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CARDIAC CONTRACTILITY: EFFECTS OF ADRENALINE, CAFFEINE AND VERAPAMIL ON THE HEARTS OF FISHES AND AMPHIBIANS AT DIFFERENT FREQUENCIES

(With 2 Tables and 7 Figures)

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الانقباضات القلبية:تاثير الأدرنالين والكافيين والفيراباميل عند ترددات مختلفة في قلب كل من الأسماك والبرمائيات

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لقد تم دراسة التأثير المتراكم للأدرنالين (عند تركيز من ١إلى ١٠ميكروجرام لكل جزيء) على قلب كل من سمك القط (القرموط والضفدعة الرقطاء الذى حفز لكي ينقبض عند معدل من الضربات المختلفة (٤٨،٣٦،٢٤،١٢ ضربة لكل دقيقة) كذلك تم دراسة تأثير ٤،٨ ميكروجرام لكل جزيءمن الأدرنالين على قلب كل من سمك القط والضفدعة على التوالى ،و ٨ ميللي جرام لكل جزيء من الكافيين و١٠ ميكروجرام لكل جزيء فيراباميل على قوة الضربة ومعدل الأنقباض ومعدل الأنبساط وكذلك الوقت اللازم للضربة لكى تصل إلى قمتها وكان ذلك لتوضيح الدور الذي تقوم به الصفيحة اللحمية وقنوات الكالسيوم الموجودة على الجدار الخلوى فى تنظيم الضربات القلبية لكل من سمك القط والضفدعة الرقطاء . زيادة على ذلك تم دراسة تأثير ٢,٥ميللى جزيء لكل جرام من الكالسيوم على هذه المتغيرات القلبية وقد تبين من هذه الدراسة أن الأدرنالين قد أحدث تأثيراً إيجابياً على قوة الضربة القلبية يصل إلى أقصاه عند تركيز ٤ ، ١ ، ٣ ، ٣ ميكروجرام لكل جزئ في قلب سمكة القرموط بينما كان عند تركيز ٨ ، ٣ ، ١ ، ٣ ميكروجرام لكل جزئ في قلب الضفدعة وذلك عند معدل ضربات ١٢ ، ٢٤ ، ٣٦ ، ٤٨ ضربة لكل دقيقة لكلا الحيوانين على التوالي ، الفيراباميل قد أحدث تأثيراً سلبياً على قوة الضربة القلبية في قلب كل من القرموط والضفدعة. وهذا التأثير السلبي قد وصل إلى أقصاه عند تركيز ١٠ ميكروجرام لكل جزئ عند كل معدلات الضربات التي طبقت في هذه الدراسة ، والأدرنالين (٤ ميكروجرام لكل جزئ) قد أحدث تأثيراً إيجابياً على قوة الضربة ومعدل الانقباض ومعدل الانبساط وكذلك الوقت اللازم للضربة كي تصل إلى قمتها عند كل معدلات الضربات المطبقة في قلب القرموط بينما كان تركيز الأدرنالين الذي أحدث نفس التأثير في قلب الضفدعة هو ٨ ميكروجرام لكل جزئ ولقد وصل هذا التأثير الإيجابي في قلب القرموط عند معدل ضربات ١٢ ضربة لكل دقيقة و٢٤ ضربة لكل دقيقة في قلب الضفدعة.

والكـــافيين (٨ ميللي جزئ لكل جرام) قد أحدث نفس التأثير الإيجابي الذي أحدثه الأدرنالين عـــلى كل المتغيرات في الضربات القلبية التي تمت عند كل المعدلات الترددية في قلب كلا الحيوانيـــن ولكـــن هذا التأثير الإيجابي للكافيين أعلى من تأثير الأدرنالين في قلب الضفدعة وليـــس في قلب القرموط. والفيراباميل (١٠ ميكروجرام لكل جزئ) كمثبط لقنوات الكلسيوم فـــي الغشاء الخلوي قد أحدث تأثيراً سلبيا على الضربات القلبية في كلا الحيوانين وقد وصل هذا التأثير السلبي أقصاه عند معدل ضربات ٤٨ ضربة لكل دقيقة. وزيادة تركيز الكالسيوم مـــن ١,٢٥ إلى ٢,٥ ميلـــلي جرام جزئ أدى إلى زيادة التأثير الإيجابي لكل من الأدرنالين والكـــافيين على الضربات القلبية بينما لم يؤدي إلى إزالة التأثير السلبي للفيراباميل عند كل المعـــدلات المطبقة وذلك مقارنة بمعدل الضربات عند ١٢ ضربة لكل دقيقة قبل إضافة أي مؤثـــرات فـــي كلا الحيوانين. وعلى ذلك يمكن الاستنتاج أن قنوات الكلسيوم الموجودة في الغشاء الخلوي في قلب كل من القرموط والضفدعة يبدو أنها تدعم الضربات القلبية عند كل المعـــدلات الترددية التي اختبرت في هذه الدراسة . علاوة على ذلك فإن النتائج المستخلصة من هذه الدراسة تبين أن مشاركة قنوات الكلسيوم أكثر فعالية في تنظيم الضربات القلبية في قلب الضفدعة منها في قلب سمكة القرموط.

