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Effect of temperature on sodium-calcium exchange in sarcolemma from mammalian and amphibian hearts

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We have investigated temperature dependence of Ca^{2+} uptake by the cardiac sarcolemmal Na^+–Ca^{2+} exchanger from dog, rabbit and bullfrog. In native rabbit sarcolemmal vesicles, Ca^{2+} affinity of the Na^+–Ca^{2+} exchanger is unchanged from 7 to 37°C; however, the initial velocity of Ca^{2+} uptake declines much more steeply below 22°C than above 22°C. In native dog sarcolemma, the temperature dependence of Na^+–Ca^{2+} exchange velocity is similar to that of native rabbit. However, in frog heart the velocity of Na^+–Ca^{2+} exchange declines much more slowly with decreasing temperature at both temperature ranges. Reconstitution of the Na^+–Ca^{2+} exchanger into artificial lipid vesicles consisting of either asolectin or phosphatidylserine, phosphatidylcholine, and cholesterol has little effect on temperature dependence of Na^+–Ca^{2+} exchange velocity in any of the three species. We conclude that the lesser temperature sensitivity of the cardiac sarcolemmal Na^+–Ca^{2+} exchanger of a poikilothermic species is at least partly an intrinsic property of the transport protein.

Introduction

The cardiac sarcolemmal Na^+–Ca^{2+} exchanger has been extensively characterized by experiments using purified sarcolemmal vesicles in recent years [1,2]. However, most experiments have been done at either 37°C or at room temperature, and there has been little systematic investigation of the temperature sensitivity of this transport process. An earlier report [3] presented limited data on Na^+–Ca^{2+} exchange velocity at temperatures from 10° to 40°C, with measurements at the higher temperatures probably not reflecting initial rates. Also, most investigators have used mammalian hearts as their source for sarcolemma; previous investigation of Na^+–Ca^{2+} exchange in sarcolemmal vesicles from animals with physiologic temperatures below 37°C is limited.

In this study, we asked three questions: (1) How do the properties of cardiac sarcolemmal Na^+–Ca^{2+} exchange vary with temperature? (2) Is the temperature dependence different for non-mammalian species that normally have lower body temperatures? Is temperature dependence of Na^+–Ca exchange a property of the exchange protein itself or of its membrane lipid environment? We have attempted to answer these questions by measuring the initial velocity of Na^+–Ca^{2+} exchange in purified sarcolemmal vesicles from dog, rabbit, and frog hearts over a wide range of temperatures. We then repeated the same experiments with the Na^+–Ca^{2+} exchanger from each species reconstituted into artificial lipid membrane vesicles.

Materials and Methods

Deionized water with resistance of at least 17 Mohm $\cdot cm^{-1}$ was used for all solutions; contaminant Ca^{2+} was 2–3 μM. Phosphatidylcholine and phosphatidylserine were obtained from Avanti Polar Lipids. Asolectin was obtained from Associated Concentrates. Bio-Beads SM-2 were purchased from Bio-Rad. Amberlite XAD-2 beads and other biochemicals were obtained from Sigma Chemicals. $^{45}Ca^{2+}$ was obtained from Amersham.

Abbreviations: chol, cholesterol; EGTA, ethylene-bis(β-aminoethylether)-*N,N,N',N'*-tetraacetic acid; Mops, 3-(*N*-morpholino)propanesulfonic acid; *p*NPPase, *p*-nitrophenylphosphatase; PC, phosphatidylcholine; PS, phosphatidylserine.

