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INTERACTIVE EFFECT OF CAFFEINE AND VERAPAMIL ON THE MYOCARDIUM OF THE CATFISH (*CLARIAS GARIEPINUS*) AT THE PHYSIOLOGICAL FREQUENCIES

(With 2 Tables and 6 Figures)

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التأثير المتداخل للكافيين والفيراباميل على عضلة قلب سمك القط (القرموط) عند معدل الضربات الفسيولوجية

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لقد تم دراسة تأثير الكافيين والفيراباميل وكذلك التأثير المتداخل للكافيين مع الفيراباميل على الضربات القلبية (قوة الضربة ومعدل الانقباض ومعدل الانبساط) التي تنمو عند معدل الضربات الفسيولوجية (١٢ ، ٢٤ ضربة في الدقيقة) في بطين قلب سمك القط (القرموط) عند ٢٠ درجة مئوية . علاوة على ذلك تم دراسة تأثير زيادة الكالسيوم الخارجي على تلك المتغيرات وقد تبين من ذلك أن : الكافيين (٨ ملي جرام لكل جزئ) كمثبط للكالسيوم المأخوذ بواسطة الصفيحة اللحمية قد أحدث انخفاضاً في قوة الضربات القلبية عند كلا المعدلين من الضربات ولكن كان هذا الانخفاض ملحوظاً عند معدل ١٢ ضربة في الدقيقة. الفيراباميل (مثبط قنوات الكالسيوم في الغشاء الخلوي) عند التركيزات المختلفة (٥، ٩ ، ١٣ ميكروجرام لكل جزئ) كان له نفس تأثير الكافيين على قوة الضربات القلبية إلا أن التأثير السلبي للفيراباميل على الضربات القلبية كان أكثر من تأثير الكافيين . أيضاً كان التأثير السلبي للفيراباميل ملحوظاً عند معدل ضربات ٢٤ ضربة في الدقيقة وعند تركيز ١٣ ميكروجرام لكل جزئ). سبب الكافيين متحدا مع الفيراباميل أيضاً إنخفاضاً في قوة الضربات القلبية عند كلا المعدلين من الضربات. وعلى العكس من تأثير الفيراباميل وحده ومتشابهاً مع تأثير الكافيين كان التأثير السلبي للكافيين مع الفيراباميل عند معدل ١٢ ضربة في الدقيقة. ازالت الزيادة في تركيز الكالسيوم الخارجي كل التأثيرات السلبية للكافيين والفيراباميل والكافيين متحداً مع الفيراباميل على قوة الضربات القلبية عند كلا المعدلين من الضربات . والخلاصة أن قنوات الكالسيوم في الغشاء الخلوى يبدو أنها تدعم عملية تنظيم الضربات في قلب سمك القط (القرموط) أكثر من الصفيحة اللحمية عند المعدلات الفسيولوجية من الضربات كما أن فعل الفيراباميل يعتمد على معدل الضربات القلبية .

SUMMARY

The influences of caffeine, verapamil and caffeine combined together with verapamil on the cardiac contractility (contractile force, the rate of contraction; df/dt and the rate of relaxation; -df/dt) developed at the physiological rates of contraction (0.2 and 0.4 Hz) in the ventricular preparations of the catfish (*Clarias gariepinus*) were studied at 20°C. Furthermore, the effect of increased extracellular calcium (2.5 mM) was also, investigated. Caffeine (8.0 mM), which inhibits the uptake of calcium by the sarcoplasmic reticulum (SR) caused a decrease in the cardiac contractility at the both rates of stimulation, but this decrease was marked at the stimulation rate of 0.2 Hz. Verapamil (an inhibitor of the sarcolemmal Ca^{2+} channels) at the different concentrations (5, 9 and 13 μM) have the same effect on the cardiac contractility like that of caffeine. However, the negative inotropic effect of verapamil on the cardiac contractility was higher than that of caffeine. Also, the lowering effect of verapamil was marked at 0.4 Hz and 13 μM. Caffeine combined together with Verapamil also caused a decrease in the cardiac contractility at both rates of Stimulation. In opposite to the effect of verapamil only and like that of caffeine on the cardiac contractility, caffeine combined together with verapamil had its marked effect at the stimulation rate of 0.2 Hz. Increased extracellular calcium removed the negative inotropic effect of caffeine combined together with verapamil on the cardiac contractility at both rates of stimulation applied. In conclusion, the sarcolemmal Ca^{2+} channels appear to support the cardiac contractility developed at the physiological rates of frequency than that of the sarcoplasmic reticulum in the catfish. Also, the verapamil action seems to be stimulation rates dependent.

Keywords: *Caffeine, Verapamil, Myocardium, Calcium, Cardiac Contractility, Catfish, Clarias gariepinus.*

INTRODUCTION

The role of sarcolemma and the sarcoplasmic reticulum (SR) in the cardiac excitation-contraction coupling (E-C Coupling) seems to vary among different vertebrate species. The cardiac sarcolemma of the teleost is less developed compared with that of mammals according to ultrastructural studies (Gabella, 1978). Isolated ventricular tissue of the *Clarias gariepinus* display a negative force-frequency relationship (El-Sayed, 1994a), particularly evident as a post-rest-potentiation which is

strongly reduced by ryanodine and caffeine (El-Sayed, 1994b). A similar situation has been described for Tilapia ventricular muscle (El-Sayed, 1994). These reactions are believed to reflect an involvement of the sarcolemma in the E-C coupling and are also seen for rabbit and dog cardiac muscle in which the E-C coupling is probably sarcolemmal dependent (Ponce-Hornos et al., 1990).

