Hypothermia-induced spatially discordant action potential duration alternans and arrhythmogenesis in nonhibernating versus hibernating mammals

Yuriy V. Egorov,1* Alexey V. Glukhov,1,2* Igor R. Efimov,2 and Leonid V. Rosenshtraukh1

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Submitted 4 August 2011; accepted in final form 2 August 2012

Egorov YV, Glukhov AV, Efimov IR, Rosenshtraukh LV. Hypothermia-induced spatially discordant action potential duration alternans and arrhythmogenesis in nonhibernating versus hibernating mammals. Am J Physiol Heart Circ Physiol 303: H1035–H1046, 2012. First published August 10, 2012; doi:10.1152/ajpheart.00786.2011.—The heart of hibernating species is resistant to lethal ventricular fibrillation (VF) induced by hypothermia. Spatially discordant (SDA) cardiac alternans is a promising predictor of VF, yet its role in the mechanism of hypothermic arrhythmogenesis in both nonhibernating and hibernating mammals remains unclear. We optically mapped the posterior epicardial surface of Langendorff-perfused hearts of winter hibernating (WH, n = 13), interbout arousal (IBA; n = 4), and summer active (SA, n = 6) ground squirrels (GSs; Spermophilus undulatus) and rabbits (n = 10). Action potential duration (APD) and conduction velocity (CV) dynamic restitution and alternans were determined at 37 to 17°C. In all animals, hypothermia induced heterogeneous APD prolongation, enhanced APD dispersion, and slowed CV. In all groups, hypothermia promoted the formation of APD alternans, which was predominantly spatially discordant in GSs and SDA in rabbits (SD of APD dispersion: 4.2 ± 0.4% vs. 2.0 ± 0.3% at 37°C and 7.5 ± 1.1% vs. 3.4 ± 0.5% at 17°C, P < 0.001 for rabbits vs. the WH group, respectively). In rabbits, hypothermia significantly increased the magnitude of SDA, which enhanced the ventricular repolarization gradient, caused conduction delays (CV: 3.2 vs. 8.2 cm/s at 17°C in rabbits vs. the WH group), conduction block, and the onset of VF (0% at 37°C vs. 60% at 17°C, P < 0.01). In contrast, no arrhythmia was observed in GS hearts at any temperature. The amplitude of CV alternans was greater in rabbits (5.2 ± 0.4% versus 4.5 ± 0.3% at 37°C and 35.3 ± 4.2% vs. 14.9 ± 1.5% at 17°C in rabbits vs. the WH group, P < 0.001 at 17°C) and correlated with the amplitude of SDA. In conclusion, the mechanism underlying SDA formation during hypothermia is likely associated with CV alternans conditioned by an enhanced dispersion of repolarization. The factors of hibernating species resistance to SDA and VF seem to be the safe and dynamically stable conduction and the low dispersion of repolarization.

METHODS

Animals. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Labora-

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tory Animals (NIH Pub. No. 80-23). All procedures were approved by the Animal Care and Use Committee of the Cardiology Research Center (Moscow, Russia). All experiments followed the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes 1986 86/609/EEC.

Ground squirrels (GSs; Spermophilus undulatus) were live trapped in the Lena river valley of the Yakutsk region of Russia and shipped via air to Pushchino Research Biological Center (Moscow region, Russia) for housing. We used the following groups of animals: summer active (SA; n = 6), winter hibernating (WH; n = 13), and winter interbout arousal (IBA; n = 4). We also used rabbits (n = 10) as a nonhibernating control. The rabbit model was selected because of its well-characterized electrophysiological properties, especially those related to hibernation-associative arrhythmogenesis and cardiac alternans development (14, 15, 18, 20, 33). SA animals were housed individually at 20 ± 2°C with a 12:12-h light-dark photoperiod before experiments were conducted. The average rectal temperature of SA and IBA animals was 37.5 ± 0.5°C. To facilitate hibernation in October, when the endogenous cycle reached the hibernating phase, animals were transferred to a darkened cold room (2°C). During this time, IBA animals were placed in a temperature (37°C) and weight (0.45 kg) similar to SA animals. WH animals were kept at a normal temperature of 37°C, the heart was gradually cooled over a 20-min period to 27°C and then to 17°C and kept at each temperature for at least 10 min before being imaged. Hypothermia resulted in a decrease in the spontaneous heart rate and pacing threshold for all animal groups, as previously observed (14–16). We (14–16) have recently demonstrated that rabbit hearts experience a complete loss of excitability at cooling below 12 ± 1°C, in contrast to GSs, which were able to maintain cardiac functions at extreme hypothermia (+2°C). Therefore, in the present study, both rabbit and GS isolated hearts were subjected to cooling from 37°C down to 17°C.

