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Long-term treatment of insulin insensitive mares with cabergoline: Effects on prolactin and melanocyte stimulating hormone responses to sulpiride and on indices of insulin sensitivity

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ABSTRACT

The main experiment assessed whether the inhibitory effects of the dopamine agonist, cabergoline, on prolactin and α-melanocyte stimulating hormone (MSH) concentrations would persist throughout a longer term administration (65 days). The possible effect of cabergoline on insulin sensitivity was also studied. Ten mares known to be insulin insensitive were allotted to two groups (treated vs. control). An insulin challenge, a glucose tolerance test, and a sulpiride challenge were administered prior to treatment. On day 0, treated mares (n = 5) received an injection of 5 mg cabergoline in slow-release vehicle; control mares (n = 5) received an equivalent vehicle injection. Injections were repeated every 10 days for a total of 7 injections. Sulpiride challenges were done 1 day before each cabergoline treatment to assess possible refractoriness to the treatment. Behavior and hair coat density were also monitored. Plasma prolactin was suppressed ($P < 0.01$) to undetectable levels in mares receiving cabergoline; control mares had robust prolactin responses to each sulpiride injection. There was no indication of refractoriness to cabergoline over time. Plasma MSH concentrations after sulpiride were also suppressed ($P < 0.05$) by cabergoline. After treatment, neither the glucose response to insulin nor the insulin response to glucose differed ($P > 0.1$) between groups. No behavioral changes were noted due to treatment. Weight of hair samples indicated that cabergoline perturbed ($P < 0.05$) winter coat growth. It is concluded that 5 mg of cabergoline in slow-release vehicle administered every 10 days is an effective way of delivering dopaminergic activity to mares that results in no noticeable detrimental effects and no refractoriness to the drug.

1. Introduction

Recent research by Hebert et al. [1] indicated that the long-acting dopamine agonist, cabergoline, in a slow-release formulation suppressed plasma prolactin secretion in mares for at least 10 days after a single intramuscular injection. Moreover, the suppression was complete, even in the face of low-dose sulpiride challenges [1], which, in the absence of cabergoline, caused relatively consistent elevations in prolactin secretion in both mares and estrogen-treated geldings [1,2]. Similarly, injections of pergolide in slow-release vehicle suppressed prolactin secretion, but for a much shorter period of time [1]. Because only one injection of cabergoline was tested in the experiment of Hebert et al. [1], the possibility of long-term detrimental effects or refractoriness could not be assessed.

Hebert et al. [1] suggested that the dopaminergic effects of cabergoline observed for prolactin secretion would likely be similar for melanotrope hormonal output, primarily α-melanocyte stimulating hormone (MSH) and perhaps adrenocorticotropin (ACTH) in pituitary pars intermedia dysfunction (PPID), due to the similar physiologic control by dopamine (via the portal blood for lactotropes and via neural input for melanotropes [3,4]). Hebert et al. [1] did not include plasma MSH concentrations in their report, thus we are providing those data herein as a prelude to the main experiment. Recently, we have reported that mares displaying hyperleptinemia, hyperinsulinemia, and a diminished response to injected insulin also have exaggerated MSH responses to sulpiride and TRH [5], similar to, but not as great a magnitude of, horses displaying symptoms of PPID [6,7]. Currently, horses and ponies diagnosed with PPID are treated with pergolide mesylate, a dopamine agonist known by its trade name Prascend. Although it has been reported to have good success rate, the medication needs to be fed daily for the duration of the horse's life. [8].