Verapamil is clinically used as an antirhythmic and anti-hypertensive drug which primary action on mammalian cardiac muscle is to block membrane channels whose conduct a slow inward Ca^{2+} current (Fleckenstein and Fleckenstein-Gurn, 1984). It has been shown that this drug has an interesting effect on molluscan cardiac muscle (Devlin, 1992) as well as smooth muscle (Huddart et al., 1990). Also, verapamil decreased the contractile force, rate of contraction and the rate of relaxation in the mammalian myocardium (Ponce-Hornos et al., 1990). Also, it has been reported that increases in the verapamil concentration are followed by a lowered in the contractile force, the rate of contraction, the rate of relaxation and the time to peak tension of the *Clarias myocardium* (El-Sayed, 1999). The cause of this effect is still unclear. However, it has been shown that a blocking Ca^{2+} channels by verapamil may resulting in a diminished influx of Ca^{2+} from the extracellular space during excitation and a lowered intracellular Na^{+} activity with an outward shift of Ca^{2+} via the Na^{+}-Ca^{2+} exchange may both be involved (Chapman & Radrigo, 1987).

It has been postulated that the *Clarias ventricular* tissue displays a post-rest potentiation and also has a negative force-frequency relationship (El-Sayed, 1994). This post-rest potentiation was transformed into a post-rest decay by caffeine, which inhibits the function of the SR by interfering with the opening mechanism of its Ca^{2+} channels. In the cardiac muscle of rainbow trout (El-Sayed and Gesser, 1989) and in the frog myocardium (El-Sayed, 2000). Caffeine inhibits the post-rest potentiation developed after 5 minutes of rest. These results suggest an involvement of the SR, also in the E-C coupling. So, it is of interest to determine whether sarcolemma or the SR which has a role in the E-C coupling of the *Clarias myocardium* at the physiological frequencies (0.2 and 0.4 Hz).

The present study looks at the E-C coupling in the myocardium of the *Clarias gariepinus* with respect to the sarcolemmal dependence and the influence of the verapamil. Since the SR and the sarcolemma seem to have a role in the E-C coupling, the function of each, then both of them together were examined. The function of the SR was assessed

with caffeine which is known to prevent Ca^{2+} uptake of the SR as well as increasing the calcium influx across the sarcolemma during excitation (Kavaler et al., 1978) and the calcium sensitivity of the contractile system (Wendt and Stephenson, 1983). The function of sarcolemma was examined with verapamil which blocks the sarcolemmal Ca^{2+} transport (Devlin, 1993), and both of them (SR and the sarcolemma) with caffeine combined together with verapamil.

MATERIALS and METHODS

The *Clarias gariepinus* Weighing about 150 g. of both sexes were obtained from a canal near to Sohag City and were immediately transported to the laboratory at the Department of Zoology, in the Faculty of Science (Sohag) where they were kept in freshwater tanks at room temperature for about three weeks. After decapitation of the fish, the heart was excised and placed in an ice-cold physiological solution, where ventricular strips were prepared.

The physiological solution for the *Clarias gariepinus* heart contained (in mM) 125 NaCl, 2.5 KCl, 1.25 $CaCl_2$, 0.94 $MgSO_4$, 1.0 NaH_2PO_4, 15 $NaHCO_3$, 5.0 glucose (EL -Sayed and Gesser, 1989).

The solution was gassed with 99% oxygen and 1% carbon dioxide by a gas mixing pump (Wösthoff 1M 301/AF.). The resulting pH was 7.6 at 20 ± 0.5 °C (Cole Parmer OT 268/16, USA). Verapamil (Sigma) was dissolved in distilled water to 10 mM L^{-1} and kept frozen (-20°C) in suitable portions, so it was not thawed more than once before use. Caffeine was added as a powder.

Commonly four strips from each ventricle were run in parallel setups. This was considered important, since the fishes used were not genetically characterized and were from different patches. For recording the contractile variations, the upper end of the preparations was connected to a recorder (Grass 79G.). The lower end was tied onto one of the two platinum stimulation electrodes. The other electrode was placed in the solution just above the preparation. The preparation was stimulated to contract by electrical square pulses having duration of 5 ms and a voltage of 1.5-2.0 times the threshold for full contraction. The preparations used were never spontaneously active. The distance between the two points of fixation could be adjusted with a micrometer screw, and the preparation was stretched to produce maximal twitch force. When this had been done, the length of the preparation was 7-15 mm, but its thickness never exceeded 2 mm.

After the initial adjustment, each preparation was left at 0.2 Hz for about 30 minutes before further investigations. The contractile variables values measured in the subsequent part of the experiment were all as percentage as to those mean recorded at the end of these 30 minutes.

