Increased expression of bradykinin type-1 receptors in endothelium of intramyocardial coronary vessels in human failing hearts

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ACCUMULATING EVIDENCE IN HUMANS and in experimental animal models has suggested that the expression of bradykinin type-1 receptors (BK-1Rs) is induced during inflammation and ischemia, and, by exerting either cardioprotective or cardio-toxic effects, they may contribute to the pathogenesis of heart failure. Nothing is known about the expression of BK-1Rs in human heart failure. Human heart tissue was obtained from excised hearts of patients undergoing cardiac transplantation (n = 13), due to idiopathic dilated cardiomyopathy (IDC; n = 7) or to coronary heart disease (CHD; n = 6), and from normal hearts (n = 6). The expression of BK-1Rs was analyzed by means of competitive RT-PCR, Western blot analysis, and immunohistochemistry. Expression of BK-1R mRNA was increased in both IDC (2.8-fold) and CHD (2.1-fold) hearts compared with normal hearts. The observed changes were verified at the protein level. Expression of BK-1Rs in failing hearts localized to the endothelium of intramyocardial coronary vessels and correlated with an increased expression of TNF-α in the vessel wall. Treatment of human coronary artery endothelial cells with TNF-α increases their BK-1R expression. These novel results show that BK-1Rs are induced in the endothelium of intramyocardial coronary vessels in failing human hearts and so may participate in the pathogenesis of heart failure.

end-stage heart failure; cardiotoxic; inflammation; cytokine

MATERIALS AND METHODS

Preparation of human heart samples. Normal heart samples (n = 6) were obtained from left ventricles of organ donors who had no history of cardiac disease and had been excluded from organ donation because of age, body size, or tissue-type mismatch. The cause of death in these subjects was subarachnoid hemorrhage. The informed consent was obtained from the next of kin before organ donation. Failing left ventricles were harvested at the time of cardiac transplantation from 13 patients with end-stage heart failure (New York Heart Association functional class IV) due to either idiopathic dilated cardiomyopathy (IDC; n = 7) or coronary heart disease (CHD; n = 6) at the University Central Hospital, Helsinki, Finland. The analyzed samples are identical to the samples in our previous article (10) except for two control samples that were consumed, and therefore replaced with two new ones. All patients had been treated with a combination of drugs, including beta-blockers, ACE inhibitors, loop-diuretics, digoxin, and spironolactone. None of the patients received angiotensin II type I receptor blocker treatment or statin treatment (see Table 1). After excision, the heart tissues were immediately frozen in liquid nitrogen and stored at −70°C. Left ventricle 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. An institutional review board approved the use of normal and failing heart human samples, and the investigation conforms to the principles outlined in the Declaration of Helsinki.

Competitive RT-PCR. Total RNA was isolated from human hearts using an ultrapure TRIzol reagent (GIBCO-BRL) and a RNeasy Mini Kit (QIAGEN). Purified total RNA (0.25 μg) was transcribed into

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Table 1. Clinical characteristics of control subjects and patients in the study

<table>
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<tr>
<th>Patient No</th>
<th>Etiology</th>
<th>Gender</th>
<th>Age, yr</th>
<th>ACEi</th>
<th>ARB</th>
<th>Statin</th>
<th>EF, %</th>
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<tr>
<td>2</td>
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<td>39</td>
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<td>NM</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>NM</td>
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<td>M</td>
<td>31</td>
<td>–</td>
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<td>NM</td>
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<td>6</td>
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<td>W</td>
<td>50</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>NM</td>
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<tr>
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</tr>
<tr>
<td>Average</td>
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<td>23 ± 4</td>
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<td>–</td>
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</tr>
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<tr>
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<tr>
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<tr>
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<td>+</td>
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<td>21</td>
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<tr>
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<td></td>
<td>52 ± 4</td>
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<td>20 ± 6</td>
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Averages are means ± SE. ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin II type I receptor blocker; EF, ejection fraction; NM, not measured; CHD, coronary heart disease; IDC, idiopathic dilated cardiomyopathy; M, man; W, woman.

