Dimorphic Response of Neurons to Estrogen

Benjamin Ziebart with Dr. Damani Bryant

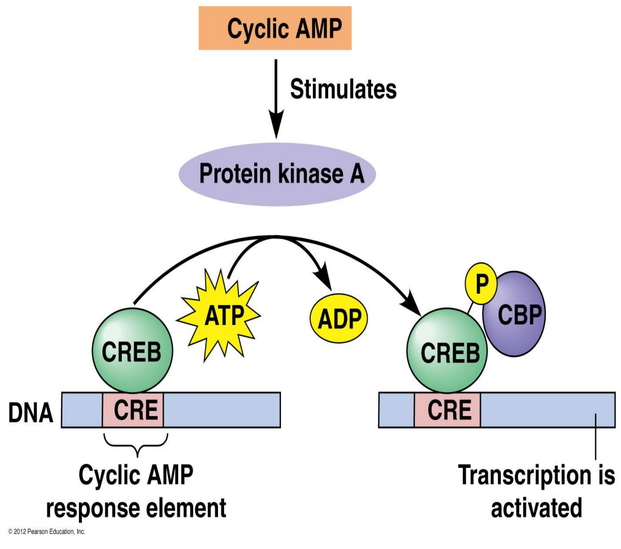
Biology Department | University of Wisconsin - Eau Claire | 2013-2014

University of Wisconsin-Eau Claire

Introduction

A large part of the damage done to the brain during a stroke is due to the toxicity of glutamate (2). Glutamate is a neurotransmitter used to signal between cortical neurons which can be fatal to neurons in large surges. 17β-estradiol (E2) levels in the brain have previously been shown to provide neuroprotection against glutamate toxicity in healthy female neurons but not male neurons. The focus of our research was to determine what role the cAMP response element binding (CREB) protein played in sexually dimorphic neuroprotection caused by E2. Findings could help indicate how female neurons can better protect themselves in the presence of glutamate, which could reduce possible damage done by a stroke.

The pathway initiated by E2 has been documented both *in vitro* and *in vivo* (1,3). Downstream from the estrogen receptor (ER-α) the CREB protein is phosphorylated (activated) which then binds to CREB binding protein (CBP). This complex binds to the cAMP response element (CRE) in the promoter sequence on the DNA. The binding of CREB and CBP complex to the CRE initiates a cellular response by regulating genes that may be required for protection. To measure the response specific to each biological sex we measured the amount of activated CREB in neurons after a 15 minute treatment of 10nM E2. It was found that there was an increase of activated CREB in female neurons. These findings were consistent with that differentiation of neuroprotection is being offered in females, in part, by the CREB protein in the estrogen signaling pathway.



Figure

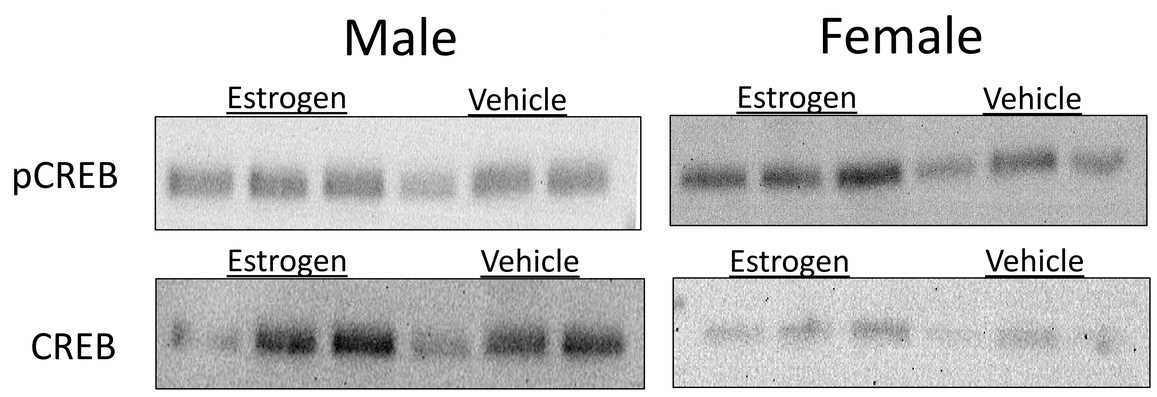
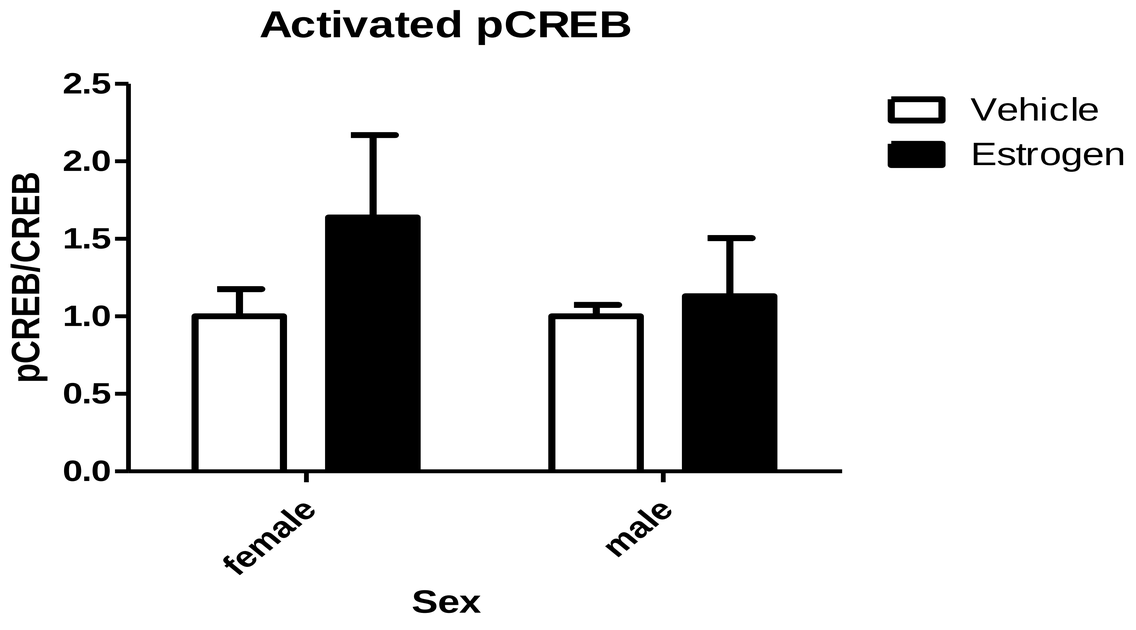


