Ryanodine in Low Concentrations Is a Ca-Release Stimulator Rather Than Inhibitor in Rat Myocardium

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Abstract. The effects of ryanodine on negative force staircase and potentiated rested-state contraction (RC) in rat myocardium were compared to the action of Ca release stimulator (caffeine) and inhibitors (local anesthetics). Only low ryanodine concentrations (0.1—0.5 μmol/l) were found to reverse anomalous mechanical patterns in rat myocardium to similar to those as generally observed in other mammalian species. Ryanodine-induced positive staircase and a weak RC were potentiated by noradrenaline. The results obtained seem to characterize ryanodine as a Ca^{2+} release stimulator rather than an inhibitor in this species and suggest different molecular substrates for ryanodine and caffeine inotropy in rat myocardium.

Key words: Rat myocardium — Rested contraction — Force staircase — Junctional Ca^{2+} channels — Ryanodine — Caffeine — Dantrolene — SKF 523A — Tetracaine

Introduction

In recent years considerable insight has been gained into mechanical response to ryanodine in various cardiac preparations (Hajdu 1969; Fairhurst 1974; Frank and Sleator 1975; Sutko and Willerson 1980; Sutko et al. 1985; Bers 1985). However, although numerous studies concerning various aspects of ryanodine inotropy have been published, the exact mechanism of its intracellular action on Ca^{2+} release from the sarcoplasmic reticulum (SR) is far from being clear. Modern cardiac pharmacology proclaimed the agent an inhibitor of Ca^{2+} release in terminal SR (Jones et al. 1979; Jones and Cala 1981; Besch et al. 1985; Feher and Lipford 1985). The concept has been mainly based on findings with high ryanodine concentrations in isolated SR: the agent was shown to increase Ca^{2+} accumulation by SR vesicles without altering Ca^{2+}

pump activity. An intriguing paradox is that the above biochemical data have been widely used to interprete mechanical effects of low ryanodine concentrations. Moreover, in many experiments on whole muscle, the inotropic effect of low ryanodine was associated with increased Ca^{2+} efflux from myocardial preparations which suggests that ryanodine may be a stimulator of Ca^{2+} release rather than its inhibitor (Frank and Sleator 1975; Hunter et al. 1983; Hilgeman et al. 1983).

The available data lead us to conclude that the current concepts of ryanodine inotropy in terms of Ca release inhibition should be revised in view of the new findings, since ryanodine remains a widely-used instrument employed in muscle physiology to clarify by role of SR in various mechanical events. A correct interpretation of ryanodine inotropy would also be important to explain the natural overload of rat myocardium as well as that of hibernating rodents both exhibiting the highest sensitivity to the agent (Sutko and Willerson 1980; Kondo and Shibata 1984; Saxon 1986). Starting this work we realized that it would not be easy to make further contribution to phenomenology of ryanodine inotropy in rat myocardium after the exhaustive information available with the readers from Sutko and Willerson's work (1980). However, for this paper, we had to revise the main features of ryanodine inotropy in rat myocardium in order to look for a new explanation. For this purpose, we compared ryanodine inotropy with that of a Ca^{2+} release stimulator (caffeine) and inhibitors (local anesthetics). The data obtained permit us to conclude that ryanodine, at least in rat myocardium, is a Ca^{2+} release stimulator possessing properties both different and common with classical agents of this type, caffeine. The present paper deals with the different features of ryanodine and caffeine inotropy in rat myocardium.

Materials and Methods

Experiments were performed on papillary muscles dissected from the right ventricle of Wistar rats (200—350 g). The animals were decapitated, the hearts quickly removed, the papillary muscles (0.2—0.8 mm in diameter) dissected in oxygenated physiological solution and mounted in a perfusion chamber for isometric force measurements. The tendinous end of the papillary muscle was tied by a nylon suture to a hook of a force-displacement tranducer (model — MX2B USSR). The resting tension was determined at which the contractile response reached 95% of maximum contraction. The preparations were allowed to equilibrate at 0.1 Hz for 1—2 h. Driving stimuli were provided either from bipolar Ag—AgCl electrodes or from field stimulation producing square waves of 5 ms duration (2 × threshold).

