

**β-ADRENERGIC STIMULATION MODULATES THE RELATIONSHIP
BETWEEN INTRACELLULAR Ca²⁺ AND SR Ca²⁺ LOADING IN
TROUT ATRIAL MYOCYTES**

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

In the teleost heart, chronotropic and inotropic effects of β-adrenergic stimulation have been described *in vivo*, *in situ* and in multicellular preparations (1). Furthermore, the stimulatory effect of the beta adrenergic agonist isoproterenol on L-type Ca²⁺ current has been described in a few teleost species (2). The influence of β-adrenergic stimulation on Ca²⁺ removal from the cytosol has, however, not been examined in the teleost heart. The aim of the present work was therefore to examine the effect of β-adrenergic stimulation on intracellular Ca²⁺ transients and Ca²⁺ uptake in the sarcoplasmic reticulum (SR).

Methods

Long depolarizations cause a steady tonic contraction and induce SR Ca²⁺ uptake in trout atrial myocytes and rapid caffeine (CAF) applications at -80 mV before and after a long depolarization can be used to determine SR Ca²⁺ loading (3). To determine the relationship between intracellular [Ca²⁺]_i and SR Ca²⁺ uptake, simultaneous measurements of ionic currents and intracellular [Ca²⁺]_i ([Ca]_i) were done in trout atrial myocytes. Depolarizations to different membrane potentials were used to induce SR Ca²⁺ uptake and rapid CAF applications were used to measure it.

Results

Simultaneous measurements of $[Ca]_i$ and ionic currents showed an elevated $[Ca]_i$ throughout the depolarization, and the SR Ca uptake rate was $120 \pm 18 \mu\text{M/s}$ at +50 mV in control conditions. In order to evaluate the effect of the β -adrenergic agonist isoproterenol (ISO) on L-type Ca^{2+} current and the amplitude of the intracellular Ca^{2+} transient, repetitive 200 ms depolarizations from -80 to 0 mV were used. With this protocol ISO increased L-type current (I_{Ca}) to $191 \pm 29\%$ of the control value and the Ca^{2+} transient to $141 \pm 10\%$ of control. Using a 3 s depolarization to +50 mV, ISO increased the SR Ca uptake rate slightly to $132 \pm 29 \mu\text{M/s}$. In contrast the average $[Ca]_i$ during a 3s depolarization decreased from $1.36 \pm 0.16 \mu\text{M}$ in control to $0.89 \pm 0.10 \mu\text{M}$ with ISO. Plotting Ca^{2+} uptake at different membrane potentials against the corresponding $[Ca]_i$ resulted in a shift in the $[Ca^{2+}]_i$, where the SR uptake rate was 50% of the rate at +50 mV, from 0.70 to 0.52 μM . Lowering of the extracellular $[Ca^{2+}]$ from 1.8 to 0.3 or 0.5 mM reduced the stimulatory effect of ISO on L-type Ca^{2+} current. The inhibitory effect of lowering the extracellular Ca^{2+} was even more pronounced on SR Ca^{2+} uptake, resulting in a shift of +26 mV in the relationship between membrane potential and SR Ca^{2+} uptake. Furthermore, ISO in the presence of low extracellular $[Ca^{2+}]$ caused a significant reduction of the SR Ca^{2+} loading during a 10s depolarization at all membrane potentials above -30 mV, when compared with control conditions.

Conclusions

These data suggest that NCX plays a central role in the regulation of SR Ca^{2+} loading in trout atrial myocytes and that ISO has a stimulatory effect on the SR Ca^{2+} pump that is not caused by an increased $[Ca]_i$ during depolarization. As phospholamban has not been characterized in trout atrial myocytes, it remains to be determined if the SR Ca^{2+} pump in this species is modulated by a direct phosphorylation of the pump or through phospholamban phosphorylation as described in mammalian species.

References

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