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Nicole Arana-Valencia
LSU Agricultural Center

Donald L. Thompson
LSU Agricultural Center

Erin L. Oberhaus
LSU Agricultural Center

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Original Research

Dopaminergic and Antidopaminergic Effects on Heart Rate in Healthy Horses When Challenged With Brief 2-minute Exercise Bouts

Nicole Arana-Valencia*, Donald L. Thompson Jr., Erin L. Oberhaus

School of Animal Sciences, Louisiana Agricultural Experiment Station, LSU AgCenter, Baton Rouge, LA 70803, USA

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ABSTRACT

Bromocriptine is a dopamine receptor agonist known to cause hypotension and bradycardia in several species. Five experiments were conducted to compare possible perturbations on heart rate (HR) in horses after a brief (2 minutes) exercise bout when exposed to either short-term or long-term treatment with bromocriptine, cabergoline, or pergolide (all commonly used dopaminergic agonists in horses) or sulpiride, a dopaminergic antagonist. For all experiments, prolactin was measured as an indicator of drug efficacy. Experiments 1 and 4 were conducted as a replicated Latin square, whereas experiments 2, 3, and 5 were double split plot designs. Experiment 1 tested changes in HR, adrenocorticotropin (ACTH), and growth hormone (GH) concentrations when geldings were pretreated with 50 mg of bromocriptine 12 hours before exercise. Bromocriptine pretreatment reduced ($P < .05$) the exercise-induced rise in HR and the ACTH and GH responses ($P < .05$). Experiment 2 assessed the daily responses of HR to exercise after intramuscular administration of 5 mg of cabergoline in vegetable oil, which diminished the rise in HR because of exercise for the first 2 days of the 7-day experiment. In experiment 3, daily feeding of 2g of pergolide top dressed over sweet feed had no effect on HR in response to exercise. Similar results were seen in experiments 4 and 5, when horses were intravenously administered .01 mg/kg BW sulpiride in saline or intramuscularly administered 1g of sulpiride dissolved in vegetable oil. Taken together, bromocriptine and cabergoline, but not pergolide or sulpiride, dampened the cardiac sympathetic response to exercise, thus, lowering the HR.

1. Introduction

The use of dopaminergic and antidopaminergic drugs is commonplace in the equine industry to treat a variety of conditions. Sulpiride, a dopamine receptor antagonist, is used for the advancement of ovulation in seasonally anestrous mares [1,2], in addition to the induction of lactation in mares [3]. The dopaminergic agonists, cabergoline and pergolide, are notably indicated for the treatment of pituitary pars intermedia dysfunction (PPID) and act by restoring dopaminergic action on melanotropes cells in the pars intermedia, thereby diminishing hormonal output by said cells [4]. Bromocriptine, another dopamine agonist, has been studied in horses [5–7]; however, it is rarely used in horses today.

Bromocriptine is currently applied in human medicine for the treatment of hyperprolactinemia, Parkinson's disease, and Type II diabetes, although it is noted to have hypotensive and bradycardic effects. Hamed et al. [8] described a significant decrease in blood pressure, heart rate (HR), and total peripheral resistance after anesthetized dogs were intravenously (IV) infused with 1 μg/kg/min of bromocriptine for 20 minutes. Similar effects are reported in humans [9–11], rats [12], and cats [13]. Because of the presence of dopamine receptors in peripheral sympathetic neurons, activation of presynaptic dopamine receptors can cause an inhibition of sympathetic nerve function [14], thereby decreasing norepinephrine secretion [15]. Moreover, cardiac sympathetic function is inhibited, causing impairments of cardiac acceleration even when

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Conflicts of interest: None.

* Corresponding author at: Nicole Arana-Valencia, School of Animal Sciences, Louisiana State University, Baton Rouge, LA 70803-4210.

E-mail address: narana1@lsu.edu (N. Arana-Valencia).

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an external stimulus is applied [14]. Similar results in cardiac activity have been reported for pergolide. Cavero et al [16] reported bradycardia, decreased blood pressure, and decreased peripheral resistance when anesthetized intact rats received 30 μg/kg IV. They similarly concluded that pergolide inhibited the sympathetic tone by stimulating dopamine receptors on peripheral nerves. Information on the sympatholytic effects for cabergoline is limited, and no reports for any dopaminergic agonists have been reported on the horse [17].

The present experiments were conducted to compare the sympatholytic effects of bromocriptine administration in horses to that stated in the literature and to determine if any effects are shared with cabergoline and pergolide administration. This information would be useful for horse owners and veterinarians treating PPID horses, as it is currently unknown whether dopaminergic agonism affects HR in horses when concurrently engaged in physical activities. This is important as changes in HR could potentially alter a horse's performance. An additional two experiments were conducted to determine if antidopaminergic activity had any effects on HR, given that in previous experiments using sulpiride, we have observed sedative-like effects in mares (personal observation) and lessening of aggressive male behavior in stallions before seminal collection [18]. In all five experiments, changes in HR in response to bromocriptine, cabergoline, and pergolide were recorded when horses were standing, walking, and trotting for 2 minutes.

2. Materials and Methods

All procedures described herein were approved by the Institutional Animal Care and Use Committee of the LSU AgCenter. All horses were long-term residents of the LSU AgCenter Horse Farm in Baton, Louisiana, and were routinely maintained outdoors on native grass pastures during the warm seasons and on winter ryegrass in winter months. Alicia Bermuda grass hay was supplemented as the availability of pasture grass diminished during the fall and winter months.

Five experiments were performed to study the effects of commonly used dopaminergic agonists and one antagonist on HR in horses. Experiments 1 through 3 assessed bromocriptine, cabergoline, and pergolide, respectively, whereas experiments 4 and 5 assessed changes in HR because of sulpiride administration under short-term and long-term conditions. All horses used in these experiments were healthy and free of any ailments. Given that the aforementioned agents have been extensively documented to affect prolactin secretion in horses [5,19–25], plasma prolactin concentrations were used in all experiments as a measure of drug efficacy.

