Effect of Temperature on the Rise in Intracellular Sodium Caused by Calcium Depletion in Ferret Ventricular Muscle and the Mechanism of the Alleviation of the Calcium Paradox by Hypothermia

M.S. Suleiman and R.A. Chapman

The effects of temperature over the range 37–10°C on the responses of isolated ferret ventricular trabeculae to the depletion and repletion of bathing calcium has been investigated. Cooling is found to reduce the rate of rise of intracellular sodium activity (measured with an ion-sensitive microelectrode) induced by depletion of bathing divalent cations, without affecting the prolonged depolarization. The rate of rise of [Na], (with the sodium pump inhibited) shows a dependence on temperature (energy of activation, 67 kJ · mol⁻¹; Q₁₀, 2.3–2.8) that increases with increasing temperature. This contrasts with previously published data for the sodium pump for which the temperature dependence falls with increasing temperature. These results offer an explanation for the alleviation of the calcium paradox caused by cooling during the period of calcium depletion, which is similar to other procedures that offer protection by limiting the rise in [Na], by having effects on the L-type calcium channel. (Circulation Research 1990;67:1238–1246)

Hypothermia is widely used in conjunction with cardioplegia to minimize tissue damage during cardiac surgery. With this, cooling is known to reduce the cellular damage associated with a number of conditions such as ischemia or subsequent reperfusion and the calcium paradox. In the case of the calcium paradox, cooling is particularly effective when applied during calcium depletion so that on calcium repletion the heart regains normal mechanical and electrical activity, shows little structural damage, and shows little fall in energy-rich phosphates; the release of protein is inhibited. However, the severity of the calcium paradox, as assessed by measuring the release of intracellular enzymes, depends on the time in calcium-free solution, so that as the period at lower temperatures is extended, the protective effect of cooling is decreased.

Work on a variety of tissues has led to a hypothesis to explain the calcium paradox, whereby on calcium depletion, the depolarization of the cells activates the L-type calcium channels, which, because they lose their ionic selectivity and do not inactivate, carry a sustained influx of Na⁺ into the cells. This influx of Na⁺ is not immediately balanced by the sodium pump, so a new elevated [Na], is established. On calcium repletion, the L-type calcium channels regain their selectivity and inactivate as the membrane at least initially repolarizes. These changes imply that calcium loading occurs predominantly via the Na/Ca exchange driven by the elevated [Na]. Calcium loading not only causes hypercontracture but also would seem to be critical in the induction of cell damage, because when intracellular buffering of calcium ions is increased by added EGTA or BAPTA, the loss of excitability and disruption of the cell membrane on calcium repletion in isolated myocytes, although Na, activity rises during calcium repletion, is prevented. The large capacity of the Na/Ca exchange, relative to the limited capacity of other calcium-regulating systems, implies that the Na/Ca exchange will maintain an elevated [Ca], until [Na], is reduced. It is evident that the degree of calcium loading, and thereby the

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extent of damage incurred during calcium repletion, is a race between the effect of [Na], on the Na/Ca exchange and principle means of reducing [Na], namely, the sodium pump.

The upshot is that the rise in [Na], during calcium depletion, which predisposes the heart to the damaging effects of the paradox on calcium repletion, and the recovery of [Na], on calcium repletion both depend on the activity of the sodium pump.\(^9,10\) Cooling is known to reduce the activity of the sodium pump,\(^15,16\) resulting in a hypothermia-induced rise in [Na],. It would be expected, therefore, that hypothermia will aggravate rather than alleviate the damaging effects of the calcium paradox.

The protective effect of hypothermia would therefore seem to be inconsistent with the hypothesis that the rise in [Na], is critical in predisposing the heart to the calcium paradox and its severity. We have therefore measured the effect of calcium-depleted solutions at four temperatures, 37\(^°\), 30\(^°\), 20\(^°\), and 10\(^°\) C, on [Na], levels, membrane potential, and the strength of the calcium-repleted contracture (as an index of the severity of the calcium paradox) in the ventricular trabeculae of ferrets.