SUMMARY

The cummulative effect of adrenaline (1.0 to 10μM) was studied on isolated heart ventricular preparation from catfish (*Clarias gariepinus*) and frog (*Bufo regularis*) after their electrical stimulation at different frequencies (0.2, 0.4, 0.6 and 0.8 Hz). The effect of adrenaline (4μM/ L for fish heart and 8 μM/ L for frog heart), caffeine (8mM) and verapamil (10 μM/L) on the contractile variable (contractile force; rate of contraction, df/dt; rate of relaxation,- df /dt and time to peak tension, TPT) were studied to investigate the role of Ca^{++} channels and the sarcoplasmic reticulum (SR) in the regulation of the cardiac variables. Furthermore, the effect of 2.5 extracellular Ca^{++} on these cardiac variables was also, studied. Relative to the stimulation rate of 0.2 Hz adrenaline had a positive inotropic effect on the cardiac force of both animals. The positive inotropic effect of adrenaline on the contractile force reached its maximal effect at 4, 1, 3 and 7 μM/L in the catfish and at 7, 3, 5 and 3 μM/L at 0.2, 0.4, 0.6 and 0.8 Hz in the frog heart. Verapamil had a negative inotropic effect on the cardiac force of both animals and it reached its maximum effect at 10 μM/L at the all stimulation frequencies applied. Adrenaline, 4 μM/L for the fish heart and 8 μM/L for the frog heart had a positive inotropic effect on the contractile variables (contractile force; rate of contraction, df / dt; rate of relaxation, - df/ dt and time to peak tension,TPT) in both animals at all

the stimulation rates. It reached its maximal effect at a frequency of 0.2 Hz and 0.4 Hz in the fish and the frog myocardium, respectively. Caffeine (8.0 mM), also, had a positive inotropic effect, like that of adrenaline, on the contractile variables developed at the different frequencies in the myocardium of both animals. In the frog but not in the fish heart, the positive inotropic effect of caffeine on the contractility was higher than that of adrenaline. Relative to the stimulation rate of 0.2 Hz, verapamil (10 μM/L), a specific inhibitor of the transarcolemmal Ca^{2+} channel, had a negative inotropic effect on the cardiac contractility of both animals and it reached its maximal effect at a stimulation frequency of 0.8 Hz. Increasing of extracellular Ca^{2+} from 1.25 to 2.5 mM increased the positive inotropic effect of adrenaline and caffeine on the cardiac contractility but it did not remove the negative inotropic effect of verapamil on the contractility developed at the stimulation rates, relative to that developed at a frequency of 0.2 Hz before addition of any treatments. Thus, the cardiac sarcolemmal Ca^{2+}channel of the catfish and the frog seems to support the contractility during adrenaline dependent developed at different frequencies examined in both animals. Furthermore, the results suggest that the contribution of the sarcolemmal Ca^{2+} fluxes in the force development in the myocardium of the frog was higher than that in the catfish myocardium.

Key words: *Adrenaline, Caffeine, Verapamil, Cardiac Contractility, Fishes, Amphibians.*

INTRODUCTION

The relative contribution of the Ca^{2+}release and uptake by the sarcoplasmic recticulum (SR) and of the Ca^{2+}fluxes across the sarcolemma in the cardiac contractility seems to differ in the ectothermic and endothermic species (Fabiato and Fabiato, 1983; Chapman, 1983). The cardiac SR and sarcolemma of the teleost is sparsely developed compared with that of mammals according to ultrastructural studies (Gabella, 1978) but, its SR appears to be well developed functionally according to an experimental study (EL – Sayed, 1994). However, the SR of the frog heart is much less developed ultrastructurally and functionally than that of the teleost (Mcleod et al., 1991).

In teleosts, an increase in frequency was found to decrease the twitch force of the cardiac muscle (Driedzic and Gesser, 1985). The *Clarias gariepinus* myocardium also shows a negative force-frequency

relationship (EL-Sayed 1994b). It has been suggested that the negative force frequency relationship may depend on the calcium regulation by the SR (Orchard and Lakatta, 1985). Also, it has been reported that the contribution of the SR in the force development at physiologically relevant frequencies appears to be less important in the teleosts heart (Driedzic and Gesser, 1988; Hove–Madsen and Gesser, 1989). Furthermore, caffeine also decreased the force developed at different frequencies, especially at a relevant physiologically frequency (El-Sayed, 1994 b). However, verapamil which is generally used as an inhibitor of sarcolemmal Ca^{2+} flux (Devlin and Smith 1996) caused a decrease in the contractile variables at different frequencies in the clarias myocardium indicating also the possible contribution of the sarcolemmal Ca^{2+} flux in the regulation of the cardiac force (El-Sayed, 1999).

In ectothermic species, it is thought that the contractions of the cardiac muscle depend on Ca^{2+} fluxes across the sarcolemma while the SR seems to be of relatively little importance (Santer, 1985; Morad and Cleeman, 1987). In accordance with that it has been claimed that the cardiac SR of frog is less developed than that of the teleost heart (Page and Niedergerke, 1972). Furthermore, frog heart exhibits post – rest decay of force after rest interval of 5 minutes which is strongly increased by caffeine, an inhibior of the SR function (El–Sayed, 2000). Verapamil, an inhibitor of the sarcolemmal Ca^{2+} channels, slightly decreased this decay in the cardiac force developed after the 5 minutes of rest in the frog heart, also. These reactions are believed to reflect an involvement of the transarcolemmal Ca^{2+} in the regulation of force.

Adrenaline enhanced twitch force developed at a steady pacing rate by a similar factor in the atrial and ventricular tissues of rainbow trout (Gesser, 1996). This factor was furthermore not affected by ryanodine and seems, therefore, not to depend on the SR. Also, in the teleost heart adrenaline increased the potentiation in the force developed after 5 minutes of rest (El– Sayed, 2000), whereas in amphibian heart, it decreased the post–rest decay in the contractility indicating that the regulation of force may depend on the Ca^{2+} fluxes across the sarcolemma. In mammals as in the teleost hearts, adrenaline increased the myocardial contractility (Brückner et al., 1985 and keen et al., 1993).

The aim of the present study is to examine the influence of adrenaline on the contractility developed at the different frequencies in the myocardium of the catfish and the frog with respect to the SR and the sarcolemmal Ca^{2+} channel dependences. Furthermore, the

experiments were extended to investigate the effects of caffeine and verapamil on the cardiac variability of both animals to evaluate the role of Ca^{2+} channels in cardiac contractility . In addition , the effect of extracellular Ca^{2+} on cardiac contract ile wac investigated.

