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Original Research

Effects of Estradiol-17β or Dihydrotestosterone on Cell Types in Equine Pituitaries Staining for Prolactin, Growth Hormone, or Both

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ABSTRACT

Ovariectomized pony mares were treated with vehicle (controls), estradiol-17β (estradiol), or dihydrotestosterone (DHT) daily for 21 days in summer, and the pituitary content of prolactin and growth hormone (GH) and the numbers of cells staining immunocytochemically for prolactin (lactotropes), GH (somatotropes), or both hormones (mammosomatotropes) were assessed. Treatments did not alter ($P > .1$) plasma prolactin concentrations over the 21 days. Estradiol treatment increased ($P < .05$) the pituitary content of prolactin several-fold relative to controls and DHT-treated ponies. The number of lactotropes was also increased ($P < .05$), as was the number of mammosomatotropes ($P < .05$). Treatment with DHT did not alter ($P > .1$) any prolactin characteristic. The number of somatotropes was not altered by either treatment ($P > .1$) nor was the pituitary content of GH ($P > .1$). Given that the mass of prolactin accumulation in the pituitary in response to estradiol was several-fold greater than the approximate doubling of the number of lactotropes and mammosomatotropes, it appears that estradiol administration to ovariectomized pony mares stimulates not only the number of prolactin-producing cells but also the amount of prolactin production per cell.

1. Introduction

We previously described [1] the morphological characteristics of cells in adenohypophyseal tissue (AP) of pony mares that stained immunocytochemically for prolactin (lactotropes) and those that stained for growth hormone (GH; somatotropes) as well as a third cell type that stained positive for both hormones (mammosomatotropes). In that report [1], we also described two distinct morphological forms of lactotropes, referred to as type I and II. Type I cells were larger and contained large, dense, polymorphic granules scattered throughout the cytoplasm; type II cells were smaller and contained small granules that were predominantly found in peripheral areas of the cytoplasm. The relative importance of these two types of lactotropes to prolactin secretion is unknown.

Direct estrogenic stimulation of plasma prolactin concentrations and pituitary content in ovariectomized pony mares was previously reported by Thompson et al [2]. Several experiments since that first report have confirmed the stimulatory effect of estradiol on prolactin production and secretion in horses [3–5], and application of the results from those experiments led to the development of a estradiol cypionate-sulpiride (or domperidone) combination treatment for inducing follicular growth and ovulation in seasonally anovulatory mares in winter [6,7]. The stimulation of prolactin via the estradiol-dopaminergic antagonist combination is synergistic (reviewed by [8]) and is dependent on both factors being present at the same

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time; interestingly, other prolactin secretagogues, such as exercise or thyrotropin releasing hormone (TRH), do not synergize with estradiol pretreatment [5].

Testosterone, like estradiol, is known to stimulate prolactin secretion in other species [9,10], likely due to its ability to be aromatized to estradiol in peripheral tissues. Dihydrotestosterone (DHT), another potent androgen, cannot be aromatized to estrogen [11], thus serves as an androgen alternative to testosterone in experimental paradigms. The purpose of the present experiment was to examine the effects of estradiol and DHT treatment on the relative proportions of lactotropes, somatotropes, and mammosomatotropes in the adenohypophysis of ovariectomized pony mares.

2. Materials and Methods

2.1. Animals and Treatments

All procedures described herein were approved by the Institutional Animal Care and Use Committee of the LSU Agricultural Center. Twelve adult (6–12 years of age) long-term (>2 years) ovariectomized mixed-breed pony mares weighing between 168 and 323 kg were kept on pasture and fed grass hay when needed to maintain good body condition throughout the year. The mares were randomly allotted to one of three treatment groups (n = 4/group) which were injected daily with: (1) 1 mL of corn oil (controls); (2) estradiol-17β (estradiol; Sigma Chem. Co., St. Louis. MO), 35 μg/kg bodyweight in 1 mL of corn oil; or (3) DHT (Sigma), 150 μg/kg bodyweight in 1 mL of corn oil. All injections were administered subcutaneously in the neck region and were repeated at 08:00 each day for 21 days. The doses of estradiol and DHT were derived from previous reports from this laboratory [2], and the effects on pituitary hormone characteristics were well documented.