The present (main) experiment was designed primarily to test the long-term effects of repeated cabergoline injections (every 10 d for a total of 7 injections) on prolactin and MSH concentrations. Insulin insensitive mares were monitored for any overt detrimental effects to cabergoline injection (e.g., behavioral changes), for any sign of refractoriness to cabergoline, and for any changes in hair coat that might be predicted from previous reports in which inadvertent immunization of pony mares against prolactin in the winter delayed hair shedding later in the spring [9]. In addition, given the similarity in MSH response to secretagogue [5] between the insulin insensitive horses first described by Gentry et al. [10] and subsequently characterized by Cartmill et al. [11] and Caltabilota et al. [12], and horses either displaying or testing positive for PPID, we also evaluated whether cabergoline injections would the insulin sensitivity (i.e., increase the glucose response to insulin or reduce the insulin response to glucose infusion) in these insulin insensitive mares as part of our ongoing study of their characteristics.

2. Materials and methods

Procedures used in these experiments were approved by the Institutional Animal Care and Use Committee of the Louisiana State University Agricultural Center.

2.1. Preliminary experiment

2.1.1 Mares and treatments.

Selected plasma samples collected from two groups (of three) in the experiment of Hebert et al. [1] were used to assess the effect of a single 5-mg injection of cabergoline on the

MSH response to a low dose of sulpiride administered 10 d after cabergoline injection. Briefly, ten mares ranging in age between 5 and 16 years old, weighing between 480 and 616 kg, with body condition scores [13] between 5 and 8 were used. On October 21, 2011 (day 0), five of the mares received a single intramuscular injection of cabergoline (Attix Pharmaceuticals, Toronto, Ontario, Canada) in 1.0 mL of a proprietary mixture of hydrophobic, oily liquids designed to slow down and produce a sustained release of drug over time. Five other mares received an equivalent injection of vehicle at the same time and served as controls.

Small doses of sulpiride (2 µg/kg of body weight [BW] of the racemic mixture; Sigma Chemical Co., St. Louis, MO) were administered to each mare via intravenous injection in saline on days -2, -1, 0, 1, 2, 3, 4, 6, 8, and 10 relative to cabergoline or vehicle injections. Jugular blood samples were collected from each mare immediately before and at 10, 20, 40, and 60 min after sulpiride injection. Heparinized plasma was harvested and subsequently stored at -15°C.

2.1.2 Sample and data analyses.

Plasma from the day -1 and day 10 sulpiride challenges were selected for measurement of MSH with commercially available kit reagents (Euria α-MSH RIA, Immuno-Biological Laboratories, Minneapolis, MN). Estimates of the limit of detection (concentration of hormone equivalent to the mean number of counts per minute of the assay zero standard tubes minus two standard deviations of those counts from the mean) of the assay and the intra-assay coefficient of variation were 1.4 pmol/L and 6.6% for the single MSH assay.

Data for MSH concentrations were analyzed by analysis of variance (ANOVA) using the general linear model of SAS (SAS Instit., Cary, NC). They were analyzed as a double-split-plot

115 design, with treatment as the main effect, repetitive challenges (day -1 and 10) as the first
116 repetition, and multiple sampling times within each challenge as the second split. Treatment was
117 tested with the mare within treatment term, and each subsequent split was tested with the
118 appropriate interaction of mare within treatment for that split. Differences between groups within
119 time periods were assessed by the least significant difference test [14].
120

121 **2.2. Main experiment**

122

123 **2.2.1. Mares and treatments.**

124

125 Ten light horse mares between the ages of 11 and 22 yr, weighing between 486 and 584 kg, and
126 with body condition scores [13] of 6 to 8 were selected from the resident herd due to their
127 continual testing as insulin insensitive, based on the technique described by Caltabilota et al.
128 [12], over at least three different trials; the latest assessment was completed in early August,
129 2011. Such mares are also hyperleptinemic and hyperinsulinemic, and display an exaggerated
130 MSH response to sulpiride and TRH stimulation [5]. All mares were housed on pasture
131 consisting of primarily alicia bermudagrass intermixed with common bermudagrass, bahiagrass
132 and Dallis grass, and white clover. Hay prepared in summer from the same pasture grasses was
133 supplemented as the availability of pasture grass diminished. The experiment was started on
134 September 9, 2012, and concluded on November 18, 2012.

