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TEMPERATURE DEPENDENT CONTRIBUTION OF Ca^{2+} TRANSPORTERS TO RELAXATION IN CARDIAC MYOCYTES: IMPORTANT ROLE OF SARCOLEMMAL Ca^{2+}-ATPASE.

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Activities of Ca^{2+}-ATPase of sarcoplasmic reticulum (SERCA) and Na^{+}/Ca^{2+} exchanger (NCX) involved in cellular Ca^{2+} turnover greatly change in hypertrophied and failing hearts. Unfortunately, contribution of these proteins as well as of the sarcolemmal Ca^{2+}-ATPase (PMCA) to cellular Ca^{2+} turnover has been investigated almost exclusively at room temperature. PMCA is of particular interest since it may affect activity of calcineurin and nNOS. Therefore the objective of this study was to reinvestigate contribution of SERCA, NCX and PMCA to cell relaxation and the effect of PMCA on cell contraction at 37°C. Myocytes isolated from the ventricles of guinea pig and rat hearts and incubated with Indo-1 were field stimulated at the rate of 60/min. Contribution of SERCA, NCX and PMCA was calculated from the rate constants of the decaying components of electrically stimulated Ca^{2+} transients or of the transients initiated by caffeine dissolved in normal Tyrode or in 0Na, 0Ca Tyrode. Increase in temperature from 24 to 37°C increased the relative contribution of NCX from 6.1% to 7.5% in rat and from 21.3 to 51.9 % in guinea pig at the expense of SERCA. The contribution of the PMCA to relaxation in both species increased upon rise in temperature from 24 % to 37°C from negligible values to 3.7 %. In both species amplitude of Ca^{2+} transients was at 24°C nearly twice as high as at 37°C. It was nearly doubled by carboxyeosine (CE), a PMCA blocker at 37°C but was hardly affected at 24°C. The effects of CE were concentration-dependent and conformed with the degree of inhibition of activity of PMCA. Conclusions: PMCA plays an important role in regulation of myocardial contraction despite its small contribution to relaxation. In guinea pig but not in rat relative contribution of SERCA and NCX to relaxation is highly temperature dependent.

Key words: *ventricular myocytes, calcium transporters, NCX, PMCA, temperature sensitivity*

INTRODUCTION

The myocyte Ca^{2+} turnover involved in excitation-contraction coupling and cell relaxation has been the subject of extensive investigation. According to the current views (1) the main intracellular store and source of contractile Ca^{2+} is sarcoplasmic reticulum (SR). Calcium is pumped to the tubular part of the SR by its Ca^{2+}-ATPase (SERCA), and transported to and stored in the SR terminal cisternae. The Ca^{2+} release channels of the terminal cisternae are activated by increase in $[Ca^{2+}]_i$ within the diadic cleft brought about by activation of the sarcolemmal Ca^{2+} L-type channels located in the walls of the T tubules. Increase in the bulk sarcoplasmic $[Ca^{2+}]_i$ activates contraction. Relaxation is brought about by decrease in sarcoplasmic $[Ca^{2+}]_i$ due to its reuptake by SERCA (~70 -90%), and outward transport by Na^+/Ca^{2+} exchange (NCX) and sarcolemmal Ca^{2+}-ATPase (PMCA). The small Ca^{2+} uptake by mitochondria is also considered (2-7).

The presence of PMCA in cardiac myocytes and its properties were characterized by Caroni and Carafoli (8). This enzyme has high affinity to Ca^{2+} and low velocity of its transport. Therefore it has been proposed that it is mostly engaged in subtle regulation of diastolic $[Ca^{2+}]_i$, its contribution to outward Ca^{2+} transport being of minor importance. The role of the PMCA in the myocyte function could be more precisely investigated since the introduction of its specific blocker carboxyeosine (CE) (9, 10). The experiments with CE proved that the PMCA is indeed important for the control of the diastolic Ca^{2+} concentration (6, 11) and that it may contribute significantly to relaxation (2-4, 6) in ferret, rabbit and rat ventricular myocytes. It has been also shown that PMCA may affect the SR Ca^{2+} content and amplitude of Ca^{2+} transients in rat ventricular myocytes (6).

Unfortunately, the experiments on PMCA were performed exclusively at room temperature. The relative contribution of SERCA and NCX to relaxation was, to our knowledge, checked at 37°C by only one study in myocytes of rabbit, ferret and cat hearts (12), but not in rat or guinea pig. The relative contribution of Ca^{2+} transporters to relaxation under physiological conditions (37°C) may differ from that at room temperature due to differences in temperature sensitivity of the involved systems. Therefore, the objective of this study was to compare the relative contribution of SERCA, NCX and PMCA to relaxation and the role of PMCA in regulation of contraction of the isolated ventricular myocytes of guinea pig and rat hearts at 37°C and 24°C. The effect of PMCA on contraction is of particular interest since it has been recently found that this protein may regulate activity of calcineurin (13) and nNOS (14).

