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Photoperiod-dependent modulation of cardiac excitation contraction coupling in the Siberian hamster

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Dibb, K. M., C. L. Hagarty, A. S. I. Loudon, and A. W. Trafford. Photoperiod-dependent modulation of cardiac excitation contraction coupling in the Siberian hamster. *Am J Physiol Regul Integr Comp Physiol* 288: R607–R614, 2005. First published November 4, 2004; doi:10.1152/ajpregu.00612.2004.—In mammals, changes in photoperiod regulate a diverse array of physiological and behavioral processes, an example of which in the Siberian hamster (*Phodopus sungorus*) is the expression of bouts of daily torpor following prolonged exposure to a short photoperiod. During torpor, body temperature drops dramatically; however, unlike in nonhibernating or nontorpid species, the myocardium retains the ability to contract and is resistant to the development of arrhythmias. In the present study, we sought to determine whether exposure to a short photoperiod results in alterations to cardiac excitation-contraction coupling, thus potentially enabling the heart to survive periods of low temperature during torpor. Experiments were performed on single ventricular myocytes freshly isolated from the hearts of Siberian hamsters that had been exposed to either 12 wk of short-day lengths (SD) or 12 wk of long-day lengths (LD). In SD-acclimated animals, the amplitude of the systolic Ca^{2+} transient was increased (e.g., from 142 ± 17 nmol/l in LD to 229 ± 31 nmol/l in SD at 4 Hz; $P < 0.001$). The increased Ca^{2+} transient amplitude in the SD-acclimated animals was not associated with any change in the shape or duration of the action potential. However, sarcoplasmic reticulum Ca^{2+} content measured after current-clamp stimulation was increased in the SD-acclimated animals (at 4 Hz, 110 ± 5 vs. 141 ± 15 μmol/l, $P < 0.05$). We propose that short photoperiods reprogram the function of the cardiac sarcoplasmic reticulum, resulting in an increased Ca^{2+} content, and that this may be a necessary precursor for maintenance of cardiac function during winter torpor.

calcium; sarcoplasmic reticulum; torpor; action potential

BIOLOGICAL TIMING IS AN IMPORTANT requisite for an extraordinarily large number of behavioral and physiological processes. Such timing occurs in response to daily and seasonal alterations in the outputs of various clocks located centrally within the suprachiasmatic nucleus of the hypothalamus and peripherally in many diverse tissues, including the heart, gut, and lung (6, 37). Within the cardiovascular system, changes in the output of the central and local circadian clocks may, by driving circadian changes in cardiac ion channels and metabolic gene expression (34, 36), be in part responsible for the prevalence of tachyarrhythmias at particular times of day in the atria (night) and ventricle (early morning) (13, 33). Over a much longer timescale, i.e., seasonally, photoperiod-induced changes in the output of the central and peripheral circadian clocks are also capable of causing marked alterations in the cardiac physiology of certain species, as exemplified by the Siberian hamster (*Phodopus sungorus*).

In the Siberian hamster, prolonged (~12 wk) exposure to short-day lengths (SD) results in a number of physiological alterations, including weight loss, appearance of winter pelage, gonadal regression, and ultimately the occurrence of bouts of daily torpor (12, 32). During the torpor cycle, the animal's body temperature drops spontaneously by ~20°C, thereby considerably reducing energy requirements during harsh winter conditions. Unlike those species in which hibernation and torpor do not occur, cardiac contractility is maintained at these low temperatures (5, 23). In addition to exhibiting preserved cardiac contractility during exposure to low temperatures, the hearts of hibernating and torpid animals are also extremely resistant to the induction of ventricular tachyarrhythmias (10, 11). This latter effect (resistance to ventricular fibrillation) is clearly an adaptive response because it occurs in torpid animals after exposure to SD, whereas in summer [in long-day length (LD)-adapted animals], it is still possible to induce arrhythmias (10).

At the cellular level, the sarcoplasmic reticulum (SR) is pivotal to determining both the inotropic (27, 29) and arrhythmogenic (8, 14) status of the heart. Ultrastructural studies have provided evidence that the amount of SR in the Golden hamster (a hibernator) is greater than in the rat (a nonhibernator) and is increased still further during hibernation (28). Furthermore, the Ca^{2+} uptake rate and the maximal Ca^{2+} binding capacity of SR vesicles also change with photoperiod and hibernation, being higher in SD- than in LD-acclimated animals and greater still in hibernating animals (4). The enhanced Ca^{2+} binding capacity of SR in hibernators may be the result of increased glycosylation of calsequestrin (CSQ) (24). Thus available evidence suggests that reprogramming of the cardiac SR is a key step in preparing the myocardium for the rigors of hibernation and low-temperature torpor. Indeed, an increase in SR Ca^{2+} content has recently been measured in the hibernating woodchuck (*Marmota monax*), although these data were obtained after stimulation under nonphysiological conditions of temperature, stimulation rate, and square voltage-clamp pulses (35).

Thus, whereas altered SR function may be key to the adaptive responses of the heart to maintain contractility in hibernation, the increased SR Ca^{2+} content does not support the concept of increased resistance to arrhythmias (8, 14). However, the SR Ca^{2+} release channel, the ryanodine receptor (RyR), also undergoes a series of biochemical and functional alterations in the heart of hibernators, including decreased Ca^{2+} sensitivity (21) and greater RyR density (24). Although these changes increase the sensitivity of contraction in the heart of hibernators to ryanodine (19), their contribution to increased resistance to arrhythmias remains undetermined.

