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Expression of Natriuretic Peptide mRNAs in Isoproterenol-Induced Cardiac Hypertrophy in Rats

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We examined the expression of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) mRNAs upon isoproterenol (Iso)-induced cardiac hypertrophy in rats. Then, we tried to investigate the effects of sympatholytics to see if they can modulate the expression of ANP and BNP. In this study, RT-PCR technique was used to characterize the expression of ANP and BNP in right atrium (RA) and left ventricle (LV) of the hypertrophied rat heart. Histologic findings indicated that stimulation of β-adrenoceptors with Iso for 5 days was sufficient to induce cardiac hypertrophy in rats. A continuous stimulation with Iso for 7 days resulted in an increase of the ANP and BNP expression in the LV and BNP expression in the RA. The increased expressions of ANP and BNP in the LV were slightly inhibited, and the increased expressions of BNP in the RA were markedly inhibited by a continuous treatment with propranolol, metoprolol, and clonidine for 7 days. Overall, our data present a differential expression of the natriuretic peptides in Iso-induced cardiac hypertrophy, and that the mechanisms involved in this differential ANP and BNP gene expression could be mediated *via* sympathetic nervous system.

Key Words: Atrial natriuretic peptide, Brain natriuretic peptide, Cardiac hypertrophy, Isoproterenol

INTRODUCTION

Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are both cardiac hormones that exhibit a similar broad range of biological effects including natriuresis, vasodilatation, and inhibition of renin-angiotensin system (RAS). They play a major role in maintaining the homeostasis of body fluids and blood pressure. They are very much alike in their amino acid sequences and pharmacological spectra (Sudoh et al, 1988). However, the distribution, secretion, and regulation of these two peptides are known to be different (Suzuki et al, 1992; Yoshimura et al, 1993). While ANP is largely atrial in origin, BNP is predominantly secreted from the ventricular myocardium. ANP is expressed in fetal ventricles, and its expression is markedly reduced in normal adult ventricles. However, it has been reported that ANP is reexpressed in the ventricles of various pathologically dilated cardiomyopathic conditions including mitral stenosis, myocardial infarction, and congestive heart failure (CHF) (Saito et al, 1989; Takemura et al, 1991). Unlike ANP, BNP shows much species-specific variations in both its structure and tissue distribution (Kambayashi et al, 1990; Mukoyama et al, 1990; Ogawa et al, 1990). Circulating BNP levels are much lower than concurrent ANP levels in normal human (Mukoyama et al, 1991). However, in patients with CHF, the plasma levels of BNP are significantly increased as in those of ANP, and sometimes serve as better markers for the severity of cardiac dysfunction, exceeding ANP levels in some cases (Mukoyama et al, 1991; Ogawa et al, 1991; Yamamoto et al, 1996; Uusimaa et al, 1999). At the same time, Omland et al (1996) have shown recently that ANP is more closely correlated with left ventricular dysfunction (LVD) and symptomatic heart failure than BNP. Although there are some ongoing conflictions regarding which natriuretic peptide is more meaning-

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ful as a marker of LVD, both of the natriuretic peptides have been focused as markers of LVD (Yasue et al, 1994; Friedl et al, 1996).

In the present study, we investigated the mRNA expression of ANP and BNP in isoproterenol (Iso)-induced cardiac hypertrophy in rats, then subsequently studied the effects of propranolol, metoprolol, and clonidine on the expression of these natriuretic peptides.

METHODS

Preparation of animals

Male Sprague-Dawley rats weighing 270 to 320 g were used throughout the study. The experimental protocols were approved by the Ethical Committee on the Animal Care and Experimentation at Dongsan Medical Center, Keimyung University. Rats were given heparin sodium at a dose of 300 units/kg intraperitoneally 30 minutes before sacrifice by cervical dislocation. The hearts were removed immediately after opening the thoracic cavity and washed thoroughly with ice-cold phosphate buffered saline three times until all visible blood was removed.

Experimental scheme

Hypertrophy was induced by daily injections of isoproterenol (Iso; 5 mg/kg/day, ip, Sigma) for 7 days. After the induction of cardiac hypertrophy, rats received daily injections of propranolol (5 & 10 mg/kg/day, Sigma), metoprolol (5 & 10 mg/kg/day, Sigma), and clonidine (30 & 50 μg/kg/day, Sigma) for 7 days intraperitoneally.

