Effect of Orexin A on Neuropeptide Y Expression in a Novel Immortalized Hypothalamic Cell Line

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Introduction

In rodents, stimulation of food intake by the peptide hormone orexin A (OX A), hypocretin 1 is dependent in part on neuropeptide Y (NPY) signaling in the hypothalamic arcuate nucleus (Arc) [1]. Arc-like NPY cells express orexin receptors 1 & 2, and orexin neurons form synapses on Arc NPY neurons [2]. OXA increases the activity of Arc NPY neurons in vitro [3,4], and OXA stimulates increases in NPY mRNA 2 h post-treatment in vivo [5]

Arc-like NPY neurons are also regulated by leptin. An NPY neuron expresses leptin receptor (LeptR) [6]. In contrast to orexin, leptin inhibits activity of NPY cells [7] and NPY mRNA expression [8]. While orexin and leptin have apparently inverse effects on Arc NPY neurons, the specific mechanisms through which leptin and orexin signaling pathways might interact to exert short- and long-term control of NPY expression and release are not fully understood.

While in vivo rodent models have been proposed using NPY- and LeptR-expressing cell lines [10], leptin effects on NPY in this line in conflict with in vivo results, and interpretation is as the cell line used was derived from a human neoblastoma rather than a normal neuron. We present here preliminary data on an Arc-like model using a mouse cell line derived from immortalized embryonic hypothalamic neurons via retroviral transfer [10]. This cell line is derived from normal neurons rather than a tumor, it is more likely to reflect responses of differentiated neurons in vivo [10]. Our initial investigations were designed to: (1) validate this cell line as Arc-like, and (2) to begin to investigate mechanisms of OXA action in these cells.

Methods

Cell Line and Maintenance: Differentiated immortalized embryonic mouse hypothalamic cells (identified Clu 121, Fig. 1) were purchased from CELLutions-Celladine (North Carolina, USA). Cells were maintained in DMEM medium supplemented with 10% FBS at 37°C with 5% CO2. Experimental Treatment: Orexin peptide (American Peptides, Sunnyvale, CA) was dissolved in PBS and stored at -20°C until the day of use. Final concentrations were diluted in DMEM. Cells were incubated for 1, 2, 4, or 24 h in either DMEM or DMEM with 300 nM orexin. Real-time RT-PCR: Total RNA from cultured cells was isolated using a commercial extraction kit (Qiagen). Isolated RNA was measured by real-time RT-PCR using a Roche LightCycler. Primers used are summarized in Table 1. Relative mRNA levels were normalized to GAPDH using a ΔΔCT method.

Results

Gene expression for NPY, leptin receptor, and orexin receptors 1 and 2 was verified using real-time RT-PCR (Fig. 2) and is consistent with the cell line profile provided by the supplier. RT-PCR data also suggest the Clu 121 line expresses agonist-related protein (not shown). The gene expression profile observed is consistent with that of an Arc NPY neuron.

While differences did not reach significance, NPY mRNA expression shows a trend for increase at 2 h after OXA treatment (Fig. 3) consistent with in vivo data on OXA effects in the Arc. It is possible that we have not yet determined the optimal OXA dosing regimen or time course to observe significant changes in NPY.

Treatment with OXA resulted in a significant decrease in leptin receptor mRNA 1 h post-treatment (p < 0.01; Fig. 4). Leptin receptor mRNA did not differ from controls at later timepoints.

Conclusions

Data suggest that in addition to increasing NPY expression and cell firing (as demonstrated by earlier in vivo and in vitro work, and consistent with trend for increased NPY here, OXA actions in the Arc might also result in changes in leptin sensitivity. Other reports suggest that Lept and orexin receptors may have convergent intracellular second messenger pathways, such as extracellular-factor-regulated kinase and the Janus kinase(STAT3) pathway [11], which could interact to modulate activity of Arc NPY neurons. The effects of OXA on Lepr mRNA in Clu 121 cells is intriguing. Leptin results in downregulation of PI3K-P-Akt and increase in leptin in heptic stellate cells, and increased PI3K-P-Akt activation in these cells reduces Lept- and block leptin effects [12]. Orexin is known to increase PI3K-p-Akt in fat cells [13]. Although it is unknown whether orexin increases PI3K-p-Akt in neuronal tissue, our preliminary data suggests a similar mechanism might be present in neuronal cells as evidenced by the OXA-induced changes in Lept-observed here. Together our results suggest that in addition to orexin-leptin interactions in downstream signaling pathways, OXA actions in Arc might also result in short-term downregulation of Lept, thus temporarily decreasing the ability of leptin to shut down NPY production during OXA-stimulated feeding. We are currently working to further investigate interactions between leptin and orexin signaling in the Clu 121 cell line. We further plan to use organotypic punches to validate our findings in intact isolated Arc tissue before moving on to in vivo validation of our findings. Finally, as a long-term goal we hope to work towards the establishment of an Arc-specific adult cell line via immortalization of neurons extracted from macaque-dissolved Arc tissue.

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References