Phosphorylation of Myocardial Proteins in Hibernating 13-lined Ground Squirrels

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The hibernation model of 13-lined ground squirrels is characterized by 1-to-2-week periods of torpor with heart rates of 3-5 bpm, interrupted by intermittent bouts of arousal (IBA) where heart rates are comparable to those of the summer active period (200-300 bpm). The switch between torpor and IBA occurs within hours and thus poses a significant physiological challenge. The role of two myocardial proteins and their phosphorylation states were investigated: cardiac troponin-I (cTnI) and heat shock protein 27 (HSP27). cTnI is the inhibitory subunit of cardiac troponin and its phosphorylation causes a decrease in calcium sensitivity, allowing for increased rates of relaxation during periods of increased heart rate. HSP27 is involved in regulating protein aggregation/stabilization, and ultimately myocardial stiffness. HSP27 phosphorylation could function with cTnI to regulate ventricular filling at high heart rates. Using SDS-PAGE and Western Blotting, proteins were separated and transferred to nitrocellulose membranes. The blots were then probed using antibodies specific to the phosphorylated protein of interest before being stripped and re-probed with antibodies for total protein content. Data obtained for the phosphorylation of cTnI supported the original hypothesis: the increased heart rates of summer active and IBA periods would be associated with elevated phosphorylation. Conversely, decreased cTnI phosphorylation during torpor was observed. A significant season effect ($p=0.005$) was observed using multiple comparison tests. The phosphorylated cTnI to total cTnI ratio for torpor (0.60 ± 0.60) was significantly less than summer (1.23 ± 0.30, $p=0.020$) and was also significantly less than IBA (1.37 ± 0.36, $p=0.007$). There was no significant change in ratio between IBA and summer ($p=0.812$). Data for the investigation of HSP27 is still outstanding, though it is hypothesized to show an increase in phosphorylation for the two groups summer active and IBA, as these require a reduction in stiffness with the higher heart rates. These mammals exhibit an inherent protection mechanism against ischemia and reperfusion injury, and understanding the underlying mechanism behind this protection could have clinical applications, e.g. with organ transplantation or myocardial infarctions.