2.1. Experiment 1: Bromocriptine

A preliminary trial using four Quarter Horse mares was conducted on April 15, 2016, to determine the time point at which 50 mg of bromocriptine dissolved in 1 mL ethanol (200 proof, Pharmaco-Aaper, Brookfield, CT, USA) administered IV would maximally suppress prolactin secretion. Fig. 1 illustrates the maximal suppression ($P < .01$) of prolactin concentrations 12 hours after injection. Based on these data, in the evening of April 27, 2016, six, mature Quarter Horse geldings ranging in age from 7 to 17, weighing from 480 to 520 kg, and with a body condition score ([BCS]; [26]) between 5 and 7, were brought in from pasture and pretreated IV with either 50 mg of bromocriptine (n = 3) or vehicle (1 mL ethanol; n = 3) 12 hours before the execution of the exercise protocol. All geldings were returned to the pasture until the next day. The following morning at approximately 0630 hours, all the

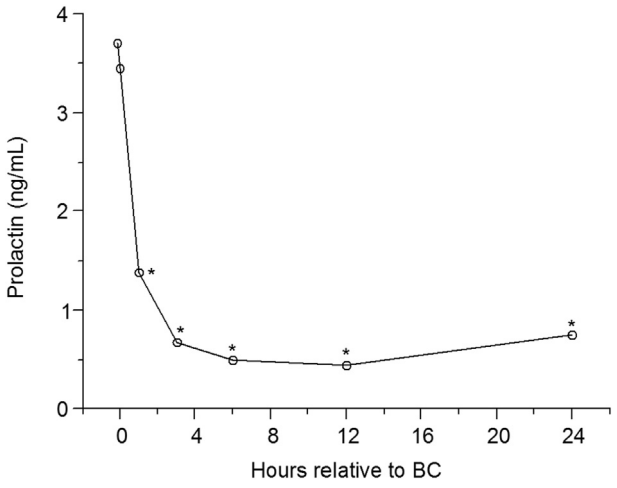

Fig. 1. Mean plasma prolactin concentrations from the preliminary trial in which mares (n = 4) were administered 50 mg of bromocriptine intravenously at time 0. Asterisks indicate differences ($P < .01$) from the time 0 mean concentration. An appropriate time for bromocriptine treatment was selected as 12 hours before exercise based on these data. Pooled SEM was 0.7 ng/mL.

geldings were quietly walked from the pasture into an outside chute. One by one, the horses were taken out of the chute into covered shed and fitted with a heart rate monitor (Polar Equine M400 Heart Rate Monitors, Kempele, Finland) attached to a surcingle. Afterward, each horse was walked to an outdoor round pen and lunged at the trot for 2 minutes. After all horses had been exercised and samples collected, they were returned to pasture until the next exercise day. Horses were allowed to rest for 7 days after which the experimental protocol was repeated with the treatments reversed (switchback design).

Heart rate was measured in the shed area for 15 minutes before lunging (resting HR), in the round pen just before lunging, immediately after lunging, and 5 minutes after lunging. Each blood sample was collected via jugular venipuncture through a 21 G × 1-inch vacutainer needle into a 6 mL evacuated plastic tube containing K_3EDTA (Vacuette, Greiner Bio-One, Monroe, NC, USA) at −15, 0, 5, 10, 20, and 30 minutes relative to the start of exercise (trotting in a round pen). All blood samples were immediately placed in an ice water bath on collection and centrifuged at the end of the day's experiment in a refrigerated centrifuge at 4°C for 15 minutes at 1200 × g. Plasma was transferred to polypropylene tubes and frozen at −20°C until later analysis.

At the end of the experiment, all frozen blood samples were thawed and analyzed for prolactin [27] and growth hormone (GH) [28] concentrations by radioimmunoassay previously validated for horse plasma. Plasma adrenocorticotropin (ACTH) was measured by radioimmunoassay with commercially available kit reagents (MP Biomedicals Inc, Costa Mesa, CA, USA). Estimates of the limit of detection of the assay and the intra- and interassay coefficient of variation were 0.2 ng/mL, 7%, and 12% for prolactin, 0.5 ng/mL, 8% and 11% for GH, and 5.7 pg/mL, 6.8%, and 10.7% for ACTH, respectively.

Data were analyzed as a replicated Latin square with repeated measures [29]. Differences between means for each sampling time were assessed by the pdiff option in SAS (least significant difference comparison) where appropriate.

2.2. Experiment 2: Cabergoline

Ten Quarter Horses were used (five mares and five geldings). They ranged in age from 8 to 21 years, weighed 420–590 kg, and

had a BCS of 5–7. Horses were randomly assorted into two groups such that gender, ages, weight, and BCS were similar between groups. Three mares and geldings were assigned as treatment and two mares and geldings were assigned as controls. Treatment consisted of 5 mg of cabergoline (Sigma Chemical Co, St. Louis, MO, USA) dissolved in 1 mL of vegetable oil (Crisco brand; J.M. Smucker Co., Orrville, OH, USA) with a few drops of DMSO (Sigma) added in order for cabergoline to go into solution. Control horses received 1 mL of DMSO/vegetable oil. All treatments were injected intramuscularly (IM) in the neck area.

The experiment was conducted from April 17, 2017 to April 23, 2017. The exercise protocol was the same as described for experiment 1 and was repeated every day, starting on day 0, for six consecutive days. In this experiment and subsequent experiments, the heart rate monitor was adjusted to continuously record HR data to better detect changes in HR while the horses were in motion. Treatments were administered on day 0 after each horse had completed their pretreatment exercise bout.

From the continuous heart monitor data, five data points were collected: shed (resting HR), walking peak HR, pre-exercise HR, peak exercise HR, and postexercise HR (immediately after stopping). Jugular blood samples were taken by venipuncture into evacuated 6 mL EDTA tubes when the horse was at rest in the shed area (denoted “shed”), before lunging in the round pen ("pre-exercise"), and immediately after lunging (“postexercise”). All blood samples were processed and assayed for prolactin as described in experiment 1.

Plasma prolactin and HR data were analyzed by analysis of variance (ANOVA) using the general linear model in SAS (SAS Institute, Cary, NC, USA). They were analyzed using a double split plot design with treatment as the main effect, day of experiment as the first split, and multiple sampling times within each day as the second split. Treatment was tested with the horse within treatment term, day and day by treatment interaction was tested with the day × horse within treatment interaction, and sampling time and its interactions were tested with residual error. Where needed, differences between treatment groups and individual time period means were tested for significance by the least significant difference test [29].