MATERIALS and METHODS

Animals:

The catfish (*Clarias gariepinus*) weighing about 150 g. of both sexes were obtained from canal near to Sohag city and were transported to the laboratory at the Zoology Department in the Faculty of science (Sohag) where there were kept in freshwater tanks at room temperature for about one month. Frogs (*Bufo regularis*) of both sexes were captured during June and July months from farms near to Sohag city and were Kept in terraria with the possibility to dwell in water. The fishes were killed by decapitation, while the frog by a blow on the head then the heart was transformed to an ice-cold oxygenated physiological solution where four strips were cut along the long axis. The four preparations from each ventricle were mounted in identical set-ups and run in parallel. It was considered important to obtain the experimental and control preparation from the same heart since several batches of fish were used.

Preparations:

Each preparation was mounted vertically. One end was tied with surgical silk to one of the two stimulation electrodes made of platinum, and the other end to a thin glass rod connected to the force transducer (Grass FTO3). The distance between the electrode and the transducer, and hence the length of the preparation, could be adjusted with a micrometer screw. The second stimulation electrode was positioned undefined cellular orientation, contractions development and changes in the contractility were normalized to that developed after the stabilization at the basic stimulation rate.

The physiological solution contained (mmol. l^{-1}): $Na\ Cl_2$ 125, $Na\ HCo_3$ 15, NaH_2Po_4 1.5, KCl 2.5, $MgS0_4$ 1.0, $Ca\ Cl_2$ 1.25, and glucose 5, for fish and frog. It was perfused with 99% 0_2 and 1% Co_2 during the experiment by gas mixing pump (wösthoff 1 M 3 o1/af). The temperature of the solution bathing the preparation was maintained at 15°C with a thermostated waterbath (Cole- Parmer OT 268/15, USA). The pH of the solution was 7.71.

Drugs:

Adrenaline – tartrate (Sigma) and verapamil (Sigma) were each dissolved in distilled water to 10 mmol / L^{-1} and kept frozen (-20C°) in suitable portions. Caffeine (8.0Mm) was added as a powder.

Experimental procedures:

Experiment I:

The relationship between adrenaline and the contractions in the myocardium of both animals was examined at different frequencies., The relationship between verapamil and the contractile variables was also investigated in the frog myocardium at different frequencies, After stabilization at basic stimulation rate (0.2 Hz), cummulative doses of adrenaline ranging from 1 to 10 µM/L were added to the bath of one of the two preparations run in parallel with 5 minutes for each dose. In another series of experiments, the stimulation frequency was increased to either 0.4, 0.6 or 0.8 Hz after a stabilization period for about 30 minutes at 0.2 Hz. Then one of the two preparations running in parallel at the new stimulation rate was exposed to cummulative doses of adrenaline. Similar experiments were repeated for frog heart to evaluate the influence of cummulative doses of verapamil ranging from 1 to 10 µM/L on the cardiac contractility at different frequencies. To assess the affinity of adrenaline and verapamil, the changes in the contractile variables(contractile force; rate of contraction, df/dt; rate of relaxation, -df/dt and time to peak tension,TPT) at each adrenaline and verapamil dose were normalized to that in the absence of adrenaline and verapamil at 0.2 Hz(control).

Experiment II:

To evaluate the impact of adrenaline, caffeine and verapamil on the contractile variables (contractile force; rate of contraction, df/dt; rate of relaxation, -df/dt and time to peak tension,TPT) in the cardiac muscle of both animals at 0.2, 0.4, 0.6 and 0.8 Hz, four preparations from each ventricle were run in parallel at 0.2 Hz and at 15°C where the force was allowed to stabilize. After stabilization, the stimulation frequency was continued at 0.2 Hz in one series of experiments and was increased to either 0.4, 0.6 or 0.8 Hz in another series of experiments. After stabilization at the new stimulation frequency, the first preparation was exposed to 4 µM of adrenaline for fish and 8. µM/L for the frog, the second to 8 mM caffeine, the third to 10µM verapamil for both animals whereas the fourth preparation was maintained at control conditions.

Experiment III:

After 10– 15 minutes of these changes, the four preparations were subjected to 2.5 mM Ca_o^{2+}, i.e. the extracellular Ca^{2+} was increased from 1.25 to 2.5 mM.

Results are given as Means ± SD. The level of significance was estimated by student-t test for either paired or unpaired samples. Differences were considered significant when $P < 0.05$.

RESULTS

Adrenaline dose – response:

The relationship between adrenaline and the contractile force in the myocardium of the catfish and of the frog was examined at different frequencies. Fig.1 shows that 1 µM adrenaline caused a significant increase in the contractile force of the fish myocardium at different frequencies, 0.2, 0.4, 0.6 and 0.8 Hz, relative to the control (in absence of adrenaline) at 0.2 Hz. With increasing the concentration of adrenaline, the cardiac force increased until it reached its maximal effect at 4, I, 3 and 7 µM/L at a stimulation frequency of 0.2, 0.4, 0.6 and 0.8 Hz respectively (Fig. 1 a, b, c and d). In the frog myocardium 1µM adrenaline, also led to a significant increase in the contractile force at the different frequencies (Fig. 2). As in the fish heart, increasing of the adrenaline concentration led to an increase in the cardiac force of the frog to reach its maximal effect at 7 µM/L at a stimulation rate of 0.2 Hz (Fig. 2a), whereas it had its marked effect on the (Fig. 2b,c & d). contractile force at 3, 5 and 3 µM at 0.4 , 0.6 and 0.8 Hz respectively

Verapamil dose-response:

The influence of cummulative doses of verapamil on the contractile force of the frog heart was examined at different frequencies. One µM of verapamil led to a significant decrease in the cardiac force (Fig.3) The lowering in the cardiac force increased with increasing of verapamil concentrations until it reaches its maximal effect at 10 µM at all the stimulation frequencies applied, 0.2, 0.4, 0.6 & 0.8 Hz (Fig.3a–d).