RNA preparation

Total cellular RNA was extracted with RNAzol™ B (Biotecx laboratories, INC.) protocol. The integrity of RNA was checked by formaldehyde/agarose gel electrophoresis.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total cellular RNA of each sample was reverse-transcribed into cDNAs, which in turn were amplified using primers specific for ANP and BNP. The ANP primers were 5'-ATGGATCTCCAGAAGGTGCT-3' and 5'-CTGCATCGTGGATTGTTCTG-3'. The BNP primers were 5'-ATGGATCTCCAGAAGGTGC-3' and 5'-CTAAAACAACCTCAGCCCG-3'. The PCR products were 116 bps (ANP) and 366 bps (BNP) in size. Both reverse transcription and PCR amplification were performed in the Perkin-Elmer GeneAmp PCR system 2400.

H & E stain

The dissected heart was fixed in 10% buffered formalin, and was transversely sectioned at 5 mm from the ventricle apex. The heart tissues were embedded in paraffin and stained with haematoxylin & eosin.

Data analysis

Samples (10 μl) of the PCR products were separated by electrophoresis in 1.5 and 2% agarose gels in the presence of ethidium bromide (0.5 μg/ml), followed by the quantitation using a video densitometer (Gel Doc 1000, Bio-Rad). Tissue concentrations of ANP and BNP mRNA were normalized to the GAPDH mRNA concentration in each sample to correct for differences in the amount of RNA applied. Statistical analysis was done using Student's *t*-test, and significance was assumed when $P < 0.05$.

RESULTS

Histology

Histological findings indicated that stimulation of β-adrenoceptors with Iso for 5 days was sufficient to induce cardiac hypertrophy in rats. Iso-induced cardiac hypertrophy presents myocyte hypertrophy, fibrosis, and some chronic inflammatory findings on haematoxylin & eosin stained sections. As compared with the untreated control, the LV cavity was reduced in size due to compression by concentrically hypertrophied left ventricular wall (Fig. 1 & 2).

Effect of isoproterenol on the ANP and BNP expression

Induction of hypertrophy by Iso (5 mg/kg/day for

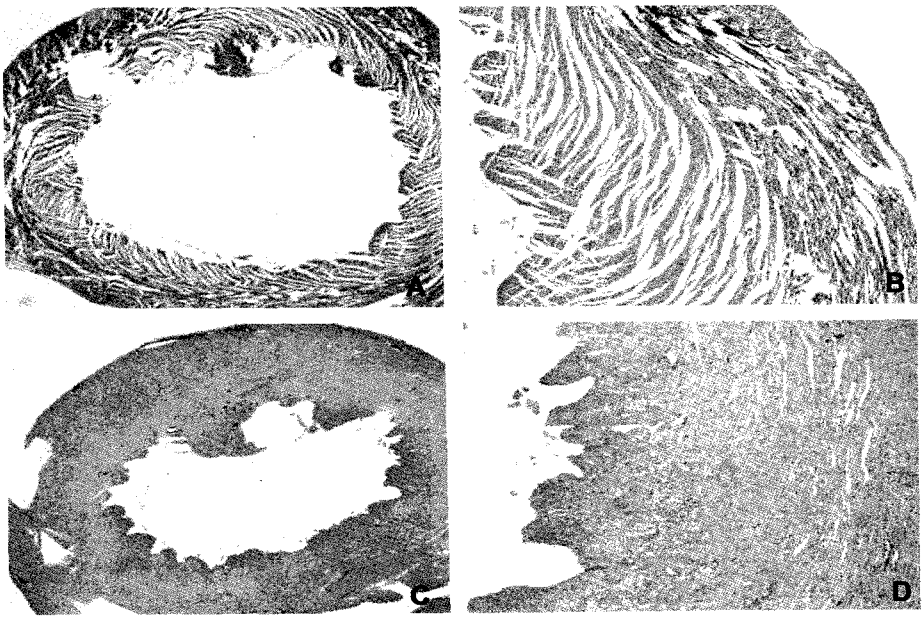

Fig. 1. Transverse sections of left ventricle of rat heart. A and B, untreated control. C and D, the histologic appearance demonstrating hypertrophy induced by isoproterenol, 5 mg/kg/day for 5 days. As compared with the untreated control, the LV cavity was reduced in size due to compression by concentrically hypertrophied left ventricular wall. There are subendocardial and adjacent mural plaques localized to near the ventricular surface of septum and anterial wall. A peculiar loss of myocytes is observed as well as the interstitial and replacement fibrosis with infiltration of macrophages and chronic inflammatory cells (original magnification, A & C; ×12.5, B & D; ×40, H & E stain).