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Dog cardiac sarcolemmal vesicles were purified as described [4] with minor modifications [5]. For the reported experiments, purification of the sarcolemmal marker K^+-*p*-nitrophenylphosphatase (K^+-*p*NPPase) was 59 ± 9-fold, with sarcolemmal activity being 31 ± 5 $\mu mol \cdot mg^{-1} \cdot h^{-1}$. Rabbit cardiac sarcolemma was purified as described [6] with minor variations: deoxyribonuclease treatment was with 2000 U/g heart; the final sucrose density gradient contained membranes suspended in 45% sucrose layered under 27% and 11% sucrose, and all layers included 0.2 mM NaCl; vesicles were harvested from the 11–27% sucrose interface after 2 h centrifugation at $204000 \times g$ max. For these experiments, the K^+-*p*NPPase purification was 25 ± 2-fold with sarcolemmal activity being 12 ± 1 $\mu mol \cdot mg^{-1} \cdot h^{-1}$. Frog hearts were obtained from bull frogs; eight hearts were pooled for each sarcolemmal purification. Frog heart sarcolemma was purified using the same method as for dog heart but scaled down to smaller volumes (about 6 g rather than 60 g myocardium). Also, in the final sucrose density gradient, the frog heart sarcolemma was spread over a wider range of sucrose densities; fractions from 11 to 29% sucrose were pooled. For frog hearts, purification of K^+-pNPPase was 40 ± 4-fold, with sarcolemmal activity being 32 ± 8 $\mu mol \cdot mg^{-1} \cdot h^{-1}$. Sarcolemmal vesicles of all species were diluted into 140 mM NaCl, 10 mM Mops (pH 7.4 at 37°C), sedimented, and resuspended in the same solution at 2–5 mg/ml. Vesicles were then stored in liquid nitrogen; transport properties were unchanged even after several months.

Na^+–Ca^{2+} exchange was measured in sarcolemmal vesicles as Na_i^+-dependent $^{45}Ca^{2+}$ uptake using a rapid quenching device to allow initial velocity measurements [6]. Sarcolemma previously stored in 140 mM NaCl, 10 mM Mops (pH 7.4) was incubated in the same solution at 37°C for at least 10 min to ensure complete loading with Na^+ prior to cooling to the temperature used for the subsequent Ca^{2+} uptake measurement. After equilibration at the new temperature, 5 μl of sarcolemma containing 7–10 μg protein were diluted into 245 μl uptake medium containing 140 mM KCl, $^{45}Ca^{2+}$ 0.1–0.2 μCi, variable Ca^{2+} as $CaCl_2$, valinomycin 0.4 μM, and 10 mM Mops (pH 7.4) adjusted at the same temperature at which Na^+–Ca^{2+} exchange was to be measured. For native vesicles, the Ca^{2+} uptake was stopped by the rapid addition of 30 μl of 1 mM $LaCl_3$ in 140 mM KCl via a solenoid driven pipettor. The vesicles were then harvested by filtration through 0.45 μm Millipore filters and washed twice with 3 ml of 140 mM KCl/0.1 mM $LaCl_3$. For reconstituted vesicles, Na^+–Ca^{2+} exchange was stopped by the rapid addition of 30 μl of 10 mM EGTA in 140 mM KCl followed by the addition of 1 ml of 1 mM EGTA in 140 mM Kcl at 4°C. The reconstituted vesicles were harvested on 0.20 μm filters and washed twice with 3 ml of 140 mM KCl/1 mM EGTA at 4°C. For all experiments, passive uptake of Ca^{2+} independent of the Na^+ gradient was measured by substituting 140 mM NaCl for 140 mM KCl in the uptake medium. The passive Ca^{2+} uptake has been subtracted for all the results presented below.

In some experiments, sarcolemmal vesicles were solubilized and reconstituted into artificial lipid membrane vesicles consisting of either asolectin or phosphatidylserine (PS), phosphatidylcholine (PC), and cholesterol in a ratio of 5 : 3 : 2 by weight. Vesicles were first solubilized by mixing 1 vol. sarcolemma (1–3 mg protein/ml) with 4 vol. 500 mM NaCl 20 mM Mops (pH 7.4 at 22°C), 1.875% Triton X-100, and 10 mg/ml asolectin or PS-PC-cholesterol, with a final exogenous lipid-to-protein ratio of at least 13 : 1. Insoluble material was removed by centrifugation for 20 min at $178000 \times g$ (max) at room temperature in a Beckman Airfuge. The solubilized protein and lipid were reconstituted into sealed vesicles by incubation with shaking for 10–20 min twice with either Bio-Beads SM-2 or Amberlite XAD-2 beads to adsorb detergent. The beads had been prewashed extensively with methanol, then water, and finally 500 mM NaCl. Beads were removed by centrifugation at $2500 \times g$ (max) for 5 min. Reconstituted vesicles were pelleted at $178000 \times g$ (max) in the Airfuge for 30 min or at $140000 \times g$ (max) for 90 min in a Beckman 42.1 rotor, and pellets were resuspended in the usual 140 mM NaCl/10 mM Mops (pH 7.4) at 37°C prior to measurement of recovered protein and Na^+–Ca^{2+} exchange.

