Insights into cardioprotection obtained from study of cellular Ca\(^{2+}\) handling in myocardium of true hibernating mammals

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Yatani, Atsuko, Song-Jung Kim, Raymond K. Kudej, Qian Wang, Christophe Depre, Keiichi Irie, Evangelia G. Kranias, Stephen F. Vatner, and Dorothy E. Vatner. Insights into cardioprotection obtained from study of cellular Ca\(^{2+}\) handling in myocardium of true hibernating mammals. *Am J Physiol Heart Circ Physiol* 286: H2219–H2228, 2004. First published February 12, 2004; 10.1152/ajpheart.01096.2003.—Mammalian hibernators exhibit remarkable resistance to low body temperature, whereas nonhibernating (NHB) mammals develop ventricular dysfunction and arrhythmias. To investigate this adaptive change, we compared contractile and electrophysiological properties of left ventricular myocytes isolated from hibernating (HB) woodchucks (*Marmota monax*) and control NHB woodchucks. The major findings of this study were the following: 1) the action potential duration in HB myocytes was significantly shorter than in NHB myocytes, but the amplitude of peak contraction was unchanged; 2) HB myocytes had a 33% decreased L-type Ca\(^{2+}\) current (I\(_{Ca}\)) density and twofold faster I\(_{Ca}\) inactivation but no change in the current-voltage relationship; 3) there were no changes in the density of inward rectifier K\(^{+}\) current, transient outward K\(^{+}\) current, or Na\(^{+}/Ca\(^{2+}\) exchange current, but HB myocytes had increased sarcoplasmic reticulum Ca\(^{2+}\) content as estimated from caffeine-induced Na\(^{+}/Ca\(^{2+}\) exchange current values; 4) expression of the L-type Ca\(^{2+}\) channel \(\alpha_{1C}\)-subunit was decreased by 30% in HB hearts; and 5) mRNA and protein levels of sarc(endo)plasmic reticulum Ca\(^{2+}\)-ATPase 2a (SERCA2a), phospholamban, and the Na\(^{+}/Ca\(^{2+}\) exchanger showed a pattern that is consistent with functional measurements: SERCA2a was increased and phospholamban was decreased in HB relative to NHB hearts with no change in the Na\(^{+}/Ca\(^{2+}\) exchanger. Thus reduced Ca\(^{2+}\) channel density and faster I\(_{Ca}\) inactivation coupled to enhanced sarcoplasmic reticulum Ca\(^{2+}\) release may underlie shorter action potentials with sustained contractility in HB hearts. These changes may account for natural resistance to Ca\(^{2+}\) overload-related ventricular dysfunction and point to an important cardioprotective mechanism during true hibernation.

woodchuck; cardiac myocyte; calcium current; sarcoplasmic reticulum; action potential; potassium; exchanger

**SIGNIFICANT INSIGHT** into the understanding of the pathogenesis of heart failure has been gained from studies of patients with heart disease or animal models (18, 28, 29, 46). Alternatively, insight can also be obtained from animals that face major environmental stresses. For example, naturally hibernating (HB) animals such as woodchucks, hedgehogs, or chipmunks undergo pronounced cardiovascular changes as they enter into hibernation: heart rate and blood pressure decrease, and body temperature decreases from 37°C when awake to 16–10°C (25). Under these conditions, the hearts of nonhibernating (NHB) animals develop serious ventricular arrhythmias or cease contraction (16). However, the hearts of hibernators exhibit remarkable tolerance and resistance to the impact of these stresses. Despite the dramatic adaptation of HB myocardium, the cellular basis for the adaptive mechanisms in HB animals is not well known (49).

A few studies (22–24, 27, 48) compared the source of Ca\(^{2+}\) for contraction in cardiac muscle from HB animals with that in NHB animals. Experimental studies have shown that intact papillary muscles isolated from HB animals exhibit an altered excitation-contraction coupling during hibernation. As in most mammalian NHB species, cardiac muscle contraction of the HB heart is dependent on both transsarcolemmal Ca\(^{2+}\) influx and intracellular Ca\(^{2+}\) stores (7). In contrast, muscle contraction during hibernation is mainly regulated by the sarcoplasmic reticulum (SR) Ca\(^{2+}\) release (16, 22, 23, 27, 48). Furthermore, ryanodine caused a transient positive inotropic effect only in HB animals that was similar to cardiac muscle with increased SR Ca\(^{2+}\) load (6, 30). However, there is no indication of ventricular arrhythmias such as afterdepolarization or aftercontraction that commonly occur in Ca\(^{2+}\)-overloaded myocardium (22). These observations suggest that a coordinated remodeling of cellular Ca\(^{2+}\) handling during hibernation may contribute to forceful contraction and the marked resistance to ventricular dysfunction.

