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The role of sarcoplasmic reticulum and sarcolemma in the regulation of cardiac contractility at different times and higher extracellur potassium in the cardiac muscle of catfish and toad.

Mohamed F. El-Sayed; Ahmed S. Ibrahim; Hanan A. Okil and Doa'a A. Abdel-karim
Department of Zoology, Faculty of Science, Sohag University Egypt

ABSTRACT

The relative contribution of sarcoplasmic reticulum (SR) and sarcolemma in the regulation of cardiac contractility (twitch force, the rate of contraction and the rate of relaxation) at different time periods (10, 20 and 30 minuted) and at elevated extracellular potassium (5.omM) was assessed in the ventricular tissues of catfish and toad using adrenaline as an activator of $SRCa^{2+}$ uptake, caffeine as an inhibitor of $SRCa^{2+}$ uptake and verapamil as an inhibitor of sarcolemmal Ca^{2+} exchange.

Cardiac contractility (twitch force, rate of contraction "df/dt" and the rate of relaxation – "df/dt") tended to decrease with increasing of the time periods from 10 to 20 and 30 minutes in the catfish and toad ventricular tissues at 2.5 and 5.omMK^+, Elevated extracellular K^+ had a positive inotropic effect on the twitch force developed at 20 and 30 minutes in the catfish cardiac muscle, but it had non significant negative effect in the toad cardiac muscle at the same periods. The df/dt developed after 30 minutes was significantly lower in the presence of 5.omMK^+ than that developed at 2.5mMK^+ in the catfish cardiac muscle, whereas the effect of elevated K^+ on df/dt was similar to that of 2.5mMK_O at all the time periods applied in the toad cardiac muscle. Elevated K^+ had a negative inotropic effect on the-df/dt developed at all time periods applied in the catfish cardiac muscle, but it had no effect on the-df/dt developed in the toad cardiac muscle at the same time periods.

Adrenaline, like caffeine had a positive inotropic effect on the twitch force, df/dt and – df/dt developed after all the time periods applied at 2.5 and 5.omMK^+ in the ventricular tissues of both animals, relative to that of control. But, the positive inotropic effect of caffeine on the cardiac contractility was significantly lower than that of adrenaline at 2.5 and 5.omM$K^+{}_O$ and at the different time periods applied (specially after 30 minutes) for both animals. Verapamil had a significantly negative inotropic effect on the cardiac contractility developed after all time periods applied at 2.5 and 5.omMK^+ in both animals, relative to that of control. However the negative inotropic effect of verapamil on the cardiac contractility was significantly higher at elevated K^+ than at 2.5mMK^+.

The twitch force developed after all time periods applied in the toad cardiac muscle was significantly lower than that in the catfish cardiac muscle after the same periods in either 2.5 or 5.omMK^+. In the presence of adrenaline, the twitch force developed in the toad cardiac muscle after each period applied was non-significantly higher than that developed in the catfish cardiac muscle at 2.5 and 5.omMK,$^+$ whereas it was significantly higher than that of catfish cardiac muscle in the presence of caffeine at 2.5 and 5.omMK^+ after the same time periods. In the presence of verapamil, the twitch force developed after each time periods in the toad cardiac muscle was significantly and non- significantly higher than that of catfish cardiac muscle at 2.5 and 5.omMK^+ respectively. The df/dt developed after each period applied in the catfish cardiac muscle was to somewhat similar to that developed in the

toad cardiac muscle at 2.5 and 5.omMK$^+$, but in the presence of adrenaline, it was significantly lower than that of toad cardiac muscle at 2.5 and 5.omMK$^+$, whereas in the presence of caffeine, it was non-signifantly higher than that of toad cardiac muscle. In the presence of verapamil, the df/dt developed in the catfish cardiac muscle after the time period applied was significantly lower than that of toad cardiac muscle at 2.5 and 5.omMK$^+$, also. The - df/dt developed in the catfish cardiac muscle after the time periods applied was similar to that of toad cardiac muscle at 2.5mMK$^+$, but it was non-significantly lower than that of toad cardiac muscle at 5.omMK$^+$. In the presence of adrenaline, the - df/dt developed after the time periods applied in the catfish cardiac muscle was significantly lower than that of the toad cardiac muscle at 2.5 and 5.omMK$^+$, whereas the opposite situation was recorded in the presence of caffeine. In the presence of verapamil at 2.5mMK$^+$, the-df/dt developed after the time periods applied in the catfish cardiac muscle was signifantly lower than that of the toad cardiac muscle, whereas it was similar in the cardiac muscle of both animals at 5.omMK$^+$.

So, it can be concluded that the cardiac contractility in the catfish and toad ventricular tissues is time dependent. Also, it seems that the sarcolemmal Ca^{2+} exchanges partake in the regulation of cardiac contractility in the ventricular tissue of both animal But, the contribution of sarcoplasmic reticulum in the regulation of cardiac force was higher in the toad cardiac muscle than that of catfish cardiac muscle. Elevated extracellular K^+ had a positive inotropic effect on the cardiac contractility in both animals.

Keywords: Sarcoplasmic reticulum, sarcolemm, cardiac contractility, extracellur potassium, cardiac muscle, catfish and toad.

INTRODUCTION

In myocardial cell, the proposed pathways for calcium ions (Ca^{2+}) influx, activated by membrane depolarization, include the sarcolemmal calcium ion channels (Yue, 1987; Hove-Madsen and Tort, 1998) and Na^+-Ca^{2+} exchange (Fibiato, 1985; Hove-Madsen *et al.*, 2000) and intracellular space (Yue, 1992; El-Sayed and Abd El-Rahim, 2003). It is thought that cardiac force in the ectothermic hearts depends on Ca^{2+} fluxes across the sarcolemma (Hove-Madsen, 1992 and Hove-Madsen et al., 2000), while the SR appears to be of little importance. El-Sayed (2003) indicated that the transsarcolemmal Ca^{2+} fluxes and SR play a key role in the regulation of the cardiac force in the ectothermic animals. Contraction of fish's heart is thought to depend strongly on the Ca^{2+}-release from the sarcoplasmic reticulum at lower rate of stimulation frequency (0.003 Hz) (El-Sayed and Gesser, 1989; El-Sayed, 2000). Whereas at the physiological rate of stimulation frequency (0.2 Hz) and at the relatively higher frequencies (0.4 and 0.6 Hz), it was found that the cardiac contraction in fishes depend more on Ca^{2+} fluxes across the sarcolemmal Ca^{2+} channels (Coyne *et al.,* 2000; El-Sayed and Abu-Amra, 2003). The same situation had been reported, for amphibian and reptilian hearts (El-Sayed, 1995; El-Sayed, 2001; Gina *et al.*, 2006).

