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Expression of kininogen, kallikrein and kinin receptor genes by rat cardiomyocytes

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Abstract

To ascertain the existence of the kallikrein-kinin system in the heart, we have studied in vivo and in vitro whether rat cardiac tissue expresses kininogen, kallikrein and kinin receptor mRNAs. The reverse transcription-polymerase chain reaction demonstrated that the ventricular myocardium of adult male rats expressed mRNAs for T- and low-molecular-weight (L-) kininogens, tissue kallikreins such as true kallikrein and T-kininogenase, and bradykinin B2 receptor, but not those for high-molecular-weight kininogen and B1 receptor. Lipopolysaccharide (LPS; 0.5 mg/kg, i.v.) increased the levels of mRNA for T-kininogen at 12 h and the bradykinin B1 receptor at 24 h without affecting that for other components. All of these mRNAs for the kallikrein-kinin system were also detected in cultured cardiomyocytes derived from neonatal rat ventricles; dibutyryl cyclic AMP, LPS or inflammatory cytokines such as interleukin-1 and tumor necrosis factor, up-regulated mRNA expression of T-kininogen, T-kininogenase, or B1 receptor in these cells in vitro. These results suggest that there are two kinin-generating systems in rat myocardium comprising T-kininogen/T-kininogenase and L-kininogen/true kallikrein respectively, and that the former may be relatively important in inflammatory diseases or conditions in which cAMP levels increase in cardiomyocytes. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Kininogen; Kallikrein; Cardiomyocyte; Rat; Lipopolysaccharide

1. Introduction

Numerous studies have demonstrated that angiotensin-converting enzyme (ACE) inhibitors can improve survival in patients or animals with severe heart failure, delay the development of heart failure in patients or animals with a symptomatic left ventricular dysfunction, and reduce short- and long-term mortality in patients or animals surviving myocardial infarction [1–3]. These beneficial effects of ACE inhibitors have been attributed to not only a decrease in the local cardiac formation of angiotensin II, but also a potentiation of kinin activity [4] and/or inhibition of its degradation [5–11].

Bradykinin or its analogues are released from kininogens by the proteolytic action of kallikreins, exert their activities through binding to B1 or B2 receptors, and show a broad spectrum of biological effects including vasodilation, blood pressure reduction, smooth muscle relaxation and contraction, pain

Abbreviations: H-, high-molecular-weight; L-, low-molecular-weight; LPS, lipopolysaccharide; RT-PCR, reverse transcription-polymerase chain reaction; Bt_2cAMP, dibutyryl cyclic AMP; PMA, phorbol 12-myristate 13-acetate; IL-6, interleukin-6; IL-1, interleukin-1α; TNF, tumor necrosis factor α; DME, modified Eagle's medium; FBS, fetal bovine serum; ACE, angiotensin converting enzyme

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induction, and inflammation [12,13]. Since concentrations of kinins in blood are very low, the formation of kinin by the kallikrein-kinin system has been considered to operate mainly at the local tissue level [14]. Consequently, a reliable method for the measurement of kinin levels in tissue has demonstrated levels much higher than in the circulation in rats, suggesting the formation at local sites [15].

The role of kinins as possible mediators for the endogenous cardioprotective mechanisms as well as the therapeutic benefits of ACE inhibitors in heart failure is based on the existence of local kallikrein-kinin system in cardiac tissues. Nolly et al. found that slices of rat heart secreted a kininogen-like protein from which an immunoreactive kinin was released by trypsin digestion [16]. They also demonstrated that tissue kallikrein mRNA was present in both atrial and ventricular tissues of rat heart by reverse transcription-polymerase chain reaction (RT-PCR). Chao et al. reported that rat heart contained a kininogen-like protein whose levels elevated on the induction of acute inflammation [17]. In addition, heart tissues and cardiomyocytes express functional B2 receptors, suggesting that cardiomyocytes are not only a source of kinins, but also a target for kinin-mediated effects [18–21].

The present study has been carried out to characterize mRNA expressions of kininogens, kallikreins and bradykinin receptors in rat heart in vivo and in isolated cardiomyocytes in vitro. The results indicate that rat cardiomyocytes as well as cardiac tissues express mRNAs for these components and that various factors, such as lipopolysaccharide (LPS), cyclic AMP and cytokines, influence their levels.