Because of the time needed for tissue processing after pituitary removal, one mare from each treatment group was started on treatment on one of four consecutive days and all mares were killed on the 22nd day of their treatment. The experiment was performed between July 11 and August 4. Daily blood samples were collected via jugular venipuncture immediately before the daily treatment or control injections. These samples were drawn into evacuated, heparinized tubes; plasma was harvested by centrifugation at 1,200*g* and stored at −15°C for later analysis.

2.2. Pituitary Preparation for Hormone Analysis

Pituitaries were removed within 7–12 minutes after euthanasia (by electrocution and exsanguination, performed by the personnel of the LSU School of Veterinary Medicine), were rinsed with saline, and were cut in half on the midsagittal plane. One half was placed in ice-cold 0.01 M phosphate-buffered saline (0.15 M NaCl; PBS) and was stripped of its surrounding capsule. The neurohypophyseal tissue was separated from the AP, and the halved AP was weighed. This AP tissue was then homogenized in 100 mL of PBS in a Waring 7011 blender (Dynamics Corp. of America, New Hartford, CT) at maximum setting for 30 seconds. Another 100 mL of PBS was used to rinse out the blender jar. The homogenate and rinse were mixed together, and 10 mL aliquots were centrifuged at 1,200*g* for 30 minutes at 4°C. The resulting supernatants were harvested and frozen for later analysis of hormone concentrations.

At the end of the experiment, each AP homogenate was diluted 1:500 in PBS containing 0.1% gelatin. Concentrations of prolactin and GH were estimated in twofold serial dilutions (from 0.2 to 0.0125 mL) of each homogenate by radioimmunoassay as described previously [12,13]. Concentrations of prolactin in the stored plasma samples were estimated in duplicate 0.2 mL aliquots in the same assay. Concentrations of GH in daily samples were not determined due to the episodic nature of GH secretion in horses. The interassay and intraassay coefficients of variation and limit of detection of the assays were 10%, 7%, and 0.2 ng/mL for prolactin and 11%, 6%, and 0.5 ng/mL for GH.

2.3. Tissue Preparation for Electron Microscopy

After a rinse with saline, the remaining half of the pituitary was placed in freshly prepared 4% formaldehyde (Electron Microscopy Sciences, Fort Washington, PA) plus 0.1% glutaraldehyde (Polysciences, Warrington, PA) buffered with 0.1 M sodium cacodylate (pH 7.3; Electron Microscopy Sciences). While in the fixative, the tissues were freed of the surrounding capsule and the neural lobe was removed. The AP tissues were then cut into approximately 1-mm^3 pieces and left in the fixative for another 2 hours. After fixation, 10 randomly selected pieces from each mare were removed and cut into smaller pieces, which were then used for embedding.

Tissue pieces were washed several times in 0.1 M sodium cacodylate buffer to remove any residual aldehyde and were then dehydrated in 50% ethanol (EtOH) for 15 minutes, 70% EtOH for 20 minutes, and 90% EtOH for 30 minutes. Some of the tissue pieces were processed for ultrastructure study using routine Epon-Araldite embedding for transmission electron microscopy as previously described by Poolsawat [14]. Tissues used for immunocytochemistry were infiltrated and then embedded with 60% LR Gold (LRG; Electron Microscopy Sciences) monomer: 40% EtOH for 30 minutes; 70% LRG:30% EtOH for 30 minutes; 100% LRG monomer for 2 hours; 100% LRG monomer plus 0.1% benzil (initiator) for 2 hours; and finally overnight in 100% LRG monomer plus initiator. All procedures were performed at room temperature. The tissues were then embedded in small gelatin or large BEEM capsules (Electron Microscopy Sciences). The LRG was polymerized under UV light at −15°C for 24–36 hours. Ultrathin, 80–90 nm light gold-colored sections were cut on a Sorvall MT2-B ultramicrotome and sections were mounted on 400 mesh gold grids.

2.4. Immunogold Labeling

Sections were first incubated for 10 minutes on a drop of 0.05 M Tris (pH 7.3) containing 0.02 M glycine to quench any aldehyde left in the tissue. They were then incubated