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Differential role of kinin B1 and B2 receptors in ischemia-induced apoptosis and ventricular remodeling

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Abstract

We investigated the role of kinin receptors in cardiac remodeling after ischemia/reperfusion (I/R). Bradykinin injection improved cardiac contractility, diastolic function, reduced infarct size and prevented left ventricular thinning after I/R, whereas des-Arg⁹-BK injection had no protective effects. Bradykinin, but not des-Arg⁹-BK, reduced cardiomyocyte apoptosis and increased Akt and GSK-3 β phosphorylation. Furthermore, myocardial infarct size was similar between wild type and B2 knockout mice after I/R, but significantly reduced in kinin B1 receptor knockout mice. These results indicate that the kinin B2 receptor, but not the B1 receptor, protects against I/R-induced cardiac dysfunction by inhibiting apoptosis and limiting ventricular remodeling.

Keywords

B1 receptor; B2 receptor; Bradykinin; Ischemia/reperfusion; Remodeling

1. Introduction

The responses to vasoactive kinin peptides are mediated by the activation of bradykinin receptor B1 (B1R) and B2 (B2R) according to the relative potencies and affinities to their agonists [14]. B2R is constitutively expressed in most tissues and responsive to intact bradykinin (BK) and kallidin (Lys-BK). In contrast, B1R which has a higher affinity for des-Arg⁹-BK(DBK) and des-Arg¹⁰-kallidin, is weakly expressed under physiological conditions but induced by pathological stimuli such as inflammation and tissue injury [15]. BK through B2R activation has been shown to limit infarct size in the ischemic heart in the late phase of preconditioning [8]. In contrast, B1R might play a detrimental role in the ischemic heart after IR, as infarct size was significantly reduced in B1R^{-/-} mice compared with wild-type mice after myocardial infarction (MI), and administration of B1R antagonist significantly reduced ischemic injury in wild-type mice [10]. Paradoxically, another study demonstrated that B1R^{-/-} mice had a larger LV diastolic chamber dimension initially and after 4–8 weeks compared with wild-type mice after MI, indicating that B1R may play protective role in regulation of structural homeostasis at the early stage after cardiac injury [20]. However, the cardioprotective role of B1R is uncertain because this study demonstrated a similar degree of myocardial injury and remodeling between wild-type mice and B1R^{-/-} mice after myocardial infarction. Furthermore, B2R has been implicated in accentuation of infarction-induced inflammation as blockade of B2R by an antagonist after MI exerts no effect on infarct size after MI but attenuates inflammatory cell reaction both inside and outside infarct area, thus resulting in reduction of

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fibrosis and collagen deposition [7]. Therefore, the role of B2R and B1R in the ischemic myocardium after MI remains to be further investigated.

In this study, we compared the effects of B1R and B2R agonists on cardiac function, cardiomyocyte apoptosis and LV remodeling in short and long-term I/R models of rats. In addition, we investigated the role of B1R and B2R in cardiac remodeling using B1R and B2R knockout mice. Using both pharmacological approaches and genetic animal models, our results demonstrate that kinin B2 receptor, but not B1 receptor, activation preserves cardiac function by inhibiting apoptosis and limiting deteriorated left ventricular remodeling after I/R is associated with Akt and GSK-3 β phosphorylation.

2. Material and methods

2.1. Animals

Wistar rats (male, 240–260 g, Sprague–Dawley Harlan, Indianapolis, IN) were employed in this study. All procedures complied with the standards for care and use of animal subjects as stated in the *Guide for the Care and Use of Laboratory Animals* (National Academy of Sciences, Bethesda, MD). Two breeding pairs of B1 kinin receptor knockout mice (B1R $^{-/-}$) on a C57/J6 genetic background were obtained from Dr. Michael Bader's laboratory (Max-Delbrück Center for Molecular Medicine, Berlin-Buch, Germany) and bred in our animal facilities. C57BL/J6 $^{+/+}$ (wild type) and B2 kinin receptor knockout mice (B2R $^{-/-}$, derived from breeding pairs of C57BL/6 mice on a 129/J genetic background) were purchased from Jackson Laboratories (Bar Harbor, ME). Aged matched male mice (body weight 25–30 g) were employed for the study.

