B. LEWARTOWSKI, B. WOLSKA

THE EFFECT OF THAPSIGARGIN ON SARCOPLASMIC RETICULUM Ca\textsuperscript{2+} CONTENT AND CONTRACTIONS OF SINGLE MYOCYTES OF RAT VENTRICULAR MYOCARDIUM

Department of Clinical Physiology, Medical Centre of Postgraduate Education, Warsaw, Poland

The effect of Thapsigargin (TG) shown to block selectively the Ca\textsuperscript{2+}-ATPase of sarcoplasmic reticulum (SR) on the SR Ca\textsuperscript{2+} content and contractions of single rat cardiomyocytes was investigated. In 80% of cells 10\textsuperscript{-6} M TG blocked Ca\textsuperscript{2+} uptake by SR at rest, depleted the SR Ca\textsuperscript{2+}, inhibited postrest potentiation and changed the pattern of response to premature contractions. Amplitude of steady state contractions was reduced to 11 ± 4% of pre-TG control. In 20% of cells TG had similar effect on the SR function, however, amplitude of contractions was reduced to only 50 ± 7% of control. It is concluded that the results obtained in 80% of the cells are compatible with the wide-spread notion that contractions of rat cardiac myocytes are activated predominantly by Ca\textsuperscript{2+} released from SR. However, population of myocytes of ventricular myocardium of this species is not homogenous in this respect. In 20% of them contractions are activated by both sarcolemmal and SR Ca\textsuperscript{2+} contribution being approximately equal. These cells occupy intermediate position between guinea-pig cells and the bulk of rat cells.

Key words: rat cardiomyocytes, excitation-contraction coupling, sarcoplasmic reticulum, calcium, thapsigargin.

INTRODUCTION

According to general consensus the main source of Ca\textsuperscript{2+} activating contraction of the cardiac myocytes is sarcoplasmic reticulum (SR) (for the recent review see ref. 1). The Ca\textsuperscript{2+} is released from SR through the release channels activated by small, but rapid increase in the sarcoplasmic [Ca\textsuperscript{2+}] brought about by activation of the sarcolemmal Ca\textsuperscript{2+} channels (2). However, recent experimental data (3) confirm the long-standing belief that dependence of activation of contraction on the SR Ca\textsuperscript{2+} varies from species to species and even from part to the part of the heart. For example we (4) found previously that it is possible to activate contraction of single myocytes of guinea-pig heart...
the SR of which had been deprived of Ca\(^{2+}\) by means of prolonged superfusion with 0.1 \(\mu\)M ryanodine. These results had been recently confirmed in experiments in which we blocked the SR Ca\(^{2+}\) uptake with sesquiterpine lactone Thapsigargin (TG) (5). The agent had been shown to block selectively the Ca\(^{2+}\)-ATPase of SR (5—8). Despite complete depletion of the SR Ca\(^{2+}\) amplitude of contractions of guinea-pig cardiomyocytes decreased only by 30%. As TG does not stimulate \(I_{\text{Ca}}\) (9), the results suggest that in normal cells contractions may be activated predominantly by sarcolemmal Ca\(^{2+}\).

In the present work we used TG in order to check the pattern of excitation-contraction coupling in the myocytes isolated from ventricular muscle of rat heart.

**METHODS**

Single myocytes of rat hearts were enzymatically isolated as described in detail elsewhere (10). The cells were placed in the Petri dish (Falcon, negatively charged) and became attached to its bottom. The dish was placed on the stage of an inverted microscope (Nikon, Diaphot) with the TV camera attached to its lateral port. The video edge-tracking system enabled continuous recording of the cell length. Changes in the cell shortening were expressed in % of control amplitude of contractions. Scales in the figures calibrate amplitude of contractions in % of resting length. This is used only as a reference enabling comparison of various records. The cells were rapidly superfused by means of a system modified from Rich et al. (3). Briefly, the selected cells were superfused by the stream of solution flowing at the bottom of the dish between the inflow plastic tube and outflow suction tube 1.5 mm in diameter. A set of miniature magnetic valves enabled direction of one of the 4 solutions of various composition through the inlet tube. The solutions flowing over the cells could be completely exchanged within 350 ms. Two platinum electrodes placed on both sides of the investigated cell were used for field stimulation.

**Solutions**

The Tyrode solution of the following composition was used throughout this work (in mM): NaCl 144.0; KCl 5.0; MgCl\(_2\) 1.0; NaH\(_2\)PO\(_4\) 0.43; HEPES 10.0; glucose 11.0. The pH of the solution was adjusted with NaOH to 7.30 for isolation of the cells or to 7.40 for experiments. In the experiments CaCl\(_2\) was added to concentration of 1.0 mM.

