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The Pharmacological Studies on the Origin of Calcium Ion in Myocardial Contraction*

Chang Mann Ko[1], Kyung Hwan Kim[2]

[1]*Department of Pharmacology, Yonsei University Wonju College of Medicine, Wonju, Kangwon-Do 220-701, Korea,*
[2]*Department of Pharmacology, Yonsei University College of Medicine, Seoul 150-752, Korea*

ABSTRACT

Na-Ca exchange transports calcium ion either into (reverse mode Na-Ca exchange) or out of the cell (forward mode Na-Ca exchange) according to the direction of driving force produced by the changes in ratio of intra- and extra-cellular Na concentrations. Thus, Na-Ca exchange is regarded as the regulator of myocardial contraction. However, the existence of reverse mode Na-Ca exchange and its role in myocardial contraction is still questioned. Present study was performed to identify the presence of reverse mode Na-Ca exchange and its possible involvement in the regulation of myocardial contraction in rat heart. Using the left atria of rat, contraction was induced by electrical field stimulation (EFS, 0.5 msec duration and supramaximal voltage). Changing of the stimulation frequencies from resting 4 Hz to 0.4, 1 or 8 Hz, caused typical negative staircase effect in twitch tension, but ^{45}Ca uptake showed bimodal increase. When the stimulation frequency was abruptly changed from 4 Hz to 0.4 Hz, the atrial twitch tension showed three phased-enhancement, that is, the initial rapid increase (the first phase) followed by rapid decrease (the second phase) and stabilization (the third phase). ^{45}Ca uptake was equivalent to tension, i.e. initial significant increase in first 30 second and then decrease. Benzamil treatment abolished the first phase of increase in a dose dependent manner from 10^{-5} to 3×10^{-4} M. Bay k 8644 (3×10^{-5} M) treatment enhanced the inotropy induced by frequency reduction and abolished the second and third phase decreases. Benzamil treatment also suppressed the contraction stimulated by Bay K 8644. Although the contraction at 4 Hz stimulation was completely abolished by verapamil 3×10^{-5} M pretreatment, the contraction reappeared as soon as the stimulation frequency was changed into 0.4 or 1 Hz and interstingly, ^{45}Ca uptake were significantly higher than no treatment.

From these results, it is concluded that reduction of stimulation frequency causes calcium influx by the reverse mode Na-Ca exchange, resulting in initial rapid increase of twitch tension. then it turns into forward mode exchange to efflux the calcium, resulting in decrease of the twitch tension in left atria of rat.

Key Words: Left atria, Negative staircase effect, Na-Ca exchange, ^{45}Ca uptake, Benzamil

INTRODUCTION

Myocardial contraction is triggered by the interaction of free calcium ion and Troponin C (Fozzard, 1977). All the mechanisms or intracellular structures which transport, store or release the calcium ion can regulate the myocardial contraction. Calcium channel, calcium pump and Na-Ca exchange have long been considered as the mechanism or structure which transport calcium ion (Deitmer and Ellis, 1987; McDonald, 1982). The

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[1]To whom all correspondence should be addressed.

sarcoplasmic reticulum or mitochondria has been regarded as the intracellular calcium storing organelles (Endo, 1977; Fabiato, 1983). The mechanisms or intracellular structures among those listed above which increase the intracellular free calcium ion can trigger or enhance the myocardial contraction. The opposite will be also true (Chapman, 1983).

L-type calcium channel is considered as one of the most important source of calcium ion triggering the myocardial contraction (Reuter, 1967). From the beginning of the myocardial action potential, calcium ions move into myocardial cytosol through L-type calcium channel. These calcium ions can induce the calcium release from sarcoplasmic reticulum (calcium-induced calcium release; CICR; Fabiato, 1983). In reality, the calcium ions released from sarcoplasmic reticulum by this mechanism would be the 90% of the initial free calcium ions in myocardium (Beuckelman & Wier, 1988; Callewaert *et al.*, 1988; Nagasaki & Fleiischer, 1988; Wier, 1990). On the other hand, the other mechanism is recently suggested to be involved in CICR. Reverse mode Na-Ca exchange is the most probable candidate (LeBlanc & Hume, 1990).