RESULTS

Human left ventricles were analyzed for their mRNA levels of BK-1Rs by competitive RT-PCR. As shown in Fig. 1, A and B, the mRNA levels of BK-1Rs were significantly increased in left ventricles from patients suffering from either IDC (2.8-fold) or CHD (2.1-fold). The calculated approximate average number of BK-1R mRNA copies was 4,300, 9,000, and 12,000 molecules/1 µg of total RNA in normal, CHD, and IDC heart samples, respectively. Because the calculated approximate average number of BK-2R mRNA copies in normal, CHD, and IDC heart samples was 79,000, 32,000, and 25,000 molecules/1 µg of total RNA, respectively (10), the ratio of BK-1R/BK-2R was highly increased in both IDC and CHD compared with normal hearts (Fig. 1C).

The observed differences in the BK-1R mRNA levels in normal and failing human hearts were further verified at the protein levels by Western blot analysis. As shown in Fig. 2, the BK-1R translation product was significantly increased in both IDC and CHD hearts compared with normal hearts. To further localize the site of BK-1R expression in the myocardium, frozen sections of normal and failing left ventricles were subjected to immunohistochemical analysis with polyclonal antibodies against BK-1R. As shown in Fig. 3A, the expression of BK-1Rs in normal left ventricles was very weak. However, in left ventricles from both IDC (Fig. 3B) and CHD (Fig. 3C) hearts, BK-1Rs were significant and mainly expressed in endothelial cells of intramyocardial vessels, indicating that the increase in BK-1R expression, as observed by Western blot analysis, is specific for the vessel endothelium.

To further investigate the possible mechanisms by which BK-1Rs are induced in the intramyocardial vessel endothelium of the failing hearts, we studied the expression of TNF-α, a potent cytokine known to induce BK-1R expression and to be involved in heart failure. Expression of TNF-α was significantly increased in both CHD and IDC hearts (Fig. 4A). Furthermore, using Pearson’s correlation test, the BK-1R ex-
pression was shown to significantly correlate with TNF-α expression in the heart samples (Fig. 4B, R = 0.58, P = 0.015). As shown in Fig. 4C, the immunoreactivity of TNF-α is strongly increased in the intramyocardial vessel walls of the failing hearts but also present in the myocardial interstitium and the myocytes (middle and right, arrows). Similar to BK-1Rs, TNF-α is significantly expressed in the vessel endothelium of the failing hearts (Fig. 4C, middle and right). In addition, the endothelial cells of failing hearts also express TNFRI (Fig. 4C, middle and right, insets), suggesting that a TNF-α-mediated signaling pathway is present and colocalizes with BK-1Rs in the failing hearts. To further examine whether TNF-α is able to induce BK-1R expression, we studied human coronary artery endothelial cells (HCAECs) in culture. Cultured HCAECs express only low basal levels of BK-1R mRNA and protein. However, culturing the cells in the presence of recombinant TNF-α (10 pg/ml) for 24 h significantly induced the expression of both BK-1R mRNA (~10-fold) (Fig. 5, A and B, left) and BK-1R protein (~3-fold) (Fig. 5B, right).

DISCUSSION

Our present results, showing a differential expression of BK-1Rs in normal and failing human hearts, are the first to

Fig. 1. Quantitative RT-PCR analysis of bradykinin type-1 receptors (BK-1Rs) and BK-2Rs in human hearts. A: representative picture showing the mRNA expression levels of BK-1Rs in 3 samples from separate individuals with normal, idiopathic dilated cardiomyopathic (IDC), and coronary heart disease (CHD) hearts. Arrowheads indicate the positions of the receptor target and its competitor (Comp). M, marker. B: mRNA levels of BK-1Rs are shown as molecules/μg total RNA in the 3 subject groups. **P < 0.01, †P = 0.07. C: ratio of BK-1R/BK-2R expression is calculated on the basis of the approximate average number of BK-2R mRNA molecules/μg of total RNA in normal (79,000 molecules), CHD (32,000), and IDC (25,000) heart samples (10).