2. Materials and methods

2.1. Materials

The following chemicals were obtained from commercial sources: LPS (*Staphylococcus typhosa* 0901) from Difco (Detroit, MI); angiotensin II (AII) and bradykinin from Peptide Institute (Osaka); dibutyryl cyclic AMP (Bt_2cAMP) from Boehringer-Mannheim-Yamanouchi (Tokyo); phorbol 12-myristate 13-acetate (PMA) from Nacalai tesque (Kyoto); recombinant mouse interleukin-6 (IL-6; 1×10^8 U/mg) from Genzyme. Recombinant human interleukin-1α (IL-1; 2×10^7 U/mg) and tumor necrosis factor α (TNF; 3×10^6 U/mg) were donated by Dainippon Pharmaceutical (Osaka).

2.2. Animals and LPS treatment

Sprague-Dawley male rats, weighing 140–160 g, were injected intravenously with LPS at a dose of 0.5 mg/kg, then sacrificed at 12, 24, and 48 h. Ventricles of the heart were isolated.

2.3. Culture of neonatal rat cardiomyocytes

Two- to 4-day-old Sprague-Dawley rats were used for the isolation of cardiac myocytes and nonmyocytes. The ventricular cardiac cells were dispersed in Hanks' balanced salt solution containing 0.04% collagenase II and 0.06% pancreatin (Gibco) as previously reported [22]. Cardiac myocytes and nonmyocytes were separately collected by the discontinuous Percoll gradient method [22]. Briefly, a discontinuous gradient of Percoll consisting of 40.5% and 58.5% was prepared in the Hanks' balanced salt solution, and cardiac cells were suspended in a layer of 58.5% Percoll. After centrifugation at $850\times g$ for 30 min at 15°C, myocytes migrated to the interface of discontinuous layers, and nonmyocytes to the surface of the layer of 40.5% Percoll. Purified cardiomyocytes or nonmyocytes were plated at a density of 3×10^4 cells/cm^2 in Dulbecco's modified Eagle's medium (DME) containing 10% fetal bovine serum (FBS). After culture for 24 h, the medium was replaced with fresh DME containing 10% FBS with or without various agents. After a further 24 h culture, cells were harvested to extract total RNA. Using this method, we routinely obtained contractile myocyte-rich cultures with 90–95% myocytes as assessed using immunocytochemistry with a monoclonal antibody against β-myosin heavy chain.

2.4. Detection of kininogen and tissue kallikrein mRNAs in myocardium and cardiomyocytes

Total RNA was extracted with acid guanidinium-phenol-chloroform from isolated myocardium or cultured cardiomyocytes [23]. We used RT-PCR followed by Southern blotting for detecting mRNAs

of kininogens and tissue kallikreins, such as high-molecular-weight (H-), low-molecular-weight (L-) and T-kininogens, true kallikrein (*rKLK1*), and T-kininogenase (*rKLK10*), as described previously [24].

2.5. Detection of bradykinin B1 and B2 receptor mRNAs in myocardium and cardiomyocytes

Levels of B1 and B2 receptor mRNAs in myocardium and cardiomyocytes were determined by RT-PCR followed by Southern blotting as follows. Total RNA (1 μg) was reverse transcribed in a 20 μl reaction mixture containing 50 pmol of the reverse primer, 2 μl of 10 mM dNTP, 2 μl of 10×PCR buffer (0.1 M Tris-HCl, pH 8.3, 500 mM KCl), 4 μl of 25 mM $MgCl_2$, 1 unit of RNase inhibitor, and 1 unit of cloned Moloney murine leukemia virus reverse transcriptase (GeneAmp RNA PCR kit; Takara, Japan). The RT reaction mixture was incubated at 42°C for 15 min, 95°C for 5 min, then 4°C for 5 min to allow synthesis of the first strand of cDNA. The cDNA was amplified in a 100 μl reaction mixture containing 50 pmol of the forward primer, 8 μl of 10×PCR buffer, 4 μl of 25 mM $MgCl_2$, and 2.5 units of *Taq* DNA polymerase. Thirty-five (B2 receptor) or 40 (B1 receptor) cycles proceeded as follows: denaturation at 95°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min. Then 8 μl aliquots were Southern blotted and autoradiographed using a Fuji Film Bio Imaging Analyzer BAS1000 (Fuji Film, Tokyo). The forward and reverse primers for B1 receptor were 5′-AGAAACCTCCCAAGACAGCA-3′ (nucleotides 26–45) and 5′-AGGAATGTGGGGA-TGCTCAA-3′ (nucleotides 679–698), respectively. The forward and reverse primers for B2 receptor were 5′-GGACCATGAAGGACTACAGG-3′ (nucleotides 891–910) and 5′-TAGGCCACGTAGGA-ACTGAT-3′ (nucleotides 1250–1269), respectively. A specific oligonucleotide for B1 receptor (5′-AGTA-CAGGAGGTCAAAT-3′) was used as an internal probe for hybridization. The probe for Southern hybridization of B2 receptor was a 400 bp fragment of B2 receptor cDNA.

