Temperature dependence of cardiac sarcoplasmic reticulum and sarcolemma in the ventricle of catfish (Clarias gariepinus)

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

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

Received 16 February 2015; accepted 19 May 2015
Available online 18 June 2015

KEYWORDS
Catfish (Clarias gariepinus); Temperature; Cardiac force; Sarcolemmal Ca$^{2+}$; SR-Ca$^{2+}$ release; Frequency rate

Abstract The present study was undertaken to examine the relative contribution of the SR-Ca$^{2+}$ release and sarcolemmal Ca$^{2+}$ channels in developing the cardiac force at two different temperatures (20 and 30°C) in the catfish (Clarias gariepinus).

The sarcolemmal Ca$^{2+}$ contribution of activator Ca$^{2+}$ was greater at a test temperature of 30°C as assessed by verapamil. Whereas the SR-Ca$^{2+}$ contribution was higher at 20 and 30°C and a frequency rate of 0.2 and 0.4 Hz as assessed by caffeine and adrenaline, respectively. Bradykinin potentiating factor (BPF$_7$) which was isolated from jelly fish (Cassiopea andromeda) decreased the cardiac force developed at a frequency rate of 0.2 Hz and a temperature of 20°C, whereas it increased the force developed at frequency rates of 0.2 and 0.4 Hz at 30°C. These results indicate that BPF$_7$ may act like verapamil in reducing the cardiac force through blocking the sarcolemmal Ca$^{2+}$ channels at low temperature and like adrenaline in an increase of the cardiac force developed at warm temperature and the high frequency rate through stimulation of SR-Ca$^{2+}$ activator. Therefore, this study indicates that the sarcolemmal Ca$^{2+}$ influx and the SR-Ca$^{2+}$ release contributors of activator Ca$^{2+}$ for cardiac force development in the catfish heart were significantly greater at warm temperature and at the pacing frequency rates of 0.2 and 0.4 Hz as assessed by verapamil, adrenaline, caffeine and BPF$_7$. However, the relative contribution of the sarcolemmal Ca$^{2+}$ influx in the development of cardiac force in the catfish heart was greater than that of SR-Ca$^{2+}$ release.

ª 2015 The Egyptian German Society for Zoology. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

During cardiac excitation–contraction (E–C) coupling, Ca$^{2+}$ enters the cell through depolarization-activated L-type Ca$^{2+}$ channels as an inward current. Sarcolemmal Ca$^{2+}$ entry induces Ca$^{2+}$ release from the sarcoplasmic reticulum (SR) through activation of the ryanodine receptors or SR Ca$^{2+}$ channels and this released Ca$^{2+}$ raises the free intercellular Ca$^{2+}$ concentration which leads to contraction. For relaxation to occur, the free intercellular Ca$^{2+}$ must be removed from the cytosol either through Na$^{+}$–Ca$^{2+}$ exchange or sarcolemmal Ca$^{2+}$-ATPase (Carafoli, 1987; Bers, 2002).

In fish heart, extracellular Ca$^{2+}$ entry through L-type sarcolemmal Ca$^{2+}$ channels is generally the major source of
Ca$^{2+}$ used during cardiac contraction (Tibbitts et al., 1992; Tiitu and Vornanen, 2002). However, the SR can play an important role during cardiac E-C coupling in some fishes (Landeira-Fernandez et al., 2004; Castilho et al., 2007; El-Sayed et al., 2012).

