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Original Research

Effects of Various Methods of Sulpiride Administration on Prolactin Release in Horses

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ABSTRACT

Four experiments assessed factors affecting prolactin responses to sulpiride administration in horses. Experiment 1 compared the efficacy of the (−) enantiomer of sulpiride to that of the commonly used (+/−) racemic mixture. Mares were used in an 8 × 8 Latin square to compare the prolactin responses to four doses of levosulpiride to four corresponding doses of the racemic mixture at twice the dose. Responses at each dose indicated equal and similar ($P > .1$) responses. Experiment 2 compared the efficacy of 1 gram of orally administered racemic sulpiride to 100 mg of intramuscularly injected sulpiride in oil in mares primed with 50 mg of estradiol cypionate (ECP). Prolactin responses in groups receiving sulpiride were robust but similar in magnitude with minor differences in timing. In experiment 3, ECP-primed geldings received subcutaneous injections of 1.8 grams racemic sulpiride in vegetable shortening in one of three sites: the neck, the back below the withers, or the lower girth region; control geldings received no sulpiride. Prolactin responses to sulpiride lasted a minimum of 96 hours. In experiment 4, prolactin responses to 3 g of racemic sulpiride in vegetable shortening were compared to similar injections (3 g) in 5 mL of sucrose acetate isobutyrate (SAIB; SucroMate) or just SAIB (control) in ECP-primed geldings. Controls had no prolactin response to SucroMate, whereas both treatment groups had extended prolactin responses lasting at least 10 days. It is concluded that prolactin responses to sulpiride in horses can be greatly extended by using hydrophobic vehicles like vegetable shortening or SAIB.

1. Introduction

Antidopaminergic drugs are commonly used in the horse industry for problems such as fescue toxicosis (domperidone [1]) and induction of ovulation in seasonally anovulatory mares (sulpiride [2] and domperidone [3]). Some of these treatments have gone on to be commercially available for administration either orally or by injection; however, their vehicles are proprietary to the formulating company. Sulpiride, a dopamine antagonist, was first reported as a secretagogue for prolactin in horses by Johnson and Becker [4], who used the racemic mixture ([+/−] sulpiride) dissolved in saline and administered intramuscularly (IM). Colborn et al [5] administered 500 mg of sulpiride subcutaneously (SQ) to stallions in winter in 2 mL of vegetable shortening, which was described as soft but solid at body temperature. Prolactin concentrations peaked in treated animals within 4 days and remained elevated for the duration of the experiment (13 days). In addition, Arana Valencia et al [6] reported the use of a propriety mixture of oily liquids for the long-term administration of sulpiride to geldings. In that study, injections were given every 5 days as the vehicle was found to prolong the effect of sulpiride on prolactin for approximately five days. Although the changes in plasma prolactin concentrations over time to a single injection of sulpiride have been well documented for IM administration in saline [4] or oil [7] and IV injection in saline [8,9], the time course of prolactin secretion for the vehicles designed for slower release of sulpiride has not.

The purpose of the experiments described herein was to compare various factors that affect the prolactin response to sulpiride, including enantiomer composition, oral administration, and various vehicle formulations, with the goal of developing a single-injection protocol with high efficacy and an extended period of

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Conflict of interest statement: The authors declare no conflicts of interest.

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stimulation on plasma prolactin concentrations to aid in equine reproductive research. Four experiments were performed to study (1) the relative activity of racemic mixture of sulpiride ([+/−] sulpiride) to the (−) enantiomer (levosulpiride), (2) the relative efficacy of sulpiride administered orally to that injected IM in oil, (3) the effect of site of injection for sulpiride injected SQ in vegetable shortening, and (4) the efficacy of sulpiride injected IM in SucroMate (a commercially available suspension of deslorelin acetate in sucrose acetate isobutyrate [SAIB]) compared to injection in vegetable shortening. SucroMate was used for its SAIB vehicle because medical grade SAIB was not readily available for research at the time this research was performed.

2. Materials and Methods

All procedures described in these experiments were approved by the Institutional Animal Care and Use Committee of the Louisiana State University Agricultural Center. Horses used in the described experiments were long-term residents of the LSU AgCenter Horse Farm in Baton Rouge, Louisiana, and were routinely maintained outdoors on native grass pastures during the warm seasons and on winter, ryegrass when available. Alicia bermudagrass hay was supplemented when the availability of pasture grass diminished during the fall and winter months.

2.1. Animals

Mares in experiments 1 and 2 were of light horse breeds (primarily Quarter horse, Thoroughbred, and Arabian), ranged in age from 5 to 15 years, had body condition scores (BCS) between 5.5 and 8, and weighed between 485 and 615 kg. Geldings in experiments 3 and 4 were of similar breeds as mares and ranged in age from 7 to 16 years, had BCS between 5 and 7.5, and weighed between 480 and 585 kg.

