Enhanced Blood Pressure Sensitivity to Deoxycorticosterone in Mice With Disruption of Bradykinin B$_2$ Receptor Gene

Costanza Emanuelli, Edwin Fink, Anna Franca Milia, Maria Bonaria Salis, Milena Conti, Maria Piera Demontis, Paolo Madeddu

Abstract—The renal kallikrein-kinin system is activated under conditions of mineralocorticoid excess. To evaluate whether endogenous kinins exert a protective role against the development of mineralocorticoid-induced hypertension, we studied the cardiovascular effects induced by long-term administration of deoxycorticosterone (DOC; 0.3 μmol/g body wt SC once per week for 6 weeks) or vehicle in transgenic mice (Bk2r$^{-/-}$) lacking the bradykinin B$_2$ receptor gene and in wild-type controls (Bk2r$^{+/+}$). Under basal conditions, Bk2r$^{-/-}$ mice showed higher systolic blood pressure (tail-cuff plethysmography) than wild-type Bk2r$^{+/+}$ and heterozygous Bk2r$^{+/-}$ mice (121±2 versus 114±2 and 115±2 mm Hg, respectively; P<0.05 for both comparisons). Heart rate was higher in Bk2r$^{-/-}$ and Bk2r$^{+/-}$ than in Bk2r$^{+/+}$ (459±12 and 418±7 versus 390±7 bpm; P<0.05 for both comparisons). Systolic blood pressure was increased by DOC in transgenic as well as in wild-type mice, whereas no change was induced by the vehicle. The pressor response to DOC was more rapid and pronounced in Bk2r$^{-/-}$ than in Bk2r$^{+/+}$ and Bk2r$^{+/-}$ (30±5 versus 15±4 and 6±3 mm Hg, respectively, at 3 weeks; P<0.01 for both comparisons). The difference in systolic blood pressure was consistent with that detected by direct intra-arterial measurements of mean blood pressure. Neither DOC nor its vehicle altered heart rate or gain in body weight over time. Under basal conditions, urinary sodium excretion did not differ between strains. During DOC administration, cumulative urinary sodium excretion was lower in Bk2r$^{-/-}$ than in Bk2r$^{+/+}$ (2.59±0.15 versus 3.31±0.22 mmol, respectively, during the first week; P<0.05). Urinary kinin excretion was increased by DOC in both Bk2r$^{-/-}$ (from 0.65±0.17 to 4.27±0.80 pmol/24 h; P<0.01) and Bk2r$^{+/-}$ (from 0.55±0.09 to 6.27±1.48 pmol/24 h; P<0.05). The increase in urinary kinin excretion was similar between strains. These results show that integrity of the bradykinin B$_2$ receptor is essential for regulation of blood pressure and heart rate under basal conditions. In addition, they indicate that activation of the kallikrein-kinin system represents a compensatory response against the development of hypertension induced by mineralocorticoid excess. (Hypertension. 1998;31:1278-1283.)

Key Words: mineralocorticoids ■ blood pressure ■ kallikrein-kinin system

K

inins, the enzymatic products of kininogen cleavage by kallikrein, induce vasodilatation, diuresis, and natriuresis by promoting the release of endothelium-derived relaxing factors and prostaglandins.1-4 They act as local hormones by activating specific receptors named B$_1$ and B$_2$, with most of the cardiovascular and renal effects being mediated by the B$_2$ receptor.5 It has been hypothesized that a dysfunction of the KKS, leading to unbalanced predominance of vasoconstrictor and antinatriuretic systems, may contribute to the pathogenesis of arterial hypertension. Interestingly, at variance with other models of genetic or experimental hypertension characterized by a defective renal synthesis and excretion of kallikrein,6-10 clinical forms of primary aldosteronism as well as experimental hypertension induced by DOC show an activated KKS.11-13 The possibility that this alteration represents a compensatory response to counteract the development of hypertension induced by DOC is based mainly on pharmacological studies in rats,14,15 showing that the hypertensive effect of mineralocorticoid administration is enhanced by the concomitant blockade of B$_2$ receptors with icatibant, a long-acting analogue antagonist of bradykinin.16,17 Unfortunately, receptor antagonists are not devoid of unspecificity. Recent advances in molecular biology have allowed the development of genetically engineered animals in which the relevant receptor gene has been disrupted. This novel approach has the potential to overcome some of the limitations related to the use of antagonists. Studies on a mouse strain (Bk2r$^{-/-}$) lacking the coding sequence for the B$_2$ receptor demonstrated that a normally functioning B$_2$ receptor is necessary for the maintenance of cardiovascular homeostasis.18-20 In addition to elevated basal levels of BP and HR, these transgenic mice show exaggerated BP sensitivity to acute or chronic administration of angiotensin II or moderate salt loading.20 To test the hypothesis of a protective role of kinins against the development and maintenance of mineralocorticoid hypertension, we evaluated the BP response to long-term administra-

