Energetics of Na^+–Ca^{2+} Exchange in Resting Cardiac Muscle

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ABSTRACT The energetic effect of extracellular Na^+ removal and readmission (in a nominally Ca^{2+}-free perfusate) in Langendorff-perfused ventricles of transgenic mice (TM), which overexpress the sarcolemmal Na^+–Ca^{2+} exchanger; normal mice (NM); young (7–12 days old) rats (YR); and older (13–20 days old) rats (OR) was studied. In all heart muscles, extracellular Na^+ removal induced an increase in heat production (H_1). Na^+ readmission further increased heat production to a peak value (H_2) followed by a decrease toward initial values. These effects were more marked in the YR and TM as compared with the OR and NM groups, respectively. Caffeine (1 mM), ryanodine (0.2 μM), and verapamil (1 μM) decreased H_1 and H_2 in both rat groups. EGTA (1 mM) decreased H_1 and H_2 in the YR but not in the OR group. Thapsigargin (1 μM) decreased H_1 and H_2 in all four hearts preparations. A possible interpretation is that Na^+–Ca^{2+} exchange acts as an energy-saving mechanism to prevent Ca^{2+} accumulation at the junctional sarcoplasmic reticulum zone (JSR) and thus prevents further release of Ca^{2+}. Extracellular Na^+ removal lead to Ca^{2+} accumulation in the JSR inducing further SR–Ca^{2+} release and increased energy release. Na^+ readmission removes the accumulated Ca^{2+} at the JSR (cleft) zone by exchanging Ca^{2+} with Na^+ producing a transitory increase in energy release due to Na^+–K pump activation.

INTRODUCTION

Excitation-contraction (EC) coupling in mammalian myocardium involves the participation of a minimum of two Ca^{2+} sources: Ca^{2+} that enters the cell across the sarcolemma (SL) and Ca^{2+} that is released from the sarcoplasmic reticulum (SR). The first component involves Ca^{2+} movement through a sarcolemmal channel (Tsien, 1983; Cheng et al., 1996), and possibly the operation of a Na^+–Ca^{2+} exchange system (Langer, 1982; Philipson, 1985; Levi et al., 1994). The second component seems to be dependent on the entrance of Ca^{2+} to a specific area close to the terminal cisternae of the SR to induce the release of more Ca^{2+} from the SR (Fabiato, 1983, 1985). The steady state requires the removal of the Ca^{2+} released during EC coupling and return of this Ca^{2+} to its original storage sites or removal from the cell. The removal of Ca^{2+}, either through the SR or the SL Ca^{2+} pumps or via the Na^+–Ca^{2+} exchanger, uses metabolic energy that is partially degraded to heat (Ponce-Hornos, 1990; Ponce-Hornos et al., 1992). The steady-state resting condition is the result of the balance between these two processes, that is, the balance between the entrance and the removal of Ca^{2+}. Different activities have been proposed for the various mechanisms during the resting state, but it has been difficult to study their relative roles (Gibbs, 1978; Loiselle, 1987; Ponce-Hornos, 1990). Current models predict that Na^+–Ca^{2+} exchange is responsible for maintenance of steady-state Ca^{2+} at rest, but there are no direct experimental measurements of the energetic cost of this process in the intact cell. For instance, there has been much discussion of the role of the Na^+–Ca^{2+} mechanism and its activity during the contraction–relaxation cycle (Langer, 1982; Levi et al., 1994). In addition, although it is widely speculated that Na^+–Ca^{2+} exchange functions to keep junctional Ca^{2+} low, there have been no direct measurements of the energy profile and costs of this process in the intact cell. Such information is important because Ca^{2+} removal from the cytoplasm can have different energetic costs (Ponce-Hornos, 1990). Ca^{2+} removal via the Na^+–Ca^{2+} exchanger uses one ATP per Ca^{2+} removed (because of the 1Ca:3Na stoichiometry of the Na^+–Ca^{2+} exchanger and the one ATP hydrolyzed for every three Na^+ removed via the Na^+–K pump). In contrast, although the removal of Ca^{2+} via the SL Ca^{2+} pump (Carafoli, 1987) has the same energetic requirements (one ATP per Ca^{2+} removed), the SR Ca^{2+} pump has a stoichiometry of two Ca^{2+} removed per ATP hydrolyzed (Tada and Inui, 1983). Consequently, one might conclude that an increase in the activity of sarcolemmal mechanisms over the SR Ca^{2+} pump could result in an increase in energy expenditure. The present study, with the use of relatively specific probes (Ca^{2+} channel blockers, ryanodine [Ry], caffeine [Caff], and thapsigargin [TPG]), evaluates the ability of the cell to manage changes in the transsarcolemmal Na^+ gradient together with the effects of interventions that affect Ca^{2+} movements. The contribution of the Na^+–Ca^{2+} exchanger to Ca^{2+} homeostasis in four different cardiac preparations is analyzed. First, adult transgenic mouse cardiac muscles enriched with sarcolemmal Na^+–Ca^{2+} exchanger (Adachi-Akahane et al., 1997) were compared with adult normal (nontransgenic) mouse cardiac muscles. Second, taking advantage of the different degree of sarcolemmal development (Fabiato, 1982; Vornanen, 1997) young (7–12-day-old) were compared with older (13–20-day-old)

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rat cardiac muscles. The results suggest that the presence of Na^+ in the extracellular space is of importance in the maintenance of a low Ca^{2+} concentration at the cleft zone via Na^+–Ca^{2+} exchange-mediated Ca^{2+} efflux. The maintenance of a low Ca^{2+} concentration in the cleft, would avoid a further release of Ca^{2+} by Ca^{2+}-induced Ca^{2+} release, thereby contributing to the maintenance of the resting state at low energetic cost.

