Journal of Equine Veterinary Science 33 (2013) 683-689

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Repeatability of Prolactin Responses to a Small Dose of Sulpiride in Estrogen-Primed Geldings in Spring and in Mares During the Estrous Cycle in Summer

Rebekah C. Hebert MS, Donald L. Thompson Jr. PhD, Pamela B. Mitcham PhD, Jeanne D. Lestelle MS

School of Animal Sciences, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, Louisiana

ARTICLE INFO

Article history:
Received 20 July 2012
Received in revised form
1 November 2012
Accepted 20 November 2012
Available online 14 March 2013

Keywords:
Prolactin
Mares
Geldings
Sulpiride
Repeatability

ABSTRACT

Two experiments were conducted to assess the repeatability of prolactin responses to a small dose of sulpiride in estrogen-primed geldings in spring and in mares during the estrous cycle in summer. Six long-term geldings each received a single intramuscular injection of 100 mg of estradiol cypionate on March 31, 2011, and were then challenged with an intravenous injection of dl-sulpiride (5 μg/kg of body weight of the racemic mixture) every other day for a total of 8 days. Jugular blood was collected at 0, 10, 20, 40, and 60 minutes after the injection of sulpiride for prolactin measurement. The experiment was repeated with six mares during the summer (July), except that the number of challenges was extended to 15 over 30 days so that any effect of estrous cycle stage could be assessed. Prolactin responses in geldings during April were robust and were varied in a quadratic manner ($P < .003$) over the eight sulpiride injections, increasing linearly to a plateau by the fourth injection. Mares also displayed robust prolactin responses to sulpiride injections in July, and there was no effect ($P > .1$) of day of injection and no effect of stage of estrous cycle (follicular phase, early diestrus, or late diestrus). We concluded that prolactin responses to this dose of sulpiride were sufficiently robust and repeatable for use as a paradigm for studies of the relative competitive efficacy and duration of action of various dopaminergic compounds and their vehicular formulations.

1. Introduction

Prolactin and α-melanocyte-stimulating hormone (MSH) are produced in the equine pituitary gland in the anterior and intermediate lobes, respectively [1-3]. The cell types that produce these hormones are under tonic negative control from dopaminergic input from the tuberoinfundibular and periventricular hypophyseal neurons of the hypothalamus [3,4]. Lactotropes respond to antagonism of that inhibitory input (e.g., after an injection of sulpiride or domperidone) with an immediate increase in the secretion rate of prolactin and a rapid increase in measurable hormone in jugular blood [5,6]. Moreover, both lactotropes and melanotropes respond to exogenous dopaminergic suppression (e.g., treatment with bromocriptine or pergolide, both of which are dopamine receptor agonists), with a reduction in hormonal secretion as seen by a decrease in circulating hormone concentrations [5,7].

Melanotropes, which produce MSH, have been implicated as the source of adrenocorticotropin (ACTH) or some other peptide with ACTH-like activity in pituitary pars intermedia dysfunction (PPID) in horses [8]. One current treatment for PPID is pergolide therapy, which suppresses the activity of the melanotropes in affected horses, thus reducing the aberrant ACTH activity and hence the resulting hyperadrenalism [7]. Other dopaminergic agonists have been used in human medicine and may also be applicable

Corresponding author at: Donald L. Thompson Jr., PhD, School of Animal Sciences, Louisiana State University, Baton Rouge, LA 70803-4210.
E-mail address: dthompson@agctr.lsu.edu (D.L. Thompson).

0737-0806/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2012.11.003

to the treatment of PPID in horses [9]. To expedite the testing of such drugs for efficacy and duration of action, measurement of prolactin, in lieu of MSH, might serve as an indicator for dopaminergic activity in horses. Such an approach would provide researchers with an alternative to expensive reagents involved with MSH measurement. In the first step toward validation of such an approach, we conducted two experiments to determine whether a small dose of sulpiride, given at regular intervals, would produce relatively constant prolactin responses over a few days or weeks. Measurement of unstimulated prolactin concentrations might also be useful but could be confounded by spontaneous surges known to occur in horses [10,11]. Moreover, the naturally low prolactin concentrations in winter would be problematic for the assessment of dopaminergic suppression of prolactin secretion; this could be overcome by pretreatment with estradiol cypionate (ECP), which greatly enhances prolactin secretion in response to sulpiride [12,13]. Thus, the first experiment, started in March 2011, assessed the repeatability of the prolactin responses to eight every-other-day injections of a small dose of sulpiride in geldings pretreated with ECP. The second experiment tested the repeatability of prolactin responses to 15 every-other-day injections of a small dose of sulpiride in mares in July 2011; this duration allowed us to assess possible estrous cycle effects (estrus versus diestrus) on the responses.