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In the present study, we utilized an animal model that exhibits low-temperature torpor after prolonged exposure to SD to determine whether photoperiod per se results in a reprogramming of the cellular mechanisms controlling cardiac excitation-contraction coupling, cellular Ca^{2+} homeostasis, and the cardiac action potential. We assessed these using conditions of temperature, stimulation frequency, and minimal intracellular dialysis, which mimicked the physiological situation as closely as possible. Our data show that in response to SD acclimation profound changes in cardiac excitation-contraction coupling occur, including an increase in the amplitude of the systolic Ca^{2+} transient and an enhanced SR Ca^{2+} content. We propose that, as a result of SD photic entrainment, the function of sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) is increased, resulting in the increased SR Ca^{2+} content and enhanced systolic Ca^{2+} transient, and that such changes may be a necessary prerequisite to the maintenance of cardiac function during periods of low-temperature torpor and hibernation. Understanding the molecular switches that induce such changes may have future therapeutic benefits in protecting the hearts of nonhibernating species against cardiac arrhythmias.

MATERIALS AND METHODS

All experimental procedures involving animals comply with the United Kingdom Animals (Scientific Procedures) Act, 1986.

Animal model. Siberian hamsters (*Phodopus sungorus*) were maintained under controlled laboratory conditions (21 ± 1°C, 80% relative humidity) as described previously (2). Experimental animals were group housed in light-controlled environmental chambers lit by a 70-W fluorescent white strip (100–400 lux) with continuous dim red light (<1 lux) throughout. Animals were either entrained to an SD photoperiod of 8 h of white light [Zeitgeber time (ZT) 0–8] and 16 h of dim red light (ZT 8–24) or an LD photoperiod of 16 h of white light (ZT 0–16) and 8 h of dim red light (ZT 16–24) for a period of 12 wk.

Cardiac myocyte isolation. Animals were killed by cervical dislocation at midlight phase (ZT 4 for SD and ZT 8 for LD), and single ventricular myocytes were isolated by a modified collagenase and protease digestion technique (29). The heart was rapidly excised and placed in warmed (37°C) Ca^{2+}-free medium containing (in mmol/l) 134 NaCl, 11 glucose, 10 HEPES, 4 KCl, 1.2 $MgSO_4$, and 1.2 Na_2HPO_4 (pH 7.34 with NaOH). The heart was then cannulated via the aorta and retrogradely perfused with the Ca^{2+}-free medium for 6–7 min before 0.5 mg/ml collagenase H (Boehringer) and 0.05 mg/ml protease XIV (Sigma) were recirculated through the heart until the ventricles became flaccid (~9 min). The heart was then perfused with a taurine solution for 15 min before the ventricles were dissected, minced, and single myocytes released into the taurine solution by gentle trituration. The taurine solution contained (in mmol/l) 113 NaCl, 50 taurine, 11 glucose, 10 HEPES, 4 KCl, 1.2 $MgSO_4$, 1.2 Na_2HPO_4, and 0.1 $CaCl_2$ (pH 7.34 with NaOH).

Measurements of intracellular Ca^{2+} concentration. Cells were loaded with the membrane-permeant acetoxymethyl ester of the Ca^{2+}-sensitive fluorescent indicator fluo 3 at a final concentration of 5 μmol/l for 5 min at room temperature (9, 29) before resuspension in the experimental modified Tyrode solution containing (in mmol/l) 140 NaCl, 10 glucose, 10 HEPES, 4 KCl, 2 probenecid, 1 $CaCl_2$, and 1 $MgCl_2$ (pH 7.34 with NaOH). Cells were allowed to deesterify for at least 30 min before being placed in the experimental chamber of an inverted fluorescence microscope (Nikon), and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured as described previously, assuming a K_d for $[Ca^{2+}]_i$ of 864 nmol/l (9, 16, 29). The temperature of the superfusate was maintained at 37 ± 0.1°C by a microperfusion apparatus (Cell Microcontrols).

Action potentials and SR Ca^{2+} content. Cells were voltage clamped by using the perforated patch technique with amphotericin B (240 μg/ml) as previously described (9, 29). The switch-clamp facility of the Axoclamp-2B voltage-clamp amplifier (Molecular Devices) was used to overcome access resistance problems of the perforated patch method (rate of 2–3 kHz). Action potentials were measured in bridge mode and elicited with a 2-ms duration depolarizing current pulse (1–2 nA). SR Ca^{2+} content was measured after steady-state current clamp stimulation by rapidly switching from bridge to voltage-clamp mode and holding membrane potential at −80 mV before applying 10 mmol/l caffeine to the superfusate to discharge the SR Ca^{2+} store. Integration of the resulting inward Na^+/Ca^{2+} exchange current was performed to assess the SR Ca^{2+} content as described previously (9, 29, 31) assuming a surface area-to-volume ratio of 6.76 pF/pl. Glass microelectrodes had resistances of <3 MΩ when filled with (in mmol/l) 125 KCH_3O_3S, 20 KCl, 10 NaCl, 10 HEPES, and 5 $MgCl_2$ (pH 7.2 with KOH).

Statistics. All values are presented as means ± SE for n experiments. Differences between groups were tested with either Student's t-tests or by two-way repeated measures ANOVA or two-way ANOVA where appropriate and considered significant when $P < 0.05$.

RESULTS

At the time of cell isolation, none of the SD animals was acutely torpid. Furthermore, to the best of our knowledge, none of the animals had exhibited bouts of torpor before the time of death. The data in Table 1 demonstrate that, in agreement with previous studies (2, 26), the body weights of the SD animals decreased during the 12-wk acclimation period, whereas those of the LD animals increased. During this time, all of the SD animals developed a white winter pelage, whereas the entire LD group retained their summer agouti pelage.

