EVIDENCES OF A FUNCTIONAL SARCOPLASMIC RETICULUM UNDER PHYSIOLOGICAL CONDITIONS IN NEOTROPICAL FISH

Ana L. Kalinin

Department of Physiological Sciences, Federal University of São Carlos
Via Washington Luiz, km 235. 13565-905 - São Carlos, SP, Brazil
Phone: ++55 16 2608314 - Fax: ++55 16 2608328
e-mail: akalinin@power.ufscar.br

Luciano Rivaroli, Luiz C. Anelli-Junior, Claudio D. Olle, Monica J. Costa, Francisco T. Rantin
Department of Physiological Sciences, Federal University of São Carlos

EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Most of the studies in teleost cardiac myocites have shown that E-C coupling is a ryanodine-insensitive mechanism at physiological frequencies and temperatures, implying an important role for transarcolemmal calcium movement. Nevertheless, there are some indications of an increased functional importance of the cardiac sarcoplasmic reticulum (SR) in trout at high experimental temperatures and low contraction frequencies (Keen *et al*. 1994) and in "athletic" fish, like tunas (Keen *et al*., 1992, Aho & Vornanen, 1998). Different results were obtained by Shiels & Farrell (1997), that found a significant Ca^{2+} contribution from the SR in trout at physiological pacing frequencies and by Hove-Madsen et al. (2001), that demonstrated a relevant contribution of the SR (~ 40% of total Ca^{2+} required to activate contraction) in this species, independently of the experimental temperature. However, little is known about tropical species.
In order to understand the role of the sarcoplasmic reticulum (SR) and extracellular calcium concentration in the contractile myocardium system, experiments *in vivo* and *in vitro* were conducted with 4 tropical teleost species from different habitats and distinct modes of life: the traira, *Hoplias malabaricus*, a sedentary fresh water fish characteristic of lentic environments, the armored catfish, *Hypostomus regani*, a sedentary species

living in fast-stream rivers, the pacu, *Piaractus mesopotamicus*, a migratory freshwater species from lotic environments and the curimbata, *Prochilodus scrofa*, an "athletic" freshwater fish. The results were compared to previous studies conducted with the embore, *Bathygobius soporator*, a teleost inhabiting tide pools (Rantin *et al*., 1998) and the Nile tilapia, *Oreochromis niloticus*, (Costa *et al*., 2000).

Material and Methods

In vivo heart rate (f_H - bpm) was obtained by electrocardiography. ECG electrodes were placed in a ventral position between the gills and the heart and in a ventral position close to the pelvic fins. A reference electrode was located in the water of the experimental chamber. The electrode set was connected to a universal coupler of a Narco recorder. After a recovery period of 12 h, the f_H was measured at 25 ^oC. For the *in vitro* preparations, pairs of strips with a thickness of maximally 1 mm were excised from the ventricle. The preparations were connected to a NARCO F-60 isometric force transducer with surgical silk and placed around a platinum electrode. The temperature of the muscle bath was maintained at 25 ^oC. The maximal capacity of hearts to develop force was assessed trough the addition of Ca^{2+} to bathing medium. The force development upon the first stimulation following a rest period was determined at 25 ^oC to examine the capacity for the storage of intracellular Ca^{2+} during resting period. After a steady-state condition at 12 bpm was achieved, stimulation ceased for a period of 5 min. Force development of the first contraction following the resting period was compared to the last contraction in a steady-state train. These experiments were conducted with and without 10 μM ryanodine in the medium.

Results and Discussion

All the species showed resting heart rate (f_H) values similar to those exhibited by temperate species (ranging from 30 to 70 bpm), differing from *B. soporator*, which presented f_H values similar to those of mammals (~ 150 bpm at 25 ^oC and 300 bpm at 40 ^oC). Increasing extracellular Ca^{2+} concentration caused a significant increase of force contraction (Fc), which reaches maximum values at 8.5 mM Ca^{2+} for all the experimental species (figure 1).

In *B. soporator* (Rantin *et al*., 1998) and *O. niloticus* (Costa *et al*., 2000), the post rest potentiation was not influenced by ryanodine (figure 1), an inhibitor of the SR, indicating that this organelle is not important to the excitation-contraction coupling irrespective of temperature (25 and 40 ^oC). This is an interesting result since both fish, particularly *B. soporator*, are frequently subjected to acute changes in temperatures. Furthermore, *B.*

soporator presents very high f_H values, suggestive of a functional SR. On the other hand, the post rest potentiation of *H. malabaricus*, *H. regani*, *P. mesopotamicus* and *P. scrofa* (figure 1) was strongly inhibited by ryanodine at both physiological temperature (25 ^{o}C) and stimulation frequency (12 bpm). At these experimental conditions, *H. malabaricus*, *P. mesopotamicus* and *P. scrofa* hearts derive a large portion (60%, 30% and 25 %, respectively) of activator calcium from intracellular stores (figure 2). Curiously, *H. malabaricus* is the most sedentary species in this group. These results indicate that other factors than high temperatures and activity can determine the level of functionality of the sarcoplasmic reticulum in tropical fish.

