

## Enhanced renal function in bradykinin B<sub>2</sub> receptor transgenic mice

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**Wang, Danzhao, Hideaki Yoshida, Qing Song, Lee Chao, and Julie Chao.** Enhanced renal function in bradykinin B<sub>2</sub> receptor transgenic mice. *Am. J. Physiol. Renal Physiol.* 278: F484–F491, 2000.—The tissue kallikrein-kinin system has been recognized as a paracrine and/or autocrine hormonal system that regulates arterial pressure, renal hemodynamics, and electrolyte excretion. We have created a transgenic mouse model overexpressing human bradykinin B<sub>2</sub> receptor, and the mice developed lifetime hypotension. With this animal model, we further analyzed the potential role of B<sub>2</sub> receptors in regulation of renal function. Baseline urinary excretion, urinary potassium excretion, and pH were significantly increased in transgenic mice, whereas urinary sodium excretion and serum sodium concentration were unaltered. Transgenic mice exhibited increased renal blood flow, glomerular filtration rate, and urine flow. Enhanced renal function was accompanied by significant increases in urinary nitrate/nitrite, cGMP, and cAMP levels with unaltered urinary kinin levels in transgenic mice compared with control siblings. Renal cGMP and cAMP content was also significantly increased in transgenic mice. Because the renin-angiotensin system exerts vasoconstriction buffering vasodilation of the kallikrein-kinin system, expression of renin-angiotensin components was examined by Northern blot analysis. We found a significant increase in hepatic angiotensinogen expression with no changes in renal renin and pulmonary angiotensin-converting enzyme mRNA levels in B<sub>2</sub> receptor transgenic mice. These studies showed that overexpression of B<sub>2</sub> receptors in transgenic mice resulted in hypotension and enhanced renal function through activation of nitric oxide-cGMP and cAMP signal transduction pathways.

nitric oxide; guanosine 3', 5'-cyclic monophosphate; adenosine 3',5'-cyclic monophosphate; kinin; human bradykinin B<sub>2</sub> receptor

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THE TISSUE KALLIKREIN-KININ system plays important roles in cardiovascular and renal functions (33, 43). Tissue kallikrein cleaves low-molecular-weight kininogen to release potent vasodilator kinins, and intact bradykinin exerts its bioactive functions via binding to the G protein-coupled bradykinin B<sub>2</sub> receptor (24). Acute bradykinin administration could induce a rapid natriuresis, diuresis, and blood pressure reduction (43). Extensive clinical studies showed that urinary kal-

likrein levels are inversely correlated with blood pressure (34, 35, 54). Because urinary kallikrein originates from the kidney, the correlation between high blood pressure and reduced urinary kallikrein levels suggests the participation of renal kallikrein in blood pressure homeostasis. Although these clinical studies have implicated a role of renal kallikrein in hypertension, the results are based on random population samples that do not lend themselves to rigorous genetic analyses. A large Utah family pedigree study showed that a dominant-allele kallikrein gene expressed as high urinary kallikrein excretion may be associated with decreased risk of essential hypertension (6). Reduced urinary kallikrein excretion has also been described in a number of genetically hypertensive rats (2, 17, 20, 34). To investigate the role of the kallikrein-kinin system in blood pressure regulation, we employed gain-of-function strategies by developing transgenic mice overexpressing the system components.

We altered the expression of tissue kallikrein by creating transgenic mouse models that expressed the human tissue kallikrein gene under the control of mouse metallothionein metal-response element or the albumin enhancer/promoter. These transgenic mice were permanently hypotensive throughout their lifetime compared with their control littermates (47, 52). Administration of aprotinin, a tissue kallikrein inhibitor, or icatibant, a specific B<sub>2</sub> receptor antagonist, to the transgenic mice restored their blood pressure to normal levels. These results suggest that the expression of functional tissue kallikrein can permanently alter the hemodynamics in the transgenic mice and that the hypotensive effect is kinin mediated (10). These studies provided the first direct evidence to link the kallikrein gene expression with blood pressure regulation. To further reinforce the protective effect of this system in the pathogenesis of hypertension, we created a transgenic mouse model overexpressing human bradykinin B<sub>2</sub> receptor (51). The B<sub>2</sub> receptor transgenic mice had sustained lifetime hypotension, and administration of icatibant restored their blood pressure to normal levels. The transgenic mice also displayed enhanced vasodepressor responses to a bolus intra-aortic injection of kinin and increased uterine contractile responses to kinin. These results were further supported by studies that reported increased blood pressure and enhanced blood pressure sensitivity to high-salt loading and deoxycorticosterone acetate-salt challenge in B<sub>2</sub> knockout mice (3, 16). In the present study we evaluated the

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effects of B<sub>2</sub> receptor overexpression in renal hemodynamics and potential second messengers in mediating the hypotensive effects. The aim of this study was to test the hypothesis that overexpression of B<sub>2</sub> receptor might result in enhancement of renal function via activation of nitric oxide (NO)-cGMP and/or cAMP signal transduction pathways in transgenic mice.

