Regulation of Sarco/Endoplasmic Reticulum Ca^{2+} ATPase mRNA as a Mechanism to Prevent Ca^{2+} Cytotoxicity

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Introduction

- Neuron excitation causes Ca^{2+} influx through ligand-gated Ca^{2+} channels and voltage-gated channels.
- Neuron excitation may result in excitotoxic amounts of Ca^{2+} without proper Ca^{2+} regulation.
- The Sarco/Endoplasmic Reticulum Ca^{2+} ATPase Pump (SERCA2 and SERCA3) plays a critical role in intracellular Ca^{2+} dynamics. One function is to store intracellular Ca^{2+} in the endoplasmic reticulum, thus acting to prevent Ca^{2+} induced excitotoxicty. (Figure 1)
- The Plasma Membrane Ca^{2+} ATPase Pump (PMCA2) also plays a critical role in intracellular Ca^{2+} dynamics. It functions in the plasma membrane as a channel through which Ca^{2+} is pumped out. (Figure 1)
- We investigated how androgens can potentially aid in upregulation of these pump-like structures.

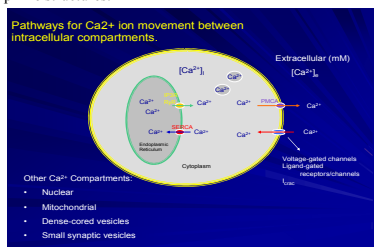

Figure 1. Ca^{2+} ions move between intracellular compartments in order to partition Ca^{2+}. SERCA2, SERCA3, and PMCA2 are all Ca^{2+} regulation mechanisms that work in a pump-like fashion to move Ca^{2+} between compartments.

Hypothesis

Androgens upregulate SERCA2 mRNA in order to decrease cytotoxic levels of Ca^{2+}

Materials and Methods

Quantitative RT-PCR was utilized to assess the expression of SERCA2, SERCA3, and PMCA2 mRNA in vitro and in vivo. Primers specific to the SERCA2, SERCA3, or PMCA2 gene were designed and utilized for the RT-PCR experiments. Hippocampus was dissected on embryonic day (E) 17 and neurons were dissociated. Primary hippocampal cultures were maintained in vitro for 14 days. Cells were treated with 1nM Dihydrotestosterone (DHT) or vehicle (control) for 48 hours. Cells were harvested, total RNA was isolated, cDNA was generated by reverse transcription (RT), and SERCA2, SERCA3, and PMCA2 mRNA was measured using quantitative RT-PCR. Quantitative RT-PCR was also utilized to assess the expression of SERCA2, SERCA3, and PMCA2 in vivo. Adult male Sprauge-Dawley rats were gonadectomized and adrenalectomized. They were then injected (s.c.) for 4 days with either DHT-P (8mg/kg) or vehicle (oil). They were sacrificed and hippocampus was harvested, total RNA was isolated, cDNA was generated by RT, and SERCA2, SERCA3, and PMCA2 mRNA expression was measured using quantitative RT-PCR. GAPDH was measured as an internal control.

Results

Calcium Pumps are Selectively Modulated by Androgens in vitro

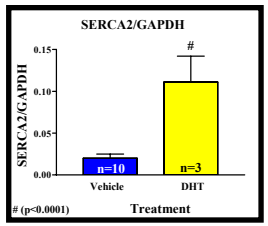

Figure 2. SERCA2 mRNA expression was significantly upregulated in primary hippocampal neurons that were treated with 1 nm DHT compared to vehicle treated controls. Quantitative RT-PCR was used to generate data.
 # indicates a significant ($p<0.0001$) increase in SERCA2 mRNA expression in DHT treated primary hippocampal neurons compared to controls.

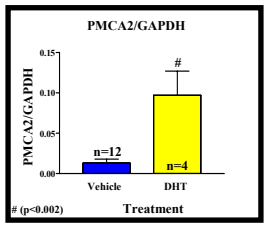

Figure 3. PMCA2 mRNA expression was significantly upregulated in primary hippocampal neurons that were treated with 1 nm DHT compared to vehicle treated controls. Quantitative RT-PCR was used to generate data.
 # indicates a significant ($p<0.002$) increase in PMCA2 mRNA expression in DHT treated primary hippocampal neurons compared to controls.

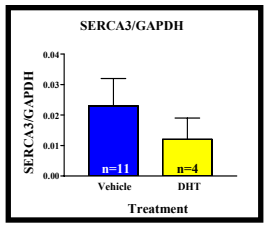

Figure 4. SERCA3 mRNA expression shows no significant difference between the two treatment groups of 1 nm DHT and vehicle in primary hippocampal neurons. SERCA3 is therefore not regulated by administration of androgens in vitro. Quantitative RT-PCR was used to generate data.

Calcium Pumps are Selectively Modulated by Androgens in vivo

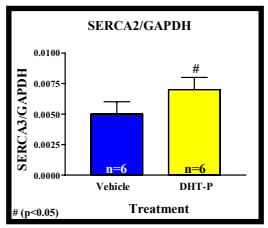

Figure 5. SERCA2 mRNA expression was significantly upregulated in the hippocampus of animals that were treated with DHT-P compared to vehicle treated controls. Quantitative RT-PCR was used to generate data.
 # indicates a significant ($p<0.05$) increase in SERCA2 mRNA expression in DHT-P treated rats compared to controls.

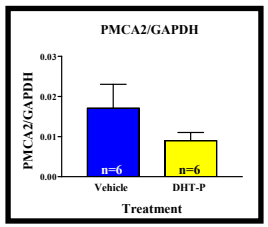

Figure 6. PMCA2 mRNA expression shows no significant difference between the two treatment groups of DHT-P and vehicle on animals. PMCA2 is therefore not regulated by administration of androgens in vivo. Quantitative RT-PCR was used to generate data.

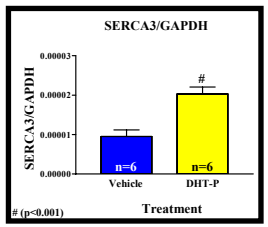

Figure 7. SERCA3 mRNA expression was significantly upregulated in the hippocampus of animals that were treated with DHT-P compared to vehicle treated controls. Quantitative RT-PCR was used to generate data.
 # indicates a significant ($p<0.001$) increase in SERCA3 mRNA expression in DHT-P treated rats compared to controls.

Summary

- SERCA2 mRNA is significantly upregulated in DHT treated primary hippocampal neurons compared to vehicle treated controls.
- PMCA2 mRNA is significantly upregulated in DHT treated primary hippocampal neurons compared to vehicle treated controls.
- SERCA3 mRNA expression shows no significant difference between DHT and vehicle treated primary hippocampal neurons.
- SERCA2 mRNA is significantly upregulated within the hippocampus of DHT-P treated animals compared to vehicle treated controls.
- PMCA2 mRNA expression shows no significant difference between DHT-P and vehicle treated controls.
- SERCA3 mRNA is significantly upregulated within the hippocampus of DHT-P treated animals compared to vehicle treated controls.

Conclusions

Androgens may prevent neuronal excitotoxicity of Ca^{2+} by upregulating SERCA2 within the hippocampus.