EDITORIAL COMMENTARY

The hidden secrets of the hibernator's heart may protect against arrhythmias

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In this issue of *Heart Rhythm,* Fedorov et al[1] describe the activation pattern and other electrophysiologic features of the heart of *Citellus undulatus* (also known as *Spermophilus undulatus*), the Siberian ground squirrel, both in summer active and in winter hibernating animals at temperatures varying from 3°C to 37°C and compared those with data in the rabbit heart as a nonhibernating species. The summary of the data can be simple. There is a substantial decrease in conduction velocity from 70–80 cm/s in the Siberian ground squirrel at 37°C to 5–10 cm/s at 3°C. However, the conduction patterns remain normal and do not show areas of substantial conduction block, which might herald arrhythmias. Interestingly, conduction velocity was significantly higher in hearts obtained from winter hibernating animals than from summer active animals at both 37°C and 3°C. Data at such low temperatures cannot be obtained from the hearts in nonhibernating species such as the rabbit in the present study, but also not in man, because their hearts become quiescent or develop ventricular fibrillation at temperatures below 20°C.[1,2] Moreover, the authors show that there is an up-regulation of connexin43 (Cx43) in working ventricular myocardium of hibernating animals, in line with previous research,[3] and an appearance of connexin45 (Cx45) in working ventricular myocardium, although the expression of the latter normally is restricted to the conduction system.[4]

This study underscores the presence of differences not only between hibernating and nonhibernating species but also between hibernating and nonhibernating individuals of the same species, with all the interesting implications for regulatory mechanisms.

Physiologic background and implications

We would like to point out first that hypothermia impairs conduction velocity by a combined effect on the underlying membrane currents and on gap junctions. The conductance of gap junctions is reduced by about 70% when the temperature drops from normal to 0°C at least in cell pairs of neonatal rat heart.[5] Whether the inward Na^+ current is operational at temperatures well below 10°C is questionable because it is seriously impaired at much smaller drops in temperature than those observed in hibernators.[6,7] Liu et al[8] reported that the L-type Ca^{2+} current virtually disappears at 10°C in rat ventricular myocytes, although rat ventricle is relatively resistant against low temperature compared with other mammals. In contrast, the L-type Ca^{2+} current has the same amplitude in hedgehog papillary muscle in the temperature range from 10°C to 35°C.[8] It is not possible to decide whether conduction remains intact, although impaired, in hibernators at temperatures well below 10°C because their membrane currents are relatively resistant against low temperature or because their gap junctions function better at low temperature compared with nonhibernators. Of note, in this study arrhythmias did not develop despite conduction velocities of 5 cm/s in the hearts from summer active animals at 3°C and conduction velocities of 9 cm/s in the hearts from winter hibernating animals at the same temperature (see Figure 4D in Federov et al[1]). Because some of the hearts isolated from animals that woke up during hibernation ("interbout arousal") did develop arrhythmias, it cannot be excluded that the "significant" difference in conduction velocity between the hibernating hearts and summer active hearts at 3°C, is functionally important. At this time, the question of whether the impressive 45% up-regulation of Cx43 during hibernation and the appearance of Cx45 in the working ventricular myocardium have any functional relevance must remain unanswered in the absence of functional measurement of gap junction behavior. During embryogenesis, Cx45 is more widely distributed throughout the developing heart, including the ventricular myocardium.[9] Therefore, it appears that hibernators use more "primitive" connexins in adapting to hypothermia. However, even at normothermia, the relation between gap junction density and conduction velocity is not well established and is far from linear,[10] let alone at low temperature, as we pointed out previously.[11] A substitution of Cx43 by Cx45 seems to decrease rather than increase conduction velocity, at least at normothermia,[12] but addition of Cx45 to increased levels of Cx43 as demonstrated in this study[1] may help support conduction at low temperature.