METHODS

Heart preparation

Experiments were performed on four different cardiac preparations: adult normal (nontransgenic) mouse cardiac muscle (NM); adult transgenic mouse (TM) cardiac muscle with the sarcolemma enriched in Na^+–Ca^{2+} exchanger (Adachi-Akahane et al., 1997); young (7–12-day-old) rat cardiac muscle (YR); and older (13–20-day-old) rat cardiac muscle (OR). Animals were killed by cervical dislocation. Hearts were rapidly excised, cannulated through the aorta, and perfused at room temperature with control solution. The perfused heart was then mounted in the inner chamber of a calorimeter (Ponce-Hornos et al., 1982, 1995). At the end of each experiment, the tissue was removed from the calorimeter, weighed, dried at 110°C and dry weight determined. Unless otherwise indicated, results included in the present work are given per gram dry weight.

Solutions and protocols

The heart was perfused at a constant rate (1 ml/min) with a solution (control solution) containing (in mmol/l): 0.3 $MgCl_2$, 133 NaCl, 1.0 $CaCl_2$, 3.6 KCl, 16.0 dextrose, 10.0 2,3 butanedione monoxime (BDM), 5.0 Tris. After about 1 h (approximately the time required for calorimeter equilibration), the perfusion was changed to a solution of the same composition as the control solution but which was nominally free of Ca^{2+} (0Ca) until steady state was reached. Thereafter, the muscle was perfused for variable periods (from 1 to 6 min) with a solution free of Ca^{2+} and Na^+ (0Ca0Na solution) in which NaCl was replaced by an equimolar amount of choline chloride, with atropine (1 mg/l) added. After the 0Ca0Na intervention, the muscle was exposed again to a 0Ca solution until steady state was achieved. In some experiments, the effects of the presence of EGTA (1 mM), TPG (1 μM) ryanodine (0.2 μM), verapamil (1 μM) or Caff (10 mM) in the perfusate on the previously described interventions were studied. All solutions were adjusted to pH 7.3.

Mechanical and heat measurements

The technique for the measurement of heat production of isolated heart muscle has been described previously in detail (Ponce-Hornos et al., 1982, 1995). Briefly, the calorimeter was submerged in a constant temperature bath in which the different perfusate solutions were also equilibrated. The present calorimeter used two thermoelectric modules (two insulated ceramic modules (Melcor Thermoelectrics, Trenton, NJ) with 127 thermosensitive junctions each). The calorimeter response to maintained heat liberation follows a diffusion-type function that considers that the heat released approaches equilibrium exponentially at the peltier units (Ponce-Hornos et al. 1982, 1995). Under the perfusion conditions used for the present experiments, the calorimetric half time was less than 30 s. Calorimeter calibration was accomplished by passing a 2.1-kHz sine wave through the muscle by means of two electrodes in the inner chamber of the calorimeter. Calorimetric output of the thermosensitive units was either recorded in a Grass (model 7, Braintree, MA) Polygraph recorder or logged by an analog-to-digital converter (DT 2808, Data Translation) into an AT 386 Desk computer. With this method, it was possible to continuously record heat production of the preparation under the various experimental conditions. For the mechanical measurements, the base of the Langendorff perfused muscle was held by two stainless steel hooks and the apex attached to an isometric force transducer (Gould-Stathan UC3, Oxnard, CA) and recorded in a Grass Polygraph recorder. An optimal resting force value was established functionally with the muscles perfused in the presence of Na^+ and Ca^{2+} and during the equilibration period. To achieve the optimal resting force value, the muscles were electrically stimulated at 0.16 Hz, and resting force was gradually increased until force development showed no detectable increase at regular gain and was kept constant thereafter.

Statistical analysis

Data are presented as mean ± standard error of the mean (SE) and statistical significance determined at $p < 0.05$ level. For paired comparisons, the paired *t*-test was used. For multiple comparisons, a one-way analysis of variance test was applied.

RESULTS

Energetic effect of Na^+ removal

Rat heart muscles were perfused with nominally Ca^{2+}-free perfusate (0Ca) until steady heat production was achieved. As shown in Fig. 1, under these conditions the removal of extracellular Na^+ (0Na0Ca perfusate) induced an increase in heat production (H_1). Readmission of Na^+ induces a further and transitory increase in the rate of heat production up to a maximum value (H_2) followed by a decrease toward the rates observed before the removal of Na^+. The magnitudes of these effects were independent of the length of the previous 0Ca-perfusion period or on the number of times that the intervention was repeated. In some experiments, the same intervention was repeated up to five times in the same preparation without changes in the observed H_1 and H_2 values. Figure 2 shows that the magnitude of H_1 was dependent on the length of the intervention.

As shown in Fig. 2, in the OR group, H_1 and H_2 increased with the length of the 0Na intervention without reaching a plateau, at least within the longest period of 0Na intervention tested (6 min). In contrast, in the YR group, both H_1 and H_2 values rapidly increased, reaching their maximum values 2 or 3 min after the 0Na intervention (Fig. 2 *a*). The observed values for any selected period were lower for the

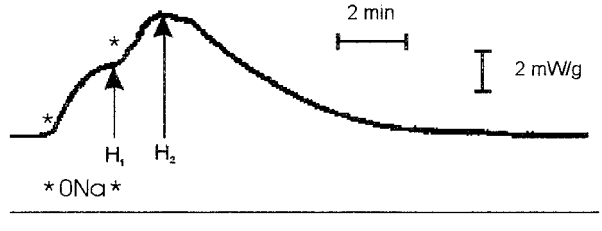

FIGURE 1 Typical myothermal response induced by extracellular Na depletion (0Na) and repletion in the absence of extracellular Ca^{2+}. The asterisks indicate the time at which solutions were switched. H_1 represents the rate of heat production achieved after the extracellular depletion of Na^+. H_2 represents the rate of heat production achieved after the restoration of Na^+ over the basal heat production (i.e., before the 0Na intervention). No corrections were performed for conduction delay or heat loss.