Adrenaline and contractility:

To evaluate the influence of adrenaline on the regulation of force of contractions in the myocardium of the catfish and of the frog at different frequencies, the variations of contractile force; rate of contraction, df/dt; rate of relaxation, -df/dt and time to peak tension,TPT with adrenaline were studied. Fig. 4A shows that the contractile force decreased with increasing of the stimulation frequency in the

myocardium of the fish (Fig. 4A) and of the frog (Fig. 4B). Adrenaline (4 μM/L) caused an increase in the contractile force developed at different frequencies in the fish myocardium, but its effect decreased with the increasing of frequency (Fig. 4A). The effect of adrenaline on the df/dt (Fig. 5A) and on the – df/dt (Fig. 6A) was similar to the effect on the contractile force, whereas it had a negative inotropic effect on the TPT at different frequencies (Fig. 7A). Also, the effect of adrenaline on TPT decreased with increasing of the stimulation frequency as happened with the contractile force, df/dt and – df/dt. Thus, there is a significant correlation (t=2.1) between force and both df/dt and – df/dt. As shown in Table 1, increasing of extracellular Ca^{2+} had a positive inotropic effect on the increasing of the contractile variables caused as a result of the addition of adrenaline.

The influence of adrenaline (8 μM) on the contractile variables developed at different frequencies in the frog myocardium (Fig. 5B, 6B and 7B.) was similar to that on the fish myocardium. In contrast to that in the fish myocardium, adrenaline had a positive inotropic effect on the TPT (Fig. 7B). As in the fish myocardium, 2.5 mM Ca^{2+} had a positive inotropic effect on the increased contractile variables caused by adrenaline (Table 2). It should be noted that the effect of adrenaline on the contractility of both animals was similar at 0.2 Hz. However the influence of adrenaline on the contractile force was higher in the frog myocardium at 0.2 Hz and lower at 0.6 and 0.8Hz than that of the fish myocardium respectively.

Caffeine and contractility:

The next question addressed was the influence of caffeine on contractility to explore the role of the SR in the excitation- contraction coupling (E-C coupling) at different frequencies. In a series of experiments, the effect of caffeine (8.0 mM) on the contractile variables produced at the different stimulation rates (0.2, 0.4, 0.6 and 0.8 Hz) in the myocardium of both animals was examined. Caffeine (8.0 mM),

like adrenaline, had a positive inotropic effect on the contractile force of the fish (Fig. 4A) and of the frog myocardium (Fig 4B). The effect of caffeine on the df/dt and – df/dt in the fish heart (Fig 5A, 6A) and on the frog heart (Fig. 5 B, 6B) was similar to the effect on the contractile force. In the fish heart, caffeine had a positive inotropic effect on TPT at all the stimulation frequencies applied except at 0.8 Hz, it was negative (Fig. 7A). Also, in the frog heart caffeine had a positive inotropic effect on TPT at a stimulation rate of 0.4, 0.6 and 0.8 Hz but

this effect was negative at 0.2 Hz (Fig. 7B). It should be pointed out that the effect of caffeine on the contractile variables was higher in the frog than that in the fish heart at all frequencies applied. Also, the influence of adrenaline on the contractility in the fish heart was higher than that of caffeine at a stimulation rate of 0.2 and 0.4 Hz whereas, in the frog heart the influence of caffeine on contractility was higher than that of adrenaline at all frequencies applied except at 0.2 Hz, it was similar.

Verapamil and Contractility:

The influence of verapamil on the contractile force, df/dt, - df/dt and TPT in the cardiac muscle of the catfish and the frog was also studied to investigate the role of the transarcolemmal Ca^{2+} in the regulation of force at different frequencies. Verapamil led to a significant decrease in the cardiac force of the catfish (Fig. 4A) and of the frog (Fig. 4B). This decrease was increased with increasing of the stimulation rate in the heart of both animals. The effect of verapamil on the df/dt (Fig. 5E) on the -df/dt (Fig. 6A) and on the TPT (Fig.7A) in the fish heart was similar to the effect on the contractile force, except at a stimulation rate of 0.2 Hz, the decrease in the contractile variables caused by verapamil was insignificant. Whereas, the effect of verapamil on the df/dt (Fig. 5B) on the – df/dt (Fig. 6B) and on the TPT (Fig. 7B) in the frog heart was similar to the effect on the contractile force (Fig. 5B). It should be noted that the effect of verapamil on the contractility in the frog heart was higher than that in the fish heart.

DISCUSSION

This study shows that the contractile variables (contractile force; rate of contraction, df/dt; rate of relaxation, -df/dt) and time to peak tension,TPT developed in the catfish and the frog hearts at different stimulation rates were increased by adrenaline and caffeine, whereas these contractile variables were decreased by verapamil, also, in both animals.

In ectothermic species such as rainbow trout (Gesser, 1996) as well as in mammalian species such as guinea pig (Drake– Holland et al., 1992), adrenaline stimulated the contractile force developed at a steady stimulation rate. In agreement with these data, adrenaline had a positive inotropic effect on the contractility in the cardiac muscle of both animals. This increase in the catfish and the frog ventricles contractility may be due to the contribution of the sarcoplasmic reticulum to the force development, since it has been shown that adrenaline enhances the

sequestration of calcium ions (Ca^{2+}) from the myocardial contractile proteins into the sarcoplasmic reticulum (Hasselbach, 1964) which facilitates relaxation. It has been suggested by many authors that this will increase the amount of calcium recirculated within the cell and increase contractility (e.g. Boller and Pott, 1989). The findings that the increase in the contractility caused by adrenaline in the catfish and the frog hearts decreased with the increasing of the stimulation frequency suggest that the contribution of the SR in the regulation of the contractility at the physiologically relevant frequencies i.e. above 0.2 Hz seems to be of less importance. In accordance with this, the catfish exhibits a rest potentiation of force (EL-Sayed, 1994 b) which is thought to be a sensitive indicator of the function of the SR (Bers, 1985) at a lower stimulation rates. In supporting of this suggestion, the contractile force correlates with the df/dt despite an insignificant decrease in "active state" (TPT) at a stimulation rates of 0.2 and 0.4 Hz. However, the positive inotropic effect of adrenaline on the contractile force in the catfish was higher than that in the frog heart at higher stimulation rates (0.6 and 0.8 Hz), while the duration of active state (TPT) in the frog was higher than that in the catfish. This finding indicates that the role of the frog cardiac SR in the regulation of force is small. This agrees with the studies suggesting that the contribution of the SR in the development of force in the amphibian heart is of little importance (Chapman, 1983).