Restitution protocol. The pacing site was located on the anterior left ventricular (LV) epicardium midway between the apex and the base. The pacing current was at least two times the pacing threshold. Dependencies of both APD and CV on the preceding DI (restitution curves) for each heart were determined using an S1-S1 stimulation protocol (dynamic protocol), as previously described (3, 14, 25). Briefly, hearts were paced with a basic cycle length (CL) equal to 400–300 ms at 37°C, 750 ms at 27°C, and 2,000 ms at 17°C. After 50 stimuli had been delivered at a basic CL, pacing was stopped, and APD at 50% repolarization (APD50/CV) of the last paced AP was measured. Pacing was then reinitiated at a shorter CL, and APD50/CV was determined after 50 stimuli had been delivered at the new CL. The CL was shortened in steps of 50 ms for CL >200 ms at 37°C (350 ms for 27°C and 600 ms for 17°C) and further in steps of 10 ms until 1:1 capture failed or ventricular tachyarrhythmias, including VF, occurred. Arrhythmias that continued for >1 min were electrically defibrillated by a custom-made LV implantable defibrillation lead and defibrillator (Gold SM 1211). The shortest pacing S1 interval to capture without Wenckebach periodicity was deemed the ventricular functional refractory period (VFRP). We observed APD/CV alternans at short CLs. Restitution slopes were obtained by analytically computing the derivative of the linearly fitted portions of the restitution curve, which contains segments of data without APD alternans (3). APD alternans was quantified by computing the AADP over two consecutive beats. The APD/CV alternans was considered to be present when relative differences in consecutive beats were ≥5% of the mean APD/CV at each temperature (41). These threshold values gave us a reliable measure of alternans spatially and temporally across all experiments.

To characterize the spatiotemporal pattern of repolarization, the mean APD at each temperature was calculated for each of the 256 mapped sites and then averaged throughout the mapped area (all 256 epicardial sites), and maximum dispersion (Dmax) was measured or calculated for all animal groups at different temperatures. Dmax was calculated as the difference between the minimum and maximum values of APD at each temperature (41). These threshold values gave us a reliable measure of alternans spatially and temporally across all experiments.

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Excitation light (525 nm) was applied with two 16 × 16 light-emitting diode (SSL-LX5055UTGPIC125, Lumex, Pataleul, IL) arrays. The fluorescent light emitted from the preparation was long-pass (>610 nm) filtered before it reached the camera. The entire anterior ventricular epicardial surface was mapped with a 127 × 128-pixel charge-coupled device camera (Dalsa) with a field of view of 16 × 16 mm. To reduce noise, we applied a moving average filter to three to seven frames in time, or median filtration of 8 × 8 × 3, i.e., 8 × 8 pixels in space and three frames in time.

Isolated Langendorff-perfused hearts were equilibrated at 37°C for 40–60 min before being imaged. After measurements were obtained at a normal temperature of 37°C, the heart was gradually cooled over a 20-min period to 27°C and then to 17°C and kept at each temperature for at least 10 min before being imaged. Hypothermia resulted in a decrease in the spontaneous heart rate and pacing threshold for all animal groups, as previously observed (14–16). We (14–16) have recently demonstrated that rabbit hearts experience a complete loss of excitability at cooling below 12 ± 1°C, in contrast to GSs, which were able to maintain cardiac functions at extreme hypothermia (+2°C). Therefore, in the present study, both rabbit and GS isolated hearts were subjected to cooling from 37°C down to 17°C.