Verapamil (a blocker of the sarcolemmal Ca^{2+} channel) had a negative inotropic effect on the twitch force, the rate of contraction and the rate of relaxation developed at 0.2 Hz in the teleosts and amphibian cardiac muscle (El-Sayed, 1999). In mammalian ventricular myocytes 20 μM of verapamil blocked L-type Ca^{2+} current and reduced the cardiac contraction (Wasserstorm *et al*., 2000).

Adrenaline, which is known as classical, rapidly acting and non-peptide transmitter that is released in response to depolarization (Sims and Owen, 1993), affects cardiac contraction and relaxation in vertebrate animals. In fishes, adrenaline increased the cardiac contraction developed at a steady pacing rate (Gesser, 1996). This effect of adrenaline was not affected by Ryanodine and seems, therefore, the cardiac contraction not to depend on the Ca^{2+} release

channel of the SR. This situation was different when the steady state was interrupted by extrastimulations. As in mammalian cardiac tissue (Drake-Holland *et al.,* 1992), adrenaline enhanced mechanical restitution in trout atrial muscle. However, the application of higher contraction (10 mM) of adrenaline after Ryanodine led to a significantly increase in the rate of contraction and relaxation (Shiels et al., 1998). Also, adrenaline had a positive inotropic action on the cardiac contractions developed at stimulation frequencies of 0.2 Hz in the ventricular tissue of the catfish and toad (El-Sayed, 2002). The action of adrenaline on the cardiac contractility can be explained on the basis that adrenaline is the predominant catecholamine involved in the modulation of cardiac contractility (Farrell and Jones, 1992). Adrenaline stimulate the β-adrenaline receptors, causing the phosphorylation of sarcolemmal L-type Ca^{2+} channels via cyclic AMP and protein kinase A pathway (Tibbits *et al.*, 1992). This phosphorylation increases the open probability of the L-type Ca^{2+} channel (Reuter, 1983), allowing for greater transsarcolemmal Ca^{2+} influx with each depolarization (Shiels and Farrell, 1997; Li *et al.*, 2000).

Gesser and Hoglund (1988), have been noted that caffeine has a similar action on the cardiac sarcoplasmic reticulum in various vertebrate groups. Caffeine, as one of its effects, appears to block the Ca^{2+} uptake of SR not only in the mammalian myocardium but also in that of teleosts, amphibian and birds (Chapman and Miller, 1974; Busselen and Van Kerkhove, 1978; Niedergerk and Page, 1981; Nayler, 1963; Blinks *et al.*, 1972; Ibraheem, 1996; Takashima *et al.,* 1980; Cooper and Lewartowski, 1985) display positive inotropic response.

An elevation of extracellular potassium is normally considered harmful to cardiac function. In cardiac muscle, a high extracellular potassium tends to decrease the force developed upon activation i.e. the cardiac force. In fishes, the increased (K°) led to a negative inotropic effect on the cardiac force developed at 2.0 Hz (El-Sayed and Gesser, 1989). Also, it has been reported that the increased extracellular potassium significantly reduced the twitch force in amphibian and reptilian hearts (Nielson and Gesser, 2001; Kalinin and Gesser, 2002; Andersen and Wang, 2003). The finding that high (K°) had a negative inotropic effect on the twitch force appears to be due this increased extracellular potassium lowered the amount of Ca^{2+} participating in the excitation-contraction coupling (Chapman and Rodrigo, 1987). On the other hand, high extracellular potassium has a depolarized effect, which, according to the model for the Na^{+}- Ca^{2+} exchange, should cause an inward shift of Ca^{2+} with increase in cellular Ca^{2+} activity as a result. This was suggested as one possible reason for the increase in resting metabolism in isolated rat papillary muscle exposed to an increase in extracellular potassium (Holroyed *et al.*, 1990). The aim of this study was to investigate the contribution of the sarcolemmal Ca^{2+} channel and the SR in the regulation of contractility at the physiological frequency (0.2 Hz) in the cardiac muscle of the catfish (*Clarias gariepinus*) and the toad (*Bufo regularis*) at the different time periods (10, 20 and 30 minutes) and at high extracellular potassium (5.0 mM). The function of the SR and the sarcolemmal Ca^{2+} channel was assessed using adrenaline, caffeine and verapamil.

MATERIALS AND METHODS

Animals:

Catfish (*Clarias gariepinus*) weighing 200-300 g. of both sexes were kept at room temperature in tanks with recirculating fresh water. Toad (*Bufo regularis*) was kept in terraria at room temperature with the possibility to dwell in water. The fish were killed by decapitation while frog by a blow on the head. Then the heart of both animals was removed while still beating and placed in an ice-cold Ringer's physiological solution in which ventricular preparations were prepared.

Physiological solution:

The constitution of the standard physiological solution for the ventricular preparations of both animals in mM: NaCl 125, KCl 2.5, $CaCl_2$ 1.25,$MgSo_4$ 0.94, $NaHCo_3$ 15 and glucose 5. The solutions were gassed with 99% O_2 and 1% Co_2 by a gas mixing pump (Wösthoff 1M 301/af). The experimental temperature was 20±0.5

(Cole-parmer OT 286/16 USA). The resulting PH at this temperature was 7.8 for fish and toad. In some experiments the composition as to potassium was altered. The desired K^+concentration was increased by adding 1mM KCl.

Drugs:

Adrenaline-tartrate (sigma) was added from a 10mM stock solution prepared immediately before use. Caffeine (sigma) 8.0 mM was added as a powder. Verapamil (sigma) was dissolved in distilled water to 10mmol/L and kept frozen (-18c°) in suitable portions until use.

