The effect of varying levels of estradiol on mRNA expression in GnRH neurons

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Abstract (revised)

Gonadotropin hormone releasing hormone (GnRH) is the primary regulatory molecule that controls the hypothalamic-pituitary-gonadal axis, which controls reproductive function. Secretion from GnRH neurons regulates the production of gametes and gonadal hormones and maintains the reproductive axis in both sexes. Gonadal hormones, such as estradiol (E_2), provide feedback to regulate the production and secretion of GnRH. Estradiol typically has a negative effect on GnRH secretion, however, little is known about the mechanisms by which estradiol regulates GnRH release. The object of this project was to determine the effect of varying estradiol concentrations *in vivo* on mRNA expression in GnRH neurons. We studied these effects on a transgenic mouse model that expresses enhanced green fluorescent protein (eGFP) only in GnRH neurons. We hypothesized that the maximal efficacy of estradiol on GnRH neuronal gene expression would be reduced in neurons from old animals, and that this maximal response would be reached with an intermediate dose of estradiol. Our preliminary results indicate that even doubling the amount of estradiol administered to an old animal does not affect GnRH mRNA expression.

Acknowledgments

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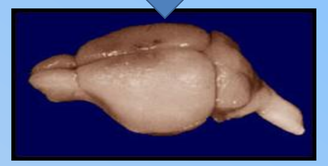
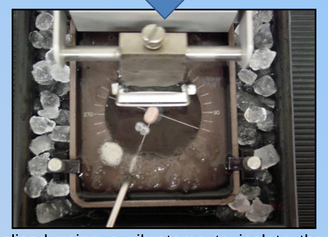
Materials and Methods

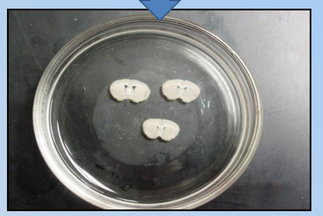
Transgenic mice that express eGFP in GnRH neurons were used. We ovariectomized old females (15-18 months old) to minimize natural sources of estradiol. Capsules were implanted in the mice containing either a placebo of corn oil, 0.625 µg of E_2 (1xE), 1.25 µg of E_2 (2xE), or 0.3125 µg of E_2 (0.5xE).



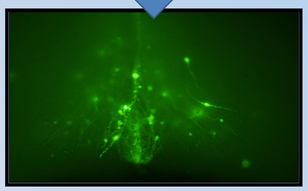
Five to seven days later, the mice were sacrificed and the brains were harvested.



The brain was sliced using a vibratome to isolate the areas with the highest GnRH concentration.



Sliced brain sections are then dissociated to isolate GnRH neurons.



GnRH neurons from the brain sections fluoresce. Individual neurons were harvested, total RNA was isolated, then quantitative RT-PCR with primers specific for GnRH was used to determine the amount of mRNA originally present in the GnRH neuron.

Results

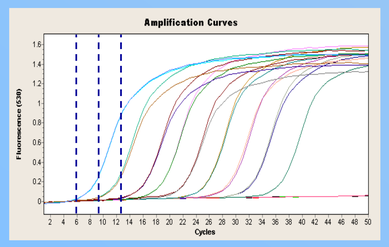


Fig. 1 Example of an amplification curve from a real-time PCR run. The crossing point (C_T) is the point at which enough fluorescence is detected after a certain amount of amplification has taken place during the run. It is determined by the number of cycles needed to reach this level, indicated by the X-axis. Thus, a higher C_T reflects a greater number of cycles, which indicates that less DNA (mRNA) is present. The more mRNA present, the earlier the C_T will be recorded. This point is shown here for the first three samples by the blue dotted lines.

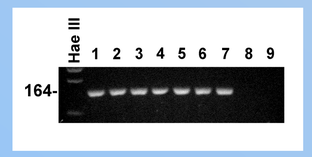


Fig. 2 Representative gel demonstrating a positive GnRH amplicon from single cells. Cell # 1-4 are from a young OVX mouse, 5-7 an old intact mouse, and 8-9 are negative RT controls.

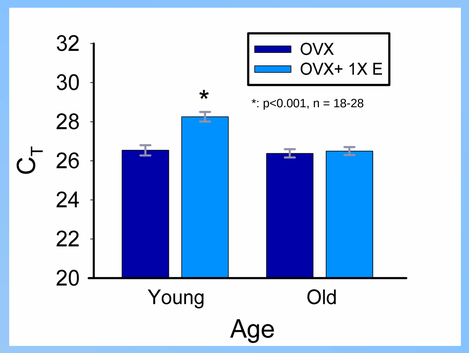


Fig. 3 Previous data of the C_T of young and old mice, treated with either the control or 1xE concentration. A higher C_T reflects a reduced concentration of mRNA present in the GnRH neuron. This graph suggests that in young mice, estradiol administered to the animal reduces the amount of GnRH mRNA expressed on a single cell basis. However, in the old mice, estradiol seems to have no affect on the C_T or activity of the GnRH neuron.

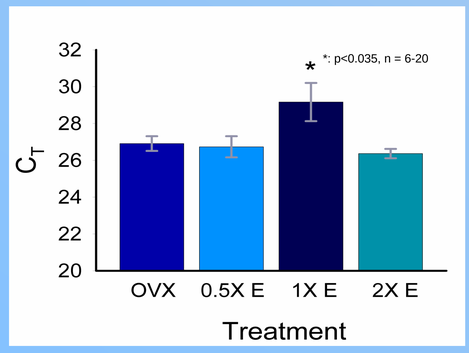


Fig. 4 C_T of each treatment group of the old mice. A higher C_T reflects a reduced concentration of mRNA present in the GnRH neuron. Changing the estradiol concetration administered to the old animal appears to have no effect on GnRH mRNA expression between the control, 0.5xE, and the 2xE. However, this preliminary data suggests that the same 1xE dose of estradiol administered in previous experiments does have an effect.

Conclusions

This study was undertaken to determine why estradiol did not affect GnRH mRNA in single GnRH neurons from aged animals. Specifically, we tested whether increasing the amount of estradiol administered to the animal would cause the same response seen in young animals. Our preliminary results indicate that even doubling the amount of estradiol administered to an old animal does not affect GnRH mRNA expression. This suggests that the results seen in our previous experiments are due to a lack of effect by estradiol on the GnRH cells themselves, not due to metabolism or animal weight. However, our current data does not agree with our previous data, because we do see an effect of 1X estradiol. There are several possibilities for this result: this data is preliminary, and more data may yield different results, technical issues with the harvest or PCR, or perhaps the previous data was incorrect. Further work is necessary to completely describe the effect of estradiol at all concentrations in the old mouse.