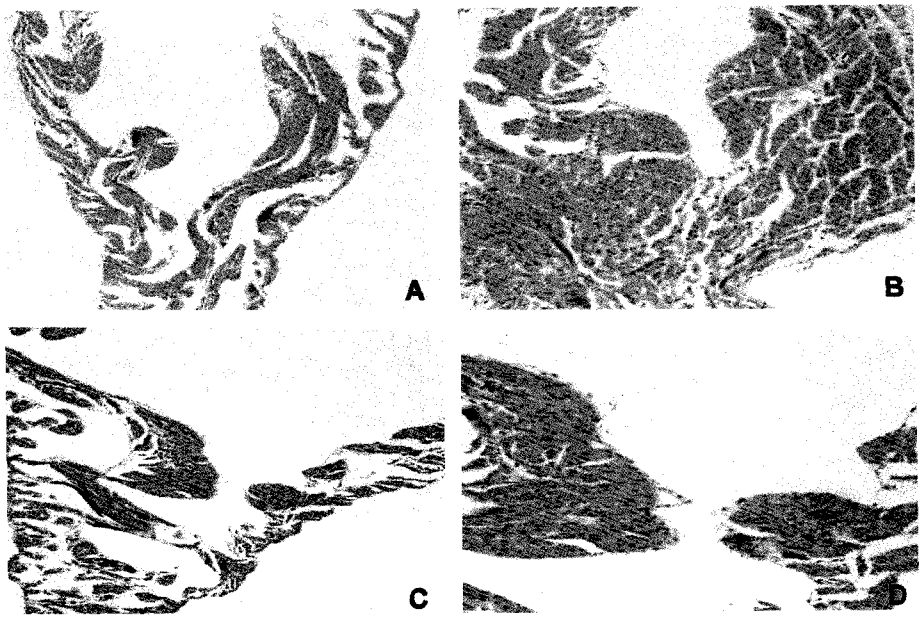

Fig. 2. Transverse sections of right atrium of rat heart. A and B, untreated control. C and D, the rat heart treated with isoproterenol, 5 mg/kg/day for 5 days. As compared with the untreated control, the RA shows negligible changes of myocardium (original magnification, A & C; ×12.5, B & D; ×40, H & E stain).

7 days) resulted in an increased ANP and BNP expression in the LV and BNP expression in the RA. The extent of the elevation in BNP levels was more marked in the RA than LV (Fig. 3 & 4).

Effects of propranolol, metoprolol, and clonidine in LV of hypertrophied rat heart

In the LV, the increased levels of ANP and BNP were slightly inhibited by propranolol, metoprolol, and clonidine (Fig. 3).

Effects of propranolol, metoprolol, and clonidine in RA of hypertrophied rat heart

In the RA, the increased levels of ANP were not significantly affected by propranolol, metoprolol and clonidine, but the BNP expression was markedly blocked by the sympatholytics used (Fig. 4).