**Materials and Methods**

Ferrets were heavily anesthetized by intraperitoneal injection of Sagatal (May & Baker Ltd., UK), and their hearts were removed and placed in oxygenated Tyrode’s solution. Trabeculae, 100–600 \(\mu\)m in diameter, were then isolated from the right ventricle and mounted in a chamber that allowed the rapid exchange of the perfusing solution; the exchange time for 10–90\% was 1.25±0.25 seconds (as measured with an ion-selective microelectrode). Solutions were stored in Mariotte bottles and ran through an internal tube, which was surrounded by another tube (containing circulating water that would be controlled at various temperatures) and an outer insulation. Before the solutions entered the experimental chamber, they passed through a modulatory tap at a flow rate of 7 ml/min either to the chamber or to waste. When solutions of different temperatures were allowed to flow through the external chamber, the time for a change of 10\(^°\) C (30\(^°\) to 20\(^°\) C) was completed in 3.5±0.3 seconds for 10–90\%. Experiments were performed at 37±1\(^°\), 30±1\(^°\), 20±1\(^°\), and 10±1\(^°\) C. Techniques used to monitor membrane potential, intracellular ionic activities, and tension development were as described elsewhere.\(^13\) The sodium-sensitive microelectrodes were made as previously described.\(^13\) Because the sodium resin showed some sensitivity to changes in temperature, electrodes were calibrated at each working temperature. Normal Tyrode’s solution contained the following (mM): NaCl 135, sodium pyruvate 5, MgCl\(_2\) 0.5, CaCl\(_2\) 5, KCl 2.5, KOH 2.5, and HEPES 5 (pH 7.4). Tyrode’s solution used during calcium depletion contained no added MgCl\(_2\). HEPES was chosen because it has a low pH change with temperature.

Solutions in which the activity of the ionic calcium was reduced were made by omitting CaCl\(_2\) and adding a mixture of 4 mM CaEGTA and 4 mM Tris-EGTA in the appropriate ratios. Values of various pCas were calculated using the appropriate binding constant. This was adjusted for the different temperatures and pH 7.4 by using a measured calcium association constant of EGTA of 6.43 M\(^{−1}\) at 20\(^°\) C and pH 7.2, as described by Chapman et al.\(^17\) Acetylstrophanthidin was purchased from Sigma Chemical Ltd., Poole, UK, and used from a stock solution of 50 mM in ethanol.

The effect of calcium repletion has been assessed in a variety of ways. In our experiments, we have used the strength of the contracture induced on calcium repletion to monitor calcium loading. The consequences of calcium repletion may be irreversible, and therefore, it is necessary to compare the responses of different preparations. As already noted, the strength of the contraction of the trabeculae shows a marked variability when expressed simply in terms of force per unit cross-sectional area,\(^13\) a feature possibly associated with the variable myofibrillar contents of these preparations.\(^18\) We have therefore adopted the procedure whereby the contracture data are expressed as a ratio of the previous maximum twitch tension, which reduces the variability.\(^13\) The data are expressed as the mean±SEM, and \(n\) refers to the number of individual experiments. Statistical analysis was carried out using STAT VIEW II ANOVA (Abacus Concepts Inc., Berkeley, Calif.). Multiple comparison tests (Scheffe’s \(F\) test, Fisher PLSD test, and Dunnett’s \(t\) test) were performed at a 95\% level of probability.

