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Studies on the Regulation of Calcium Activity in Myocardial Contraction*

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ABSTRACT

Influences of trigger calcium on myocardial contraction from several sources were investigated on the frequency reduction-induced changes of contraction in rat left atria driven by electrical field stimulation. Rat atria elicited characteristic three phase-changes according to frequency reduction: the first rapid rise in twitch tension, the second transient fast decrease in tension and the third maintenance of twitch tension at about 200% of resting tension during high frequency. Caffeine treatment enormously suppressed the frequency reduction-induced twitch tension increase. The atrial contraction during high frequency vanished after verapamil treatment. But, during low frequency, atrial contraction revived in the presence of verapamil. Ouabain treatment and sodium depletion in superfusing solution abolished the characteristic second phase with slow frequency. These results suggest that slow calcium channel is an indispensable calcium entry route and calcium release from sarcoplasmic reticulum is an major source for trigger calcium in cardiac contraction. And sodium-calcium exchange has a modulatory roles in the regualtion of trigger calcium according to the changes of intracellular sodium concentration.

Key Words: Intracellular calcium, Sarcoplasmic reticulum, Sodium-calcium exchange

Abbreviateion: SR; sarcoplasmic reticulum, $[Na^+]_i$; intracellular sodium concentration, $[Na^+]_o$; extracelluar sodium concentration.

INTRODUCTION

In cardiac muscle comparing with smooth or skeletal muscle, a very different situation may be prevailed because the heart must repeat the contraction and relaxation process continuously in order to achieve the cyclic contraction. It is the cytosolic free Ca^{2+}, during activity, entering the cytosol from extracellular fluid and being released into the cytosol from intracellular stores that plays a central role in excitation-contraction coupling (Fozzard, 1977; Fabiato, 1983). So, the regulation of intracellular Ca^{2+} concentration is obviously fundamental to cardiac muscle function.

It is well known that several types of systems can contribute to the regulation of intracellular Ca^{2+} concentration, which triggers muscle contraction. Ca^{2+} movement through Ca^{2+}-selective channels (McDonald, 1982) and reverse mode Na^+/Ca^{2+} exchange (Deitmer and Ellis, 1978), Ca^{2+} release from sarcoplasmic reticulum (SR) (Endo, 1977) will be the major mechanisms that can increase the concentration of cytosolic free Ca^{2+} (Chapman, 1983). On the other hand, Ca^{2+} flux by sarcolemmal Ca^{2+}-ATPase system (Reeves and Sutko, 1980) and Ca^{2+} uptake by SR (Endo, 1977) can be the major mechanisms for reduction of cellular free Ca^{2+} concentration.

Of course, these systems cooperate at the same time in the regultion of trigger Ca^{2+} for contraction in every beat. But, there are some reports on the specific enrolement of each system especially in the

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generation of contraction. One of such is on the leading roles of Ca^{2+} released from SR in generation of contraction (Somlyo *et al.*, 1981). Because the diffusion pathway in intracelluar space is so complex and the contractile units are, histologically, closely related with Ca^{2+} releasing sites of SR, the Ca^{2+} released from SR is more fundamental than Ca^{2+} from other sources in muscle contraction (Morad and Goldman, 1973; Somlyo *et al.*, 1981; Fabiato, 1985). So, it is interesting and important to find out if each system may have any specific participation or any sequential order in beat generation and beat-to-beat regulation.

Lederer and Sheu (1983) reported that when the stimulation rate was reduced from 1 to 0.1 Hz in the sheep cardiac Purkinje fiber, $[Na^+]_i$ fell progressively and the effect was reversed when the stimulation frequency was increased. Whereas the changes in $[Na^+]_i$ in response to changes in frequency were monophasic, but the tension responses elicited biphasic changes, i.e. an immediate increase and following decrease in twitch tension. The oppositie was true when the frequency was increased. Those immediate increase in twich tenison was believed to be achieved by the increased release of Ca^{2+} from SR after the SR filled Ca^{2+} more completely because of enlarged beat-to-beat interval (Orchard and Lakatta, 1985). And the decrease in $[Na^+]_i$ modified the Na^+/Ca^{2+} exchange (Langer, 1983).