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described (15). We used WH and rabbit hearts. The dynamic restitution S1-S1 protocol was applied as described above.

**Statistical analysis.** Continuous variables are expressed as means ± SD. Continuous variables were evaluated with an unpaired Student t-test for successive measurements using a Bonferroni correction or ANOVA. Comparisons between different animal groups were performed using t-tests for samples with different sizes with the Bonferroni correction. Spearman’s rank correlation was used as shown in Fig. 3, C and D. Differences were considered significant at P < 0.05.

**RESULTS**

**APD alternans during hypothermia.** Temperature reduction led to a progressive prolongation of APD in all groups (Fig. 1A). Although rabbit hearts had a longer APD at all temperatures applied (Fig. 1B), APD normalized to 37°C was larger in GS hearts (Fig. 1C). Figure 2 shows representative examples of APs obtained by microelectrodes used to validate optical sig-

![Fig. 1. Action potential (AP) duration (APD) changes during hypothermia. A: representative optical AP recordings at 37°C (black) and 17°C (gray) from rabbit hearts and summer active (SA), interbout arousal (IBA), and winter hibernating (WH) ground squirrel (GS) hearts. B: summarized APD at 70% repolarization (APD70) data measured at 37 to 17°C in all animal groups. C: APD70 normalized to 37°C. *P < 0.05 and **P < 0.01 vs. rabbits at 17°C; #P < 0.05 and ##P < 0.01 vs. values at 27°C within the animal group.](image)

![Fig. 2. Microelectrode recording of restitution curves. A: microelectrode recording of APs obtained from WH GS and rabbit papillary muscles at 37°C during the restitution protocol. B: representative examples of APD restitution curves [APD was plotted as a function of diastolic interval (DI); DI = S1S1 − APD90] measured using microelectrodes for WH GS and rabbit hearts at 37 and 27°C. C: averaged maximum slopes of APD restitution curves measured using microelectrodes. *P < 0.05 vs. WH GSs.](image)
nals. Microelectrode AP morphology, characteristics, and pacing CL dependence (Fig. 2A) were similar to those obtained by optical mapping (Fig. 1, A and B) and to those previously observed (15). Typical restitution curves measured using microelectrodes from individual WH and rabbit papillary muscles at 37 and 27°C as well as the averaged maximum slopes of APD restitution curves are shown in Fig. 2, B and C, respectively.

To explore the effect of hypothermia on APD alternans, we applied the dynamic restitution protocol at all temperatures studied. In all animal groups, hypothermia induced significant prolongation of APD70 and, thus, an upward shift of APD restitution curves (Fig. 3A). A significant difference in restitution dynamics was revealed between GSs and rabbits. The characteristics of APD restitution curves averaged throughout all animals within each group.

The gradual shortening of the pacing CL led to alternans of APD between neighboring beats (Fig. 4, A and B). In GSs, deep hypothermia (17°C) enhanced both basic APD alternans measured at slow rhythm (Fig. 4D) and maximum APD alternans measured at fast rhythm (CL equal to VFRP; Fig. 4C). In rabbits, maximum APD alternans was bigger compared with GSs at 37°C; however, the magnitude did not change significantly during hypothermia. It should be noted that APD alternans started earlier in rabbits than in GSs (Fig. 4E). When correlated with the maximum APD restitution slope, no direct correlation between the APD restitution slope and alternans amplitude was observed in rabbit hearts (Fig. 3, C and D). However, based on Spearman’s rank correlation coefficient, there was statistical (P < 0.001) correlation between the alternans amplitude and APD restitution slope in all GS groups.