In the present study, to examine the cellular basis for the coordinated mechanisms, we compared action potential, L-type Ca\(^{2+}\) current (I\(_{Ca}\)), K\(^{+}\) channel currents, and Na\(^{+}/Ca\(^{2+}\) exchange current (I\(_{Na/Ca}\)) in left ventricular (LV) myocytes isolated from NHB and HB woodchucks. In addition, the profiles of cellular Ca\(^{2+}\) regulatory proteins in LV myocardium from NHB and HB woodchucks were assessed by Western blotting and mRNA analysis.

Our data indicate that myocytes isolated from HB hearts exhibit significantly shorter action potential duration (APD) and higher SR Ca\(^{2+}\) content due to the higher SR Ca\(^{2+}\) uptake function, which may provide a coordinated cardioprotective mechanism to prevent Ca\(^{2+}\) overload in HB animals.

**EXPERIMENTAL PROCEDURES**

Animal model. A total of 19 woodchucks (10 NHB and 9 HB animals) were used in this study. Telemetry transmitters (model...
TL11M2-D70-PCT) were implanted in a subcutaneous pocket in the
animal’s left flank to measure temperature and ECG. HB woodchucks were
killed after displaying deep hibernation, i.e., heart rate < 25
beat/min and without external stimuli. NHB animals consisted of “age-matched controls,” i.e., animals having undergone
hibernation that were killed between March and April. The animals
were maintained and the experiments were approved by the New
York Medical School Animal Care Committee.

Electrophysiology. After completion of the in vivo measurements,
LV myocytes were isolated from NHB or HB woodchucks hearts
using a method described previously (32, 51). Cell contraction and
Ca2+ transients were measured as previously described (52). Briefly,
isolated LV myocytes were perfused with Tyrode solution that con-
tained (in mmol/l) 120 NaCl, 2.6 KCl, 1.0 CaCl2, 1.0 MgCl2, 11
glucose, and 5 HEPES (pH 7.3) and were held at -2°C. Myocyte contractile and relaxation functions were measured using a
video motion-edge detector at 32 ± 2°C. For the Ca2+ transient
measurements, cells were loaded with 5 M fura 2 acetoxymethyl
ester (AM) at room temperature for 30 min, and intracellular free
Ca2+ was monitored as the fura 2 fluorescence ratio at 340- to 380-nm
wavelengths using the Photocasan dual-beam spectrophotometer
(Photon Technology). The changes in Ca2+ transient were evaluated
by direct reading of the fluorescence intensity. The time for 70% decay
of the Ca2+ transient (TRC 70) was calculated.

Action potential and membrane currents were measured using the
whole cell variation of the patch-clamp technique (20, 32, 33, 51) at
room temperature (22 ± 1°C). Membrane capacitance was measured
using voltage ramps of 0.8 V/s from a holding potential of -50 mV.

Ica values were recorded with an external solution that contained
(in mM) 2 CaCl2, 1 MgCl2, 135 tetraethylammonium chloride, 5
4-aminopyridine, 10 glucose, and 10 HEPES (pH 7.3). The pipette
solution contained (in mM) 100 cesium aspartate, 20 CsCl, 1 MgCl2,
2 MgATP, 0.5 GTP, 5 EGTA, and 5 HEPES (pH 7.3). These solutions
provided isolation of Ica, from other membrane currents such as Na+
and K+ channel currents and also from Ca2+ flux through the
Na+/Ca2+ exchanger (NCX). For action potential and K+ current
recordings, myocytes were perfused with normal Tyrode solution that
contained (in mM) 135 NaCl, 1.8 CaCl2, 1 MgCl2, 5.4 KCl, 10
glucose, and 10 HEPES (pH 7.3). The pipette filling solution for action potential recordings contained 200 μM/ml amphotericin and
(in mM) 140 KCl, 2 MgCl2, 10 NaCl, 2 ATP, and 5 HEPES (pH 7.3). For
the K+ current measurements, 10 μM nifedipine was added to block
Ica, and patch-pipette solution contained (in mM) 110 potassium
aspartate, 20 KCl, 2 MgCl2, 2 ATP, 0.5 GTP, 5 EGTA, and 5 HEPES
(pH 7.3). For measurements of Iacaw, the external solution contained
(in mM) 150 NaCl, 2 CsCl, 2 MgCl2, 1 CaCl2, 0.001 nifedipine, 0.02
ouabain, and 5 HEPES (pH 7.3). The pipette solution contained (in
mM) 20 NaOH, 110 CsOH, 50 aspartic acid, 1 MgCl2, 2 MgATP, 42
EGTA, and 5 HEPES (pH 7.4). The concentration of free internal
Ca2+ was adjusted to 67 nM by adding CaCl2 (15). To activate Iacaw,
the cells were held at -40 mV and the external solution was rapidly
switched to one in which equimolar LiCl was substituted for NaCl.
Exposure of the myocytes to Na+-free solution produced outward
Na+ extension through the NCX (21, 33).