**Protocols**

All experiments were performed at room temperature (23°C). The cells were stimulated at the rate of 25—30/min. The 10.0 mM caffeine was superfused for 1s in order to rapidly release SR Ca\(^{2+}\) and initiate contracture. The transient caffeine contractures were used as an indirect index of the SR Ca\(^{2+}\) content (for review see ref. 4). The details of the protocols will be given in the Results section.
Statistical analysis

All results are given as means ± SD. The significance of differences between the means was evaluated by means of the Student test for paired or unpaired samples. P < 0.02 was accepted as a level of significance.

RESULTS

The cells were electrically stimulated to the steady state. One s superfusion with caffeine at the conclusion of stimulation initiated contracture the amplitude of which ranged from 150 to 400% of that of steady state electrically stimulated contractions (ESCs). A second application of caffeine a few s later initiated a weak contracture which shows that the first one depleted largely the SR Ca\(^{2+}\) (Fig. 1A). Next caffeine superfusion applied after 2 min of rest initiated contracture the amplitude of which was larger than that of the second one in all tested cells (Fig. 1A). As suggested by Shattock and Bers (11) and proved by Lewartowski and Zdanowski (10) recovery of caffeine contractures in the resting rat myocytes results from Ca\(^{2+}\) influx by the reversed Na/Ca exchange and subsequent uptake of this Ca\(^{2+}\) by the SR. Thus the cell in which the SR Ca\(^{2+}\) has been depleted by the first superfusion of caffeine provides an excellent model to study the SR Ca\(^{2+}\) uptake and its modifications by the experimental interventions.

Electrical stimulation following the third caffeine application initiated weak contractions in most cells hardly visible in the records. Inhibition of ESCs results from depletion of the SR Ca\(^{2+}\) by caffeine. Pre-caffeine amplitude was attained over 15—35 following beats. (Fig. 1A)

TG in 2 \(\times\) 10\(^{-7}\)M concentration superfused during 2 min of rest between the caffeine applications decreased the amplitude of postrest caffeine contractures by 12 ± 7% (n = 8) of control (not shown). Post-caffeine electrical stimulation to steady state resulted in contractions the amplitude of which attained 19 ± 8% of pre-TG control. Caffeine superfused then for 1 s instead of one dropped electrical stimulus initiated contracture the amplitude of which attained 31 ± 6% of steady state pre-TG control. Thus TG concentration which was sufficient for complete block of the SR Ca\(^{2+}\) uptake and SR Ca\(^{2+}\) depletion in the guinea-pig cardiac myocytes (5) exerted only a partial effect in rat.

Increase in concentration of TG superfused during 2 min of rest between the caffeine applications to 10\(^{-6}\)M resulted in reduction of the postrest caffeine contractures by 35 ± 9% (n = 8) (Fig. 1B). During 2 min of rest before TG superfusion SR was able to take up Ca\(^{2+}\). Thus TG in higher concentration
effectively blocked the SR Ca\(^{2+}\) uptake. This resulted in the drop of amplitude of post-TG steady state ESCs to 15 ± 6% of control and amplitude of caffeine contractures initiated instead of one ESC to 10 ± 3% of pre-TG control (Fig 1B). It was still much larger than that of ESCs.

In 3 out of 19 tested cells superfusion of 10\(^{-6}\) mM TG resulted in drop of amplitude of steady state ESCs to only 50—63% of pre-TG control and caffeine contractures were completely inhibited (Fig. 1C). A small deflection which appeared during the last caffeine superfusion shown in the figure is a motion artifact seen also at the feet of the preceding caffeine contractures.
In all the experiments described above each cell served as its own control. Since the effects of TG are irreversible this required that in order to investigate the next cell we had to wash the system, to change the dish and use new population of cells as well as to use more TG solution. Hence it was difficult to look this way for more cells showing this pattern of response to TG. However, looking at many cells at a time under a low magnification when the effect of TG was fully developed, we noticed that some of them still contracted strongly whereas contractions of most of them were hardly visible. After finding strongly beating cell we recorded its contractions, responses to caffeine, to rest and to premature stimuli. We found that none of these cells responded to caffeine with a clear contracture, which shows that their SR Ca\(^{2+}\) was depleted. Extent of shortening during steady state stimulation attained in these cells 6.8 ± 2.5\% (n = 9) of the resting cell length whereas extent of shortening of the cells which were not treated with TG was 13.1 ± 3.6\% of the resting cell length (P < 0.05, n = 51). Thus it seems likely that if control contractions of these cells could be recorded their amplitude would drop by 50\% under the effect of TG. Time to peak contraction in the control cells was 290 ± 59 ms and time of relaxation was 402 ± 98 ms (n = 19). In the cells treated with TG these values increased to