Fig. 2. Immunoblotting of BK-1Rs in human hearts. Protein extracts (30 μg/lane) from normal and failing human hearts (IDC, CHD) were subjected to gradient gel electrophoresis and processed for immunoblotting as described in MATERIALS AND METHODS.* P < 0.05, **P < 0.01.

Fig. 3. Immunohistochemistry of BK-1Rs in human hearts. Frozen sections of left ventricle were subjected to immunostaining with polyclonal antibodies against BK-1R. Equal amounts of nonimmune serum or PBS were used to control the specificity of the antibodies. The immunohistographs are representatives of 3 separate samples of both normal and failing hearts. *Vessel lumen. Arrows point to BK-1R-positive staining in endothelial cells.
BK-1Rs in human heart failure

Because the intramyocardial endothelial cells directly affect cardiac metabolism, growth, and contractile performance by producing and secreting a variety of auto- and paracrine metabolites, such as NO (8, 24), the specific localization of BK-1Rs to the endothelium further suggests the involvement of BK-1Rs in the pathogenesis of heart failure.

BK-1R knockout mice, in contrast to BK-2R knockout mice (5), do not spontaneously develop LVH or heart failure (12), indicating that BK-1Rs are not involved in the physiological response to kinin-mediated regulation of myocardial growth. However, the BK-1R knockout mice do show altered inflammatory responses, suggesting that BK-1Rs play an essential role in the initiation of inflammation (12). Thus the role of BK-1Rs may become evident under pathological conditions, such as in the host defense response to ischemic injury (4). Indeed, the expression of BK-1Rs is significantly upregulated in an experimental rat model of myocardial infarction (30), and in the spontaneously hypertensive rat model during the transition from stable compensated hypertrophy to heart failure (11). Moreover, in human embryonic lung fibroblasts, activation of BK-1Rs induces collagen I synthesis and fibrogenesis via stabilization of connective tissue growth factor mRNA (18).

Importantly, our present results showing a significant increase in BK-1Rs in both IDC and CHD patients suggest that the BK-1Rs as potential mediators of inflammation and fibrosis may be linked to the pathogenesis of human heart failure via this mechanism.

Recent results (3) using BK-2R knockout mice show that BK-1Rs are markedly expressed in the absence of BK-2Rs, and further suggest that they contribute to vasodilation by inhibiting a vasoconstricting product of the arachidonic acid cascade acting via the PGH2/TxA2 receptor. Furthermore, in a rat model of myocardial infarction, the BK-1Rs were suggested to contribute to the cardioprotective effects mediated by an angiotensin II type-1 receptor (AT-1R) antagonist irbesartan (31).

BK-1Rs to the endothelium further suggests the involvement of BK-1Rs in the pathogenesis of heart failure.

Fig. 4. Expression of TNF-α in human hearts. A: mRNA levels of TNF-α are shown in the 3 subject groups. *P < 0.05, **P < 0.01. B: scatter plot showing the Pearson’s correlation between BK-1Rs and TNF-α mRNA levels. C: frozen sections of left ventricle were subjected to immunostaining with polyclonal antibodies against TNF-α and TNF-α receptor I (TNFRI) (middle and right, insets). Asterisks in insets show a vessel lumen, and all arrows point to TNFRI-positive staining in endothelial cells. Equal amounts of nonimmune serum or PBS were used to control the specificity of the antibodies. Bar = 10 μm.