Experimental procedures:

For recording the cardiac contractility (force, rate of contraction "df/dt" and rate of relaxation "-df/dt"), a ventricular preparation with a diameter of about 0.5 mm was prepared and it was mounted for recording isometric contractions as described before (El-Sayed, 2000)

Experiment (1):

To examine the effect of adrenaline, caffeine and verapamil on the cardiac contractility (force, df/dt and –df/dt) of the catfish heart stimulated to contract at 0.2Hz and at 2.5 and 5.0 mMK^+, and after different periods (10, 20 and 30 minutes), two independent series of experiment were carried out. The first experiment was conducted to examine the effect of adrenaline, caffeine and verapamil on the cardiac contractility of the catfish at 2.5 mM K^+ and after different periods (10,20 and 30 minutes). Four ventricular preparations from the catfish heart were run in parallel at stimulation rate of 0.2Hz and at 2.5 mM K^+. After stabilization for about 30 minutes, the first preparation was subjected to 4.0µM adrenaline, the second preparation was subjected to 8.0mM caffeine, the third preparation was subjected to 10µM verapamil, whereas the fourth preparation was served as control. The changes in the cardiac contractility were recorded after 10, 20 and 30 minutes for the four preparations were normalized to that recorded at the end of the stabilization period (30 minutes). The above experimental protocol was repeated in the second series of experiment except that after stabilization at a frequency of 0.2Hz, K^+ was elevated to 5.0 mM K^+ and the ventricular preparations were left to stabilize for 15min. at the elevated K^+ concentration. The changes in the cardiac contractility were normalized to that recorded at the end of the new stabilization period (15min.).

Experiment (2):

The changes in the cardiac contractility in the toad heart as a result of the addition of 8.0µM adrenaline, 8.0mM caffeine and 10µM verapamil were also examined at 2.5 and 5.0mMK^+, and after different periods (10, 20 and 30 min.). The experimental protocol of the two independent series which carried out in the experiment (1) was repeated except that 8.0µM adrenaline was added to the first preparation instead of 4.0µM for catfish heart.

RESULTS

From the results depicted in figs 1, 2 and 3 it is seen that the twitch force, df/dt and -df/dt developed at frequency of 0.2 Hz in the catfish cardiac muscle decreased significantly with increasing of the periods from 10 to 20 and 30 minutes in 2-5 and 5-0 mMK^+. However, the decreasing in the twitch force in the presence of 2.5 mMK^+ was non-significantly higher (0.043) than that at 5.0 mMK^+ after 20 and 30 minutes respectively. Whereas df/dt and –df/dt developed in 2.5 mMK^+ were non-significantly lower than that in 5.0 mMK^+ after 20 and 30 minutes, respectively.

Adrenaline cased a significant increase in the twitch force (0.0002), df/dt (0.19) and –df/dt (0.001) developed after 10, 20 and 30 minutes in 2.5 and 5.0 mMK^+, relative to that developed after the same periods of control (Figs. 1, 2 and 3). But, the increase in the twitch force, df/dt and–df/dt developed as a result of addition of adrenalin was non-significantly lower, (0.07), significantly higher (0.001) and significantly higher in 5.0 mMK^+ than in 2.5 mMK^+ respectively (Figs. 1, 2 and 3).

Caffeine, like adrenaline, led to a significant (0.002) increase in df/dt and a significant (0.005) increase in –df/dt developed in 2.5 and 5.0 mMK^+ after 10, 20 and 30 minutes, relatively to that at control (Figs. 1, 2 and 3). However the increase in the twitch force as a result of application of caffeine was significantly higher in 5.0 mMK^+ than that in 2.5 mMK^+ after each period applied. Also the same situation was observed for df/dt and–df/dt (Figs. 1, 2 and 3).

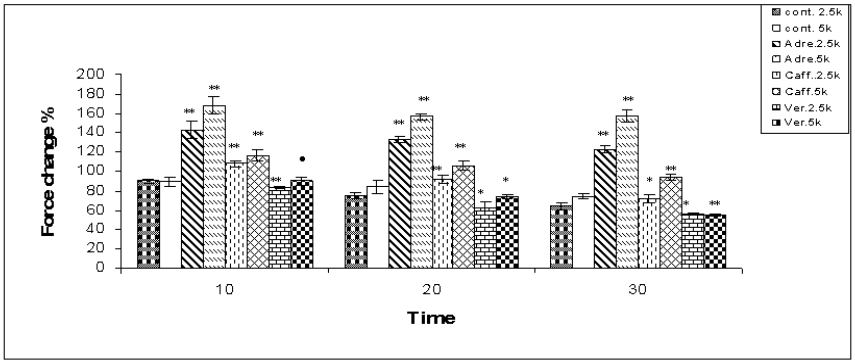

Fig (1): Change in the twitch force of catfish myocardium developed at a astimulation rate of 0.2Hz after 10, 20 and 30min. under different conditions 2.5mMK+( ), 5.0 mMK+( ), Adr. at 2.5mMK+ ( ), Adr. at 5.0mMK+( ), Caff. at 2.5mMK+( ), Caff. at 5.0mMK+( ),Ver. at 2.5mMK+( ), Ver. at5.0mMK+( ).

The number of ventricles in each series was 6
●Non significant
*P<0.05 significant
** P<0.01 highly significant as compared with control

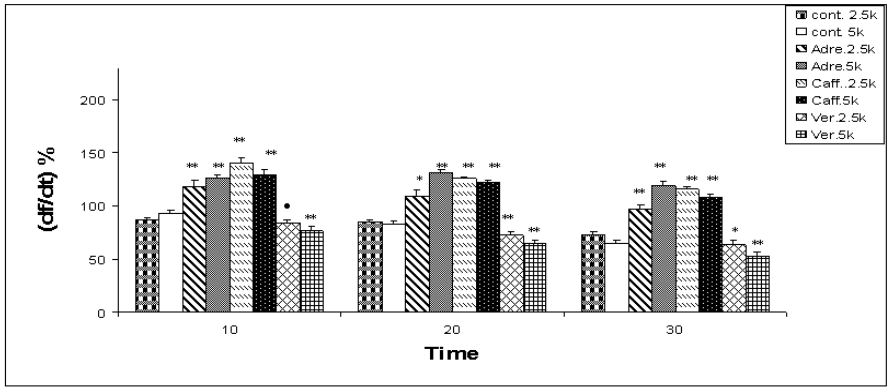

Fig (2): Change in the rate of contraction (df/dt) in the cardiac muscle of catfish developed at a stimulation rate of 0.2Hz after 10,20 and 30min. under different conditions 2.5mMK+( ), 5.0 mMK ( ), Adr. at 2.5mMK+( ),Adr**at 5.0mM K^+( ), Caff. at 2.5mMK+( ),Caff. at 5.0mMK+( ) ,Ver. at 2.5mMK+( ), Ver. at 5.0mM K^+( ).