We found that in guinea pig myocytes the relative contribution of SERCA to relaxation decreases whereas that of NCX increases greatly with increasing temperature. This effect, although much smaller, was also present in the rat myocytes. We also found that contribution of PMCA to relaxation is in both species highly temperature dependent and that its low activity at 24°C plays important role in the mechanism of temperature inotropy. Moreover, we found

that even small changes in rate of Ca^{2+} transport by this enzyme have a very strong influence on the amplitude of Ca^{2+} transients and contractions. Thus, the PMCA may be an important factor in the control of myocyte contractility.

MATERIAL AND METHODS

Cells isolation and superfusion

Ventricular myocytes of rats and guinea pigs of both sexes were isolated as described in detail elsewhere (15). Cells were rapidly superfused by the modified method of Rich et al. (16). The superfusion chamber was mounted on the stage of Nikon Diaphot inverted microscope equipped for epifluorescence.

Recording of Ca^{2+} transients and cells shortening

For estimation of changes in intracellular Ca^{2+} concentration (Ca^{2+} transients) cells were incubated for 15 min with 10 µM Indo-1 acetoxymethyl ester as described by Spurgeon et al. (17). A Nikon mercury lamp was used as a source of illumination for epifluorescence. A concentric diaphragm enabled illumination of a small fragment of a cell. The fluorescent light was passed to the 405-nm DE35 and 495-nm DE20 photomultipliers mounted in the holder attached to the side port of the microscope. The ratio of 405 to 495 nm fluorescence was obtained from the output of Dual Channel Ratio Fluorometer (Biomedical Instrumentation Group, University of Pennsylvania). No attempt was made to convert the fluorescence ratio to Ca^{2+} concentration. Cell shortening was recorded with video edge detector designed and built by J. Parker (Cardiovascular Laboratories, School of Medicine, UCLA). The fluorescence or cells shortening signals were recorded by the computer and stored for further analysis by the ISO2 Multitask-Patch-Clamp Softwere designed by M. Friedrich and K. Benndorf, University of Dresden.

Estimation of relative contribution of SERCA, NCX and PMCA to cells relaxation.

Contribution of the Ca^{2+} transporters to relaxation was estimated from the rate constants (r) of decaying component of respective Ca^{2+} transients according to Choi and Eisner (6). Two protocols were used in two separate groups of myocytes (*Fig. 1*).

1. Cells superfused at 24°C or 37°C with normal Tyrode (NT) solution were stimulated at the rate of 60/min and Ca^{2+} transients recorded. The rate constant of their decline (r1) reflects the combined activities of SERCA, NCX and slow transporters (i.e. PMCA and mitochondrial uptake). Thereafter stimulation was stopped and 10 mM caffeine dissolved in NT solution superfused. Caffeine releases Ca^{2+} from the SR and prevents its reaccumulation. Therefore the rate constant of decline of the transient initiated by caffeine (r2) reflects the combined activities of NCX and slow transporters (*Fig. 1A*).
2. In order to eliminate NCX cells were superfused for 60 s between stopping of stimulation and caffeine application with 0Na, 0Ca Tyrode solution to which 1 mM EGTA was added. This solution blocks NCX and prevents Ca^{2+} loss from the resting cell. The rate constant of the decline of the transient initiated by caffeine (dissolved in 0Na, 0Ca Tyrode) (r3) reflects under these conditions the activity of the slow transporters (*Fig. 1B*). In some experiments 1µM FCCP was added to the 0Na, 0Ca Tyrode in order to inhibit the mitochondrial Ca^{2+} uptake.

The rate constant of the component of relaxation dependent on SERCA (r_{SERCA}) was calculated by subtracting r2 of each myocyte from its r1 (r_{SERCA} = r1 - r2) (*Fig. 1A*).

In order to evaluate the contribution of NCX to decline of the caffeine induced Ca^{2+} transients, r3 should be subtracted from r2 (r_{NCX}= r2 - r3). However, the r2 and r3 were measured in different groups of cells (*Fig. 1A* and *B*) in order to avoid more then one caffeine application in a cell. Therefore for subtraction of r3 from r2, cells, the r1 of which did not differ or was very close (differences less than by 5%), were paired.

The r3 was accepted as an index of the rate of decline of Ca^{2+} transient dependent on the PMCA since it was not affected by FCCP (r_{PMCA}= r3).

