CARDIAC FUNCTION AND CALCIUM MANAGEMENT IN THE SOUTH AMERICAN LUNGFISH, *Lepidosiren paradoxa*

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EXTENDED ABSTRACT ONLY-DO NOT CITE

Introduction

Contractile mechanisms (i.e., actin-myosin interaction and its regulation by calcium) appear to be similar in all vertebrate hearts (Driedzic and Gesser, 1994). However, anatomical and ultra-structural distinctions between hearts of different species underlie important physiological differences, particularly to the regulation of calcium delivery to contractile apparatus (Tibbits et al., 1992).

Among these differences, the putative contribution of the sarcoplasmic reticulum (SR) calcium stores to excitation-contraction coupling (E-C coupling) has a profound impact on calcium management, since this organella is able to reduce significantly difusional distances and accelerates both contraction and relaxation. In most lower vertebrates studied, the main source of calcium activator is the extracellular space by its influx across the sarcolemma on a beat to beat basis, although the functional relevance of the cardiac SR in supplying calcium varies with temperature, stimulation frequency and species (Bers, 2001).

Material and Methods

Active lungfish were obtained from water filled clay pits in Brazilian Pantanal area and acclimated at 25 ^{o}C. Pairs of ventricle strips ($\phi \cong 1$ mm) were excised from the ventricle and placed into a bathing medium containing (in mM) 100 NaCl, 5 KCl, 1.2 $MgSO_4$, 1.5 NaH_2PO_4, 27 $NaHCO_3$, 2.5 $CaCl_2$ and 10 glucose and bubbled with a gas mixture of 98% O_2 and 2% CO_2 throughout the experiment. Preparations were connected to an isometric force transducer and to a stimulator delivering electrical square pulses with a voltage 50% above that of the threshold value. Twitch tension was allowed to stabilize for about 30 min at 0.2 Hz before each protocol.

In order to analyze the main source of calcium-activator, contraction frequency was increased at different temperatures (15, 25 and 35 ^{o}C) in Control preparations (physiological calcium concentration - 2.5 mM), High Calcium (supraphysiological calcium concentration - 10.5 mM) and Ryanodine (2.5 mM of calcium + 10 μM of ryanodine). When applied, ryanodine was added to the medium at least 40 min before changes in frequency. Moreover, *in vitro* frequencies were also compared to *in vivo* heart rate obtained to *L. paradoxa* by Costa et al. (2002).

Results

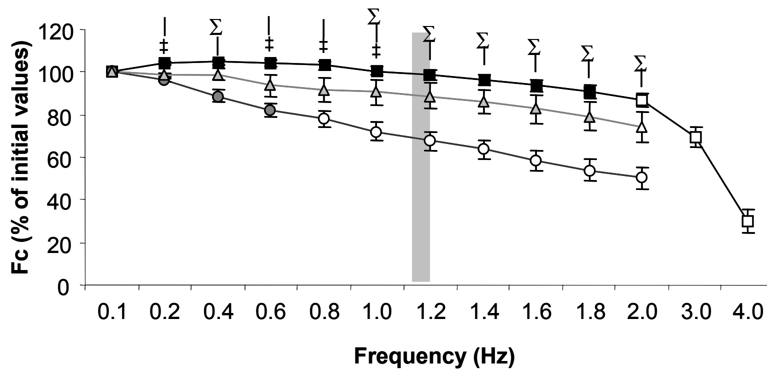
The dashed area in figure 1A indicates that the twitch force (Fc) of control preparations showed a significant increase ($p < 0.05$) when physiologically relevant stimulation frequencies (0.29 ± 0.01 Hz) were reached at 15 ^{o}C. However, when treated with ryanodine, the strips could not contract regularly to reach the *in vivo* frequency range (force-frequency relationship shifted to the right), while it was observed a decrease in force development (relationship shifted downwards) at this stimulation range when strips were tested in a supraphysiological calcium concentration.

At acclimation temperature (25 ^{o}C), when force-frequency responses were analyzed at frequencies inside the *in vivo* frequency range (0.54 ± 0.03 Hz; dashed area in figure 1B), ryanodine treatment resulted in a significant reduction ($p < 0.05$) in twitch force, which was further reduced in response to exposure to a supraphysiological calcium concentration.

