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A moderate decrease in temperature inhibits the calcium signaling mechanism(s) of the regulatory volume decrease in chick embryo cardiomyocytes

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Abstract

Chick cardiomyocytes, when submitted to hyposmotic swelling, exhibit a partial regulatory volume decrease (RVD). A Ca^{2+} influx by stretch-activated channels signals a taurine efflux and the RVD at 37°C. We evaluated the cell's performance at room temperature. Cardiomyocytes isolated and cultured from 11-day-old chick embryos were submitted to a hyposmotic solution (180 mOsm/kg H_2O) at 37°C and at room temperature (26°C). Under these conditions we measured the changes in cell volume as well as the intracellular free Ca^{2+} (using fura-2). During hyposmotic swelling, cells at 37°C displayed a peak relative volume of 1.61 ± 0.03 and recovery to 1.22 ± 0.04 (N = 14), while cells at 26°C presented a peak swell relative volume of 1.74 ± 0.06 and did not recover (1.59 ± 0.09, N = 9). Transient increases in intracellular Ca^{2+}, which are characteristic of the normal RVD, were observed at both temperatures (29.1 ± 4.5% (N = 8) and 115.2 ± 42.8% (N = 5) increase at 37° and 26°C ($P<0.05$), respectively). A delay in the Ca^{2+} transient increase was also observed when the cells were at 26°C (109 ± 34 s compared to 38 ± 9 s at 37°C, $P<0.05$). At room temperature the RVD does not occur because the calcium transient increase, which is an early event in the signaling of the RVD, is delayed. Also, free calcium is not cleared as in the 37°C RVD. In the normal RVD the free calcium returns to baseline levels. The very high and persistent free calcium levels seen at room temperature can lead to unregulated enzyme activities and may promote irreversible injury and cell death.

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- RVD
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- High free calcium

Volume regulatory mechanisms play a critical role in the maintenance of structural integrity, and thus in the proper function of living cells. These regulatory processes involve signal transduction pathways and cellular transport systems.

Cultured chick cardiomyocytes, when submitted to hyposmotic swelling (from 290 to 180 mOsm/kg H_2O) exhibit a partial regulatory volume decrease (RVD) mediated by a loss of amino acids (mostly taurine). There are no significant changes in inorganic ion contents in this condition (1). This RVD is dependent on the influx of Ca^{2+} by stretch-

activated channels which in turn signals a taurine efflux and the RVD at 37ºC (2-5).

Studies with chick cardiomyocytes under isosmotic conditions at low temperatures reveal a dissipation of the Na^+ gradient and low levels of Na^+/K^+ ATPase activity, with the Na^+/Ca^{2+} exchanger functioning only minimally (6). On the basis of these considerations, we evaluated the RVD under hyposmotic swelling at low temperature.

Cardiomyocytes isolated and cultured from 11-day-old chick embryos (7) were transferred from isosmotic (290 mOsm/kg H_2O) to a hyposmotic solution (180 mOsm/kg H_2O) at 37ºC and at room temperature (26ºC). The cells were perfused at a rate of ~4 ml/min with HEPES-buffered saline solution (142.2 mM NaCl, 5.4 mM KCl, 1.0 mM NaH_2PO_4, 10 mM HEPES, 5.6 mM dextrose, 0.8 mM $MgSO_4$, and 1 mM $CaCl_2$; the hyposmotic solution contained 50% of isosmotic NaCl) on the heated (or not) stage of an inverted microscope (Nikon Diaphot, TMD, Tokyo, Japan). Measurements of cell area from video images of spherical cells (model KP MIL camera, Hitashi Denshi, Ltd.; model PVM-137 monitor, SONY, 1000X resolution) were made using JAVA system software (Jandel Scientific, San Rafael, CA, USA) and were then converted into cell volume. Values were normalized to the last measurement made in the isosmotic solution before volume challenge.

