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Increased JNK, AP-1 and NF-κB DNA Binding Activities in Isoproterenol-induced Cardiac Remodeling

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Y. Takemoto, M. Yoshiyama, K. Takeuchi, T. Omura, R. Komatsu, Y. Izumi, S. Kim and J. Yoshikawa. Increased JNK, AP-1 and NF-κB DNA Binding Activities in Isoproterenol-induced Cardiac Remodeling. *Journal of Molecular and Cellular Cardiology* (1999) **31**, 2017–2030. The *in vivo* signal transduction pathway, responsible for isoproterenol-induced cardiac hypertrophy or remodeling, remains to be clarified. The purpose of this study was to examine c-Jun NH2-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK), activator protein-1 (AP-1) and nuclear factor-κB (NK-κB) DNA binding activity, which seem to be important in a signal transduction cascade upstream of the increased level of mRNA expression observed in isoproterenol-induced cardiac remodeling. Rats were continuously infused with saline and isoproterenol by intravenous injection (a short period; 0.5 μg/kg/min) and an osmotic minipump (a long period; 0.5 or 3 mg/kg/day). Cardiac morphology was measured by echocardiography. JNK and ERK were measured by in gel kinase assay. AP-1 and NF-κB DNA binding activity was determined using an electrophoretic mobility shift assay. Echocardiogram showed that the thickness of the left ventricular anterior wall (AW) and left ventricular posterior wall (PW) increased at day 1 in low doses, and at day 1 in high doses. Isoproterenol significantly increased ERK and JNK activity at 15 min after intravenous infusion of 0.5 μg/kg/min isoproterenol. At late phase about JNK and ERK activity, only a high dose of isoproterenol increased JNK. AP-1 DNA binding activities spurred by low or high doses of isoproterenol administration increased at 12 h, reached their peak of 24.1- and 37.1-fold ($P<0.01$), respectively, at 24 h, and thereafter decreased. Although low doses of isoproterenol did not change the level of NF-κB DNA binding activities, high doses increased it to 10.9-fold ($P<0.01$) at day 2. This study showed increased JNK, ERK, AP-1 and NF-κB DNA binding activities in isoproterenol-induced cardiac remodeling. AP-1 may contribute to the isoproterenol-induced cardiac remodeling, and JNK or NF-κB may also play some roles in it.

Key Words: Isoproterenol, Myocardial hypertrophy, Cardiac remodeling, Activator protein-1 (AP-1), Nuclear factor-κB (NF-κB), c-Jun NH_2-terminal kinase (JNK), Extracellular signal-regulated kinase (ERK), β-adrenoceptor.

Introduction

Cardiac hypertrophy is a major risk factor for development of heart failure and sudden cardiac death (Levy *et al.*, 1990). Elucidating the mechanisms of cardiac hypertrophy is therefore important to reduce the mortality from cardiovascular causes. Multiple lines of evidence indicate that stimulation of β-adrenoreceptor plays a key role in the development of pathological cardiac hypertrophy (Collins *et al.*, 1975; Knufman *et al.*, 1987; Stanton *et al.*, 1969). Continuous infusion of isoproterenol to rats causes myocardial hypertrophy and fibrosis, so called cardiac remodeling, especially high dose

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Table 1 Comparison of blood pressure and pulse rate in saline infused rats and in low dose and high dose isoproterenol infused rats

Blood pressure (mmHg)	Before	Day 2	Day 4	Day 6
Saline infused	124±0.8	122±1.4	120±1.0	125±1.6
ISO-infused (0.5 mg/kg/day)	120±1.2	126±2.0	121±1.1	128±1.3
ISO-infused (3.0 mg/kg/day)	122±1.5	115±2.2*	125±2.0	128±2.0
Pulse rate (beat/min)	**Before**	**Day 2**	**Day 4**	**Day 6**
Saline infused	324±3.6	323±5.8	320±6.5	325±5.0
ISO-infused (0.5 mg/kg/day)	330±7.2	446±11.6**	434±20.5**	468±17.6**
ISO-infused (3.0 mg/kg/day)	330±8.9	488±19.2**	482±15.0**	468±17.6**

Each value is mean±S.E.M. ($n=5$). Abbreviations are: saline infused, saline infused rats; ISO-infused (0.5 mg/kg/day), isoproterenol infused rats at a dose of 0.5 mg/kg/day; ISO-infused (3.0 mg/kg/day), isoproterenol infused rats at a dose of 3.0 mg/kg/day. *$P<0.05$, **$P<0.01$ compared with saline infused rats.

isoproterenol induces severe myocardial hypertrophy accompanied with myocardial injury (Benjamin *et al.*, 1989). Isoproterenol infusion increases mRNA expression of atrial natriuretic peptide (ANP), α-skeletal actin and cardiac fibrosis-related genes such as transforming growth factor β1 (TGF-β1), collagen type I and III (Boluyt *et al.*, 1995; Omura *et al.*, 1994). Such genomic responses require transcriptional factor, activator protein-1 (AP-1) that bind to specific recognition elements in upstream promoter regions (Angel *et al.*, 1987; Bishopric *et al.*, 1992; Kim *et al.*, 1990). As well as AP-1, nuclear factor-κB (NF-κB) plays a central role in the expression of a large number of genes involved in the inflammatory and immune response (Baeuerle and Henkel, 1994; Sen and Baltimore, 1986). These findings suggest the possibility that AP-1 and NF-κB may be involved in the onset and development of isoproterenol-induced cardiac remodeling.

c-Jun NH_2-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) are protein serine/threonine kinases and belong to the mitogen activated protein kinase (MAPK) family (Davis, 1994; Derijard *et al.*, 1994). MAPKs are regulated by different upstream activators and play a central role in cell growth (Gotoh *et al.*, 1990) or apoptosis (Perlman *et al.*, 1997) and the regulation of various transcription factor such as AP-1 (Karin, 1995) and NF-κB (Schulze-Osthoff *et al.*, 1997). Previous reports on the activity of MAPKs were observed soon after stimulation and returned to control levels for a short period (Komuro *et al.*, 1996; Seko *et al.*, 1996). Recently when the activities of MAPKs were analyzed for long time periods in *in vivo* models, ERK and JNK were shown to be activated (Izumi *et al.*, 1998; Yano *et al.*, 1998). However, activity of JNK and ERK on isoproterenol-induced cardiac remodeling in *in vivo* models has not been examined.

To elucidate the role of MAPKs and transcription factors in the development of cardiac remodeling induced by isoproterenol, we examined JNK and ERK, AP-1 and NF-κB DNA binding activities and serial morphological changes by echocardiography.

Materials and Methods

The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996).

Animal model

Eight-week-old male Wistar rats (purchased from Clea Japan Co., Inc., Osaka, Japan), weighing 240–270 g, were used in this study. Animals were given standard rat chow containing 0.3% Na (CE-2; Clea Japan Co., Inc., Osaka, Japan) and water *ad libitum*. Rats were anesthetized with pentobarbital (40 mg/kg i.p.), and an osmotic minipump (Alzet, model 2001 or 2001D, Alza Corp., Palo Alto, California, USA), containing saline or isoproterenol dissolved in saline with 0.1% ascorbic acid, was subcutaneously implanted into the back. Rats were divided into three groups, including: (1) saline infusion; (2) isoproterenol infusion at a dose of 0.5 mg/kg/day; and (3) isoproterenol infusion at a dose of 3 mg/kg/day. The continuous infusion was performed for a period of 6 h, 12 h, 24 h, 2 days, 3 days or 7 days.