2.3. Experiment 3: Pergolide

Nine horses from experiment 2 were used in this experiment except for one mare that was withdrawn because of issues unrelated to the previous experiment. One gelding and two mares were added to this experiment, for a total of 12, six mares and six geldings. Horses were assigned to either treatment or control such that gender, age, weight, and BCS were fairly equally distributed. Their ages, weights, and BCS were similar to those in experiment 2. The experiment was conducted from June 10, 2017 to June 17, 2017.

Because of time limitations, the experiment was carried out in two equal replicates, staggered by one day. On the first day of each replicate, the horses were exercised, blood samples taken, and HR monitored as described in experiment 2. After each horse finished exercising, they were individually given of 2 mg of pergolide mesylate (Prascend, Boerhinger Ingelheim Vetmedica Inc., Duluth, GA, USA) top dressed on 0.23 kg of sweet feed (Crossroads Feeds All Stock, Purina Animal Nutrition LLC, Shoreview, MN, USA) before returning back out to pasture. Controls were given only the sweet feed. Treatment was repeated every day at 0800 hours until day 8, and exercise was repeated on days 5 and 8. On days 5 and 8, when treatment and exercise coincided, horses were given their treatment first and then allowed to rest for 30 minutes so that treatment could take effect before starting exercise protocol. Statistical analyses for prolactin and HR were performed as described for experiment 2.

2.4. Experiment 4: Short-Term Sulpiride Treatment

The same horses used in experiment 3 were used in experiment 4 after a one-week period of no activity. Horses that were treated in experiment 3 were reassigned as controls and vice versa.

The experiment was conducted from June 24, 2017 to July 2, 2017. Again, due to time restrictions, the experiment was performed in two equal replicates. The exercise protocol was the same as described in experiment 1, and the blood sampling and heart rate monitoring were conducted as described in experiment 2 with two exceptions. Because of the short half-life of sulpiride (Sigma) in saline, treatment (0.1 mg/kg BW sulpiride as the racemic mixture in saline, IV, or saline only) was administered immediately after turning on the heart rate monitor, and each animal was allowed to rest 10 minutes before proceeding to the round pen. An additional blood sample and HR data point were taken at 10 minutes post sulpiride administration and before walking to the round pen. One week later, on July 1, 2017, treatment groups were switched, and the experiment was repeated so that each horse served as its own control.

Heart rate was analyzed as a replicated 2 × 2 Latin square with treatment, replicate, horse within replicate, and day within replicate as factors in the ANOVA. Plasma prolactin concentrations were analyzed as a replicated 2 × 2 Latin square as the first split with each individual factor tested with the four-way interaction and the time point and treatment by time point in the second split tested with the residual.

2.5. Experiment 5: Long-Term Sulpiride Treatment

Experiment 5 began July 5, 2017, 3 days after finishing experiment 4. Previous research from our lab has shown prolactin levels return back to baseline 1 hour after sulpiride administration in saline [30]. Therefore, it was assumed that 3 days of washout period for treated animals in the previous experiment was sufficient to attain a proper baseline for the current experiment. Horses that were assigned as treated in the switchback were reassigned as controls, and those that were controls were assigned to treatment.

All 12 horses were exercised as described in experiment 1 on days 1, 2, 3, 7, and 11. Horses were administered 1 g of racemic sulpiride dissolved in 3 mL of vegetable oil intramuscularly or vehicle alone, after exercise on day 1 and then again on days 4 and 8. Blood sampling and heart rate monitoring were conducted as described in experiment 2. Statistical analyses for prolactin and HR were performed as described for experiment 2.

3. Results

3.1. Experiment 1: Bromocriptine

Mean plasma prolactin concentrations around the time of exercise (12 hours after bromocriptine or vehicle treatment) in geldings are presented in Fig. 2. Prolactin increased ($P < .05$) in response to exercise in control geldings, whereas previous bromocriptine treatment reduced ($P < .01$) prolactin concentrations by 90%; no prolactin response to exercise was noted in this group.

Heart rate increased ($P < .001$) in controls when they were walked to the exercise area (Fig. 3.). This increase in HR as they were walking to the round pen was not seen ($P = .12$) in bromocriptine-treated geldings. Moreover, throughout this period, HR in bromocriptine-treated geldings were lower than controls ($P = .011$). Trotting for 2 minutes increased ($P < .001$) HR in controls as well as in geldings treated with bromocriptine (Fig. 3.); however, bromocriptine reduced ($P = .0002$) the postexercise HR relative to

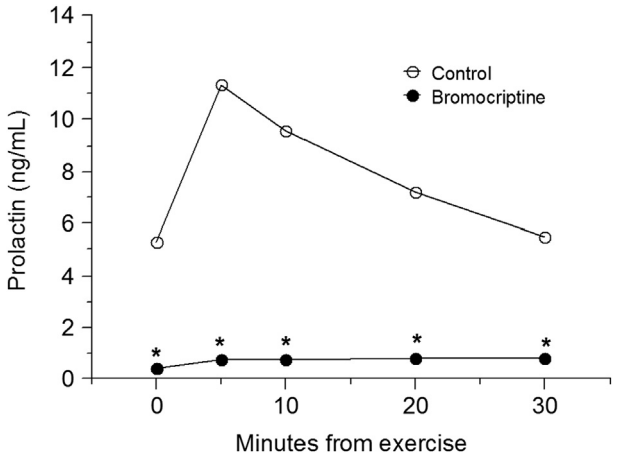

Fig. 2. Mean plasma prolactin concentrations in control geldings (vehicle-treated) during an exercise bout and in geldings previously administered 100 mg of bromocriptine intravenously 12 hours before onset of exercise. Exercise induced a rise in prolactin concentrations in controls ($P < .05$), whereas prolactin concentrations after bromocriptine treatment were suppressed and did not respond to exercise. Pooled SEM was 1.3 ng/mL.

