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Effect of Interleukin-1β on Cardiac Hypertrophy and Production of Natriuretic Peptides in Rat Cardiocyte Culture

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E. Harada, O. Nakagawa, M. Yoshimura, M. Harada, M. Nakagawa, Y. Mizuno, Y. Shimasaki, M. Nakayama, H. Yasue, K. Kuwahara, Y. Saito and K. Nakao. Effect of Interleukin-1β on Cardiac Hypertrophy and Production of Natriuretic Peptides in Rat Cardiocyte Culture. *Journal of Molecular and Cellular Cardiology* (1999) **31**, 1997–2006. This study was designed to examine the effects of interleukin-1β (IL-1β) on myocyte (MC) hypertrophy and the production of A-type natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) in rat ventricular cardiocyte culture, and to investigate the role of nonmyocyte (NMC) in this process. We examined the effects of IL-1β on the production of ANP and BNP in comparison with the effects of endothelin-1 (ET-1) by using two types of neonatal rat cardiocyte culture; MC-enriched culture and MC-NMC coculture. In the MC-enriched culture, the increase in secretion of ANP and BNP was small in treatment with IL-1β (1000 pg/ml), while ET-1 (10 nM) markedly augmented the secretion of ANP and BNP. In the MC-NMC coculture, IL-1β and ET-1 each significantly augmented the secretion of ANP and BNP. The degree of the increase of ANP and BNP was equivalent between IL-1β and ET-1. As for the morphological changes of MCs, IL-1β induced the star-shaped MC hypertrophy characterized by elongation and pointed edges only in the MC-NMC coculture, while ET-1 induced the MC hypertrophy characterized by shapes of squares, triangles or circles in both cultures. This study shows that IL-1β induces unique cardiac hypertrophy and the marked secretion of ANP and BNP, and that NMC is indispensable when treated with IL-1β.

Key Words: Interleukin-1β; A-type natriuretic peptide; B-type natriuretic peptide; Neonatal rat; Myocyte-enriched culture; Myocyte-nonmyocyte coculture; Endothelin-1; Hypertrophy.

Introduction

A-type or atrial natriuretic peptide (ANP) and B-type or brain natriuretic peptide (BNP) are cardiac hormones with a wide range of potent biological effects, including natriuresis, diuresis, vasodilation, and inhibition of the renin-angiotensin-aldosterone system and sympathetic nervous system (de Bold, 1985; Levin *et al.*, 1998).

ANP is mainly produced in and released from

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the atrium, and its plasma levels are increased in patients with congestive heart failure (CHF) (Burnett *et al.*, 1986). In addition, ANP is secreted from not only the atrium but also the ventricles in increased amounts as heart failure advances (Yasue *et al.*, 1989). BNP is predominantly produced in and secreted from the cardiac ventricles (Yasue *et al.*, 1994), and its plasma levels are markedly increased in patients with CHF and surpass those of ANP in severe cases (Yoshimura *et al.*, 1993).

We also reported that the plasma levels of BNP but not ANP are rapidly and markedly increased in the early phase of acute myocardial infarction (AMI). In that study, we reported that the plasma levels of BNP had no correlation with any hemodynamic parameters (Morita *et al.*, 1993). The findings suggest that not only hemodynamic parameters but also some humoral factors may regulate the secretion of BNP. Up to now, several humoral factors such as endothelin-1 (ET-1) (Shubeita *et al.*, 1990), phenylephrine (Simpson *et al.*, 1982a), angiotensin II (Baker *et al.*, 1990), cardiotrophin-1 (Pennica *et al.*, 1995) and transforming growth factor β (MacLellan *et al.*, 1993) have been shown to induce cardiac hypertrophy and the production of ANP and BNP.

Interleukin-1β (IL-1β) is a proinflammatory cytokine with a wide variety of effects on many different cell types (Bankers-Fulbright *et al.*, 1996). It has been reported that the plasma levels of IL-1β were increased in the early phase of AMI and that the increases were observed prior to the peak plasma levels of BNP (Blum *et al.*, 1994; Guillen *et al.*, 1995). Also, the expression of IL-1β mRNA has been observed in the infarct region of the heart (Ono *et al.*, 1998). Thus, we hypothesized that IL-1β might induce BNP secretion and cardiac hypertrophy in AMI or heart failure.

It is well known that cardiocyte culture is a useful model for studying cardiac hypertrophy and that the production of ANP and BNP is recognized as a marker of cardiac hypertrophy (Chien *et al.*, 1993). Research on the function of myocytes (MCs) was the greater part of previous studies and the effects of nonmyocytes (NMCs) were not necessarily greatly discussed (Simpson *et al.*, 1982a; Shubeita *et al.*, 1990; Baker *et al.*, 1990; MacLellan *et al.*, 1993; Pennica *et al.*, 1995). However, MCs comprise only approximately one-third of total cell numbers in the adult heart (Nag, 1980), and it has recently been reported that MC-NMC cross-talk is important for MC hypertrophy (Long, 1996; Harada *et al.*, 1997).

In this study, we examined the effects of IL-1β in comparison to ET-1 on cardiac hypertrophy and the production of ANP and BNP by using both MC-enriched culture and MC-NMC coculture, and found that IL-1β induces unique cardiac hypertrophy and the marked secretion of ANP and BNP, and that NMC is indispensable when treated with IL-1β.

Materials and Methods

Materials

Pancreatin and penicillin–streptomycin were obtained from GIBCO Laboratories (Grand Island, NY, USA), Percoll and bovine serum albumin (BSA) from Sigma Chemical Co. (St Louis, MO, USA), collagenase II from Worthington Biochemical Corp. (Freehold, NJ, USA), Dulbecco's modified Eagle's medium (DMEM) from Nikken Bio Laboratory (Kyoto, Japan), fetal calf serum (FCS) (Hazleton Biologics, Lenexa, KS, USA), bromodeoxyuridine (BrdU) from GmbH Mannheim (Germany), and culture plate and Culture Slide from Becton Dickinson (NJ, USA). The balanced salt solution was made up of 116 mM NaCl, 20 mM HEPES, 12.5 mM NaH_2PO_4, 5.6 mM glucose, 5.4 mM KCl, and 0.8 mM $MgSO_4$ (pH 7.35). The FCS (+) medium was supplemented with 10% FCS, antibiotics (100 U/ml penicillin and 100 mg/ml streptomycin) and 0.1 mM BrdU. The FCS (−) medium was supplemented with 0.1% BSA, antibiotics (100 U/ml penicillin and 100 mg/ml streptomycin) and 0.1 mM BrdU. BrdU was used to prevent NMC proliferation without MC toxicity as previously reported (Simpson *et al.*, 1982b).

Cell culture

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. Apical halves of cardiac ventricles from 1-day-old Wistar rats were recovered and minced in a chilled balanced salt solution. Ventricular cardiocytes were dispersed in the balanced slt solution, containing 0.04% collagenase II and 0.06% pancreatin, with agitation for 20 min at 37°C as previously reported (Nakagawa *et al.*, 1995). This digestion step was repeated six times and the collected cell suspensions were mixed with 1/10 vol of chilled FCS and pelleted by centrifugation. The pellets were combined in chilled FCS and kep at 4°C.

To prepare MC-enriched culture, MCs were selectively collected by the Percoll gradient method