Na-Ca exchange is a different calcium ion transporting mechanism. The changes of internal or external Na ion concentrations, hence the changes of ratios between internal and external Na ions are the underlying requirements for driving calcium ion to move. Using the electrochemical potential difference caused by the ratio as the driving force, calcium ion is cotransported with Na ion movement in reverse direction by a specific carrier (Blaustein, 1974). So, calcium ion is either transported into (reverse mode Na-Ca exchange) or out of the cell (forward mode Na-Ca exchange) according to direction of driving froce caused by two Na ion concentrations. As the intracellular calcium ion is either increased or decreased by the Na-Ca exchange mechanism, this mechanism can not only trigger and enhance but decrease or relax the myocardial contraction (Philipson, 1985). But, until date, the existence of reverse mode Na-Ca exchange is questioned, because the extracellular Na concentration is much higher than intracellular Na concentration. So, the relaxation of myocardial contraction by decrease in intracellular calcium through the forward mode Na-Ca exchange or the enhancement by increase in intracellular calcium through inhibition of this mechanism, is mainly considered and generally accepted (Bers, 1985, Bers *et al.*, 1990). Present study was performed to identify the presence and to investigate the possibility of involvement of the reverse mode Na-Ca exchange in triggering or enhancing myocardial contraction using the well known negative staircase effect of rat heart.

MATERIALS AND METHODS

Left atrial strip

Sprague-Dawley rat of either sex weighing 200g was 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 8 ml organ bath. KHB was bubbled with mixed gas of 95% O_2 and 5% CO_2 to maintain pH 7.4. Left atrial contraction was driven by electrical field stmulation (EFS) delivered through platinum electrodes in square wave pulses of 4Hz, 0.5 msec pulse duration with supramaximal voltages by digital stimulator (STM-1000, Hansung). Contraction of left atria were recorded on Polygraph (Model 7, Grass) via force displacement transducer (FT. O3, Grass). After equilibration for 30 minutes, the 4 Hz, stimulation (High frequency) was abruptly reduced to 0.4, 1Hz (Low frequency) or increased to 8Hz for 5 miuntes and then returned to 4Hz again to investigate the frequqncy-tension relationship. But, in other experiment, stimulation frequency was changed into 0.4Hz only for 5 minutes. The changes in twitch tensions and contraction shape with different frequencies were recorded and the peak tensions or the last twitch tensions obtained at the end of the third phase were calculated as % of the norepinephrine 10^{-6}M induced-peak tension at 4Hz stimulation. The composition of KHB solution were as follows; NaCl 118.8, 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 (mM).

^{45}Ca uptake

During the experiment described above, calci-

um uptake into left arial strip was measured. 1 μ Ci/ml of $^{45}CaCl_2$ was introduced 30 seconds before changing the stimulation frequency and incubated for 5 minutes in frequency-tension experiment. In ohter experiment, 1 μCi/ml of $^{45}CaCl_2$ was incubated for 10 minutes with the 5Hz EFS. Then the stimulation frequency was changed. After 30 seconds, 1, 2 and 5 minutes, the atrial strip was washed in cold KHB (4℃) and immersed in the cold lanthanum solution for 60 minutes (Karaki & Weiss, 1979). The atrial strip was dried overnight at 60℃. The radioactivity was measured by liquid scintillation counter (Beckman). The ^{45}Ca uptake was calculated as nmole/g of cell H_2O. The composition of lanthanum solution was as follows; $LaCl_3$ 80.0, Glucose 11, Tris base 6 (mM), pH=6.8 titrated with 1 N Maleic acid.

RESULTS

Frequncy-tension relationship

Rat left atria elicited typical negative staircase effect as the EFS frequencies were abruptly changed from resting 4 Hz to 0.4, 1 or 8Hz. When the EFS frequency decreased, the twitch tension was increased, and the twitch tension was decreased while the EFS frequency increased. Although the tensions showed inverse linear relationship, ^{45}Ca uptake elicited bimodal increases. ^{45}Ca uptake was increased while the EFS frequency increased. But, ^{45}Ca uptake increased again as the EFS frequency decreased (Fig. 1).