Figure 1: Preliminary data suggests treatment in 10nM E2 solution increased activated CREB in females but not males. Female and males were subjected to 5 hour starvation in minimal glucose medium and then treated with 10nM E2 or vehicle 70% EtOH (n=3). There is a statistically significant difference (p<.05) for an increase in activated CREB in females but not in males.



Materials & Methods

The procedures used to gather neurons used in our experiment conformed to guidelines established by the National Institutes of Health and University of Wisconsin Eau Claire for the humane treatment of animals. Primary cultures of cortical neurons were prepared from Sprague Dawley rat pups on embryonic day 18 (E18) as described in Brewer et al. (1993) and Brewer & Price (1996). Cortices were sorted by biological sex and dissected in Hibernate-E media (Brain Bits Inc, Springfield, Il) containing B27 supplement. Dissected cortices were incubated in Papain (20units/ml) in Hibernate-E minus calcium for 30 minutes at room temperature. Neurons were subsequently dissociated by manual trituration in Hibernate/B27. Neurons were counted and plated on poly-d-lysine coated plates in Neurobasal media containing 2% B27, 1% Glutamax and penicillin-streptomycin (Invitrogen, Carlsbad California). Cultures were maintained in a 5% CO_2 atmosphere for 10 days *in vitro* (DIV) in a humidified incubator. The neurons were then incubated at a low density concentration in well plates and "starved" in an unsupplemented low glucose media for 5 hours. Neurons were next treated with 10 nM estradiol or the control treatment of 70% ethanol for 15 minutes. After treatment neurons were lysed by ice-cold buffer and boiled for one minute. Samples were subsequently centrifuged and placed in a 10 well Tris-Acetate SDS-PAGE gel and CREB proteins were isolated. The proteins were transferred to a PVDF membrane by electrotransfer. The membranes were then blocked with 5% milk for 1 hour and immunoblotted with antibodies against pCREB and CREB (1:1000) overnight. The primary antibody was then labeled by a fluorescently labeled secondary antibody for 1 hour. This allowed us to quantify the amount of activated and total CREB existing in the neuron sample based on the amount of fluorescence radiating from the CREB bands on the membrane.

Discussion & Future Studies

The preliminary data, in figure 1, shows that there is a greater amount of activated CREB in females than in males in response to E2. This indicates that sex differentiation results in an increased response to E2 signaling through the CREB protein. If future studies show a significantly higher amount of activated CREB in females than males, then we will determine whether blocking the activation of the CREB protein will prevent neuroprotection against a high concentration of glutamate surrounding the neuron.

Acknowledgements

Funded by National Science Foundation

Special thanks Seyeon Kim, Luke Mike and DJ Ferrise for providing samples for study and also to Dr. Herman for enabling us to use his Lab's UVP EpiChemi Darkoom machine

Citations

1. Szego, E., Barabas, K., & Balog, J., et al. (2006). Estrogen induces estrogen receptor α-dependent cAMP response element-binding protein phosphorylation via mitogen activated protein kinase pathway in basal forebrain cholinergic neurons In vivo. *The Jornal of Neuroscience*.
2. Roof, R. and Hall, E. (2000). Gender differences in acute CNS trauma and stroke neuroprotective effects of estrogen and progesterone. Journal of Neurotrauma. volume 17.
3. Zhou, Y., Watters, J., & Dorsa, D. (2013). Estrogen rapidly induces the phosphorylation of the cAMP response element binding protein in rat brain. *Endocrine Press*, *137*.