Spatially concordant versus discordant APD alternans. Cardiac alternans may be spatially concordant (SCA), when all regions of tissue alternate in phase, or spatially discordant (SDA), when adjacent regions alternate out of phase, separated by a nodal line at which no alternans occurs (9, 18, 33). SDA is more arrhythmogenic than SCA because it can amplify the spatial dispersion of repolarization enough to cause unidirectional conduction block and onset of reentry (7, 37, 38).

Comparison of the spatial patterns of repolarization during concordant and discordant alternans observed in the rabbit heart at 17°C are shown in Fig. 5. During slow rhythm (CL = 2,000 ms), the APD distribution pattern had a stable base-to-apex gradient (Fig. 5A), which reached ~30% of the apical APD and did not changed between neighboring beats (not shown). The gradual shortening of the pacing interval to

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600 ms led to the occurrence of SCA (Fig. 5B) where apical and basal regions alternated in the same beat-to-beat manner (long-short-long), as shown on the AP recordings selected from those regions. During SCA, the pattern and dispersion of the APD distribution were slightly increased with that measured at slow rhythm (base-to-apex APD gradient reached ~30–40% of the apical APD vs. 30% at slow rhythm) and did not significantly change from beat 1 (40%) to beat 2 (30%). At a somewhat faster rate (CL = 470 ms), SDA occurred: the basal region alternated in a short-long-short manner, whereas the apical region simultaneously alternated in a long-short-long manner, as demonstrated on the AP recordings selected from these regions. During SDA, the pattern of repolarization alternans changed importantly (Fig. 5C). Beat 1 resulted in a relatively homogeneous APD distribution (Dmax: 35% vs. 30% at slow rhythm) with two prominent peaks located ~15 mm apart. At beat 2, the pattern of repolarization varied substantially, exhibiting a significantly heterogeneous APD distribution (Dmax = 80%) characterized by three prominent peaks unrelated to that at beat 1. Furthermore, during SDA (Fig. 5C), repolarization gradient was twice bigger than that during SCA (80% vs. 40% of the apical APD; Fig. 5B). SDA-induced gradients of repolarization were sufficiently large to cause secondary conduction delays (areas 1 and 2 labeled for beat 2 in Fig. 5C), resulting in conduction block (dotted line in Fig. 5D) and the induction of VF (Fig. 5D). Two activation maps (Fig. 5D, middle) reconstructed for the current (Fig. 5D, left) and subsequent beats demonstrated that the impulse was blocked in the area with prolonged APD (around the interventricular septum) and the following retrograde activation initiated a reentry circuit, which induced VF.

To characterize SDA quantitatively, we used the SD of APD dispersion (SD-Dmax) calculated for consecutive beats. As shown in Fig. 5C, the pattern of APD distribution was significantly varied during SDA: Dmax reached 35% of the apical APD at beat 1 and 80% at beat 2, whereas during SCA (Fig. 5B), Dmax was relatively stable and reached ~30–40% of the apical APD. Once APD alternans became SDA, APD dispersion started to oscillate in a beat-to-beat manner, mainly due to an unstable APD distribution pattern: SD-Dmax was 7% (Fig. 5B) vs. 32% (Fig. 5C). As shown in Fig. 5D, SD-Dmax measured at a slow rate did not differ between animal groups. However, when paced faster, SD-Dmax measured in rabbit hearts was significantly enhanced compared with GSs, indicating SDA in rabbits. In all animals, temporal oscillations in the dispersion of repolarization were not substantially increased over baseline values during SCA while being cooled to 17°C (SD-Dmax measured at fast pacing, top part of the column). In GS hearts, fast pacing induced significant changes in SD-Dmax only at 17°C. In contrast, rabbit hearts in hypothermia resulted in a more than a four-fold increase in the amplitude of SDA (SD-Dmax measured at fast pacing, top part of the column)
compared with the amplitude of SCA (SD-D\textsubscript{max} measured at slow pacing, bottom part of the column), which correlated with an increased probability of pacing-induced VF (0\% at 37°C vs. 60\% at 17°C, \(P < 0.01\)). Unlike rabbit hearts, no arrhythmia was observed in GS hearts at any temperature.

