knockout mice or 129Sv/J control mice. Increasing dietary salt intake did not affect mean arterial blood pressure and heart rate. Accordingly, pressure-natriuresis curves, pressure-diuresis curves, renal blood flow, and glomerular filtration rate were not different between B$_1$ receptor knockout and 129Sv/J mice. Increasing dietary salt intake to 4% increased renal blood flow to levels between 8.41 and 9.50 mL/min per gram kidney wet weight in 129Sv/J mice, whereas in B$_2$ receptor–deficient mice, renal blood flow was not affected and ranged between 6.85 and 7.88 mL/min per gram kidney wet weight. Other renal function parameters were not affected. Absence of B$_2$ receptor function was verified in B$_2$ receptor knockout mice with bradykinin infusion. These data suggest that the absence of B$_2$ receptor function does not necessarily make B$_2$ receptor knockout mice hypertensive or induce salt sensitivity. Presumably, differences in the genetic background or an adaptation to the loss of B$_2$ receptor function may account for these results, in contrast with earlier reports involving B$_2$ receptor knockout mice. We hold the latter possibility to be more likely and to be a fruitful possibility for future research. (Hypertension. 2001;37:1473-1479.)

Key Words: bradykinin ▪ mice ▪ natriuresis ▪ kidney ▪ sodium, dietary

The kallikrein-kinin system plays an important role in regulating cardiovascular and renal function. Bradykinin, the major effector of the kallikrein-kinin system, acts through at least 2 receptors. The bradykinin type 2 (B$_2$) receptor is believed to mediate most of the physiological functions, including vasodilatation, the natriuresis-diuresis relationship, and effects on cardiovascular structure. There is evidence that the kallikrein-kinin system is involved in hypertension. Patients with essential hypertension have lower kallikrein levels in their urine, and kininogen-deficient Brown Norway Katholiek rats develop salt-sensitive hypertension. Mutant mice lacking the B$_2$ receptor also exhibit salt-sensitive hypertension, according to earlier reports. On the other hand, transgenic mice overexpressing the human B$_2$ receptor are hypertensive. Blocking the B$_2$ receptor with icatibant in these mice restores the blood pressure to normal. The importance of the B$_2$ receptor has also been investigated in pharmacological studies. For instance, B$_2$ receptor blockade blunts the natriuretic response to volume expansion. The local infusion of bradykinin into the renal medullary interstitium increases sodium and water excretion. The pharmacological blockade of the B$_2$ receptor shifts pressure natriuresis and diuresis curves rightward and induces arterial hypertension associated with sodium and volume retention. Given the important role of the kidney in blood pressure regulation, we investigated the pressure-diuresis-natriuresis mechanism in B$_2$ receptor knockout mice given a usual laboratory diet and at a high salt diet.

Methods

B$_2$ receptor knockout mice, obtained from breeder pairs supplied by the Pharmacology Department, University of Sassari (Sassari, Italy), are described in detail elsewhere. The 129Sv/J mice that were used as controls were derived from the Jackson Laboratory (Bar Harbor, Me). All mice were bred in our animal facility. The mice were allowed free access to standard chow (0.25% sodium) and drinking water ad libitum, or they received mouse chow with 4% NaCl by weight (SNiff Spezialitäten GmbH). We used in our experiments the F4 to F6 generation of the breeder pairs. The experimental protocol was approved by the local council on animal care, whose standards correspond to those of the American Physiological Society. All experiments were conducted in mice aged 15 to 17 weeks. Genotypes were verified by polymerase chain reaction (PCR) and pharmacologically by a bolus of bradykinin (30 nmol/100 g body wt) to confirm the absence of the B$_2$ receptor. Telemetry was performed in 3 B$_2$ receptor knockout mice, in 8 control mice, and in 4 B$_1$ receptor knockout mice as an additional control; the latter mice are described elsewhere. The body weights averaged 28±1, 34±1, and 32±2 g before surgery in the 3 respective strains. The fewer numbers
of B2 receptor knockout mice is related to the difficulty of implanting the TA11PA-C20 blood pressure device in mice weighing <30 g. The telemetric techniques we used are described in detail elsewhere. The mice were synchronized to a light/dark schedule of 12/12 hours, with lights on at 6:00 AM. All mice were allowed at least 9 days of recovery before any measurements were made. Thereafter, baseline values were continuously recorded for 7 days, and the last 3 days of this period were used for statistical analysis. Then, the mice were given the 4% NaCl diet, and measurements were obtained at weekly intervals for 3 weeks. Once again, the last 3 days of each period were used for statistical analysis. The dietary salt load used is the same as that used when a test was made for salt sensitivity in these mice in an earlier study. All values were sampled every 5 minutes for 10 seconds continuously day and night, with a sampling rate of 1000 Hz. Values are shown as 24-hour means. For further evaluation of cardiovascular function, the baroreceptor heart rate reflex was investigated by using spontaneous changes in blood pressure and heart rate during a 2-hour period in which signals were sampled beat by beat. Baroreceptor heart rate reflex sensitivity was calculated by the sequence method. The number of sequences per 10,000 heart beats was used as an index of baroreceptor activity.