The SR Ca2+ content was evaluated by a train of 10 conditioning
pulses (100 ms at 0.5 Hz, from -70 to +10 mV) to load SR Ca2+ (26, 33, 41, 45). The cell was then superfused rapidly with an external
solution that contained caffeine (10 mM). The external solution
contained (in mM) 150 NaCl, 2 CsCl, 2 MgCl2, 1 CaCl2, and 5
HEPES (pH 7.3). The pipette solution contained (in mM) 150 CsCl,
1 MgCl2, 2 MgATP, 0.1 EGTA, and 5 HEPES (pH 7.3).

Western blotting. Quantitative immunoblotting was performed as
previously reported (19, 20). Antibodies to phospholamban (PLB),
calsequestrin (CSQ, sarco(endo)plasmic reticulum Ca2+ -ATPase 2a,
(SERCA2a), and the NCX were obtained from Affinity Bioreagents
(Golden, CO), and antibodies to PLB were obtained from Cyclacel
(Dundee, UK). The LV tissue was homogenized on ice with a
Polytron in buffer [50 mM Tris-HCl (pH 7.5) and 10 mM histidine]. The
protein content was then diluted in an equal volume of lysis buffer.
Protein concentration was measured with bicinechonic acid (Pierce).

RESULTS

In our prior study (25), we found that NHB woodchucks
have a body temperature of 36 ± 0.5°C and heart rate of 86 ± 4
beats/min, whereas in HB woodchucks heart rate decreased to
27 ± 8 beats/min and temperature decreased to 13 ± 0.6°C. In the
present study, we confirmed these data, i.e., the HB
woodchucks had a decreased heart rate (17 ± 5 beats/min) and
decreased body temperature (13 ± 1.0°C), which are the
conditions whereby the hearts of NHB animals would develop
ventricular fibrillation and stop functioning (16).

Figure 1 shows twitch contraction and Ca2+ transients in
myocytes isolated from NHB and HB hearts under steady-state
conditions (1.0 Hz). The amplitudes of myocyte contraction
and Ca2+ transient are similar between the two groups. The
pooled data (right) indicate that the maximum rate of contraction
(−dL/dt) was similar in NHB and HB myocytes, but relaxation
rate (+dL/dt) was slower in HB myocytes; however, the
TRC70 time was faster in HB myocytes. These results are
consistent with previous muscle contraction data with electrical
stimulation on hibernators, which showed that the magnitude of
muscle contraction remains at a relatively high level during
hibernation (49). Furthermore, the data suggest that the availability
of activator Ca2+ for contraction is not altered in HB
myocytes.

Action potentials. Action potentials were recorded in NHB
and HB myocytes in Tyrode solution (Fig. 2). NHB myocytes
typically had a rapid upstroke and a plateau before repolariza-

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in NHB myocytes, and HB myocytes isolated from HB or NHB hearts with signif-
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guinea pig, rabbit, or dog (35, 51, 53). HB myocytes displayed a shorter action potential compared with NHB myocytes. APD values quantified at 50% and 90% repolarization (APD50 and APD90, respectively) were significantly shorter in HB compared with NHB myocytes (Table 1). There were no significant differences in the resting membrane potentials and overshoot potentials between the two groups.

**K+ channel currents.** The characteristic shape and duration of an action potential are determined by the intricate balance of the depolarizing and repolarizing currents (6, 39, 50). Woodchuck myocytes are rarely used for electrophysiological studies, and variations in the expression of membrane currents in LV myocytes have not yet been characterized. We found that 4-aminopyridine-sensitive transient outward K+/H+ currents, which could contribute to action potential repolarization (34, 37), are present in woodchuck myocytes (Fig. 3B, inset). The K+ channel blocker tetraethylammonium chloride (10 mM) had no significant effect (not shown). As shown in Fig. 2, there was no significant change in amplitude between the two groups.