135 The ten mares were allotted to two groups of five such that ages, body conditions, leptin
136 concentrations, and insulin sensitivities (based on an insulin challenge [12] described below)
137 were similar between groups. Three pre-treatment assessments were done prior to cabergoline

138 treatment (day 0): a sulpiride challenge (day -5) to assess baseline prolactin response of each
139 mare, an insulin challenge (day -3), and a glucose infusion test (day -1). The day before each
140 assessment, the mares were brought up from pasture and were held in small pens with minimal
141 grass but with free access to water. No effort was made to rid the area of grass due to its paucity
142 in the pens. At approximately 08:00 the next morning, the mares were walked to an outdoor
143 chute and were loosely tethered at intervals to minimize stress and contact with each other. Upon
144 completion of each assessment, the mares were returned to pasture.
145
146 **2.2.2. Assessments of treatment effects.**
147
148 Sulpiride in saline was administered intravenously at a dose of 0.01 mg/kg of BW to each
149 mare in the morning, and jugular blood samples were drawn via 21-gauge needles into evacuated
150 tubes containing sodium heparin immediately before injection and then at 5, 10, and 20 min after
151 injection. Plasma was harvested by centrifugation at 1200 x *g* and was stored at -15°C for later
152 measurement of prolactin.
153 An insulin challenge was conducted on the morning of day -3, in which each mare was
154 administered 50 mU/kg BW of recombinant human insulin (Sigma Chem. Co.) in sterile saline
155 intravenously after a pre-injection (-10 and 0 min) determination of resting blood glucose
156 concentration by use of a hand held glucometer (Precision Xtra, Abbot Laboratories, Abbot Park,
157 IL). The percentage decrease in blood glucose concentrations was determined at 40 and 60 min
158 post-injection as described by Caltabilota et al. [12]. The greatest percentage (either at 40 or 60
159 min, whichever was greater) decrease in blood glucose concentration was used as an index of
160 insulin sensitivity.

On the morning of day -1, all mares were administered glucose (50% aqueous solution; Durvet Inc., Blue Springs, MO) through a 16-gauge needle inserted into the left jugular vein after collection of two blood samples 10 min apart (pre-glucose samples). Glucose was infused at a dose of 100 mg/kg of BW, and infusions typically took less than 1 min. Blood samples were drawn from the opposite jugular vein via 21-gauge needles at 5, 10, 15, 20, 25, and 30 min relative to completion of the glucose infusion. Mares tolerated the small gauge needle insertions very well and showed no sign of anxiety or refusal. Plasma was harvested and stored frozen for later measurement of insulin.

In the morning of the first treatment day (day 0), the two groups of mares, which had been established based on the criteria mentioned above, were randomly assigned as treatment and control. The five treated mares each received a 1-mL intramuscular injection of cabergoline (5 mg) in a slow-releasing vehicle [1]. The remaining five mares (controls) received a 1-mL injection of the vehicle in the same manner. The vehicle was a proprietary mixture of hydrophobic, oily liquids designed to slow down and produce a sustained release of drug over time [1]. After injections were completed, each mare had a 5- x 5-cm patch of hair on the shoulder shaved with clippers with a fine blade, and the hair saved for later assessment of total weight.

On day 9, and every 10 days thereafter through day 49 and again on day 60, the following procedure was repeated. All mares were brought in from pasture the evening before, held in small pens overnight, and then challenged with sulpiride in the morning as previously described for day -5 (including blood sampling). The mares were then returned to pasture until the following morning, at which time they received their next injection of cabergoline or vehicle. Thus, each treatment injection (10 days apart) was preceded by a sulpiride challenge so that any

change in responsiveness (i.e., refractoriness to the cabergoline) could be detected. The total number of injections per mare was seven. Shaving of a hair patch from the shoulder was repeated (from a novel area each time) on days 30 and 61. Assessments of behavior (such as signs of unusual anxiety or fear or change in social rank or treatment by other mares) were subjective and were made each day the mares were brought in from pasture. Observations were also made on the mares while in the pasture during the first week of treatment and again during the last week of treatment. Any unusual activity was noted for later consideration.

