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Induction of Functional Bradykinin B₁-Receptors in Normotensive Rats and Mice Under Chronic Angiotensin-Converting Enzyme Inhibitor Treatment

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Background—The physiological effects of ACE inhibitors may act in part through a kinin-dependent mechanism. We investigated the effect of chronic ACE-inhibitor treatment on functional kinin B₁- and B₂-receptor expression, which are the molecular entities responsible for the biological effects of kinins.

Methods and Results—Rats were subjected to different 6-week treatments using various mixtures of the following agents: ACE inhibitor, angiotensin AT₁-receptor antagonist, and B₁- and B₂-receptor antagonists. Chronic ACE inhibition induced both renal and vascular B₁-receptor expression, whereas B₂-receptor expression was not modified. Furthermore, with B₁-receptor antagonists, it was shown that B₁-receptor induction was involved in the hypotensive effect of ACE inhibition. Using microdissection, we prepared 10 different nephron segments and found ACE-inhibitor-induced expression of functional B₁-receptors in all segments. ACE-inhibitor-induced B₁-receptor induction involved homologous upregulation, because it was prevented by B₁-receptor antagonist treatment. Finally, using B₂-receptor knockout mice, we showed that ACE-inhibitor-induced B₁-receptor expression was B₂-receptor independent.

Conclusions—This study provides the first evidence that chronic ACE-inhibitor administration is associated with functional vascular and renal B₁-receptor induction, which is involved in ACE-inhibitor-induced hypotension. The observed B₁-receptor induction in the kidney might participate in the known renoprotective effects of ACE inhibition. (*Circulation*. 2002;105:627-632.)

Key Words: angiotensin-converting enzyme ■ bradykinin ■ kidney ■ blood pressure

It is now well recognized that the kallikrein kinin system acts at both the vasculature and in peripheral tissues, including heart, kidney, lung, and brain.¹ Many tissues have the capacity to generate de novo kallikrein kinin system expression and therefore to locally produce bradykinin (BK). BK and its carboxypeptidase M product, des-Arg⁹[BK] (DBK), exert their biological effects by binding respectively to the G-protein-coupled B₂- and B₁-receptors.² The main known difference between B₂- and B₁-receptors is that the B₂-receptor is constitutively expressed, whereas the B₁-receptor is weakly detectable under physiological conditions but strongly expressed in pathological states.³ Thus, until now, most studies have focused on the effects of B₂-receptor activation, whereas the B₁-receptor has received less attention.

In blood pressure (BP) regulation, it has been proposed that the kallikrein kinin system counterbalances the vasoconstrictor renin angiotensin system.² The major link between these 2 systems is angiotensin-converting enzyme (ACE), because this enzyme is able to generate the vasoconstrictor peptide

angiotensin II (Ang II) and to inactivate the vasodilator peptide BK.⁴ The vasodilator action of BK via activation of its B₂-receptor has been well demonstrated.^{1,2} Activation of the inflammation-induced B₁-receptor also generally results in hypotension.⁵

ACE inhibitors are efficient therapeutic agents in the management of hypertension, heart failure, and diabetic nephropathy.^{6–8} An important point concerning ACE inhibition that should be taken into account is that ACE-inhibitor treatment not only decreases Ang II and increases BK concentrations but also favors the generation of the B₁-receptor agonist DBK by the enzyme kininase I.³ Despite convincing data showing that ACE-inhibitor treatment increases the circulating BK concentration⁹ and potentiates the hemodynamic effects of exogenous BK administration,¹⁰ as well as that the short-term hypotensive effects of ACE inhibitors in normotensive and hypertensive subjects are BK dependent,^{7,11} the respective roles of B₁- and B₂-receptors in these protective effects induced by ACE inhibitors have not been determined. The majority of available data report

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involvement of the B₂-receptor in the protective effects of ACE inhibitors in cardiovascular pathologies¹² and diabetic nephropathy.^{13,14} To the best of our knowledge, the role of the B₁-receptor in the effects of ACE inhibitors has not been studied.