2. Materials and Methods

All procedures described herein were approved by the Institutional Animal Care and Use Committee of LSU Agricultural Center. The mares and geldings used were of light horse breeds and were long-term residents of LSU Agricultural Center horse farm in Baton Rouge, LA. They were routinely kept on native grass pasture most of the year and on winter ryegrass pasture when native grasses were dormant; grass hay was provided in transitional periods when grasses were insufficient to maintain body conditions. They remained on pasture except when experimental procedures were being performed.

2.1. Experiment 1

Six long-term geldings were used that were between 6 and 20 years old, weighed between 410 and 526 kg, and had body condition scores between 5 and 8 [14]. On March 31, 2011, all geldings received a single intramuscular injection of 100 mg of ECP (Biorelease estradiol cypionate LA, 50 mg/mL; BetPharm Pharmacy, Lexington, KY) to stimulate prolactin. Sulpiride challenges were started on April 5, 2011, and were continued every-other-day through April 19, 2011.

For each day of treatment and blood sampling, the geldings were brought in from pasture the evening before and were kept in a small lot with native grass hay and water available ad libitum. At approximately 8:00 AM on the morning of blood sampling, the geldings were tethered loosely either in an outdoor chute or under an open-sided shed. A single sample of jugular blood was obtained via venipuncture from each of the geldings, and then dl-sulpiride was injected intravenously. The dose of the racemic mixture of sulpiride (Sigma Chemical Co., St. Louis, MO) was 5 μg/kg of body weight (BW). Sulpiride was dissolved in sterile saline with sufficient NaOH added to result in complete solubilization; the final concentration of the solution was 0.5 mg/mL. This dose of the racemic mixture is approximately 50% of the dose reported by Clavier et al. [15] required to produce the half-maximal prolactin response in horses.

Samples of jugular blood were collected subsequently at 10, 20, 40, and 60 minutes after sulpiride injection. All blood samples were collected through 22-gauge needles into 7-mL tubes containing 100 units of sodium heparin. Samples were placed at 5°C until centrifugation (within 1 hour) at 1200 × *g* for 15 minutes; plasma was harvested and stored at −15°C.

The procedures described above were repeated on alternate days for a total of eight sulpiride challenges. When all plasma samples had been collected, prolactin concentration was measured by radioimmunoassay [16]. Intra- and interassay coefficients of variation and assay sensitivity were 7 and 12% and 0.2 ng/mL, respectively. From the raw prolactin data, two additional dependent variables were derived: 1) net changes in prolactin concentration from time 0, and 2) net areas under the response curve. The net change in concentration, which should be proportional to the amount of prolactin released in the first few minutes after sulpiride injection, was the difference between the highest prolactin concentration achieved after sulpiride injection (usually the 10-minute sample) minus the time 0 (preinjection) concentration. The net areas under the response curve were calculated by first subtracting the preinjection concentration from all subsequent values and then summing the concentration × time increments (rectangle summation). Net differences and net areas were analyzed by analysis of variance (ANOVA) with SAS software (SAS Institute, Cary, NC) with horse and day as the main factors: the horse × day interaction served as the error term. Differences among challenges were assessed by the least significant difference test [17]. Linear and quadratic trends in means for days were assessed by appropriate contrast statements in the SAS program.

2.2. Experiment 2

Six light horse mares with histories of normal estrous cycles were used. They ranged in age from 6–15 years old, weighed between 498 and 556 kg, and had body condition scores between 6 and 8.0.

Beginning on July 3, 2011, and continuing every other day thereafter through July 31, 2011, all mares received an intravenous injection of sulpiride in saline in the morning. For each day of injection, the mares were brought in from pasture the evening before and kept in a small lot with native grass hay and water available ad libitum. At approximately 8:00 AM on the morning of blood sampling, the mares were tethered loosely either in an outdoor chute or under an open-sided shed. A single sample of jugular blood was obtained via venipuncture from each of the mares, and then sulpiride was injected intravenously. The dose of the racemic mixture of sulpiride (Sigma) was 5 μg/kg BW and was prepared as described in experiment 1.