



Original Research

Inhibitory Effects of Pergolide and Cabergoline Formulations on Daily Plasma Prolactin Concentrations in Geldings and on the Daily Prolactin Responses to a Small Dose of Sulpiride in Mares

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ABSTRACT

Two experiments were conducted to assess the efficacy and duration of action of two dopaminergic compounds, pergolide and cabergoline, on daily prolactin secretion in geldings and on prolactin responses to a small dose of sulpiride over 10 days. In the first experiment, oral administration of 2 mg of pergolide was compared to a single injection of 2 mg of pergolide in a slow-release vehicle and a single injection of 5 mg of cabergoline in slow-release vehicle. Controls received vehicle only. All drug treatments reduced ($P < .05$) prolactin concentrations relative to that in controls but differed substantially in duration of action (oral pergolide approximately 6 hours or less, injected pergolide 6 to 24 hours, and injected cabergoline at least 6 days). In the second experiment, repeated small doses of sulpiride (2 $\mu\text{g}/\text{kg}$ of body weight intravenously) were used to stimulate prolactin release in mares, and the ability of seven daily injections of pergolide (2 mg each) and a single injection of cabergoline (5 mg) in slow-release vehicle to suppress this release were compared. Control mares receiving vehicle injections had robust prolactin responses to the sulpiride injections on all days of injection (days 1, 0, 1, 2, 3, 4, 6, 8, and 10 relative to treatment). Prolactin responses were muted ($P < .05$) by pergolide and cabergoline treatments on the first day of injection (day 0, 30 min after treatment) and were basically absent on days 1 to 8. The single injection of cabergoline continued to be suppressive through day 10, whereas mares previously treated with pergolide (through day 6) had begun to recover a prolactin response by day 10. We conclude that either daily 2-mg pergolide injections in slow-release vehicle or a single injection of 5 mg of cabergoline in slow-release vehicle is an effective way to apply dopaminergic activity to horses for approximately 7 to 10 days and may have application in the treatment of pituitary pars intermedia dysfunction in affected horses.

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1. Introduction

Pergolide is a dopamine receptor agonist that was removed from the US market for human use due to its association with heart valve dysfunction [1]. However, it has

recently been approved for use in horses as a treatment for pituitary pars intermedia dysfunction (PPID) in horses [2].

Cabergoline is another dopamine receptor agonist that is highly active on dopaminergic type D2 receptors [3]. It was also commercially available for human use and went off patent in 2005 but has the same potential side effects as pergolide [1]. It may be a potential replacement for pergolide for use in horses due to its long-acting nature [4].

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In the first phase of the current research [5], we demonstrated that either estrogen-primed geldings in spring or cyclic mares in summer provided a possible paradigm for the assessment of the efficacy and duration of activity of dopamine agonists for suppression of the prolactin responses to repetitive small doses of sulpiride. Prolactin secretion from the lactotropes of the anterior lobe of the adenohypophysis is controlled by tonic suppression by hypothalamic dopamine input in the same manner as α -melanocyte stimulating hormone (MSH) secretion from the melanotropes of the intermediate lobe [6,7,8]. Thus, we proposed that measuring drug effects on prolactin secretion could serve as an alternative to monitoring MSH concentrations, thereby providing researchers flexibility in their experimental approach.

Two experiments were conducted herein. The first experiment was designed to determine and compare the effects of the current drug of choice, pergolide, in two possible formulations (oral administration and intramuscular injection) to those of cabergoline (injected) on unstimulated daily plasma prolactin concentrations in geldings. Based on those results, the second experiment compared the efficacy of daily pergolide injections to a single injection of cabergoline for suppression of prolactin responses to small doses of sulpiride in mares.

2. Materials and Methods

All procedures described herein were approved by the Institutional Animal Care and Use Committee of the LSU Agricultural Center. The mares and geldings used were of light horse breeds and were long-term residents of the 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. The horses remained on pasture except when experimental procedures were being performed.

2.1. Experiment 1

Sixteen light horse, long-term geldings were used. They ranged in age from 6 to 20 years old, weighed between 410 and 616 kg, and had body condition scores [9] between 5 and 8.