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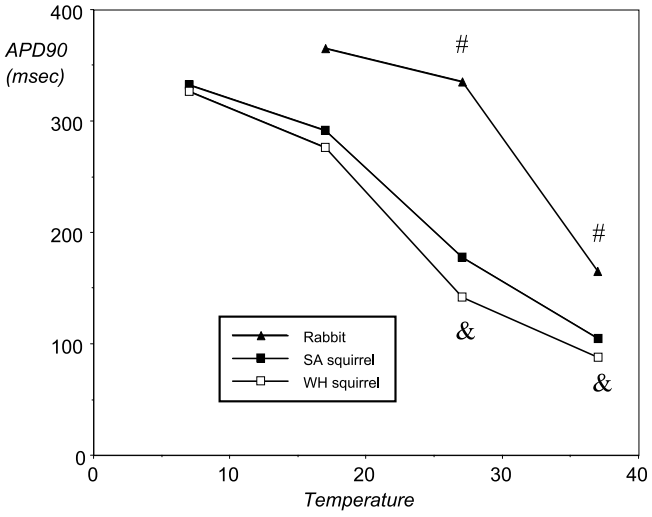

Figure 1 Ventricular action potential duration at 90% of repolarization (APD_{90}) as a function of temperature. Compiled from Table 2 in Federov et al.[1] SA = summer active; WH = winter hibernating. #Rabbit APD_{90} significantly longer than data in both squirrel groups; &APD_{90} in WH squirrels significantly shorter than in SA squirrels.

Regulatory and molecular aspects

Some mammalian species have evolved specific strategies to counter the harsh winter conditions in their natural environments. Some have adapted to become hypothermic during a daily torpor, such as the dwarf hamster species *Phodopus sungorus,* whereas others undergo longer bouts of deep hibernation, such as Syrian hamsters and ground squirrels.[13] The winter adaptations of hibernators, which include a wide variety of responses such as change in pelage, reproductive quiescence, and increased capacity for nonshivering thermogenesis, are primarily induced by reduced photoperiod rather than low temperatures *per se.* Seasonal variations in day length modify the various circadian clocks located centrally within the suprachiasmatic nucleus of the hypothalamus and peripherally in many tissues, including the heart.[14] Entering hypometabolism seems to precede the hypothermic state.[15] Interestingly, there were animals in this study[1] that were subjected to the hibernation-inducing regimen but escaped hibernation itself. They demonstrated similar adaptations as the true hibernating animals, underscoring the primary regulatory effect of day length. It will be interesting to modify the dark–light and winter–summer temperature regimens independently and to find the trigger for up-regulation of connexins, possibly related to "safe conduction."

Several studies have investigated cardiac adaptations to hypometabolism and hypothermia at the molecular level. In contrast to other tissues, a substantial number of hibernation-inducible genes have been found in the heart.[15] This is, beyond doubt, related to the necessity of continuous functioning of the heart and requires molecular adaptations. Some of these molecular adaptations relate to the switch from carbohydrates to lipid-based energy sources. The contractile apparatus also changes substantially. Finally, altered calcium handling, with an increase in calcium transient amplitude and reuptake in the sarcoplasmic reticulum, provides another basis for enhanced contractile function under hypothermic conditions.[16]

Cx43 is not expressed in the working myocardium of nonmammalian species. The evolutionary "late" appearance of Cx43 in the mammalian working myocardium may point to physiologic adaptation to euthermia. To see whether hibernating species have subsequently adapted their Cx43 to encounter the specific demands of hypothermia, we have screened the Cx43 protein sequences of 12 different mammals, including true hibernators among which is the European ground squirrel *Spermophilus citellus,* a close relative of the Siberian ground squirrel used in this study.[1] Furthermore, we included a species that undergoes daily torpor, *Phodopus sungorus,* and several nonhibernating species. Our analysis provided no evidence for specific amino acid adaptation in hibernators vs nonhibernators.[17] In fact, given the strong sequence conservation among the different mammalian Cx43s, it is unlikely that qualitative differences in Cx43-mediated conduction are underlying the observations made by the authors.[1] Alternatively, it appears that quantitative adaptation, that is, Cx43 up-regulation, is used instead. Several transcription factors and cofactors involved in other cardiac adaptations may also play a role in the up-regulation of Cx43 in hibernating hearts. Examples are PPARγ,[18] PGC-1,[18] and CREB.[19] The latter may be active via the evolutionary conserved CRE promoter element.[20] Furthermore, more active p38 MAPK is found in hibernating hearts.[19]