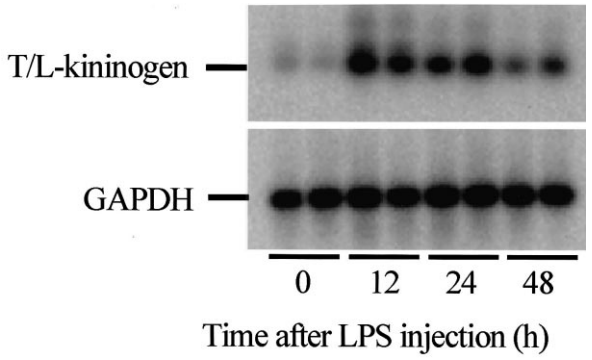

Fig. 1. Southern blot analysis of cDNA products generated from rat ventricular myocardium by RT-PCR using primers and probe common for both T- and L-kininogen. Rats were injected with LPS (0.5 mg/kg, i.v.), then four rats each were sacrificed at 12, 24 and 48 h by bleeding under ether anesthesia. Four untreated animals were sacrificed as an untreated control. Ventricular myocardium was isolated and total RNA was extracted. One μg RNA samples were analyzed by RT-PCR (40 cycles) followed by Southern blotting using T-kininogen cDNA as a probe, and two blots from two animals for each time point after LPS injection are shown as representative of the results. Bands in the upper panel represent a mixture of T- and L-kininogen cDNAs. The bottom panel shows Southern blots of the RT-PCR products amplified (25 cycles) for glyceraldehyde dehydrogenase (GAPDH) cDNA.

3. Results

3.1. Detection of kininogen mRNAs in rat ventricular myocardium

Three types of kininogens have been found so far in mammalian species; these differ by molecular weight and susceptibility to various kininogenases [25]. Two of them, H- and L-kininogen, are present throughout the mammalian lineage, whereas the third type, T-kininogen, is considered to be unique for the rat [25]. We attempted to detect H-, L-, and T-kininogen mRNAs in rat myocardium by RT-PCR and found a faint signal corresponding to T- and L-kininogen cDNAs (Fig. 1), while no signal corresponding to H-kininogen mRNA (data not shown). Since we used a set of primers common for T- and L-kininogen mRNAs, the RT-PCR products seemed to be a mixture of cDNAs amplified from both T- and L-kininogen mRNAs. Induction of systemic inflammation following i.v. injection of LPS resulted in increased levels of T/L-kininogen mRNA in rat ventricle within 12 h (Fig. 1), while there was no induction of the H-kininogen mRNA during 48 h after the LPS treatment (data not shown). To determine