To investigate the influence of caffeine, verapamil and caffeine combined together with verapamil on the contractility at 0.2 Hz and 0.4Hz, four strips from ventricular tissue were run in parallel at 0.2 Hz at 20°C where the force was allowed to stabilize. After stabilization, the stimulation rate was either continued at 0.2 Hz in one series of experiments or increased to a stimulation rate of 0.4 Hz in another series of experiments. After stabilization at either 0.2 or 0.4 Hz, first strip was exposed to 8.0 mM caffeine, the second strip was exposed to either 5, 9 or 13 μM verapamil, the third strip was exposed to 8.0 mM caffeine combined together with either 5,9 or 13 μM verapamil whereas the fourth strip was maintained at control condition. 10-15 minutes after these changes, the four strips were subjected to 2.5 mM Ca^{2+} to examine the influence of the extracellular Ca^{2+} on the cardiac contractility.

The results are given as means ± SD. The level of significance was estimated by student's ± test for either paired or unpaired samples. The limit of significance was set at $P< 0.05$.

RESULTS

Caffeine and contractility:

Caffeine (8.0 mM) which is generally used as a blocker of the Ca^{2+} uptake of the sarcoplasmic reticulum led to a decrease in the contractile force (Fig. 1 A and B), the rate of contraction (Fig. 2 A and B) and the rate of relaxation (Fig. 3 A and B) at both rates of stimulation (0.2 and 0.4 Hz) applied. It should be noted that the decrease in the cardiac contractility (Force, df/dt and – df/dt) was significantly lower at 0.2 Hz than those at 0.4 Hz.

Verapamil and contractility:

Verapamil at different concentrations, (5, 9 and 13 μM) caused a significant decrease in the contractile force (Fig. 1 A and B), the rate of contraction (Fig. 2 A and B) and the rate of relaxation (Fig. 3 A and B). However, the effect of verapamil on the contractile force at 0.4 Hz was significantly greater than that at 0.2 Hz, whereas no significant differences between the effect of verapamil on the rate of contraction at

0.2 and 0.4 Hz could be documented. But, the decrease in the rate of relaxation as a result of addition of 13 μM verapamil was significantly lower at 0.4 Hz than that at 0.2 Hz.

Caffeine combined together with verapamil and contractility:

Caffeine (8.0 mM) combined together with different concentration of verapamil (5, 9 and 13 μM) had a negative inotropic effect on the contractile force (Fig. 1 A and B), the rate of contraction (Fig. 2 A and B) and the rate of relaxation (Fig. 3 A and B) at both rates of stimulation (0.2 and 0.4 Hz). In contrast to the effect of caffeine and of verapamil, caffeine combined together with verapamil had a markedly negative effect on the cardiac contractility at the rate of stimulation of 0.2 Hz than that of 0.4 Hz. Furthermore, the negative inotropic effect of caffeine combined together with the different concentration of verapamil was maximum at 13 μM of verapamil at both rates of stimulation.

It should be pointed out that the negative inotropic effect of 8.0 mM caffeine combined together with 13 μM of verapamil on the cardiac contractility was significantly greater than that of 13 μM verapamil only at a stimulation rate of 0.2 Hz. Whereas, at the stimulation rate of 0.4 Hz the negative inotropic effect of 13 μM of verapamil on the cardiac contractility was significantly higher than that of 8.0 mM caffeine combined together with 13 μM verapamil.

Increased extracellular Ca^{2+} (2.5 mM) and contractility:

The results presented suggest that the E-C coupling in the *Clarias gariepinus* heart may depend on the calcium transport through the sarcolemmal Ca^{2+} channels. If so, the diminished contractile force, the rate of contraction and the rate of relaxation as a result of addition of verapamil should be counteracted by the increasing in the extracellular calcium concentration. Therefore, the effects of increasing extracellular Ca^{2+} on the contractile force, the rate of contraction and the rate of relaxation in the presence of caffeine, verapamil and caffeine combined together with verapamil were examined. Increasing of the extracellular calcium in the range of 1.25 to 2.5 mM stimulated the contractile force, the rate of contraction and the rate of relaxation. As shown in Fig. 4 A and B, 2.5 mM extracellular Ca^{2+} removed the negative inotropic effect of verapamil on the contractile force (Fig.4 A and B), the rate of contraction (Fig. 5 A and B) and the rate of relaxation (Fig. 6 A and B) at both rates of stimulation. A similar effect of 2.5 mM of extracellular Ca^{2+} on the contractile force, rate of contraction and the rate of relaxation in the presence of caffeine and caffeine combined together

with different concentration of verapamil (Table 1 and 2) at both rates of stimulation. However, it should be noted that the increased extracellular calcium removed the negative inotropic effect of caffeine, verapamil and caffeine combined together with verapamil on the cardiac contractility and turned them into values near to or below those of control value (1.25 mM Ca^{2+}).

DISCUSSION

In the present study, caffeine, verapamil at different concentration and caffeine combined together with verapamil had a negative inotropic effect on the contractile force, rate of contraction and the rate of relaxation at the physiological rates of stimulation (0.2 and 0.4 Hz) in the myocardium of the *Clarias gariepinus*. Furthermore, the negative inotropic effect of verapamil and caffeine combined together with verapamil on the cardiac contractility was greater than that of caffeine at both rates of stimulation.