Because the kidney is a major target organ in hypertensive and diabetic pathologies and could be considered a predictor of cardiovascular risk,¹⁵ the main objective of the present study was to analyze whether chronic ACE inhibition affects renal B₁- and B₂-receptor expression in rats and mice. The availability of specific BK-receptor antagonists and B₂-receptor knockout mice (KOB2) allowed us to answer this question clearly. We report here for the first time that chronic ACE inhibition can induce functional renal B₁-receptor expression in normotensive rats and mice, and we present evidence that the hypotensive effect of ACE inhibition involves B₁-receptor activation. B₂-receptor expression under these conditions was not modified.

Methods

Animal Treatments

Rats

Twelve groups (n=7 animals per group) of male Sprague-Dawley rats (weight 205 to 212 g) were used. They received a normal sodium diet (UAR A.40, 104 mmol of Na⁺ per kilogram). Food and water intake were monitored daily. The ACE inhibitor ramipril (Hoechst) and AT₁-receptor antagonist irbesartan (Sanofi) were added to drinking water, whereas both B₁- and B₂-receptor antagonists were injected daily by a single subcutaneous injection for 6 weeks. All experiments were conducted as stated in the *NIH Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Academy Press, Washington, DC) and were approved by a local animal care and use committee. At the end of the experimental period, mean arterial BP was monitored as described previously.¹⁶ Under our experimental conditions, we verified that anesthesia with inactine did not produce a depressor effect and that direct mean BP determined under anesthesia was correlated to the tail-cuff systolic BP. Body weight, ACE activity, and blood glucose level were also determined. ACE activity in plasma was measured as described previously.¹⁷ Blood glucose was determined with the kit Glu 1108 (Test Strips, One Touch, Lifescan).

Mice

KOB2 mice were generously provided by Dr Fred Hess (Merck & Co Inc, Rahway, NJ).¹⁸ KOB2 mice were originally on a mixed genetic background (J129sv X C57Bl/6J). We have backcrossed (10 times) the KOB2 mice to C57Bl/6J and therefore used C57Bl/6J as control mice. The mice were housed in a pathogen-free environment (SPF). Each group contained 10 mice, and ramipril was given in drinking water at 10 mg · kg⁻¹ · d⁻¹. Measurements of BP were performed in unanesthetized mice by the tail-cuff plethysmography method.

Microdissection of Rat Nephron Segments

Microdissection was performed as previously described by our laboratory.¹⁹ The following nephron segments were isolated: glomeruli, efferent arteriole, proximal convoluted tubule, proximal straight tubule, medullary thin descending limb, inner medullary thin limb, medullary thick ascending limb, distal tubule, and cortical and outer medullary collecting ducts. Glomerular and tubular surfaces were measured as described previously.¹⁹

mRNA Expression Analysis

Total RNA was extracted with the RNeasy kit (Qiagen). RNase protection was performed on 200 μg of total RNA from kidney

tissue, carotid artery, aorta, and left ventricle with the RPAIII kit (Ambion). Semiquantitative B₁- and B₂-receptor and GAPDH mRNA expression in microdissected nephron segments (1 glomerulus, surface 480 ± 80 μm², or a total surface of 480 ± 89 μm² of each renal tubule segment) was analyzed and semiquantified by reverse transcription-polymerase chain reaction (RT-PCR) followed by Southern blot analysis as described previously.¹⁹

Quantitative Competitive PCR

Quantitative competitive PCR (QC-PCR) was performed with an internal competitive template of 304 bp according to a previously described protocol.²⁰ QC-PCR contained 1 μL of 500, 100, 50, 10, 5, and 1 fg and 500 ag of a synthetic DNA competitor. The primer sequences for the mouse B₁-receptor were as follows: upstream primer, 5'-CGGAAGCCTGGGATCTGCTGTG-3' (nucleotides 173 to 194, Genbank U47281); downstream primer, 5'-CCAGCAACCTGTAGCGGTCC-3' (nucleotides 507 to 526). PCR products were analyzed on 2% agarose gels stained with ethidium bromide and quantified by densitometric analysis. B₁-receptor mRNA concentration was calculated by determining where the logarithmic ratio of endogenous B₁-receptor mRNA expression (amplified 354 bp) and competitor expression (304 bp) was equal. PCR-amplified products were sequenced to confirm that the PCR bands corresponded to B₁-receptor mRNA.