**Results**

**Effect of Hypothermia on Membrane Potential, Na, Activity, and Stimulated Twitches**

Consistent with previous reports,\(^19,20\) cooling causes depolarization and a rise in Na, activity in mammalian ventricular muscle (Table 1). At 37\(^°\) and 30\(^°\) C, the mean resting membrane potentials are similar and not statistically different from that at 20\(^°\) C, but at 10\(^°\) C, there is a significant depolarization. The effect of cooling to 10\(^°\) C is, however, more variable, with the lower values of membrane potential usually observed after a gradual decrease in membrane potential followed by a sharp drop (associated with a twitch) to the new resting value. Otherwise, the new membrane potential stabilizes after a few minutes following the change to a lower temperature, which is accompanied, particularly at 10\(^°\) C, by a cooling contracture.\(^19,21\) At 37\(^°\), 30\(^°\), and 20\(^°\) C, the measured values of Na, activity, in Tyrode’s solution in resting muscle, are not significantly different, while at 10\(^°\) C, a significant rise occurred (Table 1).

The effect of cooling on the regularly evoked heartbeats (0.17 Hz) is also variable (Table 1). Cooling to 20\(^°\) from 30\(^°\) C induced an increase in the measured average peak twitch to 114±48% of control, but the variability is large, a feature noted
TABLE 1. Effect of Temperature on Resting Membrane Potential and Na⁺ Activity and Twitch Tension Development, Measured in Normal Tyrode's Solution

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37</td>
</tr>
<tr>
<td>Membrane potential (mV)</td>
<td>85.4±1 (8)</td>
</tr>
<tr>
<td>Na⁺ activity (mM)</td>
<td>9.9±0.7 (8)</td>
</tr>
<tr>
<td>Twitch tension (% of 30°C)</td>
<td>87±8 (6)</td>
</tr>
</tbody>
</table>

Values are mean±SEM, and the number of experiments is shown in parentheses. The difference in resting membrane potentials and twitch tension observed at 10° and 37°, 30°, and 20° C is statistically significant at 95%.

Effect of Hypothermia on Membrane Potential and Na⁺ Activity During Calcium Depletion

As shown in Figure 1, when calcium depletion (pCa 7.4) is performed at 20° C, a depolarization of the membrane to values around -20 mV occurs, which are similar to those observed at 30° C. The membrane depolarizes slowly around -45 mV (usually associated with a series of action potentials), followed by a sustained depolarization that stabilizes at different values, depending on the pCa of the bathing fluid (-31±3, -18±3, -20±2, and -16±3 mV [n=6] for pCa 5.4, 6.4, 7.4, and 8.4, respectively). At pCa 5.4, depolarization beyond -40 mV is sometimes not observed, while on other occasions, particularly at 20° and 10° C, the spontaneous action potentials become progressively prolonged and the overshoot falls until the membrane stabilizes at around -30 mV. When calcium depletion is made at all temperatures, the membrane depolarization is accompanied by a rise in Na⁺ activity. The rate of rise in Na⁺ activity decreases with decreasing temperature, and a clear delay becomes apparent (Figure 1). As a result, the values of Na⁺ activity reached after 10 minutes of calcium depletion fall with decreasing temperature (Figure 2, upper panel), but after a period sufficient to allow Na⁺ activity to reach equi-
librium, the final steady-state value of Na\textsubscript{i} activity falls with temperature to reach a nadir at 20°C and then rises again on further cooling to 10°C (see Figure 4, bottom panel). At 10°C, the distribution of Na\textsuperscript{+} approaches, but does not reach, electrochemical equilibrium, and the addition of 200 \( \mu \)M strophanthinidin then has no further effect on Na\textsubscript{i} activity (Figure 2, lower panel). From this figure, it can be seen that the apparent \( K_{eq} \) at pCa~6 is similar at 37°C, 30°C, and 20°C, but is shifted to the right (pCa 4) at 10°C. At all tested temperatures, perfusion at the lower pCa levels (less than 6) followed by perfusion at higher pCa levels (greater than 7) induces further small depolarization and rise in Na\textsubscript{i} activity.

