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The Mode of the Staircase Phenomenon in Relation to Ryanodine Sensitivity in Rat Papillary Muscle, Bullfrog Atrium and Frog Ventricle

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ABSTRACT. Frog ventricular muscle, when repetitively stimulated at an interval of 1 sec, showed a gradual increase in contraction strength; i.e., the positive staircase phenomenon. The tension of rat papillary muscle, on the other hand, was maximum in the first contraction, after which it decreased gradually during repetitive stimulation; the negative staircase phenomenon. The tension of bullfrog atrial muscle was large in the first contraction, but decreased in the next 15 to 20 contractions and then increased in the following ones. The tension was small if the stimulation interval was prolonged in frog ventricular muscle (positive relationship between contraction strength and stimulation interval). This relationship was reversed in rat papillary muscle. The optimum Ca^{2+} concentrations for maximum tension development at a stimulation interval of 5 sec were 7.2-9.0 mM in frog muscles and 3.6 mM in rat muscles. Ryanodine inhibited the contraction in rat papillary muscle at lower concentration than those in bullfrog atrial muscle, and it had much less effect on frog ventricular muscle. These results indicate that tissue having high sensitivity to ryanodine exhibits the negative staircase phenomenon, a negative relationship between interval and strength, and low dependency on external Ca^{2+} concentration.

Key words : heart muscle contraction — staircase phenomenon — ryanodine

Contraction of the cardiac muscle is regulated by cytosolic Ca^{2+}, which is released from the sarcoplasmic reticulum (SR) through the Ca^{2+}-induced Ca^{2+} release mechanism.[1] The density of SR and its function, however, are not always consistent among the cardiac tissues of different animals. Some of the contraction properties of cardiac muscles are related to differences in the developmental stages of SR. Rat atrial and ventricular muscle cells, for example, are provided with well matured SR,[2] and display the negative staircase phenomenon, post-arrest potentiation, and a negative relationship between contraction strength and the stimulation interval[3-5] (the longer the interval the stronger the contraction), and the maximum tension development at slightly elevated $[Ca]_o$.[6] On the other hand, frog ventricular cells which usually lack SR,[2] but rarely are provided with SR, exhibit the positive staircase phenomenon and a positive strength-interval relationship.[3]

Atrial muscle is considered to have more matured SR than the ventricular muscle. In atrial cells, the action potential is shorter and the amount of Ca^{2+}

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entry is smaller than in ventricular cells. Accordingly, an effective amplification mechanism of Ca^{2+} concentration may be required. This mechanism is none other than the Ca^{2+}-induced Ca^{2+} release occurring in SR.

Recently, ryanodine, an insecticidal alkaloid, has attracted attention because it inhibits the contraction of cardiac and smooth muscles. It has now been elucidated that ryanodine has no effect on inward Ca^{2+} current,[7] but does inhibit Ca^{2+} release from SR through a mechanism by which it locks Ca^{2+} channels of the SR membrane into an open state, allowing the leakage of Ca^{2+} from SR into cytoplasma, which finally brings about exhaustion of stored Ca within SR.[8–13] According to Ciofalo,[14] however, frog ventricle is resistant to ryanodine.

The present study was carried out to determine the influence of stimulation intervals and external Ca^{2+} concentrations on the contraction strength in frog ventricular muscles, bullfrog atrial muscles and rat papillary muscles. These muscles are supplied by SR in different developmental stages, which were estimated from the sensitivity to ryanodine. Based on its contraction properties, the relative role of SR in regulating myoplasmic Ca^{2+} concentration was discussed.

METHODS

Ventricular muscles from frogs (*Rana nigromaculata*), atrial trabecullae muscles from bullfrogs (*Rana catesbeiana*) and papillary muscles from Wistar rats were used as the experimental materials. Frog ventricular strips were prepared by cutting the ventricle in a circumferential direction into a piece 1 mm wide and 3 mm long. Several trabecullae muscles were isolated from the bullfrog atrium and tied into a bundle 1 mm in diameter and 4–5 mm in length. Rat papillary muscles were prepared from the left ventricle and were 0.3–0.5 mm $\times$ 4–5 mm.

