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☐ Original Article ☐

Effects of Ryanodine on the Intracellular Na^+ Activity and Tension and Action Potentials of Rat and Guinea Pig Cardiac Ventricular Muscles

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Ryanodine has different effects on the contractility of rat and guinea pig ventricular muscle. Thus we investigated the effect of ryanodine on the intracellular Ca^{2+} and Na^+ activities of the rat and guinea pig ventricular myocytes with two specific aims; whether there are any differences in intracellular Na^+ activities between rat and guinea pig ventricular muscle cells, and if any, how the differences in intracellular Na^+ activities are related to the effect of Na^+-Ca^{2+} exchange on the action potential configuration and excitation-contraction coupling of the rat and guinea pig ventricular myocytes. Ryanodine (10^{-7} M) diminished the slow repolarization phase of the rat ventricular action potential while the duration of the rapid repolarization phase increased. Ryanodine (10^{-7} M) significantly increased the plateau of the action potential. At the steady state of 0.2 cps, intracellular Na^+ activities (a^i_{Na}) of the rat and guinea pig ventricular myocytes were 8.7 ±5.2 mM (n=16, 4 rats) and 10.0±4.1 mM (n=25, 7 guinea pigs) respectively, but there were no statistically significant differences. The contractility of the rat ventricular muscle nearly disappeared due to ryanodine (10^{-7} M) with little changes in a^iNa. Monensin (10 mM) not only increased the resting tension but also remarkably increased a^i_{Na} from 2.0 mM to 20 mM. Ryanodine (10^{-7} M) continuously decreased a^i_{Na} of the guinea pig ventricular muscle after the contraction ceased to decrease. Monensin increased the contractility as well as a^i_{Na}. These results suggest that the contractility of rat and guinea pig ventricular myocytes is determined by the change in the action of the Na^+-Ca^{2+} exchange mechanism depending upon the plateau of action potential and the intracellular Na^+ and Ca^{2+} activities. So ryanodine could decreases the contractility via its effect on Na^+-Ca^{2+} exchange transport which could be one of possible mechanisms of negative inotropism by ryanodine.

Key Word: Ryanodine, Na^+-Ca^{2+} exchange transport, rat, guinea pig, ventricular myocytes

The rat and guinea pig ventricular muscles have different electrical and mechanical characteristics as well as different responses to various drugs. The action potential of the rat ventricular muscle is composed of two plateaus. One is a very short plateau at 0 mV, and the other is a long plateau (slow repolarization) at about −40 to −50 mV. The action potential of the guinea pig ventricular muscle, however, has a single plateau of long duration at a membrane potential above 0 mV (Coraboeuf and Vassort, 1968; Mitchell *et al.* 1984; Cohen and Lederer, 1988). The contractility of the rat ventricular muscle shows a phasic pattern and has a membrane potential dependence like the slow inward current (Leoty, 1974). On the other hand, the contractility of the guinea pig ventriculrar muscle has a phasic as well as a tonic component (Ochi and Trautwein, 1971).

These dissimilarities suggest that mobilization of Ca^{2+} for the activation of contraction would be different between the two heart tis-

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sues. In the rat ventricular muscle, Ca^{2+} influx via the slow inward current releases Ca^{2+} from the intracellular store and induces contraction, whereas the tonic tension of the guinea pig is induced by the Ca^{2+} influx across the cellular membrane via the Na^+-Ca^{2+} exchange transport (Chapman, 1983). Recent measurements of action potential and contraction of isolated ventricular muscle cells showed that the Ca^{2+} influx across the cellular membrane as well as the Ca^{2+} release from the intracellular store determines the magnitude of the contractility. In the rat, the Ca^{2+} released from the intracellular store has a greater role in determining contractility. In the guinea pig, however, the Ca^{2+} influx across the cellular membrane plays a predominant role (Mitchell *et al.* 1987).

It is reported that the slow repolarization of the action potential measured from atrial muscle cells of various animals as well as the rat ventricular myocytes, accompanied with strong contractility (Simurda *et al.* 1981), is significantly decreased in its magnitude by low Na^+ solutions (Mitchell *et al.* 1984), and presumably consists of the inward current produced by Na^+-Ca^{2+} exchange (Schouten and ter Keurs, 1985).

