Modulation of cardiac A₁-adenosine receptors in rats following treatment with agents affecting heart rate

Nissim Balas,¹ Michael Arad,² Babeth Rabinowitz² and Asher Shainberg¹

¹The Gonda-Goldschmied Medical Research Center, Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan; ²Heart Institute, Sheba Medical Center, Sackler School of Medicine, Tel-Aviv University, Israel

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Abstract

Effects of chronic treatment affecting heart rate on A₁ adenosine receptor levels and their functions were studied. Treatment of rats with isoproterenol for 10 days accelerated heart rate and increased the level of adenosine receptors, in both the atria and ventricles. Negative dromotropic response of isolated heart to adenosine was enhanced in isoproterenol-treated rats. Similar results were obtained following treatment with atropine sulfate, or swimming training but not after treatment with thyroxine. On the other hand, treatment with amiodarone, which normally causes a decrease in heart rate, also increased the level of adenosine receptors in both atria and ventricles. The sensitivity of the isolated heart to the negative dromotropic and chronotropic effects of adenosine was not enhanced in the amiodarone treated rats. Similar results were obtained following treatment with propranolol, while treatment with PTU (6-n-propyl-2-thiouracil) increased adenosine sensitivity in the isolated heart. It was concluded that the levels of A₁ adenosine receptors in the heart correspond to heart rate, and to cardiac efficiency. While an increase in heart rate was followed by up-regulation of A₁ adenosine receptors, a decrease in heart rate caused a moderate elevation of these receptors. (Mol Cell Biochem 231: 107–116, 2002)

Key words: adenosine, 8-cyclopentyl 1,3-dipropylxanthine, ligand binding, heart rate

Introduction

Adenosine regulates a variety of physiological functions and is an important endogenous mediator of the cardiovascular system. Adenosine acts to reduce cardiac work whenever cardiac O₂ supply-to-demand balance becomes inadequate [1, 2]. It is believed that adenosine exerts its effects via four adenosine receptor subtypes termed: A₁, A₂A, A₂B and A₃ [3, 4]. Adenosine decreases O₂ demand by reducing the heart rate (a negative chronotropic effect), atrioventricular conduction (a negative dromotropic effect) and contractile force (a negative inotropic effect). It increases O₂ supply by causing coronary vasodilatation [2]. These are the direct effects of adenosine, which are mediated by two classes of cell surface adenosine receptors: A₁ subtype, located on cardiac cells, and A₂ subtype, located on vascular smooth muscle cells [3]. The indirect effects of adenosine also reduce O₂ demand by attenuating the stimulatory effects of catecholamines [5]. Adenosine produces direct effects by activating a particular class of K⁺ channels [6], coupled with A₁-receptors, via inhibitory G proteins [7, 8]. This activation causes the opening of K⁺ channels and hyperpolarization which slows the firing of pacemaker cells [7]. The indirect effects of adenosine, mediated by the inhibition of adenylyl cyclase, are also presumably linked to A₁-receptors, via one or more G proteins [9]. Chronic treatment with A₁ adenosine receptor antagonists, or agonists, is capable of regulating the density of these receptors in the heart [10–15], brain [16, 17], and adipose tissue.

Address for offprints: A. Shainberg, Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel (E-mail: shaina@mail.biu.ac.il)
[18–20]. Adenosine receptors in various tissues show a pattern of up- or down-regulation in the presence of antagonists, or agonists, respectively. Adenosine receptor levels were evaluated also by their physiological function in the cardiovascular system. However, the electrophysiological responses to adenosine were measured in the isolated heart (of a guinea pig) only in one of these studies [21]. In all other studies, the responses were measured in isolated atria. In the intact heart, this regulation pattern with correlation to heart rate has not yet been tested. We have shown in the past that treatment with agents accelerating heart rate in culture caused an up-regulation of A₁ adenosine receptors, whereas treatment with agents reducing heart rate caused a down-regulation of these receptors [22–24]. In this study we extended the investigation to in vivo experiments. Our study indicates that A₁ adenosine receptors in the heart correspond not only to heart rate but also to cardiac efficiency.