2.2. Sample Collection and Hormone Analysis

Throughout all experiments, blood sampling was performed via jugular venipuncture with 21-gauge needles into 10-mL evacuated glass tubes containing 143 USP units of sodium heparin (Becton, Dickinson & Co., Franklin Lakes, NJ, USA). Samples were routinely placed on ice until centrifugation at 1,200 × g for 10 minutes at 5°C. Plasma was harvested and stored frozen (−15°C) until the completion of a given experiment. Plasma prolactin concentrations were assayed in all samples; luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were also measured in experiment 4. All hormones were measured by radioimmunoassay as described previously (prolactin [10], LH [11], and FSH [12]). Interassay and intraassay coefficients of variation and limits of sensitivity were 7%, 12%, and 0.2 ng/mL for prolactin, 6%, 9%, and 0.2 ng/mL for LH, and 7%, 11%, and 1.4 ng/mL for FSH.

2.3. Experiments

2.3.1. Experiment 1: Comparison of Levosulpiride to the Racemic Mixture

Eight mature, light horse mares were used in a 8 × 8 Latin square design to test four doses each of levosulpiride (0.5, 1.25, 3.25, and 7.8 μg/kg of body weight) and the racemic sulpiride mixture (1.0, 2.5, 6.25, and 15.6 μg/kg of body weight) (both products purchased from Sigma Chemical Co., St Louis, MO, USA). Doses for the racemic mixture were based on data from Clavier et al [8] on dose-response curves of prolactin using the same agent. Theoretically, the racemic mixture should contain equal amount of both positive and negative enantiomers; thus, levosulpiride doses were exactly half of the racemic sulpiride. The experiment was carried out on 8 separate days starting on February 12, 2014, and ending March 2, 2014; there was at least 1 day of no treatment separating days of treatment. On each day of treatment, the mares were brought in from pasture in the morning and held in a dry lot for at least 1 hour; treatments were started around 1000 hours. For treatment, each mare was loosely tethered inside a large shed, and a 5 mL sample of jugular blood (time 0) was drawn. Her assigned treatment for that day (in 3 mL of saline) was then administered IV via the left jugular vein, and postinjection blood samples were collected at 10, 20, and 30 minutes after injection.

2.3.2. Experiment 2: Oral Administration versus IM Administration

Fifteen light horse mares were used in the fall of 2010. On November 1, all mares were administered a single IM injection of 50 mg of estradiol cypionate (ECP; BET Pharm BioRelease Estradiol Cypionate LA, USA; www.betpharm.com). On November 6, mares were brought in from pasture at 1600 hours and held overnight in an outdoor pen without access to feed but with *ad libitum* access to water. The following morning at 0700 hours, the mares were tethered loosely in sheltered pens for blood sampling and treatment. Blood samples were drawn at approximately 0800 hours and again 30 and 60 minutes later. Immediately after the last sample was drawn, five mares each were administered one of three treatments: (1) 100 mg of sulpiride (racemic mixture) in 5 mL of vegetable oil injected IM in the neck [7], (2) 1 g of sulpiride (racemic mixture; 10× dose of sulpiride in vegetable oil) in molasses fed as a top dressing on 0.5 kg of a commercially available sweet feed (Crossroads Feeds All Stock, Purina Animal Nutrition LLC, Shoreview, MN, USA), or (3) controls (no sulpiride). For each treatment, mares also received the appropriate placebo injection (2 mL of vegetable oil) and feeding (feed plus molasses but no sulpiride). Feed was offered in individual buckets, and all mares consumed the feed within the first 5 minutes. Posttreatment blood samples were drawn from each mare at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours.

2.3.3. Experiment 3: Effect of Site of Injection of 1.8 g Sulpiride in Vegetable Shortening

A preliminary trial comparing the prolactin responses to an injection of 1.5 g of sulpiride in canola oil (Crisco brand; J.M. Smucker Co., Orrville, OH, USA) either IM or SQ to a similar injection in vegetable shortening (Crisco; J.M. Smucker Co.) in ECP-treated geldings indicated that the shortening vehicle resulted in a longer period of elevated prolactin concentrations than did either oil injection. Experiment 3 was performed as a result of that preliminary data.

Fifteen light horse long-term geldings were used in the fall of 2014. They were allotted to the treatment groups described below such that average age, weight, and BCS were similar in the groups. All geldings were administered 50 mg of ECP on November 4 as an IM injection in the neck. Six days later (November 10), 12 of the geldings were administered 1.8 g of sulpiride (saturating dose of racemic mixture) in vegetable shortening as a 5-mL SQ injection; three controls received shortening only in the same manner (in the neck area). Of the 12 geldings, three groups of four received their sulpiride injections in (1) the neck region (triangle area) usually used for injections, (2) in the back, behind the rear border of the withers, and about 6 inches down the side, and (3) in the thoracic area behind the elbow, about 6 inches up the side (Fig. 1).

For the three injection sites, the neck region served as the “usual,” given that it is the site of most (at least many) IM or SQ injections given to horses. The withers and girth areas were chosen due to their potentially lower blood perfusion in the skin of those areas. This was determined from a generic thermograph of a resting