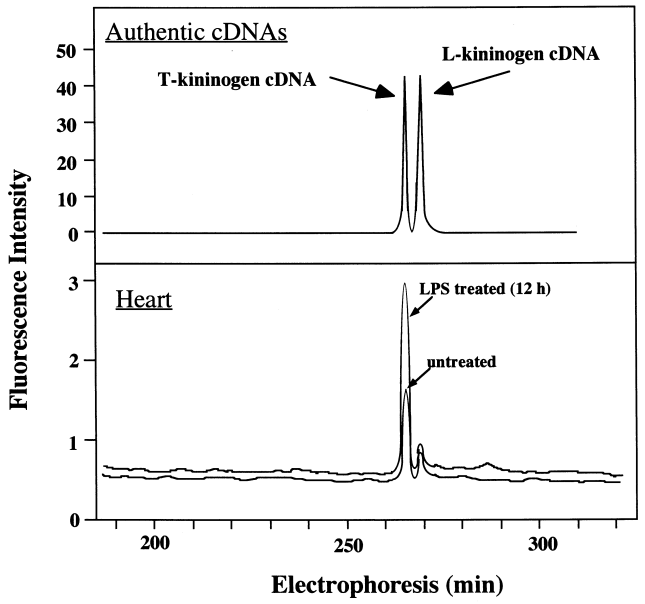

Fig. 2. Separation of RT-PCR products of T- and L-kininogen mRNAs in rat ventricular myocardium by polyacrylamide gel electrophoresis. Total RNA samples were prepared from myocardium of untreated or LPS-pretreated rats (24 h, 0.5 mg/kg, i.v.). The cDNAs transcribed from these samples were amplified by 18 cycles using a fluorescence-labeled 5′-primer and non-labeled 3′-primer which were specific for both T- and L-kininogen cDNAs. The labeled products were separated by polyacrylamide gel electrophoresis and analyzed using an ALFred DNA sequencer (Pharmacia; lower panel). The retention times for authentic T-kininogen and L-kininogen cDNAs were 264 and 269 min, respectively (upper panel). Experiments were carried out with RNA samples from four untreated and four LPS-treated animals, and representative results are shown.

whether RT-PCR products corresponding in size to T- and L-kininogen cDNAs were actually composed of these cDNAs, RT-PCR was carried out using a fluorescent Cy5-labeled forward primer common for both T- and L-kininogen cDNAs, then labeled cDNA products were resolved by an ALFred DNA sequencer (Pharmacia) on the basis of a 6 bp difference in cDNA products between T-(315 bp) and L-(321 bp) kininogens as described previously [24]. The RT-PCR products of untreated rat hearts revealed two faint peaks corresponding to T- and L-kininogen cDNAs (Fig. 2). cDNA products of LPS-treated rat hearts comprised a clear large peak of T-kininogen cDNA and a faint peak of L-kininogen cDNA (Fig. 2). Thus it was concluded that the rat myocardium expressed both T- and L-kininogen mRNAs and that LPS exclusively stimulated an expression of the T-kininogen gene.

3.2. Detection of kininogen mRNAs in rat cardiomyocytes in vitro

We recently reported that T-kininogen mRNA was present in rat fibroblasts [26] and vascular smooth muscle cells [24], and found that the expression by fibroblasts was stimulated by Bt_2cAMP and cytokines such as TNF, IL-1 and IL-6, while that by vascular smooth muscle cells was enhanced by LPS, PMA and angiotensin II. Thus it may be that these cells rather than cardiomyocytes are the source of kininogen mRNA in myocardium. To determine which type of cell in rat myocardium expresses kininogen mRNA, cardiac cells from neonatal rat heart were separated into myocytes and nonmyocytes, then cultured in vitro. As shown in the representative autoradiogram in Fig. 3, although no signal corresponding to T/L-kininogen mRNA was detected in cultured myocytes, a distinct signal for T/L-kininogen mRNA was observed in myocytes after 24 h culture with LPS (0.5 μg/ml) or Bt_2cAMP (1 mM), while not with PMA (10 μM) and cytokines, such as TNF (500 U/ML), IL-1 (500 U/ml) and IL-6 (500 U/ml). In contrast, a faint signal corresponding to T/L-

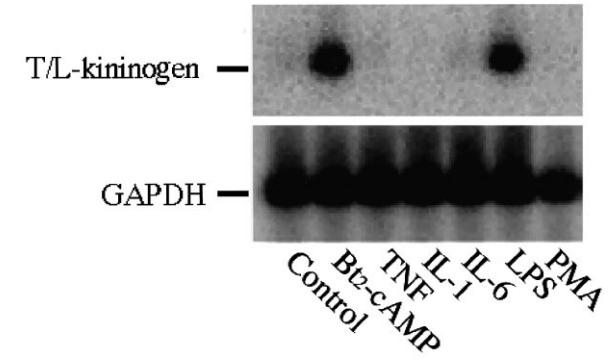

Fig. 3. Detection of kininogen mRNAs in rat cardiomyocytes by RT-PCR followed by Southern blotting. Rat cardiomyocytes were cultured with or without Bt_2cAMP (1 mM), LPS (0.5 μg/ml), PMA (10 μM), TNF (500 U/ml), IL-1 (500 U/ml) or IL-6 (500 U/ml) for 24 h. Total RNA was extracted and subjected to RT-PCR for T/L-kininogen mRNA (40 cycles) or H-kininogen mRNA (40 cycles), then Southern blotting of the cDNA products was carried out using T-kininogen or H-kininogen cDNA as a probe, respectively. Experiments were carried out 4 times for each treatment, and representative results are shown.