The % contribution of individual transporters to cell relaxation was calculated by dividing respective rate constant times 100 by r1 (which reflects combined activities of all transporters). For example: r_{SERCA}x100/r1.

SERCA contributes to relaxation by intracellular Ca^{2+} uptake. NCX and PMCA contribute to relaxation by outward Ca^{2+} transport. The % contribution of NCX or PMCA to the total outward Ca^{2+} transport was calculated by dividing r_{NCX} or r_{PMCA} times 100 by r2 (reflecting combined activities of both transporters).

Solutions

For cells isolation and throughout the experiments we used Tyrode solution (NT) of the following composition (in mM): 144 NaCl, 5 KCl, 1 $MgCl_2$, 0.43 NaH_2PO_4, 10 N-2-hydroxyethylpiperazine-N'-2-etanesufonic acid (HEPES), 11 glucose. The pH of NT was adjusted with NaOH to 7.3 for cells isolation and to 7.4 for experiments. In experiments $CaCl_2$ was added to concentration of 1 mM (rats) or 2 mM (guinea pigs). In order to block the NCX we used the 0 Ca^{2+} Tyrode in which Na^+ was replaced with Li^+. 1mM EGTA was added in order to accelerate Ca^{2+} removal. The pH of this solution was adjusted with KOH to 7.4.

Statistical evaluation of the results

The quantitative results are presented as means ±SE. Student's t-test for unpaired samples was used to determine the significance of differences between the means. To justify the use of Student's t-test normal distribution of data was proved by Shapiro-Wilk test. $P<0.05$ was accepted as a level of significance.

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996),

RESULTS

Guinea pig

The effect of temperature on the relative contribution of SERCA and NCX to cells relaxation

Table 1 shows the rate constants of decay of Ca^{2+} transients stimulated electrically or by 10 mM caffeine added to NT or 0Na, 0Ca Tyrode at 37°C or 24°C according to protocols shown in *Fig. 1.*

Table 1 Rate constants (s^{-1}) of decay of Ca^{2+} transients of guinea pig and rat myocytes

		r1	r2	r3
Guinea pig	37°C	11.17±0.43 n=26	6.01±0.43 n=26	0.39±0.04 n=26
	24°C	5.67±0.24 n=13	1.24±0.07 n=13	0.06±0.01 n=13
Rat	37°C	10.90±0.44 n=23	1.19±0.06 n=23	0.38±0.03 n=23
	24°C	6.61±0.36 n=11	0.41±0.05 n=11	0.04±0.01 n=11

r1 - rate constant of decay of Ca^{2+} transients stimulated by electrical pulses; r2 - rate constant of decay of Ca^{2+} transients stimulated by application of caffeine dissolved in normal Tyrode solution; r3 - rate constants of decay of Ca^{2+} transients stimulated by caffeine dissolved in 0Na, 0Ca Tyrode solution. Means±SE.

