Positive Inotropic Effect of Ryanodine on Rabbit Ventricular Muscle: Dependence on the Intracellular Calcium Load

R. Z. Gainullin and M. E. Saxon

Institute of Biological Physics, Academy of Sciences of the USSR, 142292 Pushchino, Moscow Region, USSR

Abstract. Two types of electrical and mechanical responses to 1 μmol/l ryanodine, depending on the intracellular calcium load, were observed in rabbit papillary muscles. In a normal calcium solution, ryanodine induced a transient decline followed by a stable increase in the developed force (by 20 ± 5% of the pretreatment level; n = 30) and prolonged the action potential (AP). The positive ryanodine response showed an increased time-to-peak force and was completely suppressed by 2 μmol/l nifedipine, partially blocked by 50 μmol/l tetracaine (Ca²⁺ release blocker), but greatly potentiated by 20 mmol/l CsCl or (—) Bay R 5414 which prolonged the AP. The prolonged time-to-peak force of the positive ryanodine response was shortened by procedures raising the content of Ca²⁺ in the sarcoplasmic reticulum (SR). It is suggested that the initial decline in the force amplitude results from Ca²⁺ leakage from the SR which is further compensated for by an elevation of both the transmembrane Ca²⁺ entry and intracellular Ca²⁺ release. In calcium overloaded myocardium, 1 μmol/l ryanodine caused irreversible contracture and dramatic AP shortening, explained by a massive Ca²⁺ release from the overloaded SR into the cytoplasm. It is concluded that the calcium content in the SR is the main modulator of the electrical and mechanical effects of ryanodine in ventricular myocardium.

Key words: Rabbit myocardium — Ryanodine — Ouabain — Tetracaine — Nifedipine — (—) Bay R 5414 — CsCl — Ca²⁺ release — Action potential

Introduction

The elucidation of the mechanism of ryanodine inotropy is one of the most exciting problems in muscle pharmacology. Ryanodine has long been known to produce a contracture in skeletal muscles (Edwards et al. 1948; Jenden and Fairhurst 1969) and depress the force contraction in various cardiac preparations (Nayler 1963; Sleator et al. 1964; Haydu 1969; Penefsky 1974; Frank and
Sleator 1975; Rumberger 1976; Sutko and Willerson 1980; Eisner and Valdeomillos 1986; Bers 1985; Kenyon and Sutko 1987; Wier et al. 1985; Horackova 1986; Saxon and Kobrinsky 1988). However, in some cases ryanodine has been reported to have positive instead of negative inotropic effect (Ciofalo 1973; Sutko et al. 1979; Valdeomillos and Eisner 1985). The reasons for this discrepancy in the ryanodine effect on skeletal and cardiac muscles are far from being understood.

The aim of the present work was to try to explain the different patterns of ryanodine inotropy from the recent views on ryanodine as a potent stimulator of \( \text{Ca}^{2+} \) release and on the key role of \( \text{Ca}^{2+} \) ions in the regulation of ryanodine activity (Fleischer et al. 1985; Pessah et al. 1986, 1987; Meissner 1986; Lattanzio et al. 1987).

Materials and Methods

Experiments were performed on papillary muscles (0.5—0.7 mm) from the right ventricular muscle of adult New-Zealand rabbits. A standard technique for recording the isometric tension and transmembrane potentials was used (Saxon and Safronova 1982; Saxon and Kobrinsky 1988).

The preparations were superfused at a constant rate of 5 ml/min, the temperature was maintained at 35° ± 0.5 °C; pH 7.4. Normal physiological solution contained (in mmol/l): Na\(^+\) 150; K\(^+\) 4.0; Ca\(^{2+}\) 2.5; Mg\(^{2+}\) 1.0; HCO\(_3\) 12; H\(_2\)PO\(_4\) 1.8; Cl\(^-\) 148.4; glucose 11. Under these conditions the electrical and mechanical activity of the preparations were stable during 7—8 h. The muscles were stabilized for 1—1.5 h at a driving rate of 1 Hz before starting the measurements. Driving stimuli were provided from bipolar Ag-AgCl electrodes by square-wave pulses of 5 ms duration and of the intensity of two thresholds. Addition of 20 mmol/l CsCl decreased the muscle excitability and voltage of higher intensity was applied to elicit the slow action potential.