![CONTROL vs Tg](image)

*Fig. 2.* The effect of 10\(^{-6}\)M Tg on premature contractions (A) and postrest potentiation (B) in a single myocyte of the rat heart. Same cell as in Fig 1C, D, E.
890 ± 95 ms and 675 ± 87 ms, respectively (P < 0.05) (Fig. 1D, E). None of these cells showed any postrest potentiation typical for rat cardiomyocytes (Fig. 2B). In contrast to the normal cells extent of shortening during premature contraction was larger than during the steady state contractions after TG superfusion (Fig. 2A).

**DISCUSSION**

In the previous paper (5) we found that 2 × 10⁻⁷M TG blocks completely SR Ca²⁺ uptake in single myocytes of guinea-pig heart. This results in complete exclusion of SR from the cell function manifest in total depletion of the SR Ca²⁺ and inhibition of postrest and postextrasystolic potentiation. However, amplitude of steady state ESCs dropped by 30% only, showing that contraction in these cells is predominantly activated by sarcolemmal Ca²⁺ influx, which is not affected by TG (9). No Ca²⁺ uptake by SR can be observed in these cells at rest (5, 10).

In contrast to the guinea-pig cardiomyocytes in 80% of the rat cells depletion of SR Ca²⁺ by TG resulted in almost complete inhibition of ESCs (Fig. 1B). This result proves that in most of rat cardiomyocytes contractions are activated predominantly by Ca²⁺ released upon depolarization from SR. Indeed, as suggested by Shattock and Bers (11) and proved by Lewartowski and Zdanowski (10) the main source of activator Ca²⁺ in rat cells is Na/Ca exchange operating in this species between the beats and at rest in „reversed” mode (Ca²⁺ in). The Ca²⁺ is then taken up by SR and released by small I<sub>Ca</sub> during next AP to activate contraction. Increase in [Ca²⁺]<sub>i</sub> during Ca²⁺ transient upon depolarization reverses Na/Ca exchange to the “Ca²⁺ out” mode which enables extrusion of this ion equivalent to its total influx. The effects of TG in the present experiments are compatible with this concept. In control runs (Fig. 1A) the SR Ca²⁺ stores depleted by caffeine were recovered over rest due to Ca²⁺ influx by Na/Ca exchange. TG largely prevented uptake if this Ca²⁺ by SR which resulted in its depletion and inhibition of contractions (Fig. 1B). The effect of TG was not as rapid and strong as in guinea-pig cells (5) and its concentration had to be increased 5 times in order to obtain comparable inhibition. This may result from much greater availability of the sarcoplasmic Ca²⁺ in rat then in other species (10).

A striking result of this paper is a finding that 20% of the rat cells responded to inhibition of the SR Ca²⁺ uptake in the way similar to that of guinea-pig myocytes i.e. contractions decreased by 50% only. This result
suggests that in these cells contractions are activated by both sarcolemmal and sarcoplasmic reticulum Ca$^{2+}$, contribution of these sources being approximately equal.

In conclusion, guinea-pig and rat occupy probably the flank positions in the species ordered with respect to their mode of myocardial excitation-contraction coupling. In guinea-pig contraction is activated predominantly by sarcolemmal Ca$^{2+}$ influx activated during AP. No net Ca$^{2+}$ influx occurs between the beats. In most of the rat cardiomyocytes contraction is activated predominantly by Ca$^{2+}$ released from the SR, Na/Ca exchange reversed between the beats being its main source. I$_{Ca}$ activated during AP serves mainly as a trigger for SR Ca$^{2+}$ release rather than a source. However, a fraction of the myocytes of rat ventricular myocardium reveals properties intermediate between bulk of rat cells and guinea-pig cells. This shows that important differences in the mode of excitation-contraction coupling may occur not only between the species but also between the cells of apparently homogenous population isolated from one part of the heart of the given species.

Acknowledgements: This work has been supported by grant No 416729101 of The National Committee for Research.

REFERENCES


Received: February 22, 1993
Accepted: April 14, 1993

Author's address: B. Lewartowski, Department of Clinical Physiology, Medical Centre of Postgraduate Education, Marymoncka 99, 01-813, Warsaw, Poland.