2.2. Myocardial I/R animal models

Myocardial I/R surgery was performed as previously described [22]. Rats were anesthetized with pentobarbital (50 mg/kg, intraperitoneally), intubated, and mechanically ventilated. Myocardial I/R was established by occlusion of left anterior descending (LAD) coronary artery for 30 min followed by either a 1-day (short-term) or a 7-day (long-term) reperfusion. The short-term study was designed to examine apoptosis and the long-term study was designed to focus on ventricular remodeling. Rats were randomly divided into four groups. In the sham group, the chest was opened and saline was injected into the myocardium ($n = 6$). In the I/R groups, rats were divided into three groups. Control group ($n = 6$) received intramyocardial injection with saline, and the second and third groups received intramyocardial injection of BK or DBK (5 μ g in 150 μ l saline) at 10 min after LAD ligation ($n = 7$). In the long-term study, BK and DBK (5 μ g in 150 μ l saline) were not only administered locally after LAD occlusion, but also received intraperitoneal implantation of osmotic mini-pumps containing BK or DBK at 1 μ g/h ($n = 7$). For induction of myocardial I/R in wild-type, B1R $^{-/-}$ and B2R $^{-/-}$ mice, animals were anesthetized with ketamine/xylazine (50 mg/kg, intraperitoneally). I/R was surgically induced by ligation of the LAD for 30 min followed by reperfusion for 24 h.

2.3. In vivo hemodynamic measurements

Hemodynamic parameters were measured upon the completion of reperfusion in rats as previously described [22]. Heart rate (HR), mean arterial pressure (MAP), left ventricular enddiastolic pressure (LVEDP), left ventricular end-systolic pressure (LVESP) and cardiac contractility (dP/dt maximum and dP/dt minimum) were recorded and analyzed by a polygraph system (BIOPAC).

2.4. Histological studies

Infarct size was analyzed with planar morphometry in the heart sections stained with triphenyltetrazolium chloride (TTC) (Sigma) and expressed as a ratio of the left ventricular area. Each heart was cut into five biventricular sections of similar thickness, which were incubated in a 1.5% TTC solution (diluted in PBS, pH 7.4) at 37 °C for 5 min for rat sections and 15 min for mouse sections. Both sides of each slice were photographed with a digital camera (Kodak 4500) connected to a stereomicroscope. The boundary of the unstained area (infarcted tissue) was traced in a blinded fashion and quantified with dedicated software (NIH Image). Masson's trichrome staining was performed to determine the infarct size and collagen deposition in rat cardiac sections.

DNA fragmentation was determined using a terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay in 4 µm-thick paraffin embedded sections as previously described [22]. The procedure was performed using an *in situ cell* death detection kit (Roche, Indianapolis, IN) according to the manufacturer's instructions. TUNEL-positive cardiomyocytes in the ischemic myocardium were carefully distinguished from TUNEL-positive non-cardiomyocytes and evaluated under double-blinded conditions. The ratio of TUNEL-positive cardiomyocytes to the total number of cardiomyocytes was calculated.

2.5. Western blot

Heart tissues in the infarct area were homogenized and cultured cardiomyocyte were harvested in the extraction buffer. Western blot analysis was performed to detect the total and phosphorylated forms of Akt and GSK-3β (Cell Signaling). Membranes were incubated with secondary antibody conjugated to LumiGLO chemiluminescent reagent. Bound antibodies were visualized by ECL chemiluminescence using an ECL-Plus kit (Perkin-Elmer) and visualized by Kodak X-ray film. The bands were quantified by densitometry.

2.6. Statistics analysis

Data were expressed as mean ± S.E.M. and were compared between experimental groups with the use of one-way ANOVA followed by Fisher's PLSD. Probability values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. BK, but not DBK, reduces infarct size and improves cardiac function after acute I/R

Table 1 shows the effects of BK and DBK on cardiac function after 24-h reperfusion. HR and MAP were not changed among all groups. BK injection significantly improved impairments in contractility (dP/dt_{max}) and diastolic function (LVEDP and dP/dt_{min}) after I/R, but DBK did not exert any protective effects. Moreover, representative TTC staining at 1 day after I/R showed that treatment with BK but not DBK reduced myocardial infarct size as compared to I/R control (Fig. 1A). Quantitative analysis showed that BK injection significantly reduced infarct size in the left ventricle compared with the I/R control group (21.5 ± 6.1% versus 49.7 ± 9.2%, $n = 6-7$, $P < 0.01$), whereas DBK did not have a significant effect on infarct size (Fig. 1B). No alterations were observed in left ventricular infarct thickness among all the groups subjected to I/R (data not shown). Taken together, these results indicate that activation of kinin B2 receptor, but not B1 receptor, reduced infarct size and improved cardiac function at the early stage after myocardial I/R.