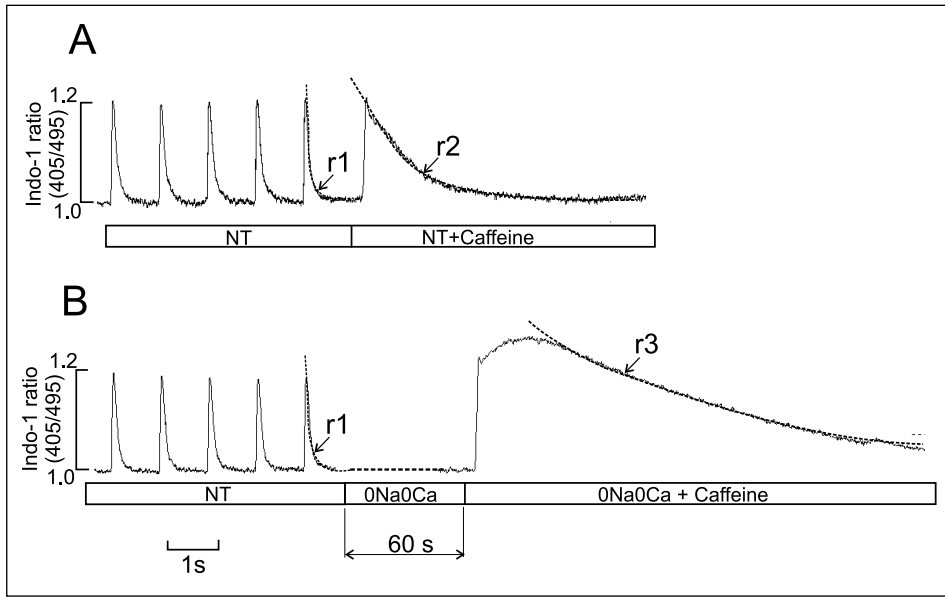

Figure 1 Experimental protocols. Ca^{2+} transients of two ventricular myocytes of rat heart. **A**: myocyte superfused with normal Tyrode solution (NT) stimulated at the rate of 60/min. Caffeine (dissolved in NT) superfused instead of electrical pulse. The monoexponentials with rate constants r1 and r2 were fitted to the decaying part of Ca^{2+} transients stimulated electrically or by caffeine, respectively. **B**: myocyte superfused with 0Na, 0Ca solution between stopping of stimulation and caffeine (dissolved in 0Na,0Ca solution) application. The monoexponentials with rate constants r1 and r3 were fitted to the decaying part of Ca^{2+} transients stimulated electrically or by caffeine, respectively.

The calculated rate constants of the components of relaxation dependent on SERCA (r_{SERCA} = r1- r2) or NCX (r_{NCX} = r2 - r3) are shown in *Table 2*. The r_{SERCA} increased upon rise in temperature 1.16 times whereas r_{NCX} increased 4.76 times.

The relative contribution of SERCA and NCX to relaxation is shown in *Fig. 2A* (top panel). The relative contribution of NCX to relaxation (r_{NCX} x 100 / r1)

increased upon rise in temperature from 21.3±1.4% to 51.9±4.2% at the expense of relative contribution of SERCA (r_{SERCA} x 100 / r1), which dropped from 77.7±1.4% to 44.4±4.4%. The values obtained at 24°C were similar to those reported by others in guinea pig myocytes at 22°C (18).

The contribiution of PMCA to relaxation.

Rate constant of decay of the Ca^{2+} transients initiated by caffeine in cells superfused with 0Na, 0Ca solution (r3) (*Fig. 1B*) reflexes the rate of outward Ca^{2+} transport by PMCA and possible mitochondrial Ca^{2+} uptake. In order to dissect PMCA contribution we measured the r3 in cells (n=10) in which mitochondrial Ca^{2+} uptake was blocked by FCCP (1 μm) added to 0Na0Ca solution. The r3 was not affected by FCCP (not shown), which suggests that mitochondrial uptake is insignificant under our experimental conditions. Therefore the r3 was accepted as an index of the rate of decline of Ca^{2+} transient dependent on the PMCA (r_{PMCA}). In order to check this, the protocol presented in *Fig. 1B* was repeated in cells preincubated for 15 min with 5 μM carboxyeosine (CE), a blocker of PMCA (*Fig. 3*). At both temperatures CE blocked decay of Ca^{2+} transients induced by caffeine in cells superfused with 0Na, 0Ca Tyrode. As shown in *Table 2*, r3 (r_{PMCA}) increased more then 7 times upon rise in temperature from 24 to 37°C.

The relative contribution of the PMCA to relaxation (r_{PMCA} x 100 / r1) was 1.0±0.1% at 24 and 3.7±0.5% at 37°C (*Fig. 2A*, top panel). Its contribution to the outward Ca^{2+} transport was 4.8±0.6% and 7.1±0.7%, respectively (Fig. 2B, top panel).

The effect of blocking of PMCA on amplitude of Ca^{2+} transients.

The amplitude of the Ca^{2+} transients electrically stimulated at 37°C was by 76% lower then at 24°C. CE (5μM) increased the amplitude of Ca^{2+} transients recorded at 37°C by 87% and hardly affected amplitude of the transients recorded at 24°C. Thus in cells pretreated with CE, the amplitudes of Ca^{2+} transients recorded at both temperatures were nearly equal (*Fig. 4A*, top panel) in spite of large difference in contribution to relaxation of SERCA and NCX at these

Table 2 Rate constants (s^{-1}) of the components of decay of Ca^{2+} transients dependent on SERCA, NCX and PMCA calculated from rate constants shown in *Table 1.*

		r_{SERCA} r1-r2	**r_{NCX} r2-r3**	**r_{PMCA} r3**
Guinea pig	37°C	5.16±0.59 n=26	5.62±0.41 n=26	0.39±0.04 n=26
	24°C	4.43±0.25 n=13	1.18±0.08 n=13	0.06±0.01 n=13
Rat	37°C	9.71±0.43 n=23	0.81±0.08 n=23	0.38±0.03 n=23
	24°C	6.20±0.38 n=11	0.38±0.05 n=11	0.04±0.01 n=11