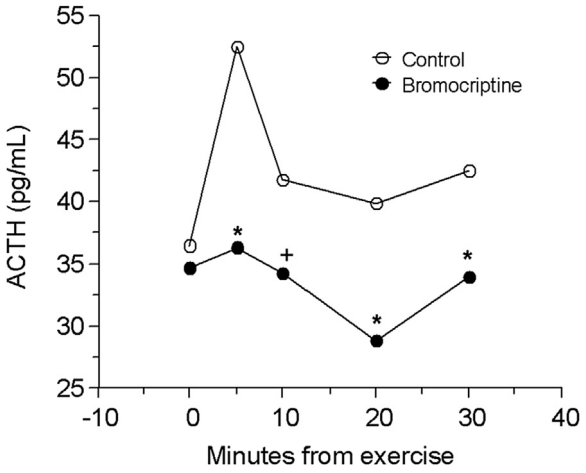

Fig. 4. Mean plasma adrenocorticotropin (ACTH) concentrations in geldings during control (vehicle-treated) exercise bouts and during bouts 12 hours after administration of 100 mg of bromocriptine intravenously. The experiment was performed as a switch-back design; thus, each mean includes 10 data points. Exercise induced a rise ($P < .05$) at 5 minutes in ACTH in control bouts; this rise was not present when geldings received bromocriptine. $^{*}P < .05$; $+P < .1$. Pooled SEM was 4.0 pg/mL.

control bouts. After 5 minutes of recovery, HR decreased ($P < .0001$) and was not different ($P = .26$) between control and treated horses.

Treatment with bromocriptine precluded the exercise-induced increase ($P < .05$) in plasma ACTH concentrations observed when geldings were treated with vehicle (Fig. 4.). Plasma GH concentrations were higher ($P < .05$) before exercise and at 5 and 10 minutes after exercise when geldings were treated with bromocriptine (Fig. 5.), but the magnitude of the exercise-induced responses ($P < .05$) was similar for both control and treatment exercise bouts.

3.2. Experiment 2: Cabergoline

Because of inclement weather, no data were collected on day 3 of the experiment. In addition, one mare's prolactin and HR data were 2–3 times higher than all the other horses and met the criterion as outliers; therefore, her data were excluded from the analyses.

Mean plasma prolactin concentrations in control and cabergoline-treated horses are presented in Fig. 6. The treatment by time interaction for prolactin secretion indicated a near complete suppression ($P < .0001$) of prolactin in cabergoline-treated animals, thereby confirming dopaminergic activity throughout the duration of the experiment.

Mean HR by day and mean net HR by time point and day in treated and control horses are presented in Fig. 7. When all time points for treated and control animals were averaged and compared by day, the overall response in HR to a short bout of exercise was reduced significantly ($P < .05$) 24 hours after cabergoline treatment (Fig. 7A). By day 2, overall HR tended to differ between groups ($P = .067$), though no more differences were noted on subsequent days. By chance, the mean initial HR in treated and control groups before treatment tended to differ ($P = .13$);

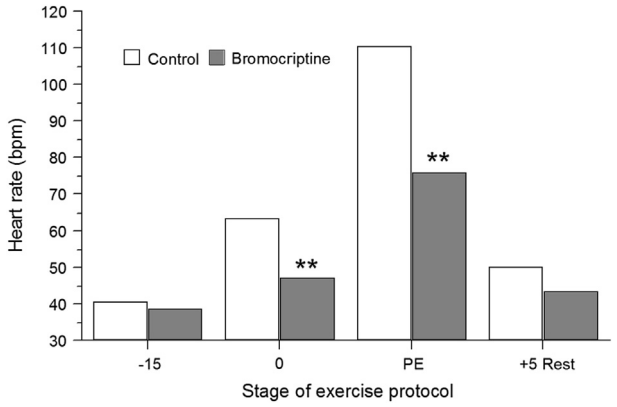

Fig. 3. Mean heart rates in geldings during control (vehicle-treated) exercise bouts and during bouts 12 hours after administration of 100 mg of bromocriptine intravenously. The experiment was performed as a switch-back design; thus, each mean includes 10 data points. Exercise induced a rise in HR in all bouts; HR was lower at time 0 ($P = .011$) and immediately after exercise (PE; $P = .0002$) when geldings were treated with bromocriptine relative to control bouts (asterisks). Pooled SEM was 7.0 bpm.

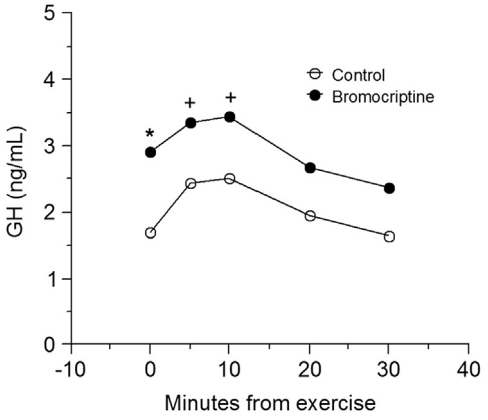

Fig. 5. Mean plasma growth hormone (GH) concentrations in geldings during control (vehicle-treated) exercise bouts and during bouts 12 hours after administration of 100 mg of bromocriptine intravenously. The experiment was performed as a switch-back design; thus each mean includes 10 data points. Exercise induced a rise ($P < .05$) in GH in both control bouts and those after bromocriptine treatment. Mean GH concentrations were higher after bromocriptine treatment than after vehicle treatment; $^{*}P < .05$; $+P < .1$. Pooled SEM was 0.7 ng/mL.

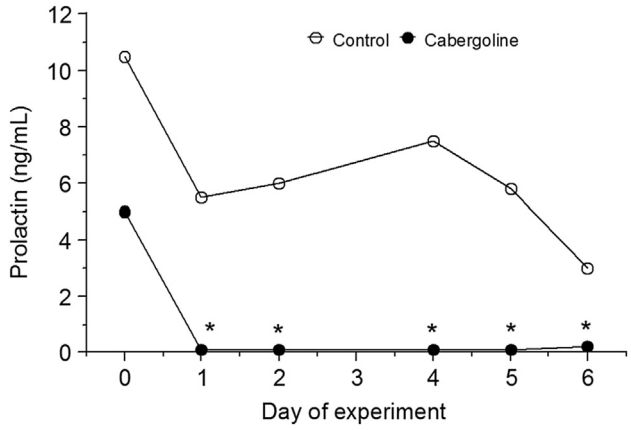

Fig. 6. Mean plasma prolactin concentrations averaged by day in horses administered 5 g of cabergoline in 1 mL of vegetable oil (n = 5) or vehicle (control; n = 4) IM. Prolactin concentrations were inhibited ($P < .0001$) in cabergoline-treated horses relative to day 0 (asterisks). Pooled SEM was 0.35 ng/mL.

therefore, to better detect differences between groups, the mean net change in HR from day 0 for each treatment group at each time point was calculated and analyzed in the ANOVA (Fig. 7). When treatment groups were compared in this manner on each day of the experiment, changes in HR because of treatment became much more evident (Fig. 7B-F). Cabergoline treatment significantly reduced ($P < .05$) the response in HR because of physical activity, be it walking or actively trotting, for at least 5 days.