All of the preparations were immersed horizontally into a muscle chamber ($5\times5\times40$ mm). One end of each preparation was tied to a hook and the other end was connected to a strain-gauge transducer. The muscle was allowed to equilibrate for 2 hrs during which "healing over" was completed. Meanwhile, the muscle was subjected to repeated stretching and release and the initial length was adjusted so as to achieve maximum tension. The muscle was stimulated by a rectangular current pulse of 3 msec duration and 2x supramaximal strength through Ag-AgCl plate electrodes. The intervals between pulses were set at 1, 2, 5 and 10 sec. The developing tension was monitored on a cathode ray oscilloscope and recorded on an ink writing pen recorder (RJG 3201, NIHON KOHDEN). Ringer solution for the frog heart contained (mM) NaCl 117, KCl 2.0, $CaCl_2$ 1.8, glucose 10 and HEPES-NaOH buffer 8.0 (pH=7.3), Tyrode solution for the rat heart contained (mM) NaCl 145, KCl 5.0, $CaCl_2$ 1.8, $MgCl_2$ 1.0 glucose 10 and HEPES buffer 8.0 (pH=7.3). When $[Ca^{2+}]$ was lowered below 1.8 mM, $MgCl_2$ was added so that the sum of $[Ca^{2+}]$ + $[Mg^{2+}]$ would be 2.0 mM. Experiments on the frog heart were carried out at room temperatures of 20–24°C, while those on rat heart were performed at 33–34°C. In both series, the muscle chamber was perfused continuously with the standard solutions bubbled with 100% O_2 at a rate of 5 ml/min. Ryanodine (Progressive Agri. System Inc, PA) was dissolved in DMSO (dimethyl sulfoxide acid) solution at 10^{-2} M and then further diluted in Ringer or Tyrode solution at a final concentration of 10^{-4}–10^{-8} M.

RESULTS

1. Contraction strength vs stimulation interval

When the frog ventricular muscle was stimulated repetitively at a stimulation interval of 1 sec, the tension increased progressively. This tension increase is known as the positive staircase phenomenon (Fig. 1A). In rat papillary muscle, on the contrary, the negative staircase phenomenon appeared ; i.e., the tension of the first contraction was large, but it decreased during repeated stimulation (Fig. 1C). If the stimulation was interrupted for several seconds, post-arrest potentiation occurred. The response of the bullfrog atrial muscle was intermediate between those of the frog ventricle and rat ventricle; that is, the contraction was strong at first, then was gradually depressed and then increased again after 20–30 stimulations. The staircase, therefore, changed from negative to positive.

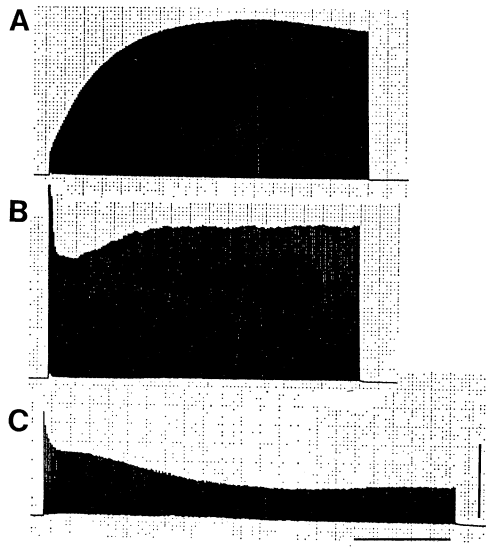

Fig. 1. The staircase phenomenon in frog ventricular muscle (A; 21°C), bullfrog atrial muscle (B; 20°C), and rat papillary muscle (C; 34°C). The stimulation interval was 1 sec. Calibrations were 0.5 g and 1 min.

The relationship between contraction strength in a steady state and the stimulation interval is shown in Fig. 2. The frog ventricular muscle was characterized by a decrease in tension as the stimulation interval was prolonged. This is called a positive relationship between contraction strength and the stimulation interval. The rat papillary muscle exhibited a condition opposite to that of the frog ventricular muscle ; namely, a negative relationship between contraction strength and the stimulation interval. Frog atrial muscle showed intermediate characteristics. Tension was maximum at 2 or 5 sec intetvals and decreased at either shorter or longer intervals than the optimum. Contractions elicited at shorter intervals than 1 sec were not studied in detail, because changes in the shape of the action potential affected the contraction strength as the stimulation interval was shortened.