The electrical and mechanical measurements were photographed from the screen of a memory oscilloscope (Tectronix 5III, USA).

The drugs used were: ryanodine (Merck, Sharpe and Dohme, Rahway N. Y., USA, kindly supplied by Dr. E. Rumberger, Hamburg), cesium chloride, ouabain, tetracaine chloride, nifedipine (Merck AG, Darmstadt, FRG); a pure agonistic enantiomere (—) Bay R 5414 (kindly provided by Bayer, AG, FRG). Nifedipine and (—) Bay R 5414 were dissolved in dimethylsulphoxide (DMSO) and subsequently diluted in Tyrode solution to achieve the final concentration required. The control muscles studied in parallel were exposed to the same concentration of DMSO. The experiments with dihydropyridines were conducted in a dark room. Means ± S.E.M. of pooled data are presented.

Results

Biphasic inotropic effect of ryanodine in normal calcium solution

In the presence of 2.5 mmol/l extracellular \( \text{Ca}^{2+} \) (\( \text{Ca}^{2+}_o \)) and at a stimulation rate of 1 Hz 1 \( \mu \)mol/l ryanodine evoked biphasic changes in the contractility: an
Fig. 1. Effects of ryanodine on the action potential and contraction of rabbit papillary muscle at steady-state stimulation by 1 Hz. (a) Superimposed transmembrane action potentials and accompanying isometric contractions before and after exposure of the muscle to 1 μmol/l ryanodine for 40 min. (b) Biphasic time-dependent changes in the force of contraction in the same muscle superfused with ryanodine for 40 min (continuous recording): the left-hand white arrow denotes the negative inotropic phase, the right-hand the positive one.

initial rapid (within 6—8 min) decline in force amplitude (by 30 ± 5%; n = 30) followed by a slowly developing force rise above the pretreatment level (by 20 ± 5%; n = 30). Fig. 1b shows three distinct peaks of contraction: 1—corresponding to the control level, 2—the transient negative phase (left, white arrow), and 3 — the slowly developing positive phase (upward, white arrow). The positive inotropic effect of ryanodine was complete within 25—30 min.

Fig. 1a, lower panel, illustrates a typical positive inotropic response to ryanodine. The time-to-peak force of the response was markedly increased (Table 1) and its duration was prolonged always causing a shift of the isometric contraction curve to the right (the so-called delayed ryanodine response).

Fig. 1a, upper panel, shows an example of simultaneous recording of AP in rabbit ventricular muscle before and during prolonged ryanodine treatment. The duration of the AP at a 30% repolarization level increased by 40 ± 5% (Table 1).
Table 1. Effects of nifedipine, (—) Bay R 5414, CsCl, tetracaine and ouabain on the positive inotropic response to ryanodine in rabbit papillary muscles

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Action potential duration at 30% repolarization level</th>
<th>Changes in isometric tension amplitude</th>
<th>Duration of isometric tension at 70% relaxation level (ms)</th>
<th>Time-to-peak force (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Control</td>
<td>80 ± 20 ms</td>
<td>180 ± 15 ms</td>
<td>140 ± 15 ms</td>
<td></td>
</tr>
<tr>
<td>Ryanodine</td>
<td>130 ± 10 ms</td>
<td>increase by 20 ± 5% over control level</td>
<td>230 ± 10 ms</td>
<td>200 ± 20 ms</td>
</tr>
<tr>
<td>1 µmol/l, 30 min (n = 30)</td>
<td>25 ± 5 ms decrease by 85 ± 5%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nifedipine</td>
<td>25 ± 5 ms completely eliminated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 µmol/l, 20 min (n = 5)</td>
<td>190 ± 10 ms increase by 180 ± 10% over control level</td>
<td>320 ± 10 ms</td>
<td>250 ± 15 ms</td>
<td></td>
</tr>
<tr>
<td>CsCl, 20 mmol alone 40 min (n = 4)</td>
<td>70 ± 5 ms decrease by 23 ± 3% below control level</td>
<td>160 ± 5 ms</td>
<td>125 ± 5 ms</td>
<td></td>
</tr>
<tr>
<td>Tetracaine</td>
<td>125 ± 10 ms no changes after 30 min</td>
<td>210 ± 5 ms</td>
<td>190 ± 5 ms</td>
<td></td>
</tr>
</tbody>
</table>
### Pharmacological properties of the delayed ryanodine response