The 16 geldings were randomly assigned to one of four treatment groups ($n = 4/\text{group}$): control (received 2 mL of vehicle), pergolide injection (received 2 mg in 2 mL of vehicle), cabergoline injection (received 5 mg in 1 mL of vehicle), and oral pergolide administration (received one 2-mg capsule). Pergolide capsules were obtained from BET Pharm (BETpharm.com) and used as supplied. Pergolide mesylate (USP; Letco Medical, Decatur, AL; letcomedical.com) and cabergoline (Attix Pharmaceuticals, Toronto, Ontario, Canada) were each formulated in a proprietary mixture of hydrophobic, oily liquids designed to slow down and produce a sustained release of drug over time, similar (but not identical) to the vehicle used for LA 300 progesterone (BioRelease; BETpharm.com) [10]. Control injections were vehicle only.

All geldings were treated at 8:00 AM on August 20, 2011. All injections (treatments and vehicle) were given intramuscularly and pills (oral pergolide) were administered with the aid of a pill gun; geldings not receiving oral treatment had the pill gun placed into their mouth to equalize stress levels across treatments, and geldings given oral pergolide received an injection of vehicle. The geldings were kept in a small pasture close to the site of treatment so they were easily accessible for blood collection (to avoid stress or running before blood collection). On the morning of treatment and for every blood collection that day, they were loosely tethered in an outdoor chute.

Blood samples were obtained via jugular venipuncture into heparinized, evacuated tubes 12 and 24 hours before treatment and then immediately before injection (time 0 on day 0); then at 1, 3, 6, 9, and 12 hours after injection; and every 12 hours thereafter until the morning of day 6. Plasma was harvested from all samples by centrifugation ($1200 \times g$ for 15 min) and was stored at -15°C . Prolactin was measured in all plasma samples as described by Colborn et al [11]. Briefly, the assay was based on a rabbit anti-porcine prolactin antiserum and a highly purified equine prolactin preparation used for radioiodination and the reference standard. Intra- and interassay coefficients of variation and limit of detection were 7%, 12%, and 0.1 ng/mL, respectively.

Prolactin concentrations were analyzed using one-way analysis of variance (ANOVA) with repeated measures (sampling times) with treatment and time as main effects (SAS software; SAS Institute, Cary, NC). Treatment effect was tested with the animal-within-treatment term, and time and interaction were tested with residual error. The significance of differences between groups for each time period was tested by the least significant difference test [12].

2.2. Experiment 2

Fifteen light horse mares were used for experiment 2. They ranged in age from 5–16 years old, weighed between 480 and 616 kg, and had body condition scores between 5 and 8. Mares were initially assigned to one of three groups of five based on their ages, body weights, and body condition scores, such that the means for those characteristics in the three groups were similar. The groups were then randomly assigned to (1) controls (vehicle injected); (2) daily pergolide injections (2-mg injections daily for 7 days); and (3) a single injection of cabergoline (5 mg in vehicle). Control mares received single intramuscular injections of vehicle daily from day 0 through day 6. Cabergoline-injected mares received the single intramuscular injection of cabergoline in slow-release vehicle on day 0 and then injections of vehicle from days 1 through day 6. Pergolide-injected mares received single daily intramuscular injections of pergolide in slow-release vehicle on days 0 through 6. All injections were given in the morning between 7:00 and 8:00 AM.

The small-dose sulpiride challenges (2 $\mu\text{g}/\text{kg}$ of body weight of the [dl]-racemic mixture in saline administered intravenously) were started on day -2 (October 19, 2011) and were repeated on days $-1, 0, 1, 2, 3, 4, 6, 8,$ and 10 . The original experimental protocol called for daily sulpiride challenges through day 10 but several mares became averse

