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# EFFECTS OF ADRENALINE, CAFFEINE AND VERAPAMIL ON THE CARDIAC CONTRACTILITY AFTER REST INTERVAL IN TELEOSTS AND AMPHIBIANS

(With 8 Figures)

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## تأثيرات الأدرينالين والكافين والفيرابابيل على الانقباضات القلبية بعد فترة راحه في الأسماك العظيمه والبرمائيات

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أجريت هذة الدارسه لإختبار تأثير الأدرينالين و الكافين والفيراباميل على الانقباضات القلبيه في أنسجه بطين الأسماك العظيمه ( القرموط) و البرمائيات ( الضفدعه )وذلك لمحاولة معرفه دور الصفيحه اللحميه و الغشاء الخلوى في هذه الانقباضات و قد تبين مايلى. بالمقارنه مع قوة الضربه عند ١٢ ضربه لكل دقيقه وجد أن فترة راحه لمدة خمس دقائق أدت إلى إزدياد ونقصان في قوة الضربه في الاسماك و الضفادع على التوالى. أربعه ميكروجزى جرام من الادرينالين أدى إلى نقصان الإضمحلال الذى حدث بعد فترة الراحه في قلب الضفدعة ولكن ٢,٥ ميللى جزى جرام من أيون الكالسيوم أدى إلى زيادة التحفيز بعد فترة الراحه في قلب السمكه وأزالة الاضحلال في قلب الضفدعه. الكافين ( ٨مليلىجزئ جرام) أدى إلى زيادة في إضمحلال الضربات القلبية بعد فترة الراحه في قلب الضفدعه والى تحويل التحفيز في الضربات القلبيه إلى إضمحلال في قلب السمكه هذا الإضمحلال أزيل بإضافة ٢,٥ ميللى جزئ من الكالسيوم مما أدى إلى زيادة في الضربات القلبيه لكل سمكه و الضفدعه. فيراباميل (١٠ ميكرو جزئ جرام) أدى إلى تحويل التحفيز في الضربات القلبية للسمكة إلى إضمحلال بينما أدى إلى نقصان الاضمحلال في الضربات القلبية لقلب الضفدعة بينما أدى زيادة أيون الكالسيوم من ١,٢٥ – ٢,٥ ميللى جزئ جرام إلى إزالة التأثير السلبى للفيراباميل وبطريقة أدت إلى زيادة في الضربات القلبية للسمكة والضفدعة. والخلاصة أن الصفيحة اللحمية في قلب سمكة القرموط يبدو أنها تدعم الضربات القلبية باعتمادها على الادرينالين بينما يبدو أيضا أن الغشاء الخلوى يلعب دوراً بسيطاً في تنظيم الضربات القلبية . أما في قلب الضفدعة يبدو أن الذى يدعم الضربات القلبية هو الكالسيوم المتدفق من خلال الغشاء الخلوى بينما تلعب الصفيحة اللحمية دوراً ، (صغيراً) في تنظيم تلك الضربات.

## SUMMARY

This study was conducted to examine the influence of adrenaline, caffeine and verapamil on the cardiac contractility in the ventricular tissues of teleost (*Clarias lazira*) and of amphibian (*Bufo regulgris*), regarding the role of sarcoplasmic reticulum and sarcolemma. Compared with the steady state twitch force at a stimulation rate of 0.2 Hz, a 5 min. rest led to an increase and to a decrease in the cardiac contractility of fish and frog; respectively. Adrenaline (4 µM/L) increased the post-rest potentiation of contractility in fish, but 8 µM/L of adrenaline decreased the post – rest decay of the cardiac contractility in frog. 2.5 mM of extracellular calcium ($Ca_o$) increased the post-rest potentiation and removed the post-rest decay caused by adrenaline in fish and frog; respectively. Caffeine (8 mM) increased the post-rest decay in the cardiac contractility in the frog ventricular tissue, whereas it was transformed the post-rest potentiation into post-rest decay in the ventricular tissue of fish. These post – rest decays were removed by 2.5 mM $Ca_o$ which increased the cardiac contractility in both tissues, also verapamil (10 µM/L) transformed the post-rest potentiation of the cardiac contractility in fish into a post – rest decay, whereas it decreased the post –rest decay of the cardiac contractility in frog. Increasing of $Ca_o$ from 1.25 to 2.5 mM removed the negative inotropic effect of verapamil in such a way that increased the cardiac contractility in the ventricular tissues of both animals. In conclusion, the cardiac sarcoplasmic reticulum of the *Clarias lazira* seems to support cardiac contractility during adrenaline dependent, whereas sarcolemmal $Ca^{2+}$ transport plays a minor role. In amphibian, the situation is opposite since it seems that the sarcolemmal $Ca^{2+}$ channels transport play an important role in the regulation of the cardiac contractility developed after a 5 minutes of rest.