Left atrial contraction during frequency reduction

Isolated rat atria elicited regular contractions

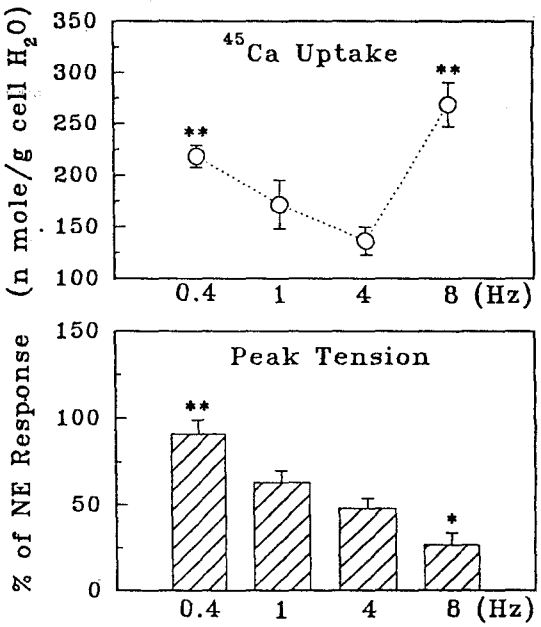

Fig. 1. Frequency-Tension and Frequency-^{45}Ca uptake relationships in rat left atria.
Atrial contraction was induced by EFS (0.5 msecs in duration, supramaximal voltage). Stimulation frequency was abruptly changed from resting 4Hz to 0.4, 1 or 8 Hz for 5 minutes (*$p<0.05$, **$p<0.01$ compare with 4 Hz).

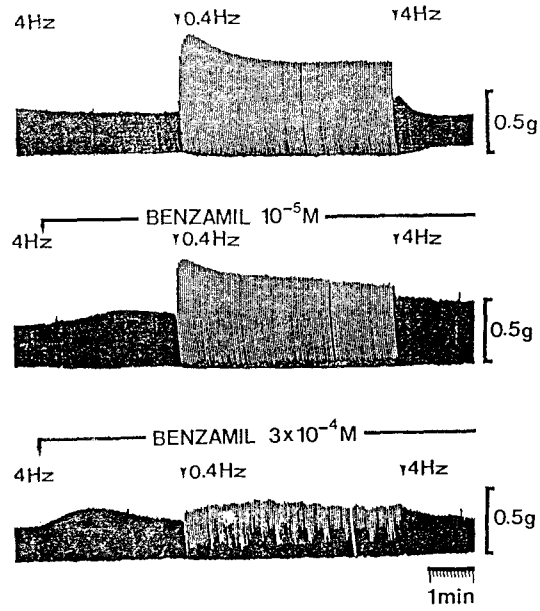

Fig. 2. Effect of benzamil on the increase in twitch tension by abrupt stimulation frequency reduction for 5 minutes in rat left atria.
Atrial contraction was induced by EFS (0.5 msec in duration, supramaximal voltage). Stimulation frequency was abruptly changed from resting 4Hz to 0.4Hz for 5 minutes.

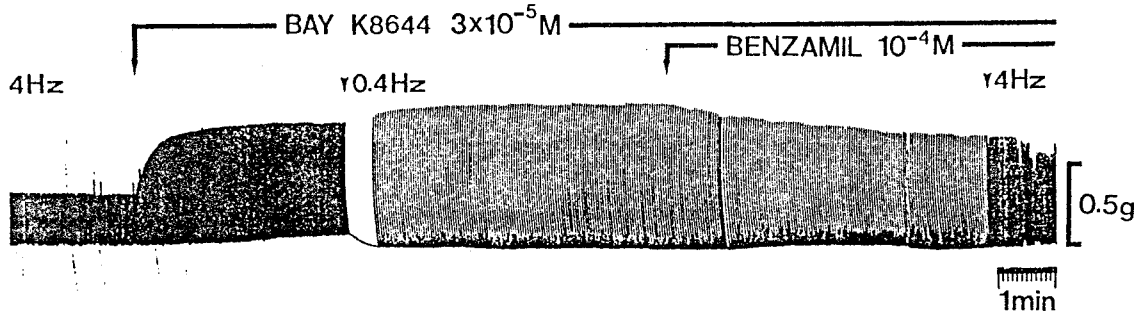

Fig. 3. Enhancement of Bay K 8644 and inhibition of benzamil on the increase in twitch tension by rapid frequency reduction for 5 minutes in rat left atria.
Legends are same as in Fig. 2.

