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Effect of changes in temperature on the force–frequency relationship in the heart of catfish (*Clarias gariepinus*)

Mohamed F. El-Sayed *, El-sabry Abu-Amra, Ahmed Badr

Department of Zoology, Faculty of Science, Sohag University, Egypt

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Abstract An isometric ventricular preparation was used to investigate the effect of changes in temperature (10, 15, 20, 25 and 30 °C) on the cardiac contractility produced by increasing of frequency in the catfish heart. The ability of the ventricular preparation to develop the cardiac force at 10 °C continued regularly until a frequency of 1.0 Hz, whereas at 15, 20, 25 and 30 °C, it continued developing the cardiac force until 2.0 Hz. The contractile force, the rate of contraction and the rate of relaxation (cardiac contractions) decreased significantly as contraction frequency increased. The decreases in the cardiac contractility with the increasing of the contraction frequency from 0.2 to 2.0 Hz were significantly higher at 15, 20 and 25 °C than that at the same frequency at 30 °C and at 10 °C in the range of frequency between 0.2 and 1.0 Hz. The percentage changes in the contractile force at a contraction frequency of 2.0 Hz at 15, 20 and 25 °C were 42 ± 2.7, 32 ± 2.5 and 32 ± 3.3, respectively; whereas it was 61 ± 1.3 at 30 °C, and at 10 °C, it was 60 ± 1.1 at a frequency of 1.0 Hz. So, it can be concluded that the catfish myocardium, like most fish hearts exhibits a negative force–frequency relationship. But, this relationship is highly affected by the changes in the temperature in a way that the lower temperature (10 °C) and the higher temperature (30 °C), may provide a protective mechanism against the depressive effects of higher stimulation frequency. This may be due to the differences in the handling of the activator Ca^{2+} to the contractile system via the transsarcolemmal Ca^{2+} channels and/or Na^{+}–Ca^{2+} changes, and the sarcoplasmic reticulum Ca^{2+} release.

Introduction

The force–frequency relationship is an important intrinsic regulatory mechanism of cardiac contractility (Endoh, 2004). This relationship was studied in multicellular and single cell preparations to understand the functional property of the heart, namely, its ability to develop force at different frequencies (Shiels et al., 2002a). Therefore, by using the pharmacological drugs and by extrapolation of the results to in vivo contraction frequencies, the force–frequency relationship is still providing useful information to the cardiac physiologist. This relation-

* Corresponding author.
E-mail address: m_mohamed6000@yahoo.com (M.F. El-Sayed).

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ship was classified as positive, primary-phase negative, secondary-phase negative or overall negative (Endoh, 2004). In mammals, the force–frequency relationship varied with the species, temperature and even between cell populations within the same species (Frampton et al., 1991). Larger mammals have a positive force–frequency relationship (Frampton et al., 1991). Among ectothermic animals, most fishes showed a negative force–frequency relationship (Keen et al., 1994; Shiels and Farrell, 1997; Mercier et al., 2002; Shiels et al., 2002a). Amphibians and reptiles (including turtles, lizards and snakes) tend to show a secondary-phase negative response that is flat or positive at low frequencies and negative at high frequencies (Rumberger and Riechel, 1972; Driedzic and Gesser, 1985; Galli et al., 2006). It has been postulated that changes in the force development are directly related to the changes in the intracellular Ca^{2+} transient (Yue, 1992). The Ca^{2+} transient is the transient rise and fall of cytosolic Ca^{2+} that links excitation of the myocyte membrane to contraction of the myofilament via a process of excitation–contraction coupling (Bers, 2001, 2008). Negative force–frequency relationship may be related to a reduction in the Ca^{2+} influx into the cytoplasm as a result of either a reduction in sarcolemmal Ca^{2+} influx (via either the L-type Ca^{2+} channels or in exchange for Na^{+}), or a reduction in sarcoplasmic reticulum Ca^{2+} release at high frequencies (Shiels et al., 2002a). In mammals, which show a positive force–frequency relationship, the sarcoplasmic reticulum Ca^{2+} cycling represents the major supply of Ca^{2+} for excitation–contraction coupling. The contribution of each component (sarcolemmal Ca^{2+} channels, Na^{+}–Ca^{2+} exchange and sarcoplasmic reticulum Ca^{2+} cycling) in the excitation–contraction coupling depends on factors including temperature (Shattock and Bers, 1987; Warren et al., 2010), species (Bers, 1985) and tissue type (atrial or ventricular) (Walden et al., 2009). Changes in temperature have a remarkable effect on physiological and biochemical processes in ectothermic animals. The temperature-dependency of the heart rate, cardiac ion channel function and Ca^{2+} sources and transport during excitation–contraction coupling of the heart are of particular interest during temperature changes (Shiels et al., 2002a,b; Farrell, 2009).