3.3. Experiment 3: Pergolide

Daily pergolide treatment, administered as a top dressing fed with sweet feed, inhibited ($P < .0001$) prolactin secretion in treated horses compared to control horses through out the sampling period (Fig. 8). Mean changes in HR after pergolide administration and in response to an acute bout of exercise are presented in Fig. 9. A day effect ($P = .024$) and a day by treatment interaction ($P = .02$) were observed at the shed time point (rest). Further analysis with the least significant difference test between day by treatment revealed a decrease ($P = .0002$) in HR in treated horses compared to the control group on day 7. At the walking time point, there was a day effect ($P < .0001$) presenting as a downward trend of HR as days progressed; however, further analysis of the day by treatment interaction did not reveal significant differences when using the least significant difference test. There were no further differences in HR at each of the other time points.

3.4. Experiment 4: Short-Term Sulpiride Treatment

Mean plasma prolactin concentrations and HR are presented in Fig. 10. Sulpiride treatment increased ($P < .0001$) prolactin

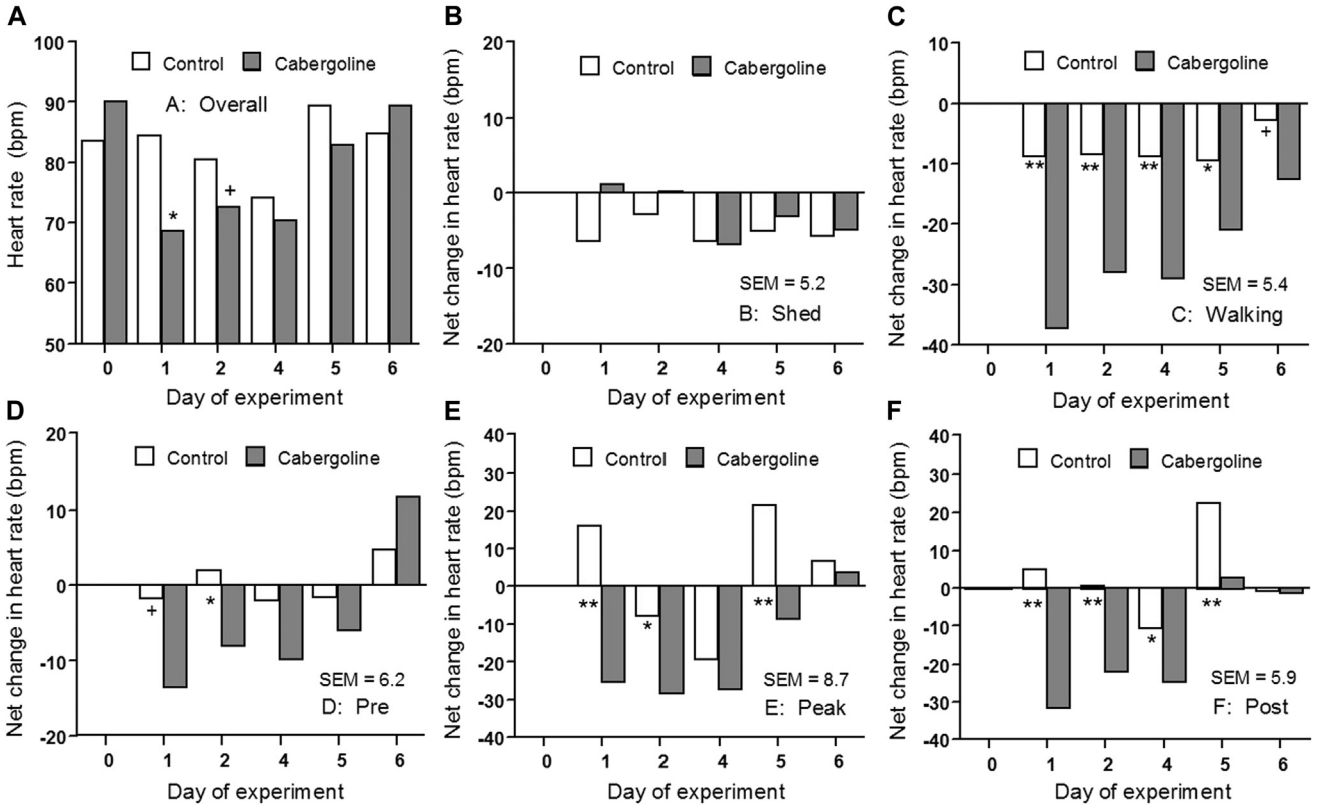

Fig. 7. (A) Mean heart rates averaged over all time periods of horses administered 5 mg of cabergoline (n = 5) or vehicle (Controls; n = 5) intramuscularly on day 0 after the exercise protocol was completed. Differences for each group from its day 0 mean are indicated: $+P = .067$; $^{*}P < .001$. Overall SEM = 2.1 bpm. (B–F) Mean net change in HR (relative to individual day 0 data) of horses administered 5 mg of cabergoline (n = 5) or vehicle (controls; n = 5) intramuscularly on day 0 after the exercise protocol was completed. Heart rates were assessed in the shed (resting, inactive), then when walking to the round pen, again just before exercise started (pre), at the peak during the 2-minute exercise bout (trotting), and then immediately after exercise was stopped (post). Mean heart rates averaged over all horses for day 0 were 43.9 in the shed, 92.0 while walking, 56.4 in the pre period, 136.4 at peak rates, and 110.0 in the post period. Differences between groups for each day are indicated: $^{*}P < .05$; $^{**}P < .001$. Standard error of the mean for each time point is presented in each graph.