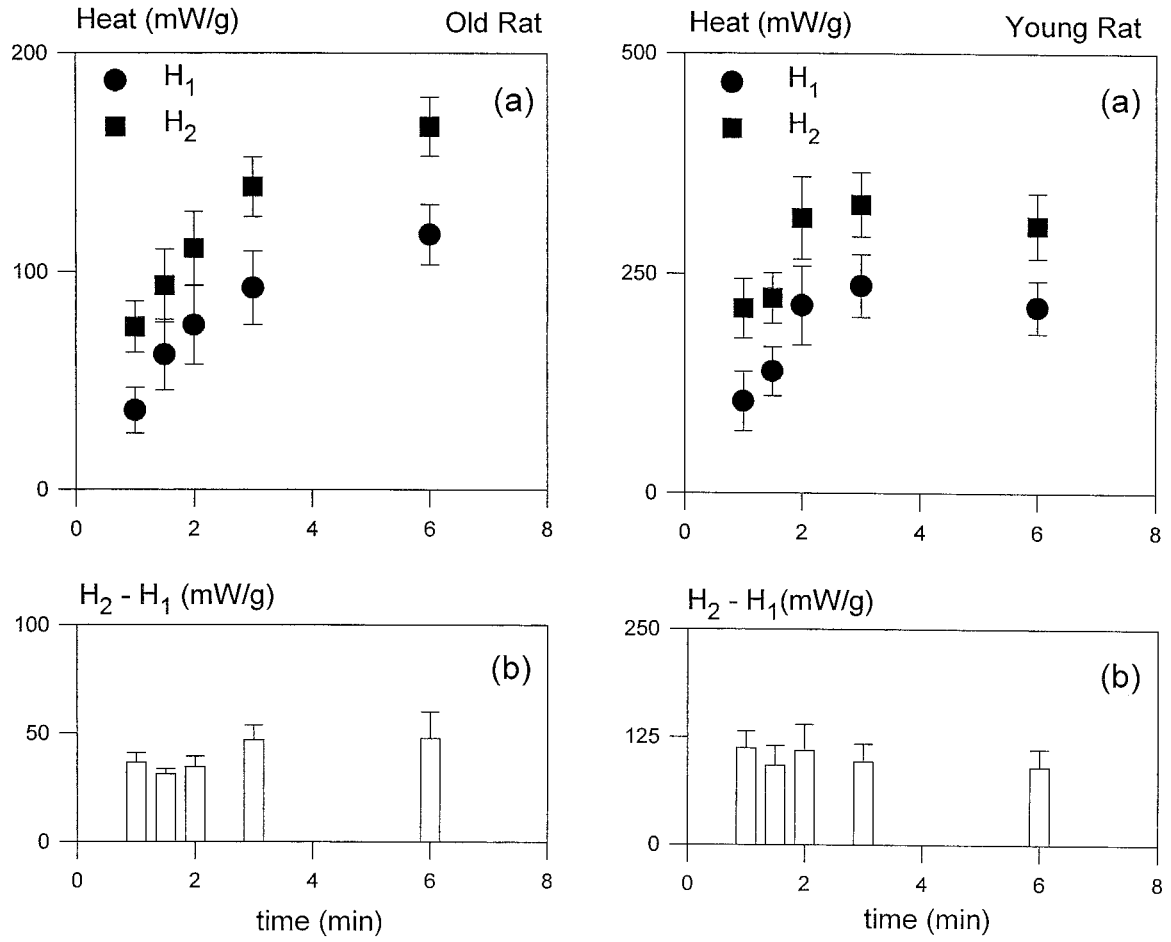

FIGURE 2 (*a*) Changes in resting heat production from old (n = 17) and young (n = 17) rat heart muscles due to extracellular Na^+ depletion (H_1) followed by Na^+ repletion (H_2) in the absence of extracellular Ca^{2+} plotted against perfusion time in the absence of extracellular Na^+. ●, Difference between the heat value reached under 0Ca0Na perfusion (Na^+ depletion) and the steady heat value under 0Ca perfusion. ■, Difference between the peak value of heat production reached under 0Ca perfusion after the 0Ca0Na intervention (Na^+ repletion) and the steady heat value under 0Ca perfusion (see Fig. 1). (*b*) Heat production due to the Na^+ repletion (i.e., $H_2 - H_1$) from old and young rat heart muscles. For all panels, vertical bars represent ±1 SE.

OR than for the YR group. Furthermore, if the data for the OR group is fitted to a hyperbola-type function, the fitted function yielded maximum heat production values of 179 ± 21 and 225 ± 8 mW/g for H_1 and H_2, respectively. Note that both values were significantly lower than the average maximal values found for the YR group (Fig. 2 *a*). The difference between H_2 and H_1 was significantly higher in the YR group than in the OR group (Fig. 2 *b*), and, in both groups, those values were independent of the length of the 0Na intervention (ANOVA F = 1.117 and overall mean = 32 mW/g for the OR group, and F = 0.21 and overall mean = 95 mW/g for the YR).

Previous studies have shown that exposure of isolated heart cells to 0Na0Ca isolates a cellular compartment of Ca^{2+} that is sensitive to the operation of the Na^+–Ca^{2+} exchanger (Langer and Rich, 1992; Post et al., 1993; Langer et al., 1995). Because the Na^+–Ca^{2+} exchanger was turned on and off by the addition or removal of Na^+ from the perfusate (Langer and Rich, 1992; Post et al. 1993; Langer et al. 1995), it was possible that the observed changes in heat production were related to the operation of the exchanger. To further test this hypothesis, a series of similar experiments were performed in a TM group, in which their sarcolemma was enriched with the Na^+–Ca^{2+} exchanger protein, and the results compared with those obtained from an NM group. As shown in Fig. 3, the magnitudes of the observed changes in heat response were doubled in the hearts enriched with the Na^+–Ca^{2+} exchanger protein when compared with the NM group (Fig. 3 *a*). As shown in Fig. 3 *b*, the additional heat released due to the return of Na^+ to the perfusion media (i.e., $H_2 - H_1$) was independent of the duration of the 0Na intervention (ANOVA F = 0.569 and overall mean = 69.04 mW/g for the NM group, and F = 0.869 and overall mean = 95.52 mW/g for the TM group).

