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Introduction

Adenosine (ADO) is an endogenous purine derived in vivo from ATP, ADP, and AMP in response to adrenergic stimulation, hypoxia, ischemia, and other unfavorable oxygen supply-demand ratio situations. An elevated interstitial concentration of adenosine is known to attenuate the effects of β-agonist stimulation of myocardium. β-adrenergic receptor stimulation of the heart results in increases of contractility and metabolic state. Adenosine binds to the extracellular A1 receptor subtype and activates an inhibitory G-protein that reduces the cyclic AMP excitatory cascade caused by β-adrenergic stimulation. Therefore, one role of adenosine is to act as a negative feedback modulator of β-adrenergic responses in the heart, essentially protecting the myocardium from the potentially deleterious effects of overstimulation. However, the cascade of events and mediators connecting adenosine to its effects is not completely understood.

Nitric oxide (NO) has been shown to depress cardiomyocyte response to β-adrenergic stimulation in some studies. In addition, preliminary research data from this laboratory (J.M. Lee, et al. 2001) indicates that extracellular NO levels increase with stimulation by ADO (figure 1). Therefore, this investigation was designed to determine if NO is expressed as an anti-adrenergic mediator in response to ADO stimulation. This study used a NO-synthase inhibitor, N-nitro-L-arginine-methyl-ester (NMMA), to determine if blocking NO production affects the anti-adrenergic effect of ADO when hearts are stimulated by a β-agonist, isoproterenol (ISO).

Methods

Hearts were obtained from 3-4 month old Sprague-Dawley rats. Immediately after isolation, hearts were retrograde constant flow perfused at 60 mmHg with physiological saline (PS; 37° C) containing (mM): 118 NaCl, 4.7 KCl, 2.5 CaCl2, 25NaHCO3, 1.2 MgSO4, 1.2 KH2PO4, and 10 glucose. The pH was maintained at 7.4 by bubbling the PS with a 95% O2 – 5% CO2 mixture. Hearts were paced at 400-475 contractions/min via platinum were electrodes, depending on the need for overdrive pacing to suppress arrhythmia. The developed left ventricular pressure (DLVP) was determined by an intraventricular water-filled balloon-tipped cannula attached to a pressure transducer with the balloon inserted into the left ventricle via the atrium. The balloon pressure was set at 5 mmHg initially and kept between 5-10 mmHg throughout the experiment. Contractile function is reported as the maximum rate of development of left ventricular pressure (+dP/dtmax). DLVP, +dP/dt, and –dP/dt were continuously recording using a Gould 2600 recorder and abstracted into a Microsoft Excel spreadsheet. The data was then analyzed and graphed using GraphPad Prism 4.0.

Protocols
Two different protocols were used during the experiment. First, using each heart as its own control, contractile response to ISO was compared with and without ADO perfusion, while using PS initially without NMMA and later with NMMA. After allowing the hearts to stabilize for 20 minutes, ISO was infused over 10 seconds into PS without NMMA through the tubing close to the heart at a rate, adjusted for PS flow, calculated to deliver a concentration of $2.5 \times 10^{-8}$ M. After allowing the contractility to return to baseline, approximately 10 minutes, ISO stimulation was repeated. Following 3 cycles of ISO infusion, ADO was infused at a constant rate calculated to deliver a concentration of $10^{-5}$ M, followed by 2 cycles of ISO infusion. Thereafter, ADO was withdrawn and the PS was quickly switched to PS with NMMA at $10^{-6}$ M. After approximately 10 minutes, 2 cycles of ISO stimulation were performed and the ADO was restarted. Following 2 cycles of ISO infusion with ADO and PS with NMMA, ADO was stopped, and 2 more ISO cycles were performed to gauge to post-test performance of the heart. (figure 2)

Second, two series of hearts were studied using PS with or without NMMA throughout the experiments, but with increasing levels of ADO infusion, in order to establish a dose-response curves. After allowing the hearts to stabilize for 20 minutes, ISO was infused over 10 seconds into PS without NMMA through the tubing close to the heart at a rate, adjusted for PS flow, calculated to deliver a concentration of $2.5 \times 10^{-8}$ M. After allowing the contractility to return to baseline, approximately 10 minutes, ISO stimulation was repeated. Following 3 cycles of ISO infusion, ADO was infused at a constant rate calculated to deliver a concentration of $10^{-9}$ M, followed by 2 cycles of ISO infusion. Thereafter, ADO was increased one order of magnitude ($10^{-8}$, $10^{-7}$, $10^{-6}$, $10^{-5}$) and after approximately 10 minutes, 2 cycles of ISO stimulation were performed. The entire cycle was repeated until the maximum study concentration of ADO was reached at $10^{-5}$ M. Following 2 cycles of ISO infusion at the maximal ADO concentration, ADO was stopped, and 2 more ISO cycles were performed to gauge to post-test performance of the heart. The same protocol was repeated on another series of hearts using PS with NMMA at $10^{-6}$ M. (figure 3)

**Summary**

1. As assessed by the parameters of myocardial contractility, the NO-synthase inhibitor NMMA prevented the antiadrenergic action of ADO.
2. There was an unexpected initial increase in contractility observed with NMMA infusion at lower concentrations of ADO.

**Conclusion**
These results indicate that nitric oxide plays a role in the anti-adrenergic effects of adenosine in isolated, perfused rat hearts.