3.2. BK, but not DBK, inhibits I/R-induced cardiomyocyte apoptosis

Apoptotic cardiomyocytes were detected by TUNEL staining in the myocardium at 1 day after I/R. Representative TUNEL-staining showed that I/R injury induced apoptosis in the ischemic

myocardium. BK treatment reduced the level of apoptotic cells, but DBK had no apparent effect on I/R-induced cardiomyocyte apoptosis (Fig. 2A). Quantitative analysis showed that the ratio of TUNEL-positive cardiomyocytes to total number of cardiomyocytes in the BK-treated group was significantly reduced as compared to the control ($23.1 \pm 2.5\%$ versus $45.9 \pm 3.0\%$, $n = 6$ and 7 , $P < 0.01$). However, there was no difference between DBK-treated and I/R control group ($42.7 \pm 6\%$ versus $45.9 \pm 3.0\%$, $n = 7$ and 6) (Fig. 2B). We further examined the effect of BK and DBK on activation of Akt-GSK-3 β signaling in the ischemic heart by Western blot analysis (Fig. 3). BK treatment resulted in increased phosphorylation of Akt and GSK-3 β in the I/R-injured myocardium as compared to the control and DBK-treated myocardium. Total Akt and GSK-3 β levels did not change among all the groups. These combined data indicate differential effects of BK and DBK on cardiomyocyte apoptosis.

3.3. BK, but not DBK, limits cardiac remodeling at 7 days after I/R

We further evaluated the effects of BK and DBK on ventricular remodeling at 7 days after reperfusion. Masson's trichrome staining showed characteristic LV free wall thinning after I/R, which was prevented by BK but not by DBK (Fig. 4A). Quantitative analysis confirmed that BK significantly restored LV wall thinning (2.4 ± 0.33 mm versus 1.05 ± 0.08 mm, $n = 6-7$, $P < 0.01$), but DBK had no effect on LV wall thickness (Fig. 4B). Measurements of hemodynamic parameters showed impaired contractility (dP/dt_{max}), and diastolic function (LVEDP and dP/dt_{min}) along with reduced heart rate and MAP at 7 days after I/R (Table 1). These parameters were worse compared to those at 1 day after I/R. BK prevented deterioration of both systolic and diastolic function in conjunction with ameliorated LV remodeling. Consistent with the LV remodeling findings, DBK exerted no improvement of cardiac function. Collectively, these results indicate that kinin B2 receptor activation but not B1 receptor activation prevents impaired ventricular remodeling and effectively improves cardiac function at the late stage after I/R injury.

3.4. Myocardial infarct size in B1 and B2 receptor knockout mice after I/R

To further verify the findings obtained from the rat model after I/R injury, we compared the infarct size of B1R and B2R knockout mice at 1 day after I/R. Representative TTC staining of the heart sections and quantitative analysis showed that LV infarct size of B1R $-/-$ mice was significantly smaller compared with wild-type mice after I/R ($21.5 \pm 6.1\%$ versus $49.7 \pm 9.2\%$, $n = 6-7$, $P < 0.01$), whereas there was no difference of infarct size between B2R $-/-$ and wild-type groups (Fig. 5A and B). These data provide evidence that kinin B2 receptor but not B1 receptor mediates the protective effects in myocardium after I/R injury.

4. Discussion

In the present study, we demonstrated that the kinin B2 receptor, but not B1, receptor exerts cardioprotective effects against myocardial I/R injury by using both pharmacological approaches and genetic animal models. Our results show that administration of BK, but not DBK, attenuates cardiomyocyte apoptosis, reduces infarct size and improves cardiac function and remodeling in rats after I/R injury. Furthermore, the anti-apoptotic effect of BK is correlated with increased Akt and GSK-3 β phosphorylation. It should be noted that BK and DBK have very short half-lives. In addition, BK can be catabolized to DBK by endogenous kininase I, although the amount of DBK produced should not be sufficient enough to detract from the specific effects of BK. Our results showed that BK, but not DBK, reduced apoptotic cardiomyocyte and improved cardiac function and LV remodeling, indicating that the short duration of BK can still cover the critical time in which apoptosis is initiated after ischemia. The role of kinin B2 receptor in protection against I/R-induced myocardial infarction was further confirmed using kinin B2 receptor and B1 receptor knockout mice. Our findings provide

significant insights regarding the differential role of kinin B2 and B1 receptors in cardiac protection.