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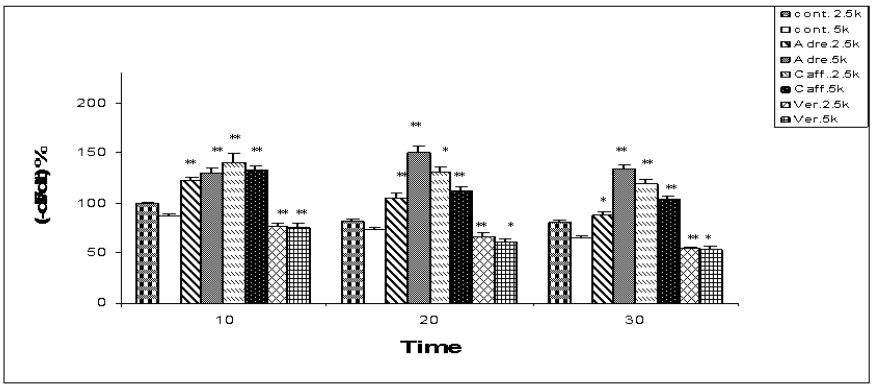

Fig (3): Change in the rate of relaxation (-df/dt) in the cardiac muscle of catfish developed at a stimulation rate of 0.2Hz after 10,20 and 30min. under different conditions 2.5mMK+( ), 5.0 mMK+( ), Adr. at 2.5mMK+( ),Adr. at5.0mMK+( ) Caff. at 2.5mMK+( ),Caff. at 5.0mM K^+( ),Ver. a2.5mMK ( ), Ver. at 5.0mMK+( ).

The number of ventricles in each series was 6
●Non significant
*P<0.05 significant
** P<0.01 highly significant as compared with control

Verapamil cased a significant decrease in the twitch force, df/dt and –df/dt developed in 2.5 and 5.0 mMK^+ after each period applied, relative to that developed after the same period of control (Figs. 1, 2 and 3). However, the decrease in the twitch force as a result of addition of verapamil was significantly higher in 2.5 mMK^+ than that in 5.0 mMK^+ after 10 and 20 minutes, but the decrease in df/dt was significantly higher in 5.0 mMK^+ than in 2.5 mMK^+ after each period applied, whereas no significant difference in -df/dt developed at 2.5 and 5.0 mMK^+ could be documented (Figs. 1, 2 and 3).

As in the catfish cardiac muscle, the twitch force, df/dt and–df/dt developed at a physiological frequency in the toad cardiac muscle in 2.5 and 5.0 mMK^+ tended to decrease with increasing of the time period from 10 to 20 and 30 minutes (Figs. 4, 5 and 6). However, the decreasing in the twitch force was significant, but the decreasing in df/dt and –df/dt were non-significant with the increasing of the time periods from 10 to 20 and 30 minutes in 2.5 and 5.0 mMK^+ also. Moreover, the twitch force, df/dt and –df/dt developed in 2.5 mMK^+ at 10, 20 and 30 minutes did not change too much from that developed at the same periods in 5.0 mMK^+ (Figs. 4, 5 and 6).

Adrenalin and caffeine caused a significantly increase in the twitch force, df/dt and – df/dt developed after 10, 20 and 30 minutes in 2.5 and 5.0 mMK^+ , relative to that developed after the same periods at control (Figs. 4, 5 and 6). The increasing in the twitch force developed after 30 minutes in 5.0 mMK^+ as a result of addition of adrenaline was significantly lower than that developed after the same period in 2.5 mMK^+, whereas the increasing in the twitch force developed after 10, 20 and 30 minutes in 5.0 mMK^+ as a result of addition of caffeine was significantly lowe than that developed after the same periods in 2.5 mMK^+ (Fig. 4). The increasing in df/dt and –df/dt developed after 10 and 20 minutes in 5.0 mMK^+ as a result of addition of adrenaline was significantly higher than that developed after the same periods in 2.5 mMK^+ , whereas it was significantly lower in 5.0 mMK^+ than that in 2.5 mMK^+ after 30 minutes (Figs. 5 and 6). The increase in df/dt developed in 2.5 mMK^+ after 30 minutes as a result of addition of caffeine was significantly lower than that in 5.0 mMK^+ after the same periods, while the df/dt developed in 2.5 mMK^+ was significantly higher than that developed in 5.0 mMK^+ after 30 minutes also, (Figs. 5 and 6).

Verapamil led to significant increase in the df/dt and –df/dt developed after 10, 20 and 30 minutes in 2.5 and 5.0 mMK+ , relative to that developed after the same periods at control (Figs. 5 and 6). However, verapamil caused a significant increase in the twitch force developed after each period applied in 2.5 mMK^+ and significant decrease after 30 minutes in 5.0 mMK+ (Fig. 4). The decreasing in the twitch force, df/dt and –df/dt developed after 10, 20 and 30 minutes in 5.0 mMK^+ as a result of addition of verapamil was significantly lower than that developed in 2.5 mMK^+ after the same periods (Figs 4, 5 and 6).

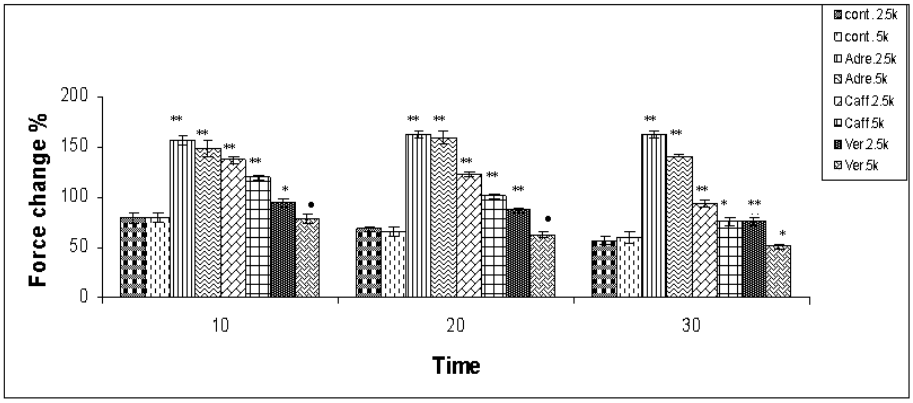

Fig (4): Change in the twitch force of toad myocardium developed at a stimulation rate of 0.2Hz after 10,20 and 30min. under different conditions 2.5mMK+ ( ), 5.0 mMK+( ), Adr. at 2.5mMK ( ), Adrat 5.0mMK+( ), Caff. at2.5mMK+( ), Caff. at 5.0mMK+( ) ,Ver. at 2.5mMK+ ( ), Ver. at 5.0mMK+( ) .