Temperature also, alters the force–frequency relationship in mammals and fish hearts. Moreover, the ability of cardiac muscle to function during an acute temperature change can be essential for survival (Shiels et al., 2002b). In most fishes, acute increases in temperature caused a decrease in force–frequency response, whereas acute decreases in temperature caused an increase in force–frequency response (Vornanen, 1989; Shiels and Farrell, 1997, 2000; Shiels et al., 1999; Titu and Vornanen, 2001). Thermal acclimation alters force–frequency relationship in such a way that it may partially compensate for the effect of acute temperature. It is clear that temperature does not modulate the open probability of the sarcoplasmic reticulum (SR) Ca^{2+}-release channel in the same manner in fish as it does in mammals. In mammals acute reduction in temperature increases the open probability of the SR-Ca^{2+} release channel (Sitsapesan et al., 1991).

Thus, the effect of the temperature changes on the force–frequency relationship in fishes is, however, still an open question. To address this, we performed our experiments *in vitro* (isometric force development) at 20 °C (acclimation temperature), as well as at 10, 15, 25 and 30 °C in isolated ventricular preparations from the catfish (*Clarias gariepinus*).

Materials and methods

Experimental animals

Catfish (*C. gariepinus*) of both sexes, weighed about 150–250 g, were kept at room temperature in glass tanks with circulating fresh water. Each experimental fish was killed by decapitation. The heart was surgically removed and immediately transferred to an ice-cold physiological solution in which ventricular strips were prepared.

Ventricular strip preparation

Pairs of strips with a maximal thickness of 1 mm were excised from the ventricle and placed into an oxygenated bathing medium containing (mM): NaCl 125, KCl 2.5, $CaCl_2$ 1.25, $MgSO_4$ 0.94, NaH_2PO_4 1, $NaHCO_3$ 15 and glucose 5. The solution was continuously bubbled with a gas mixture of 1% CO_2 and 99% O_2 by a gas mixing pump (Wöthoff 1 M 301 af, Germany) providing pH 7.8.

The preparations were suspended using surgical silk to be connected to a force transducer (Grass FT 03) placed around a platinum electrode. This electrode and another one were placed in the bath and were connected to a stimulator (Grass SD9, Quincy, MA, USA) delivering electrical square pulses with a duration of 8 ms and a voltage of 50% above the threshold value in order to provide a maximal stimulation throughout the experiment.

The stimulation frequency was 0.2 Hz (12 bpm) unless otherwise stated. Preparations were stretched to provide a twitch tension at the maximum of the length-twitch tension relation. Twitch force was allowed to stabilize for 20–30 min before experimentation.

Experimental protocol

To explore the effect of different temperatures (10, 15, 20, 25 and 30 °C) on the changes of the cardiac contractility as a result of increasing the stimulation frequency, two ventricular preparations were run in parallel at a stimulation frequency of 0.2 Hz (physiological frequency) for about 30 min. After stabilization at this frequency, the first preparation was left at 0.2 Hz to act as a control, whereas the second preparation was subjected to stepwise increases in the stimulation frequency for a period of 5 min each. The changes in the cardiac contractility (contractile force, the rate of contraction "df/dt" and the rate of relaxation "$-df/dt$") with the increase in the stimulation frequency were determined at different temperatures (10, 15, 20, 25 and 30 °C) to address the influence of different temperatures on the force–frequency relationship in the catfish heart.

Data presentation and static analysis

Results are presented as mean values ± SE. The changes imposed by the different temperatures on the cardiac contractility were normalized as a percentage (%) to that stabilized at 0.2 Hz before increasing the stimulation frequencies. In all experiments, significance levels with respect to parameters of the same experimental protocol were assessed with one way analysis of variance (ANOVA). A difference was considered significant when the P value was lower than 0.05.