The Journal of Experimental Biology 205, 2957–2962 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
JEB4318

Commentary

Adaptive mechanisms of intracellular calcium homeostasis in mammalian hibernators

Shi Qiang Wang[1,2,*], Edward G. Lakatta[2], Heping Cheng[2] and Zeng Quan Zhou[1]

[1]*National Laboratory of Biomembrane and Membrane Biotechnology, College of Life Sciences, Peking University, Beijing 100871, China and* [2]*Laboratory of Cardiovascular Sciences, National Institute on Aging, NIH, Baltimore, MD 21224, USA*

*Author for correspondence (e-mail: wangsq@grc.nia.nih.gov)

Accepted 1 July 2002

Summary

Intracellular Ca^{2+} homeostasis is a prerequisite for a healthy cell life. While cells from some mammals may suffer dysregulation of intracellular Ca^{2+} levels under certain deleterious and stressful conditions, including hypothermia and ischemia, cells from mammalian hibernators exhibit a remarkable ability to maintain a homeostatic intracellular Ca^{2+} environment. Compared with cells from non-hibernators, hibernator cells are characterized by downregulation of the activity of Ca^{2+} channels in the cell membrane, which helps to prevent excessive Ca^{2+} entry. Concomitantly, sequestration of Ca^{2+} by intracellular Ca^{2+} stores, especially the sarcoplasmic/endoplasmic reticulum, is enhanced to keep the resting levels of intracellular Ca^{2+} stable. An increase in stored Ca^{2+} in heart cells during hibernation ensures that the levels of Ca^{2+} messenger are sufficient for forceful cell contraction under conditions of hypothermia. Maintenance of Na^+ gradients, *via* Na^+–Ca^{2+} exchangers, is also important in the Ca^{2+} homeostasis of hibernator cells. Understanding the adaptive mechanisms of Ca^{2+} regulation in hibernating mammals may suggest new strategies to protect nonhibernator cells, including those of humans, from Ca^{2+}-induced dysfunction.

Key words: hibernation, Ca^{2+} metabolism, adaptation, excitation–contraction coupling, homeostasis.

Introduction

Ca^{2+} is a universal intracellular messenger that participates in numerous biological processes from neural regulation to muscle contraction, and from gene expression to cell growth and death (for a review, see Berridge et al., 2000). This incredible versatility necessitates that intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) be tightly regulated, to ensure coordination among the multiple processes involving Ca^{2+} that underlie normal cell functioning. Impaired regulation of $[Ca^{2+}]_i$ often leads to cell dysfunctioning such as occur in life-threatening diseases. For example, a transient increase of $[Ca^{2+}]_i$ is required to initiate contraction of muscle cells; inability to elevate $[Ca^{2+}]_i$ to the required level following an excitation is a major pathogenic mechanism in heart failure (Gomez et al., 2001). In contrast, excessive elevation of resting $[Ca^{2+}]_i$ is deleterious to almost all cell types, and can be associated with either necrotic or apoptotic cell death (Trump and Berezesky, 1995). Abnormal handling of $[Ca^{2+}]_i$ in the heart may induce severe arrhythmias and ventricular fibrillation (Lakatta and Guarnieri, 1993). $[Ca^{2+}]_i$ is also a key issue in transplanted organ preservation (Kim and Southard, 1998). Although a tightly regulated and constant $[Ca^{2+}]$ is not necessarily optimal under all conditions, for example when increased energy use is not an advantage (see Bickler and Buck, 1998), $[Ca^{2+}]_i$ regulation is always important for a healthy cell life. Therefore, a precise understanding of $[Ca^{2+}]_i$ regulation is tantamount not only to understanding the nature but also the development of novel therapies for treating disease.

Two different strategies for probing calcium homeostatic mechanisms are: (1) the discovery of mechanisms of $[Ca^{2+}]$ dysregulation and development of methods to prevent or reverse the defects; and (2) investigation of wild natural models that show an extraordinary capability of handling intracellular Ca^{2+}.

Hibernating mammals are one such special natural model. A mammalian hibernator, like all other mammals, can maintain its body temperature (T_b) at approximately 37°C during most of its lifetime. But in winter, hibernators can actively regulate their T_b down to only a few °C, entering into a distinct state known as hibernation (for reviews, see Lyman et al., 1982; Wang, 1988). During hibernation, circulation and respiration are well maintained, although at much lower rates than normal. T_b can be periodically, temporarily restored during hibernation (the whole period of entry, maintenance and arousal from a period of hibernation is termed ‘hibernation bout’), indicating

that neural regulation is still active, despite deep hypothermia. A complete arousal occurs either upon external stimulation or as 'scheduled' by an internal clock, during which, in ground squirrels and hedgehogs, normal T_b can be restored within 30 min by internal heat production. In this hibernation–arousal cycle, hibernators have to survive a set of extreme conditions that are fatal to humans and other non-hibernating mammals, including sustained deep hypothermia, violent shifts in T_b, highly intensified sympathetic innervation (during arousal), high viscosity and hypocoagulation of blood, and oxidative stress. As an adaptation during evolution, hibernators exhibit distinct resistance to hypothermia, arrhythmias (Johansson, 1996) and hypoxia (for a review, see Wang and Zhou, 1999a).