It is known that caffeine is generally used as an inhibitor of the sarcoplasmic reticulum function by preventing the Ca^{2+} uptake (Kavaler et al., 1978). However, the influence of caffeine on the cardiac contractility are dependent on concentration of caffeine and extracellular calcium, rate and duration of caffeine exposure, species and age (Eisner and Valdeolmillos, 1985; Jourdon et al., 1981). It has been shown that 8.0 mM caffeine and 0.5 mM extracellular calcium had a negative inotropic effect on the force developed after 5 minutes of rest in the rainbow trout (El-Sayed and Gesser, 1989) and at different frequencies in the catfish (El-Sayed, 1994b; El-Sayed, 2000). So, the decrease in the contractile force, rate of contraction and the rate of relaxation caused by caffeine (in the present study) agrees with the results obtained in the most ectothermic species (Hove-Madsen and Gesser, 1989; El-Sayed and Hassanin, 1995). Thus, it can be speculated that the sarcoplasmic reticulum (SR) may contribute in the excitation-contraction coupling (E-C coupling) of the catfish heart.

In mammalian cardiac muscle, verapamil had a negative inotropic effect on the contractile force, the rate of contraction and the rate of relaxation (Ponce-Hornos et al., 1990). However, in the smooth muscle verapamil caused an inhibition of the Ca^{2+} efflux measured by vibrating Ca^{2+} selective electrode during the process of contraction (Devlin and Smith, 1996). This efflux was in part the result Ca^{2+} influx through a channel resembling the mammalian L-Type channel because of its sensitivity to and block by the L-Type channel blocker, verapamil.

As in the mammalian cardiac muscle, verapamil caused a decrease in the contractile force, the rate of contraction and the rate of relaxation developed at a steady pacing rate of 0.2 Hz in the ventricular tissue of the catfish, *Clarias gariepinus*. (El-Sayed, 1999). This decreases in the force, the rate of contraction and the rate of relaxation was increased by the increasing in the verapamil concentrations. These results are in accordance with those obtained in the present study at the two pacing rates applied (0.2 and 0.4 Hz). Furthermore, the negative inotropic effect of verapamil on the contractile force, the rate of contraction and the rate of relaxation developed at 0.4 Hz was higher than that at 0.2 Hz, and the recovery effect of the increased extracellular Ca^{2+} (2.5 mM) on the contractions was higher at a stimulation rates of 0.2 Hz. These reactions indicate that the sarcolemmal Ca^{2+} may have a role in the regulation of cardiac force in the catfish, especially at a stimulation rate of 0.4 Hz. In agreement with these data, an elevation of frequency was found to increase the twitch force in cardiac muscle from most vertebrate species (Stemmer and Akera, 1986), and this increase is a result of increasing calcium through the sarcolemmal calcium flux (Bers, 1985; Boyett and Jewel, 1980). Verapamil acts on mammalian cardiac or smooth muscles to block Ca^{2+} influx by directly competing with Ca^{2+} for a common binding site on the outer membrane (Kohlhardt et al., 1972). With less Ca^{2+} available at the membrane surface, the slow inward Ca^{2+} current effectively reduced. This further support the suggestion that the calcium fluxes through the sarcolemmal Ca^{2+} channel is the primary Ca^{2+} source used in the regulation of the cardiac contractility of the catfish (*Clarias gariepinus*). However, the findings that the negative inotropic effect of verapamil on the cardiac contractility was greater than that of caffeine at both rates of stimulation again support the suggestion that the calcium transports through the sarcolemmal Ca^{2+} channels have a role in the regulation of the cardiac contractions of the catfish at a physiologically relevant frequencies i.e. 0.2 and 0.4 Hz.

As noted above in the present study, caffeine combined together with different concentrations of verapamil had a negative inotropic effect on the cardiac contractility developed at a stimulation rates of 0.2 and 0.4 Hz. However, the findings that the influence of caffeine combined together with verapamil on the cardiac contractility at 0.2 Hz was greater than that of caffeine only and verapamil without caffeine indicates that both sarcoplasmic reticulum and the calcium influx through the sarcolemmal Ca^{2+} channels may have a role in the regulation of the cardiac contractility developed at a stimulation frequency of 0.2 Hz in

the *Clarias gariepinus*. Moreover, the findings that the recovery of the force, the rate of contraction and the rate of relaxation with 2.5 mM extracellular Ca^{2+} was lower under the effect of caffeine combined together with verapamil than the effect of verapamil only at 0.2 Hz strongly support the contribution of both SR and the sarcolemmal Ca^{2+} channel in the regulation of force developed at 0.2Hz. As shown before in the present study, verapamil at different concentrations had a negative inotropic effect on the cardiac contractility more than that of caffeine only and caffeine combined together with verapamil at a stimulation rate of 0.4 Hz. These results suggest that the sarcolemmal Ca^{2+} channel seems to have a contribution in the regulation of force development at a relatively higher physiological frequency (0.4 Hz) in the catfish heart, since verapamil is known to inhibit the Ca^{2+} transport through the sarcolemmal Ca^{2+} channel (Devlin and Smith, 1996). The findings that the recovery of the contractile force with the increased extracellular calcium (2.5 mM) was lower under the effect of verapamil only than that of caffeine combined together with verapamil at 0.4 Hz again support the suggestion of the contribution of the sarcolemmal Ca^{2+} channels in the excitation-contraction coupling of the *Clarias gariepinus* heart at that frequency (0.4 Hz).