***Key words:*** *Effects of Adrenaline, Caffeine & Verapamil, On The Cardiac, Contractility.*

## INTRODUCTION

The role of the sarcoplasmic reticulum (SR) in the cardiac contractility seems to vary among different ectothermic vertebrate species (Tibbits et al. 1991; Driedzic and Gesser, 1994; EL-Sayed, 1997). It is probally small in amphibians (Chapman, 1983) but significant in the ventricular tissue of the cat fishes, Clarias lazira, (EL-

Sayed, 1994). The cardiac SR of the frog appears to be ultrastructurally less developed (Page and Niedergerk, 1992; Mcleod et al. 1991) than that of the Clarias lazira myocardium (EL-Sayed, 1994). Furthermore, isolated ventricular tissue of Clarias lazira differs from that of frog in displaying a post-rest potentiation, which is strongly reduced by ryanodine and caffeine (EL-Sayed 1994). Thus, it is believed that the SR participates in the excitation-contraction coupling. Also, it has been illustrated that the SR in the trout myocardium has a role in the regulation of the twitch force (Gesser, 1996). Ryanodine and caffeine specifically inhibits the function of the SR by interfering with the opening mechanism of its $Ca^{2+}$ channels. The function of the myocardial SR in the living Clarias is still unclear. It does not seem important at stable pacing frequencies within the physiological range (EL-Sayed, 1994), but it participates in the regulation of the twitch force at unphysiologically low rates of stimulation. However, studies on frog suggest that the cardiac SR is sparsely developed in comparison to that in birds and mammals (Sommer and Johnson, 1969) in which the E.C coupling is very SR dependent (Stemmer and Akera, 1986). Also, it has been reported that the cardiac SR of frog may function as a mediator for different hormones and hormone – like substances (Niedergerke and page, 1989). Adrenaline is of obvions interest in this respect. It increases myocardial contractility (Brückner et al., 1985; Drake – Holland et al., 1992; keen et al., 1993) by a number of mechanisms. In mammals, the cellular uptake of $Ca^{2+}$ is increased (Carmelict and Veereccke, 1969) as well as for the flounder (lennard and Huddart, 1989) and trout myocardia (Gesser, 1996).

The importance of the sarcolemmal $Ca^{2+}$ transport systems in the control of cardiac contractility seems to vary among different vertebrate species. In teleost the cardiac sarcolemma appears to be less developed compared with that of mammals according to ultrastructural studies (Gabella, 1978). In rabbit ventricular tissues, the force developed upon the first stimulation after the rest interval was found to be less dependent on the sarcolemmal $Ca^{2+}$ influx and more sensitive to interventions with the SR function than the force attained upon the subsequent stimulations (Bers, 1985). The cardiac excitation – contraction coupling in the clarias is highly SR dependent when the heart paced to contract at unphysiologically rates of frequency (5 min. rest), whereas, the sarcolemmal calcium channel also contribute in the regulation of force at that frequency, particuly evident as a post rest potentiation, which is reduced by verapamil, an inhibitor of sarcolemmal $Ca^{2+}$ channel, (EL-

Sayed, 1999). In amphibian myocardium, the development of the twitch force after rest intervals may relates to the $Ca^{2+}$ sequestration by the SR, but it, depends crucially on the sarcolemmal $Ca^{2+}$ transport (Chapman, 1983). Adrenaline is an important regulator of heart rate in vivo. At the cellular level, it enhances the $Ca^{2+}$ current of the action potential (Carmeliet and Vecrecke, 1969), and may stimulate SR function (Hunter et al. 1983) and the Na – K pump (Pecker et al., 1986).

The present study on heart ventricular tissue from catfish (Clarias lazira) and from frog (Bufo regularis) looks at the effects of adrenaline, caffeine and verapamil on the contractility developed after 5 minutes of rest to examine the function of the SR and the sarcolemma in the regulation of the cardiac contractility in these animals.

## MATERIALS and METHODS

Catfish, *Clarias lazira*, weighing 100 – 200g, of both sexes were kept in freshwater tanks at room temperature for about 3 weeks. Frogs, *Bufo regularis*, of both sexes were captured during June and July monthes from farms near to Sohag city and were transported to zoology department at the faculty of science (Sohag) where 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 removed while still beating and placed in an ice-cold physiological ringer solution, where ventricular strips for fish and frog were prepared.

The standard physiological solution for the fish and frog contained in mM : Na Cl 125, KCl 2.5, $CaCl_2$ 1.25, Mg $So_4$ 0.94, $NaH_2$ $Po_4$ 1, $NaHCo_3$ 15, and glucose 5-The solution was gassed by a gas mixing pump (Wösthoff 1 M 301/af). The gas mixture contained 99% $O_2$ and 1% $Co_2$ for both fish and frog. The experimental temperature was 15±0.5 (Cole-Parmer OT 268/ 16, USA) for both animals. In the experiments the composition as to calcium was altered. The desired $Ca^{2+}$ was obtained by the addition of appropriate amounts of IM Ca $Cl_2$. Adrenaline– tartrate and verapamil (Sigma) were dissolved in distilled water to 10 mM/L and kept frozen (-18C ) in suitable portions. Caffeine (sigma) was added as powder.

For the recording of the contractility, the upper end of the preparation was connected with surgical silk to a force tranceducer (Grass FT O3), which was connected to a recorder (Grass model 79G). The lower end was tied onto one of the platinum stimulation electrodes. The other electrode was placed in the solution just above the preparation.