In this brief review, we summarize the major known aspects of the adaptive mechanisms of intracellular Ca^{2+} homeostasis in hibernating mammals, and discuss their general significance and possible applications.

Enhanced capability to maintain intracellular Ca^{2+} homeostasis

The kinetics of Ca^{2+} cycling are reduced at low temperatures, so adaptive mechanisms to maintain intracellular Ca^{+} homeostasis are one of the keys to surviving hibernation. In resting cardiac myocytes from the rat, which does not hibernate, $[Ca^{2+}]_i$ increases from 140 nmol l^{-1} at 30–35°C to 200–300 nmol l^{-1} during cooling to 5–10°C (Liu et al., 1991b; Wang and Zhou, 1999b; Fig. 1). This is accompanied by a reduced amplitude of Ca^{2+} transients during cell excitation (Wang et al., 2000). These changes result in increased resting tension and a reduction of contractility (Liu et al., 1990; Wang et al., 1997b). Low temperature also causes spontaneous calcium waves (Wang et al., 1999) that are arrhythmogenic (Lakatta, 1992), which may be one reason why ventricular fibrillation is often encountered during hypothermia (Chao, 1959).

In contrast to $[Ca^{2+}]$ dysregulation in non-hibernating animals and humans during hypothermia, $[Ca^{2+}]$ regulation in hibernator cells is strikingly resistant to temperature change. At 30–10°C, resting $[Ca^{2+}]_i$ in heart cells from the ground squirrel (*Spermophilus dauricus*, a well-characterized hibernating rodent) changes very little (range 125±10 nmol Ca^{+} l^{-1}) (Wang et al., 1999; Fig. 1). The dynamic amplitude of Ca^{2+} transients following excitation is actually increased during cooling (Wang et al., 2000), which may help to retain forceful contraction despite the decreased Ca^{2+} sensitivity of myofilaments at low temperatures (Khromov et al., 1990; Liu et al., 1993). As a result, cardiac muscle from the ground squirrel and the hedgehog (another hibernating mammal) exhibited even higher contraction amplitudes at low temperatures than at normal temperatures (Liu et al., 1990; Wang et al., 1997b), which is an adaptive mechanism to ensure sufficient pumping pressure despite the consequent increased blood viscosity and peripheral resistance.

The adaptive capability to maintain intracellular Ca^{2+} homeostasis in hibernator cells is associated with stable cell function despite some pathological or stressful stimulii (Johansson, 1996). It was observed that, in hedgehog hearts, the epicardial application of aconitine, administration of high concentrations of $CaCl_2$, injection of procaine after previous adrenaline treatment, or ligation of the left descending coronary artery, each failed to induce the ventricular fibrillation that usually occurs in guinea pig hearts in response to these perturbations (Johansson, 1996). During experimental ischemia–reperfusion paradigms, ground squirrel heart showed significantly less injury, monitored by creatine kinase leakage, than rat heart, suggesting that hibernator cells are resistant to the oxygen paradox and calcium paradox (Gao et al., 1996). Brain cells of hibernating mammals are also protected against a variety of insults that are detrimental to humans and other nonhibernating species (for a review, see Drew et al., 2001), but the relationship of the neural protection to $[Ca^{2+}]_i$ regulation still needs further study.

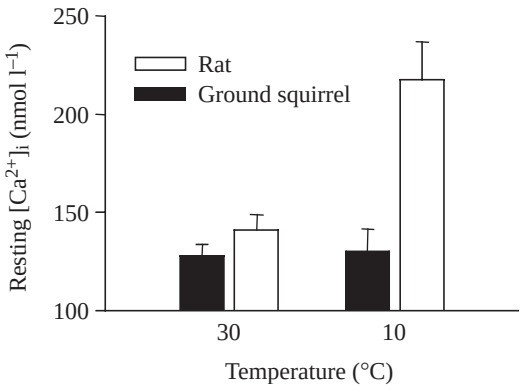

Fig. 1. Low temperature increases intracellular free $[Ca^{2+}]$ markedly in resting cardiac myocytes from the rat, but not in those from the ground squirrel. The Ca^{2+} concentration was measured using the indo-1 fluorescence ratio as described in Wang and Zhou (1999b). Values are means ± S.E.M. (N=12 for rat, N=9 for ground squirrel; P<0.01 at 10°C).