The above results suggest a role for Na^+–Ca^{2+} exchange in the heat response to the 0Na intervention. Because the contaminating Ca^{2+} in the perfusate could be playing a role in the 0Na heat response, a series of experiments was performed in which, after a set of 0Na interventions (1 and 3 min), rat heart muscles were perfused for about 30 min in

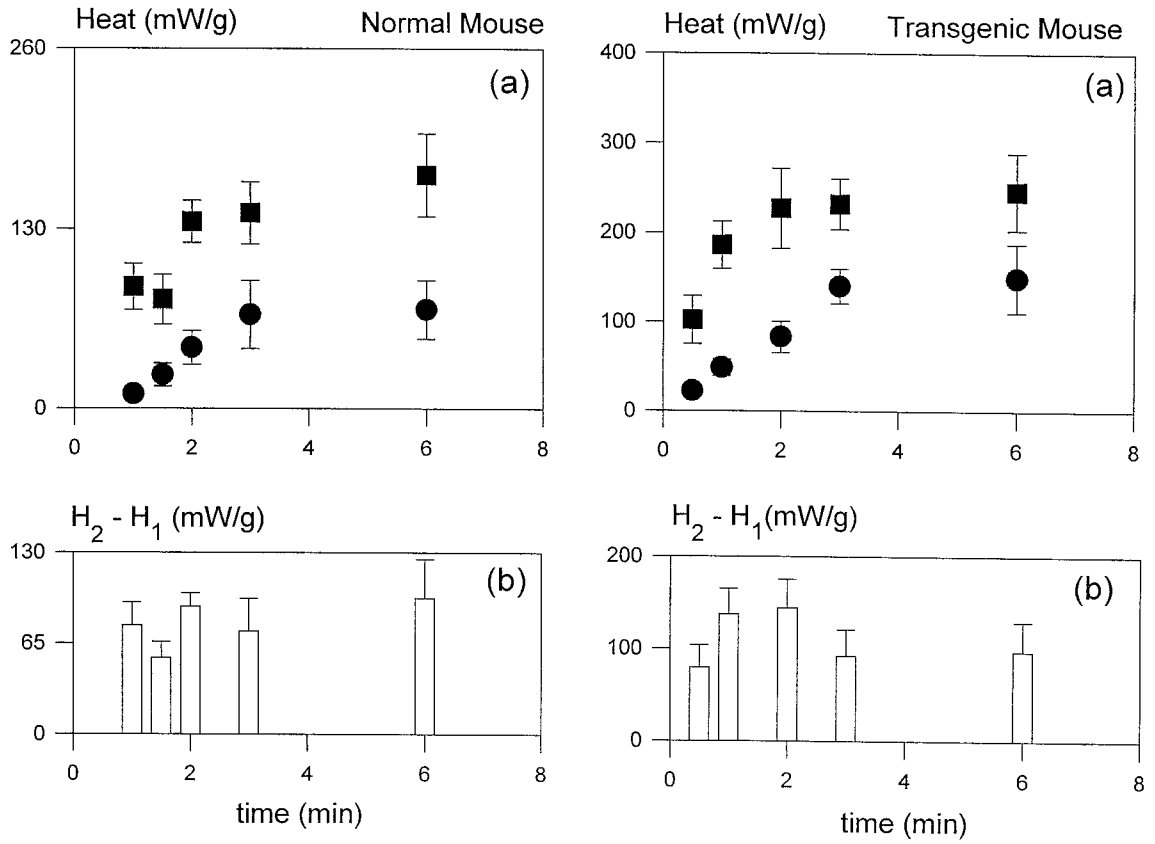

FIGURE 3 (*a*) Changes in resting heat production from nontransgenic ($n = 4$) and transgenic ($n = 6$) mouse heart muscles due to extracellular Na^+ depletion (H_1) followed by Na^+ repletion (H_2) in the absence of extracellular Ca^{2+}, plotted against perfusion time in the absence of extracellular Na^+. ● and ■ as in Fig. 2. (*b*) Heat production due to the Na^+ repletion (i.e., $H_2 - H_1$) from nontransgenic and transgenic mouse heart muscles. For all panels, vertical bars represent ±1 SE.

the presence of 1 mM EGTA. After a new steady heat production was established, another set of 1 and 3 min of 0Na intervention was repeated in the presence of EGTA in both rat preparations. In the YR group, EGTA significantly reduced the heat release associated with both the 0Na intervention and the Na^+ repletion (Fig. 4 *a*). In the OR group, no significant changes were observed for the 0Na intervention nor the Na^+ repletion (Fig. 4 *b*). The YR group behavior is consistent with a model in which the cell rapidly recaptures the Ca^{2+} removed by the exchanger, but if Ca^{2+} is sequestered (by the presence of EGTA) it cannot reenter the cell and the cycle is blocked. In contrast, the failure of EGTA to affect the heat release secondary to Na^+ removal and repletion in the OR group is consistent with a high relative participation of its intracellular sequestering system.

To further test the role of Ca^{2+} influx on the heat released by the 0Na and Na^+ restitution interventions, the effect of verapamil was tested in both preparations (Fig. 5). Verapamil decreased the heat released by both interventions, although it was quantitatively more marked in the YR group. From these results, it could be argued that, despite the resting conditions, the L type Ca^{2+} channel (due to stochastic opening), could participate in the path of reentry of Ca^{2+}.

Sarcoplasmic reticulum role

It was possible that the 0Na intervention induces an increase in cellular calcium that results in calcium cycling through the SR. Therefore, to further study the role of the SR on the Na^+ removal and repletion effects, another series of experiments was performed, in which drugs affecting the Ca^{2+} movements at the SR level were tested. The addition of caffeine (10 mM) to the perfusion media showed a significant but small effect in the YR group and a similar effect in the OR group (see Fig. 6). The addition of ryanodine (0.2 μM) induced comparable changes to those observed with caffeine in both OR and YR preparations (Figs. 6 and 7). Because both caffeine and ryanodine release Ca^{2+} from the SR without affecting the SR Ca^{2+} pump, it was possible that at least part of the Ca^{2+} released was cycling through the cytoplasm. In fact, the addition of both drugs during the 0Ca perfusion induced a sustained increase (although with somewhat different time courses) in basal heat production (data not shown). To further test the hypothesis that, under 0Na conditions, Ca^{2+} was cycling through the SR, experiments in the presence of thapsigargin were performed on all four preparations. As shown in Fig. 8, the presence of thapsigargin (1 μM) for at least 20 min in the perfusion media