CONCLUSION

Cell membranes are dynamic structures moving many different ions simultaneously in attempts to regulate Ca^{2+} distribution between intercellular and extracellular compartments. The present experiments using verapamil (Vertebrate sarcolemmal Ca^{2+} channel blocker) and caffeine (an inhibitor of the SR function) reveal that verapamil caused a significantly decrease in the contractile force, the rate of contraction and the rate of relaxation developed at a stimulation rate of 0.2 and 0.4 Hz. However, the finding that this decrease was higher at 0.4 Hz than that at 0.2 Hz suggest that the sarcolemmal Ca^{2+} channels play an important role in the excitation-contraction coupling in the cardiac muscle of the *Clarias gariepinus* at a higher rate of stimulation i.e. 0.4 Hz. Furthermore, the results suggest that both sarcolmmal Ca^{2+} channels and the sarcoplasmic reticulum in the heart of the catfish seem to contribute in the excitation-contraction coupling at a stimulation rate of 0.2 Hz since caffeine combined together with verapamil had a significantly marked effect on the contractile force developed at 0.2 Hz than that of verapamil and of caffeine.

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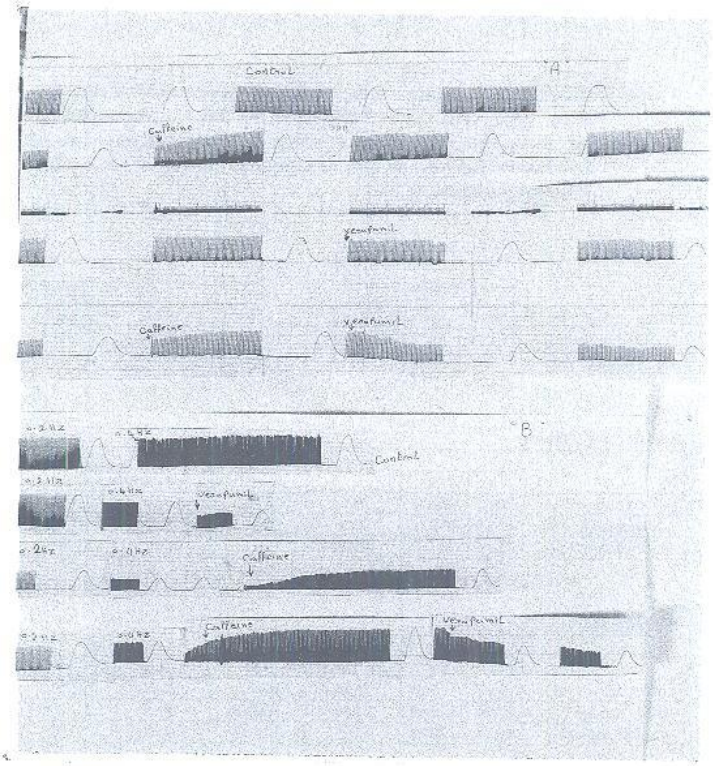

Direct recording of the changes in the cardiac force developed at 0.2 Hz (A) and at 0.4 Hz (B) in the myocardium of the catfish as a result of addition of 8.0 mM caffeine, 13 μM verapamil and 8.0 mM caffeine combind together with 13 μM verapamil. Arrows indicate start of addition of the different treatments.

Table 1: Effects of caffeine, verapamil, caffeine combined together with verapamil and 2.5 mM extracellular calcium at 0.2 Hz on force, on rate of contraction (df/dt) and on rate of relaxation (-df/dt) in the ventricullar nuscle of the catfish (*Clarias gariepinus*)

Control

		Ca^{2+}		Ca^{2+}		Ca^{2+}
Force change (%)	99±7	130±3	91±2	113±1	89±1	110±7
+df/dt change (%)	90±4	117±4	95±4	101±1	86±4	106±13
-df/dt change (%)	97±3	105±1	95±4	107±2	98±4	116±2

8.0 m M Caffeine

		Ca^{2+}		Ca^{2+}		Ca^{2+}
Force change (%)	70±2	98±3	78±7	123±2	49±4	120±1
+df/dt change (%)	76±5	110±5	70±6	101±2	50±2	107±2
-df/dt change (%)	78±6	107±2	75±5	102±1	64±3	113±3

Verapamil

	5 µM		9 µM		13 µM	
		Ca^{2+}		Ca^{2+}		Ca^{2+}
Force change (%)	61±7	115±3	55±4	104±3	50±3	103±10
+df/dt change (%)	65±6	112±4	55±4	104±3	48±8	105±7
-df/dt change (%)	70±3	118±2	65±3	109±2	54±2	91±2