Post-treatment assessments of insulin sensitivity (insulin challenge, day 62), insulin response to glucose infusion (day 64), and a final sulpiride challenge (day 65) were conducted in the same manner as the pretreatment assessments described above. Thus, the final assessment was performed within 5 days following the last cabergoline injection.

2.2.3. Sample and data analyses.

Pretreatment concentrations of leptin were measured by radioimmunoassay as described by Cartmill et al. [11]. A single plasma sample from each mare collected 10 days before allotment of mares to treatment was used. Estimate of the limit of detection of that assay and the intra-assay coefficient of variation were 0.1 ng/mL and 8%, respectively.

At the end of the experiment, all frozen plasma samples were thawed and analyzed for the appropriate hormone(s). Prolactin in the samples collected during all sulpiride challenges was measured by radioimmunoassay previously validated for horse tissues [15]. Insulin was measured in samples collected during the glucose infusions by means of commercially available kit reagents (Coat-A-Count Insulin, Siemens Healthcare Diagnostics, Tarrytown, NY). Plasma

concentrations of MSH in samples collected at the pretreatment sulpiride challenge (day -5), and at the challenges on day 39 and day 65, were measured as described in section 2.1. Estimates of the limit of detection of the assays and the intra-assay coefficient of variation were 0.2 ng/mL and 7% for prolactin; 1.2 pmol/L and 5.5% for MSH, and 0.8 mIU/L and 5.2% for insulin. Multiple assays were needed for all prolactin samples, and the interassay coefficient of variation averaged 12%.

Data for each dependent variable were analyzed by ANOVA using the general linear model of SAS (SAS Instit., Cary, NC). The percentage decreases in glucose concentrations in pre- and post insulin challenges and hair weights were analyzed by one-way ANOVA with repeated sampling [14], with treatment group as the main effect, tested with the mare within treatment term, and repetitive sampling times (pre- and post-treatment for percentage decrease in glucose and the three shaving times for hair) and the treatment-time interaction tested with the residual error term. The data for prolactin concentrations, insulin concentrations, and MSH concentrations were analyzed as a double-split-plot design, with treatment as the main effect, repetitive challenges as the first repetition, and multiple sampling times within each challenge as the second split. Treatment was tested with the mare within treatment term, and each subsequent split was tested with the appropriate interaction of mare within treatment for that split. Areas under the response curve for prolactin responses to sulpiride were calculated and subsequently expressed as percentage of pre-treatment values for each mare; these data, excluding the pre-treatment data (all 100%), were analyzed in a split-plot ANOVA. Areas for control mares were also subjected to linear regression analysis [15] in a separate analysis to assess whether the downward trend in areas over the 10-day intervals was significant. When needed, differences

between treatment groups for individual time periods were tested for significance by the least significant difference test [14].

3. Results

3.1. Preliminary experiment

Mean concentrations of MSH in control mares and in mares treated with cabergoline are presented in Figure 1. All mares had a robust MSH response in the first 10 min after injection of sulpiride on day -1, before vehicle or cabergoline injection, as did the control mares on day 10 after vehicle injection (time effect; $P < 0.01$). In contrast, mares receiving 5 mg of cabergoline 10 days earlier had little to no response to the injected sulpiride (differed from controls at times 10 and 20 min; $P < 0.05$).

3.2. Main experiment

One mare in the cabergoline treatment group developed severe lameness during the experiment and was subsequently euthanized. All of her data were excluded from the final analyses. No other cabergoline-treated mare displayed lameness or any other sign of detrimental effects due to treatment.