The preparations were superfused at a constant rate (5 ml/min). The temperature was maintained at 36.5 ± 0.5°C; pH was 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; $H_2PO_4^-$ 1.8; Cl^- 148.4; glucose 11. The contractile force measured with a transducer was monitored on an oscilloscope and recorded on film.

The chemicals used were: ryanodine (Merck, Sharpe and Dohme, Rahway N. Y., USA, kindly supplied by Dr. E. Rumberger, Hamburg), caffeine benzoate, noradrenaline, tetracaine (Serva), SKF 523-A (a generous gift of Prof. Pompeo Volpe, Padova, Italy) and dantrolene sodium (Dantrium, DAN, kindly provided by Norwich-Eaton Pharmaceuticals, Norwich, NY). DAN was dissolved at a concentration of 10^{-3} mol/l in deionized water adjusted to pH 10 with 0.1 NaOH.

The reagents were dissolved immediately before use and added directly to the solution. Values presented are means ±SEM.

Results

Steady-state tension at 0.2 Hz, force-staircase response within 0.2—2 Hz and rested contraction after 1 min rest (RC) were measured under the influence of the agents and compared to the control.

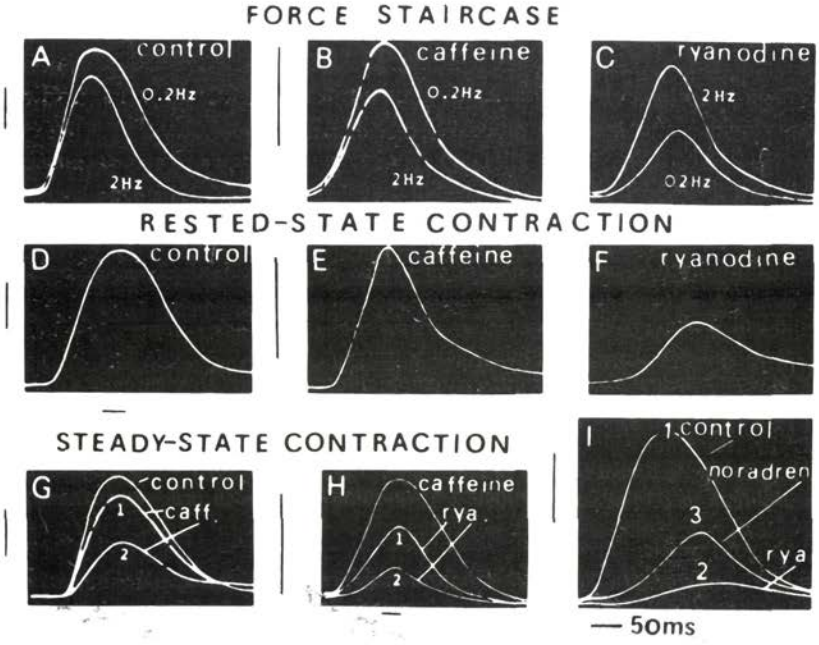

Fig. 1. Different patterns of caffeine and ryanodine inotropy in rat papillary muscle. The sequential effects of 5 mmol/l caffeine (25 min) and 0.5 μmol/l ryanodine (25 min) in the same muscle. Patterns of force staircase in the control (*A*), after the addition of caffeine (*B*) and ryanodine (*C*). Superimposed traces of twitch induced by an increase in the stimulation rate from 0.2 Hz to 2 Hz. *D*—*F*: Patterns of rested-state contraction (1 min rest) under similar influence. A subsequent negative inotropic effect of caffeine (*C*) and ryanodine (*H*) in the same preparation. *C*: 1—2 — time-dependent effect of caffeine within 1—3 min; 1—2 — time-dependent effect of ryanodine within 10—15 min. Steady-state stimulation at 0.1 Hz; *I*: another example of ryanodine-reduced rested-state contraction (1 μmol/l, 20 min, trace 2) followed by the addition of 1 μmol/l noradrenaline for 10 min. The agents were added during steady-state stimulation at 0.1 Hz. Vertical bars are twitch calibrations. Short bars: 1 g; Long bars: 500 mg.

