Journal of the American College of Cardiology
© 2002 by the American College of Cardiology Foundation
Published by Elsevier Science Inc.
Vol. 40, No. 1, 2002
ISSN 0735-1097/02/$22.00
PII S0735-1097(02)01928-9

Down-Regulation of Cardioprotective Bradykinin Type-2 Receptors in the Left Ventricle of Patients With End-Stage Heart Failure

Antti Kuoppala, MD,* Naotaka Shiota, MD, PHD,* Jorma O. Kokkonen, MD, PHD,*† Inka Liesmaa, MD,* Karam Kostner, MD, PHD,‡ Mikko Mäyränpää, MD,* Petri T. Kovanen, MD, PHD,* Ken A. Lindstedt, PHD*

Helsinki, Finland and Vienna, Austria

OBJECTIVES We sought to study the expression of bradykinin type-2 receptors (BK-2Rs) in patients with heart failure (HF).

BACKGROUND Recent work in experimental animals has suggested that bradykinin (BK) exerts cardioprotective effects through specific BK-2Rs. However, nothing is known about the regulation of BK-2R expression in the pathogenesis of human HF.

METHODS Human heart tissue was obtained from excised hearts of patients undergoing cardiac transplantation (n = 13) and from normal hearts (n = 6) unsuitable for donation. The patients had HF due to idiopathic dilated cardiomyopathy (IDC) (n = 7) or coronary heart disease (CHD) (n = 6). Tissue samples from the left ventricles were analyzed by competitive reverse-transcriptase-polymerase chain reaction and Western blotting for the expression of BK-2R messenger ribonucleic acid (mRNA) and protein.

RESULTS In both the IDC and CHD hearts, the level of BK-2R mRNA expression was found to be significantly lower (30% and 38% of control values, respectively) than that in normal hearts. Correspondingly, the BK-2R protein level was significantly reduced in both the IDC and CHD hearts (45% and 62% of control values, respectively) and apparently involved all myocardial cell types. The down-regulation of BK-2R expression in failing hearts did not correlate with decreased cellularity or with the expression pattern of other members of the G-protein–coupled receptor superfamily. However, BK-2R down-regulation in the failing hearts was associated with a decrease in endothelial nitric oxide synthase in both IDC (53% of control value) and CHD (43% of control value) hearts.

CONCLUSIONS These results are the first to suggest that a loss of BK-2Rs is involved in the pathogenesis of human HF. (J Am Coll Cardiol 2002;40:119–25) © 2002 by the American College of Cardiology Foundation

Recent work in experimental animals has suggested that kinins, notably bradykinin (BK) and kallidin, have both short- and long-term cardioprotective effects. The short-term effects include protection of the myocardium from ischemia-reperfusion injuries (1–4), whereas the long-term effects involve both a reduction in left ventricular hypertrophy (LVH) and prevention of heart failure (HF) (5–7).

The cardioprotective effects of kinins seem to be mediated by stimulation of bradykinin type-2 receptors (BK-2Rs) and involve the production and release of vasodilating nitric oxide by endothelial cells (3,8), together with both antiproliferative (9) and antihypertrophic (10) effects on fibroblasts and myocytes. Whether these cardioprotective effects are mediated by improved hemodynamics or direct effects on target cells, or both, is presently unknown.

In contrast to experimental animal models, the cardioprotective role of the kinin system, especially the role of BK receptors in the prevention of human HF, is unknown. Herein, we show that the expression levels of the cardioprotective BK-2Rs in failing human hearts are significantly reduced and that the receptor down-regulation is associated with a decrease in endothelial nitric oxide synthase (eNOS). Our novel results suggest the involvement of BK-2Rs in the pathogenesis of human HF.

METHODS

Preparation of human heart samples. Normal heart samples (n = 6) were obtained from the left ventricles of organ donors who had no history of cardiac disease and who had been excluded from organ donation because of age, body size or blood type incompatibilities. Failing left ventricles were harvested at the time of cardiac transplantation from 13 patients with end-stage HF due to either idiopathic dilated cardiomyopathy (IDC) (n = 7) or coronary heart disease (CHD) (n = 6) at the University Central Hospital, Helsinki, Finland, or at the General Hospital, Vienna, Austria. All patients were treated with a combination of drugs, including beta-blockers, angiotensin-converting enzyme (ACE) inhibitors, loop diuretics, digoxin and spironolactone. In addition, a 53-year-old man with CHD who was not treated with an ACE inhibitor was included in the

From the *Wihuri Research Institute, Helsinki, Finland; †Division of Cardiology, Helsinki University Central Hospital, Helsinki, Finland; and ‡Division of Cardiology, Allegemeines Krankenhaus, Vienna, Austria. This work was supported in part by the Finnish Heart Foundation, Helsinki, Finland (Drs. Kuoppala, Shiota, Kokkonen and Lindstedt), the Aarne Koskelo Foundation, Helsinki, Finland (Drs. Kuoppala, Shiota and Kokkonen), the Paavo Nurmi Foundation, Helsinki, Finland (Dr. Lindstedt) and the Academy of Finland, Helsinki, Finland (Dr. Lindstedt).