Temperature exerts a powerful influence upon the rate of physiological processes in ectothermic teleosts, but the effects appear to be complex. Changes in environmental temperature immediately and substantially influence cardiac function in teleost fish. In most species, the body temperature closely parallels that of the environment. Subtropical and tropical environments expose fish to over-changing temperatures which provokes large adjustments of physiological and biochemical rate processes. Moreover, seasonal and acute environmental fluctuations and the temperature-dependent regulation of cardiac performance become crucial for ectothermic fish (Kalnin et al., 2009). However, the information dealing with the effect of temperature changes on cardiac Ca$^{2+}$ management and thereby the cardiac excitation–contraction coupling in tropical and subtropical fish has been rather scarce. The Nile tilapia, subtropical and tropical fish, which acclimated to 35 °C, and then submitted to acute temperature reduction to 15 °C had a higher cardiac contractility (Maricondi-Massari et al., 1998). Also, ventricular strips from Nile tilapia (Oreochromis niloticus) and the frillfin goby (Bathygobius soporator) showed that the twitch force decreased during acute increases in temperature from 25 to 35 °C in spite of such thermal transitions in the habitat (Rantin et al., 1998; Costa et al., 2000). Nonetheless, both tilapia and frillfin goby showed an increase in heart rate when temperature was actually elevated which may increase output through a higher contractility, rather than elevation in stroke volume (Kalnin et al., 2009). As a consequence, cardiac contractility in fish can be directly influenced by temperature effects on contraction and relaxation (Bailey and Driedzic, 1990). It should be mentioned that most information on the effect of changes of temperature on excitation–contraction coupling has been carried out on cold acclimated fish. For example, it has been indicated that the isometric force of in vitro perfused heart of a cold acclimated stenothermal fish burbot (Lota lota L.) was maximum at the acclimation temperature (1 °C) and declined markedly when the temperature increased to 18 °C (Tiitu and Vornanen, 2002). The catfish, Clarias gariepinus, which is a tropical fresh water fish acclimated to 35 °C (van Vliet et al., 2013) had a negative force-frequency relationship at different temperatures, 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 (0.2 Hz). This effect may be due to the difference in the handling of the activator Ca$^{2+}$ to the contractile system via the transsarcolemmal Ca$^{2+}$ channels and/or sarcoplasmic reticulum Ca$^{2+}$ release.

Thus, the present work was aimed to investigate the temperature-dependence of cardiac Ca$^{2+}$ regulation of contractility in the catfish (C. gariepinus) to test the effects of temperature changes (20 and 30 °C) and frequencies (0.2 and 0.4 Hz) upon the significance of SR-Ca$^{2+}$ release and the transsarcolemmal Ca$^{2+}$ through L-type Ca$^{2+}$ channel contributing to ventricular force development in the catfish heart. The role of the SR-Ca$^{2+}$ release was assessed using adrenaline which is known to increase the open probability of the SR-Ca$^{2+}$ channels (Bers, 1991) allowing for greater transsarcolemmal Ca$^{2+}$. Also, it has been reported that adrenergic sensitivity of the myocardium decreases with increased temperature (Graham and Farrell, 1989). Moreover, caffeine which is known as inhibitor of SR-Ca$^{2+}$ release was used to insure the contribution of SR-Ca$^{2+}$ fluxes in force development. Verapamil which is known as a blocker of L-type Ca$^{2+}$ channels (El-Sayed, 1999; El-Sayed et al., 2010) was used to investigate the contribution of the transsarcolemmal Ca$^{2+}$ channels in the regulation of the force development in the catfish heart. Bradykinin potentiating factor (BPF$_7$) which was isolated from the jelly fish (Cassiopea andromeda) (Abu-Amra and Abdel-Rahim, 2000) was used in this study to compare its effect on the catfish cardiac muscle at different temperatures and frequencies with that of adrenaline, caffeine and verapamil.

**Material and methods**

**Experimental animals**

Catfish (C. gariepinus) of both sexes weighed about 150–250 g were brought from the fish market at Sohag Governorate and kept at room temperature in glass tanks with circulating water for 2 weeks before carrying out the experiments. 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 ventricular and placed in an oxygenated bathing medium containing (mM): NaCl 125, KCl 2.5, MgSO$_4$ 0.94, NaH$_2$PO$_4$ 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 gas mixing pump (Wöthoff 1 M 301 af, Germany) providing pH 7.8.

The recording of the twitch force developed by the ventricular strips was used as El-Sayed et al. (2012).