Mean plasma prolactin concentrations in response to sulpiride injections every 10 days in controls and cabergoline-treated mares are presented in Figure 2. There was a robust response in all mares to the first (pre-treatment) injection of sulpiride. Due to chance, because mares were

allotted to two similar groups based on other criteria as mentioned in the Materials and Methods section, the group that was randomly chosen to receive cabergoline had a lower ($P < 0.001$) prolactin response than the eventual control group. Because of this, the area data for each mare were expressed as a percentage of her pre-treatment response (set at 100%), and these percentages were analyzed as described for the original area data. The mean percentages are presented in Figure 2. The treatment by time interaction ($P < 0.0001$) reflected the almost total suppression of the prolactin response to sulpiride in cabergoline-treated mares. There was also a general linear downward trend ($P < 0.08$) in the means for the control mares over time.

Mean plasma MSH concentrations in response to the sulpiride injections on days -5, 39, and 65 are presented in Figure 3. There was a response ($P < 0.001$) in MSH concentrations for control mares at each injection. In contrast, mares in the cabergoline-treated group had a noticeable MSH response only to the pretreatment injection and differed from controls on days 39 ($P = 0.011$) and 65 ($P = 0.064$).

Plasma insulin concentrations in samples from the pre-treatment glucose infusion were high before infusion of glucose (between 50 and 600 mIU/L; for comparison, insulin concentrations before glucose infusion in the post-treatment challenge averaged 3 mIU/L in both groups) and basically decreased thereafter, indicating the horses had eaten some time before the infusions or that the samples were in some way compromised. Because the glucose challenge at the low dose of glucose used (100 mg/kg BW) requires an overnight period of feed deprivation, the pretreatment data were considered not reliable for analysis, and only the post-treatment data were used to assess the insulin sensitivity to glucose. The mean plasma insulin responses to glucose infusion conducted 3 days after the last vehicle or cabergoline injection (day 64) are presented in Figure 4. Plasma insulin concentrations increased ($P < 0.0001$) after glucose

infusion in all mares, but did not differ between control mares and those treated with cabergoline at any time before or after infusion. Similarly, the percentage decrease in blood glucose concentrations assessed before initiation of treatments and again 1 day after the last vehicle or cabergoline injection were not affected ($P > 0.1$) by treatment or time (Fig. 4).

Mean weights of the hair samples shaved on the day of first treatment (day 0) and days 30 and 61 are presented in Figure 5. There was a day effect ($P < 0.001$) and an interaction of day with treatment ($P = 0.047$) in the ANOVA. On day 30, mares treated with cabergoline had a greater weight of hair shaved ($P = 0.083$), but by day 61, controls had the greater weight of hair shaved ($P = 0.064$).

4. Discussion

Hebert et al. [1] was the first to report the efficacy of cabergoline in slow-release vehicle for the suppression of prolactin secretion in horses. In the first experiment in that report, a single intramuscular injection of 5 mg of cabergoline reduced basal (i.e., unstimulated) plasma prolactin concentrations for at least 5 days in geldings, and in a second experiment, the same injection suppressed basal and sulpiride-stimulated prolactin concentrations within 30 min and for at least 10 days. Subsequent assessment of the duration of action of the 5-mg injection in mares during the summer revealed that prolactin secretion begins to recover within 12 days after treatment [N Arana Valencia, unpublished data]. Thus, for the long-term assessment of the dopaminergic activity of cabergoline in the present experiment, a 10-day interval between injections was chosen.