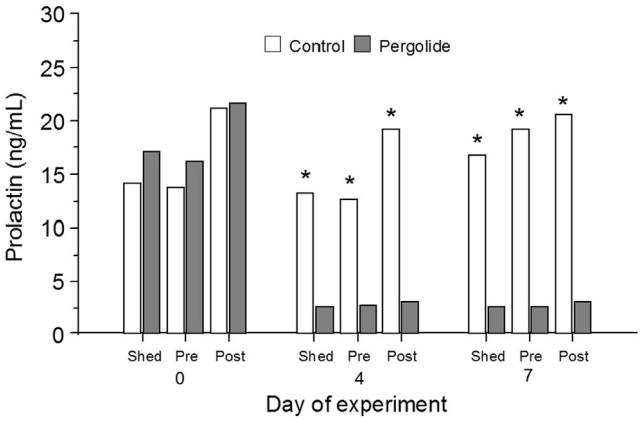

Fig. 8. Mean plasma prolactin concentrations before and after a short bout of exercise in horses administered 2 mg of pergolide (n = 6) or control (n = 6) top dressed over sweet feed daily for 8 days. Plasma prolactin was assessed on day 0 (before treatment) and on days 4 and 7 in the shed (resting, inactive), before exercise (pre), and immediately after a 2-minute exercise bout (post). Prolactin was significantly inhibited ($P < .0001$) in pergolide-treated horses relative to controls, denoted by asterisks, with the effect lasting till the end of the sampling period. Pooled SEM was 1.30 ng/mL.

concentrations within 10 minutes, which remained elevated for the entire sampling period, thereby confirming the antidopaminergic activity of sulpiride. However, mean HR at each site was unaffected because of sulpiride treatment ($P > .1$).

3.5. Experiment 5: Long-Term Sulpiride Treatment

Mean plasma prolactin concentrations in control horses and horses administered 1 g of racemic sulpiride in 1 mL of vegetable oil IM are presented in Fig. 11. Three time points were measured by day: shed (at rest), pre, and post exercise. Plasma prolactin concentrations increased ($P < .0001$) in sulpiride-treated animals, peaking by day 1 then decreasing with time; however, levels remained elevated compared to controls until the end of the sampling period on day 11.

Mean HR in response to exercise after administration of sulpiride or vehicle are presented in Fig. 12. Effect of treatment was compared at each individual time point where HR was monitored by day. There was no overall effect ($P > .1$) of sulpiride treatment or any interaction of treatment by day for any time point. Spurious differences were noted on day 0 at the shed time point ($P = .021$) and on day 11 ($P = .01$) at the walking time point. These differences were detected by the least significant difference test even though the main effect of treatment was not significant.

4. Discussion

Previous experiments [18,27,28,31–33] have reported rises in plasma prolactin and GH concentrations when horses are subjected to brief periods of stress, such as 2–5 minutes exercise bouts, twitching, teasing, and seminal collections (i.e., any activity that increases HR and ACTH concentrations). Experiment 1 was initially performed to assess the response of known stress-sensitive hormones in a low prolactin environment when horses were lunged at the trot for 2 minutes. Bromocriptine was chosen due to 1) its agonistic behavior on D2 dopamine receptors on prolactin secretion [5] and 2) for its relatively short half-life compared to cabergoline. The preliminary trial indicated that intravenous injection of 50 mg of bromocriptine maximally reduced prolactin concentrations at 12 hours after injection. Based on this information, the main experiment was performed with

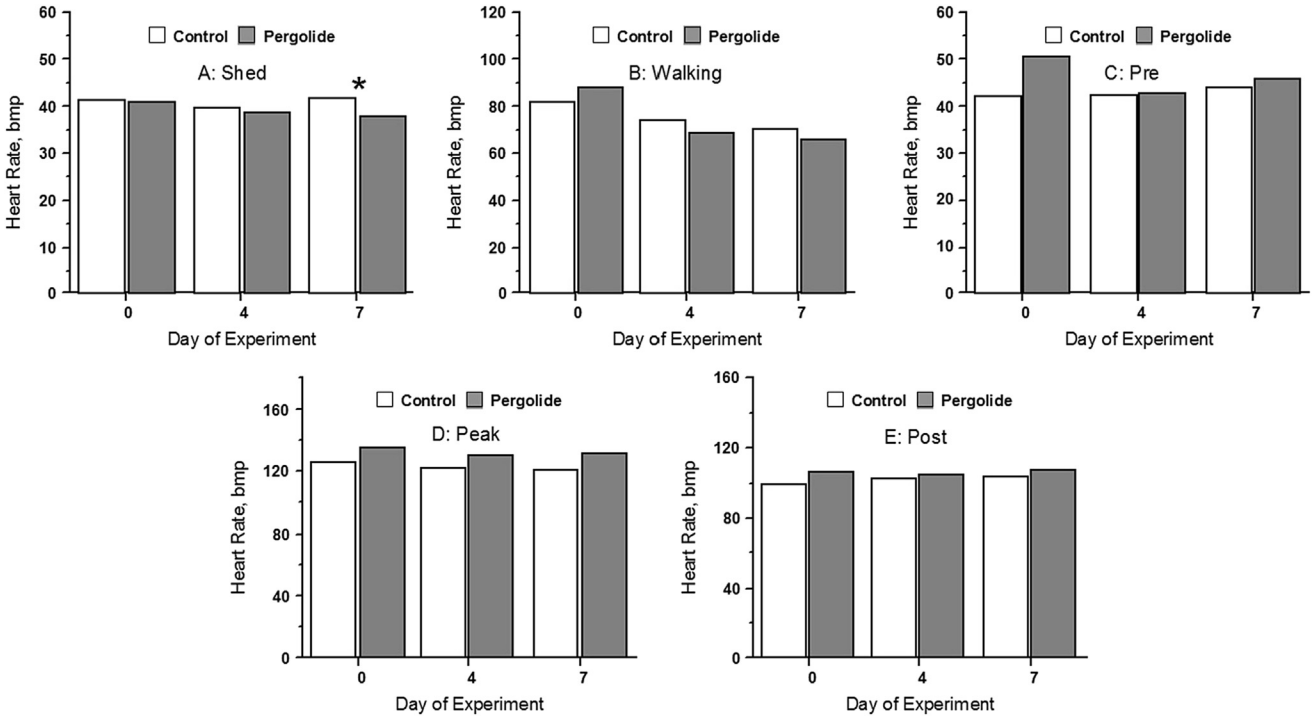

Fig. 9. Mean heart rate before and after a short bout of exercise in horses administered 2 mg of pergolide (n = 6) or control (n = 6) top dressed over sweet feed daily for 8 days. Short 2-minute exercise bouts were performed on days 0, 4, and 7 and HR was assessed at the shed (rest), walking toward the round pen (walking), immediately before trotting (pre), peak HR while trotting (peak), and immediately after trotting (post). Differences between groups are marked with an asterisk. For the walking time point, there was a day effect ($P < .0001$), denoting a downward trend of HR in both groups; however, no difference between treatment groups were present in the ANOVA. Pooled SEM was 2.7 bpm.