Difference Between Ryanodine- and Caffeine-Induced Inotropy in Rat Myocardium

In this set of experiments ($n = 10$) the sequential action of 5 mmol/l caffeine and 0.5 μmol/l ryanodine was analysed in the same muscles. The principal inotropic patterns of both agents are shown in Fig. 1. It is noteworthy that an increase in the frequency of stimulation from 0.2 Hz to 2 Hz (upper panel, A) produced a negative inotropic effect which is typical of this species. However, the resumption of stimulation of the preparation after 1 min rest activated a strong first beat (Fig. 1*D*). Such a behaviour was independent of the diameter of the preparations within 0.1—1 mm.

Both mechanical features, the negative staircase and the strong RC, were seen to be maintained in the presence of 5 mmol/l caffeine (Fig. 1*B,E*, 25 min caffeine exposure). However, both of them were readily eliminated due to the consecutive action of 0.5 μmol/l ryanodine on the same muscle. Fig. 1*C,F* is a typical example of this action. Fig. 1*C* shows an inversion of the force-staircase response from negative to positive under transient changes of stimulation rates from 0.2 to 2 Hz.

However, the maximal tension recorded in the presence of ryanodine was lower in amplitude than that initially observed before the addition of ryanodine (Fig. 3*b*). A similar effect was observed in rat muscles treated by ryanodine alone ($n = 5$). In this case (Fig. 2*a*) a tenfold increase in stimulation rate (from 0.2 to 2 Hz) also induced a gradual increase in the amplitude of tension. As can be seen in Fig. 2 1 μmol/l noradrenaline potentiated the positive staircase effect of ryanodine while noradrenaline was not effective enough to change the negative force-frequency relation in the controls ($n = 4$; data not shown).

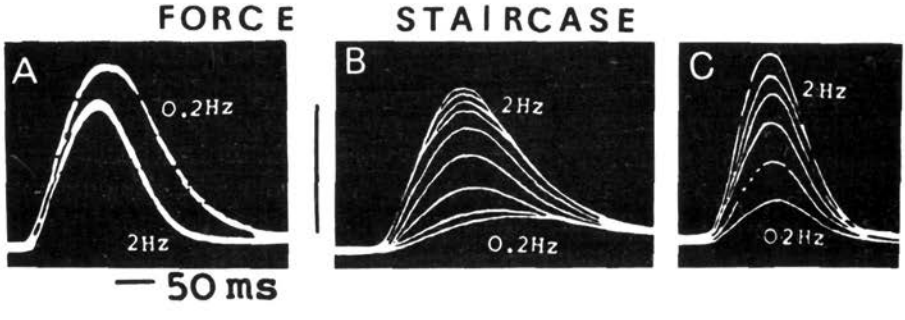

Fig. 2. Positive staircase effect of ryanodine (0.5 μmol/l, 25 min *B*) and potentiation of the phenomenon by 1 μmol/l noradrenaline (10 min *C*) in the same rat papillary muscle. Superimposed traces of subsequent beats induced by transient changes in stimulation frequency from 0.2 Hz to 2 Hz under the above influence.

It is also important that the rested contraction depressed by ryanodine became sensitive to noradrenaline (Fig. 1,I, third trace). It can be seen that in the presence of 1 μmol/l noradrenaline the amplitude of ryanodine-reduced RC increased twice.