Reduced Ca^{2+} entry through ion channels

Intracellular Ca^{2+} homeostasis requires a dynamic balance between Ca^{2+} entry into and exclusion from the cell, and between Ca^{2+} release from and re-uptake into organelles (Fig. 2). Although Ca^{2+} entry through L-type channels shows similar properties in guinea pigs (a nonhibernator) and ground squirrel (*S. richardsonii*) cardiac myocytes (Herve et al., 1992), there is ample evidence that intracellular Ca^{2+} cycling is different during hibernation. Action potentials of cardiac cells from hibernating chipmunks, ground squirrels and hedgehogs are characterized by the absence of a plateau at 0 mV (Fig. 3, arrow); both action potential and contraction become less sensitive to L-type Ca^{2+} channel antagonists such as nifedipine and Cd^{2+}, compared with the sensitivity in non-hibernating or aroused individuals (Kondo and Shibata, 1984; Kondo, 1986; Wang et al., 1995). These facts suggested that Ca^{2+} influx during excitation is reduced during hibernation. Direct

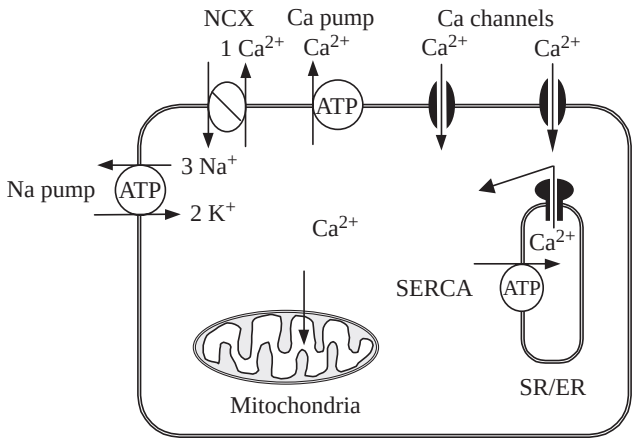

Fig. 2. General scheme of intracellular Ca^{2+} cycling. Ca^{2+} can enter the cell *via* Ca^{2+} channels and, in some situations, *via* the Na^{+}–Ca^{2+} exchanger (NCX). Ca^{2+} can be released from sarcoplasmic/endoplasmic reticulum (SR/ER) *via* Ca^{2+}-release channels, including ryanodine receptors and inositol (1,4,5)-trisphosphate receptors. Ca^{2+} is removed from the cytosol by SR/ER Ca^{2+}-ATPase (SERCA), cytolemmal Ca^{2+}-ATPase, Na^{+}–Ca^{2+} exchange and the mitochondrial uniportor.

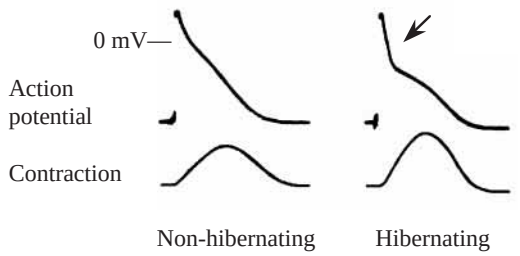

Fig. 3. A comparison of action potential and contraction between cardiac myocytes from non-hibernating and hibernating ground squirrels. The arrow indicates the absence of the action potential plateau phase due to reduced L-type Ca^{2+} currents during hibernation. Note the larger contraction amplitude in the hibernating state (Wang et al., 1995).

measurement of whole-cell current in hibernator heart cells confirmed the downregulation of L-type Ca^{2+} currents (Alekseev et al., 1996) and suggested that the suppression is due to a reduction in cAMP-independent phosphorylation of the L-type Ca^{2+} channels (Kokoz et al., 2000). The suppression of voltage-dependent Ca^{2+} entry is also observed in other hibernator tissues. The binding site density in ileal longitudinal smooth muscle from ground squirrels (*S. richardsonii*) was approximately one order of magnitude less than that from guinea pigs (Wolowyk et al., 1990). In the neural system, for example, both resting $[Ca^{2+}]_i$ and depolarization-induced accumulation of Ca^{2+} in isolated synaptosomes are significantly lower in hibernating ground squirrels (*S. tridecemlineatus*) than in cold-adapted, non-hibernating animals, owing to decreased Q-type Ca^{2+} channel activity (Gentile et al., 1996).