On the other hand, Na^+-Ca^{2+} exchange also takes part in the plateau formation of the action potential of the guinea pig ventricular muscle (Fedida *et al.* 1987). Because the increase in intracellular Ca^{2+} activity or the decrease in intracellular Na^+ activity augments the inward current produced by the Na^+-Ca^{2+} exchange (Mullins, 1981; Kimura *et al.* 1987), the difference of action potential configurations of the rat and guinea pig ventricular muscle could be due to the difference in intracellular Ca^{2+} and Na^+ activities and to the different contribution of Na^+-Ca^{2+} exchange on the action potential plateau formation.

Ryanodine, a plant alkaloid, shows different effects on the contractility of rat and guinea pig ventricular muscle. There have been various researches on the mechanism of ryanodine on contractility, but still it is not yet clearly understood (Jenden *et al.* 1969; Frank and Sleator, 1975; Besch, 1985). Recent measurements of intracellular Ca^{2+} activity with the use of fluorescence dye Quin II in Ca-tolerant rat myocytes at rested state revealed that ryanodine slowly released Ca^{2+} from the sarcoplasmic reticulum (SR) and eventually the SR became totally depleted (Hansford and Lakatta, 1987). However, since the decreasing effect of ryanodine on contractility depends upon the electric activity of the cardiac muscle cells and especially doesn't take place in resting muscle cells (Malecot and Katzung, 1987), it is not clear whether Ca^{2+} depletion by ryanodine of the SR plays a major role in decreasing the contractility. In guinea pig ventricular muscles where the Ca^{2+} influx across the cellular membrane plays a greater role, since the contractility and action potential are little affected by ryanodine, it is not clear whether ryanodine depletes Ca^{2+} out of the SR of guinea pig ventricular muscle cells. Furthermore, since ryanodine also diminishes the slow repolarization of the rat action potential (Terrar and Mitchell, 1987), assuming that the slow repolarization is via the Na^+-Ca^{2+} exchange, there could be other modes of ryanodine action; lowering intracellular Ca^{2+} activity resulting from enhanced efflux across the plasmalemmal membrane (Frank and Sleator, 1975; Suh and Sleator, 1982).

Therefore, we investigated the effect of ryanodine on the intracellular Na^+ and Ca^{2+} activities of the rat and guinea pig ventricular myocytes with two specific aims to study; whether there are any differences in intracellular Na^+ activities between rat and guinea pig ventricular muscle cells, and if any, how the differences in intracellular Na^+ activities are related to the effect of Na^+-Ca^{2+} exchange on the action potential configuration and excitation-contraction coupling of the rat and guinea pig ventricular myocytes.

METHODS

Guinea pigs of either sex ranging in size from 200 to 500 grams were sacrificed with an intraperitoneal injection of Na pentobarbital (50 mg/Kg) and heparin (2000 IU/Kg). The heart was rapidly excised and placed in oxygenated (100% O_2) Tyrode solution. Right ventricular papillary muscles were dissected, and then were transferred to a recording chamber which was continuously perfused with the oxygenated Tyrode solution. The Tyrode solution had the following composition in millimoles per liter (mM): NaCl 133.5; KCl 4.0; KH_2PO_4 0.3; $MgSO_4$ $7H_2O$ 1.2; $CaCl_2$ 1.8; HEPES 10; dextrose 10. All cardioplegic solutions had 25 mM K^+ and, NaCl

Table 1. Intracellular Na^+ activities of ventricular myocytes

Species	Intracellular Na^+ activities
Rat	8.7±5.2 mM(n=16, 4 rats)
Guinea pig	10.0±4.1 mM(n=25, 7 guinea pigs)

Mean±S.D.

was isotonically replaced with tetramethylammonium (TMA) chloride to reduce Na^+ concentration in cardioplegic solutions (Table 1). The flow rate of superfusion solutions was kept constant (about 5 ml/minute) in the way that the solution levels of solution containers were maintained constant with spring-aided small supports. The temperature in the recording chamber wasr maintained at 27.5±0.2℃. Tension was monitored by a Model 400A Force Transducer System (Cambridge Technology, Inc.) and displayed on a Philips PM 3305 Digital Storage Oscilloscope and on a Gould Brush 220 recorder along with the signal from the differential electrometer for the ion-selective electrode (ISE). A stimulating current provided by a Grass S11 stimulator was passed through a pair of platinum wires. Transmembrane action potentials were recorded between a 3 M KCl-3 % agar bridge in the bath and a standard microelectrode (RE) filled with 3 M KCl. The microelectrodes, pulled from microfiber capillary tubings (WPI, Inc.), had a typical resistance in the range of 5-20 megohms and tip diameters of less than 0.5 microns.