In order to elucidate the mechanism of the generation of the delayed ryanodine response, agents known to affect the transmembrane Ca\(^{2+}\) entry, the SR loading or the SR Ca\(^{2+}\) release were tested.

20 mmol/l CsCl prolonged the AP and augmented the positive ryanodine response. The effect paralleled the substantial AP lengthening (Table 1). In addition, CsCl further increased the time-to-peak force accompanied by an additional shift of the delayed ryanodine contraction to the right (Fig. 2b). There was no shift of the isometric contraction curve to the right despite the obvious prolongation of the AP and the isometric twitch duration in response to 20 mmol/l CsCl exposure in control preparations (Fig. 2a). Table 1 shows that the force amplitude increment and the AP prolongation were greater in ryanodine-treated preparations than in the control muscles.

Application of 20 mmol/l CsCl depolarized intact and ryanodine-treated muscles by approximately 30 mV as could be expected from the previous work (Isenberg 1976).

Nifedipine (2 μmol/l, 20 min exposure) prevented the positive inotropic effect of 1 μmol/l ryanodine in rabbit papillary muscles. Fig. 3a shows an example of simultaneous recording of the AP and isometric twitch under ryanodine treatment in the presence of nifedipine. Note that 2 μmol/l nifedipine alone shortened the early plateau of the AP, prolonged the final repolarization phase and depressed the force amplitude (Table 1). Exposure of the muscle to

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Action potential duration at 30% repolarization level</th>
<th>Changes in isometric tension amplitude</th>
<th>Duration of isometric tension at 70% relaxation level (ms)</th>
<th>Time-to-peak force (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Tetracaine washing (n = 4)</td>
<td>130 ± 10 ms</td>
<td>increase by 43 ± 3% over tetracaine level</td>
<td>230 ± 10 ms</td>
<td>205 ± 5 ms</td>
</tr>
<tr>
<td>Ouabain 1 μmol/l 20 min (n = 4)</td>
<td>35 ± 5 ms</td>
<td>increase by 200 ± 10%</td>
<td>260 ± 10 ms</td>
<td>160 ± 10 ms</td>
</tr>
<tr>
<td>Ouabain 1 μmol/l, 25 min + ryanodine, 1 μmol/l, 25 min (n = 4)</td>
<td>2 ms</td>
<td>contracture</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2. Twenty mmol/l CsCl potentiates the positive inotropic effect of ryanodine in rabbit papillary muscle. Stimulation by 1 Hz. Superimposed action potentials and corresponding contractions before and after the exposure to 20 mmol/l CsCl for 40 min in intact (a) and ryanodine pretreated (1 µmol/l, 30 min) muscles (b).

Fig. 3. Dihydropyridine agonist (−) Bay R 5414 relieves the block of the positive ryanodine response produced by nifedipine in rabbit papillary muscle. (a) Superimposed action potentials (top) and corresponding contractions (bottom) in the control and during subsequent exposure to nifedipine (2 µmol/l, 20 min), ryanodine (1 µmol/l, 30 min) and (−) Bay R 5414 (2 µmol/l, 20 min) at 1 Hz. (b) Positive inotropic effect of 2 µmol/l (−) Bay R 5414 for 20 min alone on the intact muscle at 1 Hz.

1 µmol/l ryanodine abolished its contractility without any change in the AP shape. However, subsequently added 2 µmol/l (−) Bay R 5414 antagonized the inhibitory effect of nifedipine and relieved the delayed ryanodine response.