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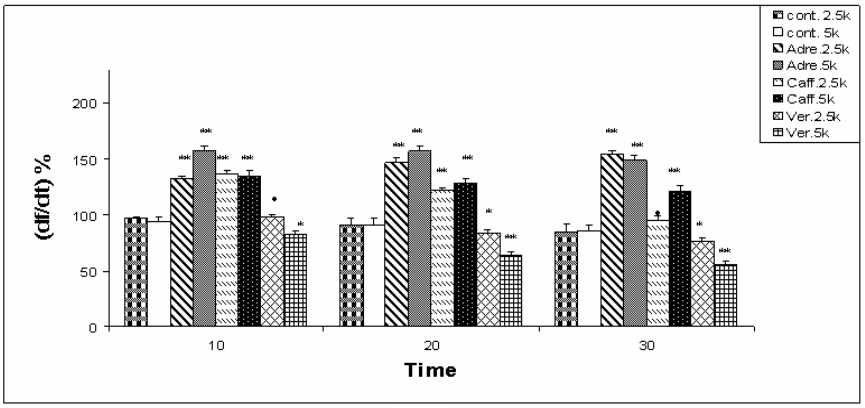

Fig (5): Change in the rate of contraction (df/dt) in the cardiac muscle of toad developed at a stimulation rate of 0.2Hz after 10,20 and 30 min.und different conditions 2.5mMK+( ),5.0 mMK+( ), Adr. at 2.5mMK+( ),Adr. at 5.0mMK+( ), Caff. at 2.5mMK ( ), Caff. at 5. 0mMK+ ( )Ve at 2.5mMK+( ), Ver. at 5.0mMK+( )

The number of ventricles in each series was 6
●Non significant
*P<0.05 significant
** P<0.01 highly significant as compared with control

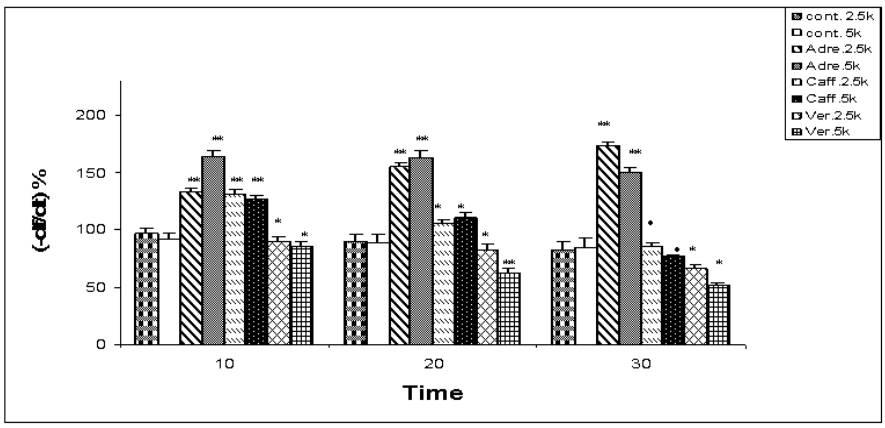

Fig (6): Change in the rate of relaxation (-df/dt) in the cardiac muscle of toad developed at a stimulation rate of 0.2Hz after 10,20 and 30min. under different conditions 2.5mMK+( ),5.0 mMK ( ), Adr. at 2.5mM K^+( ),Adr. at 5.0mMK+( ), Caff. at 2.5mMK+( ), Caff. at 5.0mMK+( ),Ver. at 2.5mMK+( ), Ver. at 5.0mMK+( ) .

The number of ventricles in each series was 6
●Non significant
*P<0.05 significant
** P<0.01 highly significant as compared with control

DISCUSSION

All the experimental protocols used in this study revealed that the twitch force, df/dt and-df/dt developed in the cardiac muscle of catfish and toad in 2.5 and 5.0 mM K^+ decreased with increasing of the time periods from 10 to 20 and 30 minutes. Also, adrenaline and caffeine had a positive inotropic effect on the twitch force, df/dt and –df/dt at all the time periods applied in 2.5 and 5.0 mM K^+ in both animals, but the positive inotropic effect of adrenaline and caffeine on the cardiac contractility was pronounced at 5.0 m M K^+. Moreover, verapamil had a negative inotropic effect on the cardiac contractility developed in the cardiac muscle of both animals at 2.5 and 5.0 mM K^+ after all the time periods applied, whereas negative inotropic effect of verapamil on the cardiac contractility developed in the cardiac muscle of toad after all the time periods applied in 5.0 mM K^+ was higher than that developed in 2.5 mM K^+ In lower vertebrates, the SR has generally been considered a poorly developed organelle of minor importance in the regulation of cardiac contractility (Driedzic and Gesser, 1994; Tibbits *et al.*, 1992). This is based mainly on the morphological and ultrastructural studies showing that the myofibers from lower vertebrates are long, cells lacking T-tubules and with an SR that is poorly developed compared with mammals (Santer 1985; Vornanen, 1992 and Vornanen *et al*., 1994). Thus, it seems that in lower vertebrates, the involvement of SR Ca^{2+} cycling in E–C coupling is of little importance (Shiels *et al*., 1999; Shiels and Farrell, 2000), whereas Ca^{2+} cycling across the sarcolemma is probably sufficient to initiate the contractility. Furthermore, in the absence of a functional SR, the sarcolemmal Ca^{2+} exchanges will become the primary transport mechanism for cardiac contractility in the lower vertebrates El-Sayed and Gesser, 1989; Gina *et al*, 2006). So, the relative contribution of the SR Ca^{2+} cycling and the sarcolemmal Ca^{2+} exchange in the E-C coupling in the ectothermic vertebrates varied with the stimulation frequency and tissue type, and appeared to correlate with maximal *in vivo* heart rates (Driedzic and Gesser, 1988;El-Sayed and Gesser, 1989; and 1994; Galli *et al*., 2006).