Previous studies reported that B1R levels are significantly higher in B2R knockout mice and vice versa [2,4]. Our results showed that infarct size and LV performance are ameliorated in B1R knockout mice with higher B2R levels compared with that of wild-type and B2R knockout mice. On the other hand, in B2R knockout mice with higher B1R levels, cardiac injury was deteriorated compared to B1R knockout mice. In the present study, the infarct size was determined by LAD-irrigated area within the left ventricle. In the B2R knockout mice, the infarct size reached the maximum in the LAD area that could not be significantly larger than wild-type control despite the protective role of the B2R. In addition, B1R activation by DBK did not protect against LV ischemic injury compared to B2R activation in rats. These combined findings further confirm the detrimental role of B1R and the protective role of B2R.

A number of studies have reported that kinin B2 receptor activation protects against myocardial injury after infarction or I/R. For example, B2R mediates cardioprotective effects of losartan and captopril in the cardiac ischemic setting. These actions can be blocked by a B2R antagonist, indicating that B2R is necessary for angiotensin receptor activation after myocardial injury [5,11,23]. Moreover, the principal mechanism of kinin's actions includes increase of regional blood flow and facilitation of preconditioning [3,4,6,9,17]. These effects are mediated through mitochondrial K_{ATP} channel opening and release of reactive oxidative species [13]. The mechanism in kinin-triggered preconditioning and inhibition of apoptosis has been shown to be mediated by Akt and GSK-3 β activation and nitric oxide (NO) formation. Akt is a key effector of PI-3-kinase in the survival pathway against apoptosis. Phosphorylation of GSK-3 β by Akt results in its inactivation [1,9,21]. Inactive GSK-3 β has been shown to be a positive regulator of cardiomyocyte apoptosis and cardiac preconditioning after ischemic injury [14,22]. In this study, we showed that BK but not DBK protects against I/R-induced cardiac dysfunction and cardiomyocyte apoptosis through activation of Akt/GSK-3 β signaling. This is consistent with our previous study that tissue kallikrein inhibits cardiomyocyte apoptosis by kinin B2 receptor activation and Akt/GSK-3 β signaling [22]. Taken together, our studies indicate that B2R contributes to cardiac protection against ischemic injury.

In contrast, DBK administration had no effect on cardiac injury after MI. In addition, infarct size of B1R-deficient mice was significantly smaller than that of B2R-deficient mice. These findings indicate that B1R does not protect against MI. Previous reports attempting to elucidate the function of B1R after myocardial ischemia are controversial. B1R has been shown to protect against myocardial ischemia as blockade of angiotensin 1 (AT1) receptor could limit infarct size and improve cardiac function, but was reversed by B1R antagonist [19]. However, B1R antagonist alone has no effect in modifying LV function after MI in rats [19]. Moreover, a recent study reported that B1R $-/-$ mice had a larger LV diastolic chamber dimension compared with wild-type mice at 8 weeks after MI. This finding suggests that B1R plays an important role in regulation of structural homeostasis at the early stage rather than remodeling at late stage after cardiac ischemic injury [20]. The role of B1R appears to be ambiguous because this study demonstrates a similar myocardial injury, cardiac function and remodeling between all female wild type and B1R $-/-$ mice.

Despite the evidence for a neutral role for B1R, recent studies indicate a detrimental role for B1R after ischemic injury. Lipopolysaccharide (LPS) induces higher levels of B1R. However, neither B1R agonist nor antagonist modifies the reduction of infarct size by LPS, suggesting that elevated expression of B1R is not involved in protective effects after myocardial ischemia [12]. In addition, Lagneux et al further reported that infarct size was significantly reduced in B1R $-/-$ mice compared with wild-type mice and administration of B1R antagonist significantly reduced I/R injury in wild-type mice [10]. Furthermore, the protective actions of

B2R are related to regulation of Na⁺/H⁺ and Na⁺/Ca²⁺ exchanger, whereas B1R is not [16]. Moreover, a recent study revealed that neutrophil migration in inflamed tissues after I/R injury is significantly reduced in kinin B1 receptor-deficient mice, and pretreatment of B2 receptor antagonist reversed this anti-inflammatory effect [18], indicating that kinin B2 receptor may prevent exacerbated tissue injury in the absence or with very low levels of the B1 receptor. Consistent with these data, our present study clearly demonstrates that B1 receptor has no apparent protective actions against cardiac injury.