Na^+-selective electrodes (NSE) were manufactured as previously described (Park and Suh, 1991). The resin used for the Na^+-selective electrodes was purchased from the Fluka Chemie AG (Fluka 71176). Glass microelectrodes were pulled from borosilicate glass capillaries (WPI 1B200F6) and placed on the top of a bottle containing a small drop of pure dichlorodimethylsilane in a oven at 200℃ for 30 minutes. The silanized pipettes were bevelled with alumina powder and filled with reference solutions (100 mM NaCl for NSE). A column of exchanger resin up to several 100 microns was forced into the electrodes by means of a partial vacuum.

The e.m.f. (electromotive force) from the NSE was measured with an electrometer (AD515 operational amplifier, Analog Devices), and membrane potential measured by the 3 M KCl filled microelectrode (RE) was electronically subtracted from the e.m.f. measured with the NSE. To improve a slow response time, the negative capacity compensation circuit was added to the head-stages of the amplifier. Na^+-selective electrodes were normally checked before and after the experiments. Na^+-selective electrodes were calibrated with mixed electrolyte solutions (NaCl 100+KCl 40; NaCl 30+KCl 110; NaCl 10+KCl 130; NaCl 3+KCl 137; NaCl 1+KCl 139; NaCl 0.3+KCl 139.7; in mM). NSE had Nernstian responses in pure NaCl calibrating solutions, and in mixed NaCl-KCl solution began to deviate from the Nernstian response at 30 mM NaCl plus 120 mM KCl.

RESULTS

Measurement of the intracellular Na^+ activity

In order to measure the effect of ryanodine and monensin on the action potential configuration and the intracellular ionic activity of rat and guinea pig ventricular muscles the change in intracellular Na^+ activity (a^i_{Na}) was measured.

After the contractility and action potential of the ventricular muscle reached a steady-state with a stimulation of 0.2 cps, a^i_{Na} of rat and guinea pig ventricular myocytes were 8.7±5.2 mM (n=16, 4 rats) and 10.0±4.1 mM (n=25, 7 guinea pigs), respectively (Table 1). a^i_{Na} of guinea pig ventricular myocytes was slightly higher than that of rat ventricular myocytes but it was not statistically significant (student's t-test).

The effects of ryanodine and monensin on the a^i_{Na} of rat and guinea pig ventricular muscle were measured. Fig. 1 is an example of the change in a^i_{Na} as the rat ventricular muscle was electrically stimulated with 0.2 cps for a long time. And as it is shown, due to the effect of ryanodine (10^{-7} M), the contractility nearly disappeared whereas a^i_{Na} decreased slightly from 1.8 mM to 1.1 mM. After the contractility was nearly diminished, monensin (10 mM) not only caused an increase in the resting tension but also remarkably increased a^i_{Na} from 2.0 mM to 20 mM.