The preparation was stimulated to contraction by electrical square pulses having a duration of 5m sec. and a voltage 1.5 – 2 times the threshold for full contraction. 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. Then the length of the preparation was 7- 15 mm whereas its thickness never exceeded 2mm.

After the initial adjustments, each preparation was left at 0.2 Hz for about 30 minutes for stabilization before further intervention. The force values measured in the subsequent part of the experiment were all normalized (%) to the twitch force recorded at the end of these 30 minutes.

To examine the effect of adrenaline, caffeine and verapamil on the contractility after rest interval of 5 minutes, four strips from each ventricles were run in parallel at a stimulation rate of 0.2 Hz for about 30 minutes. After stabilization of force, the first strip was exposed to 4 μM/L of adrenaline in fish and 8μM in frog, the second strip was exposed to 8 mM caffeine in both fish and frog, the third was exposed to 10 μM/L verapamil in both animals, whereas the fourth was maintained at control conditions. 5 – 10 minutes after these changes, the stimulation rate at 0.2 Hz was intrupted by rest intervals of 5 minutes with one concluding stimulation. After these interventions, the four strips were exposed to 2.5 mM $Ca^{2+}$ to investigate the influence of the increased extracellular calcium on the contractility after the 5 min of rest in both fish and frog hearts.

The results are presented as mean ± SD. Significances were tested with student's t – test for either paired or unpaired samples. The limit of significance was set at $P< 0.05$; n indicates number of pairs of fishes and frogs.

## RESULTS

The cardiac contractility developed after rest interval of 5 minutes in the catfish and the frog was examined under the influence of adrenaline, caffeine and verapamil to examine the role of the SR and sarcolemma in the regulation of contractility. Fig. 1 shows that the cardiac contractility after 5 minutes of rest in the catfish (*Clarias lazira*) increased relative to that at steady state at 0.2 Hz, i.e. it exhibits a post-rest potentiation, whereas it decreased i.e. it exhibits a post-rest decay in frog ventricular tissue (Fig.2). Increasing of $Ca_o$ from 1.25 to 2.5 mM led to increasing of the cardiac contractility in the catfish (Fig. 1) and to

removing of the post-rest decay and turning it into an increase in the frog cardiac muscle (Fig. 2).

**Adrenaline and contractility:**

Adrenaline (4μM/L) increased the post-rest potentiation in the contractile force of the catfish myocardium significantly (Fig. 1). However 8 μM/L of adrenaline decreased the post-rest decay in the contractile force of the frog myocardium (Fig. 2). 2.5 mM $Ca_o$ increased the potentiation in the contractile force caused by adrenaline in the fish myocardium (Fig. 1), whereas it removed the decay in the contractile force and turned it into an increase in the frog myocardium (Fig. 2).

The potentiation in the rate of contraction (df/dt) and the rate of relaxation (–df/ dt) was increased in the fish myocadium as a result of addition of adrenaline (Fig. 3, Fig.5). In frog myocardium, adrenaline increased the decay in df/dt (Fig.4), but it decreased the decay in – df/dt (Fig.6). The increasing $Ca_o$ (2.5mM) increased the potentiation of df/dt and – df/dt in the fish myocardium, and removed the decay in both df/dt and -- df/dt of the frog myocardium. It should be noted that increasing in potentiation of force, df/dt and – df/ dt in the fish myocardium as a result of the increased extracellular callcium was similar to those in the frog myocardium after removing the decay caused by adrenaline.

As shown in Fig.7 and Fig.8, the time to peak tension (TPT) developed after 5 minutes at rest in the fish and frog myocardia increased relative to that at a stimulation rate of 0.2 Hz. However, the potentiation in TPT was higher in frog than in fish myocardium. Adrenaline removed the potentiation in TPT in frog (Fig.8) and increased that in fish cardiac muscle unsignificantly (Fig. 7). 2.5 mM $Ca_o$ increased the potentiation of the TPT caused by adrenaline in the fish and frog cardiac muscle by the same amount.

**Caffeine and contractility:**

Caffeine (8 mM), an inhibitor of the SR function, transformed the post-rest potentiation in the contractile force of the fish ventricular tissue into a post-rest decay (Fig. 1) which is removed by 2.5 mM $Ca_o$. Whereas, it doubled the post-rest decay in the frog ventricular tissue which, also removed by the increased $Ca_o$ (Fig. 2).

The potentiation in the df/dt and – df/dt of the fish cardiac muscle was transformed into a decay as a result of application of caffeine (Fig. 3 and Fig. 5). In the frog cardiac muscle, the decay in the df/dt developed after 5 minutes of rest was doubled by 8 mM caffeine (Fig. 4). However, caffeine increased the decay in the – df/dt to more than three times relative to that developed after 5 minutes of rest (Fig.6).

2.5 mM $Ca_o$ removed the decay in the df/dt and – df/dt gained by caffeine in the cardiac muscle of both animals (Fig. 3, Fig. 4, Fig. 5 and Fig. 6).