Experiments with intact cells (EL-Sayed, 1994) and with the SR Ca^{2+} channels incorporated in planar lipid bilayers (Rousseau and Meissner, 1989) indicate that caffeine is thought to affect the regulation of the cardiac force by activating the sarcoplasmic reticulum Ca^{2+} release channel and thus prevent the SR from accumulating Ca^{2+}, leading to a decrease in the contractile force of the cardiac muscle. This seems to be true for the catfish heart when stimulated at unphysiologically frequencies, i.e. below 0.2 Hz (El-Sayed, 1994 b). However, the situation is the exact opposite at physiologically relevant frequencies, i.e. above 0.2 Hz, since it has been found (in the present study) that caffeine caused a highly significant increase in the contractile variables of the catfish and the frog myocardium at the higher stimulation frequencies (0.4, 0.6 and 0.8 Hz). Thus, it could be speculated that the Contractility developed at all stimulation frequencies examined does not depend on the SR in the both animals. In this respect, the frog and the catfish hearts resembles mammalian species such as rabbit and guinea pig, as well as

ectothermic species such as turtle (Lewartowski et al., 1984; Driedzic and Gesser, 1985).

The results obtained for the frog and the catfish heart indicate that verapamil, which is extensively used as a blocker of surface membrane Ca^{2+} channel (Ponce–Hornos et al., 1990) had a highly significant negative inotropic effect on the contractility of both animals at the different stimulation rates examined. These results are similar to that obtained for the mammalian species such as rabbit and dog (Ponce–Hornos et al., 1990), in which the force attained at a steady state frequency is a sarcolemmal Ca^{2+} dependence (Bers, 1985). The finding that this effect of verapamil on the contractility was not recovered by the increased extracellular Ca^{2+} (2.5mM) suggests that the contractility in both animals developed at the all stimulation frequencies examined depends on the sarcolemmal Ca^{2+} fluxes.

In conclusion, the positive inotropic effect of adrenaline and of caffeine on the contractility developed at all stimulation frequencies applied in the myocardium of the catfish (*Clarias gariepinus*) and the frog (*Bufo regularis*) indicate that the regulation of the cardiac force in these animals may depends on the Ca^{2+} flux across the sarcolemma. In supporting of this suggestion, verapamil, an inhibitor of the sarcolemmal Ca^{2+} channels, had a negative inotropic effect on the cardiac contractility of both animals. It should be noted that the negative inotropic effect of verapamil on the frog heart was higher than that on the catfish heart. This finding strongly suggests an involvement of the sarcolemmal Ca^{2+} fluxes in the regulation of the force development in the frog myocardium at all stimulation frequencies applied.

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Table 1: Effect of adrenaline, of caffeine, of verapamil and of 2.5 mM extracellular Calcium on force,df\ dt, - df\dt and TPT in the ventricullar preparation of the catfish

Control

Frequency	0.2 Hz		0.4 Hz		0.6 Hz		0.8 Hz	
		Ca^{2+}		Ca^{2+}		Ca^{2+}		Ca^{2+}
Force change (%)	103±5	162±7	82±3	104±9	76±6	88±5	58±6	69±4
df/dt change (%)	102±3	164±7	77±7	93±3	71±5	83±3	56±7	68±5
-df/ dt Chang (%)	111±2	171±66	74±4	93±3	77±4	91±3	60±8	67±7
TPT change (%)	91±4	111±1.0	77±7	78±9	59±3	61±4	55±6	56±3

Adrenaline

Frequency	0.2 Hz		0.4 Hz		0.6 Hz		0.8 Hz	
		Ca^{2+}		Ca^{2+}		Ca^{2+}		Ca^{2+}
Force change (%)	123±4	137±5	117±9	133±4	86±4	93±9	61±3	69±6
df/dt change (%)	119±5	132±6	110±11	124±8	76±3	95±4	54±4	62±5
-df/ dt change (%)	119±5	132±6	111±10	136±5	73±3	88±5	69±6	77±7
TPT change (%)	88±6	101±2	73±8	75±9	56±4	58±4	56±6	55±6

Caffeine

Frequency	0.2 Hz		0.4 Hz		0.6 Hz		0.8 Hz	
		Ca^{2+}		Ca^{2+}		Ca^{2+}		Ca^{2+}
Force change (%)	118±2	130±5	97±7	101±6	85±3	117±3	72±3	85±3
df/dt change (%)	113±4	129±7	91±7	102±7	78±3	89±3	64±2	66±2
-df/ dt change (%)	111±3	127±6	83±9	95±9	81±1.0	93±1	65±1.0	73±2
TPT change (%)	98±4	110±7	80±8	85±8	63±2	64±6	50±1	41±1

Verapamil

Frequency	0.2 Hz		0.4 Hz		0.6 Hz		0.8 Hz	
		Ca^{2+}		Ca^{2+}		Ca^{2+}		Ca^{2+}
Force change (%)	86±2	107±1.0	73±2	84±2	53±2	85±3	46±5	57±5
df/dt change (%)	101±3	115±5	73±1	84±2	50±2	85±3	50±6	52±6
-df/ dt change (%)	110±5	124±6	60±2	62±2	55±5	85±4	49±6	56±3
TPT change (%)	95±9	88±5	61±2	53±4	50±4	59±6	42±6	54±7

Four preparations from each ventricle were run in parallel at 0.2 Hz and at 15°C where the force was allowed to stabilize. After stabilization, the stimulation frequency was continued at 0.2 Hz in one series of experiments and was increased to either 0.4, 0.6 or 0.8 Hz in another series of experiments. After stabilization at the new stimulation frequency, the first preparation was exposed to 4 µM of adrenaline , the second to 8 mM caffeine, the third to 10µM verapamil whereas the fourth preparation was maintained at control conditions. 10– 15 minutes after these changes, the four preparations were subjected to 2.5 mM Ca_o^{2+}

Table 2. Effect of adrenaline, of caffeine, of verapamil and of 2.5 mM extracellular Calcium on force,df\ dt, - df\dt and TPT in the ventricullar preparation of the Frog.