Intracellular Ca^{2+} transients and cell size. In the first series of experiments, we determined whether entrainment of hamsters to SD resulted in alterations of the systolic Ca^{2+} transient. Figure 1*A* shows typical $[Ca^{2+}]_i$-frequency responses obtained from a single ventricular myocyte of an SD-acclimated animal. Qualitatively similar responses were also observed in LD-acclimated animals. With increasing stimulation frequency, diastolic $[Ca^{2+}]_i$ increased similarly in both groups (Fig. 1*B*; from 133 ± 10 vs. 123 ± 9 nmol/l at 4 Hz to 194 ± 11 vs. 207 ± 13 nmol/l at 6 Hz; n = 12–16 cells from 5–6 animals). The amplitude of the systolic Ca^{2+} transient (Fig. 1*C*) exhibited a biphasic relationship with stimulation frequency increasing between 1 and 6 Hz and then decreasing at 8 Hz. However, the amplitude of the systolic Ca^{2+} transient was greater in the SD-acclimated animals: 229 ± 31 vs. 142 ± 17 nmol/l at 4 Hz and 369 ± 46 vs. 193 ± 27 nmol/l at 6 Hz ($P < 0.001$). Finally, the rate of rise of the systolic Ca^{2+} transient during

Table 1. *Body weight changes in response to shortened day lengths*

	Week 0	Week 3	Week 6	Week 9	Week 12
Long day, g	34.3±0.9	35.7±0.6	37.4±0.8*	38.8±0.9†	39.2±1.0†
Short day, g	34.9±1.2	33.6±1.0	32.5±1.2	31.0±0.9‡	31.0±0.9‡
P (LD vs. SD)	0.65	0.15	<0.001	<0.001	<0.001

Values are means ± SE body weight changes measured every 3 wk during photoperiod acclimation; n = 7 long-day length (LD) and 6 short-day length (SD) animals. *$P < 0.05$ vs. LD initial weight. †$P < 0.01$ vs. LD initial weight. ‡$P < 0.05$ vs. SD initial weight.

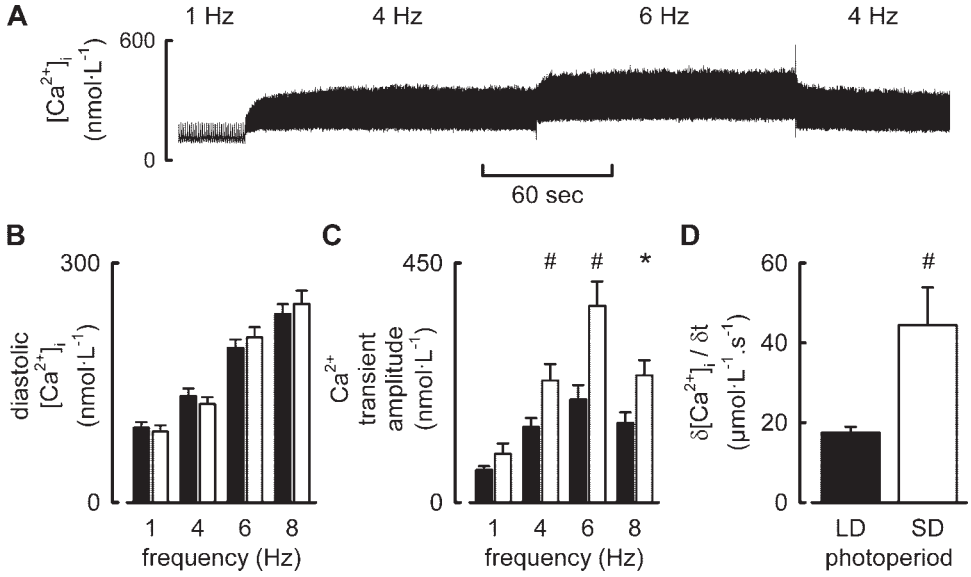

Fig. 1. Enhanced systolic Ca^{2+} transients in response to short day acclimation. *A*: time course of changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) measured in a single ventricular myocyte isolated from a short-day length (SD)-acclimated hamster. Action potentials were elicited at the frequencies shown above the record by application of a 2-ms depolarizing pulse in current clamp mode. *B*: average data for diastolic $[Ca^{2+}]_i$ at the frequencies indicated for long-day length (LD)-acclimated (solid bars) and SD (open bars)-acclimated animals. *C*: systolic Ca^{2+} transient amplitude mean data for LD (solid bars)-acclimated and SD (open bars)-acclimated animals obtained at the stimulation frequencies indicated. *D*: mean data summarizing the rate of rise of the systolic Ca^{2+} transient in LD and SD animals. Ca^{2+} transients were elicited under current clamp control at 4 Hz. $*P < 0.05$; $\# P < 0.01$.

4-Hz stimulation was determined by applying a linear regression to the rising phase of the systolic Ca^{2+} transient over the range of 10–70% of the difference between the diastolic and peak systolic $[Ca^{2+}]_i$ (Fig. 1*D*). On average, the rate of rise of $[Ca^{2+}]_i$ increased significantly from 17.5 ± 2 $\mu mol \cdot l^{-1} \cdot s^{-1}$ in LD animals to 44 ± 9 $\mu mol \cdot l^{-1} \cdot s^{-1}$ in SD-acclimated animals ($P < 0.01$).

The above changes in the systolic Ca^{2+} transient were not associated with any change in the capacitance of the myocytes, which we measured using a 10-mV hyperpolarizing pulse from a holding potential of −80 mV. On average, the capacitance was 89.6 ± 4 pF in myocytes isolated from LD animals and 96.1 ± 6 pF in SD animals (n = 25–28 cells, 7–9 hearts; $P > 0.4$).