The post-rest potentiation in TPT was transformed into a decay in the fish (Fig. 7) and was removed in the frog cardiac muscle (Fig. 8) by application of 8mM caffeine. Extracellular $Ca_o$ (2.5 mM) removed the decay in TPT in the fish myocardium and turned it into an increase (Fig. 7). In the frog myocardium, the removal in TPT caused by caffeine was turned into an increase as a result of addition of 2.5 mM $Ca_o$, also (Fig. 8).

**Verapamil and Contractility:**

Verapamil, an inhibitor of the sarcolemmal $Ca^{2+}$ channel, was used in the present study to explore the role of the sarcolemmal $Ca^{2+}$ in the regulation of the contractility in the teleost and amphibian myocardia. Verapamil (10μM/L) transformed the potentiation in the cardiac twitch force developed after 5 minuts of rest in the fish into a decay (Fig.1) which was turned into an increase by 2.5 mM $Ca_o$. Whereas, in frog cardiac muscle verapamil decreased only the post-rest decay of the twitch force. Also, as in the fish myocardium, 2.5 mM $Ca_o$ turned this decay into an increase (Fig. 2).

In the fish myocardium the post-rest potentiation in df/dt and in – df/dt was transformed into a decay by verapamil, and this decay was recovered by 2.5 mM $Ca_o$ (Fig. 3 and Fig. 5). Application of 10 μM verapamil only decreased the post-rest decay in the df/dt and – df/dt by a similar value in the frog myocardium. This decay in df/dt and – df/dt was turned into an increase by 2.5 mM $Ca_o$ (Fig. 4 and Fig. 6).

The post-rest potentiation in TPT was increased unsignificantly, in the fish myocardium, and transformed into a decay in the frog myocardium by verapamil (Fig. 7 and Fig. 8). Both increasing and decay in TPT of fish and frog myocardium were recovered and turned into an increase by 2.5 mM $Ca_o$ (Fig. 7 and Fig. 8).

It should be noted that the negative inotropic effect of caffeine and verapamil on the cardiac contractility was higher in frog than that in fish. However, adrenaline increased the post-rest potentiation of contractile variables in fishes but, it decreased the post-rest decay in frog. Also, in frog myocardium, caffeine increased the post-rest decay of force, df/dt and – df/dt more than that of adrenaline and verapamil.

## DISCUSSION

The results obtained in the present study for the Clarias heart indicate that it exhibits a post-rest potentiation. This potentiation was transformed into a decay in the presence of caffeine and ryanodine (EL-Sayed, 1994). These reactions strongly suggest an involvement of the SR. This is in accordence with that of many other vertebrate hearts (EL-Sayed and Gesser, 1989, Hove- Madsen, 1992). As should be expected from such an involvement, the frog heart, which is claimed to have a poorly developed SR (Page and Niedergerk,1992) shows a post-rest decay after 5 min of rest. The conclusion that the frog heart does not depend on the SR is strengthened by the finding that the post-rest decay is decreased by adrenaline and verapamil (an inhibitor of the sarcolemmal $Ca^{2+}$ channel.

Adrenaline enhances the sequestration of calcium ions, $Ca^{2+}$, from 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 (Morad and Goldman, 1973). In the isolated guinea pig papillary muscles, adrenaline has a positive inotropic effect on contractility may consist of two components. (1) an increase in activator circulating within the internal store, and (2) an increase in the fraction of activator recirculated from beat to beat. The speed of recireulation also appears to be increased by adrenaline (Drake-Holland et al., 1992). These results are in accordence with that obtained for *Clarias lazira* heart in the present study. As in the trout myocardium (Gesser, 1996) adrenaline enhances the force developed after 5 min. of rest in the fish myocardium (*Clarias lazira*). This action of adrenaline seems to involve an activation of the SR, as it was increased by2.5 mM extracellular $Ca^{2+}$. The frog heart is of interest because it displays a positive staircase in the presence of tetrodotoxin (Chesnais et.al., 1978). Nevertheless, this staircase seems to depend on a cellular accumulation of Na+, since the Na+ withdrawal contracture was enhanced by a preceding period at an elevated frequency (Chapman and Tunsall 1983). Adrenaline diminished the negative inotropic effect of the twitch force developed in the frog heart after 5 min. of rest. This negative influence of adrenaline may ascribed to an effect on the cellular $Na^+$ balance. It is known that adrenaline stimulates the $Na^+$ - $K^+$ pump (Pecker *et al.* 1986), and its strong inhibition of the positive inotropic effect of an elevation of frequency may consist of a

counteraction of a cellular accumulation of $Na^+$. In support of this suggestion, the $Na^+$ withdrawal, contracture of the frog heart was diminished by adrenaline (Chapman and Tunstall, 1983). This effect may be exercised via $Na^+$- $Ca^{2+}$ exchange, and consequently the amount of $Ca^{2+}$ entering across the sarcolemma during excitation. Thus, it seems that the sarcolemmal $Ca^{2+}$ transport contributes in the regulation of contractility in the frog heart. This is supported by the correlation between the effect of adrenaline on the twitch force and the rate of contraction (df/dt).

