Measuring the Activity of the SERCA Using Thapsigargin as a Ca+ Ion Pump Inhibitor

Elphias Osinde Jr., Department of Biological Sciences, College of Arts and Sciences, and Honors College
Edward Dzialowski, Department of Biological Sciences, College of Arts and Sciences

Abstract

Estivation is a means through which organisms can slow metabolism to increase chances of survival in adverse conditions such as elevated temperature, and food and water shortages. The activity of the sarcoplasmic reticulum is complex and not completely understood (Wray and Burdya 2010). An enzyme within the sarcoplasmic reticulum, known as Sarcoendoplasmic Reticulum Calcium-ATPase (SERCA) is thought to play a role in regulation of metabolism and in inducing estivation.

Chicks are ectotherms upon hatching. The point at which chicks transition from ectothermic regulation to endothermic regulation unclear. However by ~48 chicks are completely endothermic. During the 0-48 hour period the chick thermoregulates using shivering thermogenesis. Shivering is caused by the activation of the Sarcoendoplasmic Reticulum Calcium ATPase (SERCA) pump in the muscle cell. For every 2 Ca+ released ATP is broken down into ADP + P. By assaying the phosphate ions produced by SERCA, we can quantify SERCA. We chose the domestic chicken (Gallus gallus) as a model precocious chick. We will assay samples from the thigh and wing muscles in parallel tubes, with one containing the Ca+ ion pump inhibitor Thapsigargin as a negative control. Malachite green will be used as an indicator of phosphate ions. Samples will be analyzed by spectrophotometry at wavelengths of 620 and 655 nm.

Hypothesis

It is hypothesized that the amount of phosphate produced in leg muscle of estivating chicks will be significantly lower than the amount of phosphate produced by non-estivating chicks, and that the amount of phosphate present is positively correlated with concentrations of SERCA.

Literature Review

Malachite Green is a dye that has been used previously to stain bacteria and spores. It has also proven useful in assessment of SERCA productivity (Williams et al. 2008; Storey 2008). The malachite green indicator turns green in the presence of phosphate. The greater the intensity of the green color, the more phosphate present and the higher the SERCA concentrations. Analysis of SERCA concentrations have been done using this method in both snails and turtles. The snail study compared SERCA concentrations in animals fed and watered irregularly and housed at an elevated temperature, with that of a control group of snails. After ten days, both the experimental and control group were sacrificed, decapitated and the leg muscles were excised and stored at -70°C until analyzed by spectrophotometry. The SERCA productivity appears to be temperature-dependent. For an active foot muscle, SERCA levels increased as temperature increased. In an estivating foot muscle, temperature had a small change in SERCA activity.

Methodology

Malachite Green Solution Preparation
First make a solution of 4 molar HCl by mixing 66.6 ml of dH2O with 33.3 ml of 12 M HCl. Make solution C with 4M H2O2. Place 13.6 mg of KH2PO4, 2H2O; this makes a 10 mM solution (freeze in -20 Celsius). Place 1 mL of this solution into tube 1 containing 9 mL of dH2O (creating a 1 mM solution), place 1 mL of tube 1 into tube 2 containing 9 mL of dH2O (creating a 1 mM solution), place 1 mL of tube 2 into tube 3 containing 9 mL of dH2O (creating a 0.1 mM solution), and place 1 mL of tube 3 into tube 4 containing 9 mL of dH2O (creating a 0.01 mM solution). These are the cereal dilutions. Run through.

Preparing sample
After incubating the chicken eggs for 19-20 days, remove chicken from incubator to take samples of the thigh and wing. Place tubes in freeze and then pour 25% of solution into tube 1 containing 9 mL of dH2O. Then pour 25% of solution into tube 2 containing 9 mL of dH2O; this makes a 10 mM solution. After incubating the chicken eggs for 19-20 days, remove chicken from incubator to take samples of the thigh and wing. Place samples in tubes and freeze at -20 until needed.

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