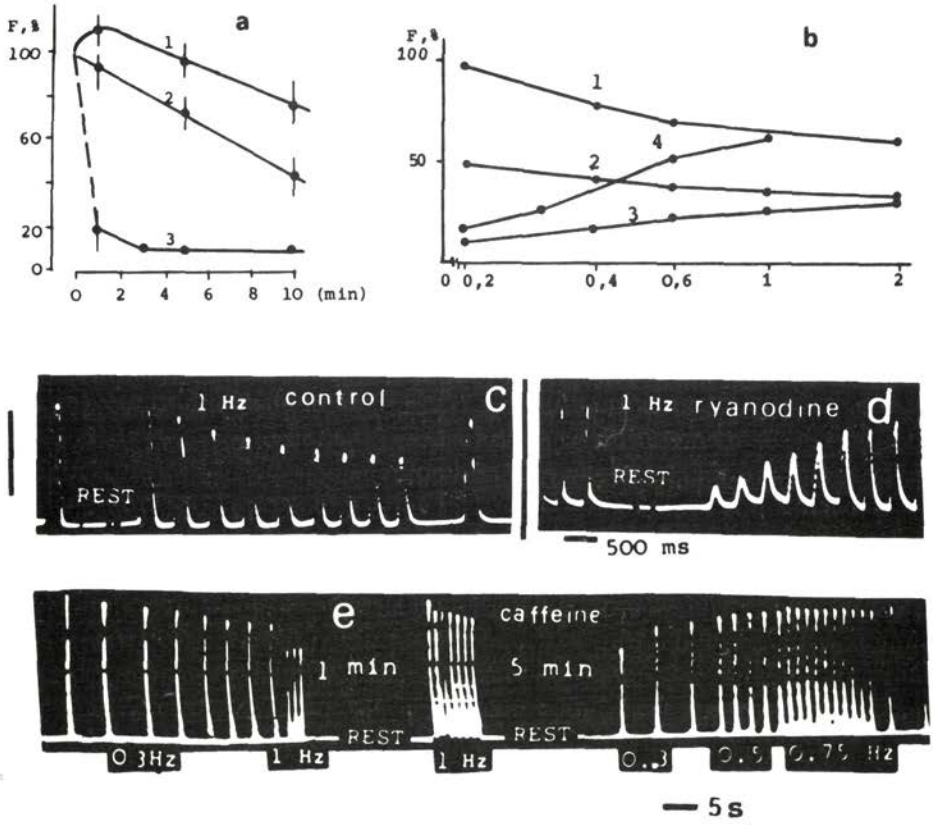

Fig. 3. *A.* Comparative effects of caffeine (curve 2, 10 mmol/l 30 min exposure) and ryanodine (curve 3, 0.5 μmol/l, 30 min exposure) on rest-dependent decay of developed force. Abscissa: duration of rest intervals (min); Ordinate: amplitude of contraction obtained following the rest intervals, expressed as a percentage of the pre-rest steady-state tension amplitude. The values shown are mean ±SE of 9 experiments. Tests of individual rest periods were separated by a period of steady stimulation at a frequency of 1 Hz to achieve a stable pre-rest contraction. Data for caffeine and ryanodine were obtained in different rat papillary muscles. *B.* Comparative staircase response of caffeine (5 mmol/l, 20 min) (curve 2) subsequently added ryanodine (1 μmol/l, 25 min) (curve 3) and noradrenaline (1 μmol/l, 5 min) (curve 4). The effects were recorded in the same muscle. Curve 1 — the corresponding control. Frequency is plotted on a logarithm scale. *E.* Influence of two rest periods (1 min and 5 min) on post-rested contractions in the presence of 5 mmol/l caffeine. *C—D.* Influence of 1 min rest intervals on post-rested contractions before (control) and after superfusion with 0.5 μmol/l ryanodine within 20 min. Stimulation frequency, 1 Hz.

For further illustration of the difference in ryanodine and caffeine inotropy, a comparison of time-dependent decay of RC in rat muscles may be of interest. Fig. 3*A* illustrates that caffeine (10 mmol/l) attenuated RC in rat myocardium only after 10 min rest (curve 2) while 0.5 μmol/l ryanodine abruptly abolished RC in the same muscle within less than 1 min. (Fig. 3*A*, curve 3). Fig. 3 *D*—*E* also show post-rested inotropy of ryanodine and caffeine in one of the preparations. Finally, to complete the comparison, the subsequent negative inotropic effect of the agents at steady-state stimulation (0.2 Hz) is shown in Fig. 1*GH*. The mean value of the tension amplitude depressed by 5 mmol/l caffeine was 65 ± 5% of the control within 2—3 min. The addition of 0.5 μmol/l ryanodine progressively declined the tension amplitude to 80% of the control within 15 min.

Difference Between Ryanodine and Ca^{2+} Release Inhibitor Effects in Rat Myocardium

In this set of experiments, the effect of local anesthetics (10—70 μmol/l) on the anomalous mechanical patterns in rat myocardium was tested. Five experiments

