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The myocardial response to adrenomedullin involves increased cAMP generation as well as augmented Akt phosphorylation

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ABSTRACT

In this work we aimed to observe (1) the changes in adrenomedullin (AM) and its receptor system – calcitonin receptor-like receptor (CRLR) and receptor activity modifying proteins (RAMPs) – in myocardial ischemic injury and (2) the response of injuried myocardia to AM and the phosphorylation of Akt to illustrate the protective mechanism of AM in ischemic myocardia. Male SD rats were subcutaneously injected with isoproterenol (ISO) to induce myocardial ischemia. The mRNA levels of AM, CRLR, RAMP1, RAMP2 and RAMP3 were determined by RT-PCR. Protein levels of Akt, phosphor-Akt, CRLR, RAMP1, RAMP2 and RAMP3 were assayed by Western blot. Results showed that, compared with that of the controls, ISO-treated rats showed lower cardiac function and myocardial injury. The mRNA relative amount of AM, CRLR, RAMP1, RAMP2 and RAMP3 in the myocardia of ISO-treated rats was increased. The elevated mRNA levels of CRLR, RAMP1, RAMP2 and RAMP3 were positively correlated with AM content in injured myocardia. The protein levels of CRLR, RAMP1, RAMP2 and RAMP3 in injured myocardia were increased compared with that of control myocardia. AM-stimulated cAMP generation in myocardia was elevated in the ISO group, and was antagonized by $AM_{22–52}$ and $CGRP_{8–37}$. Western blot analyses revealed that AM significantly enhanced Akt phosphorylation in injured myocardia, which was blocked by pretreatment with $AM_{22–52}$ or $CGRP_{8–37}$. Ischemia-injured myocardia hyper-expressed AM and its receptors – CRLR, RAMP1, RAMP2 and RAMP3 – and the response of ischemic myocardia to AM was potentiated, and the level of Akt phosphorylation was also increased, which suggests that changes in cardiac AM/AM receptor might play an important role in the pathogenesis of myocardial ischemic injury.

1. Introduction

Adrenomedullin (AM) is a multifunctional regulatory peptide and has vasodilatory, hypotensive and growth-regulating properties. AM can be synthesized and secreted from various cells of the cardiovasculature, including vascular endothelial cells, vascular smooth muscle cells (VSMCs), cardiomyocytes and fibroblasts. AM generated from cardiovascular tissues

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plays an important role in regulating cardiovascular homeostasis, not only as a circulatory hormone but also as a paracrine/autocrine factor. AM levels in plasma and cardiovascular tissues are elevated to compensate for the elevated blood pressure in cardiovascular diseases such as myocardial infarction, heart failure, atherosclerosis and hypertension [5,16,21,40]. Administration of the AM peptide or AM gene delivery has significant therapeutic effects on heart failure, hypertension and shock, protecting against cardiovascular remodeling and renal dysfunction. These results suggest that endogenous AM is an important factor in regulating cardiovascular and renal homeostasis, as a potent cardio-reno-protective factor [17,25,28,32]. However, the cytoprotective mechanism of AM in the cardiovascular system has not been completely explained. Several intracellular signal transduction pathways, such as cAMP—protein kinase A (PKA), mitogen-activated protein kinases (MAPKs), and nitric oxide-cGMP (NO-cGMP), participate in the multifunctional actions of AM [21]. It is well-known that the pattern of AM interaction with its receptors is different from that of other vasoactive peptides such as angiotensin II, endothelin and atrial natriuretic peptide.

Cardiovascular tissue is rich in AM and AM receptors [3,4,24,35]. Among which, AM may bind mainly to calcitonin receptor-like receptor (CRLR), a member of G-protein-coupled receptor (GPCR) family, stimulating the activity of adenylate cyclase. The ligand selectivity of CRLR is regulated by receptor activity modifying proteins (RAMPs). So far, the RAMP family has been shown to consist of three isoforms: RAMP1, RAMP2 and RAMP3 [20,23,39]. Co-expression of CRLR with RAMP1 resulted in the characteristics of the CGRP receptor and showed high affinity to CGRP, and sensitive to the peptide antagonist $CGRP_{8-37}$, whereas CRLR co-expression with RAMP2 or RAMP3 showed the pharmacological features of AM1 and AM2 receptor, respectively, sensitive to the peptide antagonist [19,20]. The interaction pattern of ligand–receptor-RAMPs plays a pivotal role in the function of AM.