## METHODS

**Urine and serum collection.** Male transgenic mice and age- and gender-matched control littermates were placed in metabolic cages with free access to tap water for 24 h. Twenty-four-hour urine production was collected and centrifuged in a microtube at 1,000 *g* to remove particles. The volume of the supernatant was measured and stored at -20°C until analysis for nitrite/nitrate (NO<sub>x</sub>), cGMP, cAMP, and kinin. Urinary electrolytes were measured by flame photometry. After urine collection, mice were removed from the metabolic cage and anesthetized (pentobarbital sodium, 50 mg/kg body wt ip), and blood samples were collected by direct cardiac puncture and chilled at 4°C overnight. The blood samples were centrifuged at 1,000 *g* for 20 min, and sera were removed and frozen at -20°C and subsequently used for analysis of plasma electrolytes by flame photometry.

**Measurement of renal blood flow, glomerular filtration rate, and urine flow.** Mice (male, 3–4 mo old) were anesthetized with pentobarbital sodium (50 mg/kg body wt ip). Renal blood flow (RBF), glomerular filtration rate (GFR), and urine flow were measured as described previously with modification (40). Briefly, after tracheotomy, a PE-10 tube filled with saline solution containing heparin (100 U/ml) was inserted into the carotid artery for measurement of blood pressure and blood sampling. The bladder was cannulated to allow urine collection. Hydropenic preparations were maintained by constant infusion of 5% inulin (Inutest, Laevosan, Linz, Austria) in normal saline at 0.26 ml/h and constant infusion of 5% *p*-aminohippuric acid (PAH; Merck Sharp & Dohme, West Point, PA) in normal saline at 0.26 ml/h via a PE-10 tube in the jugular vein during the experimental period. After 60 min of equilibration, three consecutive 15-min urine collections were made. Blood was sampled at the end of the first and third collections. Inulin and PAH concentrations were measured by standard anthrone and colorimetric methods, respectively (14, 18). GFR and renal plasma flow were determined from the clearance of inulin and PAH, respectively. RBF was calculated from GFR and hematocrit. Clearance data were normalized to body weight.

**RIA of urinary cGMP, cAMP, and kinin.** The assays for cGMP and cAMP were conducted according to previously described procedures (7, 21, 25). The iodination was performed by adding 20 µl of 50 mmol/l phosphate buffer to 25 µg (in 10 µl of 0.5 mol/l potassium phosphate buffer, pH 7.4) of 2'-*O*-monosuccinylguanosine or 2'-*O*-monosuccinyladenosine 3',5'-cyclic monophosphate tyrosyl methyl ester (cGMP-TME or cAMP-TME; Sigma Chemical) and then 5 µl of Na<sup>125</sup>I (0.5 mCi). Twenty microliters of 0.01% chloramine-T (Sigma Chemical) solution were added to the mixture, and the mixture was incubated for 30 s. The reaction was stopped by addition of 50 µl of 25% acetic acid. The resulting mixture was subjected to C<sub>18</sub> reverse-phase HPLC to separate the iodinated cGMP-TME or cAMP-TME from free iodine. For cGMP RIA, standards for cGMP (10, 5, 2.5, 1.25, 0.63, 0.32, 0.16, and 0.08 nmol/l) and urine samples were acetylated by addition of 20 µl of triethylamine and 10 µl of acetic anhydride to each tube. Fifty-microliter aliquots of each acetylated standard and sample, 25 µl of diluted cGMP antiserum (1:14,400), and