The present study showed that the cardiac contractility (twitch force, df/dt and –df/dt) developed at a physiological frequency (0.2 Hz) in the myocardium of the catfish and toad after 10, 20 and 30 minutes) tended to decrease with increasing of time. However, the decreasing in the cardiac contractility was gradual when the interval between the paced frequencies was 5 minutes. But, the decrease in the cardiac contractility was marked when the interval between the used frequencies was extended to 10 or 15 minutes. Also, the decrease in the cardiac contractility of the toad myocardium was higher than that in the catfish myocardium after the periods applied. So, it can be speculated that the cardiac contractility in the catfish and toad hearts is time-dependent. As the time between contractions is reduced decreased the diffusion distance for Ca^{2+} between the sarcolemma and contractile apparatus possibly increasing the impact of sarcolemmal Ca^{2+} influx. Thus Ca^{2+} cycling across the sarcolemma is probably sufficient to initiate contraction due to the large surface area of the sarcolemma of cardiac myocytes in the fish and amphibia relative to volume (Gina *et al*, 2006). So, in the catfish and a toad hearts the sarcolemmal Ca^{2+} exchanges may be important for Ca^{2+} transport and therefore for the regulation of cardiac contractility and the cardiac contractility in these animals is time-dependent.

It has been reported that increased extracellular potassium ($K_\circ$) significantly reduced the twitch force in ectothermic vertebrates (El-Sayed and Gesser, 1989; Nielsen and Gesser, 2001). However, the decrease in the twitch force in turtle ventricular preparations were modest even the ($K_\circ$) was elevated to 10 mM. But, similar levels of ($K_\circ$) almost abolish ventricular twitch force in trout (Nielsen and Gesser, 2001). In contrast to these results, increased ($K_\circ$) to 5.0 mM in the present study tend to have less effect on the cardiac contractility in the ventricular tissue of catfish and toad. It means that the effect of increased ($K_\circ$) on the cardiac contractility was similar and / or non-significant lower than that of 2.5 mMK^+. Evidence exists that an increased ($K_\circ$) by its depolarizing effect should tend to increase cellular Ca^{2+} activity via Na^+-Ca^{2+} exchange

in contracted and quiescent cardiac cells (Kalinin and Gesser, 2002). Therefore, and unlike what has been suggested for other ectothermic vertebrates (Nielsen and Gesser, 2001) high ($K_\circ$) should not reduce the cardiac contractility by lowering the amount of Ca^{2+} participating in the electro-mechanical coupling, but increase the energy demand of the cellular Ca^{2+} handling relative to that associated with the actin-myosin interaction. This seems to be true also for the catfish and toad hearts, since the cardiac contractility developed in 2.5 and 5.0 mMK^+ was similar.

The ventricular strips from the two species used in this study were highly affected by adrenaline (activator of SR function). Adrenaline led to a highly significant increase in the cardiac contractility (force, df/dt and –df/dt) of both species after each periods applied, relative to that of control. Also and as previously observed in the absence of adrenaline, the increase in the cardiac contractility decrease with the time in 2.5 and 5.0mMK^+. These findings are in agreement with the study of Gesser *et al*., (1982) who reported that adrenaline increased the contractile force in the trout ventricular tissue paced to contract at stable pacing rate of 0.2 Hz. Also, in mammalian heart (guinea pig papillary muscle), stimulated to contract at stable frequency, adrenaline caused an increase in the myocardial force (Drake-Holland *et al.*, 1992; New *et al.*, 2000). In the catfish and toad ventricular tissue which paced to contract at a steady state frequency of 0.2 Hz and 2.5 mMK^+, adrenaline led to highly significant increase in the cardiac contractility and time to peak tension (El-Sayed and Abu-Amra, 2001). It has been suggested that adrenaline enhances the sequestration of Ca^{2+} from the myocardial proteins into the SR and this will increase the amount of calcium re-circulated within and thereby increases the cardiac contractility (Drake-Holland *et al*., 1992). Adrenaline may enforce this effect by elevating the ventricular volume range through different mechanisms. It enhances the rate of relaxation (-df/dt) (Bers, 1991; Nielsen and Gesser, 2001) whereby the period of ventricular filling should be prolonged and end-diastolic volume enlarged. This seems to be also true for the catfish and toad ventricular tissue, since the rate of relaxation developed after each period applied in this study was lower than that of the rate of contraction after the same periods.

The present data shows that the rate of contraction and the rate of relaxation developed after different times in both species were higher in 5.0mMK^+ than that in 2.5 mMK^+. However, the rate of relaxation was lower than the rate of contraction in 2.5 and 5.0 mMK^+. Hence, the elevated ($K_\circ$) did not have a negative inotropic effect on the cardiac contractility even in the presence of adrenaline. These findings appears to be in contrast with the study of Nielsen and Gesser (2001) who reported that adrenaline was found to alleviate the inhibitory action of an elevation of ($K_\circ$) on trout and turtle myocardium. So, it can be speculated that ($K_\circ$), again, by its depolarizing effect should tend to increase cellular ca^{2+} activity via Na^+ - Ca^{2+} exchange, and this Ca^{2+} which fluxes through the sarcolemma induce the Ca^{2+} release from the SR as a result of the action of adrenaline which activate the Ca^{2+} in the SR and thereby handle this calcium to the myofilaments which lead to increase the cardiac contractility. Unlike to most of ectothermic species SR in the ventricular tissues of catfish and toad hearts may be participate in the regulation of the cardiac contractility (Nielsen and Gesser, 2001). In the present study, the finding that cardiac contractility in the toad ventricular tissue was significantly higher in the presence of adrenaline than that of catfish ventricular tissue can be explained on the assumption that the SR of the toad may be contribute efficiently in the regulation of cardiac contractility than that of the catfish. It means that the SR of the toad heart may be more developed than that of the catfish.