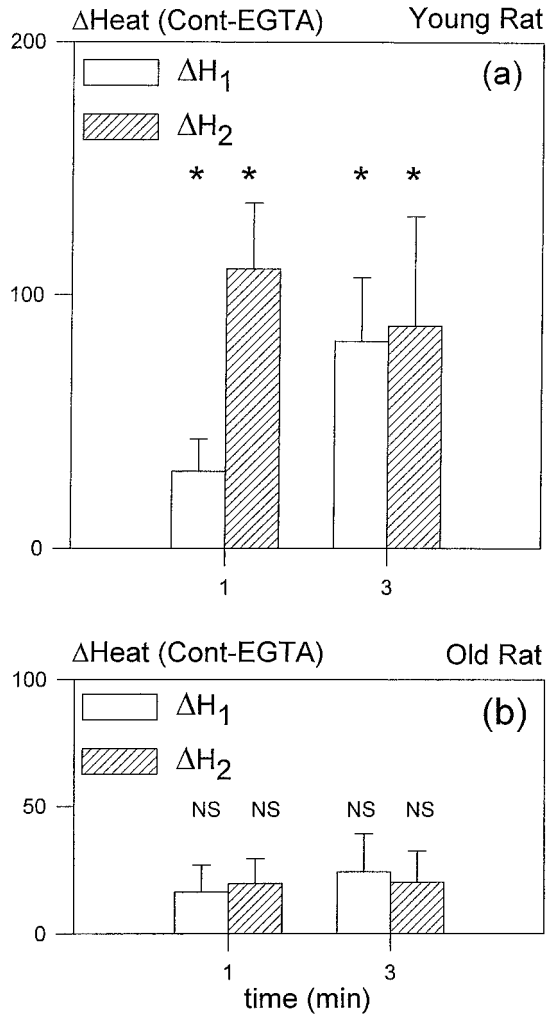

FIGURE 4 Effects of EGTA (1 mM) on the heat production induced by changes in extracellular Na^+ concentration from (*a*) young and (*b*) old rat heart muscles ($n = 7$). ΔH_1: Difference between the heat value (mW/g) reached after 1 or 3 min of 0Ca0Na perfusion in the absence (Cont) and in the presence of EGTA. ΔH_2: Difference between the peak value of heat production (mW/g) reached under 0Ca perfusion after the 0Ca0Na intervention (either 1 or 3 min) in the absence (Cont) and in the presence of EGTA. For both panels, vertical bars represent ±1 SE. ($*p < 0.05$; $**p < 0.01$).

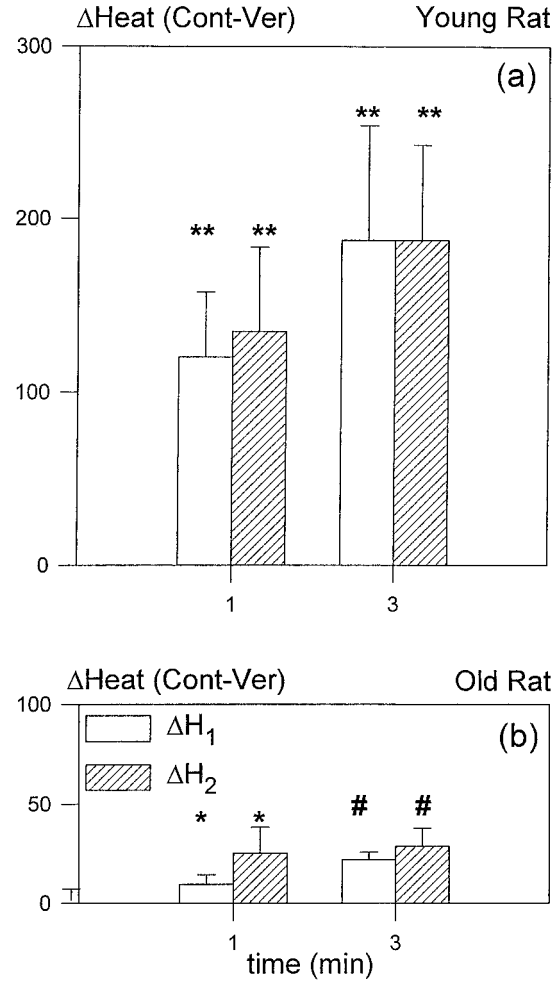

FIGURE 5 Effects of verapamil (1 μM) on the heat production induced by changes in extracellular sodium concentration from (*a*) young and (*b*) old rat heart muscles ($n = 8$). ΔH_1: Difference between the heat value (mW/g) reached after 1 or 3 min of 0Ca0Na perfusion in the absence (Cont) and in the presence of verapamil (Ver). ΔH_2: Difference between the peak value of heat production (mW/g) reached under 0Ca perfusion after the 0Ca0Na intervention (either 1 or 3 min) in the absence (Cont) and in the presence of verapamil (Ver). For both panels, vertical bars represent ±1 SE. ($*p < 0.05$; $**p < 0.025$; $^{\#}p < 0.01$).

significantly reduced the peak heat released by the $0Na^+$ and Na^+ reposition interventions in all four preparations. The percent inhibition was different (and smaller) in the rat than in the mouse, but, within any given species, the observed change was the same (over 80% in both mouse preparations and over 40% in both rat groups). The thapsigargin experiments clearly indicated that the SR Ca^{2+} pump must be active for the maintenance of the heat response to the Na^+ intervention.

Resting tension effects

To obtain an independent indication that the absence of Na^+ could induce intracellular Ca^{2+} cycling, YR and OR heart muscles were perfused in the absence of BDM and resting isometric force was measured. In the presence of Na^+, the mean values for resting force were 5.8 ± 0.5 mN and 6.8 ± 0.4 mN for the YR and OR groups, respectively. Although the mean increase in resting force induced by the $0Na^+$ intervention obtained for the YR group was higher than for the OR group, the values were not significantly different. In contrast, because the muscles from the young group were smaller than for the old group, to compare both group's resting isometric force was "corrected" by dividing isometric force by the dry weight of the muscles. Resting tension (corrected for the muscle dry weight) was significantly more affected ($p < 0.05$, $n = 6$) by the $0Na^+$ intervention in the YR group (15.1 ± 2.3 mN/g) as compared with the OR group (7.4 ± 1.9 mN/g).