Other factors relevant to avoiding arrhythmias: future research

The human heart tends to develop atrial fibrillation at 30°C, and asystole or ventricular fibrillation ensues with a further decrease in temperature.[2] Also, Langendorff-perfused canine or porcine hearts arrested by low temperature require defibrillation after they warm up to normal temperature. Although maintenance of regular conduction patterns in hibernators at low temperature is remarkable, we doubt whether this constitutes the pivotal adaptation to avoid arrhythmias. The heart of mammalian hibernators has some other intriguing characteristics.[2,21] The regulatory mechanisms involved appear to be aimed at optimal reduction of electrophysiologic inhomogeneity, probably by changing the densities of specific membrane channels and/or by changing intercellular coupling.

The older literature reports several other remarkable features of the hibernator's heart compared with that of the nonhibernator. These characteristics (including the features demonstrated in the present study[1]) are summarized as follows.

1. The hibernator's heart beats at temperatures well below 10°C.
2. The hibernator's heart does not develop atrial or ventricular fibrillation during onset of or arousal from hibernation. In fact, the ventricular myocardium in hibernators is

also extremely resistant to other treatments that induce ventricular fibrillation in other species.[2]

3. The hibernator's heart has a relatively short QT interval at normal temperature (see Figure 6 in Dawe and Morrison[22]).
4. The hibernator's heart has a relatively steep T wave at normal temperature.[23] Under certain conditions it may be described as a "T complex," based on its similarity to the QRS complex, rather than a T wave.[22,23] Alternatively, a T wave may be virtually lacking (see Figure 6 in Dawe and Morrison[22] and Figure 4 in Biürck and Johansson[23]).
5. The hibernator's heart has a relatively low maximum (driven) heart frequency at normal temperature. This frequency is lower than what would be expected based on the refractory period. It suggests postrepolarization refractoriness at normoxia (Figs. 2 and 6 in Duker et al[24]).
6. The hibernator's heart is devoid of sympathetic innervation of its ventricular (working) myocardium.[25] Sympathetic innervations seem to be restricted to the coronary vasculature.
7. The hibernator heart has a negative staircase (higher contractility at long cycle length) at normal and low temperatures (see Figure 5 in Duker et al[24]), in contrast with the nonhibernator heart, which displays lower contractility at long cycle length.

Because of the optical recording technique in the study by Federov et al,[1] we do not have ECG recordings. However, Figure 1 is a compilation of the action potential duration data at 90% repolarization (APD_{90}) as a function of the temperatures given in Table 2 of Federov et al.[1] APD_{90} is much longer in the rabbit ventricle than in the Siberian ground squirrel at all temperatures. In the rabbit, APD_{90} at 27°C is of the same order as that in the Siberian ground squirrel at 7°C! In addition, between 27°C and 37°C, APD_{90} is significantly shorter in the ventricle of the hearts obtained from winter hibernating than from summer active squirrels, underscoring the significance of seasonal regulatory mechanisms. Interestingly, comparable differences for conduction velocity were seen at even lower temperatures (see Introduction and Figure 3D in Federov et al[1]). Obviously, the hibernator's heart may hide many more intriguing enigmas that may have an antiarrhythmic potential for the human heart as well. Future research directed at the short action potential durations in combination with an assessment of their dispersion will be interesting. The roles of regional distribution of membrane currents and of gap junctions are of potential interest.

