



# Sex Difference in 17-Beta Estradiol Regulation of Akt Signaling with Estrogen Receptor Alpha Activation

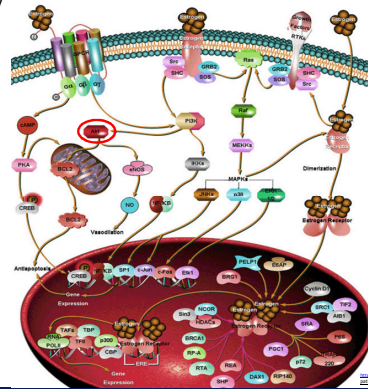
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## Introduction

There is evidence of a **sex difference** in the incidence and outcome of Alzheimer's disease and stroke (1,3,6). **Females have a better outcome than males.** However when women reach menopause, this neuroprotection in females is lost (1,5). The loss of neuroprotection in menopause suggests that **E2 regulates this sex difference in neuroprotection.** The sex differences in diseases like Alzheimer and stroke have been observed across culture, ethnic groups and socio-economic status. These observations suggest that this **sex difference is a strong predictor of neurodegenerative diseases.**

**Estrogen receptor (ER) alpha** plays an important role in E2 regulated neuroprotection. There is **more ER alpha present in female neurons** than male neurons (1,2,5). In the absence of E2, ER is bound to the Heat Shock Protein and is inactive. When an E2 molecule enters a cell and passes into the nucleus, E2 binding can regulate genes via both genomic and nongenomic signaling pathways (4). In the **genomic signaling pathway**, E2 binds to ER, translocates into the nucleus and binds to Estrogen Response Elements (ERE) to regulate the activity of different genes and to produce proteins. This pathway is slower than the nongenomic pathway of E2. The **nongenomic signaling pathway** doesn't get stimulated through binding to DNA, instead ER alpha interacts with signaling cascades such as **PI3-kinase/AKT** and cAMP/PKA/CREB (1,5).

Current understanding of E2 neuroprotection involves **rapid phosphorylation of MAPK, Akt, and CREB** (1). Bryant and Dorsa (2010) found that activation of ER alpha regulates CREB signaling in a sexually dimorphic manner in primary cortical neurons. It is possible that stimulation of ER alpha, along with CREB, regulates serine/threonine protein kinase (Akt) phosphorylation in a sexually dimorphic manner.



## Hypothesis

The purpose of this study was to test the hypothesis that there is a sex difference in 17 beta-estradiol regulation of Akt signaling with Estrogen Receptor Alpha activation in prenatal neurons.

## Methods

Prenatal neurons from rat pups were sorted by sex and dissected in Hibernate-E media. Cortices were isolated and incubated for twenty minutes in Papain in Hibernate-E minus calcium at room temperature. Sorted cortices were plated on poly-L-lysine coated glass cover slip in complete media. Twenty-four hours prior to harvesting, neurons were starved in low glucose and then treated with either diluted E2 (10nM) or diluted 70% ethanol for five or ten minutes. After treatments, they were lysed in IP buffer with protease inhibitor. Samples were boiled for three minutes and then these samples were ran in a gel electrophoresis. MOPS/MES buffer (1X) was used to run a gel. After running the gel, the gel was transferred to a membrane using transfer buffer (1X). Membranes were blocked in 5% BSA for an hour then agitated overnight in primary pAkt antibody. The antibody was diluted to 1:1000 in 5% BSA. After overnight agitation, membranes were washed with TBST five times (five minutes/one wash) and then membranes were agitated for an hour in Horse Radish Peroxidase tagged secondary antibody (1:1000). The membranes were washed with TBST five times (five minutes/one wash) then pAkt levels were detected in each membrane using software on a UVP EpiChem Darkroom machine. The sample were stripped with reblot and then the primary Akt antibody was repeated as the procedure described above. To observe the amount of pAkt and amount of Akt, we counted the number of pixels on each band.

## Citations

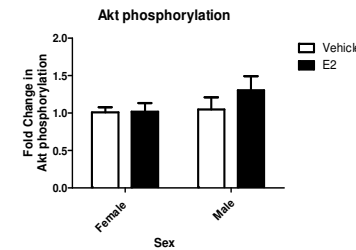
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## Figures

### 5 Minute Treatment



Figure 1. Rabbit monoclonal pAkt antibodies were used to detect pAkt using prenatal neurons that were treated for 5 minutes in vehicle and E2 (10nM).



### 10 Minute Treatment

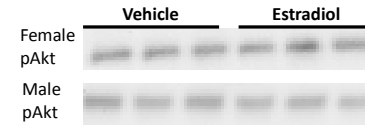
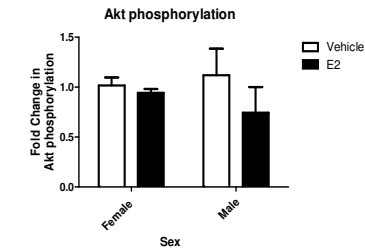


Figure 2. Rabbit monoclonal pAkt antibodies were used to detect pAkt using prenatal neurons that were treated for 10 minutes in vehicle and E2 (10nM).



## Results

- In 5 minute treatment samples, we did not observe a significant sex difference in 17 beta-estradiol regulated Akt phosphorylation (P=0.280).
- In 10 minute treatment samples, we did not observe a significant sex difference in 17 beta-estradiol regulated Akt phosphorylation (P=0.806).

## Discussion

Previous studies found more Akt phosphorylation in premenopausal women and increase in Akt phosphorylation with E2 treatment. However, we did not observe a significant sex difference in 17 beta-estradiol regulated Akt signaling in prenatal neurons.

- This contradicting result may be due to
  - Different areas of the brain
  - Different concentrations of estrogen
  - Different time of treatment exposure
  - Different ages of rats

## Future Research

This study found no sex difference in E2-regulated Akt phosphorylation in prenatal neurons. It is possible that prenatal neurons have not completed the second testosterone surge, which may have resulted in incomplete masculinization in male prenatal neurons. Completion of second testosterone surge may be the key to observe a sex difference in Akt phosphorylation. Therefore, postnatal neurons, which have completed the second testosterone surge, should be used in future research.

## Acknowledgements

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