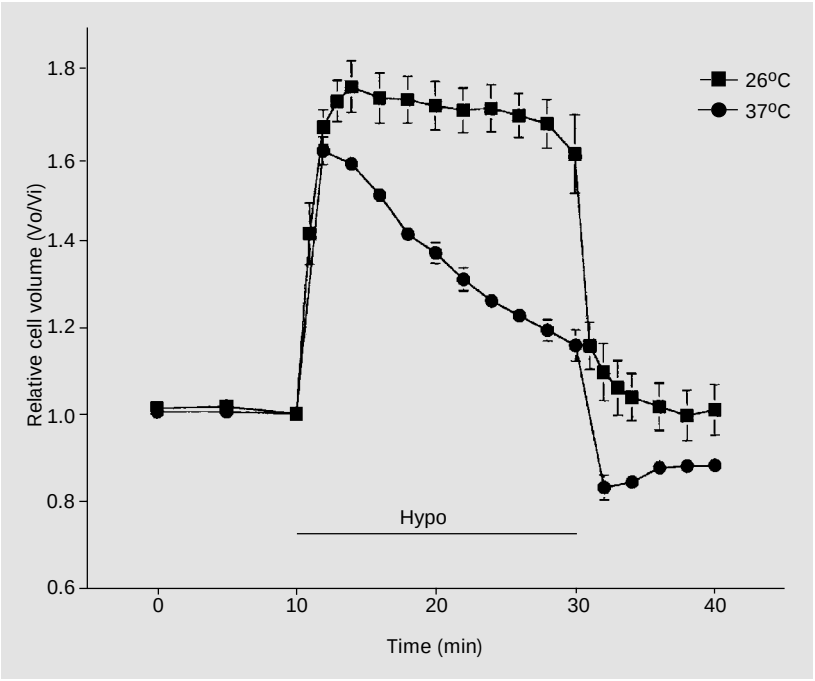

Figure 1 - Cultured chick embryo cardiomyocytes cannot activate volume regulatory processes at room temperature. The cells were exposed to a 180 mOsm/kg H_2O hyposmotic stimulus (hypo) at 37ºC (circles) and 26ºC (squares). At 26ºC the myocytes cannot recover to near control volumes as compared to the myocytes at 37ºC. Data are reported as means ± SEM, N = 14 for 37ºC and N = 9 for 26ºC. Vo: Observed volume; Vi: initial volume.

Cells microinjected with fura-2 (1 mM in 140 mM KCl, 10 mM MOPS-3-[N-morpholino] propane-sulfonic acid and passed through a chelex-100 column, pH 7.4) were used for intracellular free Ca^{2+} measurements by the ratiometric method. The excitation and emission wavelengths were 350/380 and 505 nm, respectively. The coverslips were affixed to a heated (or not) stage on a Zeiss IM35 inverted epifluorescence microscope coupled to a Spex model CM3 dual-wavelength excitation spectrofluorometer (Spex Industries, Edison, NJ, USA).

The mean and standard errors were calculated from relative values and plotted on graphs. Data for control and experimental groups were compared by the Student *t*-test.

Single cells, when submitted to a change in osmolality (from 290 to 180 mOsm/kg H_2O) at 37ºC, exhibited a peak swelling of 1.61 ± 0.03 (N = 14) within 2 min, followed by a partial RVD with an end point at 1.22 ± 0.04 (N = 14) (Figure 1). However, when the cells were exposed to the same hyposmotic condition, but at 26ºC, the peak of swelling was higher than at 37ºC (1.74 ± 0.06, N = 9; $P<0.05$) and the relative volume was not recovered (1.59 ± 0.09, N = 9; $P>0.05$) (Figure 1). Also, upon return to isosmotic saline at 30 min, the cells at room temperature returned to pre-swollen levels (relative volume of 1.0), thus further substantiating that no net loss of osmolytes and no RVD results from hyposmotic swelling at 26ºC.

When chick cardiac myocytes are submitted to hyposmotic swelling they exhibit a

transient increase in intracellular free Ca^{2+} (2,3), which was also demonstrated in our experiments. The intracellular Ca^{2+} transient increase (as measured by 350/380) was observed at both temperatures (29.1 ± 4.5%, N = 8, with a delay of 38 ± 9 s at 37°C) (Figure 2). While at 26°C the cells exhibited a 350/380 fluorescence ratio increase of 115.2 ± 42.8% (N = 5) and a delay of 109 ± 34 s ($P<0.05$, when both parameters were compared with the control temperature) (Figure 2). Post-experiment calculations were performed to calibrate and transform ratio values to Ca^{2+} concentrations. Values for the different conditions were approximately: control (0.16 µM), hyposmotic solution at 37°C (0.5 µM) and at 26°C (1.3 µM).

In order for cells to maintain a constant volume when subjected to anisosmotic environments, they must have the ability to mobilize osmolytes. The RVD response to hyposmotic swelling is usually characterized by a loss of K^+, Cl^- and some organic osmolytes (8). In chick cardiac myocytes exposed to a hyposmotic solution, there is a concurrent influx of Ca^{2+} (2,3) by stretch-activated channels (5), and it has been proposed that protein kinase C, activated by free Ca^{2+}, mediates the amino acid efflux (taurine) that leads to RVD (9). Moreover, it has been shown that cytoskeletal components are involved in the volume regulation of cardiac myocytes. Electrophysiological studies and immunolabeling assays of cytoskeletal proteins reveal suppression of a swelling current and disruption of subplasmalemmal F-actin dynamics induced by cytochalasin B and phalloidin, and the capacity of volume regulation is significantly affected (10,11).