Production and Measurement of Prostaglandin E₂ Production by Rat Microdissected Tubules

An equal surface of each renal tubule segment was transferred to a reaction tube with 55 μL of solution 1 (135 mmol/L NaCl, 1 mmol/L Na₂SO₄, 1.2 mmol/L MgSO₄, 5 mmol/L KCl, 2 mmol/L CaCl₂, 5.5 mmol/L glucose, and 5 mmol/L HEPES pH 7.4) containing the drug to be tested at 0.2 μmol/L, followed by rapid volume adjustment with solution 1 to 110 μL to give a final drug concentration of 0.1 μmol/L. Then, tubes were transferred rapidly to a 37°C water bath for 10 minutes. The incubation was stopped by freezing at -80°C until prostaglandin E₂ (PGE₂) measurements were performed. After they were thawed, the samples were centrifuged, and 60 μL of supernatant was used to measure the PGE₂ concentration with enzyme immunoassay kits (EIA, Cayman Chemical). Pellet protein concentration was measured by the dye-blue-binding method (BioRad Laboratories) after solubilization for 1 hour with 1N NaOH.

Statistical Analysis

Data are presented as mean ± SEM. Statistical analyses were performed with SPSS software (Statistical Package for the Social Sciences, SPSS Inc). ANOVA (2-way ANOVA analysis, repeated measurements) with a post hoc Tukey α-test was performed for comparison between the different groups. P < 0.05 was considered statistically significant.

Results

Efficiency of Pharmacological Treatments

The 6-week treatment with ACE inhibitor resulted in a complete inhibition of circulating ACE activity that was not changed in the presence of B₁- or B₂-antagonists (Figure 1). No significant changes were found in blood glucose (mean value for all groups 0.75 ± 0.09 g/L) and body weight (mean value for all groups 342 ± 15 g). No effect on the histological structure of the kidneys was observed (no sign of hypertrophy or of glomerular or tubulointerstitial alterations; data not shown).

Both B₁- and B₂-Receptors Are Involved in Chronic ACE-Inhibition-Induced Hypotension in Rats

Figure 2 shows BP measured at the end of the different 6-week treatments. As expected, chronic treatment with ACE inhibitors

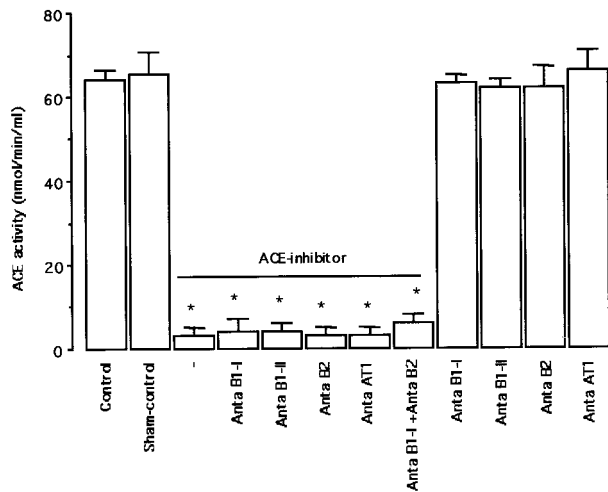


Figure 1. ACE activity in rats treated by different drugs alone or in combination as indicated. ACE inhibitor indicates ramipril (1 mg · kg⁻¹ · d⁻¹); Anta B1-I (des-Arg9Leu8[BK], 500 μg · kg⁻¹ · d⁻¹) and Anta B1-II (R715, 25 μg · kg⁻¹ · d⁻¹) are B₁-receptor antagonists; Anta B2, HOE140, B₂-receptor antagonist icatibant (500 μg · kg⁻¹ · d⁻¹); and Anta AT1, AT₁-receptor antagonist irbesartan (10 mg · kg⁻¹ · d⁻¹). Values are mean ± SEM; n=7. *P<0.01 vs control.

induced a substantial decrease in BP (-47 ± 10 mm Hg). No additional effect of ACE inhibitor plus AT₁-receptor antagonist was found. As expected, treatment with the AT₁-receptor antagonist alone decreased BP. Interestingly, the ACE-inhibitor-induced decrease in BP was partly reduced (≈50%) in the 2 groups of rats treated with 2 different B₁-receptor antagonists, des-Arg9-Leu8[BK] and R715, as well as in the group treated with the B₂-antagonist HOE140. Furthermore, the hypotensive effect of ACE inhibition was completely prevented with an equimolar mixture of both B₁- and B₂-receptor antagonists. Administration of B₁- or B₂-receptor antagonists alone was without effect on BP. These results suggest that both B₁- and B₂-receptors are involved in the hypotensive effects of ACE inhibitors.

