THE EFFECT OF EXTRACELLULAR ATP ON SPONTANEOUS CALCIUM WAVES IN ISOLATED RAT CARDIOMYOCYTES

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INTRODUCTION

Extracellular ATP (ATP_o) is often released as a co-transmitter with noradrenaline from sympathetic nerve endings including those that terminate in the heart. This ATP_o interacts with purinergic receptors on the surface membrane of cardiomyocytes, resulting in an increase in contractility.[1-3]

ATP_o is thought to exert this positive effect on cardiomyocytes by binding to either of 2 types of P_2-purinergic cell-surface receptors:

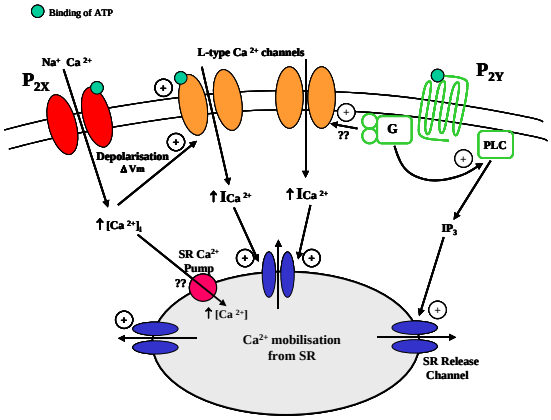


Figure 1. Overview of ATP-mediated signal transduction pathways thought to take place in in cardiac myocytes

AIMS

To observe the effects of various biological concentrations of ATP_o on internal calcium handling in isolated cardiomyocytes.

METHODS

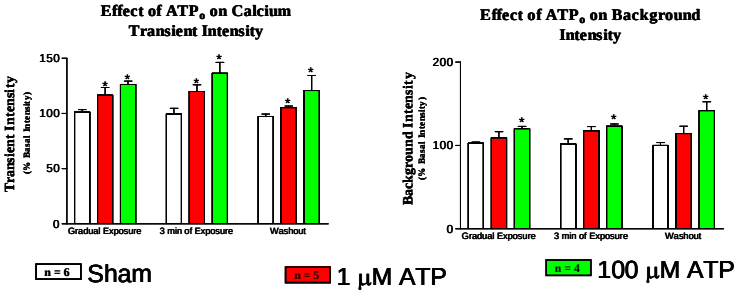
Ventricular cardiomyocytes were isolated from the right ventricle of male Sprague Dawley rat hearts using a collagenase and protease technique. Isolated cardiomyocytes were loaded with the Ca^{2+}-fluorsecent dye fluo-3 and perfused with Tyrode's (HEPES buffered) solution, containing 1.5 or 5 mmol/L $CaCl_2$ for field-stimulated calcium transients and spontaneous calcium waves respectively. Cardiomyocytes were exposed to either 1, 10 or 100 µmol/L ATP, during which Ca^{2+} transients and waves were viewed using the fast line scan mode of a confocal microscope. All experiments were performed at room temperature (~23°C).

RESULTS

All Figure values for both calcium waves and transients are expressed as a percentage of basal measurements made in control Tyrode's solution containing 5 and 1.5 mmol/L $CaCl_2$, respectively, at the beginning of each experiment (data not shown). Sham experiments represent the same time period as ATP_o experiments, except that results were obtained in the absence of ATP_o (to test the consistency of the measurements over the same time period).

Figure 2: Effect of ATP_o on Field-Stimulated Calcium Transients

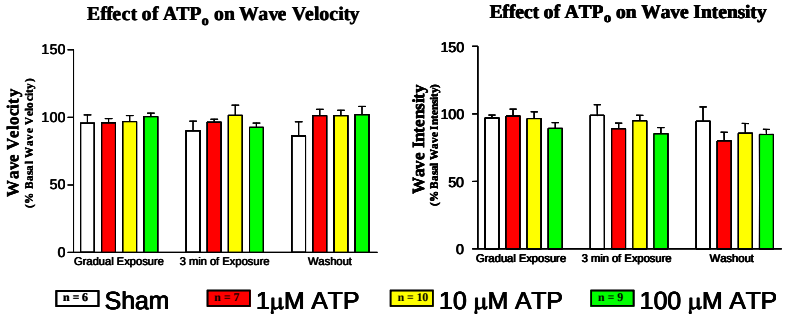
Effect of 1 and 100 µmol/L ATP_o exposure on **(A)** calcium transient intensity and **(B)** background fluorescence immediately prior to each calcium transient. (*) Indicates statistical significance when compared to sham result obtained during same time period, $P < 0{\cdot}05$.



ATP significantly increased $[Ca^{2+}]i$ transient intensity at both 1 and 100µM, and the resting $[Ca^{2+}]i$ only at 100 µ M

Figure 3: Effect of ATP_o exposure on Ca^{2+} Wave Properties

(A) calcium wave velocity and **(B)** intensity.

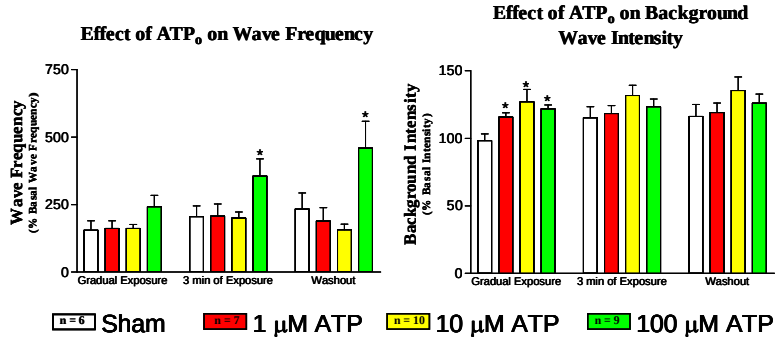


ATP did not change wave propagation velocity or wave intensity at concentrations of up to 100µM

In general, for sham experiments, mean calcium wave velocity and intensity under these control conditions did not significantly change over the experimental period (Figures 2A and B). In contrast, for the sham experiments, mean calcium wave frequency and background fluorescence immediately prior to each calcium wave (Figures 3A and B) gradually increased throughout the experimental period ($n = 6$, $P < 0.05$).

Figure 4: Effect of ATP_o exposure on Ca^{2+} Wave Properties

(A) calcium wave Frequency and **(B)** Background intensity.



ATP increased Ca^{2+} wave frequency at 100µM

Discussion

P2X-purinoceptors are thought to alter Ca^{2+} transient and wave properties by depolarising the resting membrane potential, increasing resting $[Ca^{2+}]i$ and thus loading of Ca^{2+} into the SR.

P2Y-purionceptors act via 2nd messenger systems to alter contractility either by stimulating L-type Ca^{2+} channels or SR Ca^{2+} release through the production of IP_3.[8]

In contrast, ATP may also either directly or indirectly (through changes in resting membrane potential) increase the L-type Ca^{2+} current.[4,5]

Conclusion:

Lower concentrations of ATP_o stimulate contractility through direct changes in the L-type Ca^{2+} current, and to a lesser extent through changes in the resting membrane potential or resting $[Ca^{2+}]i$.

Higher concentrations of ATP_o (100 µM) stimulate changes in contractility by interacting with P2X-purinoceptors and thus increasing resting $[Ca^{2+}]i$, but also through stimulation of a longer lasting 2nd messenger system than IP_3.

References:

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