References

1. Fedorov VV, Li L, Glukhov A, Shishkina I, Aliev RR, Mikheeva T, Nikolski VP, Rosenshtraukh LV, Efimov IR. Hibernator *Citellus undulatus* maintains safe cardiac conduction and is protected against tachyarrhythmias during extreme hypothermia: possible role of Cx43 and Cx45 up-regulation. Heart Rhythm 2005;2:966–975.
2. Johansson BW. The hibernator heart—nature's model of resistance to ventricular fibrillation. Cardiovasc Res 1996;31:826–832.
3. Saitongdee P, Milner P, Becker DL, Knight GE, Burnstock G. Increased connexin43 gap junction protein in hamster cardiomyocytes during cold acclimatization and hibernation. Cardiovasc Res 2000;47:108–115.
4. Van Veen AAB, Van Rijen HVM, Opthof T. Cardiac gap junction channels: modulation of expression and channel properties. Cardiovasc Res 2001;51:217–229.
5. Bukauskas FF, Weingart R. Temperature dependence of gap junction properties in neonatal rat heart cells. Pflugers Arch 1993;423:133–139.
6. Milburn T, Saint DA, Chung SH. The temperature dependence of conductance of the sodium channel: implications for mechanisms of ion permeation. Receptors Channels 1995;3:201–211.
7. Negatomo T, Fan Z, Ye B, Tonkovich GS, January CT, Kyle JW, Makielski JC. Temperature dependence of early and late currents in human cardiac wild-type and long Q-T DeltaKPQ Na^+ channels. Am J Physiol 1998;275:H2016–H2024.
8. Liu B, Arlock P, Wohlfart B, Johansson BW. Temperature effects on the Na and Ca currents in rat and hedgehog ventricular muscle. Cryobiology 1991;28:96–104.
9. Alcoléa S, Théveniau-Ruissy M, Jarry-Guichard T, Marics I, Tzouanacou E, Chauvin JP, Briand JP, Moorman AFM, Lamers WH, Gros DB. Downregulation of connexin 45 gene products during mouse heart development. Circ Res 1999;84:1365–1379.
10. Jongsma HJ, Wilders R. Gap junctions in cardiovascular disease. Circ Res 2000;86:1193–1197.
11. Opthof T, Rook MB. The hibernator's heart: nature's response to arrhythmogenesis? Cardiovasc Res 2000;47:6–8.
12. Alcoléa S, Jarry-Guichard T, De Bakker JMT, Gonzàlez D, Lamers WH, Coppen SR, Barrio LC, Jongsma HJ, Gros DB, Van Rijen HVM. Replacement of connexin40 by connexin45 in the mouse: impact on cardiac electrical conduction. Circ Res 2004;94:100–109.
13. Ruby FN. Hibernation: when good clocks go cold. J Biol Rhythms 2003;18:275–286.
14. Young ME, Razeghi P, Taegtmeyer H. Clock genes in the heart. Characterization and attenuation with hypertrophy. Circ Res 2001;88:1142–1150.
15. Storey KB, Storey JM. Metabolic rate depression in animals: transcriptional and translational controls. Biol Rev 2004;79:207–233.
16. Dibb KM, Hagarty CL, Loudon ASI, Trafford AW. Photoperiod-dependent modulation of cardiac excitation contraction coupling in the Siberian hamster. Am J Physiol Regul Integr Comp Physiol 2005;288:R607–R614.
17. Van der Heyden MAG, Van Eijk M, Wilders R, De Bakker JMT, Opthof T. Connexin43 orthologues in vertebrates: phylogeny from fish to man. Dev Genes Evol 2004;214:261–266.
18. Bertram JS, Vine AL. Cancer prevention by retinoids and carotenoids: independent action on a common target. Biochem Biophys Act 2005;1740:170–178.
19. MacDonald JA, Storey KB. Mitogen-activated protein kinases and selected downstream targets display organ-specific responses in the hibernating ground squirrel. Int J Biochem Cell Biol 2005;37:679–691.
20. Teunissen BEJ, Bierhuizen MFA. Transcriptional control of myocardial connexins. Cardiovasc Res 2004;62:246–255.
21. Johansson BW. Heart and circulation in hibernators. In: Fischer KC, Mammalian hibernation, vol 3. Edinburgh: Oliver & Boyd, 1967:200–218.
22. Dawe AR, Morrison PR. Characteristics of the hibernating heart. Am Heart J 1955;49:367–384.
23. Biürck G, Johansson B. Comparative studies on temperature effects upon the electrocardiogram in some vertebrates. Acta Physiol Scand 1955;34:257–272.
24. Duker GD, Olsson SO, Hechyt HH, Senturia JB, Johansson BW. Ventricular fibrillation in hibernators and non-hibernators. Cryobiology 1983;20:407–420.
25. Nielsen KC, Owman C. Difference in cardiac adrenergic innervation between hibernators and non-hibernating mammals. Acta Physiol Scand 1968;(Suppl 316):1–30.