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Intermedin$_{1-53}$ protects the heart against isoproterenol-induced ischemic injury in rats

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Abstract

Intermedin is a novel member of the calcitonin/calcitonin gene-related peptide (CGRP) family peptide, which has vasodilatory and hypotensive actions identical to those of adrenomedullin and CGRP. Cleavage sites located between 2 basic amino acids at Arg93–Arg94 result in the production of prepro-intermedin$_{95-147}$, namely intermedin$_{1-53}$. The bioactive action of intermedin$_{1-53}$ and its physiological significance are unclear. In this work, we aimed to explore the effects of intermedin$_{1-53}$ on acute myocardial injury induced by isoproterenol. Myocardial ischemia injury in rats was induced by subcutaneous injection of a high dose of isoproterenol, and the therapeutic effect of intermedin$_{1-53}$ was observed. Plasma lactate dehydrogenase activity, myocardial and plasma malondialdehyde content were higher in the isoproterenol group than that in controls. Isoproterenol-treated rats showed lower maximal rate of increase and decrease of left-ventricle pressure development (±left-ventricle dp/dt_{max}) and higher left-ventricle end-diastolic pressure (all $P<0.01$), which suggested severe heart failure and myocardial injury. Semi-quantitative RT-PCR analysis showed that the gene expression of calcitonin receptor-like receptor and receptor-activity-modifying protein (RAMP)1, RAMP2 and RAMP3 in ventricular myocardia were up-regulated by 79% ($P<0.01$), 48% ($P<0.01$), 31% ($P<0.05$) and 130% ($P<0.01$), respectively, compared with controls. In myocardial sarcolemmal membranes, the maximum binding capacity for [^{125}I]-intermedin$_{1-53}$ was increased by 118% ($P<0.01$) in the isoproterenol group compared with controls. Rats treated with low dosage intermedin$_{1-53}$ (5 nmol/kg/day, 2 days) showed 21% ($P<0.05$) higher myocardial cAMP content, 18% and 31% higher + left-ventricle dp/dt_{max} and -left-ventricle dp/dt_{max} respectively, 288% lower left-ventricle end-diastolic pressure (all $P<0.01$), and attenuated myocardial lactate dehydrogenase leakage and malondialdehyde formation (all $P<0.01$). Treatment with high dosage intermedin$_{1-53}$ (20 nmol/kg/day, 2 days) gave better results than that with low dosage intermedin$_{1-53}$. These results suggest that the intermedin receptor system was up-regulated in isoproterenol-induced myocardial ischemic injury and intermedin$_{1-53}$ might play a pivotal cardioprotective role in such injury.
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Keywords: Intermedin; Isoproterenol; Calcitonin receptor-like receptor; Receptor-activity-modifying protein; cAMP

1. Introduction

Since the discovery of calcitonin in the 1960s, many bioactive peptides such as calcitonin gene-related peptide (CGRP), adrenomedullin and amylin were discovered to be structurally related to each other and therefore grouped into the CGRP superfamily (Amara et al., 1982; Copp, 1994; Aiyar, 2001). This group of peptide hormones acts on diverse organs and tissues and regulates body homeostasis. Adrenomedullin and CGRP are important endocrine and neurocrine integrators of homeostasis in the cardiovascular, renal and respiratory systems, whereas amylin is essential for optimal glucose metabolism (Copp, 1994; Aiyar, 2001; Beltowski and Jamroz, 2004). Recently, Roh et al. (2004) identified a novel calcitonin/CGRP family peptide, intermedin, from the genomes of humans and other vertebrates. Human intermedin gene encodes a prepropeptide of 148 amino acids,

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with a signal peptide for secretion at the N terminus. Prepro-intermedin, with cleavage sites located between 2 basic amino acids at Arg100–Arg101 and Arg107–Val108, can generate a 47 amino acid mature peptide (prepro-intermedin$_{101-147}$, named intermedin$_{1-47}$) and a shorter 40 amino acid one (prepro-intermedin$_{108-147}$, named intermedin$_{8-47}$) by proteolytic cleavage at the N-terminal proximate basic residues, followed by an amidated C terminus. Preliminary studies showed that intermedin had vasodilatory and hypotensive actions identical to those of adrenomedullin and CGRP. Pharmacological analysis, furthermore, showed that intermedin exerts its effects through receptors such as the calcitonin receptor-like receptor/receptor-activity-modifying protein (RAMP) system, a common receptor system of the CGRP superfamily (Roh et al., 2004).