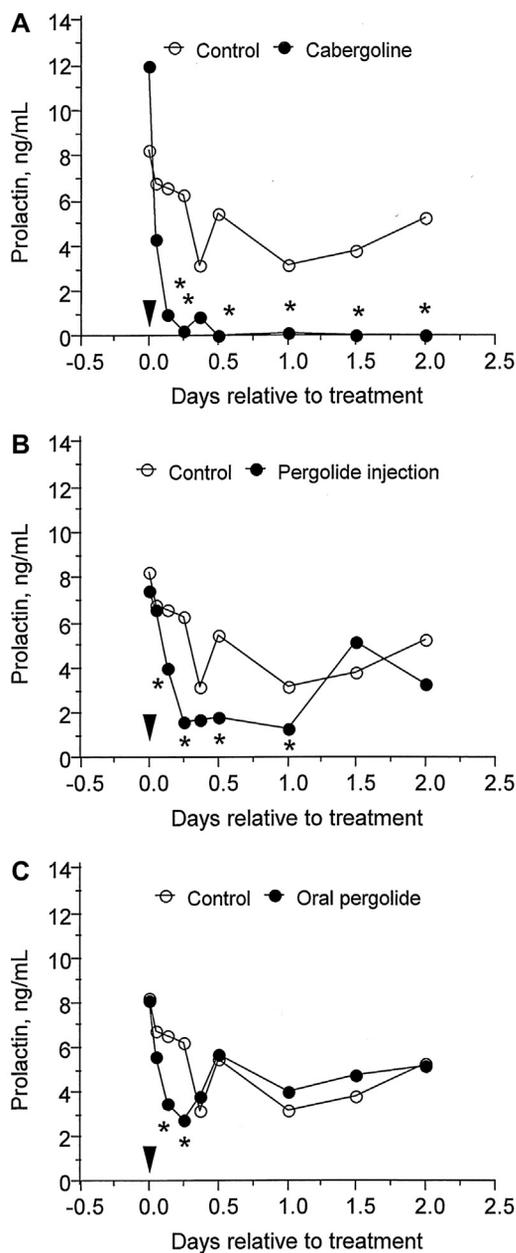


Fig. 1. Mean plasma prolactin concentrations over the first 48 hours in vehicle-treated geldings (control; shown in each panel for clarity) and those receiving an injection of 5 mg of cabergoline (A), an injection of 2 mg of pergolide (B), or oral pergolide (2 mg) (C) at time 0 (arrows) in experiment 1. Pooled SEM was 1.6 ng/mL for prolactin concentrations. *Differs from controls ($P < .05$).

to the daily injection and blood sampling regimen, and it was decided to go to an every-other-day challenge after day 4. That change alleviated the behavioral problems.

The sulpiride dose was reduced from that originally described [5] (5 $\mu\text{g}/\text{kg}$ of body weight) because the prolactin responses in those earlier experiments were more robust than needed for monitoring treatment effects and because the challenges in this experiment were to occur daily. Sulpiride (Sigma Chemical Co., St. Louis, MO) was

