Sex steroid hormones and sexual dimorphism of circadian rhythms in mice

Introduction

Nearly every cell in our bodies aligns to an internal biological clock.[2] Recently, sex steroid hormone function has been linked to this clock, governed by what is known as a circadian rhythm. These findings have implications for women who experience common conditions such as menopause or polycystic ovarian syndrome (PCOS), both of these result in dramatic reductions of estrogen levels. Additionally, PCOS is the most common cause of infertility in women and associated with common metabolic diseases such as insulin resistance[1]. My project aims to study the ways lowered estrogen physiologically affects circadian rhythms in female mice. To do this, I will monitor sleep-wake cycles and compare ovariectomized (OVX) female mice, sham-operated females, and sham-operated males.

Background and literature review

The suprachiasmatic nucleus (SCN), located in the hypothalamus, is the "master clock" of circadian rhythms in organisms, mediating endogenous light signal via the eyes and known to re-set the host organism's biological rhythm when donated.[6] The ovaries, on the other hand, are the primary site of estrogen and progesterone production in vertebrates. Patients with PCOS, for example, suffer from reduced estrogen and progesterone production and increased testosterone levels, among other endocrine symptoms. In addition, it is well known that aging results in shorter and reduced amplitude circadian rhythm patterns, suggesting a link between sex steroid hormone reduction and circadian regulation

Estrogen has been associated with various components of the biological clock. Mice lacking aromatase, an enzyme involved in metabolizing a form of estrogen known as 17β-estradiol (E2), showed changed circadian patterns.[3] Clock-dictating genes have also been shown to modulate expression levels of an estrogen receptor isoform known as ERβ.[5] Female mice that have undergone OVX have increased expression of androgen receptor (AR), a protein exemplifying sexual dimorphism in circadian components, as it is typically expressed in the male SCN but not the female.[8] Exposing OVX females to testosterone produces similar AR levels as males undergoing testosterone replacement following gonadoectomy.[7] Recent research suggests AR is implicated in regulating SCN structure, function, circadian timing system, and response to light.[4, 8]

Although some studies have demonstrated sexual differences between male and female SCNs, relevance to physiology is still unclear. Additionally, most studies on circadian rhythms have only been performed on male mice to avoid the possible complications of estrogen cycles in females. Thus, very little is known about female circadian rhythms. My project will specifically investigate the physiological function of sex steroid hormone changes in OVX female mice. I will perform OVX on female mice to remove estrogen and then compare them to sham-operated females and males, measuring their sleep-wake cycles and light sensitivity by applying the wheel-running activity measurement method.

Research question

Are there sexual dimorphisms in circadian duration patterns? Do OVX-induced "masculinization" of AR expression levels in the SCN affect the sleep-wake cycle and light sensitivity in female mice? I hypothesize that there are sexual dimorphisms in circadian duration

patterns and light sensitivity, producing masculinized patterns in OVX female mice similar to the sham-operated males.

Methodology

I will compare three groups of mice: OVX females, sham-operated females, and sham-operated males. OVX is a surgical procedure that involves removal of the ovaries, while sham-operated mice undergo the same stresses of a surgery (anesthesia, incision, etc.) without removal of any organs. It is important to use sham operation as opposed to no operation at all in order to ensure that any differences between the three groups will not be a result of stress from surgery. OVX is a reliable method for decreasing estrogen and increasing AR levels in females. Animals will have one week to recover from surgery before monitoring.

To determine sleep-wake cycles, I will monitor animals' wheel-running activity. Our cages contain devices attached to animals' wheels that electronically record the time of every spin of the wheel. This allows a simple, automatic method to monitor animals' active wake periods. I will measure this for five to six weeks: the first week under normal conditions of 12 hours of light followed by 12 hours of darkness, the next two-three under constant darkness, then the final two under constant darkness with 30-minute exposure to light once every 24 hours. I will observe their sleep-wake cycles under these conditions to determine any differences between the three groups in circadian duration, and I will spend my last week analyzing the wheel running activity data. I may expand my analysis to other groups of mice (such as females that been surgically given a testosterone pellet) if time allows.

Preparation

My preparation for this project includes working in the Turek lab since October 2010, meaning I will have completed eight quarters of working in the lab by this summer, only missing fall of 2012 to study abroad. During this time, I have been learning the techniques necessary for this project, including data analysis, wheel running activity measurement, and general mouse handling. I am on the lab's animal protocol. I am currently in training for performing OVX and am in the process of collecting preliminary comparative data between male and female mice. Additionally, my previous coursework in the biology 210 sequence and molecular biology have provided the theoretical basis of my knowledge for this project.

Conclusion

I plan to continue my research during my senior year for my senior honors thesis in biological sciences, expanding this project to include other mouse groups as well as investigating the molecular components of these effects. This research experience is important for my future, as I plan to apply to medical schools with MD/PhD programs and pursue biomedical research as my main career goal. This project will also contribute to our broader understanding of the interactions between circadian rhythms and physiology, allowing us to improve treatments for people who suffer from changed sex steroid hormone levels in conditions as common as menopause and PCOS.

References

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