References

Aho, E & Vornanen, M. (1998). *J. Exp. Biol.* 201: 525-532.

Costa, M.J. *et al.* (2000). J. Therm. Biol. 25: 373-379.

Hove-Madsen, L. *et al*. (2001). *Am. J. Physiol.* 281: R1902-R1906.

Keen, J.E. *et al*. (1992). *Can. J. Zool.* 70: 1211-1217.

Shiels, H & Farrell, A.P. (1997). *J. Exp. Biol.* 200: 1607-1621.

Rantin *et al*. (1998). J. Therm. Biol. 23: 31-39.

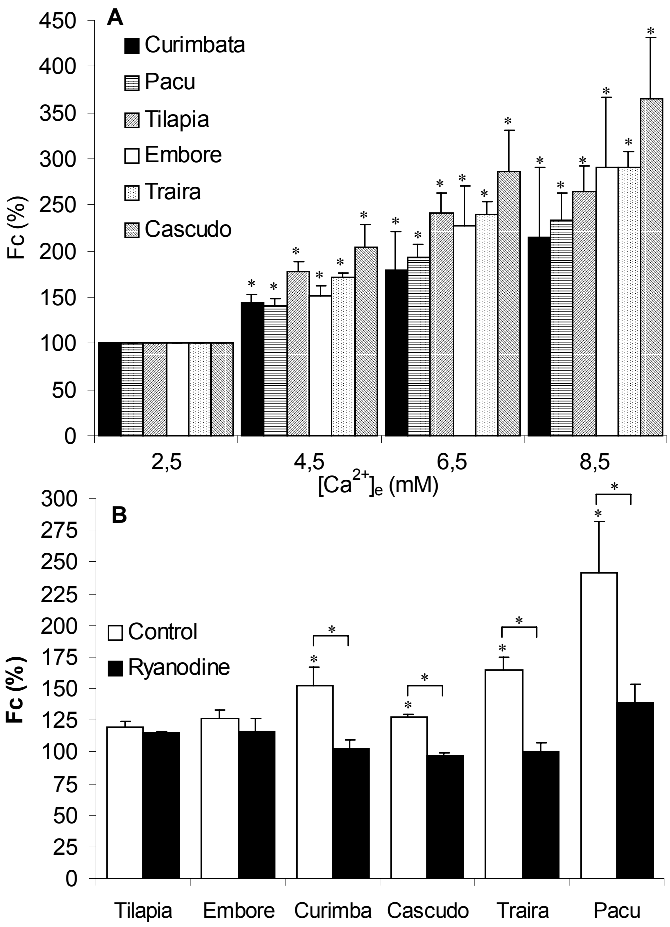

Figure 1. **A**. The effect of extracellular Ca^{2+} on twitch force (Fc - % of the initial values) of cardiac muscle of each species. **B**. Twitch force of the first contraction following a rest period. Mean values ± S.E.

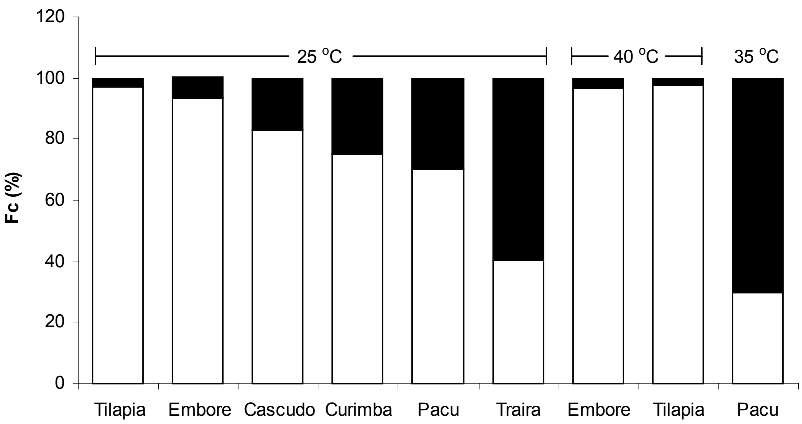

Figure 2. Estimated values of activator calcium derived from the influx across the sarcolemma (⊠) and SR stores (◫) at different experimental temperatures.