Video Article

Hormonal regulation of immortalized hypothalamic neurons: Estrogen treatment and analysis of neuropeptide Y mRNA transcript levels

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Abstract

The hypothalamus senses peripheral hormonal signals to maintain physiological homeostasis through the neuroendocrine axis. Disturbances to hormonal signaling in the hypothalamus can lead to a number of detrimental health concerns including obesity and infertility. Developing a thorough understanding of the transcriptional regulation of unique hypothalamic neuropeptides is essential in order to understand how the hypothalamus maintains physiological homeostasis. Classical in vivo approaches have been instrumental in establishing the direct synaptic connectivity between distinct hypothalamic nuclei and the functional significance of neuropeptides. However, the inherent complexity of the neuronal circuitry comprising the hypothalamus creates an unwelcoming environment to study the direct transcriptional regulation of hypothalamic neuropeptides. To this end, we have developed immortalized, hypothalamic neuronal cell lines to study the signaling events, transcriptional and secretory mechanisms responsible for the regulation of key hypothalamic neuropeptides. Here, we demonstrate a method our group and others use to study the direct regulation of hypothalamic neuropeptide mRNA transcript levels in response to hormonal treatments. Specifically, we complete a 24-hour (2-,4-,8- and 24-hour) 17β-estradiol (estrogen) treatment experiment using the mHypoE-38 neuronal cell line and isolate total RNA using a phenol-chloroform extraction method. We then completed cDNA synthesis and real time quantitative RT-PCR analysis to measure changes in NPY mRNA levels in response to estrogen treatments. The results of this experiment describe the anorexigenic and reproductive effect of estrogen on NPY gene expression that has been previously published by our laboratory (Titolo et al, Mol Endocrinol, 2006). Using similar experimental paradigms, these novel hypothalamic cell models can be also used to study the direct hormonal regulation of signaling pathways, promoter activity, secretory responses and epigenetic changes.

Disclosures

No conflicts of interest declared.