Manuscript received September 20, 2001; revised manuscript received March 25, 2002, accepted April 5, 2002.

Abbreviations and Acronyms

ACE = angiotensin-converting enzyme
AT-1R = angiotensin II type 1 receptor
AT-2R = angiotensin II type 2 receptor
BK = bradykinin
BK-2R = bradykinin type-2 receptor
CHD = coronary heart disease
DNA = deoxyribonucleic acid
eNOS = endothelial nitric oxide synthase
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
HCAEC = human coronary artery endothelial cell
HF = heart failure
IDC = idiopathic dilated cardiomyopathy
LVH = left ventricular hypertrophy
PCR = polymerase chain reaction
RNA = ribonucleic acid
RT-PCR = reverse-transcriptase polymerase chain reaction

analysis as a control for the effect of ACE inhibitors on BK-2R expression. After excision, the heart tissues were immediately frozen in liquid nitrogen and stored at −70°C. Myocardium devoid of visible scar tissue was used in the experiments. The clinical characteristics of the patients in this study are shown in Table 1. The Internal Review Committees at the corresponding hospitals approved the use of the human heart samples.

Competitive reverse transcriptase-polymerase chain reaction (RT-PCR). Total ribonucleic acid (RNA) was isolated from human heart samples using an ultra-pure TRIzol reagent (Gibco BRL) and a RNeasy Mini Kit (Qiagen).

Table 1. Characteristics of Patients

Patient No.	Etiology	Gender	Age (yrs)	ACE Inhibitor Therapy
1	Normal	M	19	No
2		M	43	No
3		M	49	No
4		M	24	No
5		M	31	No
6		F	50	No
			36 ± 5*	
7	CHD	M	61	Yes
8		M	60	Yes
9		M	55	Yes
10		M	52	Yes
11		M	60	Yes
12		M	53	Yes
			57 ± 2*	
13	IDC	M	29	Yes
14		M	60	Yes
15		M	46	Yes
16		M	56	Yes
17		M	55	Yes
18		M	56	Yes
19		M	63	Yes
			52 ± 4*	

*Average ± SEM.
ACE = angiotensin-converting enzyme; CHD = coronary heart disease; IDC = idiopathic dilated cardiomyopathy.

One microgram of purified total RNA was transcribed into complementary DNA using a Superscript pre-amplification system (Gibco BRL). The primers were as follows—BK-2R: 5′-CACCATCTCCAACAACTTCG (sense), 5′-GGTAGCTGATGACACAAGCG (anti-sense); glyceraldehyde-3-phosphate dehydrogenase (GAPDH): 5′-ACCACAGTCCATGCCATCAC (sense), 5′-TCCACCACCCTGTTGCTGTA (anti-sense); angiotensin II type 1 receptor (AT-1R): 5′-GCTTCTTGG-TGGATGAGCTT (sense), 5′-GCTGGCCCTTT-GGCAAT TAC (anti-sense); and angiotensin II type 2 receptor (AT-2R): 5′-TTCCCTTCCATGTTCTG-ACC (sense), 5′-AAACACACTGCGGA GCTTCT (anti-sense). The competitor DNA for BK-2R was obtained by inserting a 129-base pair external DNA fragment into the *Sac*I site. The polymerase chain reaction (PCR) product was verified by DNA sequencing to represent the corresponding target. The use of equal amounts of messenger RNA in the RT-PCR assays was confirmed by analyzing the expression levels of GAPDH (data not shown). The PCR products were quantitated with a Gel Doc 2000 gel-documentation system (Bio-Rad), and the logarithm of the target-to-competitor ratio was plotted against the logarithm of the competitor DNA molecules (11).