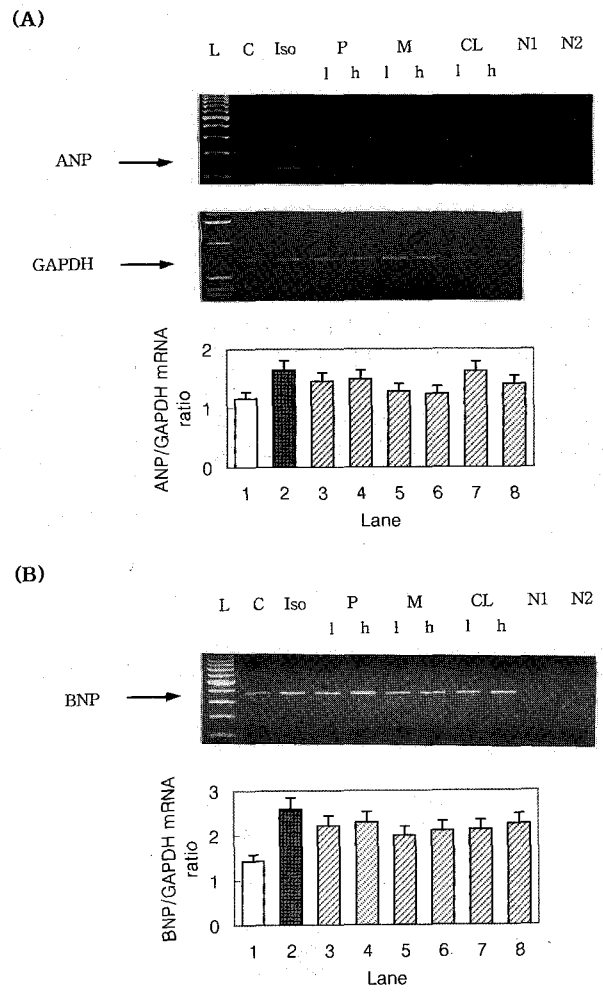

Fig. 3. Effects of propranolol, metoprolol, clonidine on the ANP (A) and BNP (B) gene expression in the left ventricle of rat heart upon isoproterenol (5 mg/kg/day for 7 days)-induced cardiac hypertrophy. L: 100 bp DNA ladder, C & lane 1: untreated control, Iso & lane 2: isoproterenol only, P & lane 3, 4: Iso+propranolol, M & lane 5, 6: Iso+metoprolol, CL & lane 7, 8: Iso+clonidine l: low dose, h: high dose, N1: RT-PCR control (no RNA), N2: RT-PCR control (no reverse transcriptase). Error bars, SE.

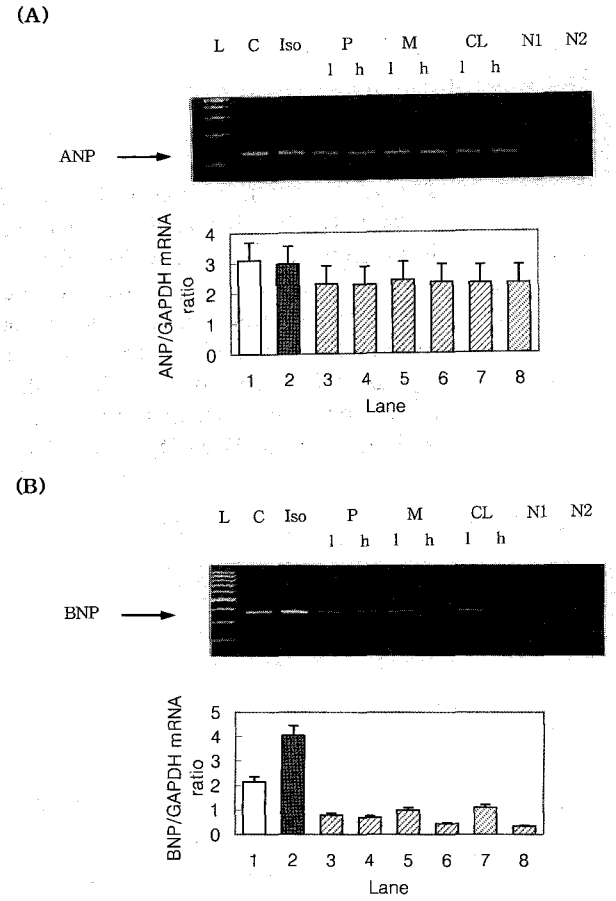

Fig. 4. Effects of propranolol, metoprolol, clonidine on the ANP (A) and BNP (B) gene expression in the right atrium of rat heart upon isoproterenol (5 mg/kg/day for 7 days)-induced cardiac hypertrophy. L: 100 bp DNA ladder, C & lane 1: untreated control, Iso & lane 2: isoproterenol only, P & lane 3, 4: Iso+propranolol, M & lane 5, 6: Iso+metoprolol, CL & lane 7, 8: Iso+clonidine l: low dose, h: high dose, N1: RT-PCR control (no RNA), N2: RT-PCR control (no reverse transcriptase). Lanes 3, 4, 5, 6, 7, and 8 in (B) were significantly different ($P<0.001$) from Lane 2 (Iso only). Error bars, SE.