During all recordings of VF, there was both an increase in APD dispersion (D\textsubscript{max} \(\approx 30\%\)) and an enhanced amplitude of SDA (SD-D\textsubscript{max} \(\approx 7\%\)). Increased APD dispersion alone was not enough for VF induction. Also, it should be noted that the profound SDA (SD-D\textsubscript{max} = 8.5\%) observed in one rabbit heart at moderate APD dispersion (D\textsubscript{max} = 19.5\%) did not result in VF.

**Role of CV restitution in the mechanism of SDA formation.** One of the mechanisms proposed for SDA is based on a steep CV restitution mechanism, i.e., when the CV of a propagating wave has a steep dependence on the preceding DI (33, 54). To estimate the role of this mechanism during hypothermia-associated SDA observed in rabbit hearts, we measured CV restitution as a function of temperature (Fig. 7, A–C).

Hypothermia induced a more substantial and heterogeneous CV slowing in rabbits compared with GSs, preferentially suppressing propagation in the transverse direction, which increased conduction anisotropy (see Fig. 7C, bottom). At 17°C, the activation pattern in rabbit hearts was disorganized and demonstrated multiple local conduction blocks (Fig. 7C, bottom).

**Fig. 5.** Transition from spatially concordant to discordant alternans during a progressive increase of pacing rate. A–D: representative examples of pseudo three-dimensional APD distribution contour maps measured at slow pacing CL (A; S1S1 = 2,000 ms), during concordant APD alternans (B; S1S1 = 600 ms), during discordant APD alternans (C; S1S1 = 470 ms), and during fast pacing-induced ventricular arrhythmia [D, ventricular fibrillation (VF)] in the rabbit heart. x- and y-axes show optically mapped tissue areas (in mm); the z-axis represents the relative APD as a percentage of the minimal APD (used as 0\%) at each pacing CL.
Hypothermia increased the amplitude of CV alternans in all animals. The amplitude of SDA well correlated with the amplitude of CV alternans (Fig. 8D).

**DISCUSSION**

APD alternans during hypothermia. Electromechanical cardiac alternans refers to the beat-to-beat alternation of APD and intracellular Ca\(^{2+}\) transients in a repeating pattern of long-short-long-short or large-small-large-small, respectively. Cardiac alternans, first observed in the form of pulse alternans (49) and later described as electrocardiographic T wave alternans (28), has been recently linked to the genesis of reentrant arrhythmias (37) and shown to be a good marker of risk for sudden cardiac death in patients (43). Cardiac alternans has been observed in a surprisingly wide variety of clinical and experimental arrhythmogenic conditions, including hypothermia (19, 20).

Cardiac alternans may be SCA (Fig. 5B) or SDA (Fig. 5C). SCA is less arrhythmogenic than SDA (37, 42). Although APD and, hence, the refractory period alternate for any given beat, the refractory period is either long or short everywhere, and thus the dispersion of refractoriness is not greatly amplified (as shown in Fig. 5C for two neighboring beats), as was observed in GS hearts at all temperatures tested (Fig. 6A). However, once APD alternans becomes SDA, the dispersion of refractoriness is greatly amplified, producing a favorable substrate for the initiation of reentry, as shown in Fig. 5, C and D. During SDA, some regions of tissue alternate in a long-short-long pattern, whereas other regions simultaneously alternate in a short-long-short pattern. These out of phase regions are separated by a nodal line in which no alternans is present (regions I and 2 on Fig. 5C). At a nodal line, the spatial APD gradient is the steepest, predisposing this region to localized conduction block (37, 42, 54). When 2:1 conduction failure occurs locally, unblocked impulses from adjacent regions can reenter the blocked region and induce figure-eight reentry (see Fig. 5D). This is the typical mechanism by which rapid ventricular pacing induces VF, as has been documented in both an experimental optical mapping study (37) and computational simulations (42).