Action potential properties. Changes in action potential duration are known to influence the amplitude of the systolic Ca^{2+} transient (17). Thus, in the next series of experiments, we sought to determine whether increases in action potential duration were responsible for the larger systolic Ca^{2+} transients observed in the SD-acclimated hamster under physiological conditions. Figure 2*A* illustrates a typical action potential obtained from an LD-acclimated animal at a stimulation frequency of 4 Hz. Figure 2*B* demonstrates that neither the resting membrane potential at 4-Hz stimulation frequency (−78 ± 1 vs. −75 ± 1 mV) nor the peak membrane potential (23 ± 2 vs. 17 ± 1 mV) was altered by photoperiod (LD vs. SD, n = 13–14 cells, 4–5 animals; $P > 0.3$). Furthermore, no differences in action potential shape were detected, as the repolarization time was similar in both groups (Fig. 2*C*).

Figure 2*D* illustrates the frequency-dependent changes in action potential morphology of a typical hamster SD myocyte; these data show that at least part of the increase in Ca^{2+}

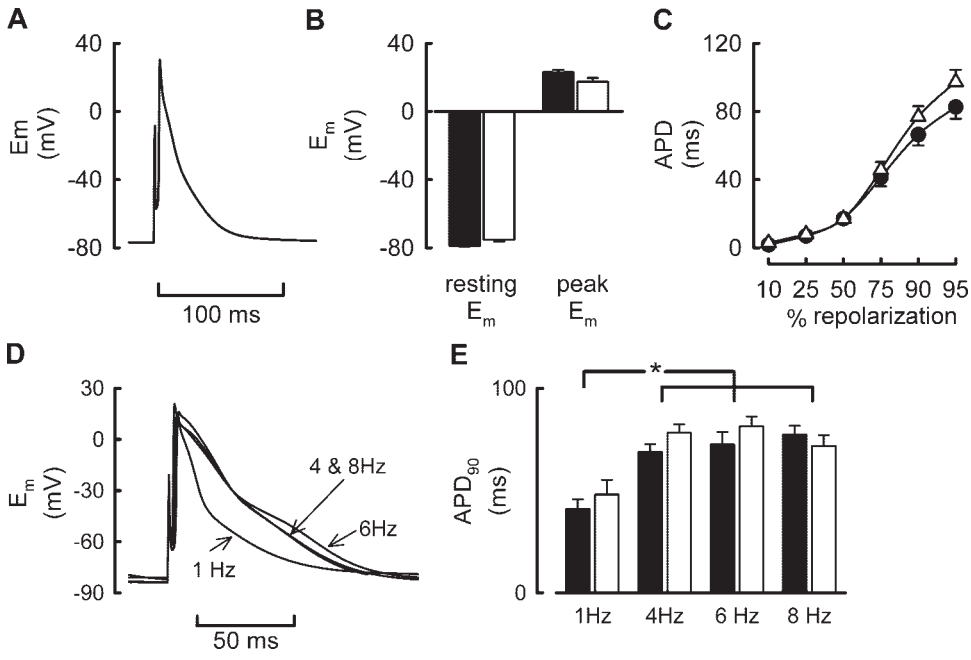

Fig. 2. Action potential properties are unaltered by photoperiod. *A*: typical action potential from an LD myocyte; stimulation rate was 4 Hz, and action potential was measured using the perforated patch technique. E_m, membrane potential. *B*: mean data summarizing resting E_m (*left*) and peak E_m (*right*) after 4-Hz stimulation in LD (solid bars) and SD (open bars) myocytes. *C*: time taken to reach varying extents of repolarization following stimulation at 4 Hz in LD (solid) and SD (open) cells. APD, action potential duration. *D*: frequency-dependent changes in action potential shape in a typical SD myocyte. *E*: mean data for time to 90% repolarization of the action potential measured at the stimulation frequencies indicated for LD (solid) and SD (open) animals. $*P < 0.05$ between 1 Hz and 4, 6, and 8 Hz.

transient amplitude on increasing stimulation frequency from 1 to 4–8 Hz is explicable in terms of longer action potential duration. However, the larger Ca^{2+} transients observed in the SD-acclimated animals cannot be explained by changes in action potential duration because this is indistinguishable in both groups at these higher frequencies (Fig. 2*E*; time to 90% repolarization at 4 Hz is 69.8 ± 8 vs. 78.2 ± 8 ms and at 8 Hz it is 77.2 ± 4 vs. 71.7 ± 5 ms in LD and SD animals, respectively; $P > 0.2$, $n = 7$–12 cells, 4–5 animals). Thus factors other than alterations to the properties of the action potential must be responsible for the differences in the systolic Ca^{2+} transient in response to altered photoperiod in the hamster.

SR Ca^{2+} content. Given the strong dependence of the systolic Ca^{2+} transient on SR Ca^{2+} content (3, 29) and the absence of any change in action potential duration to offer an explanation for the larger systolic Ca^{2+} transients observed in the SD animals, we have determined whether changes in photoperiod alter SR Ca^{2+} content. After steady-state stimulation with action potentials elicited at either 4 or 6 Hz, cells were placed under voltage-clamp control and 10 mmol/l caffeine was rapidly applied to discharge the SR store (31). The resulting Na^+/Ca^{2+} exchange current was integrated to give a measure of the SR Ca^{2+} content. Figure 3*A* shows that, in a typical SD myocyte, the amplitude of the caffeine-evoked Ca^{2+} transient, the peak inward Na^+/Ca^{2+} exchange current, and the resultant integral are larger than these results in the LD myocyte, indicating an increase in SR Ca^{2+} content. On average, SR Ca^{2+} content is 34% greater in the SD-acclimated animals (Fig. 3*B*; 110 ± 5 vs. 141 ± 15 μmol/l at 4 Hz and 139 ± 9 vs. 196 ± 11 μmol/l at 6 Hz; $P < 0.05$, $n = 6$–11 cells, 4–5 hearts).

