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Changes in plasma melanocyte-stimulating hormone, ACTH, prolactin, GH, LH, FSH, and thyroid-stimulating hormone in response to injection of sulpiride, thyrotropin-releasing hormone, or vehicle in insulin-sensitive and -insensitive mares

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ABSTRACT

Six insulin-sensitive and 6 insulin-insensitive mares were used in a replicated 3 by 3 Latin square design to determine the pituitary hormonal responses (compared with vehicle) to sulpiride and thyrotropin-releasing hormone (TRH), 2 compounds commonly used to diagnose pituitary pars intermedia dysfunction (PPID) in horses. Mares were classified as insulin sensitive or insensitive by their previous glucose responses to direct injection of human recombinant insulin. Treatment days were February 25, 2012, and March 10 and 24, 2012. Treatments were sulpiride (racemic mixture, 0.01 mg/kg BW), TRH (0.002 mg/kg BW), and vehicle (saline, 0.01 mL/kg BW) administered intravenously. Blood samples were collected via jugular catheters at −10, 0, 5, 10, 20, 30, 45, 60, 90, and 120 min relative to treatment injection. Plasma ACTH concentrations were variable and were not affected by treatment or insulin sensitivity category. Plasma melanocyte-stimulating hormone (MSH) concentrations responded ($P < 0.01$) to both sulpiride and TRH injection and were greater ($P < 0.05$) in insulin-insensitive mares than in sensitive mares. Plasma prolactin concentrations responded ($P < 0.01$) to both sulpiride and TRH injection, and the response was greater ($P < 0.05$) for sulpiride; no effect of insulin sensitivity was observed. Plasma thyroid-stimulating hormone (TSH) concentrations responded ($P < 0.01$) to TRH injection only and were higher ($P < 0.05$) in insulin-sensitive mares in almost all time periods. Plasma LH and FSH concentrations varied with time ($P < 0.05$), particularly in the first week of the experiment, but were not affected by treatment or insulin sensitivity category. Plasma GH concentrations were affected ($P < 0.05$) only by day of treatment. The greater MSH responses to sulpiride and TRH in insulin-insensitive mares were similar to, but not as exaggerated as, those observed by others for PPID horses. In addition, the reduced TSH concentrations in insulin-insensitive mares are consistent with our previous observation of elevated plasma triiodothyronine concentrations in hyperleptinemic horses (later shown to be insulin insensitive as well).

1. Introduction

Exaggerated α-melanocyte-stimulating hormone (MSH) and ACTH responses to an injection of thyrotropin-releasing hormone (TRH) have been suggested as possible indicators of pituitary pars intermedia dysfunction (PPID) in horses [1–4]. Similarly, the ACTH response to the

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dopamine antagonist, domperidone, was reported to be greater in horses affected with PPID than in normal horses [3,5,6]. In addition to MSH and ACTH, TRH administration stimulates secretion of both thyroid-stimulating hormone (TSH) and prolactin [7–9] in normal horses and was reported to inhibit the secretagogue-induced secretion of GH [10]. Domperidone, as well as sulpiride, another dopamine antagonist, also stimulates prolactin release in normal horses [8,11,12].

Gentry et al [13] first described a hyperleptinemic condition in obese mares housed on pasture side-by-side with other obese mares with normal plasma leptin concentrations. Cartmill et al [14] reported that these hyperleptinemic mares were also hyperinsulinemic and had elevated plasma triiodothyronine concentrations but did not have altered cortisol concentrations. Subsequently, Caltabilota et al [15] confirmed that the hyperleptinemic mares were in fact relatively insulin insensitive compared with normal mares of similar body condition. Approximately 60% of horses diagnosed with PPID have been found to have elevated blood insulin concentrations, indicative of insulin resistance, and elevated plasma ACTH-like activity is one of the currently accepted diagnostics tests indicative of PPID [16]. However, like the hyperleptinemic mares described by Cartmill et al [14], horses with PPID do not necessarily have elevated cortisol concentrations or signs of adrenal hyperactivity [16].