by EFS (4 Hz, 0.5 msec, supramximal voltage). When the frequency of EFS was reduced from 4 Hz (high frequency) to 0.4 Hz (low frequency), typical three phased-changes were shown in atrial contraction; an abrupt increase in atiral twitch tension (first phase), followed by a transient decline (second phase) and stabilization (third phase) at the level of over 200% of the amplitude obtained by high frequency (Fig. 2). The peak tension and last tension obtained at the end of the third phase were equivalent to 91 ± 8% and 49 ± 10% of the NE 10^{-6}M induced-peak tension, respectively (Fig. 5). ^{45}Ca uptake during 0.4 Hz stimulation elicited initial (30 seconds) significant increase and following decrease. However, it was still somewhat higher than 4Hz stimulation (Fig. 4).

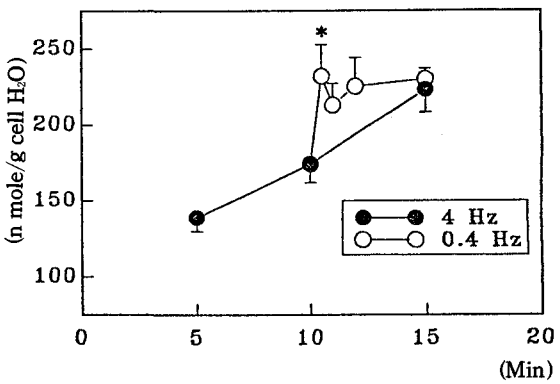

Fig. 4. Increase of ^{45}Ca uptake during abrupt stimulation frequency reduction in rat left atria.
Atrial contraction was induced by EFS (0.5 msec in duration, supramaximal voltage). Stimulation frequency was abruptly changed from resting 4 Hz to 0.4 Hz for 5 minutes (*$p<0.05$ compare with 4 Hz).

Benzamil pretreatment

Benzamil, which is known to be a Na-Ca exchange inhibitor, abolished especially the first phase increase in twitch tension during 0.4 Hz stimulation in a dose dependent manner from 10^{-5} M to 3×10^{-4} M. Severe arrhythmia was appeared after benzamil treatment more than 10^{-3} M (Fig. 2).

Bay K 8644 treatment

Calcium channel activator, Bay K 8644 increased the left atrial contraction during either 4 Hz or 0.4 Hz stimulation. Typically, the second and third phase decrease in twitch tension was abolished. Benzamil treatment decreased the twitch tension regardless of stimulation by Bay K 8644 treatment (Fig. 3).

Verapamil pretreatment

After verapamil 3×10^{-5} M pretreatment, the contraction during 4 Hz stimulation was completely abolished. As soon as the EFS frequency was changed into 0.4 or 1 Hz, the left atrial contraction reappeared, however, the developed tension was much less than without verapamil. Interestingly, the ^{45}Ca uptake during 0.4 or 1 Hz stimulation after verapamil pretreatment was rather significantly