Control

Frequency	0.2 Hz		0.4 Hz		0.6 Hz		0.8 Hz	
		Ca^{2+}		Ca^{2+}		Ca^{2+}		Ca^{2+}
Force change (%)	105± 3	132±7	80±5	111±0	61±2	75±3	43±3	62±9
df/dt change (%)	113±6	132±7	82±6	110±8	65±2	73±4	47±5	66±9
-df/ dt change (%)	110±2	135±6	78±6	103±6	61±2	72±2	51±3	66±5
TPT change (%)	93±5	102±10	72±4	76±6	65±3	68±4	60±4	82±2

Adrenaline

Frequency	0.2 Hz		0.4 Hz		0.6 Hz		0.8 Hz	
		Ca^{2+}		Ca^{2+}		Ca^{2+}		Ca^{2+}
Force change (%)	123±7	141±8	130±4	160±13	77±3	97±5	73±6	89±12
df/dt change (%)	117±5	130±6	111±11	135±5	84±5	106±2	69±6	95±10
-df/ dt change (%)	111±7	141±5	110±6	141±4	88±2	98±3	77±9	94±4
TPT change (%)	100±11	113±7	68±4	60±5	69±1.0	76±5	77±10	89±6

Caffeine

Frequency	0.2 Hz		0.4 Hz		0.6 Hz		0.8 Hz	
		Ca^{2+}		Ca^{2+}		Ca^{2+}		Ca^{2+}
Force change (%)	128±7	144±9	140±5	162±8	89±3	105±2	39±5	50±7
df/dt change (%)	118±5	140±4	131±7	151±5	90±2	103±2	48±5	60±7
-df/ dt change (%)	111±4	130±3	140±8	156±8	93±8	110±2	50±3	62±5
TPT change (%)	81±1.0	81±3	75±7	58±4	76±2	79±7	74±3	74±9

Verapamil

Frequency	0.2 Hz		0.4 Hz		0.6 Hz		0.8 Hz	
		Ca^{2+}		Ca^{2+}		Ca^{2+}		Ca^{2+}
Force change (%)	75±2	90±6	52±2	61±5	51±5	69±2	35±2	49±6
df/dt change (%)	83±7	96±8	54±3	71±3	50±6	78±7	40±2	51±4
-df/ dt change (%)	81±8	94±8	68±6	73±7	55±5	80±5	41±3	52±7
TPT change (%)	85±7	79±5	59±8	55±6	55±10	70±10	53±4	61±3

Four preparations from each ventricle were run in parallel at 0.2 Hz and at 15°C where the force was allowed to stabilize. After stabilization, the stimulation frequency was continued at 0.2 Hz in one series of experiments and was increased to either 0.4, 0.6 or 0.8 Hz in another series of experiments. After stabilization at the new stimulation frequency, the first preparation was exposed to 4 µM of adrenaline , the second to 8 mM caffeine, the third to 10µM verapamil whereas the fourth preparation was maintained at control conditions. 10– 15 minutes after these changes, the four preparations were subjected to 2.5 mM Ca_o^{2+}

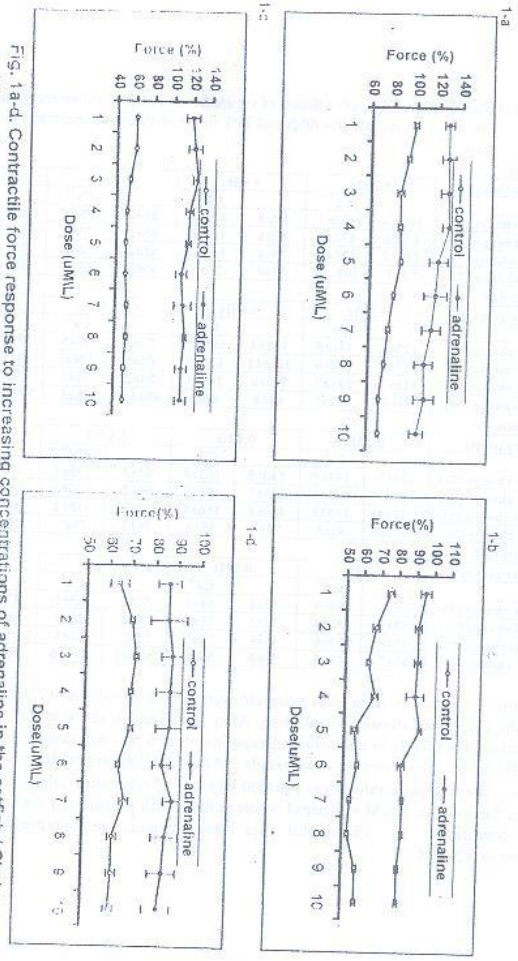

Fig. 1.a-d. Contractile force response to increasing concentrations of adrenaline in the catfish (*Clarias gariebinus*) myocardium at a stimulation frequency of 0.2 Hz (a), of 0.4 Hz (b), of 0.6 Hz (c) and of 0.8 Hz (d). The response was normalized to the response at a stimulation rate of 0.2 Hz before addition of adrenaline. The response to each of the additions of adrenaline was completed within 5-10 minutes, n=6.