Detection of BK-2Rs and eNOS by Western blotting. Triton-X-100 extracts were prepared from normal and failing heart tissues subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting (12). The BK-2Rs were detected using a monoclonal BK-2R antibody (0.25 mg/l, Transduction Laboratories), a peroxidase-conjugated goat anti-mouse antibody (0.001 mg/l, Dako) and enhanced chemiluminescence (Amersham Pharmacia Biotech), according to the manufacturers' recommendations. The eNOS was detected using a polyclonal anti-human eNOS antibody (0.2 mg/l, R&D Systems) and a peroxidase-conjugated rabbit anti-goat antibody (0.001 mg/l, Dako). The blots were quantitated with a Gel Doc 2000 gel-documentation system (Bio-Rad), and the levels of BK-2R and eNOS expression in failing hearts were expressed as the mean percentage ± SEM of control values (normal heart samples).

Expression of BK-2Rs in human coronary artery endothelial cells. Human coronary artery endothelial cells (HCAECs) (Clonetics) were cultured in endothelial cell basal medium-2 media (Clonetics) supplemented with 5% fetal bovine serum and growth factors (Bulletkit, Clonetics, CC-3202) at 37°C under 5% carbon dioxide, as recommended by the manufacturer. Subconfluent HCAECs were incubated with an ACE inhibitor (10 μM, captopril) in the absence or presence of excess BK (10 nM) for 18 h in serum-free culture media. Total RNA was isolated using an RNeasy Mini Kit (Qiagen), and the expression of BK-2Rs was determined using RT-PCR, as described previously.

Staining of normal and failing hearts. Serial frozen sections of normal and failing human hearts were stained for

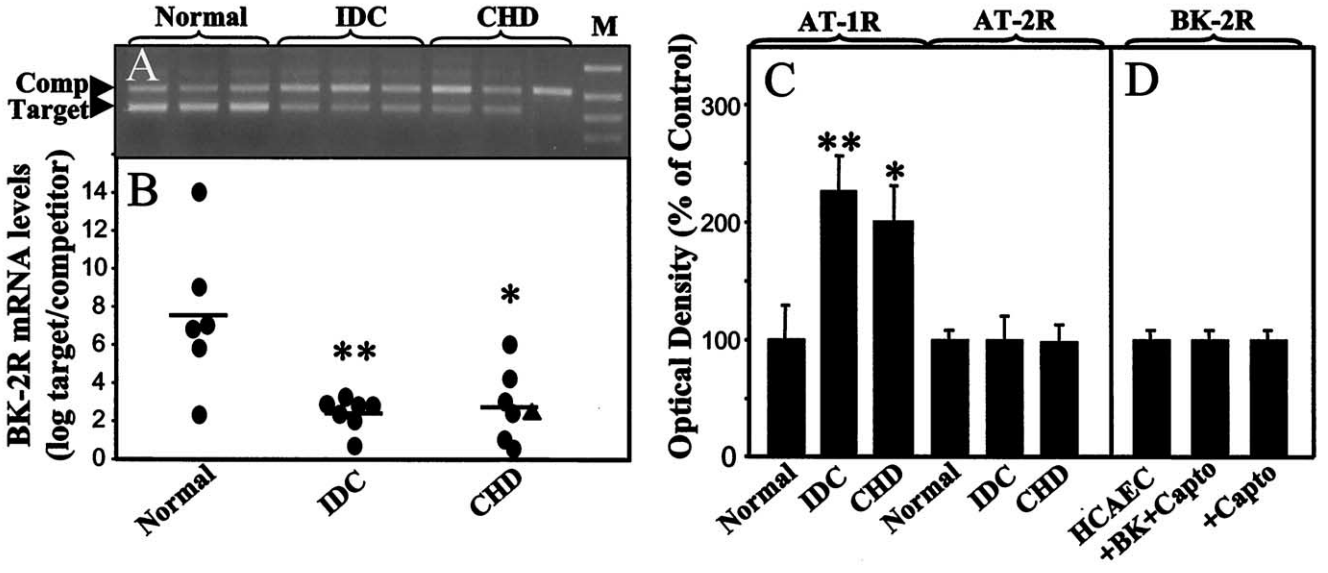

Figure 1. Quantitative reverse transcription-polymerase chain reaction of bradykinin type-2 receptors (BK-2Rs) in human hearts. **(A)** Messenger ribonucleic acid (mRNA) expression of BK-2Rs in normal and failing human hearts. **(B)** The mRNA levels of BK-2Rs are shown as the log ratios between the target and its competitor in the three patient groups. The **solid triangle** indicates a patient with coronary heart disease (CHD) who did not receive an angiotensin-converting enzyme inhibitor. **(C)** Expression levels of angiotensin II type I and II receptors (AT-1R and AT-2R, respectively) were analyzed in normal and failing hearts. **(D)** Levels of BK-2Rs were determined in bradykinin (BK)- and/or captopril (Capto)-treated cultured human coronary artery endothelial cells (HCAEC). *p = 0.01. **p = 0.003. IDC = idiopathic dilated cardiomyopathy; M = marker.

