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Posttranscriptional activation of BNP gene expression in response to increased left ventricular wall stress: role of calcineurin and PKC

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Abstract

To study the molecular mechanisms for load-induced activation of BNP gene expression, increased wall stress was imposed on isolated isovolumetrically beating adult rat hearts by distension of a fluid filled balloon within the left ventricle. The wall stress for 2 h resulted in a 1.6-fold increase in the expression of BNP gene and a 2.0-fold increase of the c-*fos* gene. The inhibition of transcription by actinomycin D significantly decreased the baseline BNP mRNA levels but the wall stretch-induced increase of the gene expression remained unaffected. In contrast, the protein synthesis inhibitor cycloheximide increased baseline BNP mRNA levels and abolished the load-induced activation of gene expression. Furthermore, we studied the effects of protein kinase C (PKC), calcineurin and protein phosphatase 2A (PP2A) inhibition to characterize the role of intracellular pathways in the stretch-induced gene expression in the left ventricle. The expression of BNP and c-*fos* genes were not influenced by calcineurin, PP2A and PKC inhibition. In conclusion, we showed that the stretch-induced activation of BNP gene expression by increased left ventricular wall stress is independent of transcriptional mechanisms and dependent on protein synthesis. Moreover, our results suggest that the load-induced activation of BNP gene expression is independent of calcineurin, PKC and PP2A.

Keywords: Heart; Hypertrophy; Natriuretic peptides

1. Introduction

The cardiac myocytes are terminally differentiated cells, which respond to increased workload by increase in size, accumulation of cellular proteins and sarcomeric assembly of myofibrils [1–3]. The hypertrophic response in the myocardium is, in addition to changes in myocyte size and structure, characterized by an alteration in the gene expression pattern. The upregulation of A-type (ANP) and B-type (BNP) natriuretic peptide gene expression as well as genes encoding the structural proteins, such as skeletal muscle α-actin and β-myosin heavy chain (β-MHC), are considered as markers of the long-term hypertrophic response [2–4]. The changes in the pattern of gene expression, on the other hand, are observed not only in the long-term hypertrophic process but also at the onset of hemodynamic overload. For example, acute hemodynamic overload and exposure of cardiac myocytes to hypertrophic agonists (such as endothelin-1 and phenylephrine) have been demonstrated to lead to a rapid increase in the expression of several proto-oncogenes, such as c-*fos* and c-*jun* [3,5]. Moreover, the expression of BNP gene takes place with many characteristics of an immediate-early gene [6–8]. Indeed, mechanical stretch of the atria and vasopressin- and phenylephrine-induced hemodynamic overload in the left ventricle have been shown to result in an increase in the BNP gene expression within 1 h [6–8]. Furthermore, it has been shown that BNP gene expression is modulated by acute mechanical stretch specifically in the atria, whereas the expression of ANP remains unchanged [9].

The transcriptional regulation of BNP gene expression has been a subject of intensive research. Hanford et al. originally reported that phenylephrine-induced early acti-

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vation of BNP gene expression in cultured ventricular myocytes is independent of protein synthesis and partially dependent on transcription [10]. The transcriptional activation of BNP gene and load-induced activation of BNP promoter have thereafter been confirmed by several other studies [11–13]. In addition, numerous transcription factors including GATA-4, myocyte-enhancer factor 2 (MEF-2), and Nkx-2.5, have been shown to be necessary for the activation of the BNP promoter both in vitro and in vivo [14]. We have previously reported that the stretch-induced activation of BNP gene expression in the atria of isolated rat heart is independent of protein synthesis but completely dependent on increased transcription [8]. In contrast, our recent study demonstrated that posttranscriptional mechanisms may play a major role in the regulation of angiotensin II-induced BNP gene expression in the left ventricle in vivo [15]. Thus, controversy still persists concerning the relative roles of posttranscriptional and transcriptional mechanisms in the regulation of BNP gene. In addition, there is no data concerning the posttranscriptional and transcriptional regulation of acute stretch-induced activation of BNP gene expression in the left ventricle of normal adult heart.

