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Prevention of RhoA activation and cofilin-mediated actin polymerization mediates the antihypertrophic effect of adenosine receptor agonists in angiotensin II- and endothelin-1-treated cardiomyocytes

Asad Zeidan · Xiaohong Tracey Gan · Ashley Thomas · Morris Karmazyn

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Abstract Adenosine receptor activation has been shown to be associated with diminution of cardiac hypertrophy and it has been suggested that endogenously produced adenosine may serve to blunt pro-hypertrophic processes. In the present study, we determined the effects of two pro-hypertrophic stimuli, angiotensin II (Ang II, 100 nM) and endothelin-1 (ET-1, 10 nM) on Ras homolog gene family, member A (RhoA)/Rho-associated, coiled-coil containing protein kinase (ROCK) activation in cultured neonatal rat ventricular myocytes and whether the latter serves as a target for the antihypertrophic effect of adenosine receptor activation. Both hypertrophic stimuli potently increased RhoA activity with peak activation occurring 15–30 min following agonist addition. These effects were associated with significantly increased phosphorylation (inactivation) of cofilin, a downstream mediator of RhoA, an increase in actin polymerization, and increased activation and nuclear import of p38 mitogen activated protein kinase. The ability of both Ang II and ET-1 to activate the RhoA pathway was completely prevented by the adenosine A_1 receptor agonist N^6-cyclopentyladenosine, the A_{2a} receptor agonist 2-*p*-(2-carboxyethyl)-phenethylamino-5′-*N*-ethylcarboxamidoadenosine, the A_3 receptor agonist N^6-(3-iodobenzyl)adenosine-5′-methyluronamide as well as the nonspecific adenosine analog 2-chloro adenosine. All effects of specific receptor agonists were prevented by their respective receptor antagonists. Moreover, all adenosine agonists prevented either Ang II- or ET-1-induced hypertrophy, a property shared by the RhoA inhibitor *Clostridium botulinum* C3 exoenzyme, the ROCK inhibitor Y-27632 or the actin depolymerizing agent latrunculin B. Our study therefore demonstrates that both Ang II and ET-1 can activate the RhoA pathway and that prevention of the hypertrophic response to both agonists by adenosine receptor activation is mediated by prevention of RhoA stimulation and actin polymerization.

Keywords Adenosine receptor activation · Cardiomyocyte hypertrophy · RhoA/ROCK pathway · Cofilin phosphorylation · Actin polymerization · p38 Nuclear translocation

A. Zeidan · X. T. Gan · A. Thomas · M. Karmazyn (✉)
Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON N6A 5C1, Canada
e-mail: morris.karmazyn@schulich.uwo.ca

A. Zeidan
Department of Anatomy, Cell Biology and Physiological Sciences, American University of Beirut, Beirut, Lebanon

Introduction

Adenosine, a product of adenine nucleotide catabolism, has been demonstrated to exert numerous effects on the cardiovascular system, which are mediated by activation of various receptor subtypes. The primary adenosine receptor in the myocardium is the A_1 subtype which is linked to Gi-mediated inhibition of adenylate cyclase although both $A_{2a/b}$ and A_3 receptors have also been identified [1–4]. There is increasing evidence that endogenously produced adenosine is an important negative regulator of the hypertrophic and remodeling processes which contribute to heart failure. Thus, from a clinical perspective, an important observation linking adenosine to the heart failure process was the report that plasma levels of the nucleoside are elevated in patients with heart failure irrespective of causative factor [5]. Moreover, the degree of elevation was dependent on the severity of heart failure according to New York Heart Association (NYHA) classification with the greatest increases (more than five-fold) observed in NYHA