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From the Departments of Farmacologia (C.E., M.P.D.), Biochimica (A.F.M., M.B.S.), and Clinica Medica (M.C., P.M.), University of Sassari (Italy); Department of Clinical Biochemistry, University of Munich (Germany) (E.F.); and National Institute of Biostructures and Biosystems, Osilo, Italy (P.M.).
Correspondence to Paolo Madeddu, MD, Clinica Medica, University of Sassari, Viale S. Pietro 8, 07100 Sassari, Italy.
E-mail madeddu@ssmain.uniss.it
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tion of DOC in mice lacking the B2 receptor gene and in wild-type controls. Because a partial deficiency rather than the complete absence of the B2 receptor is more likely to occur in humans, heterozygous animals were also studied.

Methods

Gene targeting was performed by transfecting embryonic stem cells derived from 129Sv/Ev mice with a vector designed to disrupt the entire coding sequence for the B2 receptor by homologous recombination. The Bk2r<sup>−/−</sup> mice used in the present studies were provided by Merck Research Laboratories (Rahway, NJ). They were compared with 129Sv/Ev mice (Bk2r<sup>+/−</sup>, from Jackson Laboratory, Bar Harbor, Maine) and with heterozygous mice (Bk2r<sup>+/−</sup>) obtained by breeding pairs of Bk2r<sup>−/−</sup> and Bk2r<sup>+/−</sup>. Mice were housed at a constant room temperature (24±1°C) and humidity (60±3%) for a 12-hour light/dark cycle. They had free access to chow with a normal 12-hour light/dark cycle. They had free access to chow with a normal 12-hour light/dark cycle. They had free access to chow with a normal 12-hour light/dark cycle.

Selected Abbreviations and Acronyms

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<td>Bk2r&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Bradykinin B2 receptor knockout mice</td>
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<td>Bk2r&lt;sup&gt;+/+&lt;/sup&gt;</td>
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<td>HR</td>
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<td>KKS</td>
<td>Kallikrein-kinin system</td>
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<td>MBP</td>
<td>Mean blood pressure</td>
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<td>SBP</td>
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BP and HR Measurements

SBP and HR were measured by the tail-cuff plethysmography method in unanesthetized mice prewarmed for 10 minutes at 37°C in a thermostatically controlled heating cabinet as described previously. Tail-cuff SBP is defined as the inflation pressure at which the waveform becomes indistinguishable from baseline noise. As an inclusion criterion, we required that at least 10 of 12 measurements were successful. Final SBP value was obtained by averaging 10 to 12 successful readings. Experimental measurements were performed between 10 AM and 2 PM by a single investigator (C.E.) and then judged by an independent investigator in a “blinded” fashion. HR was recorded automatically by a counter triggered by the pulse wave. To measure intra-arterial MBP, a polyethylene catheter (PE-10, Clay Adams) was inserted into the left carotid artery and advanced following day, MBP of unanesthetized, unrestrained mice was measured by connecting a Statham transducer (Gould) to the arterial volume was determined gravimetrically. Sodium concentration was measured by flame photometry.

A separate set of experiments was performed to test whether DOC affects urinary kinin excretion. Kinin levels were measured in 24-hour urine obtained from Bk2r<sup>−/−</sup> or Bk2r<sup>+/−</sup> under basal conditions and during DOC administration (n=6 per group). Urine was collected in ethanol (1:9, vol/vol), and sediment was discarded after centrifugation at 1500g for 10 minutes. Kinin determination was performed by radioimmunoassay, as described previously.