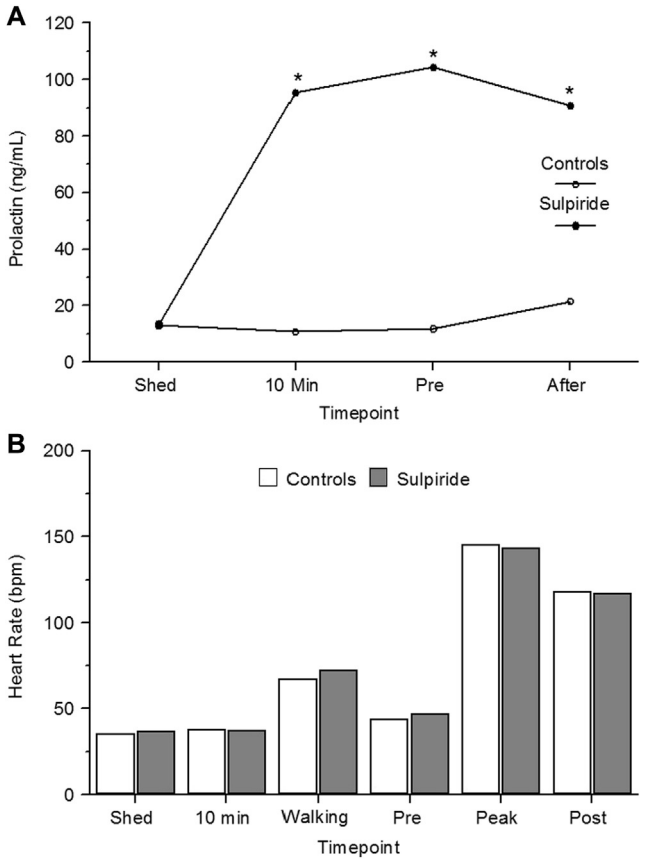

Fig. 10. (A) Mean plasma prolactin concentrations before and after a short bout of exercise in vehicle-treated and short-term sulpiride-treated (.01 mg/kg BW) horses at rest (shed), 10 minutes after sulpiride or vehicle administration (10 minutes), right before being lunged at the trot for 2 minutes (pre), and immediately after lunging (post). The experiment was performed as a single switchback with repeated measures and analyzed as a replicated 2 × 2 Latin square in the first plot with site and treatment by site interaction as the split. Concentrations were higher ($P < .0001$) in treated horses relative to controls confirming antidopaminergic activity. Asterisks indicate differences between groups for the designated sampling sites. Pooled SEM was 4.75 ng/mL. (B) Mean heart rate in control and sulpiride-treated horses at each time point that HR data was collected. The experiment was performed as a single switchback and analyzed as a replicated 2 × 2 Latin square. There was no treatment effect ($P > .1$) on HR at any given time point. Pooled SEM was 3.7 bpm.

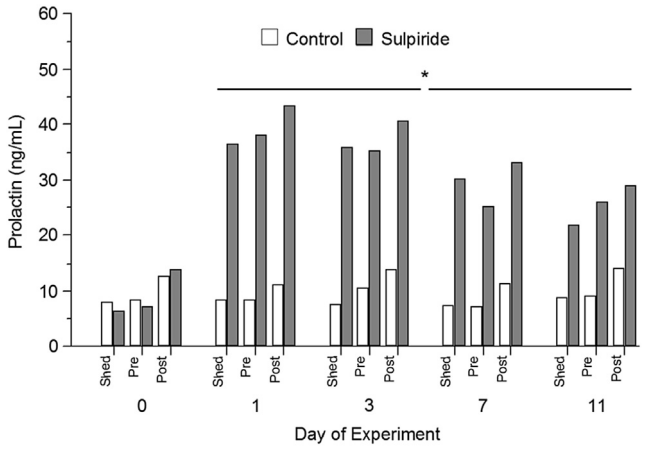

Fig. 11. Mean plasma prolactin concentrations by day of experiment and time point (shed, pre, post) in horses after long-term treatment with 1 g of sulpiride (racemic mixture) or vehicle (control) in 1 mL of vegetable oil in experiment 5. Treatments were administered on day 0, 4, and 8. Prolactin was higher ($P < .0001$) in sulpiride-treated horses than controls, denoted by asterisks, with the effect lasting till the end of the sampling period. Pooled SEM was 1.11 ng/mL.

bromocriptine administered 12 hours before exercise the following morning. It was found that bromocriptine not only reduced the exercise-induced rise in HR but also blunted the rise in exercise-induced prolactin and ACTH secretions as well. Although the overall response in GH was unaffected because of treatment, GH tended to be higher in bromocriptine-treated geldings than controls. In human studies, bromocriptine and other dopamine agonists have contrasting actions on GH secretion, in that they are generally stimulatory in healthy individuals but inhibitory in individuals with abnormal pituitary function [34–36]. Although this effect has not been previously documented in the horse, the overall GH response in this experiment was otherwise unaffected by treatment.

The bradycardic effect of bromocriptine observed in experiment 1 might be explained by its inhibitory action on peripheral sympathetic neurons [12]. Because D2 dopaminergic receptors can be found at the presynaptic end of sympathetic neurons, activation of these receptors by bromocriptine can reduce sympathetic tone by reducing norepinephrine release [11,15]. As a consequence, peripheral blood vessels dilate, which is followed by a decrease in HR, blood pressure, and peripheral resistance, though cardiac output generally remain unaffected [8].

In response to these findings, the question arose as to whether other dopaminergic agonists might cause the same effect at doses commonly used in the equine industry. Cabergoline and pergolide are two commonly used dopamine agonists for the treatment of PPID in horses. Much success has been met with their application, and pergolide is currently marketed as the treatment of choice by veterinarians. However, to our knowledge, no one has reported its effects on HR because of acute or chronic treatment in horses.