The downregulation of sarcolemmal Ca^{2+} channels may help to prevent excessive Ca^{2+} entry into cells during hypothermia. At low temperatures, ion transport becomes slow, and the cell tends to become depolarized owing to the loss of ionic gradients (Wang et al., 1997a). If depolarized to approximately –50 mV, a tonic, non-inactivating 'window' current of voltage-gated Ca^{2+} channels usually becomes activated, and may further depolarize the cell, leading to more influx of Ca^{2+}. As a result, cells become arrhythmic and calcium-overloaded. Downregulation of voltage-gated Ca^{2+} channels during hibernation decreases the chance of activation of the window current. Moreover, in hibernating ground squirrels (*S. undulatus*) the activation threshold of L-type Ca^{2+} channels shifts towards more positive potentials (Alekseev et al., 1996); the cells in hibernators can also better maintain ionic gradients (for reviews, see Willis, 1979; Wang, 1988) and thereby maintain their membrane potential (Liu et al., 1991a; Wang et al., 1997a) independently of temperature. These adaptive mechanisms effectively prevent the excessive Ca^{2+} entry and intracellular Ca^{2+} overload that would otherwise occur during hypothermia.

Remodeling of cardiac excitation–contraction coupling

Contraction of myocytes is usually initiated by their excitation. In heart cells, excitation–contraction (E–C) coupling is governed by a mechanism known as Ca^{2+}-induced Ca^{2+} release (Fabiato and Fabiato, 1975), by which Ca^{2+} influx through individual L-type Ca^{2+} channels triggers the Ca^{2+} release channels/ryanodine receptors (RyRs) in the sarcoplasmic reticulum (SR) to release Ca^{2+} (Wang et al., 2001) and initiates cell contraction. Downregulation of L-type Ca^{2+} channels in hibernating mammals decreases the magnitude of the trigger signal for E–C coupling. Normally this would result in a reduced cardiac contractility. However, neither the calcium transient nor the contraction amplitude of cardiac muscle from hibernating ground squirrels is reduced; instead, they are markedly stronger than those from non-hibernating animals (South and Jacobs, 1973; Wang, 1988; Fig. 3).

The enhanced contractility may be due either to increased Ca^{2+} transients or to an increase in myofiliment sensitivity to $[Ca^{2+}]$. It was found that the myofiliment sensitivity to $[Ca^{2+}]$ decreases as temperatures are lowered, in both hibernating and non-hibernating mammals (Khromov et al., 1990; Liu et al., 1993). Although myofiliments from hibernating ground squirrels (*S. richardsonii*) exhibit a somewhat higher Ca^{2+} sensitivity at low temperature than squirrels in the non-hibernating state, this still cannot fully explain the observed enhanced contractility over a wide temperature range.

The size of the Ca^{2+} transients has not yet been compared in animals in the hibernating and non-hibernating states, but all pharmacological evidence to date supports the idea that SR Ca^{2+} release is increased during hibernation. Blocking SR Ca^{2+} release by ryanodine or caffeine caused greater inhibition of myocardial contraction in hibernating chipmunks (Kondo and Shibata, 1984) and ground squirrels (*S. richardsonii*)

(Zhou et al., 1991) than when they were non-hibernating. This implies during hibernation, cardiac E–C coupling is remodeled so that a lower Ca^{2+} influx triggers a greater Ca^{2+} release response.

Enhanced Ca^{2+} uptake by intracellular Ca^{2+} store

Various pumps and exchangers are involved in maintaining the low $[Ca^{2+}]_i$ in cells. Plasma membrane Ca^{2+}-ATPase and Na^+–Ca^{2+} exchangers extrude Ca^{2+} from the cell, whereas sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and a mitochondrial uniporter return Ca^{2+} to intracellular organelles (Berridge et al., 2000), their relative contributions varying among cell types. In cardiac cells, Ca^{2+} uptake by SR is the dominant mechanism for rapid dissipation of intracellular Ca^{2+}, and accounts for 70–92% of total Ca^{2+} removed during each excitation–contraction cycle (Bers, 2000).

A reduction in temperature leads to a reduction in the rate of Ca^{2+} removal from the cytosol. Although the relaxation velocity of myocardial contraction decreases monotonically as the temperature is lowered in both rats and ground squirrels (*S. dauricus*), ground squirrel myocardium shows a higher relaxation velocity at any temperature between 35°C and 10°C (Wang et al., 1997b). $[Ca^{2+}]_i$ measurements indicated that the Ca^{2+} transient decays faster in ground squirrel cells than in rat cells owing to a faster Ca^{2+} uptake rate by the SR (Wang et al., 2000). This implies that the quantity and/or quality of SR and SERCA2 may be adaptively modified in hibernators.

Indeed, ultrastructural analysis has revealed that the proportional volume of SR in myocardium from hibernating ground squirrel (Rosenquist, 1970; Tang et al., 1995) and hamster (hibernator) (Skepper and Navaratnam, 1995) is double or triple that of individuals that are non-hibernating, and is mainly due to an increase in longitudinal SR, which contains abundant Ca^{2+}-ATPase and is responsible for Ca^{2+} uptake. By contrast, the content of junctional SR, where Ca^{2+}-release channels are located, changes little (Tang et al., 1995; see Table 1; Skepper and Navaratnam, 1995).