Caffeine, an inhibitor of the SR function, transformed the post-rest potentiation of contractility developed after 5 min. of rest in the cardiac muscle of the fish into a post-rest decay. These findings agree with that obtained in many vertebrate species (Hove – Madsen, 1992) and also with that shown in the previous studies (El-Sayed, 1994). This reaction is believed to reflect an involvement of the SR in the E-C coupling and are also seen for rat cardiac muscle in which the E-C coupling is very SR dependent (Stemmer and Akera, 1986). However, the situation is different for the cardiac muscle of the frog where caffeine lead to highly significant increase in the post-rest decay of contractility. It seems that the contractility in the frog myocardium does not depend on the SR, even the negative inotropic effect of caffeine on the contractility. This conclusion is strengthened by the finding that the force developed after 5 min. of rest tended to decay relative to that at 0.2 Hz.

The results obtained for the fish (*Clarias lazira*) indicate that verapamil, an inhibitor of the sarcolemmal calcium chammel (Devlin 1993), had a negative inotropic effect on the contractility (post-rest decay). It has been claimed that verapamil decreased the developed force, rate of contraction and the rate of relaxation in the mammalian myocardium (Ponce – Hornos et al., 1990). Also it is known that the sarcolemmal calcium channel mediates the transport of calcium from the extracellular space (Reuter, 1983) and this calcium is responsible for the activation of the contractile proteins and thereby the contractile force. The verapamil action probably involves an inhibition of this transarcolemmal calcium influx via the calcium channel. Thus, the post-rest decay in the contractility of the *Clarias lazira* caused by verapamil, in the present study, may be due to the impairing or reducing of calcium entry via the calcium channel which stimulate the contractile proteins. So, it can be concluded that the transarcolemmal $Ca^{2+}$ channel has to some extent a role in the cardiac E-C coupling in the *Clarias lazira*

heart. In the frog heart, verapamil only caused significant decrease in the post-rest decay of contractility. However, this decrease was abolished by the increased extracellular $Ca^{2+}$ (2.5 mM). In spite of the highly negative inotropic effect of caffeine on the contractility, this finding support the suggestion that the sarcolemmal $Ca^{2+}$ channel influx plays a major role in the cardiac E-C coupling of the frog.

In conclusion, the excitation – contraction coupling in the cardiac muscle of the *Clarias lazira* is highly dependent on the SR, particularily evident as post-rest potentiation which is increased by adrenaline and is strongly reduced by caffeine, an inhibitor of the SR function. Also, it should be noted that the sarcolemmal $Ca^{2+}$ channel has a minor role in the exitation – contraction coupling. This suggestion is strenghtened by the finding that verapamil, an inhibitor of the sarcolemmal $Ca^{2+}$ channel influx has a negative intortopic effect on contractility which is abolished by the increasing of the extracellular $Ca^{2+}$ (2.5 mM). In the frog cardiac muscle, the E-C coupling, in the opposite to that of the coadiac muscle of the catfish is highly dependent on the sarcolrmmal $Ca^{2+}$ influx. This is because adrenaline, which is known to decrease the $Na^{+}$ withdrawal in the frog heart and thereby decrease the $Ca^{2+}$ entry through the sarcolemma via $Na^{+}$-$Ca^{2+}$ exchange, still has a negative inotropic effect on the contractility. Again, this suggestion is supported by the finding that verapamil, a specific inhibitor of the sarcolemmal $Ca^{2+}$ channel, decreased a post-rest decay which is removed by 2.5 mM extracellular $Ca^{2+}$.

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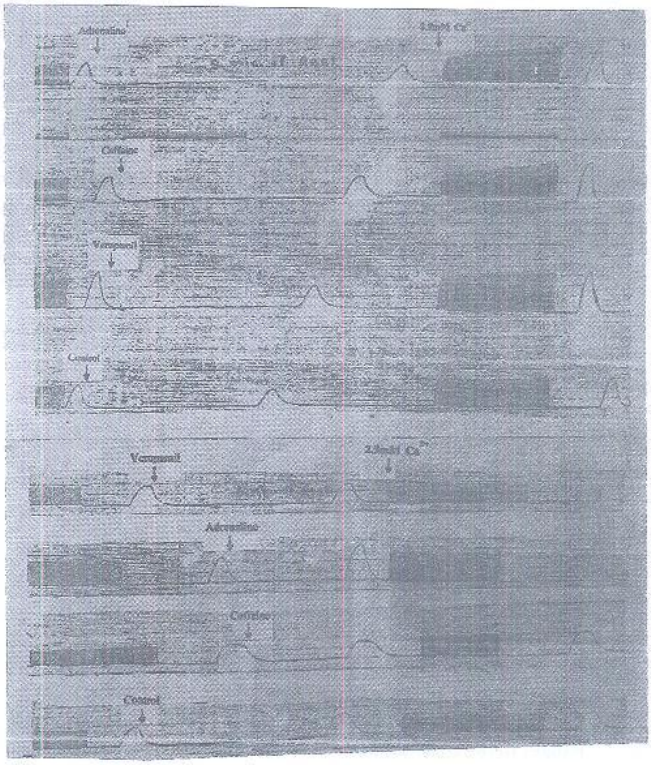


Direct recording of the changes in force developed after 5 minutes of rest under the effect of adrenaline, caffeine, verapamil and 2.5 mM extracellular Calcium in the ventricular tissues of frog (A) and Catfish (B). Arrows indicate start of addition of the different treatments.