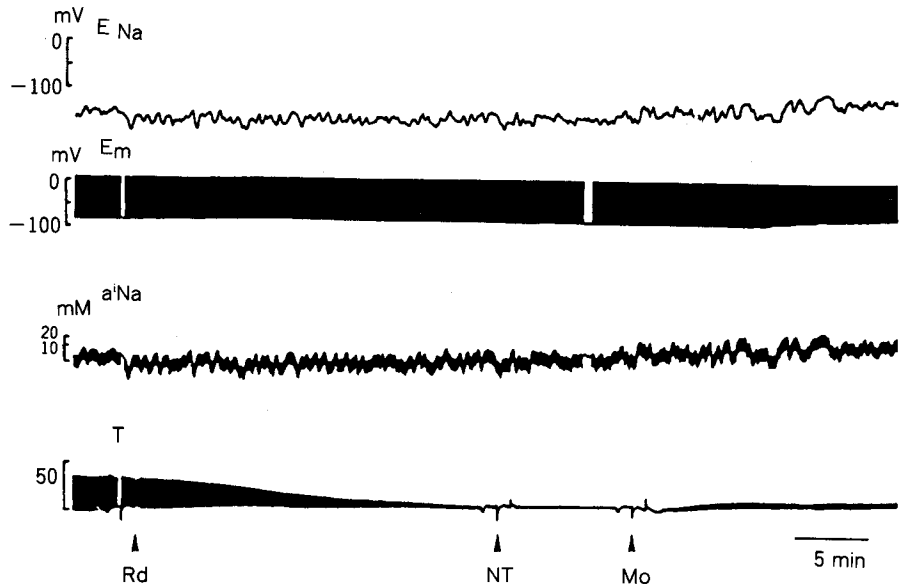

Fig. 1. *Effects of ryanodine and monensin on intracellular Na^+ activity of rat ventricular muscle. The first record is E_{Na}, the second record is Em, the third record is a^{i}_{Na}, and the fourth record is tension. When a new steady state contraction of 0.2 cps was reached with treatment of ryanodine (10^{-7} M, Rd), normal Tyrode solution (NT) was superfused for about 10 minutes, and then monensin (10 μM, Mo) was added to the superfusion solution. Stimulus was stopped for a few beats to measure a^{i}_{Na}. (See the text for details.)*

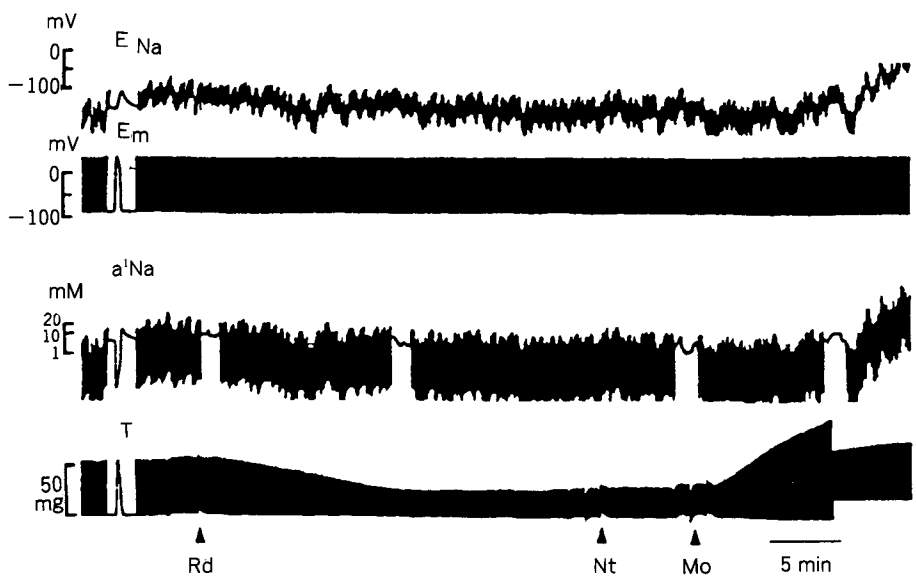

Fig. 2. *Effect of ryanodine and monensin on intracellular Na^+ activity of guinea pig ventricular muscle. When a new steady state of contraction of 0.2 cps was reached with treatment of ryanodine (10^{-7} M), normal Tyrode solution (NT) was superfused for about 10 minutes, and then monensin (10 μM) was added to the superfusion solution. Transient signal of a^{i}_{Na} was electronically filtered to measure steady-state a^{i}_{Na}. (See the legend of Fig. 1 and the text for details.).*