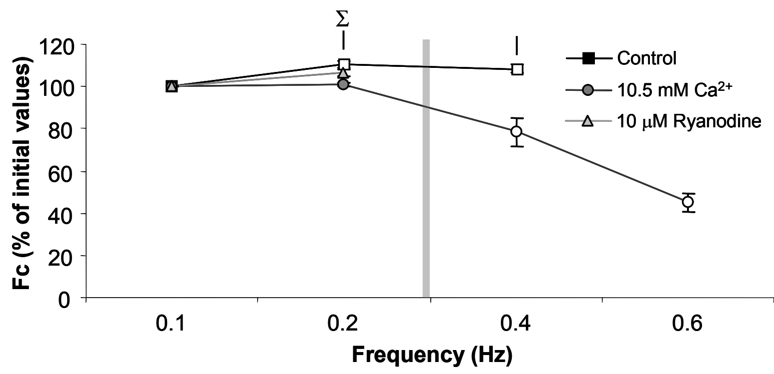
Finally, when the inotropic responses are analyzed at frequencies inside the *in vivo* range (1.17 ± 0.03 Hz; dashed area in figure 3) at the highest

temperature (35 °C), the force-frequency relationship was shifted downwards only in response to increases in extracellular calcium, while ryanodine had no effect on force development at physiologically relevant frequencies at 35 °C.

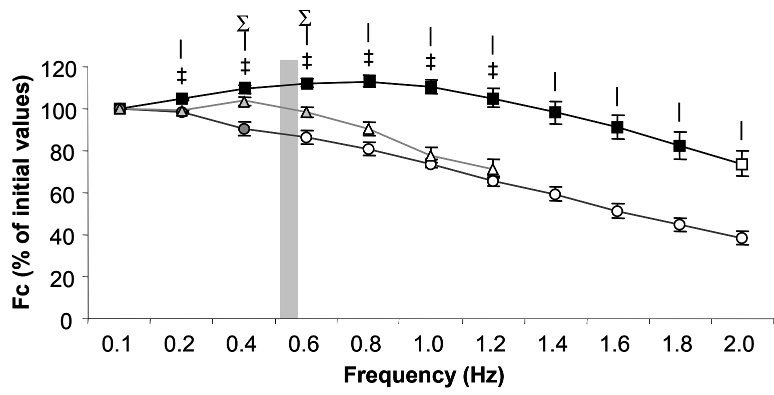
Figure 1 - Effect of increases in stimulation frequency on twitch force (Fc - % of initial values) of ventricle strips from *L. paradoxa* at different experimental conditions (Control: 2.5 mM of calcium and without ryanodine; 10.5 mM of Calcium; 10 μM of Ryanodine) at 15 °C (n = 10; A), 25 °C (n = 10; B) and 35 °C (n = 10; C). For each treatment, open~~s~~ symbols indicate a significant difference ($p < 0.05$) in relation to the Fc observed initially (at 0.1 Hz). - Control ≠ 10.5 mM of Calcium; ‡ - Control ≠ 10 μM of Ryanodine; - 10.5 mM of Calcium ≠ 10 μM of Ryanodine in the same frequency ($p < 0,05$). The dashed area indicates the heart frequency observed *in vivo* at each temperature by Costa et al., 2002. Mean values ± 1 S.E.M.



A



B



C

Discussion

The results indicate that the species, in spite of having a potentially functional and anatomically well-developed SR (Hochachaka and Hulbert, 1978), this organella probably presents a slower calcium-cycling capacity, which does not allow it to play a functional role at higher heart frequencies that are usually observed *in vivo* in response to increases in temperature.

However, when temperature is decreased, SR plays a central role in calcium regulation, since it can be observed a temperature-dependent decrease in chronotropism. Furthermore, this organella becomes essential to cardiac performance maintenance when the lowest temperature (15 ^{o}C) is reached, compensating the temperature-dependent decrease in Na^{+}/Ca^{2+} exchanger activity (Costa et al., 2002). These results contrast with those described to most fish already studied, in which ryanodine does not have any effect on force development when physiological frequencies and/or temperatures are considered (Shiels and Farrell, 1997).

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