Protein was measured by the Lowry method [7] or modified Lowry method for reconstituted vesicles [8] with subtraction of appropriate blanks.

All results presented below are expressed as mean ± S.E. of at least three experiments, with duplicate measurements within each experiment. Comparisons between groups were made by analysis of variance; $P < 0.05$ was considered statistically significant.

Results

All measurements of Na^+–Ca^{2+} exchange velocity were made at a time sufficiently brief to ensure initial velocity conditions. For the rabbit heart experiments, the reaction time was progressively lengthened from 1 s at 37°C to 2 s at 27°C, 4 s at 17°C and 8 s at 7°C to allow accumulation of more $^{45}Ca^{2+}$ while still maintaining initial velocity conditions at each temperature. At all temperatures, decreasing the reaction time to 75% of these durations did not change the measured velocity. The dog and frog heart measurements were made at 1.5 s for all temperatures. At 37°C, a 1.5 s reaction time is sufficient to measure initial velocity of Na^+–Ca^{2+} exchange in dog sarcolemma [12]. For frog sarcolemma, the same velocity was measured at 1.0 and 1.5 s (3.2 ± 0.18 $nmol \cdot mg^{-1} \cdot s^{-1}$, $n = 3$), again indicating that a

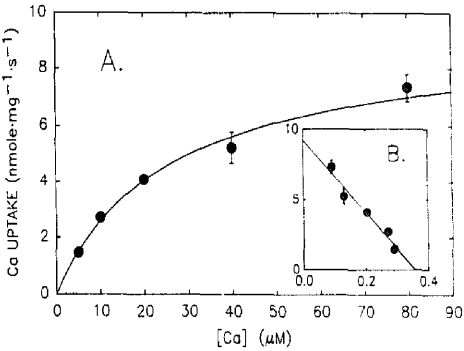

Fig. 1. Rabbit sarcolemmal Na^+-Ca^{2+} exchange velocity vs. $[Ca^{2+}]$. Panel A shows the initial velocity of Na_i^+-dependent Ca^{2+} uptake as a function of $[Ca^{2+}]_0$ at 37°C (means ± S.E.; where no error bars are visible, they fall within the plotting symbols). Panel B is an Eadie-Hofstee plot of the same data along with a linear regression ($r = 0.98$, $V_{max} = 9.3$ nmol·mg^{-1}·s^{-1}, $K_m = 25.8$ μM). Constants obtained from the linear regression are used to plot the solid line in panel A.

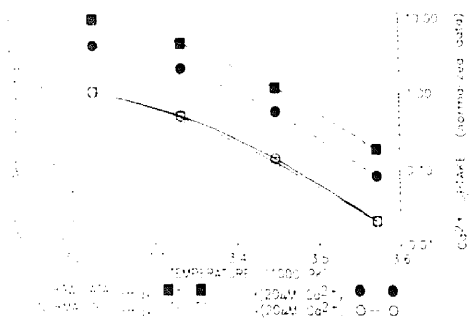

Fig. 2. Arrhenius plots of rabbit sarcolemmal Na^+-Ca^{2+} exchange velocity. Results shown in filled symbols are the V_{max} calculated from Eadie-Hofstee plots of mean data at 5–80 μM Ca^{2+} (squares) and the mean velocity at 20 μM Ca^{2+} (circles) for temperatures 7, 17, 27 and 37°C. Open symbols show the same data normalized for the values measured at 37°C. Standard errors are not shown but would fit within the plotting symbols; coefficient of variation averaged 10%, $n = 3$.

1.5 s reaction time is short enough to measure initial velocity.

With the rabbit sarcolemma, we initially measured Na^+-Ca^{2+} exchange velocity at Ca^{2+} concentrations ranging from 5 to 80 μM to determine K_m and V_{max} at temperatures of 7, 17, 27, and 37°C. Data for 37°C are shown in Fig. 1; Na_i^+-dependent Ca^{2+} uptake velocity is plotted as a function of $[Ca^{2+}]$ and replotted as an Eadie-Hofstee plot. The latter demonstrates a good fit to Michaelis-Menten kinetics. Plots for lower temperatures were similar in shape, with decreased velocities at all $[Ca^{2+}]$. Table I shows calculated V_{max} for Ca^{2+} uptake and K_m for Ca^{2+} at all four temperatures; K_m was almost constant between 7 and 37°C, ranging from 22 to 26 μM, and there was an excellent correlation coefficient for all the Eadie-Hofstee plots. Fig. 2 shows Arrhenius plots of both V_{max} and uptake velocity at 20 μM Ca^{2+}; they are parallel, and both suggest a change in slope between 17 and 27°C. After normalizing for velocity measured at 37°C, the two curves are essentially superimposable. Therefore, the remainder of the experiments were done at a single $[Ca^{2+}]$ for each species: 20 μM for rabbit and 10 μM for dog and frog.