AM has been shown to activate the Akt pathway in vascular endothelial cells [27,43]. Interestingly enough, Akt activation has been reported to lead to the prevention of myocardial injury after transient ischemia in vivo through anti-apoptotic effects [2,22,27,32]. However, the alteration of AM level and its receptor system expression in ischemic myocardia are unclear, and whether AM, a potent Akt activator, activates the Akt pathway in severe myocardial injury induced by isoproteronol (ISO) remains unknown. It was reported that supramaximal doses of ISO, a β-adrenergic agonist and well-known inducer of myocardial hypertrophy [37,38], produce subendocardial myocardial ischemia, hypoxia, necrosis and, finally, fibroblastic hyperplasia, decreased myocardial compliance and inhibition of diastolic and systolic function, which are similar to local myocardial infarction-like pathological changes, which was different from that induced by hemorrhagic shock, orthostatic collapse [38]. Our previous work [37] showed that ISO treated rats has a upregulated levels of AM, AM and RAMP2 mRNA in injuried myocarida, but the role and significance of increased AM in ISO-treated rat myocardial is unclear. In this work, we observed the changes of AM and its receptor—CRLR, RAMP1, RAMP2 and RAMP3, the potential response of myocardia to AM and Akt activation by AM in ischemic injuried myocardia induced by ISO, to explore the role and significance of AM and its receptor in the pathogenesis of myocardial ischemic injury.

2. Materials and methods

2.1. Animals and reagents

All animal care and experimental protocols complied with the Animal Management Rule of the Ministry of Health, People's Republic of China (documentation 55, 2001) and the Animal Care Committee of the First Hospital, Peking University. Male Sprague–Dawley (SD) rats weighing 180–200 g were supplied by the Animal Center, Health Science Center, Peking University. AM_{1-50} peptide, AM_{22-52} peptide and $CGRP_{8-37}$ peptide were purchased from Phoenix Pharmaceutical Inc. (Belmont, CA, USA). The [^{125}I]-RIA kit for cAMP was purchased from NEN Life Sci. Products Inc. (Boston, MA, USA); isoproterenol (ISO) and isobutylmethylxanthine (IBMX) were purchased from Sigma Chemical Co. (St. Louis, MO, USA); Trizol agent was from Gibco (Gaithersburg, MD, USA); dNTP was from Clontech (Palo Alto, CA, USA); and M-MuLV reverse transcriptase, Taq DNA polymerase, RNasin and oligo(dT)15 primer were from Promega (Madison, WI, USA). For the amplification of AM cDNA, AM Sense (5′-CTCGACACTTCCTCGCAGTT-3′) and AM antisense (5′-GCTGGAGCTGAGTGTGTCTG-3′) primers were used. For the amplification of CRLR cDNA, CRLR sense (5′-CAACTGCTGGATCAGCTCAG-3′) and CRLR antisense (5′-CATCGCTGATTGTTGACACC-3′) primers were used. For the amplification of RAMP2 cDNA, RAMP2 sense (5′-TGAGGACAGCCTTCTGTCA-3′) and RAMP2 antisense (5′-CATCGCCGTCTTTACTCC TC-3′) primers were used. For the amplification of RAMP3 cDNA, RAMP3 sense (5′-CTTCTCCCTCTGTTGCTGCT-3′) and RAMP3 antisense (5′-CACAGAAGCCGGTCAGTGT-3′) primers were used. For the amplification of β-actin cDNA β-actin sense (5′-ATCTGGACCACACCTTC-3′) and β-actin antisense (5′-AGCCAG GTC CAG ACG CA-3′) primers were used. For the amplification of RAMP1 cDNA, RAMP1 sense (5′-GCTGCTGGCTCATCATCTCT-3′) and RAMP1 antisense (5′-TACAGGATFCCCTCTGTGC-3′) primers were used. For the amplification of BNP cDNA, BNP sense (5′-TCTGCTCCTGCTTTTCCTTA-3′) and BNP antisense (5′-

Table 1 – Values for parameters of blood pressure and cardiac function of control and ISO-treated rats (each $n = 6$, mean ± S.D.)

Parameters	Control	ISO
MAP (mmHg)	99 ± 4	101 ± 16
HR (Beat/min)	414 ± 37	413 ± 27
+LVdP/dt_{max} (mmHg/s)	3520 ± 262	1765 ± 359**
−LVdP/dt_{max} (mmHg/s)	3372 ± 239	1874 ± 233**
LVESP (mmHg)	132.8 ± 16	96.5 ± 11*
LVEDP (mmHg)	4.21 ± 2.09	17.30 ± 4.96**

*$p < 0.05$; **$p < 0.01$, compared with controls. ISO: isoproterenol; MAP: mean arterial pressure; HR: heart rate; LVSEP: left ventricular end-systolic pressure; LVEDP: left ventricular end-diastolic pressure; +LVdP/dt_{max}: left ventricular peak rate of contraction; −LVdP/dt_{max}: left ventricular peak rate of relaxation.