The effects of acutely increased renal perfusion pressure (RPP) on pressure-diuresis-natriuresis relationships and on total renal blood flow (RBF) were examined in 5 B2 knockout mice weighing 24±1 g and 10 129Sv/J control mice weighing 27±0.5 g that received standard mouse chow (0.25% sodium) and in 6 B2 knockout mice weighing 24±0.4 g and 6 129Sv/J control mice weighing 28±0.1 g that received 3 to 4 weeks of 4% NaCl mouse chow. We relied on techniques described earlier, but without performing the unilateral nephrectomy. After surgery and a 30- to 45-minute equilibration period, mean arterial pressure (MAP) and RBF were recorded continuously, and urine was sampled in two 10- to 30-minute collection periods. RPP was then increased by tying off the mesenteric and celiac arteries and, thereafter, by occluding the aorta below the kidney. Blood pressure and RBF were calculated for each period by averaging all recorded values during that time period. Urinary flow was sampled and determined gravimetrically. Urinary sodium and potassium (date not shown) concentrations were determined by flame photometry (FLM3, Radiometer) or by ion-selective electrode (Konelab Microlyte 3+2). Urinary flow, sodium excretion, and RBF were normalized per gram kidney wet weight.

The effects of changes in RPP on glomerular filtration rate (GFR) and fractional excretion of sodium and of water were examined in 8 B2 knockout mice weighing 25±1 g and 8 129Sv/J control mice weighing 29±1 g that received standard mouse chow and also in 7 B2 knockout mice weighing 28±1 g and 6 129Sv/J mice weighing 31±1 g that received 4% NaCl mouse chow for 3 to 4 weeks. The mice were surgically prepared as described elsewhere. GFR was measured by inulin clearance, and for this measurement, an additional catheter (PE-10) was placed into the second jugular vein for infusion of a 1% FITC inulin in 0.9% NaCl solution (Sigma Chemical Co).

For statistical analysis, we relied on the SIGMASTAT program to perform 2-way ANOVA. When differences were found, the t test (Bonferroni) was performed. Significance was accepted at P<0.05. Data are given as mean±SEM.

Genomic DNA isolated from tissues was used to genotype the mice by PCR. The presence of the B2 receptor gene was verified by the amplification of a 360-bp fragment by using the primers IMR434 (TGTCCCTGCGGTGTTCTTTC) and IMR435 (GGGTCTGAA-CACCAACATGG), and the neomycin resistance gene was detected by a 280-bp product by using the primers IMR013 (CTTGGGTG-GAGAGGCTATTC) and IMR014 (AGGTGAGATGACAGGAGAT).

Results

Figure 1 shows the genotypic verification in B2 receptor knockout and 129Sv/J control mice. Figure 2 shows the responses to bradykinin infusion in a representative tracing from the 2 strains. Control mice showed a profound decrease in blood pressure and increase in heart rate. The B2 receptor knockout mouse, on the other hand, showed no response. Figure 3 shows 24-hour MAP, heart rate, and aortic pressure dP/dt values for B2 receptor knockout mice that received 0.25% and 4% NaCl food. The small differences between the groups were not significant. Furthermore, systolic and dia-
stolic blood pressure values (not shown) were not different. Interestingly, B2 receptor knockout mice with the lowest heart rates showed the lowest locomotion (1.5 ± 0.3 cpm), whereas the B1 receptor knockout mice with the highest heart rate values also had the highest locomotion (4 ± 0.6 cpm). Increasing the salt content from 0.25% to 4% in the chow over 3 weeks did not influence MAP, systolic and diastolic blood pressures, or the heart rate levels in B2 receptor knockout and 129Sv/J control mice. Aortic pressure dP/dt values were not different between the strains under baseline conditions and during the salt-loading procedures.