In contrast to the ubiquitously expressed BK-2Rs, the BK-1Rs are normally not expressed in significant amounts in healthy tissues. However, previous results in animal models (2, 28) and in human hearts (21) have shown a constitutive expression of BK-1Rs capable of regulating the vascular tone. Our present results showing a low but detectable level of BK-1Rs in healthy human hearts suggest that they may also exert some functions in the heart. In contrast to a low expression under physiological conditions, BK-1Rs are significantly induced under pathological conditions, such as tissue trauma, inflammation, and anoxia (16, 26), suggesting that the increased expression of BK-1Rs in failing human hearts may be used as a marker of an ongoing pathological process. Interestingly, by binding to the BK-1R, the agonist des-Arg10-Lys-BK may induce homologous upregulation of BK-1R expression in cultured human lung fibroblasts (25). Notably, the presence of BK-1R protein in normal and failing human hearts, as shown in this study, is not necessarily equivalent to receptor function. Because the intramyocardial endothelial cells directly affect cardiac metabolism, growth, and contractile performance by producing and secreting a variety of auto- and paracrine metabolites, such as NO (8, 24), the specific localization of BK-1Rs in human coronary artery endothelial cells.

Fig. 5. Expression of BK-1Rs in human coronary artery endothelial cells. A: representative RT-PCR analysis of BK-1R expression in control and TNF-α-treated human coronary artery endothelial cells (HCAECs). Arrowheads indicate the positions of the receptor target and its competitor. B: mRNA levels of TNF-α mRNA levels of TNF-α treated HCAECs. C: protein extracts (30 μg/lane) from control and TNF-α-treated HCAECs were subjected to gradient gel electrophoresis and processed for immunoblotting as described in MATERIALS AND METHODS. *P < 0.05, **P < 0.01.
The authors showed that irbesartan induced the expression of BK-1Rs, whereas AT-1R overexpression reduced the expression of BK-1Rs (31). However, our previous (10) and present studies using human failing hearts, show that both AT-1Rs and BK-1Rs are upregulated, suggesting that the reported AT-1R-mediated downregulation of BK-1Rs may be restricted to the rat model. In a recent study by Marin-Castano et al. (17), ACE inhibitors were also shown to induce the expression of BK-1Rs in experimental animal models, suggesting that the observed increase in BK-1R expression in failing human hearts may have been influenced by the fact that all patients received ACE inhibitors. Although we were unable to show any short-time effects of ACE inhibitors on the expression of BK-1Rs in cultured human coronary arterial endothelial cells (data not shown), we cannot rule out the effect of long-time ACE inhibitor treatment on BK-1R expression in the failing human hearts.

Analogous to the known opposite actions of AT-1R and angiotensin II type-2 receptors (AT-2R), a hypothetical balance between BK-1Rs and BK-2Rs may be crucial for the regulation of myocardial growth and prevention of heart failure. As shown in our present study, there is a significant shift in the BK-1R/BK-2R ratio in human end-stage heart failure. Thus apart from their individual changes, the ratio of BK-1R/BK-2R is even more dramatically increased in human failing hearts, suggesting that the balance between BK-1R- and BK-2R-mediated cell-signaling events may be important in the progression of heart failure. Interestingly, a similar increase in the BK-1R/BK-2R ratio has been observed in the failing hearts of SHR (11). In the SHR, the induction of BK-1R expression during the transition from stable compensated hypertrophy to heart failure also correlated with a rapid increase in fibrosis and diastolic dysfunction (11). However, the end-stage failing human hearts did not show a significant Pearson correlation ($R = -0.236, P = 0.437$) between BK-1R mRNA expression and ejection fraction.

What may then trigger the induction of BK-1Rs in the failing heart? Previous studies have suggested that inflammatory cytokines, such as TNF-$\alpha$, may induce the expression of BK-1Rs (1) and that the level of circulating TNF-$\alpha$ in chronic heart failure is increased (13). Similarly, in the present study, in which we have analyzed end-stage failing hearts, TNF-$\alpha$ expression was significantly increased in the intramyocardial coronary vessel wall and correlated with the increased expression of BK-1Rs. Moreover, in cultured endothelial cells derived from human coronary arteries, TNF-$\alpha$ was shown to be a potential trigger of BK-1R expression.

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REFERENCES