**Effect of Hypothermia on the Rise in Na\textsubscript{i} Activity Induced by Calcium Depletion With the Sodium Pump Inhibited**

The effect of temperature on the rise in Na\textsubscript{i} activity suggests that both sodium influx and efflux mechanisms are affected. Because the main route for sodium entry is via the calcium channels and that for sodium efflux is via the sodium pump, inhibition of the pump would allow a less ambiguous determination of the effect of temperature on sodium influx. In trabeculae, Na\textsubscript{i} activity was measured during calcium depletion (pCa 7.4) at 37°C, 30°C, and 20°C with the sodium pump inhibited by 0.2 mM strophanthinidin and compared with the data for 10°C (for which strophanthinidin has no additional effect). The level of Na\textsubscript{i} activity in normal Tyrode’s solution rises in the presence of the glycoside and is allowed to stabilize before calcium depletion. The steady-state values obtained for Na\textsubscript{i} activity on calcium depletion at 37°C, 30°C, and 20°C at pCa 7.4 are compared with that at 10°C in Figure 4 (bottom panel). In each case, the membrane depolarizes to approximately the same value of around ~7 mV. The distribution of Na\textsuperscript{+} eventually comes close to electrochemical equilibrium at 37°C and diverges as the temperature is lowered; however, statistical analysis shows that only the data for 10°C are significantly different from those at 37°C. If the buildup of Na\textsubscript{i} activity is simply due to the influx of Na\textsuperscript{+} via the calcium channels, then the time course should fit an equation for the filling of a single compartment, that is,

\[ a_{Na_i} = a_{Na_i \text{max}} (1 - \exp^{-kt}) \]

where \( a_{Na_i} \) is Na\textsubscript{i} activity, \( k \) is the rate constant, and \( t \) is time. However, the relation is initially curved on semi-logarithmic coordinates at all temperatures (Figure 3), although the rise in Na\textsubscript{i} activity can sometimes be fitted by a straight line over the last part of the curve. (In this connection, it should be noted that with large increments in Na\textsubscript{i} activity, the logarithmic response of the electrode means that small errors, caused by either calibration or subtraction of the membrane potential, will have a large effect on the estimate of Na\textsubscript{i} activity.) The simplest explanation for the observed delay would be that the influx of sodium is also affected by another process with a similar sensitivity to temperature. The measured crude overall-half-times (at \( a_{Na_i \text{max}}/2 \)) for the accumulation of sodium is (in minutes) 2.7±0.26 (n=4) at 37°C, 5.28±0.48 (n=5) at 30°C, 14±0.28 (n=5) at 20°C, and 33±5 (n=5) at 10°C. Figure 4 (top panel) shows the relation between rate constants and temperature when the rate constants were estimated as \( \ln a_{Na_i}/a_{Na_i \text{max}} \) divided by the crude half-time. By using these rate constants at different temperatures, an Arrhenius plot can be obtained (Figure 4, middle panel), where the energy of activation over the four temperatures is found to be 67 kJ·mol\(^{-1}\).

**Effect of Initiating Calcium Depletion at 30°C and Then Cooling to 20°C**

The fall in the rate of rise of Na\textsubscript{i} activity with cooling, both with and without the sodium pump
inhibited, suggests a strong temperature dependence of sodium influx, which would be consistent with the known effects of temperature on the whole-cell current through the L-type calcium channels. However, the initial delay and the nonexponential nature of the build up of $Na_i$ activity is difficult to explain in this way. In a series of experiments, the effect of initiating calcium depletion at 30°C, and cooling to 20°C after 2 minutes, on the rise in $Na_i$ activity was investigated. Figure 5 illustrates the effect of calcium depletion on membrane potential and $Na_i$ activity measured under two different conditions. The dashed line shows the effect of calcium depletion (pCa 7.4), on $Na_i$ activity measured at 20°C throughout. The solid lines represent the response to calcium depletion for 2 minutes at 30°C, which is then continued at 20°C. Although the effect on membrane potential is similar, the rise in $Na_i$ activity is accelerated by the 2-minute initial perfusion at 30°C. Figure 6 (upper panel) shows the $Na_i$ activity levels during calcium depletion (pCa 7.4) with and without a 2-minute pulse at 30°C, which shows an increase in the rate of rise in $Na_i$ activity measured over 10-minute perfusion periods. Not only is the increase in the rate of rise in $Na_i$ activity maintained, but the final steady-state values are also increased (Figure 6, lower panel). This increase is observed over the pCa range except for pCa 5.4, for which no significant difference is observed. In 80% of the experiments, a straight line results when the buildup in $Na_i$ activity (measured at 20°C after a 2-minute pulse at 30°C) is plotted on semilogarithmic coordinates; that is, the delay is abolished.