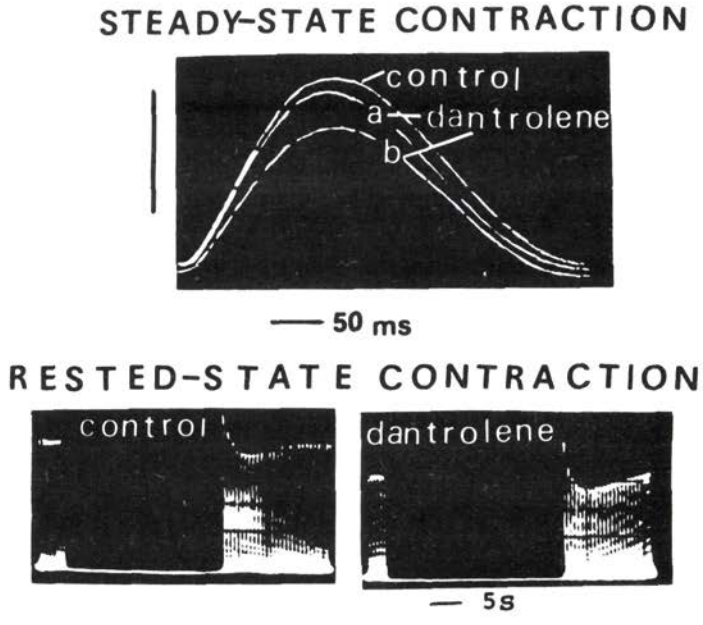

Fig. 4. Negative inotropic effect of dantrolene Na (DAN) in rat papillary muscle. *Top*: Superimposed traces of steady-state contractions at 0.2 Hz before (1) and after exposure to 10 μmol/l (*A*) and 50 μmol/l (*B*) DAN for 25 min. *Bottom*: Patterns of rested-state contraction (the first beat after a nearly 30 s rest) in control and after exposure to 50 μmol/l DAN for 30 min. Stimulation frequency, 0.8 Hz in both cases.

were performed with each compound. All the compounds (dantrolene sodium, DAN, SKF 525-A, tetracaine) are known to be blockers of various forms of Ca^{2+} release in SR vesicles (Chamberlain et al. 1984; Antonu et al. 1985).

The effect of SKF 525-A and tetracaine on twitch tension was highly dependent on the frequency of stimulation. Maximal negative inotropic effect reaching $20 \pm 3\%$ of the corresponding control was observed at 1—2 Hz. The same negative inotropic action of 50 μmol/l DAN was evident even at low rates (0.1—0.3 Hz). It was shown that neither the negative force staircase nor the strong rested contraction were reversed in the presence of the agents tested. Moreover, all of them further accentuated the negative staircase within 0.2—2 Hz. Fig. 4 shows an example of the DAN effect (previously studied in other myocardial preparations (Honerjäger and Alischewski 1983) on steady-state and rested-state inotropy in rat papillary muscle.

Discussion

Is Ryanodine A Ca^{2+}-Release Inhibitor?

A prominent feature of ryanodine inotropy observed in this and the previous works (Sutko and Willerson 1980) was the abolishing a strong RC. The action was previously interpreted as resulting from Ca^{2+} release inhibition by the compound (Sutko and Willerson 1980; Bers 1985). In this respect it should be noted that there has been no precedent in cardiac pharmacology of such an effect of Ca^{2+}-antagonists. Conceptiating on this question Bers (1985) showed that neither high Mg^{2+} (a Ca^{2+}-release inhibitor) nor La^{3+}, or Co^{2+} (Ca^{2+} antagonists) were able to affect the strong RC in rat myocardium. No depressant effect of local anecthetics, inhibitors of Ca^{2+}-release, on potentiated RC could be either observed.

On the other hand, the available evidence indicates that halothane, ether and caffeine, stimulators of Ca^{2+}-release, (Dhalla et al. 1983; Weber and Herz 1968) are potent inhibitors of RC in cat and rabbit myocardium (Bass 1976; Kamai and Rusi 1982).

Considering these indirect data, ryanodine-induced depression of RC can hardly be explained in terms of its inhibitory action on Ca^{2+}-release from SR. According to the textbook explanation (Allen et al. 1976; Johnson and Shepherd 1971) the phenomenon is rather a result of a rapid Ca^{2+} loss from SR during the short-rest period under ryanodine treatment. This conclusion is consistent with the direct observation of an increase in Ca^{2+} efflux in various cardiac preparations, including perfused rat heart, at low ryanodine concentrations (Frank and Sleator 1975; Hilgemann et al. 1983; Hunter et al. 1983). The recent observation that caffeine was not caused Ca^{2+}-release in cardiac preparations in

the presence of ryanodine (Eisner and Valdeolmillos 1985) seems to be in line with the above hypothesis.