The baroreceptor–heart rate reflex sensitivity and the baroreceptor reflex activity were not different between the strains and leveled at 2.68 ± 0.2 ms/mm Hg or 29 ± 5 sequences per 10,000 heart beats, respectively. A high salt diet increased the baroreceptor–heart rate reflex sensitivity and activity in all mice, so that these parameters leveled at 4.06 ± 0.4 ms/mm Hg or 34 ± 3 sequences per 10,000 heart beats, respectively.

Figure 4 shows the pressure-diuresis curves (left top panel), pressure-natriuresis curves (left middle panel), and RBF (left bottom panel) in B2 receptor knockout (B2-KO, filled circles), B1 receptor knockout (B1-KO, filled triangles), and 129Sv/J (open circles) mice. With usual chow (0.25% NaCl [normal Na+]) and with 4% NaCl chow (high Na+), blood pressure and heart rate were not different between the groups and were similar to the above-described results. Figure 5 shows similar data displayed in the same fashion as in Figure 4 during the high salt intake. RBF increased significantly in control mice fed a high salt diet compared with control mice given normal mouse chow. Otherwise, the pressure-diuresis and pressure-natriuresis curves of the 2 strains were not different. Finally, the hematocrits were measured under both conditions of salt intake, showing that the experiments were performed in hydrated mice.

Discussion
Contrary to what we had expected, we were not able to confirm hypertension in B2 receptor knockout mice compared with control mice. Because blood pressure values were not different, we were also not able to detect any differences in the pressure-diuresis-natriuresis relationships between the 2 strains. We challenged the B2 receptor knockout mice with a high salt intake; however, this maneuver had no effect on blood pressure or pressure-natriuresis relationships. The sole difference we found was an increase in RBF in control mice with salt loading. To avoid any confounding effects, we measured blood pressure, heart rate, and activity continuously with telemetry. We documented the absence of the B2 receptor in our mice both structurally and pharmacologically. These results are in stark contrast to those reported earlier in these same mice. We believe that these negative findings are important because they evidently underscore unappreciated physiological adjustments to major deficits that may require generations to develop.

Presumably, differences in the control strain could explain in part our results, although our knockout strain failed to show...
characteristics described earlier. Alfie et al.\textsuperscript{6,18} and Rhaleb et al.\textsuperscript{19} used SV129/SvEv and 129/SvEvTac as controls and also found no baseline blood pressure differences. However, in contrast to the present study, blood pressure increased in B$_2$ receptor-deficient mice as salt intake was increased in the earlier studies.\textsuperscript{6,7,18} However, the mice in the studies of Alfie et al.\textsuperscript{6,18} received a much higher salt load over a longer time than did the mice in our study, so that the experimental parameters were quite different from ours and may be responsible for the different results. Either an increased or no change in sensitivity to deoxycorticosterone acetate-salt has been reported for B$_2$ receptor-deficient mice.\textsuperscript{19,20} We found no increases in heart rate or altered baroreceptor control of heart rate in our animals, with or without a high salt diet, as have been described by others.\textsuperscript{21,22} We did observe an increase in baroreceptor heart rate reflex activity and sensitivity with the higher salt intake. This effect

Figure 4. Left, Relationship between RPP and urinary flow (top panel), sodium excretion (middle panel), and RBF (bottom panel) in B$_2$ receptor knockout (B$_2$-KO) and control (129Sv/J) mice with usual (0.25% NaCl) mouse chow. Pressure-diuresis and pressure-natriuresis curves and RBF were not different between the groups. Right, Relationship between RPP and fractional water excretion (% H$_2$O excretion, top panel), fractional sodium excretion (% Na excretion, middle panel), and GFR (bottom panel) in B$_2$-KO and control 129Sv/J mice with usual (0.25% NaCl) mouse chow. Curves for fractional sodium and water excretion and GFR were not different between the groups.
corresponds with data from mice and rats showing that a high salt diet increases the amplitude in the arterial pressure circadian rhythm.\textsuperscript{14,23,24} Taken together, we have no reason to suspect that early developmental processes determining heart rate in B\textsubscript{2} receptor knockout (B\textsubscript{2}-KO) and control (129Sv/J) mice with high dietary sodium intake (4\% NaCl). Pressure-diuresis and pressure-natriuresis curves were not different between the groups. High dietary sodium intake increased RBF only in 129Sv/J mice compared with 129Sv/J mice fed normal mouse chow. Right, Relationship between RPP and fractional water excretion (% H\textsubscript{2}O excretion, top panel), fractional sodium excretion (% Na excretion, middle panel), and GFR (bottom panel) in B\textsubscript{2}-KO and 129Sv/J mice with high dietary sodium intake (4\% NaCl). Curves for fractional sodium and water excretion and GFR were not different between the groups.