DISCUSSION

The β-adrenergic agonists directly induce cardiac hypertrophy in doses that do not cause hemodynamic changes (Laks et al, 1953; King et al, 1987). The factors involved in cardiac hypertrophy include sympathetic stimulations such as β-adrenergic and α_1-adrenergic receptors, cortisol, thyroid hormones, and RAS (Ikeda et al, 1991; Morgan & Baker, 1991; Sadoshima & Izumo, 1993). RAS has been implicated as the major secondary mediator in Iso-induced cardiac hypertrophy (Nagano et al, 1992). Endogenous angiotensin (Ang) II also has a major role in the maintenance of Iso-induced cardiac hypertrophy (Golomb et al, 1994). The activity of Iso that we used as a hypertrophic inducer could be achieved through both cardiac β_1-adrenergic receptors and the RAS in the kidney (Stanton et al, 1969; Allard et al, 1990; Nagano et al, 1992). The release of renin occurs in a number of organs including the kidney, lung, adrenal gland, heart, ovary, and vascular smooth muscle cells (Lindpainter & Ganten, 1991; Dostal & Baker, 1993). Therefore, Iso could be activated either by a direct interaction with its receptor in the heart or by an indirect mediation *via* secondary hemodynamic and endocrinergic changes produced by the renin of RAS.

In this study, we compared mRNA expressions of ANP and BNP in the RA and LV of Iso-induced hypertrophic rat heart. The elevated expression of ANP and BNP was observed in LV and that of BNP was notable in RA. Overall effects of the sympatholytics used on the increased mRNA levels of ANP and BNP were inhibitory. In the LV, the increased levels of ANP and BNP mRNAs were slightly inhibited by propranolol, metoprolol, and clonidine. In the RA, the elevated levels of ANP were not altered by propranolol, metoprolol, and clonidine. However, the increased levels of BNP mRNA in the RA were markedly blocked by all of the sympatholytics used.

Cardiac natriuretic peptides ANP and BNP are principal peptides released in the myocardium from each of their precursor prohormones encoded by a separate gene under atrial stretch (Mantymaa et al, 1993) and left ventricular load or stretch (Kinnunen et al, 1993). It has become evident that plasma levels of ANP and BNP serve as markers for cardiac failure or cardiac hypertrophy. Generally, ANP is produced in the atrium, and BNP is secreted from the ventricle, which is considered to reflect left ventricular function. Although the circulatory levels of both peptides are raised in ventricular dysfunction and ventricular hypertrophy, an increase of plasma BNP concentration sometimes exceeds that of ANP in severe cases. Thus, the plasma BNP levels may correlate to the severity of cardiac dysfunction (Mukoyama et al, 1991; Ogawa et al, 1991; Yamamoto et al, 1996; Uusimaa et al, 1999). Our data also indicate the predominant BNP expression in the RA.

Wei et al (1993) have reported that atrial tissue BNP is much more pronounced as compared with ANP in CHF, and therefore atria might have the capability to enhance BNP production in pathologic conditions. In comparison with ANP, BNP seems to have a different atrial processing in the circulation, especially in heart failure. The synthesis and release of ANP may be regulated by hemodynamics, whereas those of BNP are constitutive (Mukoyama et al, 1991; Wilkins et al, 1997; Levin et al, 1998). According to our data, the increased levels of BNP mRNA responded more typically to sympatholytics than the ANP in the RA. This implicates that BNP could serve as a useful marker for atrial dysfunction. In the LV, the effects of sympatholytics on the expression of ANP and BNP were not as pronounced as those of BNP in RA. Ogawa et al (1991) also have demonstrated that left ventricular BNP mRNA contents and plasma BNP levels were higher than those of ANP in hypertensive rats. They have reported that BNP response was more predominant than ANP in ventricular hypertrophy and pathophysiologic conditions. Our results correlate well with other previous reports (Mukoyama et al, 1991; Ogawa et al, 1991; Yamamoto et al, 1996; Uusimaa et al, 1999) in that BNP in RA could serve as a good diagnostic marker in cardiac hypertrophy.

In conclusion, we suggest that adrenergic system plays an important role in the regulation of the ANP and BNP expression, and BNP levels in RA could be a useful diagnostic marker in Iso-induced cardiac hypertrophy. Further studies should be conducted to provide functional roles of ANP and BNP and the mechanism underlying the differential expression of these natriuretic peptides in cardiac hypertrophy.

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