TABLE I

Kinetic parameters for Na^+-Ca^{2+} exchange in rabbit heart sarcolemma

Temp. (°C)	K_m (μM Ca^{2+})	V_{max} (nmol·mg^{-1}·s^{-1})	R^a
7	25.6	0.19	0.996
17	22.7	1.17	0.987
27	22.4	4.5	0.979
37	25.8	9.26	0.977

[a] Correlation coefficient from Eadie-Hofstee plot of mean Na^+-Ca^{2+} exchange velocities at 5 to 80 μM Ca^{2+} ($n = 3$).

A more detailed examination of the effect of temperature on Na^+-Ca^{2+} exchange velocity is shown in Fig. 3 for both native rabbit heart sarcolemma and for rabbit heart sarcolemma reconstituted into asolectin vesicles: Na^+-Ca^{2+} exchange velocity is higher in the reconstituted vesicles than in the native vesicles because of the partial purification of the exchange protein during reconstitution and the stimulation of Na^+-Ca^{2+} exchange by asolectin [12]. Therefore, velocities were normalized to the velocity at 37° before plotting. For the native vesicles, the Arrhenius plot shows a sharp break in slope at 22°C with linear portions above and below 22°C. The slope from 7 to 22°C is approximately twice as steep as that from 22 to 37°C; calculated activation energies for the two temperature ranges are shown in Table II. For native vesicles, Na^+-Ca^{2+}

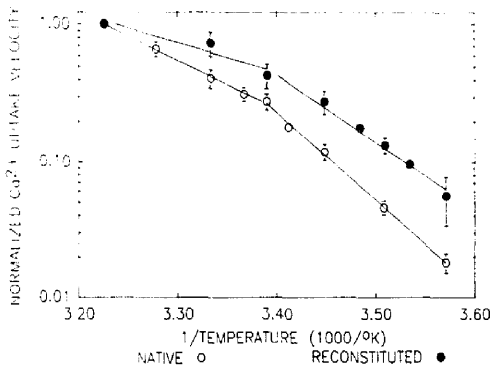

Fig. 3. Arrhenius plot of Na^+-Ca^{2+} exchange velocity at 20 μM Ca^{2+} in native (○) rabbit sarcolemmal vesicles and after reconstitution (●) into asolectin vesicles. All data are normalized for the uptake velocities at 37°C: 6.6 ± 0.8 nmol·mg^{-1}·s^{-1} for native and 15.6 ± 1.3 nmol·mg^{-1}·s^{-1} for the reconstituted Na^+-Ca^{2+} exchanger.

exchange velocity declines 2.4-fold between 37 and 27°C, while between 22 and 12°C the velocity declines 6.1-fold. The data for the reconstituted Na^+–Ca^{2+} exchanger suggest a similar breakpoint although the slopes tended to be slightly less at both temperature ranges compared to the native vesicles. The difference in slopes as a result of reconstitution was not statistically significant ($P = 0.1$, see Table II).