Role of CV restitution in the mechanism of SDA formation. Although it is unclear why neighboring cells under apparently identical conditions would respond differently with respect to their alternans phase, two possible, and not necessarily mutually exclusive, mechanisms have been proposed to explain the appearance of SDA. The first is based on a steep CV restitution mechanism, i.e., when CV of a propagating wave has a steep dependence on the preceding DI (33, 42, 54).

CV, like APD, is also sensitive to the preceding DI. CV restitution is typically flat at long DI but decreases at short DI due to the incomplete recovery from inactivation of Na\(^+\) channels. In the case of CV restitution, a sufficiently short DI causes the wave front to slow. As it slows, its distance from the wave ahead increases, resulting in a longer DI. As DI increases, the APD also lengthens. Meanwhile, the wave’s changing APD affects the DI of the wave behind it, so that the next wave’s APD will also oscillate, and so forth for each successive wave. The important consequence is that the APD of the same wave changes while propagating through the tissue, becoming short in some areas and long in others. The
CL at which CV restitution is engaged becomes a major determinant of the conversion of SCA to SDA. During hypothermia, Na\(^+\) channel recovery from inactivation becomes delayed (11, 30), enhancing the range of DI over which CV varies. This may be a major factor promoting SDA alternans and increased the risk of arrhythmia in these settings.

In the present study, we demonstrated that hypothermia slowed CV, preferentially suppressing propagation in the transverse direction, which increased conduction anisotropy (Fig. 7C). This result is consistent with our previous observations (14). CV restitution measured in rabbit hearts had a steeper dependence on the preceding DI, as characterized by a higher CV restitution curve slope (Fig. 7D). Hypothermia also increased the amplitude of CV alternans (Fig. 8C); however, these changes were more pronounced in rabbits than in GSs. Moreover, the amplitude of SDA was well correlated with the amplitude of CV alternans in all animal groups (Fig. 8D). It should also be noted that CV alternans started earlier than APD alternans. At the same time, our data demonstrate that CV disorder is not a mutually exclusive mechanism responsible for SDA formation. During all episodes of VF, there was both increased APD dispersion and enhanced amplitude of SDA. VF did not occur only at increased APD dispersion or only at profound SDA.

Role of regional ionic and Ca\(^{2+}\) cycling heterogeneity in the mechanism of SDA formation. An alternative mechanism requires the presence of preexisting heterogeneities, and SDA can be formed via an appropriately timed stimulus or a change in pacing rate (6, 37). In heterogeneous tissue, the amplitude of alternans varies spatially, making it possible to time a stimulus such that it reverses the alternans phase in only one part of the tissue. This mechanism is associated with pacing history, or the short-term memory phenomenon, which is based on the spatial heterogeneities of Ca\(^{2+}\) cycling and the sarcolemmal ionic currents that govern repolarization (33, 41). According to this mechanism, repolarization alternans arises when the heart rate exceeds the capacity of myocytes to cycle intracellular Ca\(^{2+}\) (41, 48). During APD alternans, the amount of released Ca\(^{2+}\)
can only be fully reclaimed on an alternating beat basis, giving rise to the alternation of free cytosolic Ca\(^{2+}\) levels. This, in turn, can result in cellular AP alternans through several electrogenic Ca\(^{2+}\)-sensitive sarcolemmal currents. A primary role of this mechanism is believed to be attributed to sarcoplasmic reticulum (SR) Ca\(^{2+}\) cycling. Reduced sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) expression (26) or interventions that decrease the function of SR Ca\(^{2+}\)-release channels (ryanodine receptors) (22) have been shown to promote both intracellular Ca\(^{2+}\) alternans and APD alternans.