The increase in quantity of SR during hibernation is paralleled by an increase in the Ca^{2+} uptake capacity of the SR. SR vesicles isolated from winter-hibernating ground squirrels exhibit a faster rate of Ca^{2+} uptake and a greater level of Ca^{2+} accumulation than those from non-hibernating individuals either in winter or in other seasons (Belke et al., 1991; Tang et al., 1995; Fig. 4). Even in non-hibernating ground squirrels, the Ca^{2+} uptake rate by the SR is still higher than those in rats at temperatures between 35°C and 5°C (Liu et al., 1997).

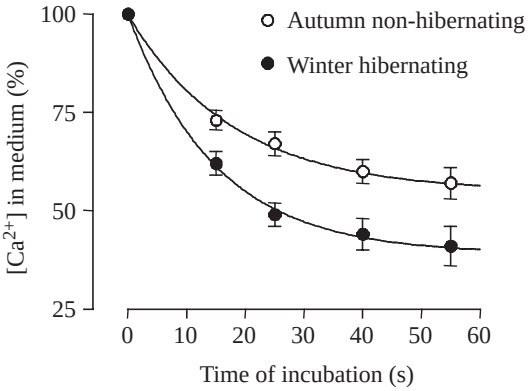

Fig. 4. Cardiac sarcoplasmic reticulum vesicles from winter-hibernating ground squirrels exhibit faster Ca^{2+} uptake than those from autumn, non-hibernating individuals. The graph shows the normalized change in $[Ca^{2+}]$ in 1 ml of reaction medium after addition of 1 mg SR protein. The initial uptake rates for non-hibernating and hibernating groups were 137±13 and 235±17 nmol Ca^{2+} min^{-1} mg^{-1} SR protein, respectively (Tang et al., 1995).

SERCA is the central protein involved in active SR/ER Ca^{2+} uptake against an electrochemical gradient. Since Ca^{2+} regulation in the SR during hibernation is enhanced, it is surprising that the enzymatic activity of SERCA is unchanged (Belke et al., 1991). Although SERCA from ground squirrels (*S. richardsonii*) is less temperature-sensitive than that from rats (Liu et al., 1997), the major mechanism for enhanced SR Ca^{2+} uptake is reliant upon the increased volume of SR present during hibernation.

Calsequestrin is a Ca^{2+}-binding protein in the SR that binds Ca^{2+} at a ratio of 40–50 Ca^{2+} per molecule, and greatly increases the Ca^{2+} storage capacity of the SR, facilitating further Ca^{2+} uptake by decreasing the free Ca^{2+} concentration in the SR lumina. Calsequestrin also directly regulates the leakage and release of Ca^{2+} *via* RyRs by the structural link between them (Sitsapesan and Williams, 1997). In an early electron microscopic study, Rosenquist (1970) noticed that the terminal cisternae of myocardial SR in hibernating ground squirrels exhibits a higher electron density than those in non-hibernating animals, which suggests an increased expression of calsequestrin during hibernation. A novel isoform of

Table 1. *The proportional volume of myocardial subcellular structures in non-hibernating and hibernating ground squirrels* S. dauricus

Groups	Sarcoplasmic reticulum			Mitochondria	Lipid droplets	Myofilaments
	Total	Junctional	Longitudinal			
Non-hibernating	14.7±1.3	1.1±0.1	13.6±1.3	324±11	2.4±0.7	510±11
Hibernating	35.8±1.9*	1.2±0.1	34.5±1.8*	280±11	10.6±0.7*	514±11

Values are parts per thousand (means ± S.E.M., N=60).
*P<0.01.

calsequestrin has been identified in isolated cardiac SR from two species of ground squirrels, with a molecular mass about 7% greater than that of cardiac calsequestrin isolated from other mammals (Milner et al., 1991). The increased molecular mass is partially due to its distinct glycosylation, which appears to include an additional carbohydrate chain that is not present in other isoforms. This molecular modification improves the binding of Ca^{2+} to calsequestrin (Milner et al., 1991) and would thus be helpful in facilitating Ca^{2+} uptake, suppressing Ca^{2+} leakage and increasing the amount of Ca^{2+} available for release; therefore, this novel isoform of calsequestrin enhances Ca^{2+}-induced Ca^{2+} release in cardiac cells during hibernation.

The role of the Na^+–Ca^{2+} exchanger

The Na^+–Ca^{2+} exchanger (NCX), an important cation antiporter in the surface membrane (and some organelles) in most cells, transports three Na^+ in exchange for one Ca^{2+}. The transport is not coupled to ATP hydrolysis directly; instead, it is driven by the total Na^+ electrochemical gradients across the cell membrane, and the Na^+ gradient is maintained by Na^+/K^+-ATPase. Depending on the transmembrane Na^+ and Ca^{2+} concentrations and membrane potential, NCX can operate in either an inward or an outward mode. In cardiac cells, NCX contributes to Ca^{2+} entry only during the early stages of cell excitation, when the membrane potential exceeds the reverse potential for NCX. In response to Ca^{2+} release from SR, NCX becomes the major driving force in extruding Ca^{2+} from cells (Bers, 2000).