8.0 mM Caffeine + Verapamil

	Caf. + 5 µM V.		Caf. + 9 µM V.		Caf. + 13 µM V.	
		Ca^{2+}		Ca^{2+}		Ca^{2+}
Force change (%)	59±2	81±1	44±2	106±5	35±2	95±6
+df/dt change (%)	64±5	78±2	58±4	98±2	32±4	104±14
-df/dt change (%)	66±7	98±2	64±5	97±4	40±2	94±3

Table 2: Effects of caffeine, verapamil, caffeine combined together with verapamil and 2.5 mM extracellular calcium at 0.4 Hz on force, on rate of contraction (df/dt) and on rate of relaxation (-df/dt) in the ventricullar nuscle of the catfish (*Clarias gariepinus*)

Control

		Ca^{2+}		Ca^{2+}		Ca^{2+}
Force change (%)	86±3	127±2	91±2	99±2	90±4	99±1
+df/dt change (%)	95±4	103±1	88±2	125±2	84±2	108±6
-df/dt change (%)	98±2	110±2	97±3	110±5	89±3	100±3

8.0 mM Caffeine

		Ca^{2+}		Ca^{2+}		Ca^{2+}
Force change (%)	80±5	105±2	74±4	110±1	64±6	112±2
+df/dt change (%)	86±1	95±2	73±6	84±2	75±1	95±2
-df/dt change (%)	90±3	102±2	89±5	110±3	86±3	103±3

Verapamil

	5 µM		9 µM		13 µM	
		Ca^{2+}		Ca^{2+}		Ca^{2+}
Force change (%)	48±3	113±2	40±4	70±2	30±1	51±2
+df/dt change (%)	61±3	112±2	61±2	81±1	50±1	93±3
-df/dt change (%)	95±3	108±2	63±1	80±2	40±2	80±2

8.0 mM Caffeine + Verapamil

	Caf. + 5 µM V.		Caf. + 9 µM V.		Caf. – 13 µM V.	
		Ca^{2+}		Ca^{2+}		Ca^{2+}
Force change (%)	65±2	87±2	51±1	81±2	45±3	73±1
+df/dt change (%)	78±2	90±1	61±1	80±2	54±3	90±3
-df/dt change (%)	84±5	99±3	63±3	80±2	56±4	80±1

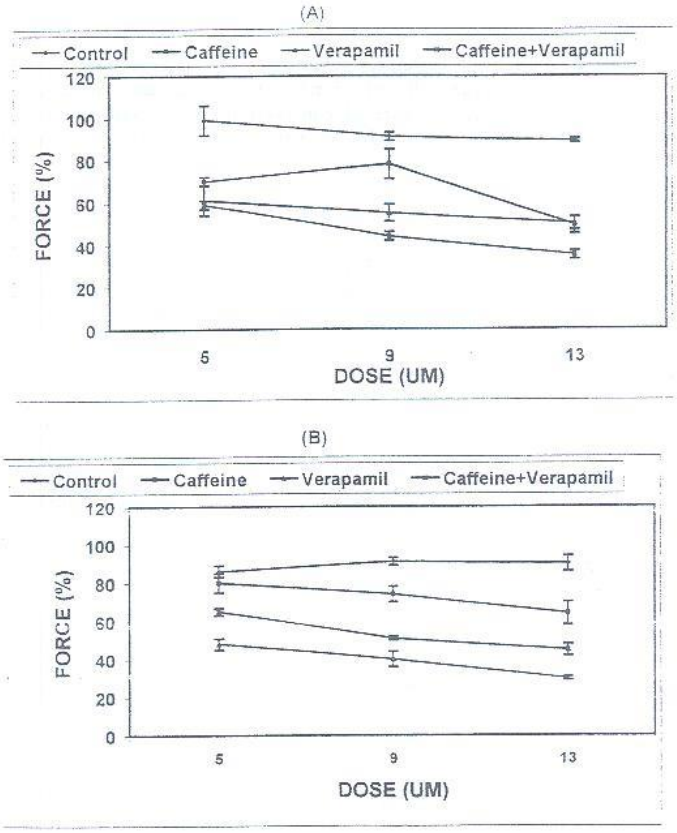

Fig .1: Changes in the contractile force at o.2Hz (A) and at o.4 Hz(B) of *Clarias gariepinus* cardiac muscle under different conditions: control (◆) ; 5, 9 and 13µM verapamil (▲);8.0 Mm caffeine(■) and 8.0 Mm caffeine combined together with 5, 9 and 13µM verapamil (✕) .n=6 in both A and B .