**Effect of Hypothermia on Tension Development After Calcium Repletion**

At 20°C and once $Na_i$ activity has reached steady-state values, the repletion of calcium induces a contraction, which in some instances is partially sustained. If the tissue is then warmed to 30°C, no additional effect is observed on the developed tension. On the other hand, when the calcium repletion contracture at 10°C is observed and becomes well sustained, elevation of the temperature to 30°C produces a phasic contraction that is followed by a slow relaxation but to a new higher baseline. In most tissues and at 10°C, a significant twitch in normal Tyrode’s solution as well as a contraction after calcium repletion could not be detected. Recovery after calcium repletion and following the tension development depends on temperature and the level $Na_i$ activity had reached during calcium depletion. Figure 7 shows the contracture/twitch ratio obtained during calcium repletion after different periods of incubation in calcium-depleted solutions (pCa 8.4) at 37°C, 30°C, and 20°C. It is clear that the effect is time dependent so that, at lower temperatures, the period of incubation in calcium-depleted solution must be extended to achieve a similar effect. Part of this shift will be due to the effect of temperature on the calcium sensitivity of the contractile proteins, as exemplified by the transient contraction that occurs on rewarming to 30°C. Furthermore, and consistent with the rise in $Na_i$ activity involving the warming pulse experiments, the developed contracture on calcium repletion is markedly augmented.

**Discussion**

The effect of temperature on the resting membrane potential, $Na_i$ activity, and the strength of the regularly evoked heartbeats of isolated ferret ventricular trabeculae perfused with normal Tyrode’s solution has been found to closely resemble results of previous work on other mammalian cardiac tissues (see Reference 20 and Table 1). The rise in $Na_i$ activity has been attributed to a greater effect of cooling on the active extrusion of Na⁺ because of the effect of temperature on the sodium-pump current. The complicated effect on the strength of the heartbeat is presumably associated not only with the change in $Na_i$ activity, but
also with effects on the membrane currents passing during the action potential, the calcium turnover in the sarcoplasmatic reticulum, and changes in the sensitivity of the contractile proteins.22,24–26

Effect of Temperature on Membrane Potential and Na⁺ Activity During Calcium Depletion and Contraction on Calcium Repletion

Cooling during the period of calcium depletion reduces calcium loading and cellular damage as assessed by the development of a contracture and loss of electrical and mechanical responses on return to normal Tyrode’s solution, an effect that is reduced if the period of calcium depletion is extended at the lower temperatures. Therefore, isolated ferret ventricular trabeculae responded in a similar way to cooling as did Langendorff-perfused hearts, when the consequences of calcium repletion are determined by a variety of parameters.2–8 In addition to confirming previous work, we have further shown that cooling affects the rise in Na⁺ activity on the removal of divalent cations (Figures 1 and 2 [upper panel]), with the rate of rise being much slowed in spite of a similar depolarization of the cell membrane. This effect on Na⁺ activity means that the protective effect of hypothermia can be explained in terms of the hypothesis that the rise in Na⁺ activity predisposes cardiac tissue to damage on calcium repletion. This slowing of the rate of rise of Na⁺ activity would explain not only the protective effect on the calcium paradox seen when the period of exposure to calcium-free media is held constant around 10–20 minutes, but also why the beneficial effects of cooling decrease with time (Figure 7). Furthermore, the finding that cooling, when made after calcium depletion, aggravates rather than alleviates the consequences of calcium repletion (Figure 5) would be predicted, because Na⁺ activity rises more rapidly and to higher levels (Figure 6). This means that the effect of hypothermia on the calcium paradox is similar to other procedures that increase the resistance of cardiac tissue to the calcium paradox by reducing the rise in Na⁺ activity, namely, exposure to organic calcium channel blockers, a reduced outside sodium concentration, foreign divalent cations, a raised extracellular magnesium concentration, and voltage clamp of the membrane potential.12,27 The effects of cooling, therefore, support rather than contradict the hypothesis that it is the rise in Na⁺ activity that is the factor that predisposes the heart to damage on calcium repletion.