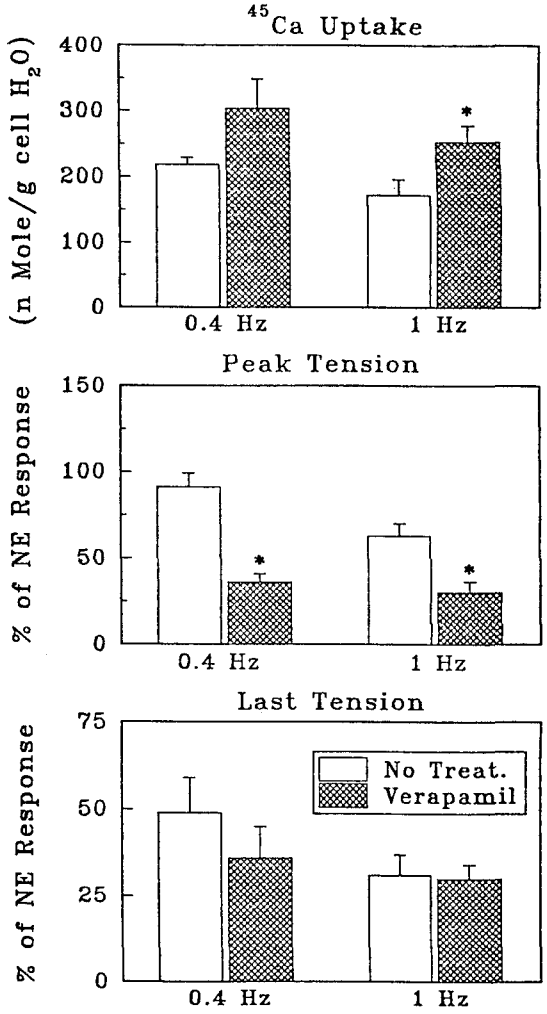

Fig. 5. Effect of verapamil on the ^{45}Ca uptake or the increase of twitch tension by abrupt stimulation frequency reduction.

Atrial contraction was induced by EFS (0.5 msec in duration, supramaximal voltage). Stimulation frequency was abruptly changed from resting 4 Hz to 0.4 or 1 Hz for 5 minutes (*$p<0.05$ compare with no treatment).

increased than without verapamil (Fig. 5).

DISCUSSION

The intracellular Na^+ concentration is regulated by the Na^+, K^+-pump. The total action time of Na^+, K^+-pump can be changed as the contractile frequency changes. During the rapid contraction, the intracellular Na^+ concentration increases, because the total action time of Na^+, K^+-pump is decreased (Woodbury, 1963). This increase in intracellular Na^+ concentration changes the ratio between the internal and external Na concentration and in turn, the Na-Ca exchange, and results in intracellular calcium concentration increase (Lederer & Sheu, 1983; Sheu & Blautein, 1986). The reverse is believed to be true. So, in most species except rat, the myocardial contractility is generally increased as the contraction frequency increases and it is decreased while contraction frequency is slowed (positive staircase effect, Langer, 1971). But, rat myocardium does not follow this rule and elicit negative staircase effect. The mechanism of negative staircase effect if still unclear.

Even in the species which elicit positive staircase effect, the initial several beats are to be enhanced, while abruptly slowing the contraction frequency (Lederer & Sheu, 1983). This enhancement was believed to be caused by the increase in calcium release from sarcoplasmic reticulum, because the more calcium is stored in sarcoplasmic reticulum during the increased diastolic interval (Orchard & Lakta, 1985). The increase in contractile tension of rat myocardium during frequency reduction is also explained by these mechanism (Ko & Hong, 1990). But, considering the present result, the other possible explanation could be suggested. As the stimulation frequency was abruptly reduced, the twitch tension elicited enhancement of the first phase. The first phase increase in twitch tension was specifically abolished by the Na-Ca exchange inhibitor, benzamil. This result shows that the first phase increase in twitch tension is believed to be induced by the Na-Ca exchange. Interestingly, the initial calcium uptake in rat left atria is also significantly increased. So, it is possible to conclude that while the stimulation frequency is reduced in rat atria, the Na-Ca exchange activity is changed and causes the calcium influx through its reverse mode. this increased calcium influx enhances the first phase twitch tension. This conclusion could be supported by the increase of calcium uptake during the fre-

quency reduction in verapamil pre-treated atria. Since the calcium channel is bloc-ked by the enough dose of verapamil, the source of increased calcium influx could only be the reverse mode Na-Ca exchange (Wier & Beuckel-mann, 1989). It is reported that the activity of Na-Ca exchange is much exagerated at low intracellular Ca concentration (Blaustein *et al.*, 1986). From the succeeding decrease in calcium uptake in left atria during the frequency reduction, it is also suggested that Na-Ca exchange is turned into forward mode and transport the calcium ion out of the cell to decrease the twitch tension (Bers, 1985) resulting the second phase decrease. Monen-sin treatment is supposed to enhance the initial calcium influx through reverse mode Na-Ca exchange and to suppress the calcium extrusion through forward mode Na-Ca exchange, thus, it can enhance the twitch tension during the whole 0.4 Hz stimulation period, abolishing the second and third phase.