Some horses presenting with symptoms of PPID seem to have alterations in cortisol concentrations that are not obvious or have no detectable changes [2,4,17]; however, reduced insulin sensitivity seems to be a common symptom of horses with equine metabolic syndrome [18] and in PPID horses that also display excessive hair growth [19]. The present experiment was conducted to determine whether the pituitary hormonal responses to sulpiride and TRH differed in normal mares relative to mares of known insulin insensitivity. The MSH and ACTH responses were monitored on the basis of their known responses in PPID horses. The remaining adenohypophyseal hormones were included 1) to confirm the biological activity of the injected compounds (eg, prolactin and TSH after TRH) and 2) to show any possible responsiveness not yet reported in the literature.

2. Materials and methods

All procedures described herein were approved by the Institutional Animal Care and Use Committee of the Louisiana State University Agricultural Center. The mares used were of light horse breeds and were long-term residents of the Louisiana State University Agricultural Center horse farm in Baton Rouge, Louisiana. They were 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.

Twelve light horse mares were used that ranged in age between 6 and 21 yr, weighed between 480 and 616 kg, and had body condition scores [20] between 5 and 8. The mares were selected from the larger herd on the basis of their relative insulin sensitivity, determined multiple times with consistent results, in previous experiments [15,21]. Six mares with low insulin sensitivity and 6 mares with normal sensitivity were then used in a replicated 3 by 3 Latin square design experiment to assess the hormonal responses to the 2 secretagogues reported to be useful in diagnosing PPID in horses (TRH and sulpiride [1–6]). All replicates of the Latin square were performed simultaneously. Mean BWs and body condition scores for the insulin sensitive and insensitive groups were similar ($P > 0.1$).

Treatments in the experiment were as follows: 1) vehicle, 0.155 M NaCl in sterile water, 0.01 mL/kg of BW, administered intravenously (i.v.), 2) TRH (obtained from Sigma Chemical Co, St. Louis, MO, USA), 0.002 mg/kg BW in saline, administered i.v., and 3) sulpiride (from Sigma Chemical Co), 0.01 mg/kg BW of the racemic mixture in saline, administered i.v. Days of the experiment were February 25, 2012, and March 10 and 24, 2012. Treatments were predetermined such that on each day of treatment 2 mares of each insulin sensitivity category were administered each treatment.

Mares were brought in from pasture the evening before each treatment day and were held in a drylot with no feed or grass but free access to water. At approximately 7:00 AM the next morning, the mares were moved to a chute and were restrained to minimize contact with each other. Each mare then received a 14-gauge jugular catheter that was held in place with cyanoacrylate glue. As long-term residents of the farm, the mares were routinely handled (moved into the lot, restrained, and catheterized) in this manner; thus, it posed no novel stimulation, and the mares displayed no signs of discomfort or anxiety.

After approximately 1 h, 2 samples of jugular blood were collected via the catheter (−10 and 0 samples) of each mare, and then the treatment for that day was injected through the catheter. Post-treatment blood samples were collected at 5, 10, 20, 30, 45, 60, 90, and 120 min relative to treatment injection. In each case, the blood sample was split between 2 tubes for processing: one 7-mL tube containing 140 U sodium heparin (Sigma Chemical Co) and one 3-mL tube containing disodium EDTA as the anticoagulant (Sigma Chemical Co; 2 mg/mL of blood) and aproptinin (Sigma Chemical Co; 600 kallikrein inactivator units/mL of blood) as a protease inhibitor. All tubes with blood were placed on ice and were centrifuged within 15 min at 1200× *g* for 10 min. Plasmas were harvested from the 2 tubes and stored at −15°C. After all blood samples had been collected for that day, the mares were returned to pasture.

In addition to the 3 treatment days, mares were also sampled between treatment days 1 and 2 (March 3, 2012) to assess their 24-h patterns of cortisol in plasma. For this sampling, the mares were kept in a drylot that abutted the chute used on treatment days and were provided water and grass hay for ad libitum consumption. Blood samples (5 mL) were collected by jugular venipuncture (21-gauge needles) every 4 h from 7:00 AM the first day until 7:00 AM the next morning; samples were collected from the left jugular vein, and no local analgesia was used. Mares were moved into the chute for each blood sampling and then returned to the