In contrast to the intracellular Ca\(^{2+}\) dysregulation observed in nonhibernating animals and humans during hypothermia (31, 50, 52), hibernating animals demonstrate an enhanced capability to maintain intracellular Ca\(^{2+}\) homeostasis (23, 50, 52). At 30 to 10°C, resting intracellular Ca\(^{2+}\) measured in cardiomyocytes from GSs (*S. dauricus*) changes very little (range: 125 ± 10 nmol/l) (50) compared with rat myocytes, where it increases from 140 nmol/l at 30–35°C to 200–300 nmol/l during cooling to 5–10°C (31, 53). Moreover, measurements of intracellular Ca\(^{2+}\) have indicated that the Ca\(^{2+}\) transient decays faster in GS cells than in rat cells owing to a faster rate of Ca\(^{2+}\) uptake and a greater level of Ca\(^{2+}\) accumulation in the SR (51). In accordance with this, ultrastuctural analysis of the hibernator myocardium revealed a double or triple increase in the content of the longitudinal SR, which contains abundant Ca\(^{2+}\)-ATPase and is responsible for Ca\(^{2+}\) uptake (46). Although the enzymatic activity of SERCA was shown to be surprisingly unchanged during hibernation, some studies have revealed an enhanced expression of calsequestrin, a Ca\(^{2+}\)-binding protein, and an increase in its Ca\(^{2+}\)-binding activity in GS hearts, which would be helpful in facilitating Ca\(^{2+}\) uptake, suppressing Ca\(^{2+}\) leakage, and increasing the amount of Ca\(^{2+}\) available for release during hibernation.

However, although these data suggest that the enhanced capability of the hibernator myocardium to maintain intracellular Ca\(^{2+}\) homeostasis could be responsible for its resistance to SDA associated with short-term memory, further experiments are needed to elucidate the mechanism precisely.

Although our data clearly demonstrate that activation time is one of the factors modifying electrical alternans, enhanced APD dispersion is also required for SDA formation. Spatial tissue heterogeneity seems to be necessary but is not sufficient for SDA formation (3, 42). Therefore, based on our data and the literature, we conclude that the mechanism underlying SDA formation during hypothermia is primarily associated with SDA alternans conditioned by an enhanced dispersion.
of repolarization. Hibernating species are resistance to SDA and VF. The safe and dynamically stable conduction and low dispersion of repolarization revealed in this study are important factors protecting the heart from the induction of arrhythmia during hibernation.

Role of electrotonic coupling in a mechanism of SDA. An additional mechanism contributing to susceptibility to discordant alternans has been proposed to be related to intracellular uncoupling (38, 54). Normal cell-to-cell coupling through gap junctions has been shown to attenuate heterogeneities between different regions (27, 40). The current flow between well-coupled neighboring cells with different intrinsic repolarization times will tend to delay recovery in cells with an intrinsically short APD and will advance recovery in cells with intrinsically long APDs. Therefore, it has been suggested that cellular uncoupling is caused by hypothermia, resulting from the down-regulation (14, 15), lateralization (14, 21), and dephosphorylation (21) of the principal gap junction protein in the ventricles, connexin (Cx)43, and may unmask intrinsic differences in APD, enhance APD dispersion (14, 20, 45), and allow neighboring myocytes to alternate with opposite phase because of differences in Ca\(^{2+}\) cycling properties and APD restitution.