**Drugs**

Adrenaline-tartrate (Sigma) was added from a 10 mM stock solution prepared immediately before use. Caffeine (Loba Chemie) 8.0 mM, was added as a powder. Verapamil (Sigma) was dissolved in distilled water to 10 m M/L and kept frozen (−18 °C) in suitable portions until use. Bradykinin potentiating factor (BPF$_7$) isolated from the jelly fish (C. andromeda) which were collected from two shallow water extracts discrete at 56 and 64 km, northern and southern of Qusayr City, respectively and aqueous extracts were centrifuged and the supernatant was frozen. The venom extracted factor (BPF) separated from jelly fish (BPF$_7$) was isolated, purified and detected according the method of Ferreira (1965). BPF$_7$ was dissolved in distilled water to 10 m M/L and kept frozen (−20 °C) in suitable portions until use.

**Experimental design**

**Experiment I**

To examine the influences of each adrenaline, caffeine, verapamil and BPF$_7$ on the cardiac force of the catfish heart at a
frequency rate of 0.2 and 0.4 Hz, and at 20 °C after 5 and 10 min, two independent series of experiments were carried out. The first experiment was conducted to examine the effect of adrenaline, caffeine, verapamil and BPF7 on the cardiac force of the catfish heart developed at a frequency rate of 0.2 Hz and 20 °C after 5 and 10 min. Five ventricular preparations from the catfish heart were run in parallel at a frequency rate of 0.2 Hz and 20 °C. After stabilization for about 30 min, the first preparation was subjected to 4 µM adrenaline, the second was subjected to 10 mM caffeine, the third was the twitch force after 10 min was highly significant (P < 0.001) lower than that developed at control. Moreover, the positive effect of caffeine was non-significant after 5 and 10 min were normalized (%) to that recorded at the end of the stabilization period. The above experimental protocol was reported in the second series of the experiment at the same temperature and durations, but at a frequency rate of 0.4 Hz instead of 0.2 Hz.

Experiment II

Experiment I was repeated to examine the influence of temperature at 30 °C on the cardiac force developed in the catfish heart at 0.2 and 0.4 Hz after 5 and 10 min.

Data analysis

Results are presented as mean values ± S.E. In the two 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 and highly significant when the P value was lower than 0.01.

Results

Adrenaline, verapamil, caffeine, BPF7, and cardiac contractions developed at a frequency rate of 0.2 and 0.4 Hz after 5 and 10 min at 20 °C

The influences of adrenaline, verapamil, caffeine and BPF7 on the twitch force developed after 5 and 10 min in the catfish cardiac muscle stimulated to contract at a frequency rate of 0.2 and 0.4 Hz, and at 20 °C were examined. Adrenaline caused a highly significant (P < 0.01) increase in the twitch force developed after 5 and 10 min at the stimulation frequency of 0.2 Hz, and at 20 °C, compared to that developed at control (Fig. 1). However, the positive effect of adrenaline on the twitch force after 10 min was non-significant, relative to that developed after 5 min. On the other hand, verapamil resulted in a highly significant (P < 0.001) decrease in the twitch force developed after 5 and 10 min, relative to that developed at control. But, the twitch force developed after 5.0 min was significantly (P < 0.05) higher than that developed after 10 min (Fig. 1). Like adrenaline, caffeine has a positive effect on the twitch force developed after 5 and 10 min, but the positive effect of caffeine was non-significant after 5 min and highly significant (P < 0.01) after 10 min, relative to that developed at control. Moreover, the positive effect of caffeine on the twitch force after 10 min was highly significant (P < 0.001) than that developed after 5 min (Fig. 1). The positive effect of caffeine on the twitch force developed after 5 and 10 min was significantly (P < 0.001) lower than that of adrenaline after the same periods. It should be noted that the effect of adrenaline, verapamil and caffeine on the twitch force is time-dependent at the stimulation frequency of 0.2 Hz and 20 °C.

Bradykinin potentiating factor (BPF7) which was isolated from the jelly fish (C. andromeda) caused a significant (P > 0.05) decrease in the twitch force developed after 5 and 10 min, relative to the control. The decrease in the twitch force developed after 10 min as a result of the effect of BPF7 was non-significant and lower than that developed after 10 min (Fig. 1).