To demonstrate that the above quantitative differences in SR Ca^{2+} content are not due to photoperiod-induced alterations in the capacitance-volume relationship, we also measured the amplitude of the caffeine-evoked Ca^{2+} transients. Figure 3*C* shows that, after steady-state stimulation at 4 Hz, the amplitude of the caffeine-evoked Ca^{2+} transient increases in the SD-acclimated animals by 42% from 465 ± 42 to 663 ± 86 nmol/l ($P < 0.05$, $n = 8$–9 cells, 4–5 hearts). The increase in this qualitative assessment of SR Ca^{2+} content is of a similar magnitude to that obtained with the quantitative method of integrating the Na^+/Ca^{2+} exchange current and suggests that there are unlikely to be any major changes in the capacitance-to-volume ratio induced by photoperiod that might otherwise invalidate the quantitative assessments of SR Ca^{2+} content.

Na^+/Ca^{2+} exchange function. At the cellular level, the arrhythmogenic potential of the heart is controlled by both the state of loading of the SR and the efflux of Ca^{2+} across the sarcolemma by the electrogenic Na^+/Ca^{2+} exchanger. Although the increase in SR Ca^{2+} content in SD-acclimated animals potentially increases the probability of spontaneous SR Ca^{2+} release and thus arrhythmias, it is possible that photoperiod may also alter the function of the Na^+/Ca^{2+} exchanger, thus negating the effects of the increased SR Ca^{2+} content. We have examined Na^+/Ca^{2+} exchange function by determining the relationship between $[Ca^{2+}]_i$ and Na^+/Ca^{2+} exchange current during the decay phase of the caffeine-evoked Ca^{2+} transient. During this period, both $[Ca^{2+}]_i$ and Na^+/Ca^{2+} exchange current should be in equilibrium (7, 30). The data of Fig. 3*D* illustrate typical examples of this relationship, and the mean data of Fig. 3*E* demonstrate that the slope between Na^+/Ca^{2+} exchange current and $[Ca^{2+}]_i$ is unchanged by photoperiod (0.32 ± 0.02 and 0.29 ± 0.05 pA·nmol^{-1}·l^{-1} in the LD and SD groups, respectively; $P > 0.5$, $n = 8$–10, 4 animals/group). Thus the apparent decreased susceptibility to cardiac arrhythmias in SD-acclimated (or hibernating) animals does not occur because of decreased Na^+/Ca^{2+} exchange function or because of diminished SR Ca^{2+} content.

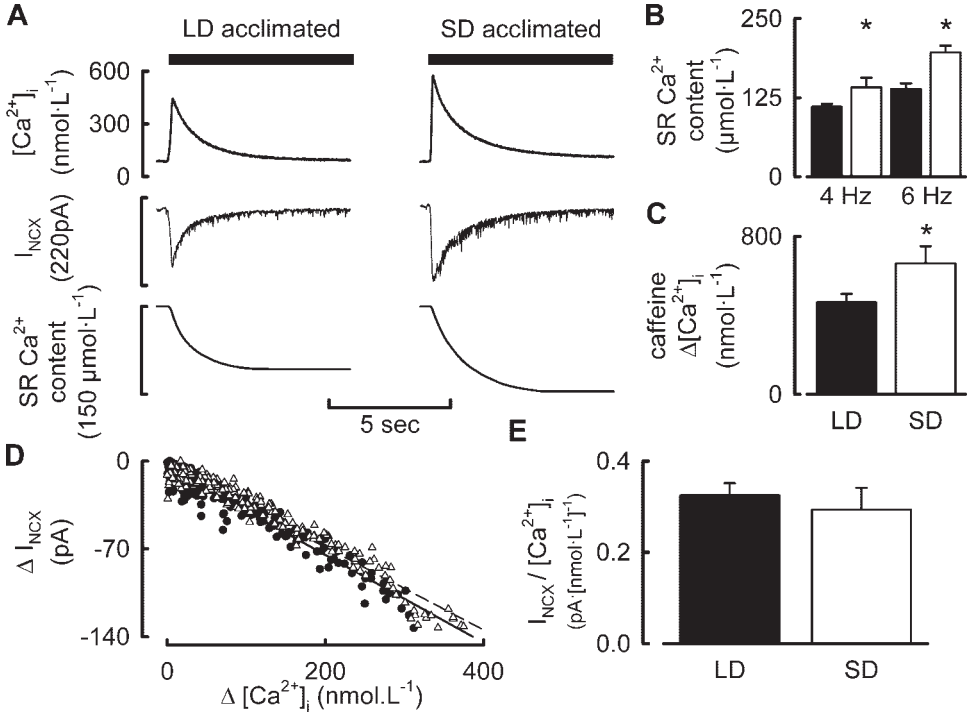

Fig. 3. Sarcoplasmic reticulum (SR) Ca^{2+} content and Na^+/Ca^{2+} exchange function. *A*: experimental method to assess SR Ca^{2+} content in typical LD (*left*) and SD (*right*) myocytes. Caffeine (10 mmol/l) was applied to the cells for the time indicated by the solid bar above the records, and the traces show $[Ca^{2+}]_i$ (*top*), Na^+/Ca^{2+} exchange current (I_{NCX}; *middle*), and integrated current trace (*bottom*). *B*: mean SR Ca^{2+} contents for LD (solid bars) and SD (open bars) cells after steady-state stimulation at the frequencies indicated. Measurements were made by the quantitative method detailed in *A*. *C*: caffeine-evoked Ca^{2+} transient amplitude mean data for LD and SD cells. *D*: relationship between Na^+/Ca^{2+} exchange current and $[Ca^{2+}]_i$ for a typical LD (solid symbol) and SD (open symbol) myocyte. *E*: mean data summarizing the I_{NCX}-$[Ca^{2+}]_i$ relationship obtained by fitting linear regressions to the data obtained in *D*. *$P < 0.05$.