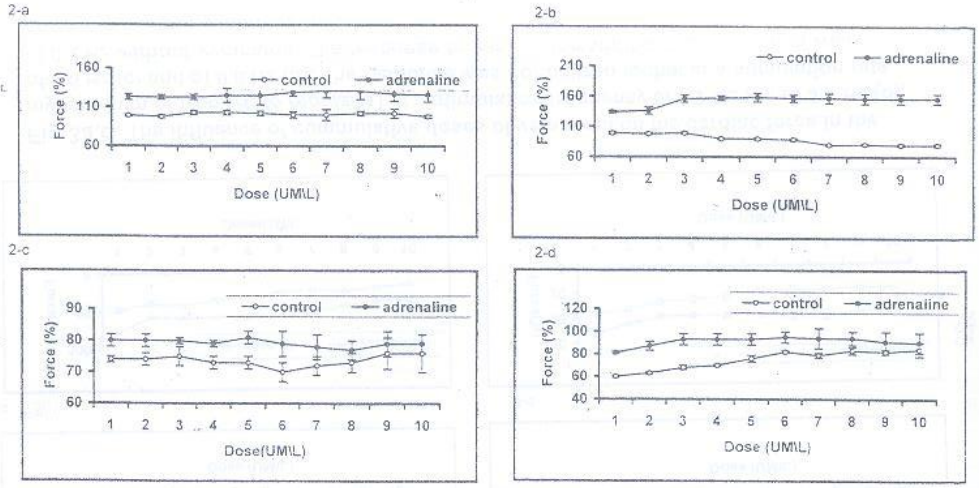

Fig.2a. d. Contractile force response to cummulative doses of adrenaline in the frog (*Bufo regularis*) cardiac muscle at a stimlation rate of 0.2 Hz (a), of 0.4 Hz (b), of 0.6 Hz (c) and of 0.8 Hz (d) . The response was normalized to that at a stimulation rate of 0.2 Hz before addition of adrenaline. The response to each of the additions of adrenaline was completed within 5-10 minutes. n=6.

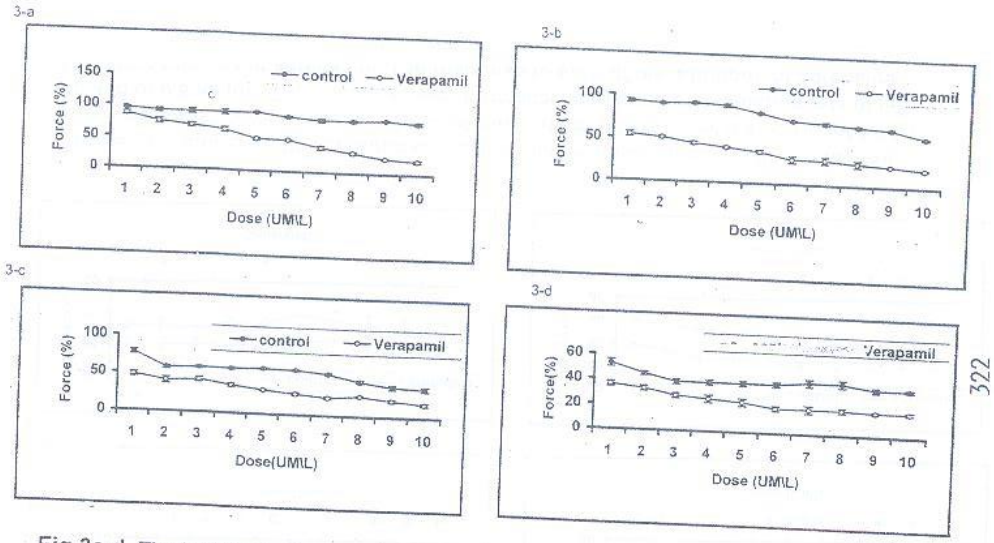

Fig.3a.d. The influence of cummulative doses of verapamil on the cardiac force in the myocardium of frog (*Bufo regularis*) at a stimulation frequency of 0.2 Hz (a), of 0.4 Hz (b), of 0.6 Hz (c) and of 0.8 Hz (d). The response was normalized to that at a stimulation rate of 0.2 Hz without verapamil. The response to each of the additions of verapamil was completed within 5 minutes. n=6.

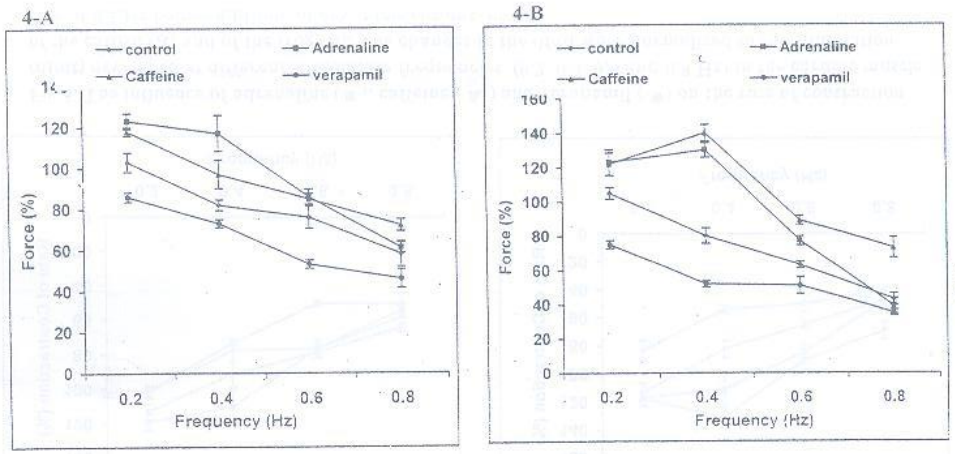

Fig.4. Effect of adrenaline (■), caffeine (▲) and verapamil (●) on the cardiac force of the catfish (A) and of the frog (B) at stimulation rates of 0.2, 0.4, 0.6 and 0.8 Hz. The changes in the cardiac force were normalized to that at 0.2 Hz before addition of any interventions. n=6.