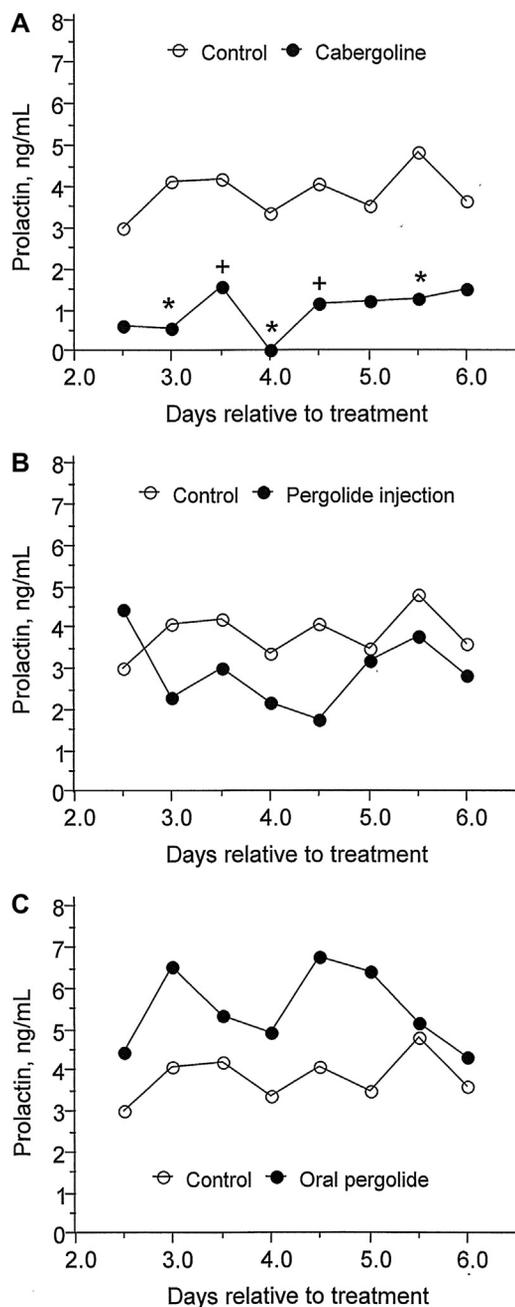


Fig. 2. Mean plasma prolactin concentrations of 2.5 to 6 days after treatment in vehicle-treated geldings (control; shown in each panel for clarity) and those receiving an injection of 5 mg of cabergoline (A), an injection of 2 mg of pergolide (B), or oral pergolide (2 mg) (C) in experiment 1. Only cabergoline reduced prolactin concentrations during this period. * $P < .05$; † $P < .1$. Pooled SEM was 1.6 ng/mL for prolactin concentrations.

dissolved in sterile saline with sufficient NaOH added to result in complete solubilization; the final concentration of the solution was 0.5 mg/mL.

For each sulpiride challenge, 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

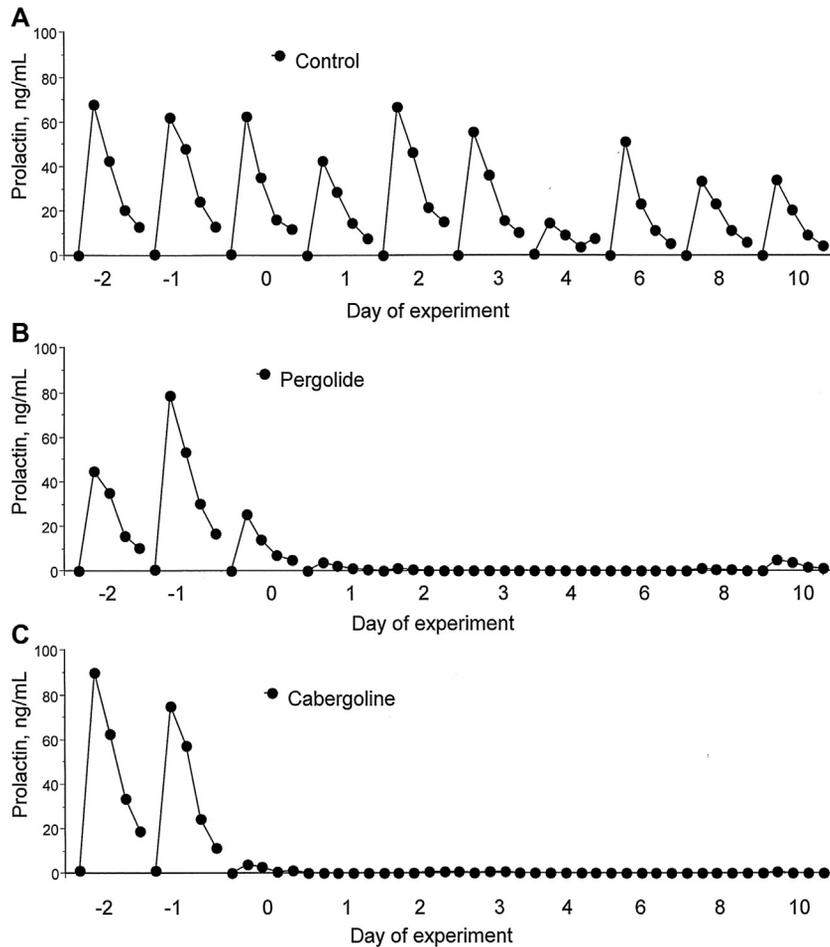


Fig. 3. Mean plasma prolactin concentrations in response to a small-dose sulpiride challenges in control mares (A) and mares receiving daily injections of 2 mg of pergolide (days 0-6) (B) or a single injection of 5 mg of cabergoline (C) in slow-release vehicle on day 0 in experiment 2. The sulpiride challenge on day 0 was started 30 minutes after the treatment injections. The pooled SEM was 6.6 ng/mL.