Materials and methods

Treatment with agents affecting heart rate

Male Wister rats (from Charles River Laboratories) weighing 250–350 g were used as experimental animals. Isoproterenol was injected 1.0 mg/kg i.p in phosphate buffer saline (PBS) twice daily for 10 days. Thyroxine (T₄) was dissolved in 0.1 N NaOH and was injected 1 mg/kg s.c daily, for 10 days. Atropine sulfate was injected 40 mg/kg s.c in PBS twice daily for 10 days. Propranolol was injected 30 mg/kg i.p in PBS twice daily, for 10 days. Control rats received injections of PBS. Amiodarone was given by oral gavaging 100 mg/kg in water once daily for 10 days. PTU (6-n-propyl-2-thiouracil) was given 0.02% in drinking water for 5 weeks. Amiodarone (25 mg/ml) and PTU (0.2%) stock solutions, were dissolved in water at 70°C and allowed to cool to room temperature. These stock solutions remained stable for up to 4 weeks, when protected from light. Swimming training was performed for 2 h in a 34°C water bath once daily for 4 weeks. The heart rate of the intact animal was measured during and at the end of each treatment by a bipolar electrocardiogram (ECG). Measurements were taken 10 min after the rats had been connected to electrodes, and placed in a small chamber enabling movement.

Thyroxine level measurements

Free thyroxine (FT₄) concentration in serum was determined by using the commercial radioimmunoassay kit (Amersham International, Amersham, UK).

Isolated perfused heart preparations

Rats were anesthetized with sodium pentobarbital (60 mg/kg i.p) and heparin (500 U i.p). The heart was quickly excised and placed in ice-cold heparinized saline [25]. The aorta was cannulated on Langendorff system and perfused in retrograde fashion. Perfusion was performed with oxygenated (95% O₂, 5% CO₂) Krebs-Henseleit solutions consisting of (in mM): NaCl, 118; KCl, 4.9; CaCl₂, 2.7; MgSO₄, 1.2; glucose, 11.1; NaEDTA, 0.5 and NaHCO₃, 25.0. The pH was maintained at 7.4 and the temperature was kept at 37 ± 1°C by water jacketing. The heart was suspended in a sealed chamber containing a silicon funnel for collection and volumetric measurement of the coronary effluent. The coronary effluent was kept at a constant flow rate by a peristaltic pump (Watson-Marlow, model 101U), providing a coronary pressure of about 55 mm/Hg. Coronary perfusion pressure was recorded through a Senso Nor 840 transducer at the level of the aortic cannula. Stainless steel electrodes were placed in the right atrium and in the apical epicardium. The heart was allowed to equilibrate for 20 min after placement of the electrodes prior to the start of the experiments. All parameters were recorded by an eight-channel recorder (Astro-Med, model MT-8800). Cardiac rhythm and coronary perfusion pressure were recorded (Microsoft C program, developed in Sheba Medical Center) and presented visually on the monitor. Atrial pacing at 300 beats/min was performed to test atrioventricular conduction when sinus heart rate fell below 200 beats/min. Atrial pacing was performed by the right atrium electrode, with the relative electrode placed on the aortic cannula. A programmable stimulator (Medtronic, model 5325) delivered stimuli in the form of square-wave pulses of 2 msec duration.

Protocols for isolated hearts

The inhibitory effects of adenosine on sinus heart rates (negative chronotropic effects) and on the atrioventricular nodal conduction (negative dromotropic effects) were measured. The electrogram was recorded after 20 min of perfusion with a Krebs-Henseleit solution and six minutes of perfusion with each of the following adenosine concentrations: 1, 3, 10, 30, and 100 µM. Measurements were taken on spontaneously-beating hearts and the longest P-R interval before the onset of second-degree AV (atrioventricular) block was considered to be the maximal response. Dose-response curves were constructed for the direct negative chronotropic and dromotropic effects of adenosine.

Membrane preparation and protein measurement

Crude atrial and ventricular membranes were prepared identically according to Wu et al. [15]. The excised heart was