fibrosis by using a commercial Masson trichrome staining kit (Accustain, Sigma Diagnostics) and for BK-2Rs by using a monoclonal anti–BK-2R antibody (5 mg/l, Transduction Laboratories). Equal amounts of nonimmune serum or phosphate-buffered saline were used to control the specificity of antibodies. Control sections were stained for smooth muscle cells (monoclonal anti–alpha-smooth muscle cell actin antibody (3 mg/l; Sigma), endothelial cells (rabbit polyclonal anti–von Willebrand factor antibody (26 mg/l; Dako) and fibroblasts (monoclonal anti-human prolyl-4-hydroxylase antibody (3 mg/l; Dako). The density of the Masson trichrome (blue color = fibrosis; red color = myocytes) and the antibody-peroxidase–generated color (reddish-brown) in the stained frozen sections were quantitated using the Image-Pro Plus (version 4.0/4.1) image analysis system (Media Cybernetics).

Statistical analysis. Data are expressed as the mean value ± SEM. All three patient groups showed normal distribution when analyzed with the Shapiro-Wilks *W* test, and comparisons between the groups of normal and failing hearts were made using two-way analysis of variance with Dunnett's post-hoc test. Statistical significance was accepted at $p < 0.05$.

RESULTS

Expression of BK-2Rs mRNA in normal and failing hearts. Figure 1A shows a representative competitive RT-PCR analysis of BK-2Rs in three individual samples randomly chosen from each subject group. The mRNA levels of the cardioprotective BK-2Rs (Fig. 1B) were found to be significantly decreased in hearts with either IDC (30% of control value, p = 0.003; middle row) or CHD (38% of control value, p = 0.01; right row), as compared with normal hearts (left row). The competitive RT-PCR assay was standardized to the expression level of GAPDH. Furthermore, the observed down-regulation was specific for BK-2Rs, because the expression of other members of the G-protein–coupled receptor superfamily in the failing hearts (Fig. 1C) was either induced (AT-1Rs) or unaffected (AT-2Rs). In addition, incubating cultured HCAECs, which express BK-2Rs in a constitutive manner, with an ACE inhibitor (10 μM, captopril) in the presence or absence of excess BK (10 nM) for 18 h did not affect their levels of BK-2R expression (Fig. 1D).

Bradykinin-2R protein in normal and failing hearts. As shown in Figure 2, the protein levels of BK-2Rs were significantly reduced both in IDC (45% of control value, p = 0.002; Fig. 2, middle bar) and CHD (62% of control value, p = 0.04; Fig. 2, right bar) hearts, as compared with normal hearts (Fig. 2, left bar). In keeping with the aforementioned results (Figs. 1 and 2), positive staining for BK-2Rs, quantitated as the extent of reddish-brown intensity, was clearly less in both IDC (42% of control value) and CHD (65% of control value) hearts, as compared with normal hearts (100%) (Figs. 3A to 3C). By staining serial tissue sections with Masson trichrome and quantitating the level of fibrosis (blue color), we found that the observed reduction in BK-2R expression was associated with a significantly higher degree of fibrosis (20.6 ± 1.7% vs. 16.0 ± 2.9% in normal samples; $p < 0.05$) in IDC hearts