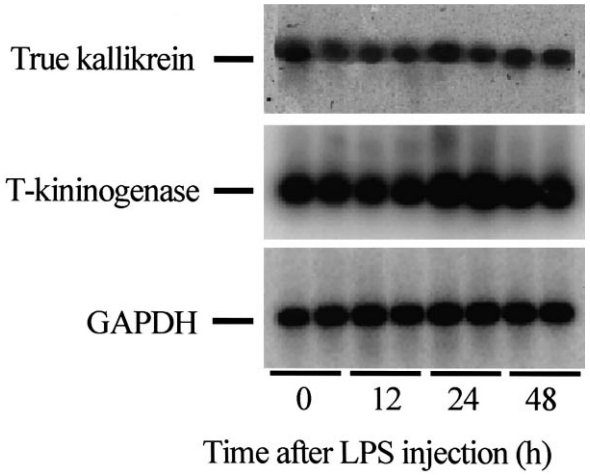

Fig. 4. Detection of tissue kallikrein mRNAs in rat ventricular myocardium by RT-PCR followed by Southern blotting. RNA samples were obtained from ventricular myocardium of untreated and LPS-pretreated rats, as described in the legend of Fig. 1. One μg of the sample was analyzed by RT-PCR followed by Southern blotting for the true kallikrein (35 cycles), T-kininogenase (40 cycles) and GAPDH (25 cycles). The figure shows two blots from two animals for each time point after LPS injection as representative of the results.

kininogen mRNA was also detected in nonmyocytes, but the levels were not influenced by treatment with LPS, Bt_2cAMP, PMA and cytokines (data not shown). Polyacrylamide gel electrophoresis of cDNA products demonstrated that rat cardiomyocytes expressed both T- and L-kininogen mRNAs, and that levels of T-kininogen mRNA but not of L-kininogen mRNA were increased by culture with LPS or Bt_2cAMP (data not shown). No detectable signal for H-kininogen mRNA was found in either cardiomyocytes or nonmyocytes (data not shown). Western blotting using a rabbit anti-T-kininogen antibody demonstrated that a T-kininogen-like protein with a molecular mass of 68 kDa was present in the conditioned medium of cardiomyocytes that had been cultured for 24 h with 1 mM Bt_2cAMP, but not in medium of untreated cardiomyocytes, suggesting that rat cardiomyocytes secrete T-kininogen in response to Bt_2cAMP (data not shown). These results indicate that rat cardiomyocytes are a source of T- and L-kininogens, and that the expression of the T-kininogen but not the L-kininogen gene is stimulated by LPS and Bt_2cAMP.

3.3. Detection of tissue kallikrein mRNAs in rat ventricular myocardium and cardiomyocytes

Rats have more than ten members of the tissue kallikrein family. Of these members, true kallikrein encoded by *rKLK1* has a kinin-liberating activity for L- and H-kininogens [13], but not T-kininogen [27], and T-kininogenase encoded by *rKLK10* has a kininogenase activity for T-kininogen [28]. Consistent with a report by Nolly et al. [16], we confirmed the presence of mRNA for true kallikreinin in rat myocardium, and that the levels did not change after an i.v. injection of LPS (Fig. 4). Since T-kininogen appeared to be a major kininogen produced by rat cardiac tissues, we tried to detect mRNA for *rKLK10*. As shown in Fig. 4, T-kininogenase mRNA was also detected in RNA samples from rat myocardium, and the levels were not altered by LPS injection. Tissue kallikrein mRNAs for *rKLK1* and *rKLK10* were detected in cultured cardiomyocytes (Fig. 5), but not in nonmyocytes (data not shown). Neither LPS (5 μg/ml), PMA (10 nM) nor cytokines (500 U/ml) influenced the levels of these kallikrein mRNAs in cardiomyocytes, but Bt_2cAMP