Finally, the MBP responses of Bk2r<sup>−/−</sup> or Bk2r<sup>+/−</sup> (n=6 per group) to acute blockade of the bradykinin B2 receptor were assessed after 3 weeks of DOC administration by injection of the B2 receptor antagonist AcLys[D-ala<sup>2</sup>]-des-Arg<sup>9</sup>-bradykinin (3 nmol/100 g body wt IV), a generous gift from Professor Domenico Regoli (University of Sherbrooke, Canada) or vehicle. MBP was recorded under basal conditions and for 10 minutes after injection.

Statistical Analysis

All data are expressed as mean±SEM. Changes in SBP from baseline are expressed as absolute values as well as areas under the BP curves. Multivariate repeated-measures ANOVA was performed to test for interaction between time and grouping factor. Univariate ANOVA then was used among groups and over time. Differences within and between groups were determined with the use of paired or unpaired Student’s t test, respectively, with the Bonferroni multiple-comparison adjustment.

Results

Under basal conditions (before vehicle or DOC administration), Bk2r<sup>−/−</sup> showed higher SBP values than Bk2r<sup>+/−</sup> and Bk2r<sup>+/+</sup> (vehicle, 121±2 versus 113±2 and 114±1 mm Hg, respectively; DOC, 121±2 versus 114±2 and 115±2 mm Hg, respectively; P<0.05 for both comparisons). HR values of Bk2r<sup>−/−</sup> and Bk2r<sup>+/−</sup> were higher than those of Bk2r<sup>+/+</sup> (vehicle, 463±8 and 415±6 versus 386±5 bpm, respectively; DOC, 459±12 and 418±7 versus 390±7 bpm; P<0.05 for both comparisons).

As shown in Figure 1, no significant change from baseline was observed regarding the SBP of groups given vehicle. By contrast, SBP was increased by DOC administration. The pressure effect was more pronounced in Bk2r<sup>−/−</sup> than in Bk2r<sup>+/−</sup> and Bk2r<sup>+/+</sup> (30±5 versus 15±4 and 6±3 mm Hg, respectively, at 3 weeks; P<0.01). In addition, the average increase in SBP, expressed as the area under the curve of the SBP increments, was significantly higher in Bk2r<sup>−/−</sup> than in Bk2r<sup>+/−</sup> and Bk2r<sup>+/+</sup> (18.0±2.1 versus 9.8±1.9 and 10.3±1.8 mm Hg/wk, respectively; P<0.05 for both comparisons). As shown in Figure 2, the effect induced by DOC on SBP was consistent with measurements of MBP at 3 weeks (Bk2r<sup>−/−</sup>, 140±2 versus 112±1 mm Hg in vehicle-treated mice, P<0.01; Bk2r<sup>+/−</sup>, 113±1 versus 102±1 mm Hg in vehicle-treated mice, P<0.05; Bk2r<sup>+/+</sup>, 112±2 versus 104±2 mm Hg in vehicle-treated mice, P<0.05). Neither vehicle nor DOC altered HR in any strain. Thus, the difference observed under basal conditions was maintained at the end of the experimental period (vehicle, 466±17 in Bk2r<sup>−/−</sup>...
and 405 ± 12 in Bk2r−/− versus 368 ± 14 bpm in Bk2r+/+. P < 0.05 for both comparisons; DOC, 464 ± 21 in Bk2r−/− and 401 ± 10 in Bk2r−/− versus 378 ± 16 bpm in Bk2r+/+, P < 0.05 for both comparisons). Body weight gain was similar among groups (data not shown).

Under basal conditions, urinary sodium excretion was similar in Bk2r−/− and Bk2r+/+ (Table). No difference regarding urinary volume was detected between strains before and during the administration of DOC. Cumulative urinary sodium excretion was increased by DOC in comparison with vehicle. This effect was less in magnitude in Bk2r−/− than in Bk2r+/+ (2.59 ± 0.15 versus 3.31 ± 0.22 mmol during the first week, respectively; P < 0.05). A shift in the relationship between natriuresis and BP in Bk2r−/− is indicated by the lower ratios of urinary sodium to BP under basal conditions (2.23 ± 0.04 versus 2.92 ± 0.05 mmol/mm Hg in Bk2r−/−; P < 0.05). A similar pattern was observed during DOC administration (2.24 ± 0.06 versus 3.09 ± 0.07 mmol/mm Hg in Bk2r−/− at 1 week; P < 0.01). As shown in Figure 3, urinary kinin excretion was increased by DOC in Bk2r−/− (from 0.55 ± 0.09 to 6.27 ± 1.48 and 5.36 ± 0.78 pmol/24 h at 1 and 4 weeks, respectively; P < 0.05 for both comparisons). A similar effect was observed in Bk2r+/− (from 0.65 ± 0.17 to 4.27 ± 0.80 and 6.79 ± 1.67 pmol/24 h at 1 and 4 weeks, respectively; P < 0.01 for both comparisons).