25 µl of iodinated cGMP (15,000 cpm) were mixed in the assay tubes and incubated overnight at 4°C. The assay was stopped by addition of 50 µl of the 5× diluted human plasma containing 4 mmol/l of EDTA and then 1 ml of cold 12% polyethylene glycol. The tubes were vortexed and incubated at 4°C for 1 h before they were spun for 20 min at 1,000 *g* at 4°C. The supernatant was aspirated, and another 1 ml of 12% polyethylene glycol was added gently to each tube. Tubes were centrifuged as described above, the supernatants were aspirated, and the tubes were counted in a gamma counter. For cAMP RIA, 100 µl of cAMP standards (40, 20, 10, 5, 2.5, 1.25, 0.64, and 0.32 ng/ml) and urine samples were acetylated by addition of 10 µl of triethylamine-acetic anhydride (2:1) to each tube. After 10 min of incubation, 900 µl of 50 mM sodium acetate (pH 6.0) were added to the acetylated mixture. One hundred-microliter aliquots of each standard and sample, 100 µl of diluted cAMP antiserum (1:20,000), and 100 µl of iodinated cAMP (10,000 cpm) were mixed in the assay tubes and incubated overnight at 4°C. The assay was stopped by addition of 50 µl of 1% γ-globulin and 500 µl of 25% polyethylene glycol. The tubes were vortexed and incubated at 4°C for 1 h before they were spun for 30 min at 1,000 *g* at 4°C. The supernatant was aspirated, and the tubes were counted in a gamma counter. Urinary kinin levels were determined by a direct kinin RIA, as previously described (45). Briefly, 100 µl of <sup>125</sup>I-labeled tyrosyl-bradykinin (BK, 10,000 cpm/100 µl), 100 µl of rabbit antiserum against BK (1:100,000 dilution), 100 µl of diluted sample, and 100 µl of 0.1% egg albumin assay buffer (0.1% egg albumin, 10 mM EDTA, 10 mM 1,10-phenanthroline in PBS, pH 7.0) in a final volume of 400 µl were incubated at 4°C overnight. After addition of 400 µl of 1% bovine γ-globulin and 800 µl of 25% polyethylene glycol in PBS to the reaction mixture, free and antibody-bound BK were separated by centrifugation at 3,500 *g* for 30 min. The standard BK used ranged from 4 to 500 pg.

**Urinary NO<sub>x</sub> measurement.** Urinary NO<sub>x</sub> content was measured by a colorimetric assay based on the Griess reaction (44). Briefly, diluted urine samples were incubated with 0.1% acidified sulfanilamide and 0.2% *N*-(1-naphthyl)-ethylenediamine for 10 min at room temperature. Nitrite accumulation was monitored spectrophotometrically at 450 nm and compared with sodium nitrite standards.

**Tissue preparation and RIA of cGMP and cAMP.** Mice (male, 3–4 mo old) were killed by cervical dislocation. Kidneys were removed and homogenized in 0.1 N HCl with a Polytron (Brinkmann Instruments, Westbury, NY). The cGMP and cAMP levels in kidney extracts of B<sub>2</sub> receptor transgenic and control mice were determined according to previously described procedures (7, 21, 25).

**Northern blot analysis of angiotensinogen, renin, and angiotensin-converting enzyme mRNAs.** Mice (male, 3–4 mo old) were killed by cervical dislocation. Kidney, liver, and lung were quickly removed from transgenic and control mice, and total RNA was isolated by guanidine-CsCl gradient centrifugation (42). cDNA probes of rat angiotensinogen, renin, and angiotensin-converting enzyme (ACE) were used for Northern blot analysis, respectively. Total RNA (20 µg) in each lane was separated by electrophoresis on a 1.5% agarose gel containing 0.66 M formaldehyde. The blot was transferred onto the Immobilon-N membrane (Millipore, Bedford, MA) and hybridized under stringent conditions to a <sup>32</sup>P-labeled cDNA probe according to the instructions of the manufacturer (Bethesda Research Laboratories, Bethesda, MD). After hybridization at 60°C for 16–18 h, the membrane was washed with 0.5× saline-sodium phosphate-EDTA-0.1% SDS at 60°C and exposed to X-ray film at -80°C. Finally, the blot was rehybridized with a human β-actin cDNA probe (Clontech

Table 1. *Physiological analysis of human bradykinin B<sub>2</sub> receptor transgenic mice*

Variables	Control	Transgenic
Blood pressure, mmHg	98.6 ± 0.5	85.4 ± 0.2†
Body wt, g	22.6 ± 1.6	19.5 ± 0.8
Urine volume, μl · g body wt <sup>-1</sup> · day <sup>-1</sup>	53.9 ± 6.1	90.8 ± 5.8†
Urinary Na output, μmol · g body wt <sup>-1</sup> · day <sup>-1</sup>	37.2 ± 2.1	34.5 ± 2.3
Urinary K output, μmol · g body wt <sup>-1</sup> · day <sup>-1</sup>	80.0 ± 10.7	160.2 ± 10.6†
Urinary pH	6.6 ± 0.2	7.0 ± 0.2*
Serum Na concentration, mmol/l	142.5 ± 0.4	145.2 ± 1.6

Values are means ± SE. Statistical significance between groups was determined by Student's *t*-test; \* *P* < 0.05; † *P* < 0.01.

Laboratories, Palo Alto, CA). Densitometric analysis of autoradiographic films was performed in NIH Image 1.60/68k with Hewlett Packard ScanJet II cx/T.

*Statistical analysis.* Group data are expressed as means ± SE. Statistical analysis was performed by unpaired Student's *t*-test. Differences were considered significant at *P* < 0.05.