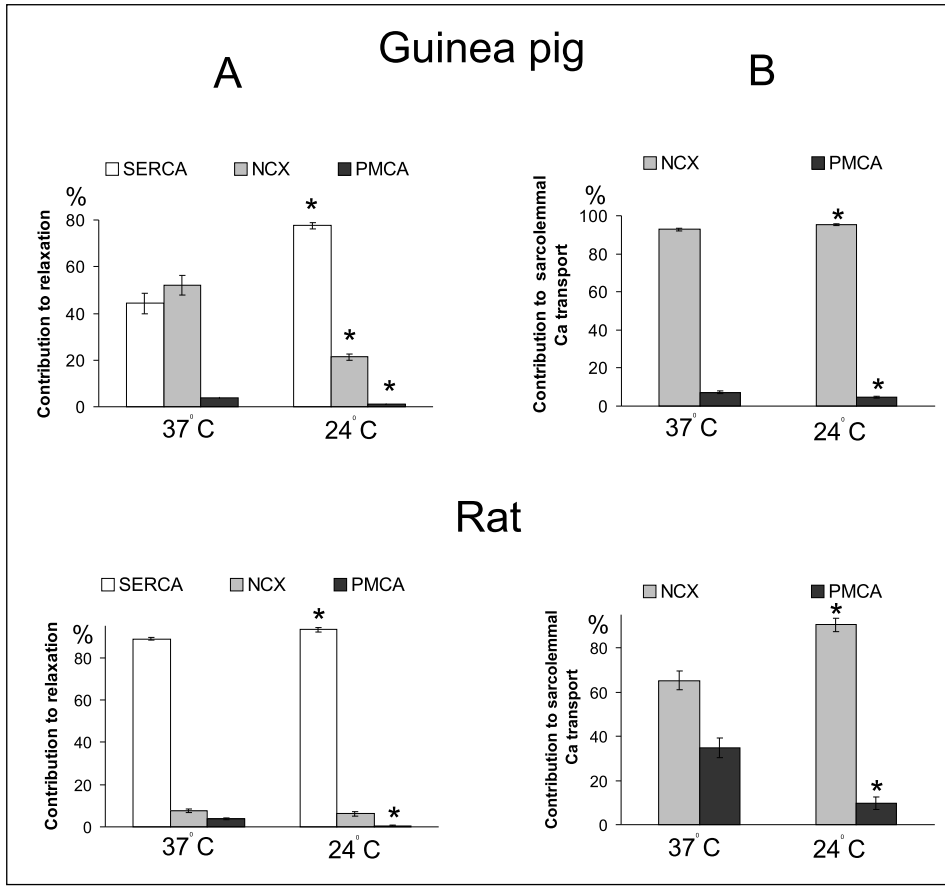

Figure 2 **A**: the effect of temperature on the relative contribution of the Ca^{2+} transporters to relaxation in ventricular myocytes. **B**: the effect of temperature on the relative contribution of Na^{+}/Ca^{2+} exchanger (NCX) and the sarcolemmal Ca^{2+}-ATPase (PMCA) to the sarcolemmal Ca^{2+} transport. Means±SE (n=11 - 26). * $p<0.05$ vs. relative contribution of the Ca^{2+} transporters at 37°C.

temperatures (*Fig. 2A*, top panel). Therefore decrease of the activity of the PMCA at 24°C may alone account for the temperature inotropy.

The concentration dependent effect of CE on amplitude of cell shortening

Myocytes superfused with NT at 37°C were field stimulated at the rate of 60/min and contractions recorded before and after superfusion for 15 min with different concentrations of CE (from 0.25 μM to 5.0 μM). Figure 5 shows the response of amplitude of cells shortening to respective CE concentrations. The 0.25μM CE increased the amplitude of cells shortening by 37%. The 1 μM CE increased the amplitude of shortening by 95%, whereas 5 μM CE increased it