JACC Vol. 40, No. 1, 2002
July 3, 2002:119–25

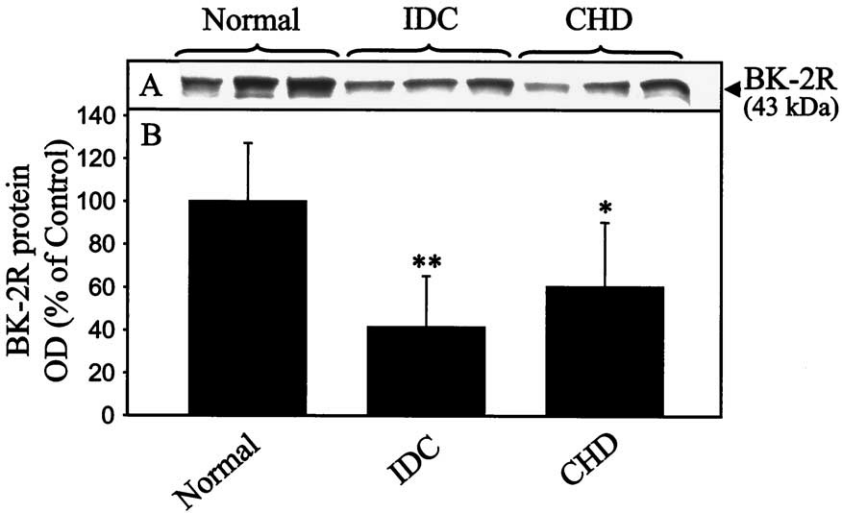

Figure 2. Western blotting of bradykinin type-2 receptors (BK-2Rs) in human hearts. **(A)** Representative Western blot showing the expression of BK-2R proteins in normal and failing human hearts. Equal amounts of protein (30 μg/lane) were loaded onto the gel. **(B)** The relative amounts of BK-2Rs were quantitated in the different patient groups by measuring the optical densities (OD) in the Western blots. *p = 0.04. **p = 0.002. CHD = coronary heart disease; IDC = idiopathic dilated cardiomyopathy.

(Fig. 3E). However, the degree of fibrosis (20.3 ± 8.3%) in the CHD samples (Fig. 3F) was not significantly different from that in the normal myocardium (16.0 ± 2.9%).

Immunohistochemical staining of BK-2Rs. To localize the BK-2Rs within the myocardial cell population, we immunostained left ventricular tissue sections by using specific antibodies for BK-2Rs and specific markers of the individual myocardial cell types. Figure 4A shows sections of normal myocardium with intramyocardial branches of coronary arteries. All of the major cell types were found to express BK-2Rs: endothelial cells (Fig. 4, upper left panel, long white arrow), smooth muscle cells (Fig. 4, upper left and middle panels, short white arrow) and fibroblasts, both in the fibrotic area surrounding the vessels (Fig. 4, upper left panel, short black arrow) and in the myocardial interstitium (upper right panel, short black arrow). Also, the myocytes were found to express BK-2Rs (Fig. 4, upper left panel, long black arrow and inset showing the selected myocyte at a higher magnification). The myocardial localization of endothelial cells (Fig. 4A, lower left panel), smooth muscle cells (lower middle panel) and interstitial fibroblasts (lower right panel) was further verified by cell-specific staining of serial sections of normal hearts. Interestingly, by grossly comparing the BK-2R–positive cells in normal and failing hearts, we found no apparent differences in their cell-specific BK-2R expression pattern (Fig. 4B).

Expression of eNOS in normal and failing hearts. Because the physiologic effects of BK, including vasodilation, inhibition of myocardial growth and anti-ischemic effects, are largely mediated through BK-2R–stimulated expression and activation of eNOS (13–15), we measured the expression level of eNOS in normal and failing hearts. As shown in Figure 5, the observed decrease in BK-2R expression in failing hearts was associated with a decrease in eNOS expression in both IDC (53 ± 5% of control value, p = 0.001) and CHD (44 ± 10% of control value, p = 0.002) hearts.

DISCUSSION

The present results, showing a differential expression of BK-2Rs in normal and failing hearts, are the first to suggest, to the best of our knowledge, that BK-2Rs may be involved in the pathogenesis of human HF. It has recently been shown that BK-2R knockout mice develop LVH and HF (16), suggesting that the BK-2R signaling system is essential for the regulation of myocardial growth and prevention of HF. These data raise the possibility that the regulation of myocardial growth and prevention of human HF may depend on the presence of an intact BK-2R signaling system. Thus, down-regulation of BK-2R expression in failing human hearts, as shown in this study, may reflect a maladaptive response in the pathogenesis of HF. Recently, a direct regulatory link between BK-2Rs and eNOS was shown in both normotensive (14) and ischemic (15) rats, suggesting that the level of eNOS and the amount of nitric oxide may partly depend on the level of BK-2Rs. Similarly, in our present study, the down-regulation of BK-2Rs in failing human hearts was associated with a significant decrease in eNOS, a finding compatible with the idea that the reduced amount of BK-2Rs may affect the level of eNOS.