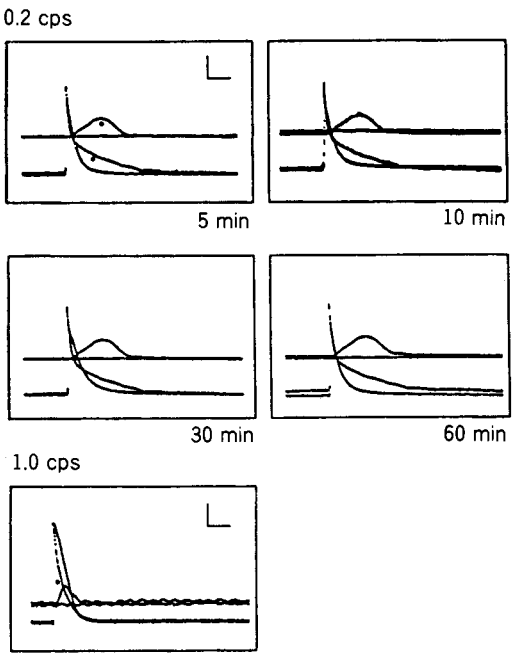

Fig. 3. *Effects of ryanodine on contractility and action potential of rat ventricular muscle. Tissue was stimulated at 0.2 cps and 1.0 cps. Action potentials and tensions after treatment of ryanodine (10^{-7} M) were compared with the control figures (normal Tyrode; closed circle). At 0.2 cps, the slow repolarization phase disappeared and the duration of the rapid repolarization phase was increased. At 1.0 cps, the duration of the rapid repolarization phase was increased. Tension decreased significantly at both frequencies tested. Vertical scale, 20 mV/div for Em and 0.1 g/div for tension. Horizontal scale, 25 msec/div.*

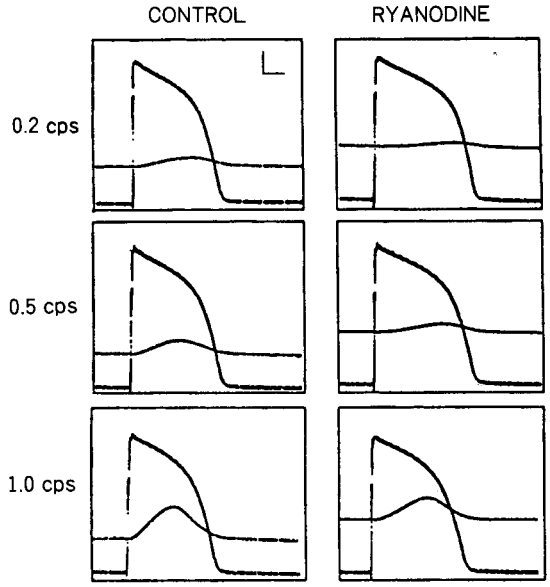

Fig. 4. *Effects of ryanodine on contractility and action potential of guinea pig ventricular muscle. Tissue was stimulated at 0.2, 0.5 and 1.0 cps. After treatment of ryanodine (10^{-7} M), the duration of action potentials was increased and tension was decreased, at all frequencies. But the degree of changes was not so prominent compared to the rat ventricular muscle (Fig. 1). Vertical scale, 20 mV/div for Em and 0.1 g/div for tension. Horizontal scale, 50 msec/div.*

According to Fig. 2, which is an example of the effect of ryanodine (10^{-7} M) on the guinea pig ventricular muscle as a^i_{Na} was continuously measured with an electric stimulation of 0.2 cps, the contractility did not disappear completely but decreased about 50%, and a^i_{Na} continuously decreased from 6.2 mM to 3.1 mM. Monensin (10 mM) increased the contractility and a^i_{Na} to about 400% and 20 mM, respectively.

The effect of ryanodine on the action potential of rat ventricular muscle

The action potential and tension of an isolated right ventricular papillary muscle of the rat was electrically stimulated with 0.2 contractions per second (cps) after being fixed in an experimental chamber. The action potential of the rat ventricular muscle in a normal Tyrode's solution (the control group) showed a rapid repolarization phase after an initial depolarization and thereafter a slow repolarization appeared (Fig. 3).

The action potential was compared with the control group after being treated with ryanodine (10^{-7} M) with a different stimulation frequency. With a stimulation frequency of 0.2 cps the slow repolarization phase nearly disappeared and the rapid repolarization phase slightly increased in its fashion. With a stimulation frequency of 1.0 cps where the slow repolarization phase didn't appear well, the du-