Fig. 3a shows that in the presence of the agonist the augmented ryanodine response included a markedly prolonged relaxation phase besides the charac-
Positive Inotropic Effect of Ryanodine

Fig. 4. Partial depression of the positive ryanodine response by tetracaine in rabbit papillary muscle. Superimposed traces of the action potentials (a) and corresponding contractions (b) upon subsequent exposure of the muscle to tetracaine (50 μmol/l, 15 min), ryanodine (1 μmol/l, 30 min) and tetracaine washing (15 min) at 1 Hz.

Figure 4 shows a representative pattern of the electrical and mechanical effects of 1 μmol/l ryanodine in the presence of tetracaine, a possible blocker of the SR Ca²⁺ release from the SR in skeletal and cardiac muscle (Antoniu et al. 1985; Chamberlain et al. 1984). Note that 50 μmol/l tetracaine alone diminished the force amplitude (by 26 ± 5%; n = 4) with a slight shortening (within 5—10%) of the AP duration. Addition of 1 μmol/l ryanodine to the muscle exposed to tetracaine resulted in an initial decline of the force amplitude (not shown) and a characteristic increase in the time-to-peak force without a secondary positive inotropic phase. However, tetracaine did not prevent the conventional AP prolongation induced by ryanodine in the presence of local anesthetics (Fig. 4a). After tetracaine washout the positive inotropic effect of ryanodine readily re-appeared. Fig. 4b shows the enhancement of ryanodine response after a 15 min tetracaine washing. It should be emphasized that the contraction was enhanced and prolonged more than it could be expected from the corresponding changes in the AP duration (Fig. 4a).

Effect of Ca²⁺ and stimulation frequency

Fig. 5b shows that upon rising Ca²⁺ from 2.5 to 5.4 mmol/l the amplitude of the positive ryanodine response is transiently enhanced followed by a constant
Fig. 5. Different effects of Ca\(^{2+}\) enriched solution and high driving rate on intact and ryanodine-treated muscles. (a) Control preparation. Superimposed contractions at 1 and 2 Hz in the presence of 5.4 mmol/l Ca\(^{2+}\). (b) Transient stimulatory effect of 5.4 mmol/l Ca\(^{2+}\) on a positive ryanodine response at 1 Hz. (c) Constant depression of the same ryanodine response at 1 and 2 Hz following a 10 min exposure to 5.4 mmol/l Ca\(^{2+}\).

depression and prolongation (Fig. 5c). In the presence of 5.4 mmol/l Ca\(^{2+}\) the stimulation at 2 Hz elicited a shift of the ryanodine response to the control position. The effect of high frequency stimulation was accompanied by an enhancement of the tonic force (Fig. 5c, left arrow) and a prolongation of muscle relaxation. Stimulation at 2 Hz caused the opposite effect in rabbit papillary muscles exposed to 5.4 mmol/l Ca\(^{2+}\) in the absence of ryanodine. In this case (Fig. 5a), the high driving rate increased the force amplitude, accelerated the mechanical relaxation and decreased the tonic tension (left arrow). The changes in ryanodine response shown in Fig. 5b—c were constant findings in other six experiments.

Electrical and mechanical effects of ryanodine on calcium overloaded myocardium

In this set of experiments, 1 \(\mu\)mol/l ryanodine was added to ouabain pretreated muscles (1 \(\mu\)mol/l for 25 min). Ouabain is known to increase the SR loading via an increase of Na\(^{+}\)—Ca\(^{2+}\) exchange due to the Na-pump inhibition (Langer et al. 1975; Akera et al. 1977). Fig. 6a shows the electrical and mechanical effects of 1 \(\mu\)mol/l ouabain alone and the changes elicited by ryanodine in the presence of ouabain. After a 20 min exposure of muscles to 1 \(\mu\)mol/l ouabain, the force amplitude increased in parallel with AP shortening (Table 1). However, the increase in the driving rate from 1 to 2.5 Hz led to a depression of the force amplitude. Fig. 6c, illustrates the negative force staircase induced by high driving rate in the presence of ouabain as compared with the ordinarily positive
Positive Inotropic Effect of Ryanodine