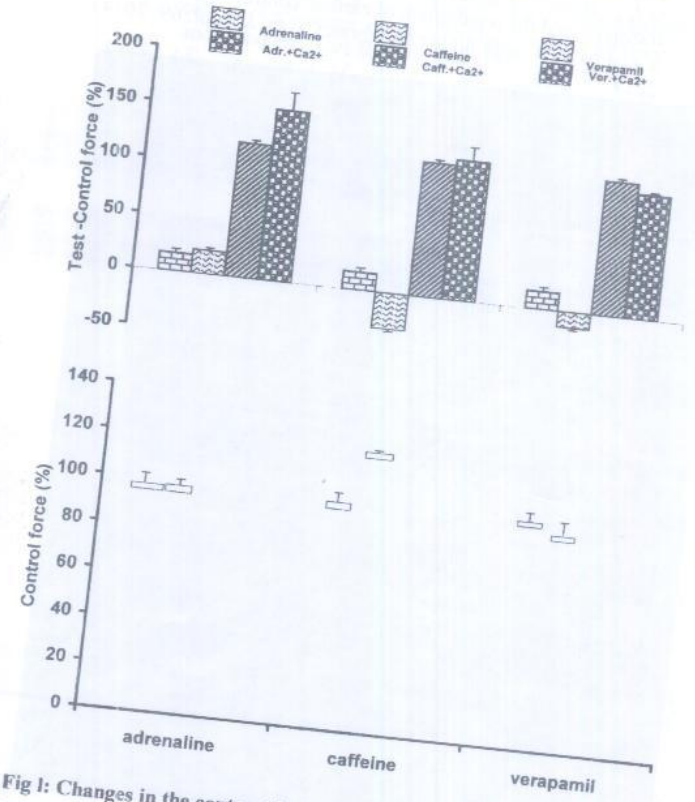


**Fig 1: Changes in the contractile force of the *Clarias lazira* cardiac muscle after 5 minutes of rest with one concluding stimulation under different conditions, control( ), control + 2.5 mM Ca2+ ( ) 4u M/L adrenaline ( ), 4u m M/L adrenaline + 2.5 mM Ca 2+ ( ), 8mM caffeine ( ), 8mM caffeine + 2.5 mM Ca 2+ ( ), 10u M/L verapamil ( ) and 10 uM/L verapamil + 2.5mM Ca +2. ( ), n=6.**

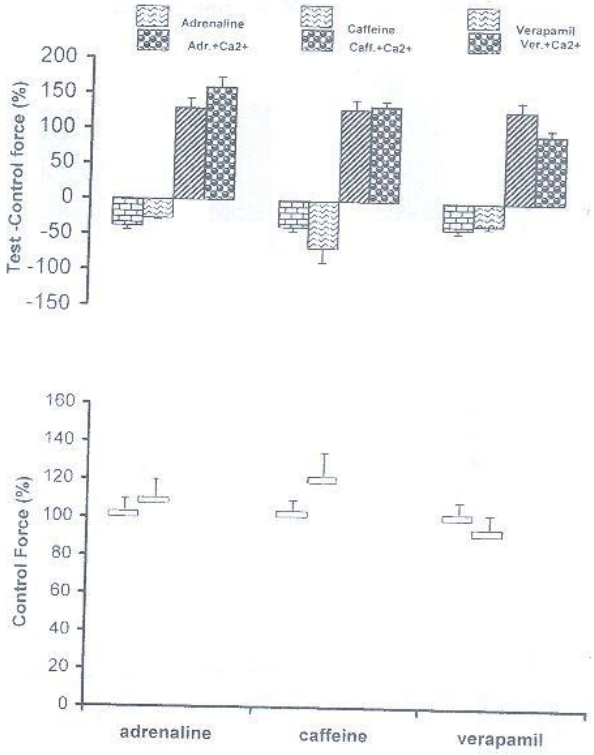


**Fig.2: Changes in the contractile force of the frog cardiac muscle after 5 minutes of rest with one concluding stimulation under different conditions. Experimental protocol as in Fig.1, except the concentration of adrenaline, it was 8 uM/L. n=6.**

