Central Progesterone Involvement in Estrogen-Induced Prolactin and Luteinizing Hormone Secretion Surges in Female Rats

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CENTRAL PROGESTERONE INVOLVEMENT IN ESTROGEN-INDUCED PROLACTIN AND LUTEINIZING HORMONE SECRETION SURGES IN FEMALE RATS

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A thesis submitted to the University Honors Program in partial fulfillment of the requirements for the Honors Degree

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May 10, 2014
Acknowledgements

I would like to thank Dr. Lydia Arbogast for her guidance as well as Amanda Barnard and Phil Jensik for their assistance and support in my research experience, of which I am greatly appreciative. I would also like to thank Research Rookies and the Honors Department for providing the means and encouragement for this participation.
# Table of Contents

Introduction..................................................................................................................................1-4

Objectives....................................................................................................................................5

Experimental Methods..............................................................................................................6-8

Results.........................................................................................................................................9-12

Summary and Conclusions.........................................................................................................13

References.................................................................................................................................14
Introduction

In a cycling female rat, ovarian follicles produce steroid hormones under the influence of gonadotropins released from the pituitary gland (3). These gonadotropins are stimulated by gonadotropin releasing hormone, which is secreted in a pulsatile manner from the hypothalamus of the brain (3). Although ovarian steroids feedback negatively on the hypothalamic-pituitary axis during most of the menstrual cycle, as the cycle of steroid synthesis and secretion increases, a spike in circulating estradiol produced by ovarian follicles under gonadotropin influence drives the process of estrogen positive feedback on the afternoon of proestrus (3). This estrogen positive feedback signals the brain to release gonadotropin releasing hormone and a prolactin-releasing factor that triggers the luteinizing hormone (LH) and prolactin surges, respectfully, from the pituitary gland on the afternoon of proestrus (3). These pituitary hormones then circulate in the bloodstream (1). LH stimulates ovarian follicular steroidogenesis, and causes ovulation (1). Prolactin modulates behaviors and eventually promotes the demise of the corpus luteum (luteolysis) to allow for subsequent reproductive cycles to continue (1). Both of these hormones are crucial for reproductive function of female rats.

When LH stimulates ovarian follicular steroidogenesis and ovulation, the ruptured follicles produce progesterone (1). However, a preovulatory progesterone level is necessary for the gonadotropin surge, and the source of this hormone has been under some controversy (2). Rats that are removed of their ovaries and adrenal glands are still able to produce an estradiol-stimulated LH surge, when administered exogenous estradiol (2). Also, antagonizing progesterone synthesis prevents the estradiol-induced LH surge (2). Moreover, an increase in
neuroprogesterone is correlated with surge levels of plasma LH (2). These recent studies have supported the theory that, in addition to these steroid hormones from an ovarian source, there is localized steroid hormone production, particularly progesterone, in the hypothalamic region of the brain that has an effect on this estrogen positive feedback (2).

Studies have also shown that the estradiol circulating from ovarian origin regulates the synthesis of neuroprogesterone in the hypothalamus, and this locally produced neuroprogesterone is involved in the initiation of the LH surge and subsequent ovulation (4). By using in vitro studies, postpubertal hypothalamic astrocyte cells were seen to respond to estradiol stimulation by producing great amounts of progesterone via a rapid initiation mechanism (4). In vivo studies also show responsiveness of neuroprogesterone to estradiol stimulation, but also include a transcriptional component, which is a hallmark of the long-term phase of neurosteroidogenesis (2).

In this study, female rats were overectomized, in order to eliminate steroid influence from the ovaries, and then given an amount of estradiol known to elicit a hormonal response. The prolactin surge was first characterized by giving no further treatment; plasma samples were collected from these rats during the afternoon and evaluated using radioimmunassays.

After characterization, in order to explore the effects of neuroprogesterone on these hormones, aminoglutethemide (AGT), the P450 side-chain cleavage enzyme inhibitor, was infused into the brains of the rats and prevented synthesis of progesterone in the hypothalamus (Figure 2). AGT has been shown to disrupt the reproductive cycles of female rats through studies of changes in vaginal cytology, ovarian follicles, and uterine characteristics (2). The control group was only injected with the vehicle used to infuse AGT into the hypothalamus. Plasma samples were collected from both the AGT and vehicle-treatment animals on the
afternoon of the experiment and evaluated using radioimmunassays specific for either prolactin or LH.

**Figure 1. Estrogen Feedback Pathway.** Gonadotropin Releasing Hormone from the hypothalamus triggers LH and Follicular Stimulating Hormone (FSH) from the pituitary. The ovaries are then stimulated to begin steroidogenesis. Prolactin is also released from the pituitary, but has other effects than ovarian stimulation. Estradiol synthesized in the ovaries then feeds back to effect release of hypothalamic and pituitary hormones.
**Figure 1. Progesterone Synthesis Pathway.** AGT inhibits the P450 side chain cleavage enzyme at the first step in the synthesis of progesterone.
Objectives

1) **Characterize the prolactin surge in our colony of rats.** Overectomized rats were given estradiol treatment in order to stimulate the afternoon prolactin surge. No brain cannulation was performed, so that no further treatment was given. Plasma samples were taken every hour and then measured using radioimmunassays and analyzed.