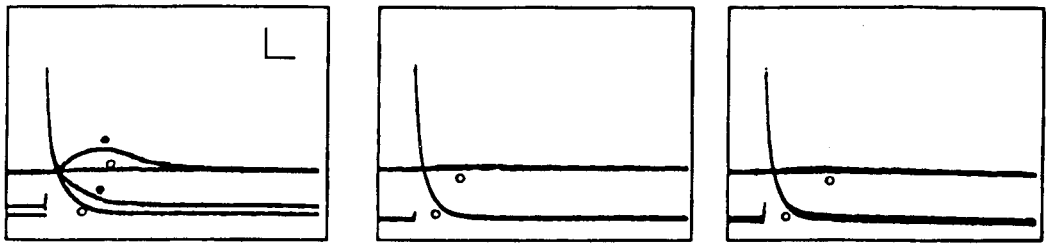

Fig. 5. *Effects of monensin on contractility and action potential of ryanodine-pretreated rat ventricular muscle. Heart tissue was stimulated at 0.2 cps. After the tissue was pretreated for 25 min with ryanodine (10^{-7} M, open circle), monensin (10 μM, Mo) was superfused (closed circle). Tension was slightly increased by monensin in 5 minutes, with little changes in action potential configuration. Sustained treatment for 10 minutes caused a tendency of recovery in the slow repolarization phase and depolarization in resting membrane potential. Vertical scale, 20 mV/div for Em and 0.1 g/div for tension. Horizontal scale, 25 msec/div.*

ration of the action potential due to the rapid repolarization phase increased. Ryanodine decreased tension in all stimulation frequencies so it was almost never recorded (Fig. 3).

The effect of ryanodine on the action potential of guinea pig ventricular muscle

The action potential of the guinea pig, compared with that of the rat, showed a single plateau at a quite higher membrane potential (Fig. 4). In all stimulation frequencies ryanodine (10^{-7} M) did not change the appearance of the repolarization pattern but the duration of the action potential markedly increased. The tension was decreased but, contrary to the rat ventricular muscle, did not disappear completely (Fig. 4).

The effect of monensin on the action potential of rat ventricular muscle

The effect of monensin on the action potential of rat ventricular muscle was measured when the slow repolarization phase almost disappeared due to ryanodine (Fig. 5). After being treated with 5 minutes of monensin (10 μM) the contractility increased slightly, but the shape of the action potential changed little and the slow repolarization phase which disappeared due to ryanodine did not recover. When treated with 10 minutes of monensin (10 μM) the slow repolarization phase recovered a little bit and the resting membrane potential was depolarized slightly.

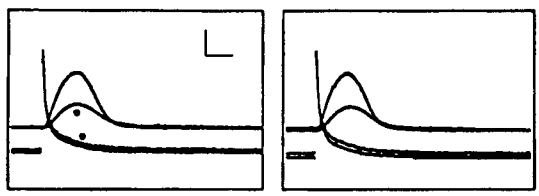

Fig. 6. *Effects of monensin on contractility and action potential of rat ventricular muscle. Heart tissue was stimulated at 0.2 cps. With 5 min.'s treatment of monensin (10 μM, closed circle). tension was significantly increased and the resting membrane potential was slightly hyperpolarized compared to the control (closed circle). The changes were more visible after sustained treatment. Vertical scale, 20 mV/div for Em and 0.1 g/div for tension. Horizontal scale, 25 msec/div.*

When the rat ventricular muscle was treated with monensin (10 μM) without being pretreated with ryanodine, the contractility increased remarkably and the resting membrane potential was slightly depolarized (Fig. 6). The depolarization of the action potential still remained even though there was no more increase in contractility with a continued treatment with monensin (10 μM).

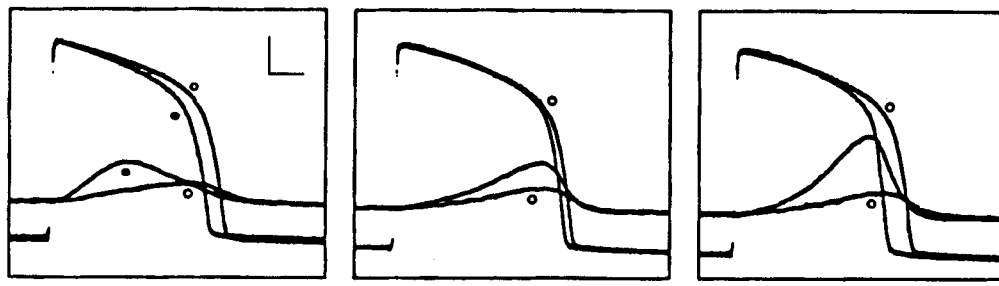

Fig. 7. *Effects of monensin on contractility and action potential of ryanodine-pretreated guinea pig ventricular muscle.*
Heart tissue was stimulated at 0.2 cps. After the tissue was pretreated for 20 min with ryanodine (10^{-7} M, open circle), monensin (10 μM, Mo) was superfused. The duration of action potentials was decreased by monensin (Mo), and recovered to the control (closed circle) in 15 min. Tension was significantly increased. Vertical scale, 20 mV/div for Em and 0.1 g/div for tension. Horizontal scale, 50 msec/div.