## RESULTS

*Physiological analysis of transgenic mice expressing human bradykinin B<sub>2</sub> receptor:* The physiological changes in bradykinin B<sub>2</sub> receptor transgenic mice were evaluated with respect to blood pressure, body weight, urine volume, urinary excretion of potassium and sodium, and serum potassium and sodium levels (Table 1). The systolic blood pressure, measured by the tail-cuff method, was 85.4 ± 0.2 (SE) mmHg (*n* = 20) for transgenic mice and 98.6 ± 0.5 mmHg (*n* = 25, *P* < 0.01) for negative siblings. The body weight did not show much difference between the two groups (19.5 ± 0.8 vs. 22.6 ± 1.6 g, *n* = 20 or 25). Urine excretion was significantly increased in B<sub>2</sub> receptor transgenic mice compared with their control littermates (90.8 ± 5.8 vs. 53.9 ± 6.1 μl · g body wt<sup>-1</sup> · day<sup>-1</sup>, *n* = 20 or 25, *P* < 0.01). Urinary potassium output of transgenic mice was increased by 100% compared with the control (160.2 ±

10.6 vs. 80.0 ± 10.7 μmol · g body wt<sup>-1</sup> · day<sup>-1</sup>; *n* = 6, *P* < 0.01), whereas urinary sodium output remained the same in these two groups. The pH for urine collected from transgenic mice was more basic than that for urine collected from control mice (7.0 ± 0.2 vs. 6.6 ± 0.2, *n* = 6, *P* < 0.05). No significant changes in serum sodium levels were observed between transgenic and control mice. Serum potassium levels were not obtained because of hemolysis during blood collection.

*Effects of bradykinin B<sub>2</sub> receptor overexpression on renal function.* To assess the effects of B<sub>2</sub> receptor overexpression on renal function of the transgenic mice, renal hemodynamics, including RBF, GFR, and urine flow, were measured in transgenic vs. control mice (Fig. 1). Overexpression of B<sub>2</sub> receptors caused a significant increase in urine flow compared with control mice (6.5 ± 0.7 vs. 4.2 ± 0.5 μl · min<sup>-1</sup> · 100 g body wt<sup>-1</sup>, *n* = 6 or 9, *P* < 0.05). RBF in B<sub>2</sub> receptor transgenic mice was increased by 1.5-fold compared with control mice (9.8 ± 1.5 vs. 6.5 ± 0.9 ml · min<sup>-1</sup> · 100 g body wt<sup>-1</sup>, *n* = 6 or 9, *P* < 0.05), and GFR increased by 1.8-fold compared with control littermates (0.7 ± 0.1 vs. 0.4 ± 0.1 ml · min<sup>-1</sup> · 100 g body wt<sup>-1</sup>, *n* = 6 or 9, *P* < 0.05).

*Effects of bradykinin B<sub>2</sub> receptor overexpression on urinary NO<sub>x</sub>, cGMP, cAMP, and kinin levels.* To investigate the potential signal transduction pathways underlying the physiological alterations in the transgenic mice, urinary kinins, NO<sub>x</sub>, cGMP, and cAMP levels were measured in transgenic and control mice (Fig. 2). Urinary NO<sub>x</sub> content significantly increased by 1.7-fold in transgenic mice compared with control mice (169.3 ± 10.9 vs. 97.8 ± 15.7 nmol · g body wt<sup>-1</sup> · day<sup>-1</sup>, *n* = 6 or 8, *P* < 0.01). Urinary cGMP levels increased by 2.7-fold in transgenic mice compared with control mice (346.8 ± 23.5 vs. 130.0 ± 21.1 pmol · g body wt<sup>-1</sup> · day<sup>-1</sup>, *n* = 6 or 8, *P* < 0.01). Urinary cAMP levels increased by 2.8-fold in transgenic mice compared with control mice (292.2 ± 53.5 vs. 102.7 ± 20.3 pmol · g body wt<sup>-1</sup> · day<sup>-1</sup>, *n* = 6 or 8, *P* < 0.01). However, urinary kinin levels remained