The roles of calcium channel is considered as the main mechanisms for triggering the myocardial contraction (Peuter, 1967). This was also proved in the present experiment. The addition of Bay k 8644 elicited potent enhancement in twitch tension during high frequency and low frequency stimulation. On the other hand, the present result of the inhibition of contractile force by benzamil treatment after Bay K 8644 possibly suggest that the Na-Ca exchange works contemporarily with calcium channel in triggering and stimulating the myocardial contraction. Furthermore, considering the report of the possible involvement of Na-Ca exchange in CICR by LeBlanc & Hume (1990) and the result which proved the complete inhibition of twitch tension enhancement after stimulation frequency reduction by ryanodine treatment (Ko & hong, 1990), Na-Ca exchange seems to be involved as an usual and important calcium source; this calcium induces the CICR contemporarily with the calcium from calcium channel. But, this cannot be concluded at the moment. More evidences will be needed to clarify this conclusion.

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=국문초록=

심근 수축에 있어서 Calcium 이온의 기원에 관한 약리학적 연구

연세대학교 원주의과대학 약리학교실, 의과대학 약리학교실

고 창 만·김 경 환

Na-Ca 교환은 calcium 이온을, 세포 내외의 Na 이온 농도차에 의해서 형성되는 원동력의 방향에 따라, 세포내로(역방향 Na-Ca 교환), 혹은 세포밖으로(정방향 Na-Ca 교환) 이동시킨다. 그러므로 Na-Ca 교환은 심근 수축 운동의 조절 기전의 하나로 받아 들여지고 있다. 그러나, 세포내의 Na 이온 농도는 항상 세포외의 농도보다 낮으므로, 역방향 Na-Ca 교환의 존재와 아울러 이의 심근 수축에 있어서의 역할 가능성에 대해 많은 연구자들이 회의를 가지고 있는 것이 사실이다. 그러므로 본 연구는 흰쥐의 좌심방을 이용하여, 역방향 Na-Ca 교환의 존재 여부와 그 역할의 존재 가능성을 추구하여 보고자 하였다. 흰쥐의 좌심방은 전기장 자극(0.5 msec, supramaximal voltage)으로 수축을 유발하고, 자극 빈도를 안정시 4 Hz에서 0.4, 1, 8 Hz로 변동시킬때 그 수축 장력에서 특징적인 역 사다리 효과(negative staircase effect)가 나타내었으나, 이때 ^{45}Ca 섭취는 저빈도로 갈수록, 또한 고빈도로 갈수록 증가되는 이원적인 증가를 나타내었다. 자극 빈도를 4 Hz에서 0.4 Hz로 변동시에는 수축 장력이 특징적인 삼단계 변환, 즉 급격히 증가하는 첫단계에 이어 급격하게 감소하는 이단계와 안정되어지는 삼단계로 나타났다. ^{45}Ca 섭취도 장력 변동과 같은 양상으로 처음 30초 동안에 현저하게 증가한 후 감소되었다. Na-Ca 억제 약물인 benzamil은 10^{-5} M에서부터 3×10^{-4} M까지 용량에 비례하여 특히 초기의 장력증가를 봉쇄하였다. Bay K-8644 (3×10^{-5} M) 처치는 자극 빈도 감소에 따른 수축력 증가를 현저하게 항진시켰으며, benzamil처치는 이때에도 억압을 나타내었다. Verapamil 3×10^{-5} M 전처치시에는 4 Hz 자극시의 수축 운동은 완전히 소실시켰으나, 0.4 혹은 1 Hz로 바꿈에 따라 수축 운동이 재현되었다. 이때 ^{45}Ca 섭취는 verapamil을 전처치하지 않은 경우보다 현저하게 항진되었다. 이상의 결과로 보아, 흰쥐의 좌심방에서 자극 빈도 감소후에는, 먼저 역방향 Na-Ca 교환에 의해 calcium이온이 세포내로 유입되어 수축운동의 항진이 유발되고, 이어 Na-Ca 교환이 정방향으로 변환되어 calcium이온을 세포밖으로 유출시킴에 따라 수축 운동이 감소된다고 생각한다.