The velocity of Na^+–Ca^{2+} exchange measured at 10 μM Ca_0^{2+} in native dog and frog sarcolemma at temperatures from 10 to 37°C and normalized to the 37°C results is shown in Fig. 4. Calculated activation energies for temperature ranges 10–22°C and 22–37°C are shown in Table II. The results for the dog heart sarcolemma are quite similar to those for the rabbit in Fig. 3; there appears to be a breakpoint around 22°C and the activation energies shown in Table II are similar to those for rabbit. In contrast, the decline in velocity with temperature for frog heart sarcolemma is much less both above and below 22°C, where there still appears to be a change in slope. For example, compared to maximal Na^+–Ca^{2+} exchange activity at 37°C, frog heart sarcolemma maintains 60% activity at 22°C and 24% at 10°C compared to 31% and 5% for dog sarcolemma at the same temperatures. Fig. 4 also shows Arrhenius plots of Na^+–Ca^{2+} exchange velocity after reconstitution the dog and frog Na^+–Ca^{2+} exchangers into asolectin vesicles and into PS, PC, cholesterol vesicles. Again velocities for both species in each type of vesicle are normalized for their respective values at 37°C. There are no differences in the slopes of the Arrhenius plots after reconstitution into either type of liposome, and the striking difference between dog and frog remains. The results are virtually identical with reconstitution into either asolectin or the PS, PC, cholesterol mixture. Thus the relative insensitivity of frog heart sarcolemmal Na^+–Ca^{2+} exchange to inhibition by decreasing temperature compared with dog heart persists when the Na^+–Ca^{2+} exchangers from both species are reconstituted into the same artificial lipid environment.

TABLE II

Activation energies for Na^+–Ca^{2+} exchange

Activation energies are calculated by linear regression of the indicated range of the Arrhenius plots in Figs. 3 and 4. $r > 0.95$ in all cases. The number of experiments is shown in parentheses for each preparation. Values for higher temperatures are different from values for lower temperatures in all conditions. Values for native and reconstituted vesicles are not significantly different in any species ($P = 0.1$ for rabbit native vs. asolectin). Activation energies for frog preparations are significantly less than corresponding values for dog and rabbit, which did not differ from each other.

Preparation (n)	Activation energy (kcal/mol)	
	Above 22°C	Below 22°C
Rabbit, native (4)	16	30
Rabbit, asolectin (5)	10	23
Dog, native (7)	14	25
Dog, asolectin (4)	14	21
Dog, PC-PS-chol (4)	11	27
Frog, native (8)	6	13
Frog, asolectin (4)	5	12
Frog, PC-PS-chol (4)	5	11

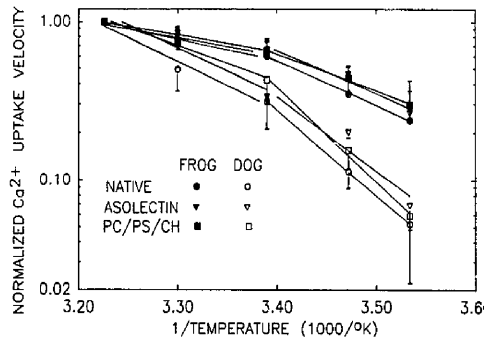

Fig. 4. Arrhenius plots of dog and frog Na^+–Ca^{2+} exchange velocity measured at 10 μM Ca^{2+}. Results are shown for native sarcolemma, after reconstitution into asolectin, and after reconstitution into PC, PS, and cholesterol (PC/PS/CH). Data are normalized for the 37°C value for each condition – native vesicles: 10.1 ± 1.1 nmol·mg^{-1}·s^{-1} for dog and 5.7 ± 0.4 nmol·mg^{-1}·s^{-1} for frog; asolectin vesicles: 24.1 ± 0.3 nmol·mg^{-1}·s^{-1} for dog and 13.9 ± 1.0 nmol·mg^{-1}·s^{-1} for frog; PS-PE-chol vesicles: $19.6 + 2.5$ nmol·mg^{-1}·s^{-1} for dog and 14.2 ± 0.2 nmol·mg^{-1}·s^{-1} for frog. Means ± S.E. are plotted. Lines are plotted from linear regressions of data from 10 to 22°C and from 22 to 37°C.

Discussion

We have investigated temperature dependence of Na^+–Ca^{2+} exchange in native cardiac sarcolemmal vesicles from dogs, rabbits, and frogs and in reconstituted lipid vesicles. In the rabbit preparation we demonstrated that K_m for Ca^{2+} of the Na^+–Ca^{2+} exchanger does not change between 7 and 37°C. In the rabbit sarcolemma, Na^+–Ca^{2+} exchange activity declined about 2-fold/10 C° between 37 and 27°C and 6-fold between 17 and 7°C, with an apparent break in the Arrhenius plot at 22°C. The V_{max} for Na^+–Ca^{2+} exchange and the velocity of Na^+–Ca^{2+} exchange at 20 μM Ca^{2+} declined in parallel with decreasing temperature. Data for temperature dependence of Na^+–Ca^{2+} exchange velocity measured at a single $[Ca^{2+}]$ for the dog sarcolemma were similar to the rabbit data.