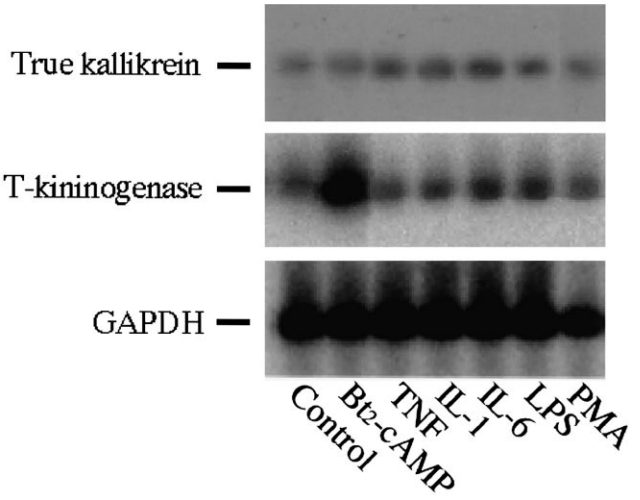

Fig. 5. Detection of tissue kallikrein mRNAs in rat cardiomyocytes by RT-PCR followed by Southern blotting. Rat cardiomyocytes were cultured with or without Bt_2cAMP (1 mM), LPS (0.5 μg/ml), PMA (10 μM), TNF (500 U/ml), IL-1 (500 U/ml) or IL-6 (500 U/ml) for 24 h, then RNA samples were amplified by RT-PCR for true kallikrein (35 cycles), T-kininogenase (40 cycles) and GAPDH (25 cycles) and Southern blotted. Experiments were carried out 4 times for each treatment, and representative results are shown.

(1 mM) caused an increase in levels of T-kininogenase mRNA (Fig. 5).

3.4. Detection of B1 and B2 receptor mRNAs in rat ventricular myocardium and cardiomyocytes

By RT-PCR Southern hybridization, we detected bradykinin B2 receptor mRNA in either rat myocardium or cultured cardiomyocytes, and the levels were not affected by the injection of or culture with LPS (Figs. 6 and 7). Although B1 receptor mRNA could not be detected in untreated rat hearts, LPS injection caused a marked induction of B1 receptor mRNA at 12–24 h (Fig. 6). B1 receptor mRNA was detectable in cultured cardiomyocytes, and the level was increased after culture with LPS (5 μg/ml), IL-1 (500 U/ml) and TNF (500 U/ml), but decreased by IL-6 (500 U/ml) (Fig. 7). These agents did not affect the levels of B2 receptor mRNA in cardiomyocytes.

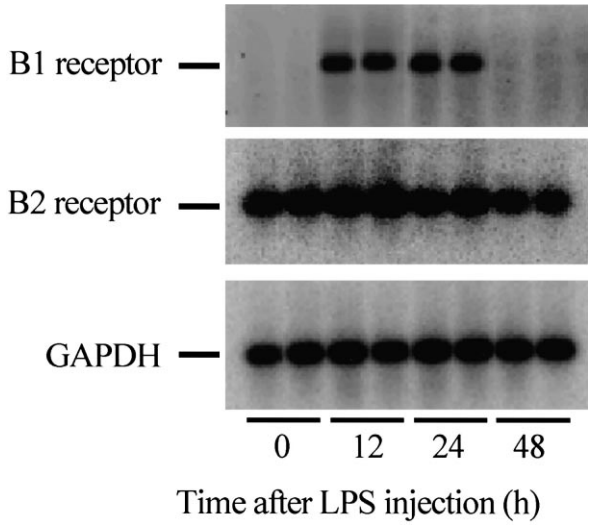

Fig. 6. Detection of bradykinin B1 and B2 receptor mRNAs in rat ventricular myocardium by RT-PCR followed by Southern blotting. RNA samples were obtained from the ventricular myocardium of untreated and LPS-pretreated rats, as described in the legend of Fig. 1. One μg of the sample was analyzed by RT-PCR followed by Southern blotting for B1 receptor (40 cycles), B2 receptor (35 cycles) and GAPDH (25 cycles). The figure shows two blots from two animals for each time point after LPS injection as representative of the results.

