Recording of calcium transient and analysis of calcium removal mechanisms in cardiac myocytes from rats and ground squirrels

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Abstract  With confocal microscopy, we recorded calcium transients and analyzed calcium removal rate at different temperatures in cardiac myocytes from the rat, a non-hibernator, and the ground squirrel, a hibernator. The results showed a remarkable increase of the diastolic level of calcium transients in the rat but no detectable change in the ground squirrel. Calcium transient of the ground squirrel, compared with that of the rat at the same temperature, had a shorter duration and showed a faster calcium removal. As indicated by the pharmacological effect of cyclopiazonic acid, calcium uptake by sarcoplasmic reticulum (SR) was the major mechanism of calcium removal, and was faster in the ground squirrel than in the rat. Our results confirmed the essential role of SR in hypothermia-tolerant adaptation, and negated the importance of Na-Ca exchange. We postulated the possibility to improve hypothermia-tolerance of the cardiac tissue of non-hibernating mammals.

Keywords: cardiac cells, calcium transient, calcium removal, sarcoplasmic reticulum, hibernation, hypothermia, confocal laser-scanning microscopy.

It is well known that the myocardium of non-hibernating mammals, including the human, cannot work at low body-temperatures, which is in contrast to the well-maintained contractility of hibernator myocardium under the same condition[1,2]. Our recent research revealed that low temperature caused a marked increase of intracellular free calcium ([Ca²⁺]i) in cardiac myocytes from the rat but not in those from the ground squirrel, a hibernator[3]. However, since these data were collected from resting myocytes, they were still insufficient to correlate intracellular calcium with the hypothermia-induced change of contractility.

Further, if hibernator cells do successfully keep from calcium overload during hypothermia, they must have special adaptation in calcium removal mechanisms. Some investigations showed that sarcoplasmic reticulum (SR) vesicles isolated from hibernator hearts had a higher calcium uptake rate than those from non-hibernators[4]. Notable increases of both cellular SR density and in vitro calcium uptake capacity of SR were found in hibernators during their entrance into hibernation[5,6]. While the evidence suggested an important role of SR in cold-adaptation of heart myocardium, some investigators, however, argued a special role of Na-Ca exchange in intracellular calcium homeostasis during hypothermia. Since Na-Ca exchange does not consume
ATP directly, it was postulated that Na-Ca exchange could help buffering the ineffective energy supply at low temperatures. This viewpoint was supported by a low-amplitude plateau of cardiac action potential in hibernating chipmunks, different intracellular ionic response to cryo-treatment in a hibernator and a non-hibernator, and high sensitivity of repolarization to extracellular Na/Ca in hibernators (unpublished data). The key point of this controversy, we believe, is that the measurement of SR uptake was done in vitro, and thus could not account for their in situ contribution, let alone compare with Na-Ca exchange.

In the present study, we recorded the temperature-dependent change of calcium transient in driven cardiac myocytes of rats and ground squirrels with confocal microscopy, analyzed their calcium removal rate by exponential fitting of the recovery phase of calcium transients, and determined the in situ contribution of SR in uptake calcium by the aid of a selective SR Ca-pump blocker cyclopiazonic acid (CPA). The results revealed the adaptation of hibernator myocardium in avoiding intracellular calcium overload during hypothermia.

## 1 Methods

### 1.1 Cell preparation

Adult rats and ground squirrels (Citellus dauricus) of either sex were used in our experiments. Ventricular myocytes were prepared and loaded with indo-1 as previously reported. Briefly, the hearts were rapidly excised from the animals under ether anesthesia and perfused for 5 min at 37°C with a calcium free buffer containing (in mmol/L): 110 NaCl, 4 KCl, 1.2 MgCl₂, 1.2 NaH₂PO₄, 20 NaHCO₃, 30 taurine, 10 glucose, aerated with 95% O₂ + 5% CO₂, pH 7.4. The tissue was then digested in a buffer containing 0.5 mg/mL collagenase (Sigma, Type 1A), 1% bovine serum albumin (Sigma, fraction V, essentially fatty acid free) and 75 μmol/L Ca²⁺. About 15 min later, the ventricle was cut into small pieces and incubated in digesting solution. Finally the myocytes were harvested and stored in Tyrode solution which contained (in mmol/L): 140 NaCl, 4 KCl, 2.0 CaCl₂, 1.2 MgCl₂, 1.2 NaH₂PO₄, 10 glucose, 5 HEPES, pH 7.4 adjusted by NaOH. Indo-1 was loaded by exposing the cells to 2.5 μmol/L indo-1/AM (Molecular Probes) in Tyrode solution for about 10 min in the dark at 37°C.

### 1.2 Temperature-controlled chamber

The experimental chamber was a glass-bottomed petri culture dish fixed inside with a circular glass canula of 1 cm diameter to form a 0.5 mL solution container (fig. 1). Temperature was controlled by perfusing the circular glass canula with a Cole-Parmer refrigerated circulator. A tiny bithermal thermistor probe was placed on the glass bottom of the dish to monitor the temperature. When there was 0.3 mL or more solution in the chamber, the temperature difference between the center and the periphery of the glass bottom was less than 0.5°C. Cells were driven by 0.5-Hz and 2-ms pulse with a pair of silver electrodes, which were placed parallelly in the solution container and connected to an electronic stimulator. In order to stabilize the cell position,