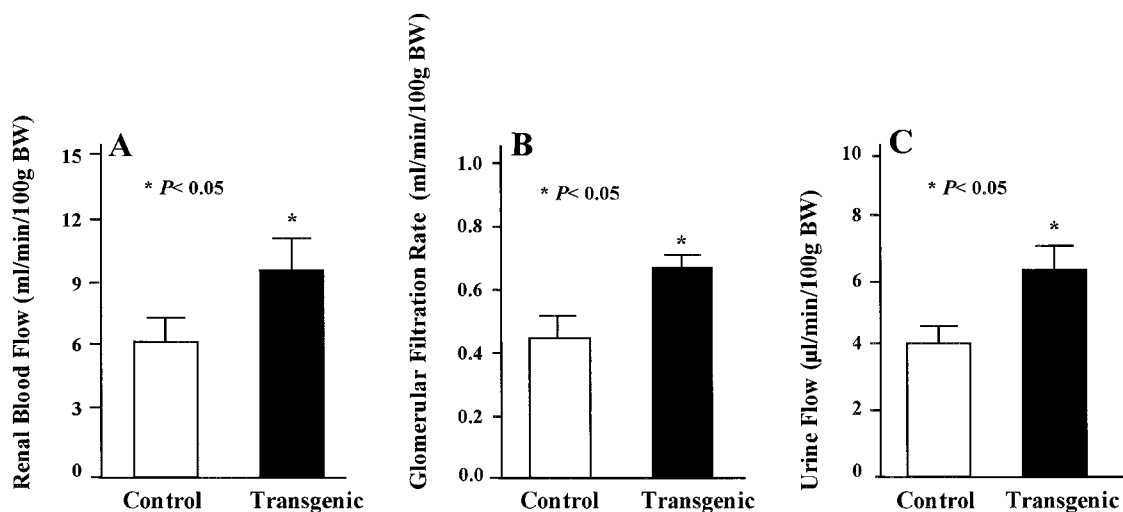


Fig. 1. Renal function of human bradykinin B<sub>2</sub> receptor transgenic mice. Renal blood flow, glomerular filtration rate, and urine flow of transgenic mice are increased compared with control littermates. BW, body wt. Values are means ± SE (*n* = 6 or 9).

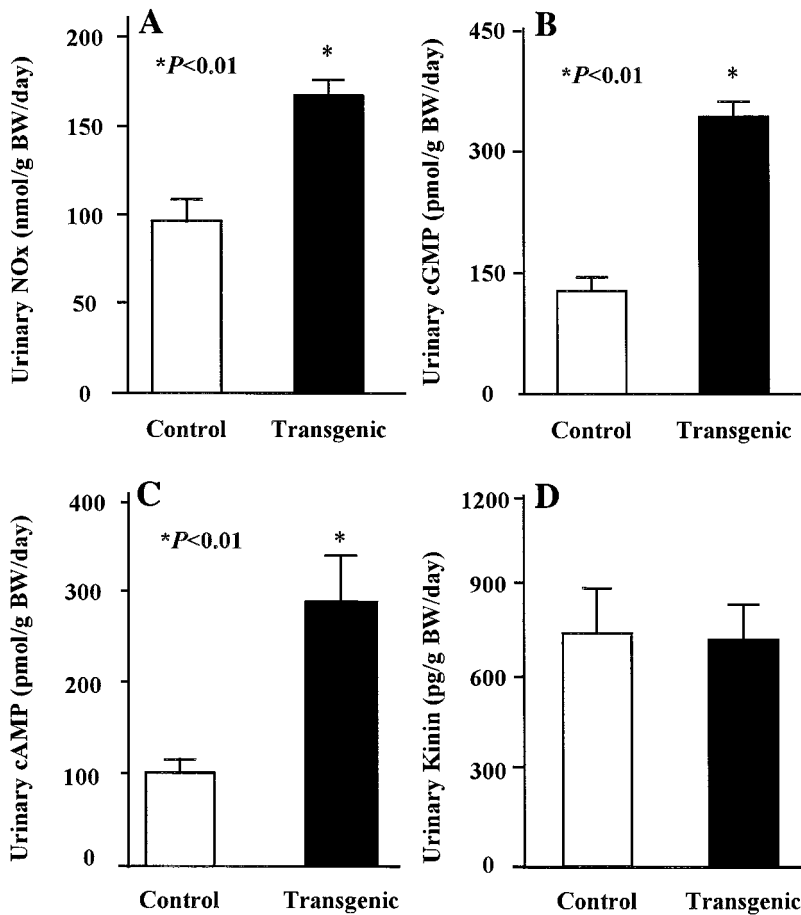


Fig. 2. Urinary nitrite/nitrate (NO<sub>x</sub>), cGMP, cAMP, and kinin levels in human bradykinin B<sub>2</sub> receptor transgenic mice. NO<sub>x</sub>, cGMP, cAMP, but not kinin levels in urine of transgenic mice are increased compared with control littermates. Values are means ± SE ( $n = 6$  or  $8$  for NO<sub>x</sub>, cGMP, and cAMP assays;  $n = 8$  or  $16$  for kinin measurement).

the same between transgenic and control groups ( $721.8 \pm 121.7$  vs.  $748.2 \pm 160.0$  pg·g body wt<sup>-1</sup>·day<sup>-1</sup>,  $n = 8$  or  $16$ ).

**Effects of bradykinin B<sub>2</sub> receptor overexpression on cGMP and cAMP levels in kidney extracts.** Renal cGMP and cAMP contents were measured by RIA. cGMP and cAMP levels in kidney extracts of transgenic mice were significantly increased (Fig. 3). Renal cGMP levels increased by 2.8-fold in transgenic mice compared with control mice ( $3.8 \pm 0.7$  vs.  $1.4 \pm 0.02$  pmol/mg protein,  $n = 6$  or  $8$ ,  $P < 0.05$ ). Renal cAMP levels increased by 1.5-fold in transgenic mice compared with control mice

( $34.6 \pm 2.6$  vs.  $22.4 \pm 4.2$  pmol/mg protein,  $n = 6$  or  $8$ ,  $P < 0.05$ ).