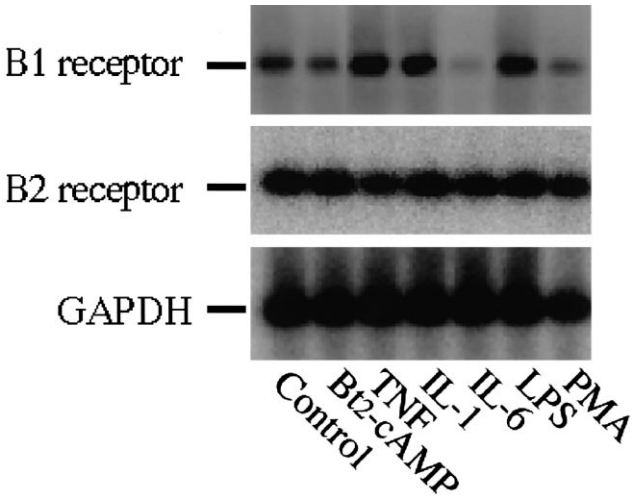

Fig. 7. Detection of bradykinin B1 and B2 receptor mRNAs in rat cardiomyocytes by RT-PCR followed by Southern blotting. Rat cardiomyocytes were cultured with or without Bt_2cAMP (1 mM), LPS (0.5 μg/ml), PMA (10 μM), TNF (500 U/ml), IL-1 (500 U/ml) or IL-6 (500 U/ml) for 24 h, then RNA samples were amplified by RT-PCR for B1 receptor (40 cycles), B2 receptor (35 cycles) and GAPDH (25 cycles) followed by Southern blotting. Experiments were carried out 4 times for each treatment, and representative results are shown.

4. Discussion

The present study provides the first evidence that rat cardiomyocytes are the source of T-kininogen, a unique kininogen in the rat, but poor sources of H- and L-kininogens. T-kininogen has been characterized as a protein highly homologous in structure to rat L-kininogen as well as one of the acute-phase proteins in the rat [27,29]. This protein is produced not only by hepatocytes but also by fibroblasts and vascular smooth muscle cells [24,26]. In fact, T-kininogen mRNA has been detected in several tissues of rats, such as the liver, lung, kidney and heart [17,30]. However, Barka and van der Noen have reported that the T-kininogen gene is not expressed in rat heart on the basis of findings that T-kininogen mRNA was not detectable by a sensitive RT-PCR method in cardiac tissue of either normal or turpentine-injected rats [31]. In contrast to their results, we detected T-kininogen mRNA in rat ventricular myocardium and found that the level was increased after injection of LPS. Furthermore, cardiomyocytes from neonatal rat hearts not only contained T-kininogen mRNA but also secreted immunoreactive T-

kininogen in culture. Since we used the same primers for detecting T-kininogen mRNA as they used, the reason for this discrepancy is not obvious. Interestingly, an addition of LPS to cultures resulted in increased levels of T-kininogen mRNA in cardiomyocytes, suggesting that LPS acts directly on cardiomyocytes to induce the T-kininogen gene rather than indirectly by LPS-induced mediators, such as IL-1 and IL-6. This may be why the T-kininogen mRNA level was not elevated in rat heart even after induction of inflammation by turpentine [31]. The fact that little T-kininogen mRNA was detected in nonmyocytes in the presence or absence of LPS in vitro suggests that cardiomyocytes are the major cells expressing the T-kininogen gene in rat heart.

When RT-PCR products amplified by primers common for T- and L-kininogen mRNAs were resolved on polyacrylamide gel electrophoresis, we observed a faint peak with a retention time corresponding to L-kininogen cDNA in RNA samples, suggesting the presence of L-kininogen mRNA in cardiomyocytes. However, the levels were very low in comparison to the T-kininogen mRNA level and did not change after LPS treatment in vivo or in vitro, making the physiological relevance unclear. In addition, we could not detect H-kininogen mRNA in either ventricular myocardium or cardiomyocytes, even by a sensitive RT-PCR method. Thus it is likely that rat cardiomyocytes are poor sources of L- and/or H-kininogens. Nolly et al. reported that rat cardiac slices secreted a kininogen which liberated bradykinin-like immunoreactivity by trypsin digestion [16], providing the first evidence for the presence of cardiac kininogen. It is well known that T-kininogen releases Ile-Ser-bradykinin by trypsin digestion and that this bradykinin analogue is recognized by polyclonal anti-bradykinin antibodies. Thus, our findings, together with the evidence described above, indicate that the cardiac kininogen in the rat is mainly T-kininogen, not H- and L-kininogens.