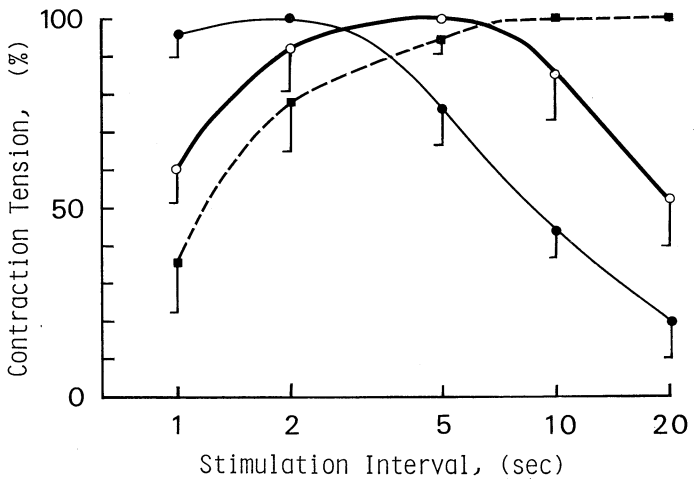

Fig. 2. Relationship between contraction strength and stimulation interval in frog ventricular muscle (●—● thin line, 20-22°C), bullfrog atrial muscle (○—○ thick line, 20—22°C), and rat papillary muscle (■—■ interrupted line, 34°C), in a standard Ringer solution containing 1.8 mM $CaCl_2$. Each point was obtained from 5 preparations of separate tissues. Vertical bars indicate + or − SE. Tension at the optimal interval was taken as 100%; 10 sec intervals for rat papillary muscle, 5 sec intervals for bullfrog atrial muscle, and 2 sec intervals for frog ventricular muscle.

2. Effect of $[Ca^{2+}]$ on contraction

A rise in $[Ca^{2+}]$ in Ringer or Tyrode solution potentiated contraction, while a fall in $[Ca^{2+}]$ inhibited it. The changes in tension, however, were not always consistent among the three kinds of heart muscle preparations. The maximum tention was obtained at a $[Ca^{2+}]$ as low as 3.6 mM in rat ventricular muscle, whereas it was at 7.2-9.0 mM in muscles from the frog atrium and ventricle (Fig. 3). The fact that tension fully developed at a lower extracellular $[Ca^{2+}]$ in rat papillary muscle indicates that the Ca amplification mechanism of the SR of rat muscle functions better than those of frog atrium and ventricle. A rise in the $[Ca^{2+}]$ beyond the optimum inhibited the contraction and slowed relaxation. The decrease in tension at a low $[Ca^{2+}]$ was not due to the change in the number of contracting cells[15] but to reduction of the contraction capacity of each cell. The curve relating tension to $[Ca^{2+}]$ for frog ventricular muscle was less steep than those for frog atrial or rat papillary muscle, suggesting that the Ca ions necessary for the initiation of contraction are coupled directly to extracellular Ca and that a self-regenerative or amplification mechanism plays a very small role in the regulation of intracellular $[Ca^{2+}]$.

3. Inhibition of contraction by ryanodine

The muscle chamber was perfused with Tyrode or Ringer solution containing 10^{-6}–10^{-4} M ryanodine. The contractions of bullfrog atrial muscles and rat papillary muscles were inhibited or abolished by ryanodine but that of frog ventricular muscle was not (not shown in Fig. 4). Fig. 4 shows inhibition of the contraction of bullfrog atrial muscle by ryanodine at two different concentrations. The inhibition was completely reversed if ryanodine was washed out.