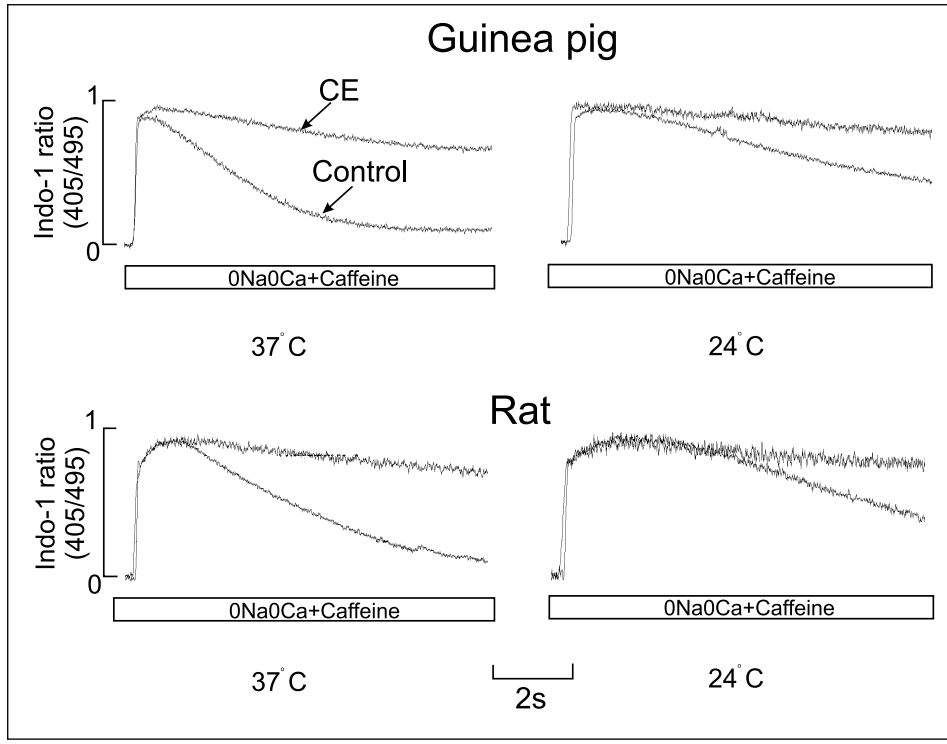

Figure 3 The effect of 5 μM carboxyeosine (CE) on the decline of the Ca^{2+} transients stimulated by 10 mM caffeine dissolved in 0Na, 0Ca solution in the ventricular myocytes superfused with 0Na, 0Ca solution. In each panel the Ca^{2+} transients stimulated by caffeine in myocytes preincubated with CE were normalized to the Ca^{2+} transients of control myocytes. Symbols pertain to all records.

by 117%. The difference between the effects of 1 μM and 5 μM CE is not significant ($p>0.05$). Therefore it may be accepted that the effect of 5 μM CE was maximal and this concentration was used throughout this work. Cells incubated with 20 μM CE used by others (4, 6) when stimulated, died very soon showing the symptoms of haevy Ca^{2+} overload. The reason of this discrepancy is not clear.

It would be important to relate the increase in amplitude of cell shortening to the degree of inhibition of the PMCA. However, the PMCA dependent decay of the Ca^{2+} transient stimulated by caffeine in cells superfused with 0Na, 0Ca solution is very slow which renders unrealistic an attempt to measure its gradual inhibition by the increasing concentrations of CE. Therefore we tested the effect of only 0.25 μM CE, which increased the amplitude of contractions by 37% (*Fig. 5*). Superfusion of cells with 0.25 μM CE decreased the r3 (r_{PMCA}) from 0.38±0.04 to 0.21±0.02 s^{-1} i.e. by 45 %, which conforms well with its effect on contractions.

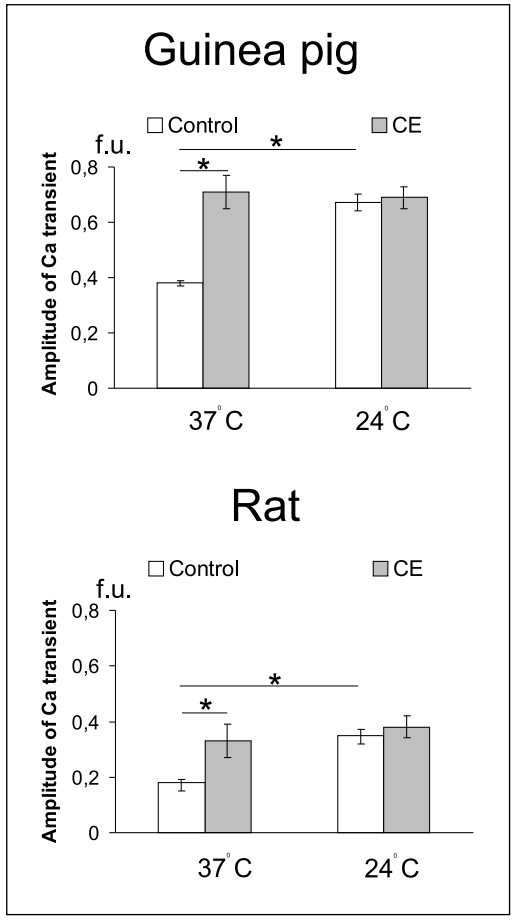

Figure 4 The effect of 5 μM carboxyeosine (CE) at 37 and 24°C on the amplitude of electrically stimulated Ca^{2+} transients. The amplitude of the Ca^{2+} transients was calculated by subtracting of the Indo-1 fluorescence ratio for diastolic and systolic Ca^{2+} concentrations and expressed in units of fluorescence (f.u.). Values are means±SE. n vary from 13 to 54 cells for different bars. * $p<0.05$