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Fig. 1. Myocardial infarct size after 30-min ischemia followed by 24-h reperfusion. (A) Representative photomicrographs show ventricular infarct size stained with TTC from I/R-injured ventricular sections of rats injected with BK or DBK. Original magnification is 10 $\times$. (B) Quantitative analysis of infarct size from I/R-injured cardiac sections. Data are expressed as mean $\pm$ S.E.M., $n = 6-7$. * $P < 0.01$ vs. other groups.

Fig. 2.

Effects of BK and DBK on cardiomyocyte apoptosis after 30-min ischemia in rats followed by 24-h reperfusion in rats. (A) Representative photomicrographs show TUNEL-positive apoptotic cardiomyocytes from I/R-injured ventricular sections of rats injected with BK or DBK. TUNEL-positive non-cardiomyocytes were excluded. Original magnification is 200 $\times$. (B) Quantitative analysis of apoptotic cardiomyocytes expressed as percentage of TUNEL-positive nuclei in cardiomyocytes. Data are expressed as mean $\pm$ S.E.M., $n = 6-7$. * $P < 0.05$ vs. other groups.

Fig. 3.

Effects of BK and DBK on Akt and GSK-3 β phosphorylation after 30-min ischemia followed by 24-h reperfusion in rats. (A) Representative Western blot of myocardial extracts from sham, I/R control, and I/R receiving BK and DBK. Histograms show relative intensity of phosphor-Akt (B) and phosphor-GSK-3 β (C), * $P < 0.05$ vs. other I/R groups, $n = 6-7$.

Fig. 4. Left ventricular remodeling after 30-min ischemia followed by 7-day reperfusion in rats. (A) Representative photomicrographs show ventricular sections stained with Masson's trichrome from sham, I/R control, and I/R receiving BK and DBK. Original magnification is 10 $\times$. (B) Quantitative analysis of left ventricular wall thickness. Data are expressed as mean $\pm$ S.E.M., $n = 6-8$. * $P < 0.01$ vs. other groups.

Fig. 5. Myocardial infarct size in wild-type, B1R^{-/-} and B2R^{-/-} mice after 30-min ischemia followed by 24-h reperfusion. (A) Representative photomicrographs show ventricular infarct size stained with TTC from I/R-injured ventricular sections. Original magnification is 10 $\times$. (B) Quantitative analysis of infarct size from I/R-injured cardiac sections. Data are expressed as mean $\pm$ S.E.M., $n = 6-7$. * $P < 0.01$ vs. other groups.

Table 1

Effects of BK and DBK on hemodynamics at 1 day and 7 days after I/R

	Sham	I/R		
		Control	BK	DBK
1 day				
HR (bpm)	353.2 ± 10.4	361.7 ± 9.8	355.7 ± 11.4	359.7 ± 12.9
MAP (mmHg)	108.0 ± 8.4	100.4 ± 5.8	102.3 ± 6.3	99.4 ± 5.6
LVEDP (mmHg)	1.45 ± 0.25 [†]	17.5 ± 2.6	8.27 ± 1.3 [*]	15.8 ± 3.3
+dP/dt (mmHg/s)	4525.7 ± 205.0 [†]	2641.4 ± 166.9	3436.1 ± 245.6 [*]	2717.2 ± 157.5
-dP/dt (mmHg/s)	3843.5 ± 210.3 [†]	2112.7 ± 186.9	3115.1 ± 237.3 [*]	2225.7 ± 184.9
7 days				
HR (bpm)	364.7 ± 14.2 [*]	309.5 ± 12.1	357.3 ± 15.3 [*]	313.6 ± 13.7
MAP (mmHg)	107.2 ± 9.0 [†]	73.3 ± 5.9	90.1 ± 9.6 [*]	70.9 ± 5.6
LVEDP (mmHg)	3.3 ± 0.65 [†]	15.5 ± 1.9	7.4 ± 1.1 [*]	12.6 ± 3.6
+dP/dt (mmHg/s)	4797.5 ± 147.8 [†]	2366.3 ± 124.9	3421.5 ± 142.1 [*]	2530.3 ± 101.8
-dP/dt (mmHg/s)	3615.8 ± 163.9 [†]	1631.4 ± 89.3	2803.3 ± 131.4 [*]	1753.3 ± 111.5

HR, heart rate; bpm, beats per minute; MAP, mean arterial pressure; LVEDP, left ventricular end-diastolic pressure; dP/dt_{max}, maximum first derivative of pressure; dP/dt_{min}, minimum first derivative of pressure.

* $P < 0.05$ vs. I/R control and I/R-DBK

[†] $P < 0.05$ vs. I/R groups.