Another feature of ryanodine inotropy in rat myocardium closely parallel to RC depression is the positive staircase response. Current models of electromechanical coupling explain the "staircase" phenomenon in cardiac muscle as resulting from cyclic changes in the amount of the activator Ca^{2+} in internal pools supplied by transmembranes Ca^{2+} influx (Koch—Weser and Blinks 1963; Morad and Goldman 1973; Johnson and Shepherd 1971; Adler et al. 1985). A more detailed study by Lewartowski (1983) has pointed to a special SR compartment with rest- and frequency-dependent volume. In rat myocardium, with a negative force-frequency relation, internal pools are suggested to be the major sources of Ca^{2+} for contraction. Consequently, transmembrane Ca^{2+} plays a minor role in the above process (Wohlfart 1982; Poggesi et al. 1984). It is noteworthy that I_{si} modulators failed to influence the negative force staircase in rat myocardium (Siegl and McNeill 1982). The only condition for the development of a positive staircase in rat papillary muscle is a lowering of the external Ca^{2+} concentration to 1.5 mmol/l (Forester and Mainwood 1974). However, the situation was cardinally changed by ryanodine which did not seem to modify either the energy metabolism, or the transport function of Ca^{2+} ATPase, I_{Ca} or Na^+/Ca^{2+} exchange in cardiac muscle (Besch 1985). It is important that the agent induced a positive staircase even at high (for rat myocardium, but physiological for other species) external $[Ca^{2+}]_0$ (2.5 mmol/l). Moreover, in the presence of ryanodine, the rat myocardium acquires sensitivity to I_{si} modulators — stimulators and antagonists (Fig. 2, and Clarke and Patmore 1984). These data imply that ryanodine induces deep changes in the source of the contraction activator Ca^{2+} increasing the contribution of transmembrane Ca^{2+} influx to the developed tension in rat myocardium. This was found to be true in direct measurements of transmembrane Ca^{2+} influx in ryanodine-treated rat papillary muscle (Bers 1985).

Possible Reason for Different Ryanodine and Caffeine Inotropy in Rat Myocardium

Judging upon indirect mechanical findings, one can deduce that despite the common affinity of the caffeine and ryanodine to terminal reticulum observed in biochemical experiments (Weber and Herz 1968; Fairhurst 1974; Williams and Jones 1983; Pessah et al. 1985) the molecular substrates of different mechanical actions of these agents in muscle as a whole are likely different. It seems likely that caffeine, promoting Ca^{2+}-release into myoplasm, decreases Ca^{2+} concentration in the release pool without substantially modifying intracellular Ca^{2+} metabolism or volume of recirculating Ca^{2+}.

The mechanical behaviour of rat myocardium following a treatment with

low ryanodine concentrations can be interpreted as resulting from stimulation of Ca^{2+} release from SR to extracellular space leading to SR depletion.

Specific sites for ryanodine action in rat myocardium according to the Hilgemann hypothesis, are special junctional Ca^{2+} channels controlling rest-dependent Ca^{2+} efflux from SR to the glycocalyx (Hilgemann 1982; Hilgemann et al. 1983). It should be stressed that the coupling structures between the sarcolemma and SR are most abundant in rat myocardium (Forssman and Giradier 1970) and skinned rat cardiac cells lose sensitiveness to ryanodine (Nayler 1973; Fabiato 1985). In view of this hypothesis, it is not difficult to imagine that some deficiency in the junctional channels would diminish the diastolic Ca^{2+} efflux from SR leading to SR overload, as exhibited by rat myocardium even at physiological $[Ca^{2+}]_0$. An opposite effect would be expected from agents facilitating Ca^{2+} efflux through the junctional channels. According to our preliminary data besides ryanodine such an ability is also characteristic of dihydropyridine Ca^{2+} agonists, CGP 28 392 and Bay K 8644.

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After the paper was accepted for publication, the direct biochemical evidence for Ca^{2+} releasing ability of a low ryanodine concentration on the Ca^{2+} release channels of cardiac sarcoplasmic reticulum has become available (Meissner G. 1986: Ryanodine activation and inhibition of the Ca^{2+} release channel of sarcoplasmic reticulum. J. Biol. Chem. **261**, 6300—6306).