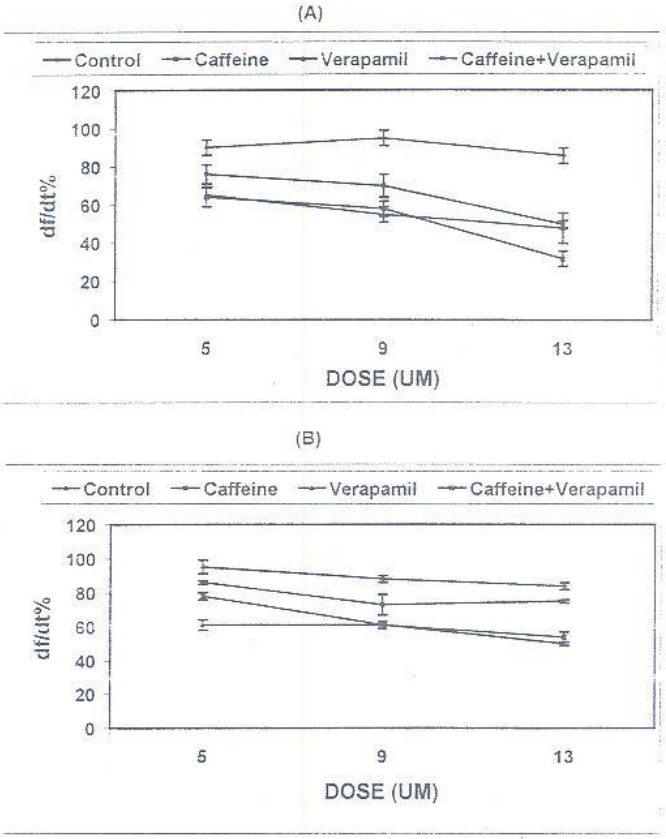

Fig .2 : Changes in the rate of contraction(df/dt)at o.2Hz (A) and at o.4 Hz(B) of *Clarias gariepinus* cardiac muscle under different conditions: control (◆) ; 5, 9 and 13 µM verapamil (▲);8.0 Mm caffeine(■) and 8.0 Mm caffeine combined together with 5, 9 and 13 µM verapamil (✕) .n=6 in both A and B .

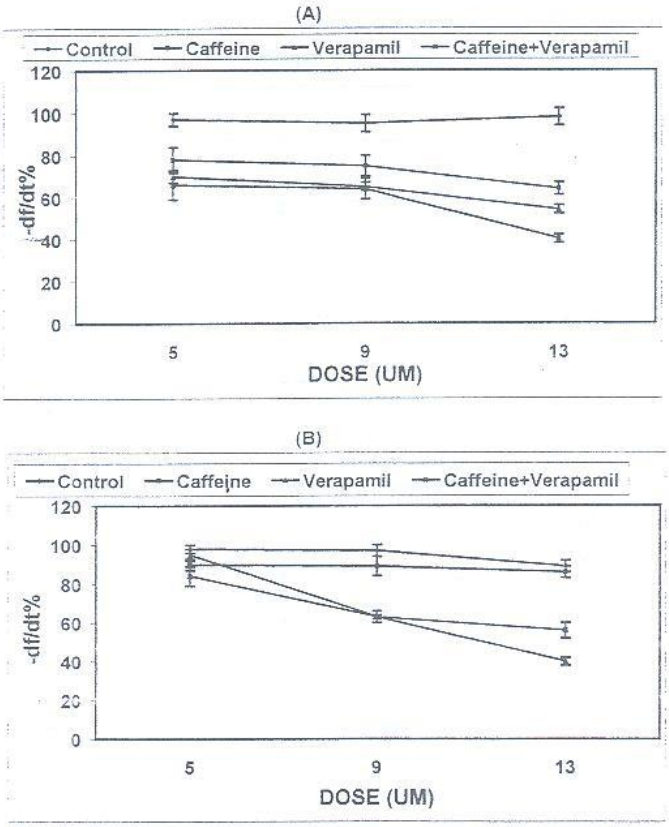

Fig .3 : Changes in the rate of relaxation (-df/dt)at o.2Hz (A) and at o.4 Hz(B) of *Clarias gariepinus* cardiac muscle under different conditions: control (◆) ; 5, 9 and 13 µM verapamil (▲);8.0 Mmcaffeine(■) and 8.0 Mm caffeine combined together with 5, 9 and 13 µM verapamil (×) .n=6 in both A and B .