Temperature and the Flux of Sodium During Calcium Depletion

The rise in Na⁺ activity during calcium depletion would seem to result from an increased influx of sodium, primarily through the L-type calcium channels, that is not immediately balanced by the sodium efflux via the sodium pump. The influx of Na⁺ has been shown to be driven by the sodium gradient, while the rise in Na⁺ activity stimulates the sodium pump.13 However, although the rate of rise in Na⁺ activity, with or without inhibition of the sodium pump, falls with cooling (Figures 2 [upper panel] and 3), the final steady-state value of Na⁺ stimulates achieved during calcium depletion shows a U-shaped dependence on temperature in the absence of 0.2 mM strophanthidin (Figure 2, lower panel), but a shallow decline with temperature when the pump is blocked (Figure 4, bottom panel). This suggests that the effect on the influx and efflux of sodium during calcium depletion is not the same over the whole range of temperatures. A comparison between 37°
and 30°C, where the steady-state value of Na activity in low-calcium media and the rate of rise of Na activity fall, suggests that the effect of cooling is greater on the sodium influx than on the sodium efflux. Between 30° and 20°C, there is little change in the final steady-state value of Na activity, although the rate of rise of Na activity falls further, so that over this temperature range, the effect of cooling on influx and efflux of Na⁺ is of a similar magnitude. At 10°C, the rise in Na activity approaches electrochemical equilibrium, which would be consistent with a greater effect on the sodium efflux than on its influx. On sodium-pump inhibition by strophanthidin, the rise in Na activity approaches electrochemical equilibrium (Figure 3), suggesting that the pump is the main mechanism for the efflux of Na⁺. In fact, it would seem that the relative temperature dependence of the mechanisms for sodium influx and the sodium pump are different, and this results in the U-shaped curve, when the sodium pump is not inhibited with the glycoside. This can be clearly illustrated by comparison of the Q₁₀ for the influx of Na⁺ on calcium depletion with the sodium pump inhibited (Figure 3), with other data available for the sodium pump.¹⁶ The values for the rise in Na activity are 2.8±0.2 (37–30°C), 2.7±0.2 (30–20°C), and 2.3±0.3 (20° and 10°C), so that the relative temperature sensitivity increases with temperature. A similar set of data for the sodium pump is not available, but in Purkinje fibers, the Q₁₀ values are 1.5 (46–36°C) and 2.3 (36–26°C) obtained from their Figure 3c. Our data suggest that at 10°C the sodium pump is fully inhibited because the K₅₀ for Na activity is shifted to the right (Figure 2, lower panel) and the addition of strophanthidin causes no further rise in Na activity in calcium-free media. This means that the relative temperature sensitivity of the sodium pump falls with an increase in temperature, while that for the sodium influx rises. The difference in the effect of temperature on the sodium influx and efflux is consistent with the suggestion that the sodium pump is the principal mechanism of sodium efflux during calcium depletion. However, if the entry of sodium is largely via the L-type calcium channels, it would be anticipated that the temperature dependence of current through the calcium channels would be similar to that for the rise in Na activity. The temperature dependence of whole-cell current when Ca²⁺ is the charge carrier over the range 23–35°C has a Q₁₀ between 2.5 and 2.9, which is similar to the figures we obtained for the rise in Na activity over the same range of temperatures. Single-channel data for L-type calcium channels from guinea pig bladder myocytes with calcium, barium, or sodium as the charge carriers has a lower Q₁₀ of 1.55 for the open channel conductance.²⁸ This difference between single-channel and whole-cell currents suggests that temperature also affects channel availability and/or open probability.