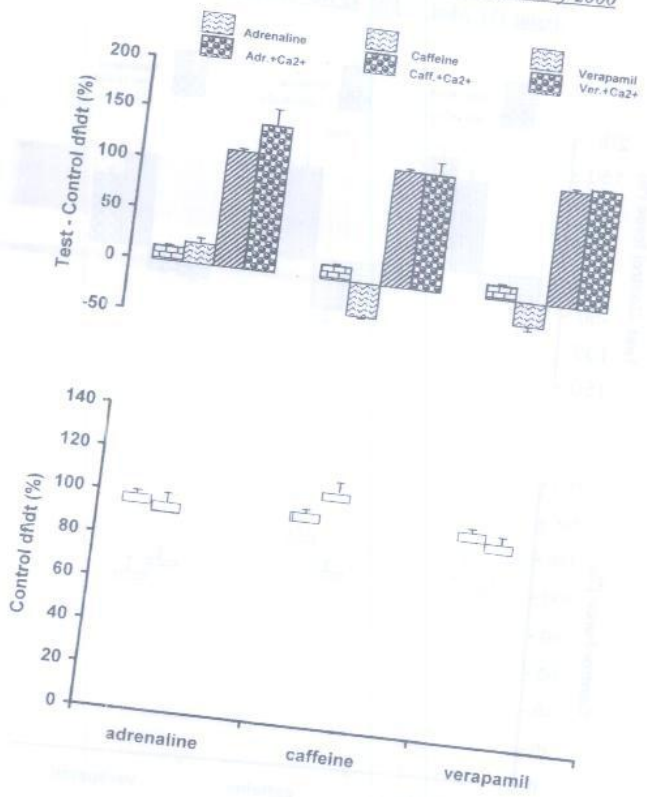


**Fig.3: Influence of adrenaline, caffeine and verapamil on the changes in the rate of contraction (df/dt) developed after 5 min.of rest in the cardiac muscle of the *Clarias lazira*. Experimental protocol as in Fg.1. n=6.**

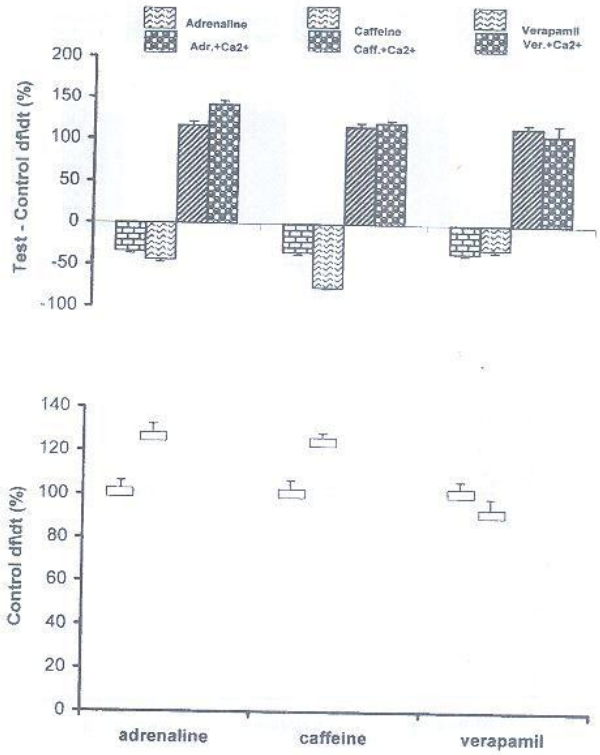


**Fig. 4. Influence of adrenaline, caffeine and verapamil on the changes in the rate of contraction (df/dt) developed after 5 minutes of rest in the frog cardiac muscle. Experimental portocol as in Fig.1. n=6.**

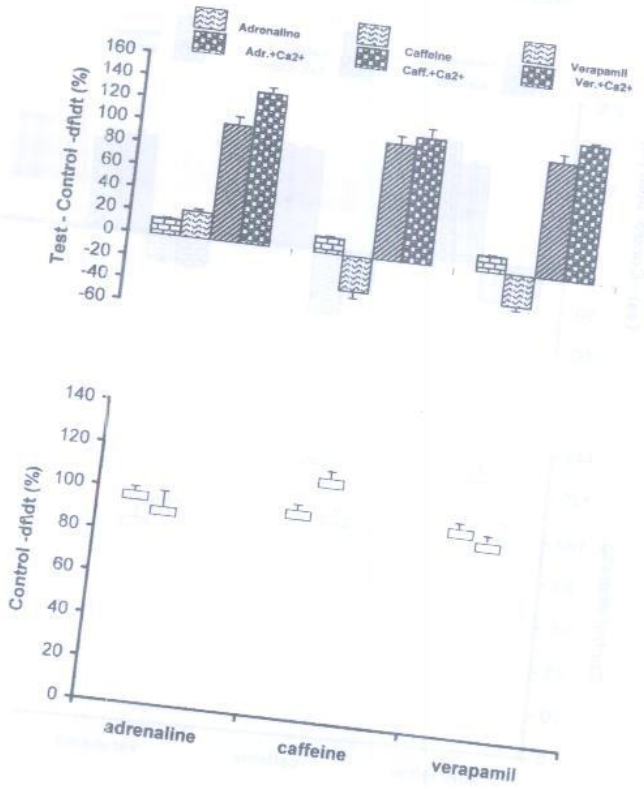


**Fig.5. Changes in the rate of relaxation (-df/dt) of the *Clarias lazira* myocardium after 5 minutes of rest with one concluding stimulation under different conditions. Experimental protocol as in Fig.1. n=6.**