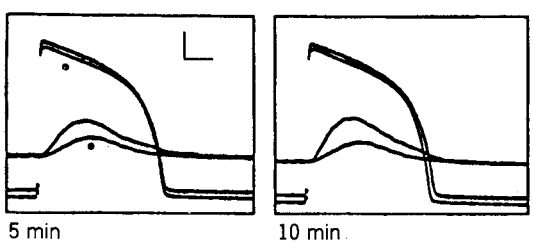

Fig. 8. *Effect of monensin on contractility and action potential of guinea pig ventricular muscle. Heart tissue was stimulated at 0.2 cps. With 5 min.'s treatment of monensin (10 μM), the duration of action potentials was slightly decreased, and resting membrane potential was hyperpolarized. Tension increased significantly. Vertical scale, 20 mV/div for Em and 0.1 g/div for tension. Horizontal scale, 50 msec/div).*

The effect of monensin on the action potential of guinea pig ventricular muscle

The effect of monensin on the action potential of guinea pig ventricular muscle was measured when the duration of the action potential increased remarkably (when the duration of the plateau of 0 mV increased from 210 to 240 msec) by the treatment of ryanodine (Fig. 7). When treated with monensin (10 μM) the duration of the action potential began to decrease and after 15 minutes recovered to 210 msec which is the action potential duration before the pretreatment of ryanodine (10^{-7} M). The contractility, however, increased to a great extent compared with the contractility before the pretreatment of ryanodine.

Monensin (10 μM) increased the contractility of the guinea pig ventricular muscle which was not pretreated with ryanodine. Monensin also decreased the duration of the action potential and hyperpolarized the resting membrane potential (Fig. 8). When monensin was removed from the normal Tyrode's solution, the increase of contractility as well as the hyperpolarization of action potential still remained.

DISCUSSION

Ryanodine-binding protein has been isolated as a Ca^{2+}-release channel of the SR membrane, and its structure has been brought to light. Ryanodine is thought to release Ca^{2+} continuously from the sarcoplasmic reticulum by regulating the Ca^{2+}-release channel (Saito *et al.* 1988; Smith *et al.* 1988; Wagenknecht *et al.* 1989). But the action mechanism of ryanodine on the decrease of contractility is not completely understood (Besch, 1985; Sitsapesan and Williams, 1990).

One possible mechanism of ryanodine on the

decrease of contractility in the cardiac muscle is that it releases Ca^{2+} slowly into the sarcoplasm, as it acts in the skeletal muscle, and eventually the sarcoplasmic reticulum becomes completely depleted of Ca^{2+} (Hansford and Lakatta, 1987). However, the degree of decreasing effect on contractility depends upon the electrical activity of the cardiac muscle and ryanodine has a minimal effect in the resting state of the cardiac muscle cells (Malecot and Katzung, 1987). It is not clear whether the decrease in contractility is surely and solely due to Ca^{2+} depletion of the SR.

For the activation of cardiac muscle, unlike the skeletal muscle, Ca^{2+} is also transported across the plasma membrane (Winegrad, 1979). So it is possible that ryanodine affects the efflux (or influx) of Ca^{2+} via the Na^+-Ca^{2+} exchange, especially during the action potential plateau, and consequently decreases the contractility. Therefore, the aim of this study is to understand the effect of ryanodine on the Ca^{2+} transport across the cellular membrane by observing the effect of ryanodine on the action potential shape along with accompanying contraction and the intracellular Na^+ activity.

The action potentials of the atrial myocardium of various animals including the rat and also the ventricular myocardium of the rat have the slow repolarization phase accompanied with strong myocardial contractility (Simurda *et al.* 1981; Mitchell *et al.* 1984). It is reported that this phenomenon is due to the inward current of the Na^+-Ca^{2+} exchange (Schouten and ter Keurs. 1985). When intracellular Ca^{2+} activity decreases, the inward current produced by Na^+-Ca^{2+} exchange is decreased and consequently the slow depolarization disappears, as shown in Fig. 3. In other cases, an increase in intracellular Na^+ activity would decrease the inward current via Na^+-Ca^{2+} exchange or would increase the outward current which results in an increase in Ca^{2+} influx. Furthermore, depending on the increase in intracellular Ca^{2+} activity, the inward current via Na^+-Ca^{2+} exchange may be reactivated.