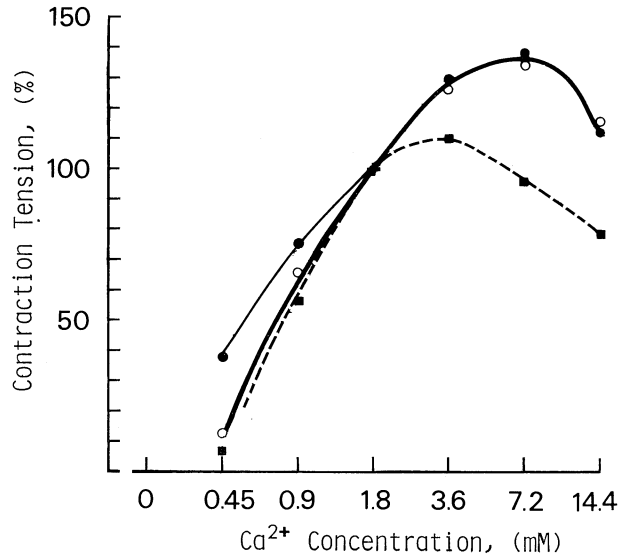

Fig. 3. Tension at different $[Ca^{2+}]_o$ in frog ventricular muscle (●—● thin line, 21-22°C), bullfrog atrial muscle (○—○ thick line, 22-24°C), and rat papillary muscle (■—■ interrupted line, 34°C). Each point was obtained from 3-4 preparations of separate tissues at stimulation intervals of 5 sec. Tension developed in the 1.8 mM $CaCl_2$ solution was taken as a control of 100%.

Sometimes an increase in tension was observed preceding inhibition (Fig. 4B). Valdeomillos and Eisner[16] reported that a transient but marked increase in tension was caused by ryanodine. In the present study, however, the tension increase was only slight and was not observed in all of the preparations. In addition, spontaneous partial recovery during ryanodine perfusion was observed in about half of the preparations examined when the ryanodine concentration was 10^{-5} M (Fig. 4A). Another finding was that ryanodine did not completely abolish the tension. A small amount of measurable tension, less than 1% of the control, still existed even if a high concentration of ryanodine was applied.

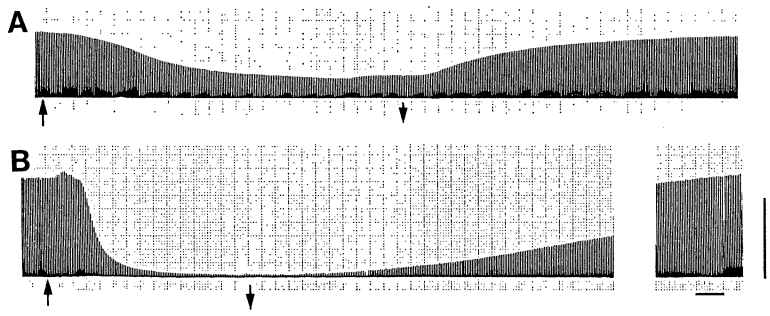

Fig. 4. Inhibition of tension by ryanodine at 10^{-5} M in A and at 10^{-4} M in B. Bullfrog atrial muscles. Ryanodine-Ringer solution was introduced at the arrow mark ↑, and the standard Ringer solution at the arrow mark ↓. Calibrations were 0.5 g and 1 min, 23°C.

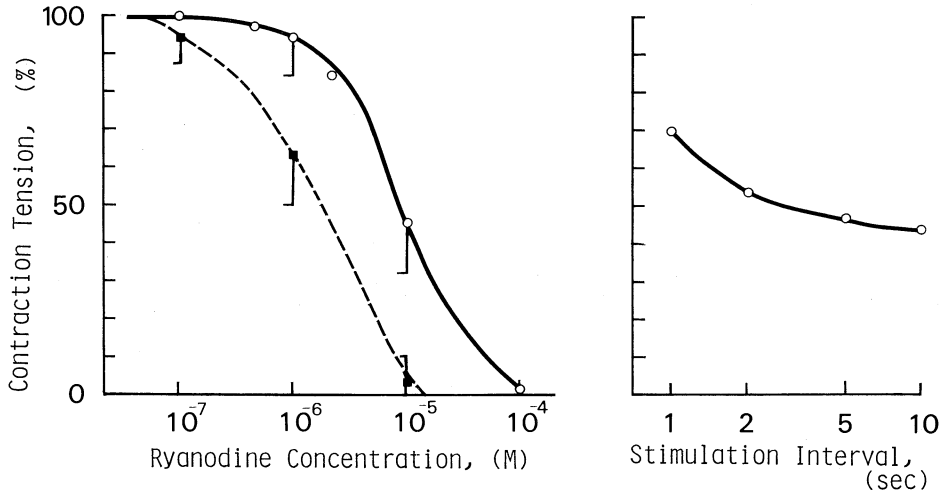

Fig. 5. A: Effect of different concentrations of ryanodine on contraction in bullfrog atrial muscle (○—○ thick line, 20-22°C) and in rat papillary muscle (■—■ interrupted line, 34°C) at stimulation intervals of 5 sec. Tension in the standard solution without ryanodine was taken as 100%. B: Effect of 10^{-5} M ryanodine on contraction at different stimulation intervals in frog atrial muscle. Tension in the standard Ringer solution at each stimulation interval was taken as 100%.