So the present study was performed to characterize the roles of SR Ca^{2+} release in the cardiac contraction and to identify the relationships between Ca^{2+} movement through Ca^{2+} channel or Na^+/Ca^{2+} exchange and SR Ca^{2+} release in the generation of cardiac contraction, using left atrial contraction driven by electrical field stimulation with fast and slow frequencies.

MATERIALS AND METHODS

Sprague-Dawley rat of either sex weighing 150-250g were used. The animals were sacrificed by cervical dislocation. The hearts were quickly excised and suspended in oxygenated Krebs-Hensleit buffer (KHB). Left atria were cautiously dissected and mounted in 5ml organ bath, which was superfused with KHB by 2 ml/min. KHB was bubbled with mixed gas of 95% O_2 and 5% CO_2. Left atrial contraction was driven by electrical field stimulation (EFS) delivered through platinum electrodes in square wave pulses of 4 Hz, 0.5 msec pulse duration with supramaximal voltages by digital stimulator (STM-1000, Hansung). Contraction of left atrium was recorded on Polygraph (Model 7, Grass) via force displacement transducer (FT .03, Grass). After equilibration for 30 minutes, the 4 Hz stimulation (high frequency) was abruptly slowed to 0.4 Hz (low frequency). The changes in twitch tensions and contraction shape with different frequencies were recorded. And the effects of drug added in the above superfusion solution on these changes were compared. The compositons (mM) of the solution used here were as follows; NaCl 118.0, KCl 4.70, $CaCl_2$ 2.52, $MgSO_4$ 1.16, $NaHCO_3$ 24.88, KH_2PO_4 1.18, Glucose 5.55, Na-Pyruvate 2.0.

Drugs used: Caffeine, verapamil HCl, tetrodotoxin, procaine HCl, ouabain $8H_2O$ were purchased from Sigma Chemical Co (USA).

RESULTS

Left atrial contraction

Isolated rat atria elicited regular contractions by EFS (4 Hz of frequency, 0.5 msec pulse duration and supramximal voltage). When the frequency of EFS was reduced from 4 Hz (high frequency) to 0.4 Hz (low frequency), typical three phase-changes were shown in atrial contraction: an abrupt increase in atrial twitch tension (first phase), was followed by a transient decline (second phase) and 2 minutes later stabilization (third phase) at the level of over 200% of the amplitude obtained by the high frequency. Upon switching from the low to high frequency, the twitch tension abruptly returned nearly to the previous level of the high frequency (Fig. 1).

Effect of $[Na^+]_o$ depletion and ouabain

When Na^+-lack KHB solution in which 50% of Na^+ was replaced by an equimolar concentration of sucrose was superfused, twitch tension was progressively increased during high frequency stimulation. But, switching to low frequency, the abrupt increase in twitch tension amplitude was similarly manifested as demonstrated in the normal KHB solution except the loss of second transient decline phase (Fig. 2).

With the treatment of 10^{-4}M ouabain, the increase in basal tone was more prominent than amplitude increase during high frequency stimulation. However, when stimulation was switched to the low frequency (0.4 Hz), the first abrupt increase in twitch tension was evidenced in association with increment in diastolic relaxation. And ouabain treatment also