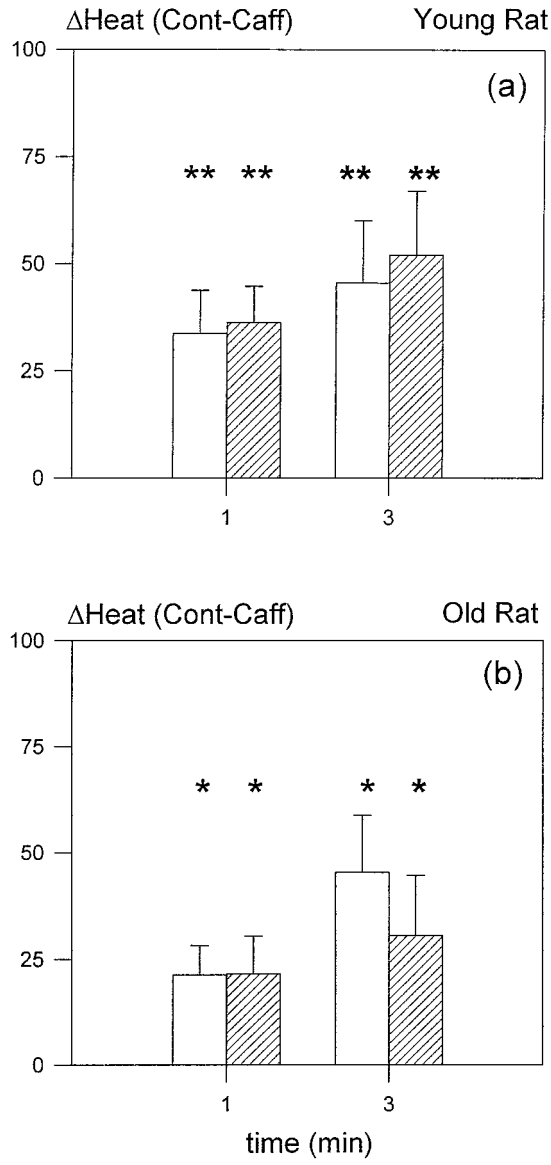

FIGURE 6 Effects of caffeine (10 mM) on the heat production induced by changes in extracellular sodium concentration from (*a*) young and (*b*) old rat heart muscles ($n = 7$). ΔH_1: Difference between the heat value (mW/g) reached after 1 or 3 min of 0Ca0Na perfusion in the absence (Cont) and in the presence of caffeine (Caff). ΔH_2: Difference between the peak value of heat production (mW/g) reached under 0Ca perfusion after the 0Ca0Na intervention (either 1 or 3 min) in the absence (Cont) and in the presence of caffeine (Caff). For both panels, vertical bars represent ± 1 SE. ($^*p < 0.05$; $^{**}p < 0.01$).

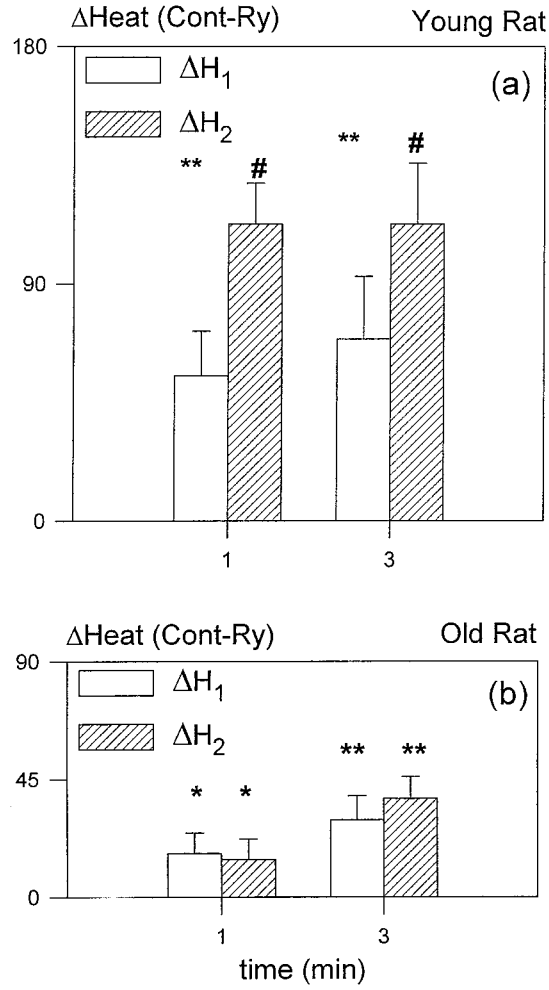

FIGURE 7 Effects of ryanodine (0.2 μM) on the heat production induced by changes in extracellular sodium concentration from *(a)* young and (*b*) old rat heart muscles. ΔH_1: Difference between the heat value (mW/g) reached after 1 or 3 min of 0Ca0Na perfusion in the absence (Cont) and in the presence of ryanodine (Ry). ΔH_2: Difference between the peak value of heat production (mW/g) reached under 0Ca perfusion after the 0Ca0Na intervention (either 1 or 3 min) in the absence (Cont) and in the presence of ryanodine (Ry). For both panels, vertical bars represent ± 1 SE. ($^*p < 0.05$; $^{**}p < 0.01$; $^{\#}p < 0.005$)

DISCUSSION

It has been shown that, in both adult and neonatal heart cells, there is a kinetically described Ca^{2+} compartment that is sensitive to the operation of the Na^+–Ca^{2+} exchanger (Langer and Rich, 1992; Langer et al., 1995). Those studies have shown that, after a period of 0Na and 0Ca perfusion, Na^+ readmission to the perfusate releases Ca^{2+} from this compartment to the extracellular space (Langer and Rich, 1992). Under resting conditions, one might expect that the removal of Na^+ from the extracellular medium would decrease the energy release. In fact, under quiescent conditions, a decrease in extracellular Na^+ by 50 mM (from 145 to 95 mM) induces a decrease in resting heat, which was demonstrated to be mainly related to a decrease in the activity of Na^+–K pump (Ponce-Hornos et al., 1987). In contrast, Na^+ readmission would remove Ca^{2+} from the above-mentioned compartment at the expense of Na^+ entry (which would have to be finally removed by the Na^+–K pump) and an increase in energy release would be expected (Ponce-Hornos, 1990).