Fig. 5A shows the dose dependency of steady tension in bullfrog atrial muscle and rat papillary muscle. For bullfrog atrial muscle, the threshold concentration was 5×10^{-7} M and the maximum concentration was 2×10^{-4} M. The curve for rat papillary muscle paralleled that for bullfrog atrial muscle but shifted by 0.5 units toward a lower concentration.

The mode of dependency of tension on the ryanodine concentration was modified by changes in the stimulation intervals. In Fig. 5B, the ratio of the tension in a 10^{-5} M ryanodine solution to that in the standard Ringer solution was plotted against the stimulation interval. First, the steady state tension at intervals of 1, 2, 5 and 10 sec was measured in the standard Ringer solution, then in the ryanodine solution, and then again in the standard solution. The tension in the ryanodine solution was compared to that in the control ; i.e., the average tension before and after ryanodine treatment. When the ryanodine concentration was set 10^{-5} M, the tension decreased by nearly half. This result

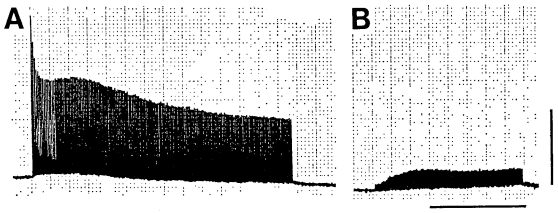

Fig. 6. The negative staircase phenomenon changed into a positive one after treatment with 10^{-4} M ryanodine. Rat papillary muscle. Between A and B, the muscle chamber was perfused with 10^{-4} M ryanodine-Tyrode solution for 15 min. The stimulation interval was 0.85 sec. Calibrations were 0.1 g and 1 min, 34°C.

indicated that the inhibitory effect of ryanodine became more pronounced as the stimulation interval was prolonged. Probably, SR can accumulate a great deal of Ca during resting periods between stimuli and is affected by ryanodine.

In rat papillary muscle, ryanodine changed the negative staircase phenomenon into a positive one, as shown in Fig. 6. The ryanodine concentration required to produce this effect was relatively high. When the concentration was lower, ryanodine simply abolished the staircase and when it was higher, the contraction was almost completely inhibited. These results also indicate that the development and function of SR are closely related to the presence of the negative staircase.

DISCUSSION

The typical positive staircase phenomenon like that first described by Bowdwich in 1871 was observed in frog ventricular muscle (Fig. 1). This phenomenon is characterized by depression of the contractile capacity during quiescent periods and improvement during the repetition of contractions. Therefore, the contraction strength decreases as the stimulation interval is prolonged (Fig. 2). The rat papillary muscle exhibited opposite or negative properties, and the bullfrog atrial muscle was characterized by intermediate ones between those of the rat papillary muscle and the frog ventricular muscle. The optimum $[Ca^{2+}]_o$ level for the development of maximum tension was lower in rat papillary muscle than in the other preparations. Ryanodine had a greater effect on the rat papillary muscle than on the bullfrog atrial muscle, and had no effect on the frog ventricular muscle. It has already been reported that the sensitivity of the mammalian ventricle to ryanodine is in the order of rat > dog > cat > rabbit.[17] The present study adds to this the results on amphibian cardiac muscles.

The differences in contraction properties, such as the mode of the staircase, the dependence on $[Ca^{2+}]$ and the sensitivity to ryanodine, appear to be due to the degree of development or maturity of SR among different tissues. Fig. 7 is a schematic diagram showing the intracellular Ca^{2+} regulation during a cycle