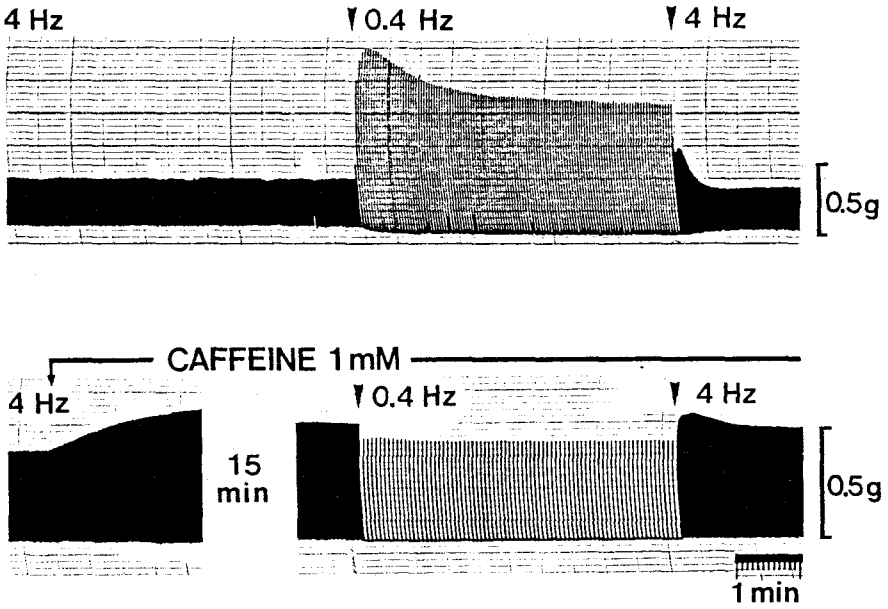

Fig. 1. Effects of caffeine (1mM) on the frequency reduction-induced changes of contraction in rat left atria driven by electrical field stimulation (0.5 msec, supramaximal voltage)

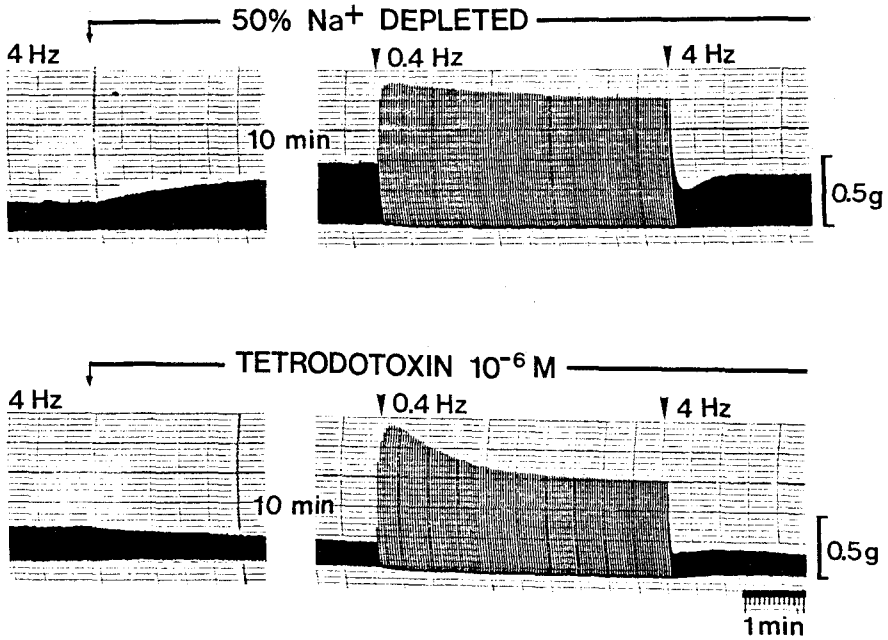

Fig. 2. Effects of $[Na^+]_o$ depletion or tetrodotoxin (10^{-6}M) on the frequency reduction-induced changes of contraction in rat left atria driven by electrical field stimulation (0.5 msec, supramaximal voltage)