Because the NCX does not consume ATP directly, it was hypothesized to be a preferred Ca^{2+} transporting system during hibernation, when ATP production is limited owing to the low temperature. The major evidence for this idea is the observation that the cardiac action potential in hibernating chipmunks (Kondo, 1987) and ground squirrels (Wang et al., 1995) is characterized by a prolonged low-level plateau that is sensitive to extracellular Ca^{2+} and Na^+ concentrations. However, this phenomenon may also be attributed to the enhanced amplitude or prolonged duration of Ca^{2+} transient at low temperatures (Wang et al., 2000), which drive NCX more quickly, but passively. Direct analysis of Ca^{2+} removal mechanisms in both ground squirrels (*S. dauricus*) and rats has failed to establish a dominant role of NCX in removing Ca^{2+}. On the contrary, the fractional contribution of NCX to total Ca^{2+} removal is significantly less in ground squirrels than in rats (Wang et al., 2000). Moreover, NCX contributes even less at lower temperatures in both species, consistent with the observation that the temperature coefficient for NCX is even greater than that for SERCA (Marengo et al., 1997). In addition, NCX is not efficient in terms of energy used. By contrast to SERCA, which transports two Ca^{2+} per ATP molecule consumed, NCX extrudes only one Ca^{2+} per ATP molecule (the three Na^+ that enter during the exchange are then pumped out by Na^+/K^+-ATPase/Na^+-pump at the expense of one ATP molecule) (Bers, 2000). Therefore, the role of NCX in Ca^{2+} removal is decreased in hibernator cells, possibly as an adaptive regulation; consequently, SERCA-based Ca^{2+} uptake becomes more important during hibernation.

Although the role of NCX is secondary in rapid Ca^{2+} removal from the cell, it is still important because it brings Ca^{2+} regulation within a global ionic homeostasis. Owing to the existence of NCX, Ca^{2+} homeostasis is tightly linked to the homeostasis of Na^+ and K^+ and to the ATP supply. An enhanced ability of hibernating animals to produce ATP and to maintain Na^+ and K^+ gradients by Na^+/K^+-ATPase (for reviews, see Willis, 1979; Lymann et al., 1982; Wang, 1988; Willis et al., 1992) is thus an essential part of intracellular Ca^{2+} homeostasis.

Significance and general discussions

We have summarized the major aspects of the adaptive Ca^{2+} regulation in hibernating mammals. Most of these studies described focused on the cardiovascular system. While Ca^{2+} homeostasis is a key issue of cardiovascular functions, Ca^{2+} regulation in other types of cells, such as neurons and endocrine cells, is also important for mammals to survive hibernation and therefore merits more study.

Based on current knowledge, the strategy used by hibernators to maintain intracellular Ca^{2+} homeostasis is to reduce Ca^{2+} entry into the cell while enhancing Ca^{2+} removal. If the SR function in ground squirrel cardiomyocytes is partially inhibited by caffeine, its resistance to hypothermia is lost, and the cells exhibit the same manifestations of Ca^{2+} overload as those observed in nonhibernator cells. Conversely, in the rat, which does not hibernate, partial blockade of Ca^{2+} entry through L-type Ca^{2+} channels prevents many effects of hypothermic Ca^{2+} overload in heart muscle (Wang et al., 1997b). These observations suggest that the Ca^{2+} homeostasis occurring under hypothermia or other similar stressful conditions is not ‘patented’ by hibernators. The underlying regulatory mechanisms may rather also be employed by non-hibernator and human cells to improve stress resistance. In this way, extending our studies on the mechanisms of hibernation may provide strategies for developing new therapies or designing new drugs, and thereby contribute to human health.

References

Alekseev, A. E., Markevich, N. I., Korystova, A. F., Terzic, A. and Kokoz, Y. M. (1996). Comparative analysis of the kinetic characteristics of L-type calcium channels in cardiac cells of hibernators. *Biophys. J.* **70**, 786-797.

Belke, D. D., Milner, R. E. and Wang, L. C. H. (1991). Seasonal variations in the rate and capacity of cardiac SR calcium accumulation in a hibernating species. *Cryobiol.* **28**, 354-363.

Berridge, M. J., Lipp, P. and Bootman, M. D. (2000). The versatility and universality of calcium signalling. *Nat. Rev. Mol. Cell. Biol.* **1**, 11-21.

Bers, D. M. (2000). Calcium fluxes involved in control of cardiac myocyte contraction. *Circ. Res.* **87**, 275-281.