The mode of Na^+-Ca^{2+} exchange transport is determined by the intracellular Na^+ and Ca^{2+} activity as well as by the membrane potential. As in the action potential of rat atrial muscle where the plateau of the action potential is kept low (−40 to −50 mV) by the fast repolarization via activation of the transient outward current, the Na^+-Ca^{2+} exchange may work mainly in a forward mode (Josephson *et al.* 1984; Giles and Imaizumi, 1988). If ryanodine is assumed to increase the activity of the Na^+-Ca^{2+} exchange mechanism in a forward mode, Ca^{2+} efflux would be increased and consequently, the slow repolarization will be disappeared as the contractility diminished as shown in Fig. 3. In rat ventricular muscle cells, the forward mode of Na^+-Ca^{2+} exchange seems persistant or at least the outward mode is not effective, perhaps due to the low membrane potential generated by the fast repolarization of the action potential. Even when the intracellular Na^+ activity, unchanged by the treatment of ryanodine (Fig. 1), was increased by monensin forcing the Na^+-Ca^{2+} exchange mechanism to act in a way for Ca^{2+} to move into the cell, the increase in contractility is minimal (Fig. 6), compared to the guinea pig ventricular muscle (discussed later).

The current via the Na^+-Ca^{2+} exchange mechanism is reported to participate in the formation of the plateau of the guinea pig ventricular action potential (Fedida *et al.* 1987; Hilgemann, 1988; Egan *et al.* 1989), which has no fast repolarization, contrary to the action potential of the rat, as shown in Fig. 4. And the inward current due to the Na^+-Ca^{2+} exchange mechanism would be augmented when intracellular Ca^{2+} activity is increased and/or intracellular Na^+ activity is decreased (Mullins, 1981; Kimura *et al.* 1987). However, due to the relatively high plateau potential, the mode of Na^+-Ca^{2+} exchange in guinea pig ventricular muscle could be outward relatively easily when intracellular Ca^{2+} activity is lowered. For instance, after intracellular Ca^{2+} activity was decreased by ryanodine (this can be accomplished due to the same mechanism in rat ventricular myocardium), Na^+-Ca^{2+} exchange transport would act in an outward mode causing Ca^{2+} influx and activating myocardial contraction. (The contraction persists during the action potential and relaxes as soon as the action potential repolarizes, as shown in Fig. 4 and 7.) The outward mode of Na^+-Ca^{2+} exchange would be augmented further when intracellular Na^+ activity was increased by monensin (Fig. 7).

Beside the difference in membrane potential at which the mode of Na^+-Ca^{2+} exchange is governed, there seems to be a difference in intracellular Na^+ activities between two tissues;

a^i_{Na} of guinea pig ventricular myocytes was slightly higher than that of rat ventricular myocytes, although it was not statistically significant (Table 1). The effects of ryanodine and monensin on the a^i_{Na} of rat and guinea pig ventricular muscle were also different. a^i_{Na} of the rat ventricular muscle was not affected so much as intracellular Ca^{2+} activity by both drugs, while a^i_{Na} of the guinea pig ventricular muscle was quite sensitive to both drugs as shown in Fig. 1 and 2. These interesting behaviors of intracellular ionic activities draw some speculations such as differences in Na^+-K^+ ATPase, and action potential configurations.

From these experimental data, it can be postulated that ryanodine's negative inotropic effect in rat and guinea pig ventricular muscle is partly mediated by Ca^{2+} movements via Na^+-Ca^{2+} exchange, depending upon the plateau potential and intracellular ionic activities of Na^+ and Ca^{2+}. Although this study shows that the decrease in contractility by ryanodine is related to intracellular Na^+ activity of muscle cells, it is not clear whether ryanodine has a direct effect on the Na^+-Ca^{2+} exchange transport. Direct measurements of the membrane current via Na^+-Ca^{2+} exchange, under the condition of dissociation from intracellular environment via the giant excise patch method, would be useful and powerful for further investigation of ryanodine effects on ionic currents.

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