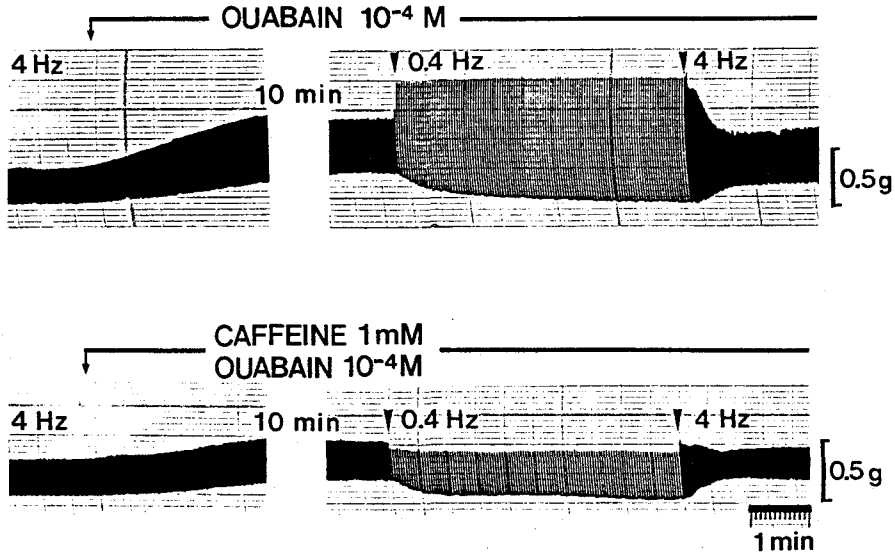

Fig. 3. Effects of ouabain (10^{-4}M) or combination of caffeine (1mM) with ouabain on the frequency reduction-induced changes of contraction in rat left atria driven by electrical field stimulation (0.5 msec, supramaximal voltage)

characteristically abolished the second transient decline phase. When returned back to the high frequency (4 Hz), all the findings shown during the low frequency were disappeared (Fig. 3).

Effects of verapamil

During the stimulation with high frequency, application of 10^{-5}M verapamil caused loss of twitch response. At 10 min of superfusion with verapamil, the frequency of the stimulation was switched to the low frequency. In this case, the twitch responses to low frequency were little affected by verapamil, slow Ca^{2+} channel blocker. The atrial contraction was reappeared. However, when the stimulation was reversed to high frequency, the twitch itself stopped. Interestingly, addition of 2mM $CaCl_2$ in the KHB solution during the low frequency stimulation further accentuated the twitch responses, while, during high frequency just before or after the low frequency period, it never caused any reappearance of contraction (Fig. 4).

Effects of caffeine

During the stimulation with the high frequency, caffeine (1mM) accentuated the twitch tension development markedly. Nevertheless, when the stimulation was switched to the low frequency, the twitch amplitudes were turned out to be decreased and constantly remained at the low level throughout 5 minutes. Uppon increasing the frequency, the twitch tension recovered to the original level (Fig. 1). Combination of caffeine (1mM) with ouabain (10^{-4}M) caused an increase in twitch response during the high frequency stimulation, but leaded to a diminution of amplitude during the low frequency stimulation. However, the increment in diastolic relaxation observed with ouabain was not affected by caffeine (Fig. 3). Alternatively, when caffeine was combined with verapamil, the characteristic reappearance of contraction during low frequency was also manifested, but the twitch tension developed and the effects of $CaCl_2$ administration were further diminshed (Fig. 4).

Effects of tetrodotoxin and procaine

Superfusion of the KHB solution containing 10^{-6}M tetrodotoxin caused slight decrease in twitch tension during the stimulation with high frequency. Under treatment with tetrodotoxin, little difference was found when compared with the control group upon application of low frequency (Fig. 2).

While, application of 3×10^{-4}M procaine caused irregular rhythme. Thus, to evoke regular rhythme, the power voltage was increased. Treatment with procaine exerted an increase in twitch amplitude during the high frequency period. Following reduction to the low frequency, the first abrupt increase in twitch