Previous studies showed pressor effects of the B1 receptor blockade in Bk2r−/− under basal conditions. At variance with these findings, acute administration of the B1 receptor antagonist AcLys[β-Nal,17Ile]des-Arg9-bradykinin did not alter the MBP of Bk2r−/− (−3 ± 2 versus −3 ± 2 mm Hg in vehicle-treated mice; P = NS) or Bk2r+/+ (−5 ± 3 versus −3 ± 2 mm Hg in vehicle-treated mice; P = NS) after 3 weeks of DOC administration. Similarly, no change in HR was observed after the administration of the antagonist or its vehicle (data not shown).

### Discussion

Recently, the presence of an altered cardiovascular phenotype in mice with disruption of the bradykinin B2 receptor gene has been documented by our group. These mice are characterized by higher BP and HR levels under basal conditions and by an exaggerated BP sensitivity to chronic excess of angiotensin II or dietary salt. In addition, the present study indicates that the lack of the B2 receptor is responsible for a more rapid and pronounced pressor response under conditions of chronic mineralocorticoid excess.

Hypertension induced by long-term administration of DOC in combination with high salt and uninephrectomy in rats is regarded as an experimental model of human hypermineralocorticoidism. Interestingly, DOC alone is not able to induce hypertension in adult rats unless they are exposed to mineralocorticoids from the very early phases of life. In comparison with rats, adult mice show a greater BP sensitivity to chronic excess of angiotensin II or dietary salt. In addition, the present study indicates that the lack of the B2 receptor is responsible for a more rapid and pronounced pressor response under conditions of chronic mineralocorticoid excess.

In our study we considered that preservation of renal integrity and maintenance of normosodic conditions would be essential to test the participation of endogenous kinins in the cardiovascular response to DOC. In fact, uninephrectomy plus salt reportedly reduces renal kallikrein release and alters the magnitude of the BP increments induced by B2 receptor blockade with icatibant in DOC-treated rats. In addition, an enhanced sensitivity to salt has been demonstrated in Bk2r−/−, and therefore high salt intake could mask an augmented BP response to mineralocorticoids.

The seminal finding that the renal KKS, a potent natriuretic and vasodilator system, is typically activated in human hypermineralocorticosteroidism as well as in rats given long-term DOC has been interpreted as a compensatory response
to counteract the vasopressor and sodium-retaining effects of mineralocorticoids.\textsuperscript{11-13} Consistent with the results obtained in other species is the finding that urinary kinins are increased in DOC-treated mice. The mechanism by which mineralocorticoids stimulate the renal KKS is not yet clear. Examination of the 5' flanking sequence of tissue kallikrein gene revealed several putative hormone binding sites.\textsuperscript{24} Factors such as steroid hormones might affect tissue kallikrein levels in various tissues by acting at the level of transcription.

The demonstration that long-term blockade of B\textsubscript{2} receptors by icatibant accelerates the development of DOC-induced hypertension favors a protective role of the KKS.\textsuperscript{14,15} Although a large body of evidence indicates that icatibant is a selective B\textsubscript{2} receptor antagonist in the rat,\textsuperscript{16,17} a recent publication has suggested that it may be able to interact with B\textsubscript{1} receptors in a bovine aortic cell line.\textsuperscript{25} In addition, residual agonist effects of icatibant have been reported in the rat.\textsuperscript{26} Therefore, caution is appropriate when the hypertensive effect induced by icatibant is attributed to selective blockade of the B\textsubscript{2} receptor. However, consistent with the evidence provided by the use of the antagonist is the report that Brown Norway Katholiek rats, a strain congenitally deficient in kininogen in plasma and devoid of kinin release in urine, exhibit a more rapid BP increase during DOC-salt treatment than normal Brown Norway Kitasato rats.\textsuperscript{27}