Figures 1–3 clearly show that Na^+ removal (from 145 to 0 mM) induces an increase rather than a decrease in the energy released under resting conditions. This apparently paradoxical result can be explained if the presence of Na^+

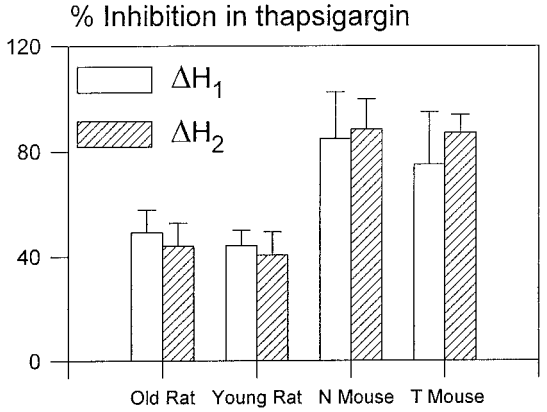

FIGURE 8 Effect of thapsigargin (TPG 1 μM) on heat production induced by changes in extracellular sodium concentration in all four-heart muscles studied. ΔH_1: Percent inhibition induced by thapsigargin in heat production after 6 min of 0Ca0Na perfusion, ΔH_2: Percent inhibition induced by thapsigargin in the peak value of heat production reached after Na^+ readmission. Vertical bars represent ± 1 SE.

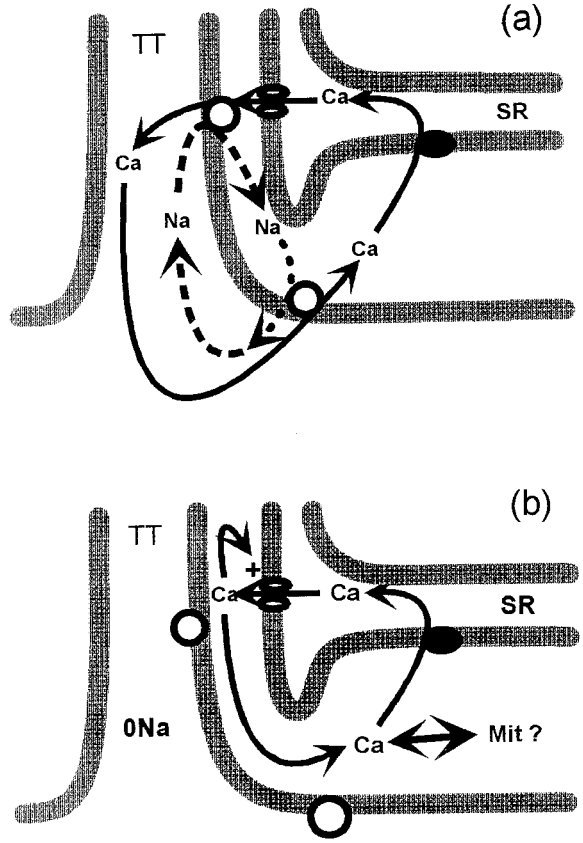

FIGURE 9 Schematic showing the calcium cycles involved in the presence and in the absence of extracellular Na^+. (TT) T tubules, (SR) sarcoplasmic reticulum, (Mit) mitochondria. (*a*) In the presence of extracellular Na^+, the Na^+–Ca^{2+} exchanger removes the Ca^{2+} leak from the SR. The fact that the Na^+ depletion–repletion can be repeated several times with the same heat response indicates that the calcium removed by the exchanger re-enters the cell and is recaptured by the SR-Ca^{2+} pump. (*b*) In the absence of extracellular Na^+, the SR Ca^{2+} leak at the cleft would not be able to leave the cell and would cycle via the cytoplasm through the SR-Ca^{2+} pump. Because the Ca^{2+} leaked at the cleft is not removed, Ca^{2+} concentration would increase, inducing a further release of Ca^{2+} by the SR. This would increase the amount of Ca^{2+} to be cycled and the energy released. As shown in the figure, the presence of the mitochondria as part of the cycle cannot be discarded (see the text for further explanation).

in the extracellular fluid is required to maintain a low concentration of Ca^{2+} at the zone where the sarcolemma is in close apposition to the junctional sarcoplasmic reticulum (JSR), the cleft space (Langer and Peskoff, 1996, 1997). This space between the JSR and the T tubule is the region in which Ca^{2+} release must occur. Therefore, it is possible that the absence of Na^+ in the extracellular media prevents Ca^{2+} removal from this zone with the result that more Ca^{2+} would be released from the JSR, resembling an excitation type of process (see Fig. 9). This Ca^{2+} accumulation would lead to an increase in the SR-Ca^{2+} pump activity, with resultant increased energy expenditure. The increase in energy expenditure could be masking a possible decrease in the energy release by the Na^+–K pump, which is seen when Na^+ reduction is not enough to alter Ca^{2+} efflux from the tissue (Ponce-Hornos, 1990). Such a sequence would indicate a central role for the Na^+–Ca^{2+} exchanger in resting metabolism. This hypothesis is supported by the fact that, in the YR group, in which the role of the sarcolemma (and hence of the Na^+–Ca^{2+} exchanger) is more marked than in the OR group, the Na^+ removal/readmission effect is greater. Furthermore, in the TM and NM experiments (Fig. 3) the greater effect was found in the TM group (with a sarcolemma enriched with Na^+–Ca^{2+} exchanger protein) as compared to the NM group.

In the presence of nifedipine (non-beating cells), Na^+–Ca^{2+} exchange-mediated flux derived from the SR is reduced but remains about 35% of control level (Wang et al., 1997). Thus, no matter what the feedback process in the ryanodine receptor, at least within the periods studied, there is a continuous resting state leak. In the present work, we measure the energetic consequences of this activation. The resting Na^+–Ca^{2+} exchange-mediated flux remains significant and the energetic measurements directly support this.