SR Ca^{2+} uptake and fractional release. The purpose of the final series of experiments was twofold: *1*) to determine whether changes in fractional release of Ca^{2+} from the SR are responsible for increased Ca^{2+} transient amplitude in SD animals and *2*) to determine at the cellular level whether SERCA function was upregulated in response to SD, thus leading to the increased SR Ca^{2+} content. SR fractional release was determined qualitatively after steady-state current clamp stimulation at 4 Hz by assessing the ratio of the amplitude of the systolic Ca^{2+} transient (indicated by a in Fig. 4*A*) to the amplitude of the caffeine-evoked Ca^{2+} transient (indicated by b in Fig. 4*A*) (3). Figure 4*B* summarizes the mean data and shows that SR fractional release increases in SD animals (0.33 ± 0.04 vs. 0.56 ± 0.1, $P < 0.05$, $n = 10–11$ cells, 4–5 hearts).

To establish whether SERCA function is upregulated, we determined the SR-dependent rate of Ca^{2+} removal from the cytosol by fitting the decay phase of the systolic Ca^{2+} transient and the caffeine-evoked Ca^{2+} transients with single exponential functions. The rate constant of decay of the 4-Hz systolic Ca^{2+} transient (K_{sys}) increased from $10.58 \pm 0.4\ s^{-1}$ in LD cells to $13.85 \pm 1.1\ s^{-1}$ in SD cells (Fig. 4*C*, $P < 0.01$), whereas the rate constant of decay of the caffeine-evoked Ca^{2+} transient following 4-Hz stimulation (K_{caff}) did not change with photoperiod ($1.41 \pm 0.1\ s^{-1}$ in LD animals and $1.49 \pm 0.1\ s^{-1}$ in SD animals; $P > 0.6$). The lack of a difference in K_{caff} indicates that the rate of removal by sarcolemmal mechanisms, principally the Na^+/Ca^{2+} exchanger, is unaltered by photic entrainment and is also consistent with the data of Fig. 3, *D* and *E*. K_{sys} represents Ca^{2+} removal from the cytosol by SERCA, the Na^+/Ca^{2+} exchanger, and the sarcolemmal Ca^{2+}-ATPase and K_{caff} Ca^{2+} removal by the Na^+/Ca^{2+} exchanger and sarcolemmal Ca^{2+}-ATPase as SERCA activity is effectively negated by the maintained presence of caffeine. Thus K_{sys}-K_{caff} represents the SR-dependent rate of Ca^{2+} removal from the cytosol. Figure 4*D* summarizes the mean data and shows that the SR-dependent rate of Ca^{2+} removal increases from $9.2 \pm 0.4\ s^{-1}$ in LD animals to $12.4 \pm 1\ s^{-1}$ in SD animals ($P < 0.01$), indicating increased SERCA function in response to SD entrainment in the Siberian hamster.

DISCUSSION

The results of the present study demonstrate that, in a mammalian species that exhibits low-temperature torpor (the Siberian hamster), there are marked alterations to cardiac excitation-contraction coupling in normothermic animals in response to prolonged exposure to SD. The principal observations are *1*) the amplitude of the systolic Ca^{2+} transient is increased in SD-acclimated animals, *2*) the SR Ca^{2+} content and fractional release of Ca^{2+} from the SR are increased in SD-acclimated animals, *3*) there are no changes in action potential duration or shape, and *4*) in SD-acclimated animals, SR-dependent Ca^{2+} removal is increased, whereas no change in the rate of sarcolemmal Ca^{2+} extrusion is detected. We propose that in the SD-acclimated Siberian hamster the increased SR Ca^{2+} content arises as a consequence of enhanced SR Ca^{2+} uptake, which then facilitates the greater fractional release of Ca^{2+} from the SR on depolarization and thus the larger systolic Ca^{2+} transient. The present results are consistent with earlier histological and biochemical studies demonstrating an increased SR density (28) and enhanced SR Ca^{2+} uptake rate (4) and suggest that alterations in the function of the SR are paramount to the continued contractile performance of the myocardium of hibernating species during conditions when the hearts of nonhibernating species would cease to function (5, 18, 23).

Systolic Ca^{2+} and photic entrainment. In the present study, cardiac cellular Ca^{2+} homeostasis was determined under conditions designed to be as physiological as possible, i.e., action potentials elicited at a stimulation frequency of 4–8 Hz (15), physiological temperature (37°C), and with minimal perturbation of the intracellular environment through use of the perforated patch technique. The key observation is that entrainment to SD resulted in an increase in the amplitude of the systolic Ca^{2+} transient (Fig. 1*C*), which decayed more quickly (Fig. 4*C*). To our knowledge, this is the first demonstration that photoperiod induces alterations in cardiac cellular Ca^{2+} homeostasis in normothermic animals. It is possible that the discrepancy among our results, earlier observations of increased force production during hibernation (38), and the data