Figure 5. Left, Relationship between RPP and urinary flow (top panel), sodium excretion (middle panel), and RBF (bottom panel) in B\textsubscript{2} receptor knockout (B\textsubscript{2}-KO) and control (129Sv/J) mice with high dietary sodium intake (4\% NaCl). Pressure-diuresis and pressure-natriuresis curves were not different between the groups. High dietary sodium intake increased RBF only in 129Sv/J mice compared with 129Sv/J mice fed normal mouse chow. Right, Relationship between RPP and fractional water excretion (% H\textsubscript{2}O excretion, top panel), fractional sodium excretion (% Na excretion, middle panel), and GFR (bottom panel) in B\textsubscript{2}-KO and 129Sv/J mice with high dietary sodium intake (4\% NaCl). Curves for fractional sodium and water excretion and GFR were not different between the groups.

Taken together, we have no reason to suspect that early developmental processes determining heart rate in B\textsubscript{2} receptor-deficient mice are different from those in the other mice. Telemetry allowed us to calculate locomotion and the aortic blood pressure velocity (dP/dt), which is an indirect indicator of stroke volume.\textsuperscript{25} Only B\textsubscript{1} receptor knockout mice exhibited increases in heart rate and locomotion under baseline conditions; otherwise, these parameters were not different between the groups. The differences in heart rate and blood pressure in B\textsubscript{2} receptor knockout mice are subtle and protocol dependent. Furthermore, differences in the genetic background of the experimental mice may be important. For instance, in angiotensin II type 2 receptor knockout mice with different genetic backgrounds, either a slightly increased blood pressure\textsuperscript{14,26} or no change in blood pressure was described.\textsuperscript{27}
The kidney controls the relationship between RPP and sodium and water excretion, a phenomenon termed pressure-natriuresis-diuresis. Recently, we showed that pressure-diuresis-natriuresis can be measured in mice. Intrarenal infusion of bradykinin causes vasodilatation, diuresis, and natriuresis. Kinins may be part of the mechanism coupling changes in arterial pressure and sodium excretion and, therefore, could contribute to long-term arterial pressure regulation. Others, on the other hand, showed that intrarenal kinins are not short-term regulators of electrolyte and water balance and are not necessarily involved in pressure-diuresis and pressure-natriuresis. A shifted pressure-natriuresis relationship has been postulated for B2 receptor knockout mice. However, in accordance with the telemetric recording of arterial blood pressure, we found that the curves of pressure-natriuresis and pressure-diuresis were not different from control curves at either level of salt intake. Thus, fractional sodium and water excretion, RBF, and GFR also showed similar values in the 2 strains. In agreement with an earlier study using SV129Sv/Ev as control mice, RBF was increased when the control mice were fed a high salt diet. This effect was not seen in B2 receptor–deficient mice and could therefore be a kinin-dependent effect, as was suggested by Alfie et al. In salt-resistant Dahl rats, endogenous bradykinin does not participate in basal blood pressure homeostasis, although it appears to play an important role in blood pressure regulation in Dahl salt-sensitive rats. Our B2 receptor knockout mice were not salt sensitive. Therefore, the vasodilatation and sodium- and water-excreting role of bradykinin must have been assumed by other systems regulating sodium and water elimination, as well as blood pressure. Under this assumption, bradykinin participates in the regulation of renal function and blood pressure only when other systems are inhibited. In summary, we showed that B2 receptor knockout mice have blood pressure and heart rate values in the range described for other mouse strains. Blood pressure and heart rate were not affected by increasing the dietary salt intake. In accordance with these results, pressure-natriuresis curves, pressure-diuresis curves, RBF, and GFR were not different between B2 receptor knockout and 129Sv/J mice. Increasing dietary salt intake increased RBF in 129Sv/J mice; other renal function parameters were not different between the groups. These data show that differences in the genetic background or an adaptation to the loss of the B2 receptor may have changed the phenotype of bradykinin B2 receptor knockout mice. We do not have reason to believe that our control mice were responsible for our failure to find differences between B2 receptor knockout mice and control mice. We suggest that redundant systems adjusted for the absence of the B2 receptor in our mice. These adjustments were evidently not operative in earlier studies and required generations to develop. Elucidating the nature of these adjustments will give important physiological insights and will expand the utility of the gene-disruption technique. One approach will be to perform microarray-assisted gene expression studies in the kidney or other relevant tissues. We are preparing to perform such studies.

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References


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