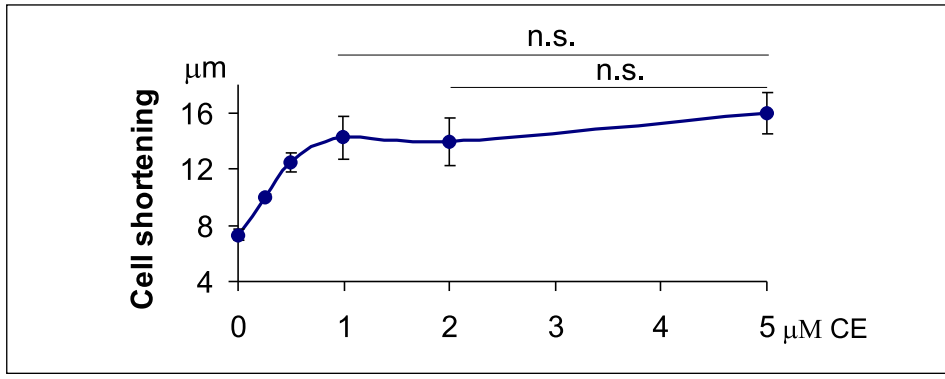

Figure 5 The effect of 15 min superfusion of 0.25 - 5 μM carboxyeosine (CE) on the amplitude of cell shortening of ventricular myocytes of guinea pig heart stimulated at the rate of 60/min. T= 37°C. Values are means±SE. n vary from 6 to 16 cells for different dose of CE and n=46 for control. n.s. - $p>0.05$.

Rat

The rate constants of decaying components of Ca^{2+} transients stimulated electrically or by 10 mM caffeine added to NT or 0Na, 0Ca Tyrode at 37°C or 24°C according to protocols presented in *Figure 1* are shown in *Tab. 1*. The calculated rate constants of the components of relaxation are shown in *Tab. 2*. The r_{SERCA} increased upon rise in temperature 1.57 times (vs. 1.16 in guinea pig) whereas r_{NCX} increased 2.10 times (vs. 4.76 in guinea pig).

Due to similar sensitivity of SERCA and NCX to temperature their relative contribution to relaxation at 24°C (SERCA - 93.3±1.1%; NCX - 6.2±1.0%) and 37°C (SERCA - 88.8±0.7%; NCX - 7.5±0.7%) hardly differed (*Fig.2A*, bottom panel). Contribution of SERCA at 24°C agrees very well with this reported by Choi and Eisner (6) and Negretti et al. (5). The contribution of the PMCA to relaxation was in rat myocytes at both temperatures similar to that in guinea pig (0.6±0.1 and 3.7±0.5% at 24 °C and 37°C, respectively) (*Fig. 2A*, bottom panel and *Fig. 3*, bottom panel). However, its contribution to the transsarcolemmal Ca^{2+} transport (r_{PMCA} x 100 / r2) was at 37°C 34.8±4.3% i.e. about 4 times greater in rat then in guinea pig, which resulted from the smaller contribution of NCX to relaxation in rat. At 24°C PMCA contribution to relaxation was 9.6±2.9%, i.e. 2 times greater then in guinea pig (*Fig. 2B*, bottom panel).

The amplitude of the Ca^{2+} transients was at 37°C in cells pretreated with CE by 94% higher then in normal cells. At 24°C both in normal cells and in cells pretreated with CE the amplitude of the transients was much higher (by 106 % and 124%, respectively) then in control cells at 37°C (*Fig. 4*, bottom panel).

DISCUSSION

This study provided results showing the species related differences in response of the Ca^{2+} transporters to changes in temperature and an important role of the PMCA in the regulation of myocyte contraction. We also found that there are important differences in sensitivity to temperature between the transporters.

The effect of temperature on the relative contribution of SERCA and NCX to relaxation

In guinea pig r_{SERCA} reflecting the rate of Ca^{2+} uptake by the SR increased 1.16 times upon rise in temperature from 24°C to 37°C whereas r_{NCX} reflecting the rate of outward Ca^{2+} transport by NCX increased 4.76 times (*Tab. 2*). This difference in temperature sensitivity could be expected considering the $Q_{10} = 2.6$ of SERCA measured in isolated myocardial vesicles (19) and $Q_{10} \sim 4$ of NCX in guinea pig cardiomyocytes (20). The small increase in r_{SERCA} upon rise in temperature may result from increased competition for Ca^{2+} on the part of NCX. Differences in temperature sensitivity of r_{SERCA} and r_{NCX} resulted in the drop of the relative

contribution of SERCA to relaxation and respective increase of relative contribution of NCX upon rise in temperature to 37°C (*Fig. 2A*, top panel).