In the present study, to examine the stretch-induced BNP gene expression in the left ventricle of adult heart, we applied an in vitro stretch model in isolated perfused rat hearts. Wall stress was imposed on isovolumetrically beating rat hearts by distension of a fluid filled balloon in the left ventricle. Previously, similar experimental setup has been used to characterize the activation of c-*fos* and c-*jun* gene expression [16]. To examine the role of transcriptional and posttranscriptional mechanisms in the regulation of left ventricular wall stress-induced activation of BNP and c-*fos* gene expression, transcription and protein synthesis inhibitors were used. In addition, we studied the effects of calcineurin, protein phosphatase 2A (PP2A) and protein kinase C (PKC) inhibition on the stretch-induced activation of BNP and c-*fos* genes by using selective pharmacological inhibitors.

2. Materials and methods

2.1. Chemicals

The chemicals used in this study were as follows: bisindolylmaleimide I, okadaic acid and FK-506 were from Calbiochem, Nottingham, UK; cyclosporine A was from Novartis International AG, Basel, Switzerland; and actinomycin D and cycloheximide from Sigma-Aldrich, St. Louis, MO, USA.

2.2. Isolated perfused rat heart preparation

Male Sprague–Dawley rats, weighing 250–320 g, from the Center for Experimental Animals at the University of Oulu were used. The experimental design was approved by the Animal Use and Care Committee of the University of Oulu and it was similar to that described previously in detail [17]. Briefly, the rats were decapitated, the aorta was cannulated above the aortic valve and the hearts were arranged for retrograde perfusion by the Langendorff technique. The hearts were perfused with modified Krebs–Henseleit bicarbonate buffer (pH 7.40) equilibrated with 95% O_2–5% CO_2 at 37 °C. The composition of the buffer was (in mmol/L) 113.8 NaCl, 22.0 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 1.1 $MgSO_4$, 2.5 $CaCl_2$, and 11.0 glucose. The coronary flow rate was set to 15 mL/min with a peristaltic pump (Minipuls 3, model 312, Gilson, Villiers, France). The heart rate was increased 15–20% above the spontaneous beating frequency by using a Grass stimulator (11V, 0.5 ms, model S88, Grass Instruments, Quincey, MA, USA). A fluid filled balloon connected to a pressure transducer (Isotec, Hugo Sachs Elektronik, Germany) was inserted through the mitral valve to the left ventricle to measure the ventricular pressure. Analog signals were digitized at a sampling frequency of 125 Hz, and all the data was recorded and analyzed with an IBM PC-compatible computer using the Ponemah data acquisition software (Gould Instrument System Inc, OH, USA). The drugs or the vehicle were added into the aortic cannula as a continuous infusion in the absence and presence of increased wall stress. At the end of the experiments, the left ventricles were immersed in liquid nitrogen and stored in −70 °C until assayed.

2.3. Isolation and analysis of RNA

The RNA extraction and Northern blot analysis were performed as described previously [17]. The RNA was extracted from the left ventricles using the guanidine–thiocyanate–CsCl method. For the Northern blot and dot blot analyses, 20 μg samples of RNA were separated and transferred to nylon membranes (Osmonics, Westborough, MA, USA). The cDNA probes complementary to rat BNP, adrenomedullin, c-*fos* or ribosomal 18S RNA were random prime labeled, and the membranes were hybridized and washed 3×20′ at +62 °C. The membranes were thereafter exposed with PhosphorImager screens (Molecular Dynamics, Sunnyvale, CA, USA), which were scanned by Biorad Molecular Imager (Bio-Rad Laboratories, Hercules, CA, USA). All results were quantitated by using Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA).

2.4. Statistics

The results are expressed as mean±SEM. For the comparison between two groups, Student's *t*-test was used. A P value of <0.05 was considered statistically significant.