Recently, growing evidence has affirmed that adrenomedullin and CGRP are potent endogenous cardio-renal-protective substances. Exogenous administration of adrenomedullin and CGRP peptides or their gene delivery is a new preventive and therapeutic strategy for cardiovascular diseases such as hypertension, myocardial ischemia, heart failure and renal failure (Kato et al., 2003; Chao et al., 2001; Niu et al., 2004). The cardiovascular protective effects of adrenomedullin and CGRP are considered to be mediated by a calcitonin receptor-like receptor/RAMP system (Fischer et al., 2002). In view of the common receptor system of the CGRP superfamily, we hypothesized that intermedin may also play a cardioprotective role through its receptor calcitonin receptor-like receptor/RAMP system. Amino acid sequence analysis showed that cleavage sites are located between 2 basic amino acids at Arg93–Arg94, resulting in the production of prepro-intermedin$_{95-147}$, namely intermedin$_{1-53}$, which might be a major endogenous degraded fragment of prepro-intermedin with bioactive actions. We synthesized intermedin$_{1-53}$ and found that it has positive inotropic effects on isolated perfused rat hearts. In the present work, we observed the binding capacity of intermedin$_{1-53}$ to its receptors in myocardia, changes of calcitonin receptor-like receptor/RAMP mRNA levels and the therapeutic effects of intermedin$_{1-53}$ on an isoproterenol-induced myocardial injury model, to explore the cardioprotective action of intermedin$_{1-53}$ and its pathophysiological significance.

2. Materials and methods

2.1. Materials

All animal care and experimental protocols were in compliance with the P.R. China Animal Management Rule (Ministry of Health, P.R. China, document no. 55, 2001) and the Animal Care Committee of the First Hospital, Peking University. Male Wistar rats (220–250 g) were provided by the Animal Department, Health Science Center, Peking University. Human intermedin$_{1-53}$ peptide and [^{125}I]-intermedin$_{1-53}$ were from Phoenix Pharmaceuticals Inc. (Belmont, CA, USA). Isoproterenol, aprotinin, *N*,*N*-dimethyl-*p*-phenylenediamine sulphate and sodium dodecyl sulfate (SDS) was purchased from Sigma Co. (St. Louis, MO, USA); Trizol was from GIBCOL (BRL, Rockville, MD, USA); and dNTP, M-MuLV RT, Oligo(dT)$_{15}$ primer, and Taq DNA polymerase were purchased from Promega Co (Madison, WI, USA). Sequences of oligonucleotide primers were as follows: calcitonin receptor-like receptor-S, 5′-CAACTGCTGGATCAGCTCAG-3′, and calcitonin receptor-like receptor-A, 5′-CAT CGCTGATTGTTGACACC-3′, used for the amplification of calcitonin receptor-like receptor cDNA; RAMP1-S, 5′-GCTGCTGGCTCATCATCTCT-3′ and RAMP1-A 5′-TACACGATGCCCTCTGTGC-3′, used for the amplification of RAMP1 cDNA; RAMP2-S, 5′-TGAGGACAGCCTTCTGTC A-3′, and RAMP2-A, 5′-CATCGCCGTCTTTACTCC TC-3′, used for the amplification of RAMP2 cDNA; RAMP3-S, 5′-CTTCTCCCTCTGTTGCTGCT-3′ and RAMP3-A, 5′-CACA GAAGCCGGTCAGTGT-3′ used for the amplification of RAMP3 cDNA; and β-actin-S, 5′-ATCTGGCACCA CACCTTC-3′, and β-actin-A, 5′-AGCCAGGTCCAGACGCA-3′, used for the amplification of β-actin for calibrating sample loading. All the above sequences of oligonucleotide primers were synthesized by Sai Bai Sheng (Beijing, China). Other chemicals and reagents were of analytical grade.

2.2. Experimental protocols

The isoproterenol-induced myocardial injury model was produced as described (Rona, 1985), with minor modifications. Briefly, isoproterenol (30 and 20 mg/kg/d) was subcutaneously injected in rats (n=15) on 2 consecutive d, respectively. The rats in the therapeutic groups, besides receiving isoproterenol, were intraperitoneally injected with low and high doses of intermedin$_{1-53}$ (5 and 20 nmol/kg/d, respectively) for 4 days (beginning 2 days before isoproterenol administration). Another 6 rats were injected with physiological levels of saline (0.2 mL/d) as a control. Twelve rats were intraperitoneally injected with low and high doses of intermedin$_{1-53}$ (5 and 20 nmol/kg/d, respectively) for 4 days low and high doses of intermedin$_{1-53}$ alone respectively. During the experimental period, all rats were housed under standard conditions (temperature 20±1 °C, humidity 60±10%, light from 6 a.m. to 6 p.m.) and given standard rodent chow and free access to water.

The experiment was stopped 12 h after the last administration of the drugs. The rats were intraperitoneally anesthetized with pentobarbital sodium (30 mg/kg). To measure arterial blood pressure and left ventricular pressure, 2 catheters filled with heparin saline (500 U/mL) were inserted into the right femoral and right carotid arteries, and for the latter, farther into the left-ventricle. The heart rate, maximal rate of increase and decrease of left-ventricle pressure development (left-ventricle dp/dt_{max}), left ventricular end-systolic pressure and left ventricular end-diastolic pressure were recorded on a Powerlab (4S, Pty Ltd., Castle Hill, Australia) as described. After hemodynamic parameters were measured, blood was collected in heparinized syringes from the left ventricular and transferred to tubes. The blood was immediately centrifuged, and plasma samples were assayed for lipid peroxide product malondialdehyde content and lactate dehydrogenase activity. Rats were killed, and hearts were removed rapidly. Histological assay of myocardial sections in the cardiac apex was performed by means of hematoxyline eosin staining.

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