**Expression of the renin-angiotensin system.** Because the renin-angiotensin system is a counteracting hormonal system of the kallikrein-kinin system, we examined whether overexpression of B<sub>2</sub> receptors causes any compensatory changes of the renin-angiotensin system components in transgenic mice. The expression of angiotensinogen, renin, and ACE mRNA levels in transgenic and control mice was measured by Northern blot analysis (Fig. 4). Quantitative densitometry of the Northern blot showed that angiotensinogen mRNA in

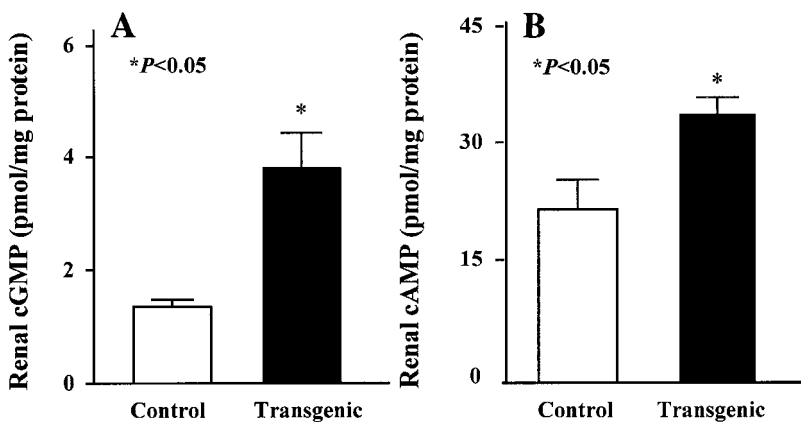


Fig. 3. Renal cGMP and cAMP levels in human bradykinin B<sub>2</sub> receptor transgenic mice. cGMP and cAMP levels in kidney extracts of transgenic mice are increased compared with control littermates. Values are means ± SE ( $n = 6$  or  $8$ ).

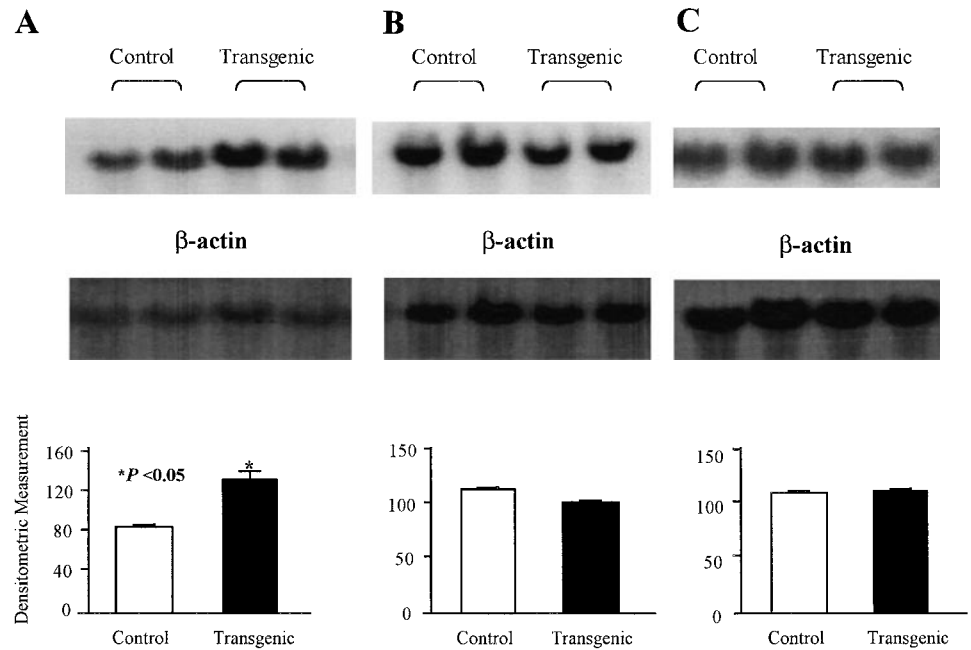


Fig. 4. Expression of hepatic angiotensinogen (A), renal renin (B), and pulmonary angiotensin-converting enzyme (C) mRNAs in human bradykinin B<sub>2</sub> receptor transgenic mice. *Top*: Northern blot autoradiography; *bottom*: densitometric plots.

the liver of transgenic mice increased by 1.5-fold compared with control mice ( $128.6 \pm 1.2$  vs.  $84.2 \pm 4.5$  densitometric units). No difference in renal renin and pulmonary ACE mRNA levels was detected between transgenic and control mice.