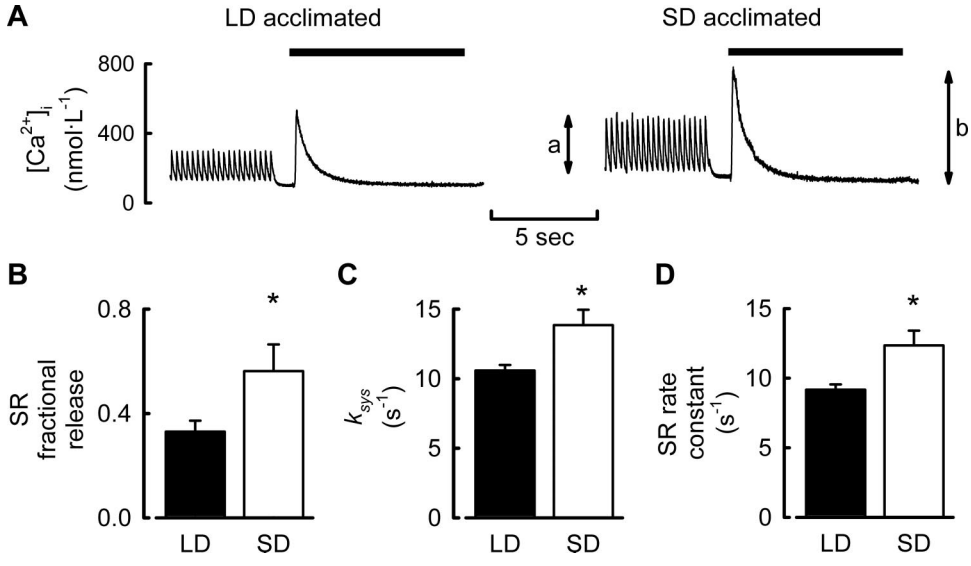

Fig. 4. Enhanced SR function in SD-acclimated hearts. *A*: time course showing systolic Ca^{2+} transients elicited at 4 Hz under current-clamp conditions in LD-acclimated (*left*) and SD-acclimated animals (right). Current clamp stimulation was stopped, and voltage clamp control was imposed before 10 mmol/l caffeine was applied for the times indicated by the solid bars above the records. Amplitudes of the systolic Ca^{2+} transients (a) and caffeine-evoked transients (b) were determined, and the ratio a/b was used to calculate SR fractional release. *B*: mean data for SR fractional release in LD and SD cells. *C*: mean data summarizing the rate of decay of the systolic Ca^{2+} transient (K_{sys}) in LD and SD animals. *D*: mean data for the SR-dependent rate of Ca^{2+} removal from the cell for LD and SD cells. $*P < 0.05$.

of Yatani et al. (35) is due to differences in temperature (32 ± 2°C) and stimulation rates (1 Hz) used by Yatani et al. Indeed, our study failed to detect a difference in Ca^{2+} transient amplitude when very slow (1 Hz) stimulation frequencies were used (Fig. 1*C*). Our finding of an increased Ca^{2+} transient amplitude in response to SD is, however, consistent with an earlier observation of increased force production in papillary muscles isolated from SD-acclimated (nonhibernating) Asian chipmunks (20) and suggests that the alterations to cardiac excitation-contraction coupling leading to increased force production during low-temperature conditions (38) are present even before the animal enters the hibernating or torpid state.

Photoperiod, the action potential, and Na^+/Ca^{2+} exchange function. In response to acclimation to SD over many weeks, we find that there are no alterations to the action potential in the Siberian hamster (Fig. 2, *B*, *C*, and *E*). The lack of change in the duration and shape of the action potential is important from at least two perspectives: *1*) the larger Ca^{2+} transient observed in the SD animals cannot be explained by an increased action potential duration (17), and *2*) it is unlikely that there will be profound changes in Ca^{2+} entry via the L-type Ca^{2+} ($I_{Ca\text{-}L}$) as balancing alterations in repolarizing K^+ currents would be required to maintain an identical action potential morphology; however, we cannot totally exclude this possibility from the data obtained in the present study. Finally, from an arrhythmogenic aspect, the lack of increase in action potential duration does not increase the likelihood of arrhythmias, nor however does it decrease it. Thus the available data suggest that the changes in cardiac excitation-contraction coupling and reduced sensitivity to ventricular tachyarrhythmias occurring in response to SD entrainment are likely the result of changes to the steps governed by intracellular sources and sinks of Ca^{2+}, i.e., the SR.

In actively hibernating animals, action potential duration is decreased (20, 35). Reductions in $I_{Ca\text{-}L}$ density, as reported in the hibernating woodchuck (35) and ground squirrel (*Citellus undulates*) (1), likely contribute to this observation. Whether changes in action potential duration occur in the acutely torpid hamster has not been determined. Because action potential duration is also largely determined by the properties of the transient outward current, it is interesting to speculate whether known circadian variations in gene expression, protein levels, and current densities of the molecular determinants of transient outward current, $K_v4.2$ and $K_v1.5$ (34), would be sufficient to lead to such reductions in action potential duration over the short time frame of torpor exhibited by the Siberian hamster.

A key electrophysiological component initiating cardiac arrhythmias is the efflux of Ca^{2+} by the electrogenic Na^+/Ca^{2+} exchanger. Here, spontaneous release of Ca^{2+} from the SR activates forward-mode Na^+/Ca^{2+} exchange, and the inward current leads to depolarization and thus potentially triggered arrhythmias. Theoretically, it is possible to increase the arrhythmogenic effect of the Na^+/Ca^{2+} exchanger either by increasing the amplitude of the initiating spontaneous release event or by increasing the amount of inward depolarizing current produced for a given change in $[Ca^{2+}]_i$. From previous studies conducted by our group (8), we know that, once a certain threshold SR Ca^{2+} content is reached and spontaneous Ca^{2+} release occurs, the amount of Ca^{2+} pumped out of the cell by the Na^+/Ca^{2+} exchanger per spontaneous release event remains constant and attempting to further overload the SR only increases the frequency of such events. In this study, we have specifically examined the latter possibility by determining the relationship between $[Ca^{2+}]_i$ and arrhythmogenic inward Na^+/Ca^{2+} exchange current (Fig. 3, *D* and *E*) and find that this relationship is not altered in the SD-acclimated animal. This method also directly assesses Na^+/Ca^{2+} exchange function in a physiological manner and accounts for any allosteric regulation of the Na^+/Ca^{2+} exchanger by changes in $[Ca^{2+}]_i$ or intracellular Na^+ concentration. Our findings are consistent with the recent demonstration of constancy of reverse-mode Na^+/Ca^{2+} exchange current density (hyperpolarizing current) produced by removal of extracellular Na^+ in myocytes isolated from the hibernating woodchuck (35). Together, these results do not implicate the Na^+/Ca^{2+} exchanger as a key element in the reduced sensitivity to ventricular tachyarrhythmias at low body temperatures.