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 mare, and then sulpiride was injected intravenously. 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 tubes containing 100 units of sodium heparin. Samples were placed at 5°C until centrifugation at $1200 \times g$ for 15 minutes; plasma was harvested and stored at -15°C. On day 0, the day of first treatment injection, the sulpiride challenge was started 30 min after the treatment injections.

Plasma prolactin concentrations were determined as in experiment 1. Plasma prolactin concentrations in response to sulpiride were analyzed in a one-way ANOVA with two repeated measures (days and minutes within days). Also, areas under the response curves for each mare on each day were calculated by first subtracting the pre-injection concentration from all subsequent values and then summing the concentration \times time increments (rectangle summation); these areas were analyzed in a one-way ANOVA with repeated measures

(days). The significance of differences between groups for each time period was tested by the least significant difference test [12].

3. Results

3.1. Experiment 1

Mean plasma prolactin concentrations in the four groups of geldings for the first 2 days after treatment are shown in Figure 1. The means for each treated group are plotted against the means for the vehicle-treated (control) geldings for clarity. There was an effect of treatment ($P = .061$) as well as a treatment \times time interaction ($P = .0062$) in the ANOVA. Relative to controls, all treatments reduced ($P < .05$) prolactin concentrations, but the treatments varied as to the degree of reduction and the duration of reduction. Oral pergolide reduced prolactin concentrations only at 3 and 6 hours, down to 2.7 ng/mL after administration, whereas the injection of pergolide reduced prolactin concentrations from 6 to 24 hours after treatment, down to 1.6 ng/mL. Cabergoline injection reduced prolactin concentrations to <1 ng/mL

for the first 2 days and to <1.6 ng/mL thereafter through day 6 (Fig. 2). Neither oral nor injected pergolide affected prolactin concentrations from day 2.5 to 6 ($P > .1$).

3.2. Experiment 2

Mean plasma prolactin concentrations in response to sulpiride challenges over the course of the experiment are presented in Figure 3. There was an effect of treatment, day, and minute of blood sampling ($P < .01$) as well as the three-way interaction ($P < .001$). Daily pergolide injections and single cabergoline injection both suppressed ($P < .05$) the prolactin response to sulpiride through day 10. There was an effect of treatment even on day 0, just 30 minutes after the first (or only) treatment injection, with cabergoline almost totally suppressing the prolactin response to sulpiride challenge. When expressed as areas under the curves (Fig. 4), the prolactin responses after cabergoline were essentially zero after day 0, whereas there was a small response in the pergolide-treated mares on day 0 and eventually on day 10 (which was 4 days after the last pergolide injection).

4. Discussion

Although a starting dose of pergolide of 0.5 to 1.0 mg/day orally is recommended for horses suspected of having PPID [2,13,14], a single dose of 2 mg was used in the present experiment as a typical dose that would be given after a gradual increase up to the effect. It is not uncommon for the dose to be ramped up to as high as 6 mg/day [2,6]. This 2-mg dose, when administered orally, had a very short-lived effect on plasma prolactin concentrations in experiment 1. If the inhibitory effect on melanotropes in the intermediate lobe of the pituitary gland were similar in degree and duration as the effect on prolactin secretion, it seems that this mode of administration of pergolide is an unnecessary waste of drug. Intramuscular injection of an equal amount of pergolide produced a full 24 hours of suppression and thus would be a more efficient and efficacious approach for treating PPID.

Cabergoline was administered as an injection and at 5 mg/horse because it in combination with the slow-release vehicle used was expected to last considerably longer than the pergolide injections. The cabergoline injections definitely produced the greatest suppression of prolactin concentrations in these geldings, and the duration of action was much longer than the pergolide injection. Prolactin concentrations were generally suppressed by cabergoline even at 5.5 days after injection. If this efficacy and duration of activity can be shown to be consistently obtained with larger groups of horses, a 5- to 6-day injection regimen would be vastly superior to daily feeding or injection of pergolide.