2) **Block progesterone synthesis in order to measure the effects of neuroprogesterone on the estradiol-induced prolactin and LH surges.** These animals were implanted with a brain cannula in order to inject either the AGT treatment or only the vehicle treatment used for infusion of AGT into the lateral ventricle. Plasma samples were again collected on the afternoon of the predicted surges. These samples were assayed for both prolactin and LH and analyzed so that the groups were comparable.
Experimental Methods

Surgical Procedures

All surgical techniques were done under isoflurane anesthesia and with sterile technique. Metacam (1.0 mg/kg) was used as an analgesic before and after all surgeries except those on Day 10, as to not interfere with the surges occurring on the following day.

On Day 1, stainless steel guide cannulas (23 gauge, 4.5 mm long) were implanted into the right lateral cerebral ventricle. The coordinates for placement were: 1.0 mm posterior from bregma, 1.2 mm lateral from midline, and 3.0 mm vertical from dura. Cannulas were fixed to the skull with stainless steel screws and dental cement, and a dummy cannula was inserted.

Placement of the brain cannula was verified at implantation with the presence of artificial cerebrospinal fluid as well as after sacrifice with the presence of methylene blue dye in the ventricle circulation. Both ovaries were also removed from rats on this day.

On Day 8, Estrogen capsules with a dose of 400 µg of β-estradiol per mL of sesame oil were inserted subdermally into rats. The estradiol is then able to enter the circulation and begin the cycle of estrogen-positive feedback to trigger LH and prolactin surges.

On Day 10, a right jugular vein cannula was inserted into rats. This allowed for a blood sample to be drawn from the rat while the animal was awake and able to freely move.

Drug Treatments

Animals were injected with either 5.0 µL of 50 nmol AGT or vehicle treatment. The vehicle used for AGT delivery was 10% hydrochloric acid in artificial cerebrospinal fluid. These treatments were given on the afternoon before and morning of the experiment through the lateral
ventricle cannula using a gastight 10 µL syringe. The injection was completed over 30 seconds, then left in place for 30 seconds. The guide cannula was then sealed to prevent leaking.

Experimental Protocol

0.4 mL of blood was drawn from each animal through use of the jugular cannula on the hour mark on the afternoon of the experiment. Samples were immediately centrifuged, and plasma was collected. The samples were then stored at -20 degrees Celsius until analysis. At the end of the experiments, rats were placed under isoflurane anesthesia, and the brain cannula were injected with methylene blue dye. The rats were sacrificed, and brain tissue was collected and tested for placement of the cannulation.

Radioimmunoassays

The underlying principle that the body is reactive to endogenous compounds allows for measurement of hormones through radioimmunoassays (1). Antibodies created by the body in response to these stimuli are highly specific, and will form hormone-antibody complexes with either the hormone from the plasma samples or additive radiolabeled hormone, thereby creating a competitive environment due to limited antibody concentrations. By use of a standard curve and gamma counter, the assays were able to quantify the variable amounts of prolactin or LH in the plasma samples through comparison of the amount of displaced radiolabeled hormone (1). This particular competitive binding assay used a known quantity of prolactin or LH labeled with $^{125}$I (radioactive isotope). Five day assays were performed for prolactin, and three day assays were performed for LH.
Statistics

Two-way analysis of variance (ANOVA) statistical analysis (Prism software) was used for repeated measures. P< 0.05 was considered statistically significant. Post-hoc analysis was performed with Bonferroni’s test.
Results

In the rats that were studied for prolactin characterization, a discernable surge was witnessed on the afternoon of the predicted surge (Figure 3). These data not only supported that the estrogen treatment given was initiating a surge of prolactin but also provided a visualization model of a surge for the following experiments.

In the AGT and vehicle-treatment rats, samples were again taken on the afternoon of the predicted surges but involved previous injections of either treatment. For prolactin, a surge was witnessed in the vehicle-treatment groups as well as a slightly delayed and decreased surge in the AGT-treatment group (Figure 4). However, time not treatment group was the only significance in the statistical analysis of these data.

For the LH surge in AGT or vehicle-treatment rats, a surge was not seen for either group (Figure 5). It is important to note the significantly smaller scale of hormone levels in these data. Neither time nor treatment group was significant in this statistical analysis.
Figure 3. Prolactin Characterization. The prolactin surge was characterized on the afternoon of the predicted estrogen surge. n= 6 per group.
Figure 4. Prolactin surges in AGT and vehicle-treatment rats. For prolactin, a surge was seen in both the AGT and vehicle-treatment rats. Although determined not significant by statistical analysis, the surge in AGT-treatment rats was decreased and slightly delayed. n= 8 or 9 per group.
Figure 5. LH surges in AGT and vehicle-treatment rats. No LH surge was witnessed in either the AGT or vehicle-treatment rats. n= 8 or 9 per group.
Summary and Conclusions

In the estrogen-treatment model used, a diurnal prolactin surge was witnessed, but no LH surge was present. Elevated prolactin levels could have contributed to the absence of an LH surge. Although not significant, the prolactin surge tended to be decreased and delayed. This was possibly mediated by AGT blockade of neuroprogesterone synthesis.

Overall, these data do not support neuroprogesterone involvement in estrogen-induced prolactin and LH surges. This dose of AGT was selected from the literature as effective in the disruption of the estrus cycle in rats (3). Further studies are needed to verify the inhibition of the synthesis of neuroprogesterone in our colony. Until further data is obtained, neuroprogesterone as they key to estrogen positive feedback will remain under question.

Presentation

These data were presented at the Undergraduate Creative Activities and Research Forum on April 7, 2014.

Funding

Funding provided by NIH grants HD045805 and HD048925.
References