Similarly, there was no significant difference in the density of the inward rectifier K+ currents between NHB and HB myocytes. The mean current densities at −100 mV in NHB and HB myocytes were −10.8 ± 0.6 (n = 15) and −10.1 ± 0.5 pA/pF (n = 12), respectively. Thus it appears that changes in repolarizing currents are not associated with action potential shortening in HB myocytes.

**L-type Ca2+ currents.** There were no significant changes in K+ channel currents in HB myocytes. Thus a change in the amplitude and/or kinetics of I_{Ca} could explain the altered action potential profile that we observed. To evaluate this, we analyzed I_{Ca} (Fig. 4, A and B). The peak inward I_{Ca} density value (peak I_{Ca} amplitude normalized relative to cell capacitance) was ~36% smaller in HB myocytes (4.4 ± 0.2 pA/pF; n = 97) compared with NHB myocytes (6.0 ± 0.3 pA/pF; n = 92). There was no significant change in the current-voltage relationship between the two groups (Fig. 4C).

As shown in Fig. 4D, the expression level of the L-type Ca2+ channel α1C-subunit, which contains the ion-conducting pore (9) in HB hearts, was reduced by 35 ± 8%; this is consistent with the decreased I_{Ca} being due to fewer Ca2+ channels (rather than altered regulation). However, because we were unable to measure protein levels owing to the unavailability of the antibody against the general β-subunit, we cannot exclude the possibility that alterations in the β-subunit levels might influence channel activity.

I_{Ca} decay kinetics are also an important parameter for Ca2+ entry. Thus we analyzed whether the time course of inactiva-

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**Fig. 1.** Typical examples of myocyte contractions (A) and fura 2 Ca2+ transients (B) recorded in left ventricular (LV) myocytes isolated from hearts of nonhibernating (NHB) and hibernating (HB) woodchucks. Left: contractions and Ca2+ transients were recorded during field stimulation at a frequency of 1.0 Hz. Right: summaries of results. Data are means ± SE of NHB (n = 40) and HB (n = 32) myocytes for contractile parameters on measurements and NHB (n = 30) and HB (n = 24) myocytes from five NHB and five HB woodchucks. *P < 0.05. TRC70%, time for 70% decay of Ca2+ transient; −dL/dt, maximum rate of contraction; +dL/dt, relaxation rate.
tion was altered in HB myocytes (Fig. 5). At the peak potential (+10 mV), \( I_{Ca} \) inactivated rapidly during maintained depolarization in both groups. Despite the smaller \( I_{Ca} \) value, HB myocytes exhibited significantly faster inactivation compared with NHB myocytes (Fig. 5A). The times to half-decay (\( t_{1/2} \)) in HB (\( n = 60 \)) and NHB (\( n = 91 \)) myocytes were 24 ± 2 and 37 ± 2 ms, respectively (Fig. 5C). Decreased \( I_{Ca} \) amplitude is often associated with prolonged inactivation due to less Ca\(^{2+}\)-induced channel inactivation (52), therefore, the more rapid inactivation could have been secondary to increased SR Ca\(^{2+}\) loading and release (20, 32, 42). Thus we measured \( t_{1/2} \) in the presence of ryanodine (10 \( \mu \)M). After the application of ryanodine, the rate of \( I_{Ca} \) inactivation as measured by \( t_{1/2} \) was increased in both groups (Fig. 5A). There was no significant difference in the inactivation rate between the two groups (Fig. 5, B and C), which indicates that Ca\(^{2+}\)-dependent inactivation was increased in HB myocytes due to enhanced Ca\(^{2+}\) release from the SR in response to \( I_{Ca} \).

Measurement of \( I_{Na/Ca} \). We next examined \( I_{Na/Ca} \) in myocytes from NHB and HB hearts (Fig. 6). The myocytes were held at −40 mV, and \( I_{Na/Ca} \) was activated by rapidly reducing the external Na\(^{+}\) concentration (21, 26, 33). When the external Na\(^{+}\) was replaced by Li\(^{+}\), the membrane current shifted to an outward direction in both groups (Fig. 6, A and B). There was no significant difference in the peak amplitude of \( I_{Na/Ca} \) between NHB and HB myocytes. Under our experimental conditions, the average \( I_{Na/Ca} \) densities in NHB and HB myocytes were 0.5 ± 0.05 (\( n = 38 \)) and 0.5 ± 0.06 pA/pF (\( n = 33 \)), respectively (Fig. 6C). The results indicate that changes in Ca\(^{2+}\) influx and/or efflux through the NCX are unlikely to occur in HB myocytes.