As demonstrated by Nolly et al., rat cardiac tissues and cardiomyocytes synthesize and secrete a tissue kallikrein corresponding to the true kallikrein [16]. In addition to this subtype of tissue kallikreins, we found the mRNA of T-kininogenase to be present in rat cardiomyocytes. Ma et al. have demonstrated that T-kininogenase is expressed in the rat at high levels in the submandibular gland and low levels in the kidney, but not at all in seven other tissues, including the heart [28]. In contrast, our present study showed that mRNA for T-kininogenase was detectable in rat cardiomyocytes as well as ventricular myocardium. Since we used the same primers for detecting T-kininogenase as reported by Ma et al., the discrepancy in the findings is probably due to the differences in conditions for RT-PCR, including temperature, reaction times and PCR cycles. Of interest, the levels of T-kininogenase mRNA in cardiomyocytes elevated after the culture with Bt_2cAMP. Thus the treatment of cardiomyocytes with Bt_2cAMP induced the gene expression of not only T-kininogen but also T-kininogenase, suggesting that the elevation of intracellular cAMP levels in cardiomyocytes results in the increased production of both T-kininogen and T-kininogenase. In fact, we observed increased levels of T-kininogen mRNA in cardiomyocytes after culture with 10^{-8} M isoproterenol (data not shown). Since T-kininogenase liberates Ile-Ser-bradykinin from T-kininogen, one may speculate that the cardiac kallikrein-kinin system in the rat comprises T-kininogen and T-kininogenase rather than H/L-kininogen and true tissue kallikrein.

There are at least two subtypes of bradykinin receptors, B1 and B2, in mammalian tissues; the majority of the classical actions of bradykinin have been attributed to an activation of B2 receptor, whereas B1 receptors, unique as an inducible receptor activated by inflammatory stimuli, appear to be involved in chronic inflammatory responses [32–34]. The existence of these two subtypes of bradykinin receptors in cardiac tissues has been confirmed by means of radioligand binding [18]. Consistent with findings by other investigators [35–37], we detected B2 receptor mRNA in ventricular myocardium of either untreated or LPS-injected rats, while detecting B1 receptor mRNA only after injection of LPS. Similar results were obtained in the in vitro experiments using rat cardiomyocytes in which mRNA levels for B1 receptor but not for B2 receptor were elevated after cultures with LPS. Ni et al. reported that the 5′-flanking region of the human B1 receptor showed promoter activity inducible by LPS and proinflammatory cytokines, such as IL-1 or TNF, in vascular smooth muscle cells [38]. As shown in the present study, the exposure of rat cardiomyocytes to LPS,

IL-1 and TNF in vitro also produced an elevation in the B1 receptor mRNA level, suggesting that the inducible process for B1 receptor seen in vascular smooth muscle cells exists in cardiomyocytes. It should be noted that B1 receptor mRNA was detectable in untreated cells, suggesting the induction of B1 receptor following the isolation and in vitro incubation of cardiomyocytes [39–42]. In contrast to IL-1 and TNF, another proinflammatory cytokine, IL-6, suppressed B1 receptor mRNA levels in cultured cardiomyocytes, suggesting a potential regulatory role for IL-6 in the upregulation of B1 receptors following tissue injury or inflammation. Further studies will be needed to clarify the importance of IL-6 to the pathophysiological role of the B1 receptor.

In conclusion, the present study has demonstrated that cardiomyocytes in rat ventricular myocardium express mRNAs for the kallikrein-kinin system, such as kininogens, kallikreins and B1/B2 receptors. However, this system in myocardium seems to be unique to the rat in that the only kininogen synthesized locally in myocardium appears to be T-kininogen, a unique kininogen in the rat. Ile-Ser-bradykinin may possibly be generated in rat heart and serve as the cardioprotective kinin. Since this kinin-generating system is not present in humans and other animals, further studies are needed to clarify the existence of the independent kallikrein-kinin system in cardiomyocytes of these species.

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