**Temperature and the Loss of Ionic Selectivity by the Calcium Channel**

So far, the results have been explained in terms of a differential effect of temperature to reduce the influx through the individual L-type calcium channels and the sodium efflux via the sodium pump. A number of observations are not entirely consistent with this scheme, namely, the significant initial delay in the buildup of Na activity that occurs even though the membrane is depolarized promptly to around −20 mV (Figure 1), the nonexponential nature of the buildup of Na activity when the sodium pump is inhibited (Figure 3), and the effect seen when calcium depletion is initiated at 30°C and then cooled to 20°C (Figures 5 and 6, upper panel). The final steady-state levels reached in these experiments, which are greater than those seen when the temperature is maintained at 20°C or 30°C (Figure 6, lower panel), suggests an increase in the sodium influx or a reduction in the sodium efflux. It would seem unlikely that a brief period of warming would have the
long-lasting effect on the sodium pump that suggests an additional effect of temperature on the sodium influx. There is evidence that this may be the case for voltage-clamped guinea pig ventricular myocytes at 20°C, in which calcium depletion causes only a weak inward current and a slow rise in Na⁺ activity. On slowly warming, there is a gradual increase in inward current and Na⁺ activity, but at around 30°C, there is a sudden increase in inward current and a marked rise in Na⁺ activity, an effect not reversed by recouling to 20°C.

Differences between the effect of temperature on single-channel currents (Q₁₀ 1.55) and whole cell currents (Q₁₀ 2.5–2.9) through L-type calcium channels suggest an effect on channel availability in addition to the effect on single-channel conductance. However, the irreversible effect of warming on both Na⁺ activity and current in ventricular myocytes and the effect of a warm pulse at 30°C (Figure 5) suggest an effect of temperature on the transition of the calcium channel from a calcium-selective to a nonselective channel. Incidentally, Glossman and Streissnig found that the dissociation of Ca²⁺ from calcium channels shows a very large temperature dependence (Q₁₀ = 5) over the narrow range of their experimental temperatures, which was 25–30°C. As the binding of calcium has been shown to regulate the selectivity of the L-type calcium channels, then a strongly endothermic reaction might contribute to the slowing of the loss of ionic selectivity on calcium depletion at low temperatures. The reduced channel conductance, a reduction in channel open probability, and the retardation in the loss of ionic selectivity might all therefore contribute to the nonexponential buildup of Na⁺ activity. A similar effect is suggested when the data of previous work are examined, evidence of a “transition temperature” at around 30°C, above which it seems hard to protect against the calcium paradox, is apparent. How this transition is expressed in channel activity remains to be resolved.

References


25. Fabiato A: Time and calcium dependence of activation and inactivation of Ca-induced release of calcium from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. J Gen Physiol 1985;85:247–289


27. Rodrigo GC, Chapman RA: The relationship between the rate of Na-loading membrane current and membrane potential in guinea pig ventricular myocytes during reperfusion with Ca and Mg free Tyrode (abstract). J Physiol (Lond) 1990;426:16P

28. Klockner U, Schiefer A, Isenberg G: L-type Ca-channels: Similar Q10 of Ca-, Ba- and Na-conductance points to the importance of ion-channel interaction. Pflugers Arch 1990;415:638–641

29. Suleiman MS, Rodrigo GC, Chapman RA: Effect of hypothermia on Na influx during the Ca-paradox, in guinea pig ventricular myocytes and isolated ventricular trabeculae of the ferret (abstract). J Physiol (Lond) 1990;427:53P


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