SR Ca\(^{2+}\) content measured as caffeine-induced \( I_{Na/Ca} \). Taken together, the electrophysiological data suggest that smaller \( I_{Ca} \) density and enhanced SR Ca\(^{2+}\) release during twitches contribute to the cardiac phenotype observed in HB myocytes. To further test for this, we measured SR Ca\(^{2+}\) content by integrating \( I_{Na/Ca} \) after a rapid application of 10 mM caffeine (8, 26, 41). Figure 7, A and B, shows the inward currents during caffeine application, which are thought to be the result of a transient increase of intracellular Ca\(^{2+}\) that is accompanied by a transient \( I_{Na/Ca} \). In both HB and NHB myocytes, caffeine activated \( I_{Na/Ca} \), but the integrated current was significantly larger in HB myocytes (Fig. 7C).

Ca\(^{2+}\) regulatory protein and gene expression. To examine changes in mRNA levels of Ca\(^{2+}\) transport proteins during hibernation, we performed RT-PCR analysis on RNA isolated from NHB and HB hearts using specific primers for SERCA2a, PLB, CSQ, and NCX (Fig. 8). The mRNA level of SERCA2a was increased threefold, whereas the transcript that encodes PLB was decreased by 50% in HB hearts compared with NHB hearts. There were no changes in CSQ or NCX gene expression.

Western blot analysis of NHB and HB hearts revealed a pattern of changes similar to gene expression of SERCA2a, PLB, NCX, and CSQ (Fig. 9): SERCA2a protein levels were increased by 300 ± 35%, whereas PLB levels decreased by 55 ± 5% in HB hearts compared with NHB hearts. Thus the ratio of PLB to SERCA2a was significantly reduced in HB hearts, which indicates that a larger portion of the pumps were in the “uninhibited state” relative to NHB hearts. There were no changes in CSQ and NCX expression levels.

DISCUSSION

In the present study, we found that 1) HB myocytes have significantly shorter APDs compared with NHB myocytes but no change in contractile amplitude; 2) there was no change in K\(^{+}\) channel currents, but \( I_{Ca} \) density was significantly decreased in HB myocytes; and 3) HB myocytes had enhanced SR Ca\(^{2+}\) uptake capacity but no change in NCX. Thus our data suggest that the significantly enhanced SR Ca\(^{2+}\) uptake capacity and shorter APD that resulted from a reduction of Ca\(^{2+}\) channels contribute to maintain normal diastolic and systolic function during hibernation (16, 25). Because electrophysiological remodeling and defects in cellular Ca\(^{2+}\) handling are a major cause of ventricular arrhythmias and fibrillation in failing hearts (28), data obtained during natural hibernation may

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<th>Table 1. Action potential characteristics recorded from NHB and HB myocytes</th>
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Values are means ± SE; \( n \), no. of cells examined from 4 hibernating (HB) and 4 nonhibernating (NHB) woodchucks. APD\(_{50}\) and APD\(_{90}\), action potential duration at 50% and 90% repolarization, respectively. *\( P < 0.05 \) vs. NHB myocytes.
Fig. 3. Transient outward currents recorded in NHB and HB myocytes. Representative families of currents were elicited by voltage steps from −60 to +60 mV in 20-mV increments from a holding potential of −80 mV (A). Current-voltage relationships for peak values were normalized to cell capacitance to yield current densities (B). Data are from six NHB (n = 17 cells) and four HB (n = 12 cells) woodchucks. Effects of 10 mM 4-aminopyridine (4-AP) on K⁺ currents (+60 mV) in HB myocytes are shown (inset).

Fig. 4. A and B: whole cell Ca²⁺ current (I_{Ca}) characteristics in NHB (A) and HB (B) myocytes. Currents were elicited from a holding potential of −50 mV to the indicated test potentials. C: current-voltage relationships in NHB and HB myocytes. I_{Ca} was normalized to cell capacitance to obtain current densities. Data are from six NHB (n = 60 cells) and five HB (n = 83 cells) woodchucks. D: Western blot analyses of L-type Ca²⁺ channel α₁l subunits for NHB and HB hearts. Averaged values for NHB (N = 6 hearts) and HB (N = 6 hearts) animals are plotted below the immunoblots. *P < 0.01.
provide unique insights into the treatment of patients with heart failure.