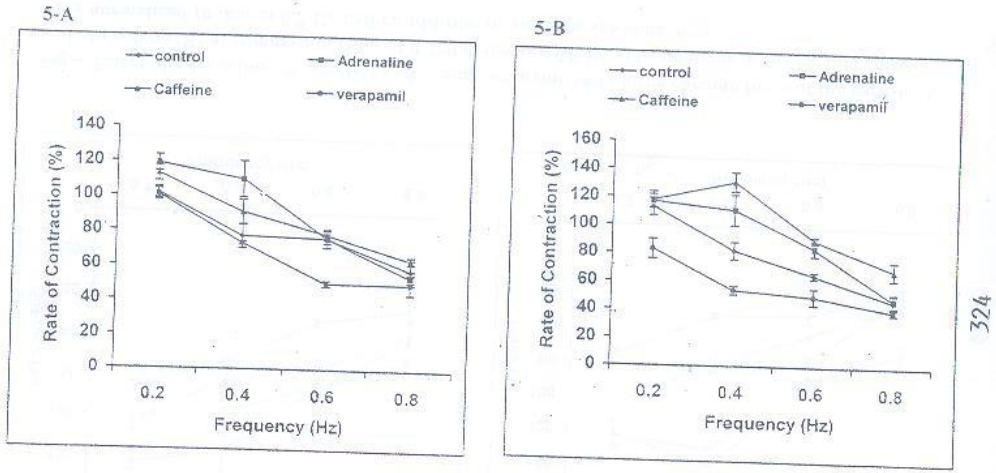

Fig.5. The influence of adrenaline (■), caffeine (▲) and verapamil (●) on the rate of contraction (df/dt) developed at different stimulation frequencies, (0.2, 0.4, 0.6 and 0.8 Hz) in the cardiac muscle of the catfish (A) and of the frog (B). The changes in the df/dt were normalized to the stimulation rate of 0.2 Hz before addition of any interventions. n=6.

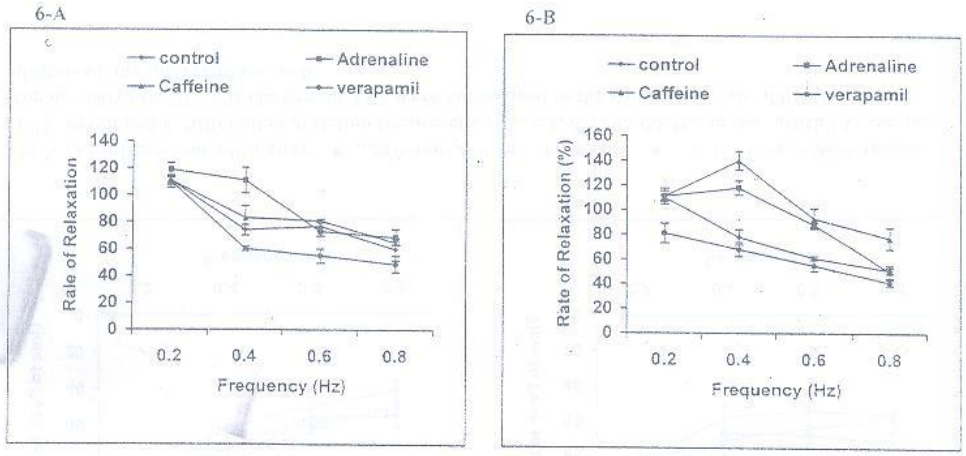

Fig.6. The influence of adrenaline (■), caffeine (▲) and verapamil (●)on the rate of relaxation, (-df/dt) developed at a stimulation frequency of 0.2, 0.4, 0.6 and 0.8 Hz in the cardiac muscle of the catfish (A) and of the frog (B). The changes in –df/dt was normalized to the stimulation rate of 0.2 Hz before addition of any interventions. n=6.

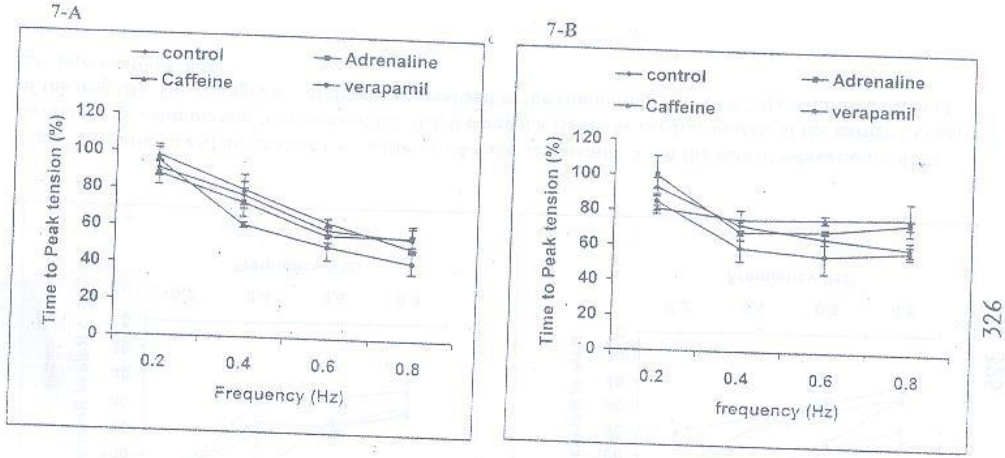

Fig.7. The influence of adrenaline (■), caffeine (▲) and verapamil (●) on the time to peak tension (TPT) developed at different stimulation frequencies (0.2, 0.4, 0.6 and 0.8 Hz) in the catfish (A) and the frog myocardium (B). The changes in TPT were normalized to the stimulation rate of 0.2 Hz before addition of any interventions. n=6.