BK-2Rs as a part of the cardioprotective system. During the early stages of a pathologic process in the myocardium that may ultimately lead to HF, LVH develops as a compensatory "remodeling" mechanism, whereas the later stages of the process are characterized by a loss of myocytes, with subsequent left ventricular dysfunction and HF. Thus, if BK-2Rs are an essential part of the cardioprotective system, as has been suggested (4,16), a logical adaptive response to the onset of such a pathologic process would be

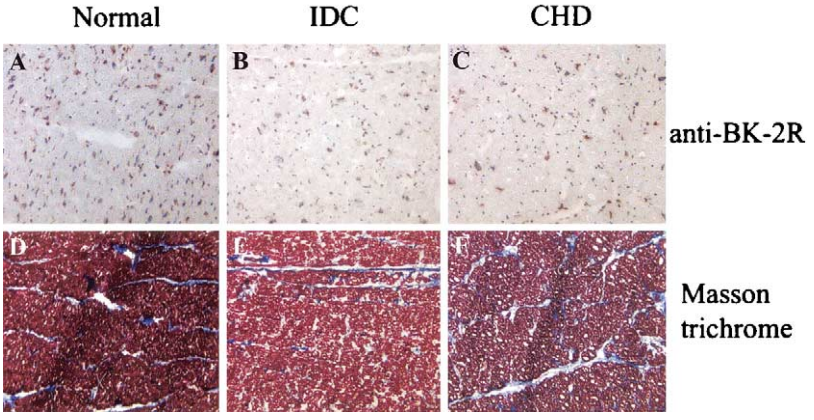

Figure 3. Detection of bradykinin type-2 receptors (BK-2Rs) and fibrosis in human hearts. Serial sections of normal **(A and D)**, idiopathic dilated cardiomyopathy (IDC) **(B and E)** and coronary heart disease (CHD) **(C and F)** hearts were analyzed for BK-2R expression **(A to C)** by using a monoclonal BK-2R antibody (magnification ×200) and for the degree of fibrosis **(D to F)** by Masson trichrome staining (magnification ×200).

an increase in BK-2R expression. Therefore, it is possible that BK-2R expression is induced during the early stages of the disease (i.e., LVH) and that the observed receptor down-regulation occurs only later, at the stage of HF. To settle this important question, serial samples of myocardial tissue should be analyzed at different time points during the pathogenesis of HF. Unfortunately, such an experimental set-up in human HF is not feasible at present.

BK-2R polymorphism. Recently, an altered frequency of a promoter (−58T/C) polymorphism of the BK-2R gene has been described in hypertensive African-American (17) and Japanese (18) subjects, suggesting that decreased transcriptional activity of the BK-2R promoter may be involved in essential hypertension. Indeed, a partial genetic deficiency (heterozygotes) of BK-2Rs in mice is already sufficient for the onset of LVH (16). Thus, we cannot exclude the possibility that patients with the phenotype of end-stage HF have a genetically determined malfunction in their BK-2R expression system, which could affect the balance between cardiac risk factors and the cardioprotective system. Further elucidation of the potential role of a hereditary dysfunction of the BK-2R gene in the pathogenesis of HF requires studies on the linkage between a HF phenotype and possible BK-2R polymorphism.

BK-2R expression and ACE inhibitors. Because ACE inhibitors are generally thought to exert their cardioprotec-