**Ionic mechanisms of APD shortening.** Previous studies of intact papillary muscles isolated from HB chipmunks or ground squirrels have reported an absence of action potential plateau but no data at the cellular level (24, 47). Our data revealed that ventricular myocytes isolated from HB woodchucks have significantly shorter APD 50 and APD 90 values.
compared with NHB myocytes. There was no significant difference in the resting membrane potential between the two groups. These data are in sharp contrast to the action potential prolongation that occurs in ventricular myocytes from failing hearts (3, 28, 43).

In cardiac hypertrophy and failure, prolongation of the action potential repolarization is often associated with a reduction in K⁺ channel currents (17, 37, 43). In the present study, we found that woodchuck ventricular myocytes express inward rectifier and transient outward K⁺ currents; however, these currents were unchanged during hibernation. Instead, we observed a significant reduction of peak I_{Ca} density in HB myocytes. Furthermore, HB myocytes exhibited significantly faster I_{Ca} inactivation compared with NHB myocytes. There was no change in the voltage-dependent activation character-
Altered Ca2+ Handling in Hibernating Hearts

A faster rate of Ca2+ uptake and a greater level of Ca2+ accumulation in cardiac SR vesicles isolated from HB ground squirrels were previously reported by Belke et al. (5). However, in that study, the high rate of Ca2+ uptake was not associated with enhanced enzymatic activity or total amount of SERCA. In the present study, using Western blotting, we demonstrated that changes in expression levels of Ca2+-regulated proteins are responsible for changes in Ca2+ homeostasis during hibernation. For example, the simple explanation for the reduced Ica in HB myocytes would be a decrease in the numbers of Ca2+-channels (instead of channel regulation). Similarly, the enhanced ability of Ca2+ uptake by intracellular Ca2+ stores in HB hearts compared with NHB hearts could be explained by a change in the relative ratio of SERCA2a (threefold increase) to PLB (55% decrease). In this regard, we found no other changes such as NCX or the Ca2+-binding protein CSQ in the SR, which increases the Ca2+ storage capacity (40). Thus the origin of activator Ca2+ for contraction is significantly shifted to SR Ca2+ release from extracellular Ca2+ entry in HB myocytes. Increased SERCA and decreased PLB appear to be critical contributors to maintenance of contraction during hibernation. The increased SR Ca2+ uptake could explain the faster rate of Ca2+ decline during twitch observed in HB myocytes.

Importantly, the changes we observed in HB myocytes are directionally opposite to those in myocytes from failing hearts. Most previous studies in cardiac hypertrophy and failure have shown that changes in Ca2+ handling are due to a diminished relative protein expression of SERCA relative to PLB (12). For example, it has been shown (12) that SERCA protein levels were significantly reduced in failing hearts while unchanged protein levels of PLB were demonstrated. These changes are often associated with compensatory upregulation of NCX (13, 14, 36).

In addition, it is well documented that ventricular contractile dysfunction after ischemia and infarction is associated with electrical remodeling and changes in Ca2+ handling at the cellular level (10, 11, 38). An abnormal increase in resting Ca2+ has also been reported in ischemia and hypoxia (2). Increasing SR Ca2+ uptake usually causes SR Ca2+ overload, which is a predisposing factor for the development of arrhythmias, but no ventricular dysfunction or arrhythmias were found in HB animals. In this study, we have not directly addressed alternative explanations for the maintained contractility during hibernation despite decreased Ca2+ influx. These include enhanced Ca2+ sensitivity of myofilaments that may be related to a slower relaxation time observed in HB myocytes, increased responsiveness of SR Ca2+ release channels, and/or enhanced functional coupling of L-type Ca2+ channels and SR Ca2+ release channels. Future studies could further delineate which cellular processes are critically involved in natural resistance to ventricular fibrillation. However, our results strongly support the hypothesis that SR Ca2+ uptake is markedly enhanced during true hibernation. This coupled with shorter APD associated with downregulation and increased Ca2+-dependent inactivation of Ica may in part provide a coordinated cardioprotective mechanism to prevent intracellular Ca2+ overload and/or metabolic demand associated with intracellular Ca2+ cycling in HB myocytes.
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GRANTS

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