Because experiment 1 was done on one day, any possible effects on HR carried over to subsequent days could not be determined. Therefore, for experiments 2 through 5, the acute and chronic effects of the selected agents were assessed. In addition, in each experiment, the expected suppression of prolactin secretion by the selected agent was in fact observed, confirming the dopaminergic activity of the treatments.

Because of the tendency of HR to differ between treatment groups in experiment 2 (cabergoline treatment), the data for subsequent days were first normalized to day 0 data, and the residuals were analyzed as such. From that analysis, it was determined that the cabergoline blunting of HR after exercise lasted through day 5 of the experiment. The blunting effect on HR was only evident in the time points where each horse was acutely exposed to a stressor, as in the case of walking to the round pen, trotting, or just finishing the 2-minute trot bout but not during periods of inactivity. The ability of cabergoline to blunt the exercise-induced rise in HR is likely because of its similar binding affinity to D2 dopaminergic receptors on presynaptic terminals [37]. In contrast, this reduction to stress-induced HR was not observed in pergolide treatment, even though plasma prolactin concentrations were suppressed as expected. Like bromocriptine and cabergoline, pergolide has a strong affinity for D2 receptors; so in theory, it should have affected the D2 receptors located on the presynaptic terminals of sympathetic neurons. It may be possible that sympathetic effects of pergolide were not noticed in this study due to its short-term treatment. Pergolide is metabolized relatively quickly in horses, exerting its dopaminergic effects for 12 hours on average [21].

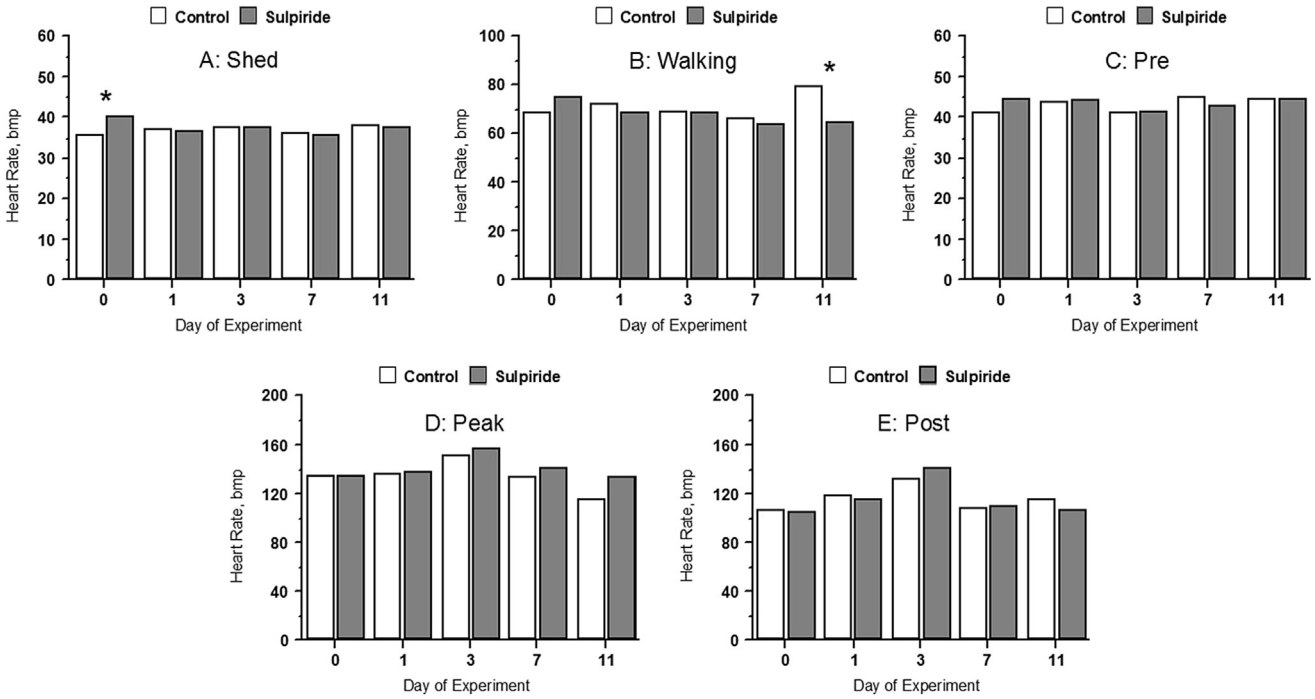

Fig. 12. Mean heart rate by time point and day of experiment in horses after administration of 1 g of sulpiride (racemic mixture) or vehicle (control) in 1 mL of vegetable oil in experiment 5. Injections were given in the neck. There was no effect ($P > .1$) of treatment or interaction of treatment by day at any time point, except where it is noted with an asterisk. The least significant difference for the shed time point on day 0 was $P = .021$ and $P = .01$ at the walking time point on day 11. Pooled SEM was 2.5 bpm.

Therefore, a longer-term study with higher doses of pergolide may be needed to assess its interaction with sympathetic effects in horses.

In human medicine, sulpiride has been used as antipsychotic, antiemetic, and antiseptic drug [38]. In past experiments, sedative effects have been noted in horses 5–10 minutes after sulpiride administration (N. Arana Valencia and D. L. Thompson, Jr., personal observations). Thomson et al. [18] also noted a slowing of aggressive male sexual behavior in stallions during seminal collections. Given the antidopaminergic nature of sulpiride, an effect opposite of bromocriptine or cabergoline might be expected on HR after exercise; however, no literature indicating such an effect was found for any species. Results from experiment 4 (short-term sulpiride) and 5 (long-term sulpiride) indicated that HR in response to exercise was similar in both treatment groups across all time points. The acute or chronic antagonistic effects on D2 dopaminergic receptors had no discernible effects on HR.

In conclusion, administration of bromocriptine and cabergoline at the doses and modes of administration presented in these experiments significantly decreased the exercise-induced rise in HR in horses. Although the focus in these was on HR, the effect of bromocriptine on ACTH secretion in the first experiment likely confirms that these drugs mute the sympathetic response to exercise similar to what has been reported for humans, rats, dogs, and cats [8–13]. The implication of this effect may be of importance for horses engaged in competitive activities and deserves further investigation.

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