In a guinea pig model of cardiac alternans, the introduction of a structural barrier to electrotonically uncoupled neighbouring cells greatly facilitated the development of discordant alternans (38). Other evidence suggests that SDA induced by ischemia in the guinea pig isolated heart could be suppressed by pharmacologically increasing the gap junction intercellular conductance (24). It has recently been shown that decreased cell-to-cell coupling by carbeneoxalone, a gap junction inhibitor, increased the amplitude and temporal synchronization of spontaneous Ca\(^{2+}\) release in the isolated guinea pig heart (39). Moreover, anisotropic downregulation of Cx43 expression and reduction of Cx43 phosphorylation together with an increased ultrastructural abnormalities, which could serve as structural barriers, result in spatially discordant CV alternans, which led to nonuniform propagation discontinuities and wavebreaks in patients with heart failure (17). Therefore, the upregulation of Cx43 expression observed in hibernators (14, 15, 44) is an additional mechanism suppressing the transition from SCA to SDA. This is in accordance with previous findings showing an increased level of alternans during different conditions of cellular uncoupling, such as ischemia (10) or hypothermia (19, 20), and supports the hypothesis that the transition from discordant to discordant alternans is affected by the degree of gap junction intercellular conductance, as suggested by experimental (38) and modeling (42) studies.

Seasonal differences of hibernators in hypothermia resistance. Another important characteristic of the hibernator heart is that the heart’s resistance to cold is significantly season dependent. Duker et al. (12) showed that hearts of WH woodchucks were completely resistant to VF-inducing agents, which was not the case for the hearts of SA animals. However, in other hibernators, the heart’s resistance to cold is season independent (5). In the present study, we found that WH and IBA animals were more resistant to hypothermia-associated SDA and arrhythmias compared with SA animals (Figs. 6B and 8C). We (14) have previously demonstrated that increased conduction anisotropy, shortened wavelength, and consequently enhanced VF induction was revealed in SA animals and rabbits but not in WH animals. Differences in stimulation threshold and AP characteristics were also observed between WH, IBA, and SA animals (15). These functional data correlate well with immunohistochemistry data showing an upregulation of both Cx43 and Cx45 during the hibernation period (14, 15). Moreover, in active animals, the leading role in the adjustment to low temperatures is played by the extracellular sources of Ca\(^{2+}\), whereas the intracellular sources of Ca\(^{2+}\) play a leading role in the regulation of force during the hibernation state (29, 35). Ca\(^{2+}\) current density was found to be significantly decreased and SR Ca\(^{2+}\) uptake capacity increased in myocytes of hibernating woodchucks compared with active woodchucks, protecting the heart from Ca\(^{2+}\) overload (52). These studies point out that the problem of adaptive mechanisms of winter-sleeping animals to low temperatures harbors a lot of mysteries, and further studies must be done to determine the molecular mechanisms underlying antiarrhythmia protection that have evolved in hibernating species through natural selection.

Study limitations. Electromechanical uncoupling drugs are necessary when high-spatial resolution video imaging systems are used to suppress motion artifacts to evaluate spatiotemporal characteristics of some electrophysiological parameters, such as APD and CV alternans. At the same time, the use of electromechanical uncouplers could affect the observed results due to partial ion channel blockage. However, our previous studies, in which we did not use BDM (14, 15) or any drugs (16), showed that BDM did not significantly change the resistance of hibernating GSs to arrhythmias associated with hypothermia. Moreover, in the present study, all animals were studied under equivalent conditions, and thus the effect of BDM should be the same for all animals.

The glass microelectrode technique was used in the present study to validate optical AP. Microelectrode AP morphology, characteristics, and frequency dependence (Fig. 2, A and B) were similar to those obtained by optical mapping (Fig. 1, A and B) and those observed in our previous study (15). The characteristics of APD restitution curves measured using optical mapping were similar to those obtained by microelectrodes (compare Figs. 2, B and C, and 3, A and B). Although electromechanical uncouplers could affect some electrophysiological parameters, their effects seem to be negligible compared with the difference between hibernating versus nonhibernating animals. We acknowledge that further studies must be done to directly determine to what extent the different electromechanical uncouplers could affect the formation and spatiotemporal characteristics of APD and CV alternans in both hibernating and nonhibernating animals during hypothermia.

GRANTS

This work was supported by the Stanley and Lucy Lopata Endowment (to I. R. Efimov), Russian Foundation for Basic Research Grant 05-04-48311, and Russian President Foundation for Scientific School Grant SS-6211.2006.7 (to L. V. Rosenshtraukh).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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