#### DISCUSSION

Hypertension is a polygenetic disease resulting from a complex interaction between genetic and environmental factors. Transgenic technology represents a powerful tool to study human genetic diseases in animal models (37). The present study showed that transgenic mice overexpressing human bradykinin B<sub>2</sub> receptor are hypotensive and exhibited augmented renal hemodynamics, as manifested by increased RBF, GFR, and urine flow. Enhanced renal functions were accompanied by increased vasorelaxation factors such as NO, cGMP, and cAMP in the kidney and urine. These studies indicated that overexpression of B<sub>2</sub> receptors in transgenic mice results in hypotension and increased renal function through activation of NO-cGMP and cAMP signal transduction pathways.

Hypertension is the major cause for development of end-stage renal failure, and limited renal injury may induce a permanent elevation of blood pressure. Therefore, reduction of blood pressure and attenuation of renal injury, as well as renal dysfunction, are equally important for the treatment of hypertensive renal diseases. The tissue kallikrein-kinin system is one of the major regulators for renal circulation (43). Previous studies employing bradykinin antagonist suggested that endogenous kinins contribute to the increase in renal blood flow through reduction of the renal vascular resistance (9, 18). Potential protective roles of the tissue kallikrein-kinin system to improve renal function have been manifested in our recent studies in which several hypertensive rat models with progres-

sive renal injury were used (11, 12, 39, 53). For example, somatic kallikrein gene delivery attenuated hypertension, slowed progression of renal damage, and significantly improved renal function in two-kidney, one-clip Goldblatt rats with renovascular hypertension, Dahl salt-sensitive rats with salt-induced chronic renal failure, and gentamicin-induced nephrotoxic rats with acute renal failure (12, 39, 53). Long-term infusion of rat urinary kallikrein via protein therapy was also reported to attenuate renal injury in Dahl salt-sensitive rats (48). The alterations induced by kallikrein infusion were potentiated by the concomitant administration of the ACE inhibitor (48). These findings lend support to the hypothesis that the tissue kallikrein-kinin system could offer a protective effect in hypertension-induced renal injury. This notion is also supported by studies demonstrating renal-protective effects of renin-angiotensin inhibition in experimental hypertensive animals (26). ACE inhibitor or ANG II (AT<sub>1</sub>) receptor antagonist ameliorated renal damage and enhanced renal function in experimental renal diseases. Conversely, ANG II infusion exacerbated renal function and progressed to glomerulosclerotic lesions (26). Taken together, these findings suggest that activation of the vasodilator kallikrein-kinin system and inhibition of the vasoconstrictor renin-angiotensin system could produce protective effects on renal function.

Our present study showed that bradykinin B<sub>2</sub> receptor overexpression enhanced renal function and significantly increased RBF, GFR, and urine flow. Protection of the kidney from renal injury and improvement of renal function have been demonstrated by somatic delivery of tissue kallikrein gene in our previous studies (11, 12, 39, 53). We examined histological sections of the adult mouse kidney and found well-preserved kidney morphology in cortex and medulla of the B<sub>2</sub> recep-

tor transgenic mice (unpublished data). It has been recognized that diabetic nephropathy is associated with increased glomerular basement membrane (GBM) thickness, glomerular volume, and total mesangial volume (4). Glomerular sclerosis in diabetes is due to glomerular hyperfiltration, resulting from high glomerular capillary blood flow and high glomerular capillary hydraulic pressure (5). High pressure challenges the GBM and, thus, causes hyperfiltration and renal injury. Increases in RBF and GFR in the early stage of diabetes followed by GBM thickening and GFR decline with the onset of proteinuria appear within 2.5 yr of onset of diabetes in humans (23). The long-term effect of high RBF and GFR in these B<sub>2</sub> receptor transgenic mice will be further studied by examining their renal histology in response to various factors including experimental diabetes.

We previously generated two transgenic animal models overexpressing human tissue kallikrein in addition to the B<sub>2</sub> receptor transgenic mice (47, 51, 52). Although all these animal models are hypotensive, a threshold on blood pressure was observed. Therefore, it is likely that compensatory mechanisms may occur in response to augmentation of the kallikrein-kinin system. Because the renin-angiotensin system is the most important system to counteract against the kallikrein-kinin system (9), we examined the expression of this system in the B<sub>2</sub> receptor transgenic mice by Northern blot analysis. Our results showed elevated expression of hepatic angiotensinogen mRNA in B<sub>2</sub> receptor transgenic mice compared with control mice, with no detectable changes in renin and ACE expression (Fig. 4). These findings indicate that the renin-angiotensin system may be involved in blood pressure threshold and sodium homeostasis in the transgenic mice overexpressing the kallikrein-kinin components. Whether other counteracting systems are also activated in these transgenic mice awaits further investigation.