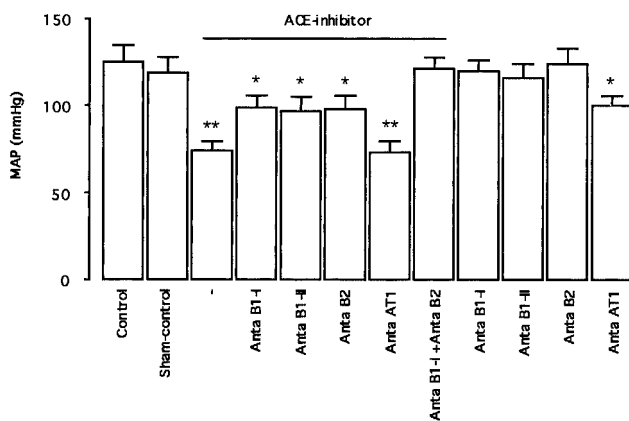


Figure 2. Mean arterial BP (MAP) in rats treated by different drugs alone or in combination as indicated. Abbreviations as in Figure 1. Values are mean ± SEM; n=7. *P<0.05, **P<0.01 vs control.

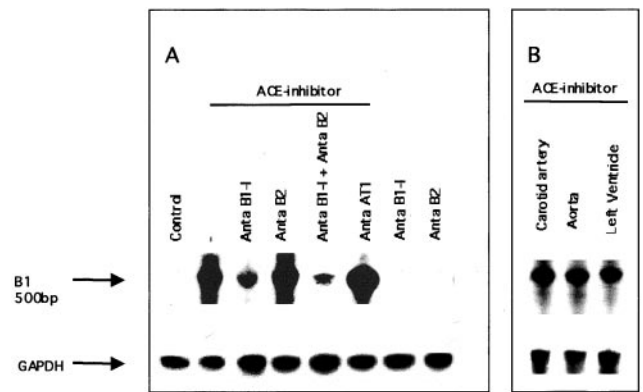


Figure 3. Detection of B₁-receptor mRNA by RNase protection in (A) whole kidney from rats subjected to different treatments and (B) cardiovascular tissues from ramipril-treated rats. Autoradiographs shown are representative of 7 independent experiments. Abbreviations as in Figure 1.

Quantitative B₁-Receptor mRNA Analysis in Whole Kidney, Carotid Artery, Aorta, and Left Ventricle

To verify whether chronic ACE inhibition in these rats modified kinin receptor mRNA expression, we performed RNase protection analysis in whole kidney (Figure 3A) and in some cardiovascular tissues (Figure 3B). Six weeks of ACE-inhibitor treatment induced significant renal B₁-receptor mRNA expression compared with control rats. Concomitant B₁-receptor antagonist treatment strongly decreased this ACE-inhibitor-induced B₁-receptor mRNA induction.

In contrast, B₂-receptor and AT₁-receptor antagonist treatment were without effect on ACE-inhibitor-induced B₁-receptor mRNA expression. Combined B₁- and B₂-receptor antagonist treatment in the presence of ACE inhibitor prevented B₁-receptor mRNA induction. Separate treatment with the B₁- or B₂-receptor antagonists was without effect on B₁-receptor mRNA expression. The different treatments were without effect on GAPDH mRNA expression. Parallel RNase protection analysis of B₂-receptor expression showed that the different treatments were without effect on B₂-receptor mRNA expression levels (data not shown). This B₁-receptor mRNA induction was not restricted to the kidney level, because significant B₁-receptor mRNA expression was observed in 3 different cardiovascular tissues (Figure 3B) after ACE-inhibitor treatment that was not observed in control rats (not shown).