Throughout the experiment, the geldings were observed while blood samples were collected for any adverse signs due to the dopaminergic agonist treatments. No adverse behaviors were noted during the 6-day period of sample collection, nor were there any signs of irritation or swelling at the site of injections. It was concluded that the pergolide and cabergoline formulations for injection

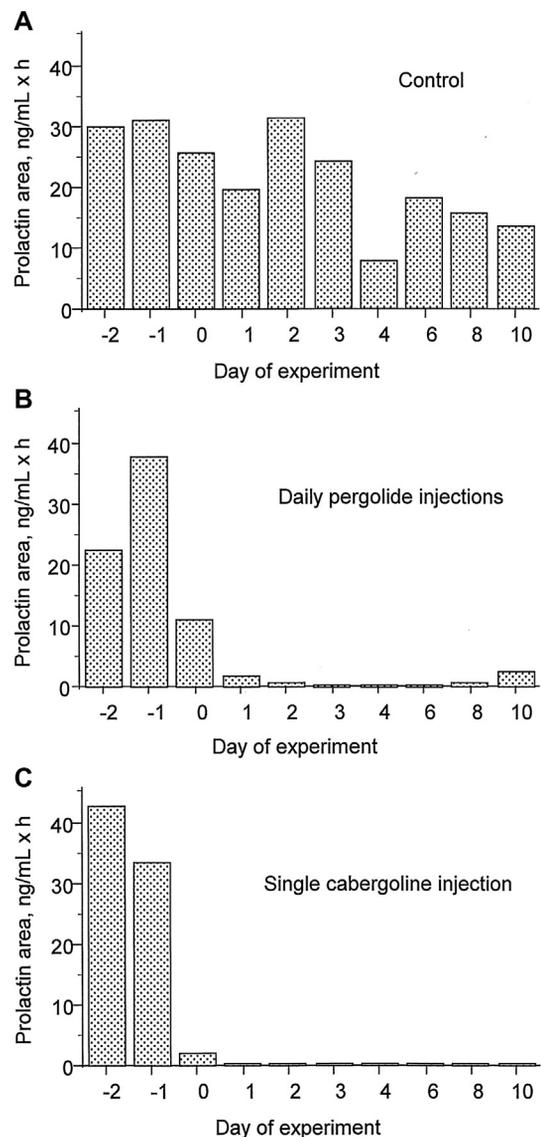


Fig. 4. Mean areas under the prolactin response curves in control mares (A) and mares receiving daily injections of 2 mg of pergolide (days 0-6) (B) or a single injection of 5 mg of cabergoline (C) in slow-release vehicle on day 0 in experiment 2. The sulpiride challenge on day 0 was started 30 minutes after the treatment injections. Both pergolide and cabergoline treatment reduced ($P < .05$) the prolactin response relative to that of controls from days 1 to 10. The pooled SEM was $5.9 \text{ ng} \cdot \text{mL}^{-1} \cdot \text{h}$.

deserved further study as potential modes for administration of dopaminergic activity.

In experiment 2, both the daily injection of pergolide and the single injection of cabergoline suppressed the sulpiride-induced release of prolactin for extended periods of time. The suppressive effects of the pergolide injections lasted at least 3 days after the last injection, and only a small prolactin response was observed on the 4th day. Given that a single pergolide injection in experiment 1 suppressed daily plasma prolactin concentrations only through 24 hours, it is likely that an accumulation of drug, or of the drug effect, occurred with

seven daily injections in experiment 2. The suppressive effects of the single injection of 5 mg of cabergoline lasted at least 10 days with no indication of recovery. Given these results, it is apparent that further study of the cabergoline formulation is needed to determine just how long the suppressive effects persist.

Dopaminergic pathways are found throughout the body and brain, including centers involved with fear and anxiety [15], and dopaminergic agonist treatment is sometimes associated with behavioral changes in patients with Parkinson's disease [16,17]; thus the mares in experiment 2, as the geldings in experiment 1, were monitored for any signs of treatment-induced behavioral changes as well as for reactions at the site of injection. The behavioral changes noted in Materials and Methods (mares becoming averse to the daily injection/blood sampling regimen) were noted in mares of each group, including controls, thus were not attributed to the drugs per se. No other side effects were noticed in the mares receiving pergolide or cabergoline.

The high degree of effectiveness and longer duration of activity of the cabergoline formulation in these experiments indicate that it might prove superior to pergolide in counteracting antidopaminergic activity of the injected sulpiride in this experimental paradigm. Whether the results obtained herein for prolactin in response to sulpiride will similarly apply to MSH and ACTH secretion in horses with PPID needs to be determined in future research.

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