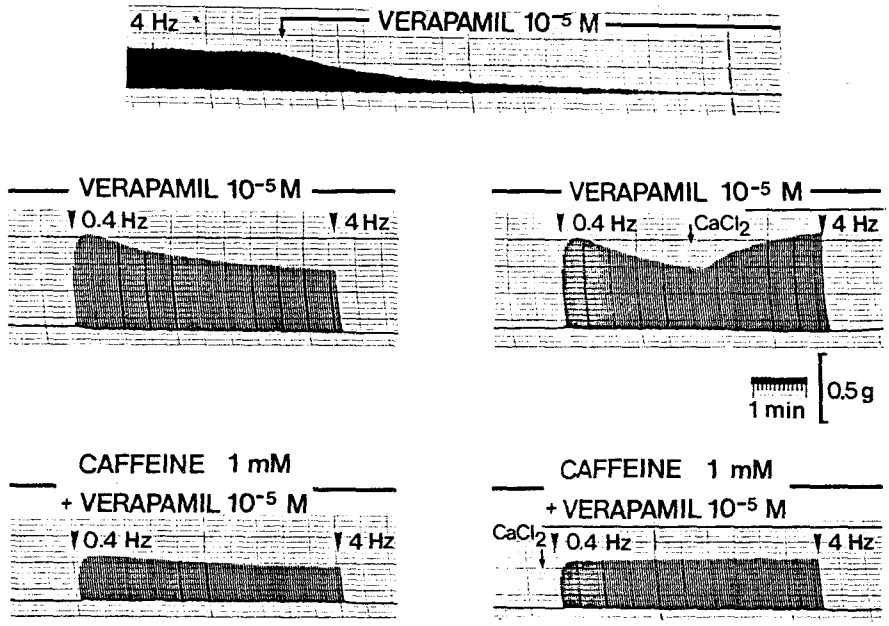

Fig. 4. Effects of verapamil (10^{-5}M) or combination of caffeine (1mM) with verapamil and influences of $CaCl_2$ addition on the frequency reduction-induced changes of contraction in rat left atria driven by electrical field stimulation (0.5 msec, supramaximal voltage)

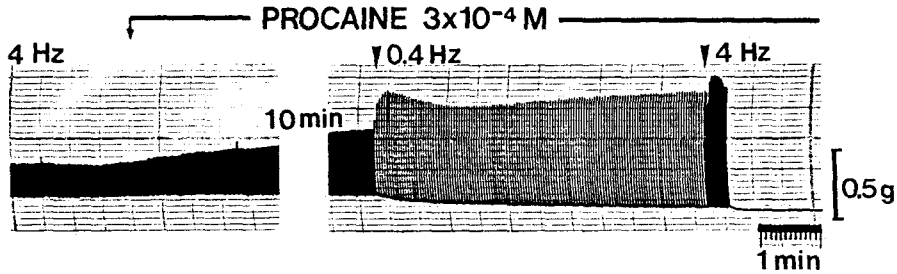

Fig. 5. Effects of procaine (3×10^{-4}M) on the frequency reduction-induced changes of contraction in rat left atria driven by electrical field stimulation (0.5 msec, supramaximal voltage)

response and second transient decline were observed but in less degree. However, in third phase, procaine treatment caused increase in the twith tension, which was never observed in other groups. When the frequency was reversed to the high frequency, the twitch response was abolished (Fig. 5).

DISCUSSION

Stimulation frequency reduction caused an immediate increase and following decrease in twitch tension in rat left atria same as in ship Purkinje fiber by Lederer and Sheu (1983). The initial rapid rise in twitch tension was believed to be the result of increased release of Ca^{2+} from SR (Orchard and Lakatta, 1985). Interestingly, caffeine abolished this first phase-increase of twitch tension after switching to slow frequency in control and ouabain treated atria. Caffeine has known to have a biphasic effect, causing Ca^{2+} release from SR in low concentrations and preventing SR Ca^{2+} uptake in higher concentrations, eventually depleting the SR of Ca^{2+} (Blinks *et al.*, 1972; Endo, 1977; Hesse and Wier, 1984). So, it is

resonable to conclude that this abolition of the first phase, initial rapid rise in twitch tension, by caffeine would be the result of Ca^{2+} depletion from SR. Furthermore, the twitch tensions achieved after caffeine treatments during slow frequency were about half and about 1/3 of the tensions without caffeine treatment in control and ouabain group, respectively. So, the free Ca^{2+} released from SR thought to have more than that much extent-contribution in the generation of atrial contraction.

After verapamil treatment, the left atrial contraction during high frequency was not abruptly but progressively disappeared. This means that the Ca^{2+} influx through calcium channel would be the most abundant or important source for trigger Ca^{2+} in cardiac contraction (Tsien, 1983). But, any other sources for trigger Ca^{2+} entry possibly remain, because the cessation of contraction was not abrupt, though the Ca^{2+} entry through slow Ca^{2+} channel would be abruptly abolished by verapamil treatment (Chapman, 1983; McDonald *et al.*, 1984). However, the contraction was revived after switching to slow frequency, although the tension developed was far less than control group. And this reappeared twitch tension was further attenuated by caffeine treatment. So, this reappeared contraction was, at least, generated by increased SR Ca^{2+} release by reduced frequency. On the other hand, addition of Ca^{2+} caused increase in twitch tension. This tension increase was possibly generated by Ca^{2+} influx through other routes than slow Ca^{2+} channel: Na^+/Ca^{2+} exchange or Ca^{2+}-induced Ca^{2+} release. But, caffeine which increases the Ca^{++}-induced Ca^{++} release (Endo, 1977) also characteristically attenuated this increase. Considering these results, it would be more probable that the route of Ca^{2+} entry which mediates those tension increments could be the Na^+/Ca^{2+} exchange. At the same time, the Ca^{2+} entered via Na^+/Ca^{2+} exchange seemed to be participated as a trigger Ca^{2+} for cardiac contraction only after released from SR because caffeine treatment significantly attenuated the tension increment by Ca^{2+} addition (Sutko *et al.*, 1986).