Caffeine, like adrenaline significantly enhanced the cardiac contractility developed after different periods of time in the catfish and toad hearts. These results are in agreement with the study of Coyne *et al*. (2000) who reported that caffeine increased the cardiac force developed at physiological frequency in rainbow trout heart. Evidence exists that Ca^{2+} is transported primarily from the extracellular space for the activation of contraction in toad ventricular tissue, and there is no trigger release of internal stores (SR) of recirculation of sequestered Ca^{2+} (Klitzner and Morad, 1983). Also, it has been

reported that caffeine, which is usually used to demonstrate the rate of contribution of the SR in the cardiac excitation-contraction coupling increases the sarcolemmal Ca^{2+} influx (Bers, 1985), enhances the Ca^{2+} entry and also myofilaments Ca^{2+} sensitivity (Wendt and Stephenson, 1983), while it decreases the SR Ca^{2+} release (Bers, 1989). Thus, the effects of activation are the net effect of at least these three single effects. So, the positive effects transiently offsetting the negative inotropic effect of caffeine on the SR–Ca^{2+} uptake and release. This explanation seems to be true with the results obtained in the present study for catfish and toad hearts since caffeine had a positive inotropic effect on the twitch force, df/dt and –df/dt developed after different periods.

The present data show that the cardiac force, df/dt and -df/dt developed after different periods in the catfish myocardium in the presence of caffeine and 2.5 mMK^+ was significantly higher than that developed in the toad myocardium after the same periods and in the presence of the same treatments. However, the situation is the exact opposite in the presence of 5.0 mMK^+, since it had been found that the cardiac contractility developed after different periods in the presence of caffeine and 5.0 mMK^+ was significantly lower than that developed after the same periods in the toad heart. Here, it must pointed out that the information about the effect of extracellular K^+ in the presence of caffeine on the cardiac contractility in the ectothermic vertebrates is scarce. Thus, the lowering positive effect of caffeine in the presence of 5.0mMK^+ on the cardiac contractility developed in the catfish heart compared with that of toad heart at the same situation could be attributed to the assumption that elevations of extracellular K^+ shift the resting membrane potential to more positive values and shorten the duration of action potential. This shorting of the action potential may be associated with a decrease in the Ca^{2+} transient activating contractility, since action potential duration determines the time available for Ca^{2+} to enter the cell through the L-channels. Furthermore, a study on toad myocardial cells suggests that the Ca^{2+} enters the cell not only via the L-channels but also via Na^+-Ca^{2+} exchange during the plateau phase of action potential (Fan *et al*., 1996).

Consistent with the previous studies on amphibian and teleost ventricular tissues, verapamil had a negative inotropic action on the cardiac contractility developed after the time periods applied in the presence of 2.5 and 5.0mMK^+ in the ventricular preparations of catfish and toad hearts (El-Sayed, 1999; El-Sayed 2000 and 2002). This negative intropic action of verapamil can be attributed to a decrease in cytosolic Ca^{2+} concentration. Verapamil impaired the transport of extracellular Ca^{2+} via the cell membrane by inhibiting the sarcolemmal Ca^{2+} channels (Devlin, 1993). So, the levels of the transsarcolemmal Ca^{2+} which is necessary to activate the cardiac contractility declines and thereby caused a decrease in the cardiac contractions. Evidence exist that verapamil, like other calcium channel blockers has an effective protection against cell damage during myocardial ischemia (Smith *et al*., 1976). In mammalian ventricular tissues, verapamil blocked L-types Ca^{2+} channel and reduced the cardiac contraction (Wasserstrom *et al*., 2000). Also, in rat cardiomyocytes, verapamil blocked completely the contractility (Estevez *et al*., 2000). Moreover in a study to examine how prolonged exposures (24–72 h) to modifiers of Ca^{2+} transsarcolemmal transport affect the cardiomyofibillar structure and myofibillar proteins in the adult guinea-pig ventricular myocytes. Verapamil 10 mM produced minor changes in myofibillar structure and proteins profiles (Harackova *et al*., 2000). These reactions are believed to reflect an involvement of the sarcolemmal Ca^{2+} channels in the regulation of the cardiac contractions in ectothermic and endothermic vertebrates. However, the contribution of the sarcolemmal Ca^{2+} channels in the cardiac contractions varies between ectothermic and endothermic vertebrates (Tibbits *et al*., 1991; Driedzic and Gesser, 1994; El-Sayed, 2000). In ectothermic vertebrates, it has been claimed that the sarcolemmal Ca^{2+} channel mediates the influx of calcium from the extracellular space and this calcium may activate the contractile proteins to develop the contractions. In teleost and amphibian hearts, the calcium necessary to support contractility is derived from the extracellular space (El-Sayed, 2000). The findings that verapamil, in the present study, caused a significantly decrease in the cardiac

contractility developed in the presence of 2.5 and 5.0 mMK^+ after different periods, relative to the control at the same conditions in the catfish and toad hearts indicates that the sarcolemmal Ca^{2+} channels have a role in the regulation of the cardiac contractility of both species. However, the decrease in the cardiac contractions as the effect of verapamil was pronounced in the catfish than that in the toad heart.

So, it can be suggested that the transsarcolemmal Ca^{2+} fluxes play a significant role in the regulation of the cardiac contractions in the catfish than that in the toad heart. Furthermore, the data in the present study revealed that the decrease in the cardiac contractions increased with the time. Thus, it can be speculated that the influence of verapamil on the cardiac contractions in both species is time–dependent.

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ARABIC SUMMARY

دور الصفيحه اللحمية والغشاء الخلوى فى تنظيم الضربات القلبية فى العضلة القلبية لكل من سمكة القرموط والتودة عند فترات زمنية مختلفة وتركيز عال لأيون البوتاسيوم المضاف خارجيا

محمد فرج السيد – أحمد سلمى إبراهيم – حنان على عقيل – دعاء عباس عبد الكريم
قسم علم الحيوان - كلية العلوم – جامعة سوهاج

لقد صممت هذه الدراسة لاستبيان دور الصفيحة اللحمية والغشاء الخلوى فى تنظيم الضربات القلبية فى أنسجة البطين لكل من سمكة القرموط والتودة عند فترات زمنية مختلفه (١٠ ، ٢٠ ، ٣٠ دقيقة) وعند التركيز العالى من البوتاسيوم (٥ مللى جزي جرام) المضاف خارجيا ٠ ولقد استعمل الأدرينالين كمحفز للكالسيوم الماخوذ و المفقود خلال الصفيحة اللحميه والكافيين كمثبط للكالسيوم الماخوذ و المفقود خلال الصفيحة اللحمية والفيراباميل كمثبط للكالسيوم المتبادل خلال الغشاء الخلوى وقد تبين أن:-