The influence of adrenaline on the twitch force developed at a frequency rate of 0.4 Hz and 20 °C was similar to that recorded at a frequency rate of 0.2 Hz and the same temperature. In contrast to that recorded at a frequency rate of 0.2 Hz, verapamil and caffeine had a negative inotropic effect on the twitch force developed after 5.0 and 10 min at a frequency rate of 0.4 Hz (Fig. 1). Also, the positive effect of adrenaline on the twitch force after 10 min was significantly (P < 0.05) lower than that developed after 5 min. Furthermore, the negative effect of verapamil on the twitch force developed after 10 min was significantly (P < 0.05) higher than that developed after 5 min, and this was opposite to that recorded at a frequency rate of 0.2 Hz and 20 °C. The negative effect of caffeine on the twitch force developed after 10 min was similar to that developed after 10 min (Fig. 1). The influence of adrenaline and verapamil on the twitch force developed at a stimulation frequency of 0.4 Hz is a time-dependent like that observed at a stimulation frequency of 0.2 Hz and 20 °C.

In contrast to that recorded at a frequency rate of 0.2 Hz, BPF7 caused an increase in the twitch force developed after 5 and 10 min at a frequency rate of 0.4 Hz. Also, the increase in the twitch force developed after 10 min was higher than that developed after 5 min at the same frequency rate (Fig. 1).

Adrenaline, verapamil, caffeine, BPF7 and cardiac contractions developed at a frequency rate of 0.2 and 0.4 Hz after 5 and 10 min at 30 °C

Adrenaline, caffeine and BPF7 had a positive inotropic effect on the twitch force developed at a stimulation frequency of 0.2 Hz after 5 and 10 min at 30 °C, whereas verapamil had a negative inotropic effect on the twitch force developed at the same conditions, relative to that developed at control (Fig. 2). These results were similar to those observed at a frequency rate of 0.2 Hz and 20 °C except that BPF7 caused an increase in the twitch force developed after 5 and 10 min. However, the positive effect of adrenaline on the twitch force developed at 30 °C was significantly (P < 0.01) lower than that developed at 20 °C, whereas the positive effect of caffeine at 30 °C was highly significant (P < 0.001) and higher than that developed at 20 °C (Fig. 1). Also, the negative effect of verapamil on the twitch force developed after 5 min at 20 °C was higher than that developed at 30 °C, while it was highly significant (P < 0.001) after 10 min at 30 °C (Fig. 1).

Changes in the twitch force developed after 5 and 10 min at the stimulation frequency of 0.4 Hz at 30 °C as a result of the addition of adrenaline, verapamil, caffeine and BPF7 (Fig. 2), were similar to those observed at 0.2 Hz and the same
temperature (Fig. 2). It means that adrenaline, caffeine and BPF7 had a positive inotropic effect, whereas verapamil had a negative inotropic effect on the twitch force. However, the positive effect of adrenaline after 5 and 10 min on the twitch force developed at a frequency rate of 0.4 Hz was higher than that developed after the same periods at a frequency rate of 0.2 Hz. But, the opposite situation was observed for the positive effect of caffeine. The negative effect of verapamil on the twitch force developed after 10 min at a stimulation frequency of 0.2 Hz was higher than that developed after the same period at 20 °C, whereas the positive effect of BPF7 on the twitch force developed after 10 min at a stimulation frequency of 0.4 Hz was slightly higher than that developed at a stimulation frequency of 0.2 Hz, whereas the opposite situation was observed after 10 min (Fig. 2).

The positive effect of adrenaline and caffeine on the twitch force developed after 5 min at a frequency rate of 0.4 Hz and 30 °C was higher than that developed at the same frequency and period at 20 °C. Also, the positive effect of caffeine and BPF7 on the twitch force developed after 5 and 10 min and a stimulation frequency of 0.4 Hz at 30 °C was higher than that developed after the same periods and frequency at 20 °C, whereas the negative inotropic effect of verapamil at 30 °C was higher than that developed at 20 °C (Figs. 1 and 2).