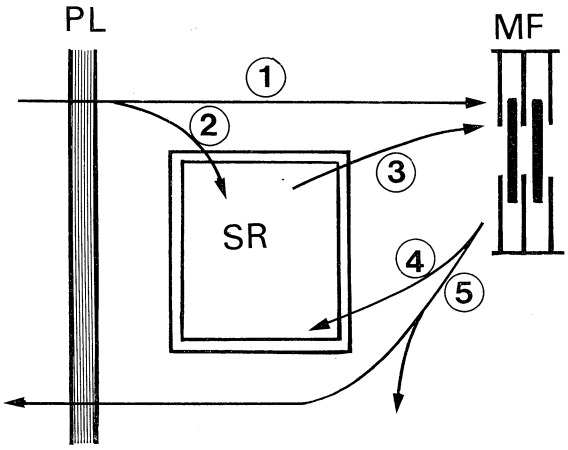

Fig. 7. Schematic diagram illustrating Ca^{2+} movement during the contraction-relaxation cycle. PL: plasma membrane, SR: sarcoplasmic reticulum, MF: myofilaments. See text for explanation.

of contraction and relaxation. It can be seen that Ca ions entering the myoplasm during action potential are directly bound to troponin to trigger the contraction (route ①), or they operate upon SR (②) and are received by troponin after having been amplified in their concentration through a Ca^{2+}-induced Ca^{2+} release mechanism (③). Relaxation follows sequestration of Ca^{2+} by SR (④) or extrusion outside the cell (⑤). In cardiac tissue in which SR is rare, such as that of the frog ventricle, the amount of stored Ca is only a fraction of that required for the maximum contraction. Accordingly, the first contraction after the resting period is very small. If the stimulation is repeated, the available Ca^{2+} increases either by enhancement of transsarcolemmal entry via ① or by filling of the storage site via ②, and consequently the positive staircase phenomenon takes place. In cardiac tissue provided with mature SR such as that of rat papillary muscle, it is assumed that there is a sufficient storage of Ca^{2+} for the development of full contraction but the rate of refilling via ② is not rapid enough to compensate for the release via ③. When the tissue is repetitively stimulated, therefore, the stored Ca progressively decreases, resulting in the negative staircase phenomenon. If the function of SR is eliminated by treatment with ryanodine, the staircase in rat papillary muscle becomes similar to that in frog ventricular muscle (Fig. 6). Thus, the mode of the staircase is clearly related to the maturity of SR.

The change in tension which occurs with the change in extracellular $[Ca^{2+}]$ may also be explained on the same basis. In rat papillary muscle, the contraction is primarily dependent on the Ca^{2+} released from SR (route ③) and extracellular Ca^{2+} modifies the amount of stored Ca^{2+} via route ②. In frog ventricular muscle, the contraction is directly related to external $[Ca^{2+}]$ (route ①). Therefore, as shown in Fig. 3, frog ventricular muscle is more dependent on $[Ca^{2+}]_o$ than rat papillary muscle.

Ciofalo[14] reported that ryanodine did not inhibit but slightly augmented the contraction of frog ventricular muscle. In the present study, no potentiating action of ryanodine on frog ventricular muscle was observed. The bullfrog atrial muscle was sensitive to ryanodine, although the concentration required to have an effect was higher than that for the rat papillary muscle. The results indicate that SR plays a significant role as a Ca^{2+} source for triggering contraction in the bullfrog atrium, as is expected from the intermediate properties it exhibits in the staircase phenomenon and from its $[Ca^{2+}]_o$ dependence. The role of SR in the bullfrog atrium has already been suggested from results with another alkaloid, caffeine.[18] Pharmacological investigation of the function of SR and of the staircase phenomenon has now been proven to be valid.[19]

One characteristic of ryanodine action is that its inhibitory effect on tension is increased if the stimulation interval is prolonged (Fig. 5). This is related to the extent of filling of SR with Ca^{2+}. As has previously been described, if the refilling process of Ca via route ② is not very rapid, the amount of stored Ca^{2+} may be fully supplied at intervals of 10 sec, whereas it may decrease at intervals of 1 sec. Ryanodine has a stronger effect on SR fully filled with Ca and inhibits tension at 10 sec intervals more markedly than at 1 sec intervals. In addition, ryanodine does not abolish tension completely but leaves a small amount of tension, i.e., as low as 1% of the control. A similar result was obtained after treatment of muscle with a calcium channel blocker, nifedipine.[20] Therefore, there must be a process which is resistant to ryanodine and nifedipine

for raising of the myoplasmic Ca^{2+} concentration. It has been postulated that the Na^{+}–Ca^{2+} exchange mechanism plays a role in controlling $[Ca^{2+}]$ to regulate the contraction-relaxation cycle.[4,21,22]

Acknowledgment

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