The EGTA experiments showed that, in the YR group, the cell would recapture the Ca^{2+} removed by the exchanger, indicating that, in the presence of Na^+, an intracellular-extracellular cycle for Ca^{2+} would be established (see Fig. 9). This is based on the fact that, if Ca^{2+} is sequestered by the presence of EGTA, the cell would empty of Ca^{2+} and the cycle triggered by the Na^+ removal intervention would be diminished. In contrast, in the OR group, in which the intracellular sequestering system has a higher participation than in the YR group, the Ca^{2+} removed by the exchanger under resting conditions is small with respect to the total cell calcium (Fabiato, 1985). Consequently, in the OR group, extracellular Ca^{2+} sequestration by EGTA does not affect the heat release due to the 0Na intervention and repletion (see Fig. 9). This also agrees with the mechanical results observed in the absence of BDM, in which resting tension is more affected in the YR than in the OR group.

As previously mentioned, the fact that verapamil diminished the effect of both Na^+ removal and repletion could be an indication that the L type Ca^{2+} channel is a part of the path for maintenance of steady-state intracellular Ca^{2+} content. In contrast, it should be pointed out that the action of verapamil on the Ca^{2+} channel requires previous activation of the channel (i.e., cell excitation), which is not present under the present experimental conditions. Therefore, the effect seen by the verapamil intervention could also be associated with a locus other than that of the channel. In this regard, it has been reported that a fraction of heat released during a contraction is verapamil sensitive and is not ascribable to its action on the channel (Consolini et al., 1997). In addition, it has been found that, under resting conditions, verapamil partially inhibits the posattium-induced increase in resting metabolism (Holroyd et al., 1990; Márquez et al. 1996). Furthermore, the blockade induced by verapamil in the YR preparation indicates that the role of this verapamil-sensitive compartment is more prominent in the YR than in the OR preparation.

The relatively small effect of caffeine and ryanodine might be related to the fact that both drugs release Ca^{2+} from the SR (Coronado et al., 1994) without affecting the SR-Ca^{2+} pump. Consequently, although the SR was partially emptied by the presence of either caffeine or ryanodine, it was possible that at least part of the Ca^{2+} released was cycling through the cytoplasm. In the presence of extracellular Na^+, the Ca^{2+} that exits the cells via the exchanger is restored to the cell keeping this circuit active (see Fig. 9 *A*). In this connection, it has been demonstrated that caffeine-induced release of Ca^{2+} is removed via the exchanger (Callewaert et al., 1989). In addition, in arrested guinea pig hearts, caffeine evokes an increase in oxygen consumption accompanied by an increase in diastolic pressure if extracellular Na^+ is reduced (Hanley et al., 1994). Therefore, when Na^+ is removed, the long circuit is interrupted and the short circuit is triggered (see Fig. 9 *B*), although it is somewhat reduced due to the decreased SR Ca^{2+} content induced by caffeine or ryanodine. The quantitative difference between YR and OR groups agrees with previous results (Post et al., 1993; Langer et al., 1995) that showed that the size of the Na^+-dependent Ca^{2+} exchange compartment is dependent on whether the Na^+ removal/readmission intervention was applied to neonatal or adult heart cells. For the neonatal cells, this Ca^{2+} compartment (about 760 μmol Ca^{2+}/kg dry cells) is 2.2 times higher than for the adult cells (350 μmol Ca^{2+}/kg dry cells). As shown in Fig. 2, the power released by the readmission of Na^+ ($H_2 - H_1$) was about 2.5 times higher in the YR group as compared with the OR group.

The thapsigargin experiments (Fig. 8) give further support to an active role of the SR in this process. The decrease in the heat released associated with the Na^+ removal–repletion intervention induced by thapsigargin clearly indicates that the SR-Ca^{2+} pump must be active for the maintenance of both Ca^{2+} cycles in all four-heart preparations. If the SR-Ca^{2+} pump is impaired by thapsigargin (Langer et al., 1995) cytosolic Ca^{2+} is accumulated in another compartment and the cycle halted.

In a simple scheme, the maximal values measured for H_1 should be related to the heat released by ATP hydrolysis (and its oxidative recovery) due to Ca^{2+} removal, and therefore, it should be representative of the cytosolic Ca^{2+} cycling. Using 80 kJ/mol ATP hydrolyzed and a Ca^{2+}/ATP ratio of 1.5, the expected Ca^{2+} flux via the SR-Ca^{2+} pump, for the highest H_1 value measured (YR group), would be about 4.7 μmol/g·s (Carafoli, 1985; Ponce-Hornos, 1990). Such a Ca^{2+} flux implies a Ca^{2+} concentration between 1 and 10 μM Ca^{2+} and, because the mitochondria has a Km close to 10 μM, it should be contributing in some extent to the heat release during the 0Na intervention. Also, such a Ca^{2+} concentration will agree with the increase in resting force induced by the removal of Na^+.

In summary, the results presented in this work are consistent with the existence of two Ca^{2+} cycles whose operation, under resting conditions, depend on the presence of Na^+ in the extracellular media. The presence of extracellular Na^+ maintains a low Ca^{2+} concentration in the diadic cleft by removing the Ca^{2+} via the Na^+–Ca^{2+} exchanger. This Ca^{2+} reenters the cell, maintaining a steady-state intracellular–extracellular Ca^{2+} cycle. If Na^+ is removed from the perfusate, Ca^{2+} accumulates in the cleft zone inducing a further release of Ca^{2+} from the SR that cycles intracellularly through the SR and increases energy release. This increase in energy expenditure can be prevented if SR Ca^{2+} load is prevented (thapsigargin experiments) or if SR-Ca^{2+} can be emptied (EGTA in the YR group). The readmission of Na^+ to the perfusate removes the accumulated Ca^{2+} at the JSR zone by exchanging Ca^{2+} with Na^+ so that a transitory increase in heat production is seen due to activation of the Na^+–K pump induced by the increased Na^+ influx. Therefore, the Na^+–Ca^{2+} exchanger seems to be acting as an energy-saver mechanism in avoiding, during the resting state, the accumulation of Ca^{2+} at the cleft zone and preventing the possibility of further Ca^{2+} release.

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