**Discussion**

The aim of this study was to examine the specific plasticity of the ventricular contractility function to temperature in the African catfish (C. gariepinus). To assess the specific thermal

\[\text{Figure 1} \quad \text{The twitch force developed at a stimulation frequency of 0.2 and 0.4 Hz after 5.0 (A) and 10.0 (B) minutes at 20 °C in the catfish myocardium against adrenaline (■), verapamil (||), caffeine (∇) and BPF7 (∇). The number of ventricular preparation in each series was 6.} \\
\text{● Non significant as compared with control.} \quad \text{*} P < 0.05 \text{ significant as compared with control.} \quad \text{**} P < 0.01 \text{ highly significant as compared with control.} \]
constrains on its cardiac contraction, this study was conducted at different temperatures (20 and 30 °C) and at different frequencies (0.2 and 0.4 Hz). This study reports in poorly studied fresh water teleost, catfish, a significant effect of acclimation temperature upon calcium handling capacity and contractility. In this study, we showed that SR-Ca$^{2+}$ release can be an important source of activation for contraction beside L-type sarcolemmal Ca$^{2+}$ channels according to (i) temperature, (ii) pacing frequencies and (iii) time.

Previous studies have extensively examined and discussed the significance of SR-Ca$^{2+}$ release to ventricular force development in marine ectothermic animals at cold and warm temperature (Shiels and Farrell, 1997; Shiels et al., 1998, 2002; Vornanen et al., 2002; Imbert-Auvray et al., 2013). Results of these studies indicate that the role of SR is secondary to that of the sarcolemmal Ca$^{2+}$ influx in supplying Ca$^{2+}$ for the force development. Our study on the fresh water catfish showed that at 20 °C, SR contribution is involved at 0.2 and 0.4 Hz pacing frequencies with cardiac force falling by approximately 21% after caffeine treatment and cardiac force increasing by approximately 40% and 18% at 0.2 and 0.4 Hz pacing frequencies after adrenaline treatment. This is in contrast to previous studies which indicated that the SR involvement is limited to low pacing frequency (0.2 Hz) (Shiels and Farrell, 1997; Shiels et al., 1998; Imbert-Auvray et al., 2013). Furthermore, our study showed that the SR-Ca$^{2+}$ release contribution is involved at

---

**Figure 2** The twitch force developed at a stimulation frequency of 0.2 and 0.4 Hz after 5.0 (A) and 10.0 (B) minutes at 30 °C in the catfish myocardium against adrenaline (adr.), verapamil (verap.), caffeine (caff.) and BPF-7 (BPF-7). The number of ventricular preparation in each series was 6.

- Non significant as compared with control.
- *P < 0.05 significant as compared with control.
- **P < 0.01 highly significant as compared with control.
30 °C with cardiac force increasing by approximately 20% and 30% after adrenaline treatment at pacing frequency of 0.2 and 0.4 Hz, respectively. This in contrast with the previous study which stated that the SR contribution was more important to force development in the ventricular strips of sea bass at low temperature, but it is in agreement with that of common sole at high temperature (25°C). So, it can be concluded that the SR-Ca²⁺ cycling contribution to force development in the catfish heart was more important at higher frequencies, and low and high temperatures.