Tissues of mammals exposed to low temperatures show alterations in intracellular ion contents (6,12). For homeotherms, hypothermia can attenuate enzymatic reactions leading to impairment of cell integrity. Considering the differences in temperature dependence for the active and passive transport components of the cell's pump-leak system, which maintains a stable intracellular ion concentration, a change in temperature represents a significant challenge to cellular functions.

Embryonic chick cardiomyocytes in isosmotic solution at low temperatures exhibit an increase in intracellular Na^+ concentration. It has been suggested that Na^+ influx from Na^+/H^+ occurs and may not be corrected by Na^+/K^+-ATPase, which in this situation shows only 15% of its original activity (6). No change was observed in intracellular K^+ concentration, and total intracellular Ca^{2+} presented a small increase at low temperature (6), while intracellular free Ca^{2+} demonstrated a substantial increase (13).

Few reports regarding cell volume regulation at low temperature are available. Malphigian tubules of a New Zealand mountain insect exposed to hyperosmotic conditions at low temperature (0°C) showed a greater change in cell volume as compared to higher temperatures (20°C) and no regulatory volume increase was observed (14). Some data about chick cardiac myocytes at low temperatures are available (10°C), but only under isosmotic conditions, and in this situation the cells shrink (6). The authors believe

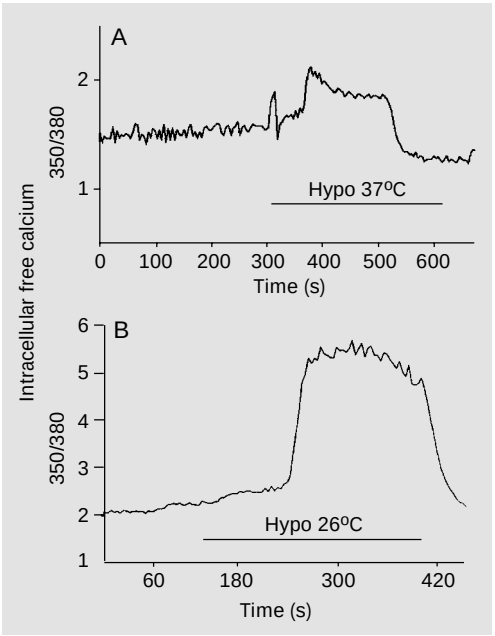

Figure 2 - Intracellular free Ca^{2+} measurements expressed as fluorescence ratio. Representative experiments showing Ca^{2+} transient increases in hyposmotic solutions (hypo) at 37°C (A) and 26°C (B).

that the shrinkage is a consequence of inhibition of the inwardly directed $Na^+/K^+/2Cl^-$ cotransporter.

Our findings reveal that embryonic chick cardiac myocytes during hyposmotic challenge at 26°C (room temperature) swell more than at 37°C, and that at room temperature no RVD is observed. Also, in this situation the intracellular free Ca^{2+} exhibits a huge increase, much more than a transient increase during hyposmotic swelling at 37°C.

As mentioned previously, during a hyposmotic challenge there is a Ca^{2+} influx for signaling the RVD which occurs via stretch-activated channels, and the rapid free Ca^{2+} transient increase at 37°C is a result of Ca^{2+} influx activating Ca^{2+} release from intracellular stores (15). At room temperature the transient is also present, but is delayed. Our findings suggest that the Ca^{2+} release mediated by receptor activities is inefficient at the lower temperature and the same lack of responsiveness may exist with calmodulin, which normally is instrumental in clearing high cytoplasmic free calcium levels through its stimulatory effect on plasma membrane Ca^{2+} ATPase (16). It is unclear at this point if low temperatures prevent the conformational changes in calmodulin which are necessary to activate Ca^{2+} ATPase, or if the activated ATPase is less efficient in hydrolyzing ATP at the decreased temperatures. However, the latter is most likely the case and may explain why Ca^{2+} ATPases in the sarcoplasmic reticulum, which are normally highly efficient in transporting calcium out of the cytoplasm, appear to be inactive at 26°C. In addition, mitochondrial uptake of Ca^{2+} through its low-affinity/high-capacity Ca^{2+} pump appears to be temperature sensitive in our model as this mechanism is known to operate when intracellular free calcium becomes perilously high (17). Also, even with abundant free Ca^{2+}, signal transduction pathways that lead to the activation of protein kinase C (and thus to the RVD) are also interrupted.

Taken together, the evidence obtained in this study as well as in other investigations on the chick embryo cardiomyocyte suggests that even moderate hypothermia is a potent inhibitor of active transport. This is manifest in the cardiomyocyte inability to mobilize osmolytes for the RVD response and to control and reduce cytoplasmic calcium concentrations to normal levels. It is well known that if the intracellular free Ca^{2+} concentration rises to high levels, it levies toxic effects on the cells. The very high and persistent free calcium seen at room temperature can lead to unregulated enzyme activities and promote irreversible cell injury and perhaps cell death.

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