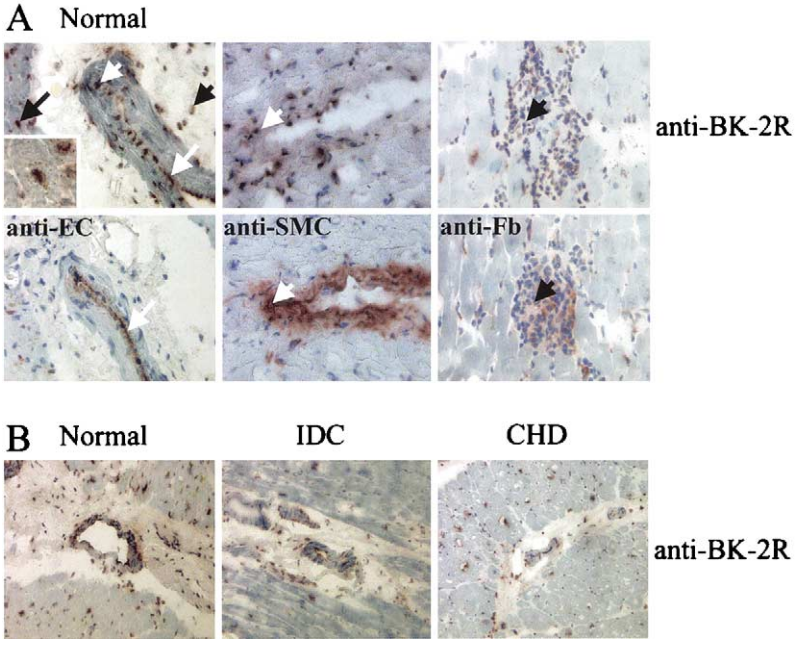

Figure 4. Detection of bradykinin type-2 receptors (BK-2Rs) and myocardial cells in human hearts. **(A)** Serial frozen sections of normal hearts were analyzed for cell-specific BK-2R expression (magnification ×400) and for specific antibodies in endothelial cells (EC), smooth muscle cells (SMC) and fibroblasts (Fb). A BK-2R–positive myocyte **(upper left panel, long black arrow and inset** showing the selected myocyte at a higher magnification [×1,000]), an EC **(long white arrow)**, a SMC **(short white arrow)** and a Fb **(short black arrow)** are also indicated in the upper left panel. **(B)** Frozen sections of normal, idiopathic dilated cardiomyopathy (IDC) and coronary heart disease (CHD) hearts were stained for BK-2R expression (magnification ×200).

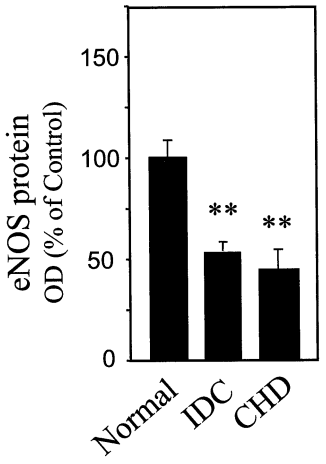

Figure 5. Expression of endothelial nitric oxide (eNOS) in normal and failing hearts. **(A)** Expression of eNOS in normal and failing human hearts was analyzed by Western blotting. Equal amounts of protein (50 μg/lane) were run on the gel, and eNOS was quantitated as described in the Methods section. **p = 0.001 for idiopathic dilated cardiomyopathy (IDC) vs. normal and p = 0.002 for coronary heart disease (CHD) vs. normal. OD = optical density.

tive effects by inhibiting the production of angiotensin II and by increasing the concentration of BK, it is relevant to ask the question whether ACE inhibition also may affect the level of BK-2R expression. Recent observations show that BK levels in the human plasma of HF patients treated with ACE inhibitors are similar to BK levels in healthy persons (19), suggesting that the down-regulation of BK-2Rs observed in the present study occurs independent of BK levels. Furthermore, the fact that the beneficial effects obtained by ACE inhibitors are inhibited by HOE-140, a specific BK-2R antagonist, does not support a suppressive role of ACE inhibitors in BK-2R expression. In the present study, we had an opportunity to analyze a heart sample from an additional patient with CHD who had not received an ACE inhibitor (Fig. 1B, black triangle), and the BK-2R expression was not different from that of patients with CHD treated with ACE inhibitors. In addition, incubating HCAECs, which express BK-2Rs in a constitutive manner, with an ACE inhibitor in the presence or absence of excess BK for 18 h did not affect their levels of BK-2R expression. However, although it appears unlikely that the observed down-regulation of BK-2R expression in the failing hearts is due to the presence of ACE inhibitors, we cannot completely exclude the possibility that long-term administration of ACE inhibitors may have an effect on the level of receptor expression.

Cell-specific expression of BK-2Rs. The observed down-regulation of BK-2Rs seems not to be cell-specific, because not only the cells in the intramyocardial vascular tree (i.e., endothelial cells and smooth muscle cells) but also the cells in the myocardial interstitium (i.e., fibroblasts and contractile cells [cardiomyocytes]) are affected. Whether the receptor down-regulation is organ-specific is not known. This question is not possible to answer because, due to ethical reasons, samples of other organs from patients with end-stage HF cannot be obtained. Because the functional role and importance of BK-2Rs are likely to vary among the different cell types, further experiments are necessary to answer the challenging questions regarding the cell-specific role of BK-2Rs in the pathogenesis of human HF.

Acknowledgments

We thank Jaana Tuomikangas, Elina Kaperi and Suvi Mäkinen for their excellent technical assistance. We are grateful to the heart transplantation teams at the University Central Hospital, Helsinki, Finland, and the General Hospital, Vienna, Austria, for supplying human heart tissue.