In fish hearts, extracellular Ca²⁺ entry through the L-type sarcolemmal channel is generally the major source of Ca²⁺ during contraction (Tibbets et al., 1992; Titu and Vornanen, 2002; Benitah et al., 2010). This seems to be in agreement with the results obtained in this study, since verapamil (blocker of the transmembrane flux of calcium via L-type Ca²⁺ channel) caused a marked reduction in the cardiac force developed at a frequency rate of 0.2 and 0.4 Hz and at 20 and 30°C in the catfish heart. In support of our results, nifedipine, which is known as L-type Ca²⁺ channel blocker caused a marked decrease in the absolute force of contraction developed at a frequency rate of 0.2 Hz and at 20°C in the ectothermic cardiac muscle (Driedzic and Gesser, 1988; Vornanen, 1989; Wasserstrom et al., 2000; El-Sayed, 2002). However, the negative inotropic effect of verapamil on the cardiac force developed at 30°C and at the two rates of frequency (0.2 and 0.4 Hz) was higher than that developed at the same rates of frequency at 20°C. Also, the negative inotropic effect of verapamil on the cardiac force developed after 10 min at the frequency rate of 0.2 and 0.4 Hz, and at 20 and 30°C was higher than that developed after 5 min. at the same frequencies and temperatures. So, these results confirm the assumption that the sarcolemmal Ca²⁺ fluxing through the L-type Ca²⁺ channels is the main source of Ca²⁺ during excitation–contraction coupling in the catfish heart. However, the information about the influence of changes in temperature and the frequency rates on verapamil action on cardiac contractions is scarce. The findings that the negative inotropic effect of verapamil on the cardiac force is higher at 30°C than that at 30°C are consistent with the observation that the L-type Ca²⁺ channels in the hearts of some ectothermic species (fishes) are temperature-dependent since the density and kinetics of the L-type Ca²⁺ channel current increased as temperature was increased, whereas it decreased as the temperature was decreased (Shiels et al., 2000). Recent studies demonstrated that the negative inotropic effect of L-type Ca²⁺ channel blocker need not be abolished by adrenaline as this agent binds irreversibly to the L-type Ca²⁺ channels. Also, at all frequencies used in the study of Methling et al. (2012), contractions were mainly supported by L-type Ca²⁺ channel influx, especially at high temperature. So, the results of this study suggest that the transsarcolemmal Ca²⁺ influx can be considered as the main source of Ca²⁺ for activation of the myofilament during cardiac contraction in the catfish, as in most fish, and also mobilization of the intercellular Ca²⁺ stores of the SR. It means that the transsarcolemmal Ca²⁺ via L-type Ca²⁺ channels is the primary source of Ca²⁺ for cardiac contraction, while SR-Ca²⁺ release is the secondary source for activator Ca²⁺. Moreover, the Ca²⁺ available for cardiac contraction through L-type Ca²⁺ channels and the SR-Ca²⁺ release is temperature, time and frequency rate-dependent. This assumption is in line with the previous studies using adrenaline, caffeine and nifedipine to assess the effect of temperature and the rate of frequency on the contribution of the sarcolemmal L-type Ca²⁺ channel fluxes and SR-Ca²⁺ release and uptake in the regulation of cardiac contractions in teleost heart (e.g. Shiels et al., 2002; Methling et al., 2012).

The findings that BPF₇ caused a slightly significant decrease in the cardiac contraction developed at a frequency rate of 0.2 Hz and 20°C, while it had a positive inotropic effect on the cardiac force developed at a frequency rate of 0.2 and 0.4 Hz at 30°C, and at 0.4 Hz and 20°C suggest that the prime action of BPF₇ may be localized on the sarcolemma at a low rate of frequency (0.2 Hz) and temperature (20°C). This may be attributed to its chemical property as a polypeptide molecule (Nassar et al., 1990) Thus, it can be concluded that BPF₇ may impair the Ca²⁺ flux through the sarcolemmal Ca²⁺ channel. In this respect its action on Ca²⁺ fluxes may resemble that of verapamil. At a higher temperature (30°C) and frequency rate (0.4 Hz), the action of BPF₇ may resemble that of adrenaline on SR-Ca²⁺ release. However, we do not at present have an explanation for the action of BPF₇ on Ca²⁺ delivered to the contractile system for cardiac contraction through the sarcolemmal Ca²⁺ channel and SR-Ca²⁺ release other than what we have stated because of very scarce information about its action on the Ca²⁺ activator for cardiac force in ectothermic and endothermic animals. So, the influence of BPF₇ on the cardiac contractions should be taken into consideration in future.

In conclusion, this study shows that, in the catfish, SR-Ca²⁺ cycling and sarcolemmal Ca²⁺ fluxes through the L-type Ca²⁺ channels are dependent on temperature, pacing frequency and time. The involvement of the SR in excitation contraction coupling was important at low temperature and low frequency rate (0.2 Hz), whereas the transsarcolemmal Ca²⁺ influx through L-type Ca²⁺ channels was more important at higher temperature and low frequency rate (0.2 Hz).

References


Temperature dependence of cardiac sarcoplasmic reticulum and sarcolemma in catfish