Kinin is a natriuretic and diuretic peptide (43). Consistent with this notion, we observed significant increases in urine excretion and potassium excretion in transgenic mice compared with their nontransgenic littermates (Table 1). However, the sodium excretion was not altered. The dissociation between RBF and sodium excretion has been shown to occur during chronic infusion of bradykinin or secretin (22, 32). Moreover, a lack of diuresis and natriuresis was observed in transgenic mice overexpressing the atrial natriuretic peptide gene (36). Regulation of renal hemodynamics and handling of salt and water balance resulted from interactions among multiple hormonal systems. The renin-angiotensin system could enhance sodium reabsorption and potassium excretion in renal tubules. When injected intravenously or locally into the renal artery, kinin induced an increase in plasma renin levels (19). Animals with chronic B<sub>2</sub> receptor blockade, B<sub>2</sub> receptor deficiency, or kininogen deficiency showed enhanced blood pressure responses to ANG II (29, 30, 31). Some of the cardiovascular effects of ACE inhibition were mediated by blockade of ANG II formation and concomitant enhancement of kinin production (28).

The renin-angiotensin system might be responsible for buffering the renal excretory function of the kallikrein-kinin system in B<sub>2</sub> receptor transgenic mice.

We showed that urinary kinin levels in B<sub>2</sub> receptor transgenic mice were similar to those in control mice (Fig. 2). This finding is consistent with the study in which transgenic mice deficient in the B<sub>2</sub> receptor excreted the same amount of urinary kinin as control mice (16). These results suggest that there was no feedback regulation on kinin formation by B<sub>2</sub> receptor overexpression or deficiency. Therefore, without increased kinin production, the hypotensive effect of B<sub>2</sub> receptor overexpression is likely to be achieved by amplification of the intracellular signaling downstream to the B<sub>2</sub> receptor. Similar to the β<sub>2</sub>-adrenergic receptor, overexpression of B<sub>2</sub> receptors might also lead to the increase of receptor spontaneous activation without the agonist and, thereafter, elevation of second-messenger levels and production of physiological responses (38).

In the present study we showed a significant increase in urinary and renal cGMP and cAMP as well as urinary NO<sub>x</sub> levels in B<sub>2</sub> receptor transgenic mice. It has been known that NO and cGMP are the vascular relaxing factors that mediated the modulation of kinin on vascular smooth muscle tone. Moreover, NO-induced cGMP accumulation was found in vascular smooth muscle cells (13). Acute infusion of icatibant, a B<sub>2</sub> receptor-specific antagonist, reduced cGMP levels in the renal interstitial fluid (46). Also, fewer vasopressor responses were elicited toward the administration of the NO synthase inhibitor N<sup>ω</sup>-nitro-L-arginine methyl ester in the B<sub>2</sub> receptor knockout mice than in control mice (30). In the vasculature, endothelial cells constitutively express bradykinin B<sub>2</sub> receptors that are coupled to multiple signal transduction pathways (8, 41). Binding of kinin to B<sub>2</sub> receptors activates phospholipase C and, thereby, hydrolyzes phosphatidylinositol 4,5-bisphosphate to the signaling mediators inositol 1,4,5-trisphosphate and diacylglycerol. Inositol 1,4,5-trisphosphate triggers a rapid increase of intracellular calcium concentrations, which stimulates NO synthesis and release (8, 14). Increased NO production enhances the formation of cGMP in vascular smooth muscle cells, which activates cGMP-dependent protein kinase. This leads to increased extrusion of intracellular calcium in vascular smooth muscle cells, inhibition of the contractile machinery, and finally relaxation of vasculature (50). Moreover, cGMP-dependent protein kinase also phosphorylates potassium channels to induce hyperpolarization and, thereby, inhibits vasoconstriction (49). Kinin has also been shown to enhance cAMP formation in cultured vascular smooth muscle cells (15). Coupling of B<sub>2</sub> receptors stimulates phospholipase A<sub>2</sub> to release arachidonic acid, the precursor of vasoactive mediator prostacyclin, and then the release of cAMP (8). cAMP is a potent vasodilator, and increased cAMP leads to extrusion of intracellular calcium and, thus, vasorelaxation (1, 49). These combined findings suggest that signal transduction pathways mediated by cGMP and cAMP are involved in blood pressure reduction and enhanced renal hemodynamics in bradykinin B<sub>2</sub> recep-

tor transgenic mice. These findings are consistent with our previous study showing that the action of the kallikrein-kinin system in blood pressure reduction after potassium supplementation was mediated by the same signaling pathways (Fig. 7 in Ref. 27).

This work was supported by National Heart, Lung, and Blood Institute Grant HL-52196.

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Received 20 May 1999; accepted in final form 19 October 1999.

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