About 50 % contribution of the outward Ca^{2+} transport to relaxation in myocytes of guinea pig heart at 37°C means that Ca^{2+} influx accounts for about 50% of Ca^{2+} activating contraction in this species. Such a large contribution of influx is probably responsible for the relative insensitivity of amplitude of Ca^{2+} transients and contractions to inhibition of SERCA by thapsigargin at room temperature (21) and at 37°C (Mackiewicz and Lewartowski, unpublished data).

In the rat myocytes the r_{SERCA} increased upon rise in temperature 1.57 times, however, the r_{NCX} increased 2.1 times (two times less then in guinea pig) (*Tab. 2*). This resulted in much smaller then in guinea pig drop in the relative contribution of SERCA to relaxation from 93.3% at 24°C to 88.8% at 37°C and insignificant change in relative contribution of NCX (*Fig. 2A*, bottom panel). These values are comparable to 87% contribution of SERCA at room temperature reported by Negretti et al. (5). The small effect of temperature on the relative contribution of SERCA to relaxation in rat is similar to that in myocytes of rabbit, ferret and cat hearts (12).

The rate constants of relaxation of steady state, electrically stimulated Ca^{2+} transients and of transients initiated by caffeine in rat cells at 24°C measured in this study are comparable to those reported previously in myocytes of adult rats (2) and of 4-6 mo old rats (22). However, rate constants of electrically stimulated cells were 3 times higher then respective rate constants reported by Choi and Eisner (6). We have no explanation for these discrepancies.

PMCA: its contribution to relaxation.

We found that activity of PMCA is highly temperature dependent since in both species r_{PMCA} increased greatly upon change from 24 to 37°C (*Tab. 2* and *Fig. 3*). The low activity of PMCA at 24°C is consistent with that reported at room temperature by Bers (1) and Bassani et al. (2). Thus, the relative contribution of PMCA to relaxation increased upon rise in temperature from negligible values (0.6% in rat and 1% in guinea pig) to almost 4% in both species (*Fig. 2A*). However, the effect of temperature on the relative contribution of PMCA to sarcolemmal Ca^{2+} transport differed in these species. It increased upon rise in temperature from ~10% to ~35% in rat and from ~5% to ~7 % in guinea pig (*Fig. 2B*) due to relatively small activity of NCX and its low temperature sensitivity in rat (Fig. 2A, bottom panel).

PMCA: the effect of its activity on Ca^{2+} transient and cell shortening

In both species the amplitude of Ca^{2+} transients electrically stimulated at 37°C was almost doubled by cooling to 24°C or by preincubation with 5 μM CE. However, it was not affected by CE at 24°C. Thus the amplitudes of Ca^{2+}

transients of control cells at 24°C and of cells at 37°C preincubated with CE did not significantly differ (*Fig. 4*).

Two observations suggest that increase in amplitude of the Ca^{2+} transients in control cells by cooling to 24°C is also related to the low rate of Ca^{2+} transport by the PMCA at this temperature. The amplitude of the Ca^{2+} transients was not further affected by CE at 24°C, and amplitude of the Ca^{2+} transients in cells pretreated with CE did not change upon increase of the temperature to 37°C despite increase in activity of NCX. Thus inhibition of PMCA could alone account for the temperature inotropy. These results suggest that PMCA could be an important factor in the control of myocytes contractility. This was further tested in experiments on the response of myocytes to various concentrations of CE.

We found that superfusion of 0.25 μM CE at 37°C resulted in increase in amplitude of cells shortening by 37% and that this increase was related to inhibition of the rate of the PMCA dependent relaxation by 45%. Superfusion with 1.0 μM CE resulted in nearly maximal increase in the amplitude of contractions. The real concentration of CE cumulating within cells is not known, however, 0.25μM CE is of similar order of magnitude as $IC_{50} \sim 0.3$ μM for PMCA in red blood vesicles and $IC_{50} \sim 1.0$μM for the cardiac PMCA reported by Gatto et al. (9).

In conclusion, changes in activty of PMCA may play an important role in regulation of myocardial contraction despite small relative contribution of PMCA to relaxation. Regulation of contraction may be exerted by PMCA by modulation of amount of available contractile Ca^{2+} or indirectly by regulation of activity of such important enzymes as calcineurin (13) and nNOS (14).

Moreover, we found that in guinea pig but not in rat increase in temperature from 24°C to physiological 37°C greatly increases the relative contribution of NCX to myocyte relaxation at the expense of SERCA.

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