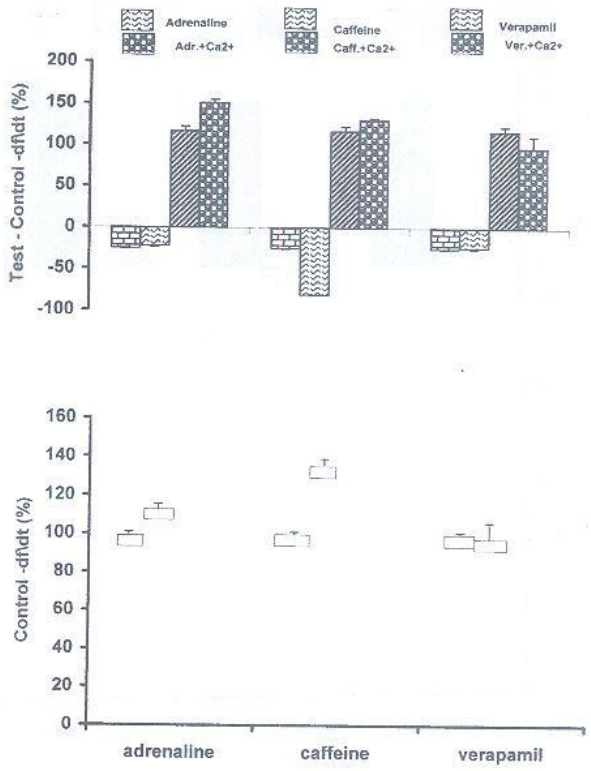


**Fig.6. Changes in the rate of relaxation (-df/dt) of the frog myocardium after 5 minutes of rest with one concluding stimulation under different conditions. Experimental protocol as in Fig.1. n=6.**

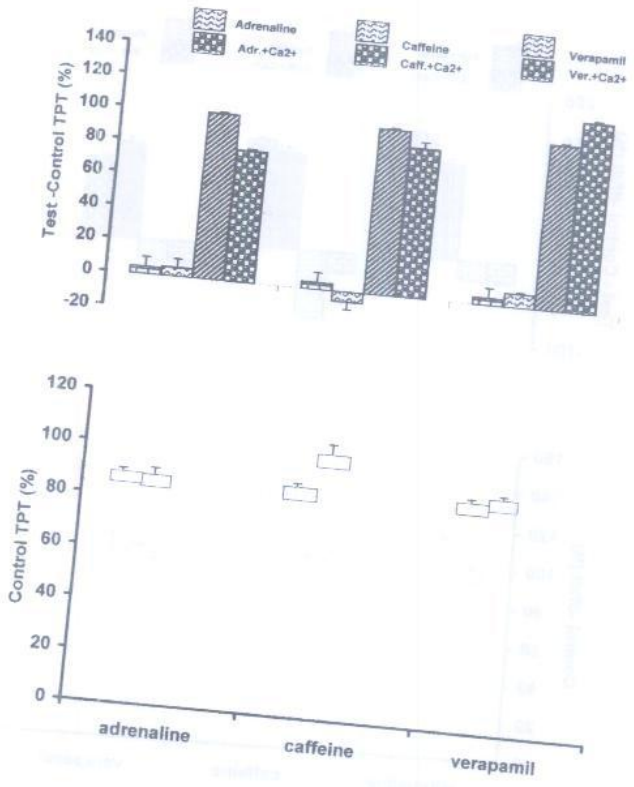


**Fig.7. Effect of adrenaline, caffeine and verapamil on the changes in time to peak tension (TPT) after 5 minutes of rest in the *Clarias lazira* heart. Experimental protocol as in Fig.1. n=6.**

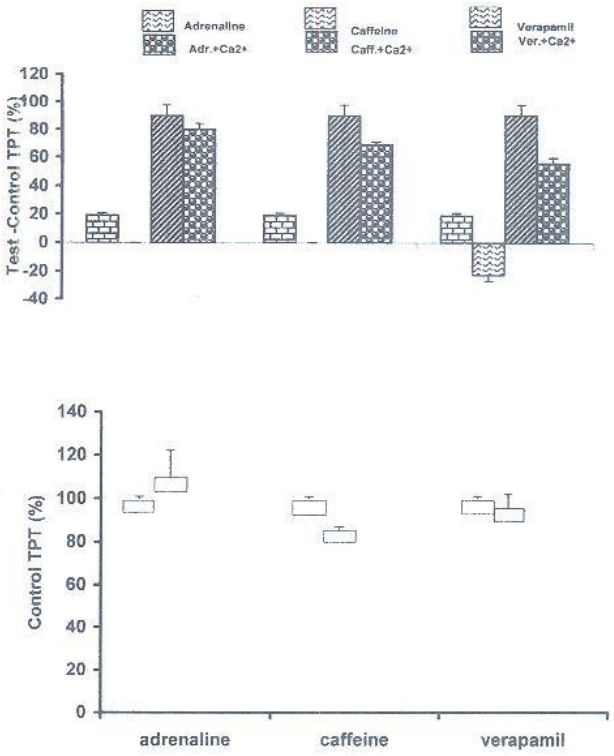


**Fig.8. Effect of adrenaline, caffeine and verapamil on the changes in TPT after 5 minutes of rest in the frog heart. Experimental protocol as in Fig.1. n=6.**