Bickler, P. E. and Buck, L. T. (1998). Adaptations of vertebrate neurons to hypoxia and anoxia: maintaining critical Ca^{2+} concentrations. *J. Exp. Biol.* **201**, 1141-1152.

Chao, I. (1959). The recovery of dogs from deep hypothermia. *Acta Sci. Nat. Univ. Pekinensis* **5**, 99-102.

Drew, K. L., Rice, M. E., Kuhn, T. B. and Smith, M. A. (2001).

Neuroprotective adaptations in hibernation: therapeutic implications for ischemia-reperfusion, traumatic brain injury and neurodegenerative diseases. *Free Radic. Biol. Med.* **31**, 563-573.

Fabiato, A. and Fabiato, F. (1975). Contractions induced by a calcium-triggered release of calcium from the sarcoplasmic reticulum of single skinned cardiac cells. *J. Physiol. (London)* **249**, 469-495.

Gao, T. L., Huang, Y. Z. and Wang, J. (1996). The resistance to ischemia-reperfusion injury of the isolated heart from hibernator *Citellus dauricus*. *Acta Sci. Nat. Univ. Pekinensis* **32**, 527-533.

Gentile, N. T., Spatz, M., Brenner, M., McCarron, R. M. and Hallenbeck, J. M. (1996). Decreased calcium accumulation in isolated nerve endings during hibernation in ground squirrels. *Neurochem. Res.* **21**, 947-954.

Gomez, A. M., Guatimosim, S., Dilly, K. W., Vassort, G. and Lederer, W. J. (2001). Heart failure after myocardial infarction: altered excitation-contraction coupling. *Circulation* **104**, 688-693.

Herve, J. C., Yamaoka, K., Twist, V. W., Powell, T., Ellory, J. C. and Wang, L. C. H. (1992). Temperature dependence of electrophysiological properties of guinea pig and ground squirrel myocytes. *Am. J. Physiol.* **263**, R177-R184.

Johansson, B. W. (1996). The hibernator heart – nature's model of resistance to ventricular fibrillation. *Cardiovasc. Res.* **31**, 826-832.

Khromov, A. S., Srebnitskaya, L. K. and Rogdestvenskaya, Z. E. (1990). Low-temperature-induced calcium sensitivity changes in ground squirrels skinned trabeculae muscle. *Cryo. Lett.* **11**, 331-336.

Kim, J. S. and Southard, J. H. (1998). Alteration in cellular calcium and mitochondrial functions in the rat liver during cold preservation. *Transplantation* **65**, 369-375.

Kokoz, Y. M., Grichenko, A. S., Korystova, A. F., Lankina, D. A., Pimenov, O. Y. and Markevich, N. I. (2000). The regulation of L-type Ca^{2+} currents in cardiac myocytes of hibernating animals. *Membr. Cell Biol.* **14**, 277-284.

Kondo, N. (1986). Excitation–contraction coupling in myocardium of nonhibernating and hibernating chipmunks: effects of isoprenaline, a high calcium medium and ryanodine. *Circ. Res.* **59**, 221-228.

Kondo, N. (1987). Electrophysiological effects of Ca antagonists, tetrodotoxin, $[Ca]_o$ and $[Na]_o$ on myocardium of hibernating chipmunks: possible involvement of Na–Ca exchange mechanism. *Br. J. Pharmacol.* **91**, 315-319.

Kondo, N. and Shibata, S. (1984). Calcium source for excitation-concentration coupling in myocardium of nonhibernating and hibernating chipmunks. *Science* **225**, 641-643.

Lakatta, E. G. (1992). Functional implications of spontaneous sarcoplasmic reticulum Ca^{2+} release in the heart. *Cardiovasc. Res.* **26**, 193-214.

Lakatta, E. G. and Guarnieri, T. (1993). Spontaneous myocardial calcium oscillations: are they linked to ventricular fibrillation? *J. Cardiovasc. Electrophysiol.* **4**, 473-489.

Liu, B., Wohlfart, B. and Johansson, B. W. (1990). Effects of low temperature on contraction in papillary muscles from rabbit, rat and hedgehog. *Cryobiology* **27**, 539-546.

Liu, B., Arlock, P., Wohlfart, B. and Johansson, B. W. (1991a). Temperature effects on the Na and Ca currents in rat and hedgehog ventricular muscle. *Cryobiology* **28**, 96-104.

Liu, B., Wang, L. C. H. and Belke, D. D. (1991b). Effect of low temperature on the cytosolic free Ca^{2+} in rat ventricular myocytes. *Cell Calcium* **12**, 11-18.

Liu, B., Wang, L. C. H. and Belke, D. D. (1993). Effects of temperature and pH on cardiac myofilament Ca^{2+} sensitivity in rat and ground squirrel. *Am. J. Physiol* **264**, R104-R108.