The characteristic second phase of twitch tension change during slow frequency was abolished, in our result, by treatment of ouabain or $[Na^+]_o$ depletion. Ouabain which inhibit the Na^+, K^+-ATPase, increases the $[Na^+]_i$ and causes the net Ca^{2+} influx via Na^+/Ca^{2+} exchange (Lee, 1985). Extracellular Na^+ depletion also causes the net Ca^{2+} influx via Na^+/Ca^{2+} exchange by the reduced ratio of $[Na^+]_o/[Na^+]_i$ (Lee *et al.*, 1980). Therefore, ouabain or $[Na^+]_o$ depletion reversed the previously mentioned $[Na^+]_i$ decrease and Ca^{2+} outflux induced by reducing the frequency rate (Lederer and Sheu, 1983; Orchard and Lakatta, 1985). So, the second phase-transient rapid decrease in twitch tension during low frequency could be related with this decrease in $[Na^+]_i$. Decrease in $[Na^+]_i$ may cause the changes in the rate of Na^+/Ca^{2+} exchange leading to net Ca^{2+} outflux and eventual tension decrements (Caroni and Carafoli, 1983; Pott, 1986).

Interestingly, procaine which inhibits the Na^+ entry reversed the third phase decline in twitch tension in contrast to tetrodotoxin, a typical Na^+ entry blocker. Of course, there would be the possibility that this result may be an artifact resulting from electrical voltage increase. Because electrical stimulation increases the production of inositol triphosphate which releases the intracellular Ca^{2+} store (Poggioli *et al.*, 1986). But, if this data is correct, it would suggest the relationship between procaine and Na^+/Ca^{2+} exchange. Anyhow, this remains to be defined.

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＝국문초록＝

심근 수축에 있어서 Calcium 작용의 조절에 관한 연구

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전기장 자극으로 수축을 유발한 흰쥐 좌심방에서, 자극 빈도 변경에 따른 수축 운동의 변동에 미치는, 여러 경로를 통한 수축 유발 calcium의 영향을 검색하므로, 각 경로가 심근 수축에 미치는 영향을 추구하였다. 흰쥐 좌심방은 자극 빈도를 급격하게 낮추므로, 특징적인 삼단계 변동을 나타내었다. 즉, 처음의 급격한 수축 장력증가와, 두번째 일시적인 빠른 장력감소, 이어서 세번째로 수축장력의 유지단계로 나타났으며, 이때 수축장력은 고빈도 자극시의 2배 정도가 되었다. Caffeine처치는 이와같은 자극빈도 하강에 따른 수축 장력의 증가를 현저하게 억압하였다. Verapamil은 고빈도 자극시 수축 운동을 완전히 소실시켰으나, 저빈도 자극으로 변경시에는 verapamil 존재하에서도 수축 운동이 소생되었다. 한편 ouabain처치나 영양액내 sodium 배제시에는 저빈도 자극으로 나타나는 특징적인 두번째 단계의 변동이 소실되었다. 이러한 결과로 보아, 심근막의 calcium 통로는 세포내 유지에 필수불가결한 경로이며, 심근 수축 유발 calcium의 주된 기원은 근 소포체로 부터 유리되는 것으로 믿어진다. 또한 sodium-calcium 교환은 세포내 sodium 농도의 변동에 따라 수축 유발 calcium양 형성에 조절 인자로서의 기능을 갖는 것으로 추측된다.