Chronic ACE Inhibition Induces B₁-Receptor mRNA Expression in Microdissected Nephron Segments

To identify more precisely the renal location of the induced B₁-receptor mRNA after ACE inhibition, we performed semiquantitative B₁-receptor mRNA expression analysis using an RT-PCR/Southern blot approach on microdissected nephron segments. Although no renal B₁-receptor mRNA could be detected along the nephron (Figure 4A) under our control conditions, ACE inhibition induced significant B₁-receptor mRNA expression in all nephron segments studied (Figure 4C). Controls (Figure 4B and D) for contaminating genomic DNA amplification and relative quantification with GAPDH were performed as described previously.¹⁹ As eval-

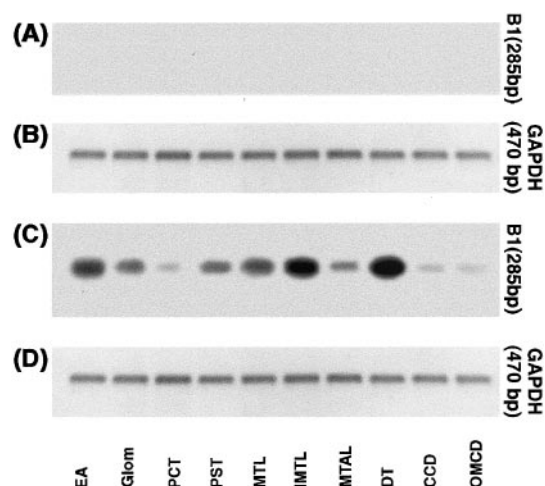


Figure 4. B₁-receptor and GAPDH mRNA expression (detected as described in Methods) in microdissected nephron segments of control (A, B) and ramipril-treated (C, D) rats. EA indicates efferent arteriole; Glom, glomeruli; PCT, proximal convoluted tubule; PST, proximal straight tubule; MTL, medullary thin descending limb; IMTL, inner medullary thin limb; MTAL, medullary thick ascending limb; DT, distal tubule; CCD, cortical collecting duct; and OMCD, outer medullary collecting duct.

uated by this method, high B₁-receptor mRNA expression was observed in the efferent arteriole, glomeruli, medullary thin descending limb, inner medullary thin limb, and distal tubule; moderate expression was found in the proximal convoluted tubule, proximal straight tubule, medullary thick ascending limb, and cortical collecting duct; and low expression was observed in the outer medullary collecting duct after ACE inhibition. As previously reported,¹⁹ expression of B₂-receptor mRNA was observed in all nephron segments of control rats, and ACE inhibition was without significant effect in B₂-receptor mRNA in the different nephron segments (data not shown).

ACE Inhibition Results in Induction of Functional B₁-Receptors Along the Nephron

To verify whether induction of B₁-receptor mRNA by ACE inhibitors is effectively translated into functional B₁-receptors, the ability of the B₁-receptor agonist DBK to stimulate PGE₂ secretion was examined on microdissected nephron segments (Figure 5). B₁-receptor stimulation had no effect on PGE₂ secretion in microdissected nephrons obtained from untreated rats compared with basal PGE₂ production. In contrast, a significant increase in PGE₂ secretion after B₁-receptor stimulation was observed in the efferent arteriole, glomeruli, proximal convoluted tubule, proximal straight tubule, medullary thin descending limb, inner medullary thin limb, distal tubule, cortical collecting duct, and outer medullary collecting duct from ACE-inhibitor-treated animals. No effect of B₁-receptor stimulation on PGE₂ production was observed in the medullary thick ascending limb. B₁-receptor-induced stimulation of PGE₂ secretion was inhibited in the presence of the B₁-receptor antagonist des-Arg⁹-Leu⁸[BK], whereas this antagonist alone was without effect on PGE₂ secretion (data not shown).