Enhanced SR Ca^{2+} content and Ca^{2+} uptake in SD-acclimated animals. The major determinant of the inotropic and lusitropic properties of the heart is the function of the SR. In the present study, we find that both the Ca^{2+} content of the SR (Fig. 3, *A–C*) and the SR-dependent rate of Ca^{2+} removal from the cytosol (Fig. 4*D*) are increased in the SD-acclimated animal. A similar increase in SR Ca^{2+} content has been very recently reported in the hibernating woodchuck (35), although no data regarding SR Ca^{2+} uptake rate were provided. Possible explanations for the increased SR-dependent rate of Ca^{2+} uptake and thus the enhanced SR Ca^{2+} content include changes in the relationship between SERCA and its inhibitory peptide phospholamban such that either phospholamban is more phosphorylated or the ratio of SERCA to phospholamban is increased. Indeed, biochemical studies support this latter hypothesis in the hibernating animal (35).

If we assume that in the hamster the relationship between the amplitude of the systolic Ca^{2+} transient and SR Ca^{2+} content is the same as we have previously reported for the rat and ferret (7, 29), then the increase in SR Ca^{2+} content between the LD and SD animals at a given stimulation frequency observed in the present study would increase the Ca^{2+} transient amplitude 2.5-fold as opposed to the 1.4-fold increase observed. There are a number of factors that could contribute to this apparent alteration in the effectiveness of the Ca^{2+}-induced Ca^{2+} release mechanism, including *1*) changes in the glycosylation of CSQ (24) and *2*) reduced RyR Ca^{2+} sensitivity (24). Although we have no direct assessments of either of these, it is worthwhile to speculate as to their potential physiological implications and role in preparing the myocardium for torpor or hibernation.

Enhanced glycosylation of CSQ by increasing the Ca^{2+} affinity of CSQ should reduce the power of the relationship between SR Ca^{2+} content and Ca^{2+} transient amplitude. Furthermore, this could also serve to increase the arrhythmogenic threshold of the myocardium by increasing the SR Ca^{2+} content required to initiate the spontaneous release of Ca^{2+} from the SR. The second possible explanation, reduced RyR Ca^{2+} sensitivity or altered coupling between sarcolemmal Ca^{2+} entry and SR Ca^{2+} release, would not in itself be expected to produce maintained changes in the amplitude of the systolic Ca^{2+} transient due to compensatory changes in net sarcolemmal Ca^{2+} fluxes and SR Ca^{2+} content (25); however, the relationship between SR Ca^{2+} content and Ca^{2+} transient amplitude would be altered in the manner observed in the

present study, and once again the threshold for spontaneous SR Ca^{2+} release and thus arrhythmias would be increased. Thus both CSQ glycosylation and reduced RyR Ca^{2+} sensitivity offer potential explanations for the paradox of increased SR Ca^{2+} content and reduced propensity for arrhythmias in hibernating animals.

Finally, we should consider that the power of the relationship between Ca^{2+} transient amplitude and SR Ca^{2+} content may simply be lower in the hamster than in the rat and ferret since comparable SR Ca^{2+} contents (4 Hz in SD and 6 Hz in LD animals) yield similarly sized Ca^{2+} transients (Fig. 1*C*). Thus there may be no need to invoke changes in the efficiency of SR Ca^{2+} release of the nature described above. Nevertheless, regardless of the mechanisms by which the relationship between SR Ca^{2+} content and Ca^{2+} transient amplitude is altered in response to photoperiod, the present study demonstrates that the increase in SR Ca^{2+} content results in a greater fractional release of Ca^{2+} from the SR of the SD-acclimated hamster (Fig. 4, *A* and *B*) and that this occurs independently of any change in action potential duration or morphology. Thus we would suggest that changes in SERCA function are responsible for the enhanced excitation-contraction coupling observed in the Siberian hamster in response to acclimation to SD.

Study limitations. In the present study, we have not directly addressed the molecular mechanisms responsible for increased SERCA function and ultimately the larger systolic Ca^{2+} transient observed in response to SD. However, there is a consensus of either direct measurements of SERCA mRNA or protein and functional measurements of SERCA turnover in vesicles to support our tenet that enhanced SR function is an adaptive response employed by hibernating or torpid animals to facilitate survival under conditions in which the hearts of nonhibernating animals would cease to function. Our data demonstrate an enhanced fractional release of Ca^{2+} from the SR, and we conclude that this arises as a consequence of the increased SR Ca^{2+} content rather than through alterations in sarcolemmal Ca^{2+} entry due to the identical action potentials in the LD and SD animals; however, of course such a conclusion regarding sarcolemmal Ca^{2+} entry requires future verification. Nevertheless, we do postulate that there may be changes in action potential duration in the acutely torpid hamster. Finally, given the increased Ca^{2+} transient amplitude and that myofilament Ca^{2+} sensitivity increases during hibernation (22), we would predict that cardiac contractility would also be increased in vivo after prolonged exposure to SD.

In conclusion, we demonstrate that prolonged changes in photoperiod cause marked alterations to cardiac cellular Ca^{2+} homeostasis in nontorpid animals. The results show the SR to be the key intracellular organelle responsible for the enhanced systolic Ca^{2+} transients. To the best of our knowledge, this is the first such study to demonstrate that many of the adaptive changes to cardiac excitation-contraction coupling required to facilitate maintained cardiac contractility during low-temperature hibernation and torpor are present before the animal enters these physiological states. We would suggest that understanding the molecular and cellular adaptations employed by animals exhibiting true hibernation and low-temperature torpor will have profound implications for our understanding of the susceptibility of humans and other nonhibernating species to ventricular tachyarrhythmias and thus be of future therapeutic benefit.

GRANTS

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