To find out whether temperature dependence of Na^+–Ca^{2+} exchange is different in a poikilothermic species, we measured Na^+–Ca^{2+} exchange velocity in bullfrog heart sarcolemma. As the temperature was decreased, the frog Na^+–Ca^{2+} exchanger maintained a much higher percentage of its maximal velocity than the mammalian preparations. Stability of Na^+–Ca^{2+} exchange at lower temperatures in the bullfrog heart seems appropriate to a poikilotherm that often lives at temperatures below 20°C. A similar contrast in the shape of

Arrhenius plots has been shown for succinate oxidation by mitochondria from rat and trout livers [9]; and mammalian mitochondria from a hibernating animal exhibited an Arrhenius plot characteristic of poikilotherms [10]. Correlation of the temperature optimum for skeletal muscle myosin ATPase with preferred body temperature has also been shown within one family of poikilotherms [11].

To find out whether the temperature sensitivity of the Na^+-Ca^{2+} exchanger is an intrinsic property of the Na^+-Ca^{2+} exchange protein or whether it is determined by the membrane lipid environment, we reconstituted the Na^+-Ca^{2+} exchangers from dog, rabbit and frog hearts into asolectin, a soybean phospholipid mixture that gives maximal or near maximal activity to the Na^+-Ca^{2+} exchanger [12]. Arrhenius plots for Na^+-Ca^{2+} exchange velocity were unaffected by reconstitution into asolectin in all three species. The same result was obtained for dog and frog preparations constituted into a PS, PC, cholesterol mixture that also allows high Na^+-Ca^{2+} exchange activity. Thus the interspecies differences in temperature dependence of Na^+-Ca^{2+} exchange appear to depend on differences in the exchange protein itself.

Alteration of membrane lipid environment is another mechanism that may regulate temperature sensitivity of membrane transport processes. For instance, within a single poikilothermic species, goldfish, adaptation to different temperatures has been correlated with changes in the temperature dependence of viscosity in synaptosomal membranes [13]. Also, synaptosomal membranes from rat brains exhibited higher viscosity at low temperatures than membranes from goldfish adapted to 25°C, the highest temperature tested [13]. However, using a reconstitution procedure to investigate the role of membrane lipids in the temperature dependence of Na^+-Ca^{2+} exchange, we conclude that there are differences in temperature sensitivity intrinsic to the Na^+-Ca^{2+} exchange protein.

Na^+-Ca^{2+} exchange activity is present in the plasma membranes of many different mammalian organs [1]. Schellenberg and Swanson have measured Na^+-Ca^{2+} exchange velocity as a function of temperature in native plasmalemma from rat brain [14] and in asolectin reconstituted vesicles [15]. Their data for Na^+-dependent Ca^{2+} uptake measured after 10 s may not accurately reflect initial velocity at higher temperatures, but the temperature dependence of Na^+-Ca^{2+} exchange is very similar for the native and reconstituted preparations. Also, like the cardiac Na^+-Ca^{2+} exchanger, velocity declines more rapidly below 21°C than above.

We have presented calculated activation energies primarily to describe the Arrhenius plots. The activation energy for a process as complex as Na^+-Ca^{2+} exchange is difficult to interpret, particularly since specific reaction steps are unknown. However, the much steeper dependence of velocity upon temperature below 22°C in all the species suggests that the reaction sequence might be altered at lower temperatures. The much smaller activation energy for the frog Na^+-Ca^{2+} exchanger also might reflect a fundamental mechanistic difference from the mammalian Na^+-Ca^{2+} exchanger.

There is little practical consequence of the temperature sensitivity of the cardiac sarcolemmal Na^+-Ca^{2+} exchanger for homeotherms at their usual body temperatures of 37–38°C. However, under artificial laboratory situations such as cold-induced contractures of the heart [16,17] or under the clinical situation of open heart surgery with cold cardioplegia, temperatures at or substantially below 22°C may be encountered. Under such a situation, Na^+-Ca^{2+} exchange would be severely inhibited. If Na^+-Ca^{2+} exchange is normally more important for sarcolemmal Ca^{2+} efflux than for influx, inhibition of Na^+-Ca^{2+} exchange at low temperatures may be part of the mechanism for cold-induced contracture of the heart.

Acknowledgements

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