- الضربات القلبية (قوة الضربة) معدل الأنقباضة ، ومعدل الأنبساط ، يميل الى النقصان مع زيادة الفترات الزمنية من ١٠ الى ٢٠ و٣٠ دقيقة فى بطين كل من سمكة القرموط والتودة عند تركيزى البوتاسيوم (٥ر٢ ، ٥ مللى جزئ جرام) ٠ التركيز المرتفع من البوتاسيوم كان له تأثيراً ايجابياً على قوة الضربة التى نمت عند ٢٠ و ٣٠ دقيقة فى العضلة القلبية لسمكة القرموط بينما هذا التركيز من البوتاسيوم كان له تأثيراً سلبياً غير معنوى على العضله القلبية للتودة عند نفس الفترات الزمنية أما معدل الانقباضة التى نمت بعد ٣٠ دقيقة فى وجود التركيز العالى من البوتاسيوم كانت أقل معنويا عنها عند تركيز ٥ر٢ مللى جزئ جرام من البوتاسيوم فى العضلة القلبية لسمكة القرموط بينما كانت متشابهة فى العضلة القلبية للتودة عند تركيزى البوتاسيوم وعند كل الفترات الزمنية التى المطبقة. أما التركيز العالى من البوتاسيوم قد أحدث تأثيرا سليبا على معدل الانبساط الذى نما عند كل الفترات الزمنية المطبقة فى العضلة القلبية لسمكة القرموط بينما لم يحدث أي تأثير للعضلة القلبية للتودة عند نفس الفترات الزمنية ٠
- الأدرينالين مثل الكافيين كان لهما تأثيرا ايجابيا على الضربات القلبية عند كل الفترات الزمنية التى طبقت وعند تركيزى البوتاسيوم فى بطين سمكة القرموط والتودة ، مقارنة بتلك التى سجلت للمجموعات الضابطة ٠ ولكن التأثير الايجابى للكافيين على الضربات القلبية التى سجلت بعد الفترات الزمنية المطبقة وعند تركيزى البوتاسيوم كانت أقل معنويا عن تلك المسجلة للادرنالين بعد نفس الفترات و عند تركيزى البوتاسيوم لكلا الحيوانين
- الفيراباميل قد أحدث تأثيرا سليبا على الضربات القلبية لكلا الحيوانين بعد كل الفترات الزمنية المطبقة وتركيزى البوتاسيوم مقارنة بتلك المسجلة للمجموعات الضابطة بينما كان التأثير السلبى للفيراباميل على الضربات القلبية أعلى معنويا عند ٥ مللى جزئ جرام من البوتاسيوم عنه عند ٥ر٢ مللى جزئ جرام بوتاسيوم ٠
- الضربة القلبية التى نمت بعد كل الفترات الزمنية المطبقة وعند تركيزى البوتاسيوم فى العضلة القلبية فى التودة كانت أقل معنويا عنها فى سمكة القرموط عند نفس الظروف واما فى وجود الأدرنالين كانت الضربة القلبية للتودة أعلى غير معنويا عنها لسمكة القرموط عند كل الفترات الزمنية وكذلك عند تركيزى البوتاسيوم بينما كانت تلك الضربة أعلى معنويا لقلب سمكة القرموط عنها للتودة فى وجود الكافيين ٠ وفى وجود الفيراباميل كانت تلك الضربة أعلى معنويا وغير معنوية فى قلب التودة عنها لسمكة القرموط عند ٥ر٢ ، ٥ مللى جزئ جرام من البوتاسيوم على التوالى ٠
- معدل الانقباضة التى نمت عند تركيزى أيون البوتاسيوم و بعد كل فترة زمنية في العلة القلبية لسمكة القرموط كانت متشابهة إلى حد ما مع تلك المسجلة للتودة عند نفس الظروف ، و لكن في وجود الأدرينالين كانت معدل الأنقباضة أقل معنوياً في قلب سمكة القرموط عنها في التودة عند تركيزي البوتاسيوم بينما في وجود الكافيايين كانت أعلى غير معنوياً عنها في قلب التودة أما في وجود الفيراباميل كانت معدل الأنقباضة التي نمت في قلب سمكة القرموط أقل معنوياً عنها في قلب التودة عند تركيزي البوتاسيوم.
- معدل الأنبساط في قلب سمكة القرموط بعد كل الفترات الزمنية المطبقة كان مشابهاً لتلك التي سجلت لقلب التودة عند تركيز ٢.٥ مللي جزئ جرام من البوتاسيوم و لكنها كانت أقل غير معنوياً عنها بقلب التودة عند تركيز ٥ مللي جزئ جرام من البوتاسيوم و لكن في وجود الأدرينالين كان معدل الأنبساط الذي نما بعد كل الفترات الزمنية المطبقة في قلب سمكة القرموط أقل معنوياً عنها في قلب التودة عند تركيزي ٢.٥ و ٥.٠ جزئ جرام من البوتاسيوم بينما كان الوضع مختلفاً في وجود الكافايين. أما في وجود الفيراباميل كان معدل الأنبساط الذي نما في قلب سمكة القوموط أقل معنوياً عنه في قلب التودة عند تركيز ٢.٥ ملل جزئ جرام من البوتاسيوم بينما معدل الأنبساط في قلب التودة كان مشابهاً لذلك في قلب سمكة القرموط عند ٥ مللي جزئ جرام من البوتاسيوم.
- و على ذلك يمكن أستخلاص أن الضربات القلبية لبطين سمكة القرموط و كذلك التودة يعتمد على عامل الوقت بين كل فترة زمنية و أخرى. كذلك بيدو أن الكالسيوم المتبادل خلال الجدار الخلوي يشارك في تنظيم الضربات القلبية لكلا الحيوانين و لكن مشاركة الصفيحة اللحمية في تنظيم الضربات القلبية كانت أعلى في قلب التودة عنها في سمكة القرموط و التركيز العالى لأيون البوتاسيوم كان له تأثيراً إيجابياً على الضربات القلبية في قلب كلا الحيوانين.