Fig. 6. Depressive electrical and mechanical effects of ryanodine on calcium overloaded rabbit papillary muscle pretreated with ouabain. (a) Superimposed traces of the action potentials (top) accompanied by the contractions in the control (bottom), during subsequent application of ouabain (1 µmol/l, 25 min) and ryanodine (1 µmol/l, 13 and 25 min) at 1 Hz and 2.5 Hz. (b) Steady state contraction at 1 and 2.5 Hz in the control preparation and following exposure of the muscle to ouabain for 20 min (c).

staircases observed in the control (Fig. 6b). Furthermore, the force amplitude slowly declined at a basal rate of 1 Hz during a long (over 20 min) superfusion of the muscles with ouabain. The events mentioned indicate that ouabain caused the intracellular Ca\(^{2+}\) overload in rabbit papillary muscles. Addition of 1 µmol/l ryanodine to the muscles exposed to ouabain for 25 min resulted in an increase in the tonic tension which continued slowly rising after a rapid initial increase. Fig. 6a, bottom, shows the time-dependent contracture recorded after 13 and 25 min of ryanodine action. It also shows that ryanodine produced a dramatic shortening of AP leading to the generation of spike-like electrical activity (Fig. 6a, top). Note that the increased stimulation frequency insignificantly affects the level of ryanodine contracture.

Discussion

Two types of electrical and mechanical responses of rabbit papillary muscles to ryanodine could be observed, depending on the SR loading: a biphasic response in normal calcium solution, and ryanodine contracture in calcium overloaded muscles.
Mechanism of the biphasic inotropic response to ryanodine

In normal calcium solution, after a transient decline of the force amplitude, ryanodine elicited a stable positive inotropic effect showing a critical dependence on the transmembrane Ca\(^{2+}\) entry. Thus, the positive response was abolished by nifedipine, but markedly potentiated by CsCl or calcium channel agonist, (−) Bay R 5414 which prolonged the AP. This can be taken to support for the conclusion that the positive inotropic effect of ryanodine is due to an increased Ca\(^{2+}\) influx through voltage-dependent Ca\(^{2+}\) channels as a result of the AP prolongation. It should be noted that the positive inotropic effect of ryanodine is absent in rodent myocardium which has a very short ventricular AP (Sutko and Willerson 1980). As to the AP prolongation, the phenomenon has been attributed to the suppression of the ryanodine-sensitive Ca\(^{2+}\)-dependent component of the transient outward current which is rather large in rabbit ventricular cells (Ito et al. 1984). It has been demonstrated that ryanodine itself has no significant effect on the calcium current (\(I_\text{Ca}\)) in ventricular and cardiac Purkinje fibers (Mitchell et al. 1984; Sutko and Kenyon 1983). The positive ryanodine response was shown to be also sensitive to the Ca\(^{2+}\) release blocker tetracaine. The local anesthetic diminished the amplitude of the response leaving AP prolonged (as a result of ryanodine acting in the presence of tetracaine). It seems likely that the positive inotropic effect of ryanodine is partially attributable to the ability of the drug to stimulate Ca\(^{2+}\) release from the SR. The phenomenon has been recently discovered in the skeletal and cardiac muscles (Fleischer et al. 1985; Meissner 1986; Imagawa et al. 1987; Lattanzio et al. 1987; Inui et al. 1988). Interestingly, while antagonizing the positive inotropic effect of ryanodine, tetracaine did not influence the initial rapid decline in the force amplitude. Based on the current concepts on calcium releasing ability of ryanodine, the negative inotropic effect can be explained by Ca\(^{2+}\) leakage from the SR through a tetracaine-insensitive Ca\(^{2+}\) release pathway. According to the Mensing-Hilgeman hypothesis, such a Ca\(^{2+}\) release pathway may operate in a junctional structure providing Ca\(^{2+}\) efflux from the SR to the extracellular space (Mensing 1979; Hilgeman 1982).