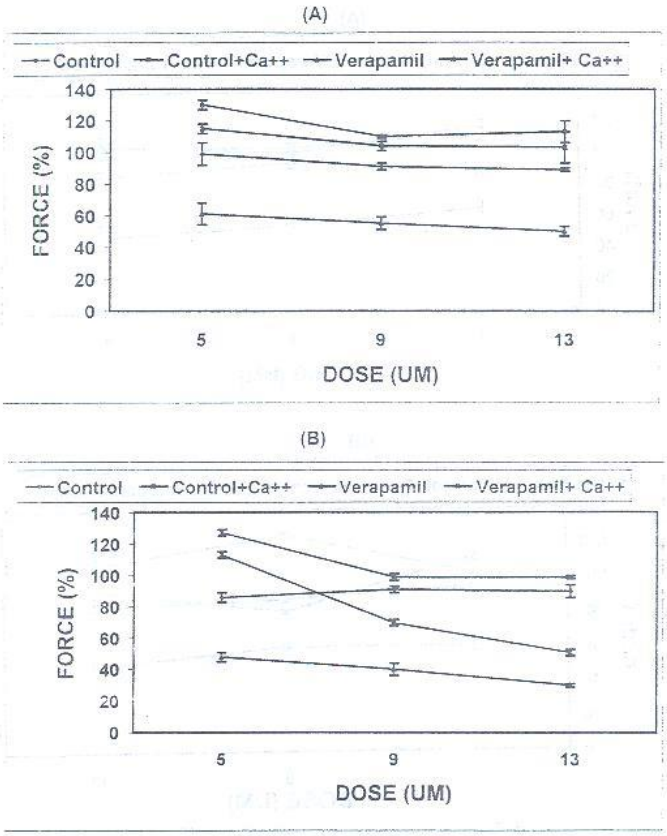

Fig .4 : Influence of 2.5 mM Ca2+on the contractile force at o.2Hz (A) and at o.4 Hz(B) of *Clarias gariepinu*s cardiac muscle in the absence and in the presence of 5, 9 and 13 µM verapamil .n=6 in both A and B .

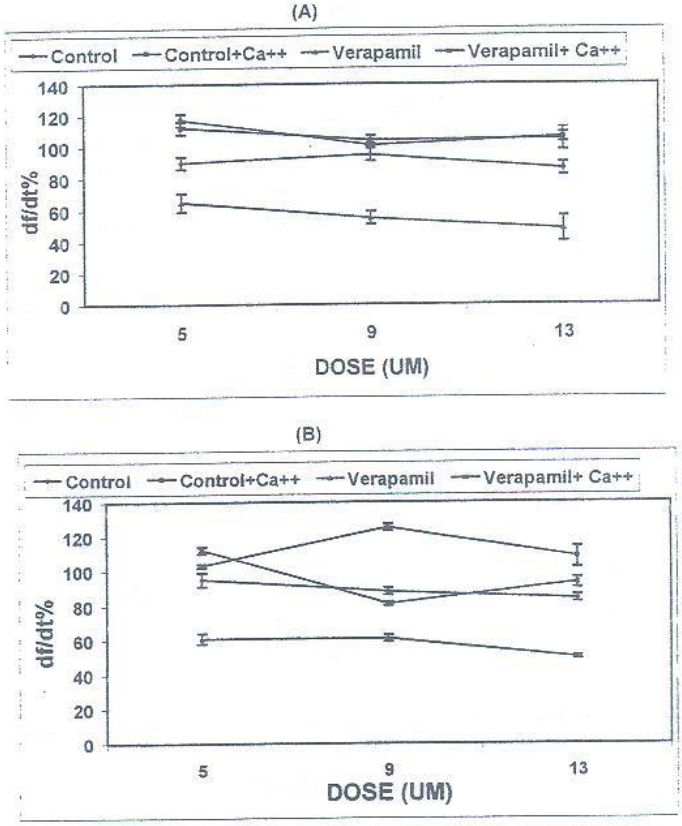

Fig .5 : Influence of 2.5 mM Ca2+on the rate of contraction(df/dt) at o.2Hz (A) and at o.4 Hz(B) of *Clarias gariepinu*s cardiac muscle in the absence and in the presence of 5, 9 and 13 µM verapamil .n=6 in both A and B .

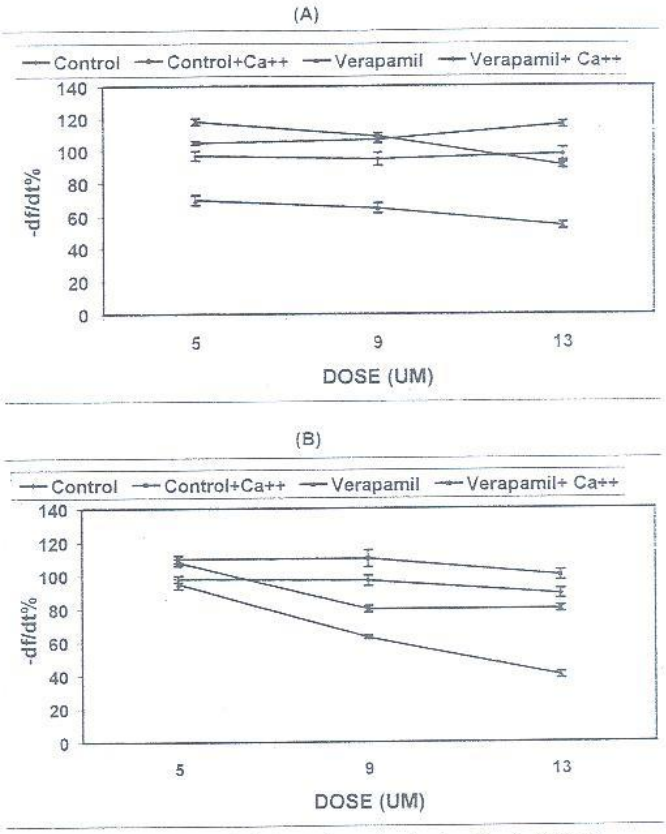

Fig .6 : Influence of 2.5 mM Ca2+on the rate of relaxation (-df/dt) at o.2Hz (A) and at o.4 Hz(B) of *Clarias gariepinu*s cardiac muscle in the absence and in the presence of 5, 9 and 13 ΜM verapamil .n=6 in both A and B .