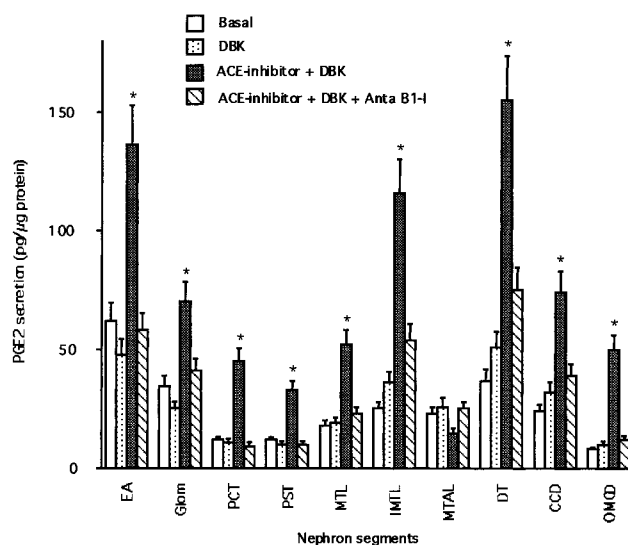


Figure 5. PGE₂ secretion by isolated nephron segments from control and ACE-inhibitor-treated rats under basal conditions or stimulated (5 minutes) by B₁-receptor agonist DBK at 0.1 μmol/L in presence or absence of Anta B1-I (des-Arg⁹Leu⁸[BK]) at 1 μmol/L. Abbreviations as in Figure 4. Values are mean ± SEM; n=7. *P<0.01 vs respective control (basal value).

Chronic ACE Inhibition Induces B₁-Receptor mRNA Expression in KOB2 Mice

The results displayed in Figures 2 and 3 suggest that induction of the B₁-receptor by ACE inhibitors can occur independently of B₂-receptor activation. This hypothesis was tested by treating KOB2 and wild-type mice for 6 weeks with the ACE inhibitor ramipril. Under physiological conditions, there was no difference in BP between control and KOB2 mice, but ACE inhibition induced a similar significant decrease in BP of both control and KOB2 mice (Figure 6A). When QC-PCR was used, no renal B₁-receptor mRNA expression was detectable in wild-type mice, whereas basal B₁-receptor mRNA expression was found in KOB2 mice (Figure 6B). ACE inhibition induced renal B₁-receptor mRNA expression in both wild-type and KOB2 mice, although this was significantly higher in KOB2 than in wild-type mice (Figure 6B). B₁-receptor mRNA induction by ACE inhibition, therefore, does occur independently of the B₂-receptor.

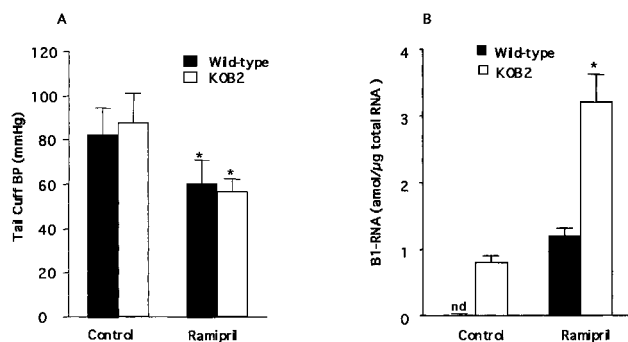


Figure 6. Effect of 6 weeks of ramipril treatment in wild-type and KOB2 mice on (A) BP and (B) B₁-receptor mRNA expression quantified by QC-PCR. Values are mean ± SEM, n=10; *P<0.05 vs respective control. nd indicates not detectable.

Discussion

The present study provides evidence for the first time that chronic ACE inhibition can induce functional renal and vascular kinin B₁-receptors. Furthermore, these newly synthesized B₁-receptors are involved in the hypotensive action of ACE inhibition. No effect of chronic ACE inhibition was observed on B₂-receptor expression.

In this article, evidence for B₁-receptor induction by chronic ACE inhibition was presented on the level of B₁-receptor mRNA expression, on the basis of renal B₁-receptor activity (PGE₂ production), and on the basis of the ability of 2 different B₁-receptor antagonists to partially reverse ACE-inhibitor-induced hypotension. The ability of B₁-receptor antagonists to reverse ACE-inhibitor-induced hypotension correlates with the often-observed B₁-agonist-induced hypotension under inflammatory conditions.⁵ Depending on the animal species and the vascular bed studied, the mechanism underlying this B₁-receptor-induced vasodilator effect involves either prostaglandins or nitric oxide.^{3,5} Studies on the short-term effect of ACE inhibition on B₁-receptor expression in rabbits gave conflicting results. In Nwator and Whalley,²¹ ACE inhibition (3 to 20 hours IV) resulted in an increased response to the B₁-agonist, whereas under similar conditions, another laboratory³ observed no effect on B₁-receptor expression (mRNA, hemodynamic response to B₁-agonist administration).