Liu, B., Belke, D. D. and Wang, L. C. H. (1997). Ca^{2+} uptake by cardiac sarcoplasmic reticulun at low temperature in rat and ground squirrel. *Am. J. Physiol.* **272**, R1121-R1127.

Lyman, C. P., Willis, J. S., Malan, A. and Wang, L. C. H. (eds) (1982). *Hibernation and Torporin Mammals and Birds*. London: Academic Press.

Marengo, F. D., Wang, S. Y. and Langer, G. A. (1997). The effects of temperature upon calcium exchange in intact cultured cardiac myocytes. *Cell Calcium* **21**, 263-273.

Milner, R. E., Michalak, M. and Wang, L. C. H. (1991). Altered properties of calsequestrin and the ryanodine receptor in the cardiac sarcoplasmic reticulum of hibernating mammals. *Biochim. Biophys. Acta.* **1063**, 120-128.

Rosenquist, T. H. (1970). Ultrastructural changes in the plasma membrane and SR of myocardial cells during hibernation. *Cryobiol.* **7**, 14-18.

Sitsapesan, R. and Williams, A. J. (1997). Regulation of current flow through ryanodine receptors by luminal Ca^{2+}. *J. Membr. Biol.* **59**, 179-185.

Skepper, J. N. and Navaratnam, V. (1995). Ultrastructural features of left ventricular myocytes in active and torpid hamsters compared with rats: a morphometric study. *J. Anat.* **186**, 585-592.

South, F. E. and Jacobs, H. (1973). Contraction kinetics of ventricular musclefrom hibernating and non-hibernating mammals. *Am. J. Physiol.* **225**, 444-449.

Tang, Y. J., Wang, S. Q. and Zhou, Z. Q. (1995). Seasonal variation in ultrastructure and Ca^{2+} uptake rate of cardiac sarcoplasmic reticulum in ground squirrels. *Acta Physiol. Sinica* **47**, 478-483.

Trump, B. F. and Berezesky, I. K. (1995). Calcium-mediated cell injury and cell death. *FASEB J.* **9**, 219-228.

Wang, L. C. H. (1988). Mammalian hibernation: an escape from the cold. In *Advances in Comparative and Environmental Physiology*, pp. 1-45. Berlin: Springer-Verlag.

Wang, S. Q. and Zhou, Z. Q. (1999a). Medical significance of cardiovascular function in hibernating mammals. *Clin. Exp. Pharmacol. Physiol.* **26**, 837-839.

Wang, S. Q. and Zhou, Z. Q. (1999b). Alpha-stat calibration of indo-1 fluorescence and measurement of intracellular free calcium in rat ventricular cells at different temperatures. *Life Sci.* **65**, 871-877.

Wang, S. Q., Feng, Q. and Zhou, Z. Q. (1995). Experimental analysis of the calcium source for cardiac excitation-contraction coupling in ground squirrel. *Acta Physiol. Sinica* **47**, 551-558.

Wang, S. Q., Cao, H. and Zhou, Z. Q. (1997a). Temperature dependence of the myocardial excitability of ground squirrel and rat. *J. Thermal Biol.* **22**, 195-199.

Wang, S. Q., Huang, Y. H., Liu, K. S. and Zhou, Z. Q. (1997b). Dependence of myocardial hypothermia resistance on sources of activator calcium. *Cryobiology* **35**, 193-200.

Wang, S. Q., Hong, Q. and Zhou, Z. Q. (1999). Temperature dependence of intracellular free calcium in cardiac myocytes from rat and ground squirrel measured by confocal microscopy. *Sci. China C Life Sci.* **42**, 293-299.

Wang, S. Q., Hong, Q. and Zhou, Z. Q. (2000). Recording of calcium transient and analysis of calcium removal mechanisms in cardiac myocytes from rats and ground squirrels. *Sci. China C Life Sci.* **43**, 191-199.

Wang, S. Q., Song, L. S., Lakatta, E. G. and Cheng, H. (2001). Ca^{2+} signaling between single L-type Ca^{2+} channels and ryanodine receptors in heart cells. *Nature* **410**, 592-596.

Willis, J. S. (1979). Hibernation: cellular aspects. *Annu. Rev. Physiol.* **41**, 275-286.

Willis, J. S., Xu, W. and Zhao, Z. (1992). Diversities of transport of sodium in rodent red cells. *Comp. Biochem. Physiol.* **102**, 609-614.

Wolowyk, M. W., Howlett, S., Gordon, T. and Wang, L. C. H. (1990). Smooth muscle contractility and calcium channel density in hibernating and nonhibernating animals. *Can. J. Physiol. Pharmacol.* **68**, 68-70.

Zhou, Z. Q., Liu, B., Dryden, W. F. and Wang, L. C. H. (1991). Cardiac mechanical restitution in active and hibernating Richardson's ground squirrel. *Am. J. Physiol.* **260**, R353-R358.