Reprint requests and correspondence: Dr. Ken A. Lindstedt, Wihuri Research Institute, Kalliolinnantie 4, FIN-00140 Helsinki, Finland. E-mail: ken.lindstedt@wri.fi.

REFERENCES

1. Yang XP, Liu YH, Scicli GM, Webb CR, Carretero OA. Role of kinins in the cardioprotective effect of preconditioning: study of myocardial ischemia/reperfusion injury in B2 receptor knockout mice and kininogen-deficient rats. Hypertension 1997;30:735–40.
2. Zhang X, Xie YW, Nasjletti A, Xu X, Wolin MS, Hintze TH. ACE inhibitors promote nitric oxide accumulation to modulate myocardial oxygen consumption. Circulation 1997;95:176–82.
3. Kitakaze M, Node K, Minamino T, et al. Inhibition of angiotensin-converting enzyme increases the nitric oxide levels in canine ischemic myocardium. J Mol Cell Cardiol 1998;30:2461–6.
4. Su JB, Barbe F, Houel R, Guyene TT, Crozatier B, Hittinger L. Preserved vasodilator effect of bradykinin in dogs with heart failure. Circulation 1998;98:2911–8.
5. McDonald KM, Mock J, D'Aloia A, et al. Bradykinin antagonism inhibits the antigrowth effect of converting enzyme inhibition in the dog myocardium after discrete transmural myocardial necrosis. Circulation 1995;91:2043–8.
6. Liu YH, Yang XP, Sharov VG, et al. Effects of angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor antagonists in rats with heart failure: role of kinins and angiotensin II type 2 receptors. J Clin Invest 1997;99:1926–35.
7. Wollert KC, Studer R, Doerfer K, et al. Differential effects of kinins on cardiomyocyte hypertrophy and interstitial collagen matrix in the surviving myocardium after myocardial infarction in the rat. Circulation 1997;95:1910–7.
8. Groves P, Kurz S, Just H, Drexler H. Role of endogenous bradykinin in human coronary vasomotor control. Circulation 1995;92:3424–30.
9. McAllister BS, Leeb-Lundberg F, Olson MS. Bradykinin inhibition of EGF- and PDGF-induced DNA synthesis in human fibroblasts. Am J Physiol 1993;265:C477–84.
10. Ishigai Y, Mori T, Ikeda T, Fukuzawa A, Shibano T. Role of bradykinin-NO pathway in prevention of cardiac hypertrophy by ACE inhibitor in rat cardiomyocytes. Am J Physiol 1997;273:H2659–63.
11. Shiota N, Fukamizu A, Okunishi H, Takai S, Murakami K, Miyazaki M. Cloning of the gene and cDNA for hamster chymase 2, and expression of chymase 1, chymase 2, and angiotensin-converting enzyme in the terminal stage of cardiomyopathic hearts. Biochem J 1998;333:417–24.
12. Lindstedt KA, Mahon MG, Foisner R, Hermann M, Nimpf J, Schneider WJ. Receptor-associated protein in an oviparous species is correlated with the expression of a receptor variant. J Biol Chem 1997;272:30221–7.
13. Hornig B, Kohler C, Drexler H. Role of bradykinin in mediating vascular effects of angiotensin-converting enzyme inhibitors in humans. Circulation 1997;95:1115–8.

14. Bachetti T, Comini L, Pasini E, Cargnoni A, Curello S, Ferrari R. ACE-inhibition with quinapril modulates the nitric oxide pathway in normotensive rats. J Mol Cell Cardiol 2001;33:395–403.
15. Cargnoni A, Comini L, Bernocchi P, et al. Role of bradykinin and eNOS in the anti-ischemic effect of trandolapril. Br J Pharmacol 2001;133:145–53.
16. Emanueli C, Maestri R, Corradi D, et al. Dilated and failing cardiomyopathy in bradykinin B(2) receptor knockout mice. Circulation 1999;100:2359–65.
17. Gainer JV, Brown NJ, Bachvarova M, et al. Altered frequency of a promoter polymorphism of the kinin B2 receptor gene in hypertensive African-Americans. Am J Hypertens 2000;13:1268–73.
18. Mukae S, Aoki S, Itoh S, et al. Promoter polymorphism of the beta$_2$ bradykinin receptor gene is associated with essential hypertension. Jpn Circ J 1999;63:759–62.
19. Cugno M, Agostoni P, Brunner HR, Gardinali M, Agostoni A, Nussberger J. Plasma bradykinin levels in human chronic congestive heart failure. Clin Sci 2000;99:461–6.