The availability of B\textsubscript{2} receptor knockout mice allowed us to evaluate whether the absence of the receptor confers a greater sensitivity to the vasopressor action of DOC. This was indeed the case, since the increase in the SBP of Bk2r\textsuperscript{−/−} was twofold that of wild-type controls (30 versus 15 mm Hg at 3 weeks). In consideration of our recent finding that the BP of untreated Bk2r\textsuperscript{−/−} increases toward elevated levels after 60 days of life, while that of wild-type controls remains stable with aging (C.E. and P.M., unpublished data, 1997), we wished to rule out the possibility that the BP difference between Bk2r\textsuperscript{−/−} and Bk2r\textsuperscript{+/−} under conditions of mineralocorticoid excess was merely attributable to a time-related effect. Therefore, experiments were conducted in Bk2r\textsuperscript{−/−} that had already reached an age (18 weeks) at which no further increase in BP occurs, as demonstrated in the present study by the lack of BP changes during the administration of vehicle.

The reduced ratio of urinary sodium to BP reflects a shift in the relationship between BP and natriuresis in Bk2r\textsuperscript{−/−},\textsuperscript{20} possibly attributable to an impaired renal excretory ability. This defect could be compensated for at the cost of higher BP levels under basal conditions and by incremental rises in BP.

### Figure 3

**Urinary kinin excretion under basal conditions (□) and during administration of DOC (0.3 μmol/g body wt SC) in Bk2r\textsuperscript{+/−} and Bk2r\textsuperscript{−/−} at 1 week (□) and 4 weeks (■) (n=6 per group).** Values are mean±SEM. *P<0.05, **P<0.01 vs basal value.
after the application of salt loading or an excess of sodium-retaining hormones.

Targeted disruption of the B2 receptor gene might have altered other components of the KKS. At variance with previous studies showing that antagonism of the B1 receptor increases the BP of Bk2r+/− under basal conditions, we found that acute administration of a B1 receptor antagonist does not alter the BP of DOC-treated mice. Since the magnitude of BP increases induced by B1 antagonist20 or DOC treatment was approximately the same, an intriguing explanation might be that, under basal conditions, activation of the B1 receptor compensates, in part, for the absence of the B2 receptor, while this compensatory mechanism could be overwhelmed under conditions of mineralocorticoid excess. Further studies are necessary to elucidate this possibility. Apart from components of the KKS, we cannot exclude that long-term adaptation or counterbalancing mechanisms taking place in animals that are submitted to higher BP during development might have contributed to amplify the pressor response to DOC in Bk2r−/−.

In Bk2r−/− we have observed higher HR levels under basal conditions.20 In the present study these animals did not show any reflex HR change in response to the BP increase induced by DOC. It is hard to reconcile the accelerated HR of Bk2r−/− with the ability of bradykinin to enhance the release of norepinephrine via the B2 receptor.28 However, similar tachycardia was observed in rats given icatibant in utero,29 thus suggesting that peripheral and/or central B2 receptors may be involved in the early developmental processes that determine the adult HR phenotype in rodents. Reduced parasympathetic activity, sympathoexcitation, and/or alteration in baroreceptor reflex sensitivity could be responsible for the alterations in HR. Consistent with the latter possibility is our previous observation that rat baroreceptor reflex response to increments in BP is reduced by central administration of icatibant.30

Heterozygous animals are useful not only because they help us to understand the effect of varying the number of functional copies of the targeted gene, but also because they resemble more closely a condition of “partial deficiency” that might occur in human hypertensive patients. We found that Bk2r+/− are indistinguishable from wild-type animals regarding BP under basal conditions as well as in response to DOC. In addition, the vasodepressor response to exogenous bradykinin is similar in Bk2r−/− and Bk2r+/−.20 These findings are compatible with a great deal of redundancy or spare capacity of B2 receptors (i.e., only a fraction has to be occupied to elicit a full response). A different pattern was observed regarding the response to other stimuli, as heterozygous animals showed mildly augmented BP response to long-term angiotensin II infusion compared with wild-type controls.28 Therefore, it is likely that the enhanced BP sensitivity to angiotensin II is related to a direct vasoconstrictor action of this peptide rather than to its ability to stimulate mineralocorticoid release.

In conclusion, our findings confirm that a normally functioning B2 receptor is essential for the regulation of BP and HR under basal conditions and indicate that endogenous kinins can protect against the development of mineralocorticoid-induced hypertension.

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References


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