Simultaneous chronic AT₁-receptor antagonism and ACE inhibition did not have an additive effect on BP, but chronic AT₁-receptor antagonism lowered BP when used alone, thereby demonstrating the effectiveness of the treatment and the drug under our experimental conditions. In general, long-term combined ACE inhibitor and AT₁-receptor antagonist treatment under pathological conditions in humans and in experimental pathologies in animals is more efficient to reduce BP than treatment with one of these drugs alone (congestive heart failure²²; essential hypertension²³; patients with hypertension, microalbuminuria, and non-insulin-dependent diabetes²⁴; and spontaneously hypertensive rats²⁵). In contrast, as observed in the present study on normotensive rats, 4 weeks of combined treatment with ACE inhibitor and AT₁-receptor antagonist in normotensive patients with diabetic nephropathy or glomerulonephritis was without further effect on BP, whereas the glomerular filtration rate was efficiently increased in this "add-on" treatment.²⁶ It thus seems that long-term ACE inhibitor and AT₁-receptor antagonist treatment are more efficient in reducing BP than separate drug treatment in several pathologies but not under normotensive conditions. In contrast to the absence of an add-on effect on BP in chronic combined ACE inhibitor and AT₁-receptor antagonist treatment under normotensive conditions, a single dose of both drugs was found to have an additive effect on BP in sodium-depleted (ie, renin-angiotensin system activation) healthy normotensive humans and lasted up to 6 hours.²⁷ An observation in the present study that might be related to the absence of an add-on effect is that ACE inhibition in the presence of both a B₁- and B₂-receptor antagonist returned BP to the value of nontreated animals. This suggests the absence or loss of the role of Ang II

blockade in chronic ACE-inhibitor-induced hypotension, possibly explaining the absence of an add-on effect.

The most important observation of the present study is the ACE inhibitor-induced functional BK B₁-receptor expression. This induction could be mediated by stimulation of preexisting B₁-receptors by increased B₁-agonist formation due to chronic ACE-inhibitor treatment,⁹ which confirms, *in vivo*, previous studies that the B₁-agonist can induce the expression of its own receptor.^{28,29} The induction is probably not due to an inflammatory reaction, at least in the kidney, because histomorphometric kidney analysis did not show signs of such a reaction after chronic ACE inhibition. ACE-inhibition-induced B₁-receptor induction is not mediated by the B₂-receptor, because we observed that ACE inhibition induced significant B₁-receptor expression in KOB2 mice. Interestingly, using QC-PCR, we observed basal B₁-receptor expression in the kidney of nontreated KOB2 mice. The B₂-receptor might thus negatively control B₁-receptor expression. Our observation using a quantitative method confirms a recent study in which nonquantitative RT-PCR analysis also showed basal B₁-receptor expression in KOB2 mice.³⁰

A large number of studies have described the protective cardiovascular and renal effects of ACE inhibitors, which are increasingly attributed, at least in part, to kinin potentiation.^{8,12-14} The beneficial effects of ACE inhibitors are not solely due to the reduction of BP, and because ACE inhibitors exert tissue protective effects in diabetic nephropathy independently of their vasodepressive effects,^{13,14} we sought to determine whether chronic ACE inhibition had an effect on renal BK receptor expression. Although there was no effect on renal B₂-receptor expression, functional B₁-receptors were induced in all nephron segments studied. What could be the role of renal B₁-receptor induction under physiological or pathological conditions? It has been reported that activation of renal B₁-receptors increases renal vascular resistance, which suggests that the B₁-receptor might be important in the maintenance of renal vasoconstriction in pathologies that lead to renal failure.^{16,31} Finally, a protective role for the B₁-receptor in the development of end-stage renal diseases has been proposed recently with the use of polymorphism analysis.³²

In addition to the well-known involvement of the B₁-receptor in inflammation and hyperalgesia, because of which B₁-receptor antagonists may be clinically useful as anti-inflammatory and analgesic drugs,³ other pathologies (mainly, cardiovascular and some renal pathologies) associated with cell enlargement and proliferation might benefit from B₁-receptor activation. Indeed, recent studies have reported the involvement of the B₁-receptor in the inhibition of neointima formation.³³ In addition to the evidence for a role of the B₁-receptor in ACE-inhibitor-induced hypotension, ACE-inhibition-induced renal B₁-receptor expression could thus participate in the other "beneficial effects" attributed to ACE inhibitors.

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