It should be noted that the initial negative inotropic phase was accompanied by an increase in the time-to-peak force. Our finding of the “delayed” ryanodine response is in agreement with the observation of the delayed and prolonged aequorin light signal reported in rabbit and ferret ventricular fibers in the presence of ryanodine (Morgan and Morgan 1984; Wier et al. 1985). We observed that the prolonged time-to-peak force of the ryanodine response could be decreased by increasing the Ca\(^{2+}\) content in the SR. Procedures such as enrichment of the solution with Ca\(^{2+}\) and high driving rate shifted the “delayed” ryanodine response to the control position with a parallel increase in the tonic
tension. This finding suggests that the increased time-to-peak force in the presence of ryanodine reflects a slow filling of the primarily depleted releasable pool by Ca$^{2+}$ entering through the cell membrane. Principally, this situation has much in common with that suggested for the explanation of the so-called “late” type of contraction which occurred after a long rest period in guinea pig ventricular myocardium (Reiter et al. 1984).

These data lead us to conclude that the biphasic inotropic effect of ryanodine is the result of an initial depletion of the releasable pool due to Ca$^{2+}$ leakage from the SR, subsequently this depletion is compensated for by a slowly enhanced transmembrane Ca$^{2+}$ influx reflected in a prolongation of the time course of the Ca$^{2+}$ release.

**Mechanism of ryanodine depressive effect on contracture of calcium overloaded myocardium**

Contracture and dramatic AP shortening were the main results of ryanodine action in the presence of ouabain. It should be emphasized that ryanodine contracture has been postulated as a specific response of skeletal muscles (Jenden and Fairhurst 1969). However, our results as well as those reported in the literature suggest that ryanodine contracture is a common response of variety of cardiac tissues (insect heart, ventricular myocytes, Purkinje fibers) exhibited under the conditions of elevated Ca$^{2+}$ in the SR (McCann and Penefsky 1981; Sutko and Kenyon 1983; Eisner and Valdeolmillos 1986). Therefore, the above differences in ryanodine response can be explained by relatively high amounts of Ca$^{2+}$ stored and mobilized by skeletal muscle under ryanodine treatment.

Since ryanodine did not influence the Ca$^{2+}$ uptake in cardiac SR (Jones et al. 1979), ryanodine contracture could be explained by a massive mobilization of Ca$^{2+}$ from the overloaded internal stores to the cytoplasm. According to recent biochemical data, ryanodine stimulates the SR Ca$^{2+}$ release by “locking Ca$^{2+}$ release channels in the open state” (Rousseau et al. 1987).

The increase in the cytosolic calcium has been shown to alter the value and inactivation of the calcium current due to a fall in the electrochemical potential for Ca$^{2+}$ to enter (Reuter 1973; Fedida et al. 1988). Evidently, these events contribute greatly to the generation of a spike-like AP during ryanodine contracture.

Finally, the data presented indicate that Ca$^{2+}$ is an important modulator of ryanodine inotropy in various types of muscles. The nature of the Ca$^{2+}$-dependent ryanodine activity was studied in biochemical experiments that showed that Ca$^{2+}$ is specifically associated with ryanodine receptors and directly affects the affinity and number of ryanodine binding sites on Ca$^{2+}$ release channels (the so-called “Ca-ryanodine receptor complex, Pessah et al. 1986, 1987).
The two types of electrical and mechanical responses to ryanodine action and their dependences on intracellular Ca\(^{2+}\) load are schematically presented in Fig. 7.

**Fig. 7.** Schematic representation of the two types of ryanodine response in rabbit papillary ventricular muscle and their dependences on the intracellular Ca\(^{2+}\) load.

**References**


Positive Inotropic Effect of Ryanodine


during contraction and relaxation of mammalian cardiac and vascular smooth muscle as detected with aequorin. Amer. J. Med. 77, 33—46
Nayler W. G. (1963): Effect of ryanodine on cardiac muscle. Amer. J. Physiol. 204, 975—978
Rumberger E. (1975): The role of the sarcoplasmic reticulum in the pure frequency potentiation: The effect of ryanodine. Pflügers Arch. 364, 203—204

Final version accepted May 31, 1989