297 Dopaminergic agonists have been tested in the past as appetite depressants, with
298 moderate success. However, one problem often encountered was gradual resistance to the drug,
299 or tolerance to its effects, such that increasing dosages were required the longer the drug was
300 used [weeks to months; 16,17] to achieve the same effects. Thus, we incorporated the standard
301 sulpiride challenges into this experiment, one day before each successive cabergoline injection,
302 to assess the ability of cabergoline to keep prolactin secretion suppressed. The prolactin response
303 to sulpiride in cabergoline treated mares was essentially zero in all challenges, including the
304 post-treatment challenge on day 65. Given that this experiment was conducted during the
305 autumn, prolactin secretion would be tending to decrease in conjunction with the decreasing day
306 lengths [18]. This was in fact reflected in the downward trend in the prolactin areas for control
307 mares in Figure 2. Although the cabergoline injections used herein were suppressive under the
308 conditions of this experiment, the efficacy of injections needs to be tested during the spring and
309 summer, when prolactin production and secretion are the highest. Moreover, the administration
310 of dopaminergic agonists for the treatment of PPID would basically be needed year around,
311 given that the cause of the disease is likely permanent changes in the dopaminergic neural input
312 to the intermediate lobe of the pituitary [4]. The efficacy of these cabergoline injections would
313 therefore need testing under those conditions.

314 The MSH response to sulpiride injection in control mares was similar in magnitude to the
315 responses we previously observed for insulin insensitive mares [5]. Treatment with cabergoline
316 in the present experiment abolished the MSH response to sulpiride injection on days 39 and 65.
317 Thus, the assumption that the suppressive effects of cabergoline on prolactin secretion and
318 response to sulpiride injection should be similar for MSH secretion, as suggested by Hebert et al.
319 [1], has been confirmed both for those samples [1], shown in Figure 1, and for the longer-term

sampling in the present experiment. Both experiments were performed in the fall, when plasma MSH concentrations are highest [19,20]. However, the possible year-round suppression of MSH, and perhaps other products from the intermediate lobe of horses with PPID [4], will need to be tested under those conditions.

The weight of hair shaved from the shoulder region was similar in mares of the two groups at the onset of treatment (day 0). By day 30, the hair weights from cabergoline-treated mares were greater than for control mares. Prolactin has been shown to be involved with hair shedding in spring in various species, including the horse [9], and a lack of prolactin at that time results in a failure to shed [9,21]. Moreover, reduction of prolactin secretion in summer hastens the onset of winter pelage growth in mink [22], whereas prolactin treatment of voles subjected to short days prevents the onset of growth of the winter hair coat [23]. Thus, greater hair weights in these mares treated with cabergoline would be expected based on the suppression of prolactin secretion. The consistent increases in hair weights in control mares from day 0 to 30 to 61 would also be expected due to the gradually decreasing prolactin concentrations occurring naturally at this time [18], reflected in the decrease in prolactin responses to sulpiride. The apparent reversal in hair weights of the treated and control groups by day 61 was basically due to the continued rise in weights of the control mares and a cessation of increase in the treated mares (i.e., the 30- and 61-day means did not differ). Whether this was a cessation due to the earlier stimulation of winter coat, or whether the treated mares had actually reached their maximum growth, cannot be determined from the available data. Continued monitoring into December may have provided insight into these two possibilities.

In conclusion, cabergoline administration at the dose and in the vehicle described in this experiment was effective in providing long-term suppression of both plasma prolactin and MSH

concentrations in insulin insensitive mares when compared to insulin insensitive controls. However, no effect of cabergoline treatment was observed for insulin sensitivity. No noticeable detrimental effects were noticed throughout the experiment, except for the perturbation of hair coat growth. Thus, cabergoline administration as described herein may offer an alternate treatment option for long-term delivery of dopaminergic activity to horses, in lieu of daily pergolide feeding, which is the current treatment for PPID in horses and ponies.

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Fig. 1. Mean plasma concentrations of melanocyte stimulating hormone (MSH) in response to an intravenous injection of sulpiride (2 µg/kg of body weight) in saline at time 0 in control mares (n = 5) and mares treated intramuscularly with 5 mg of cabergoline in slow-release vehicle (n = 5) in the second experiment of Hebert et al. [1]. Sulpiride injections were administered before treatment (Pre) and 10 days after treatment (day 10). Plasma MSH concentrations were suppressed ($P < 0.01$) on day 10 in cabergoline-treated mares at 10 and 20 min after sulpiride injection. Pooled standard error of the means was 16 pmol/L.

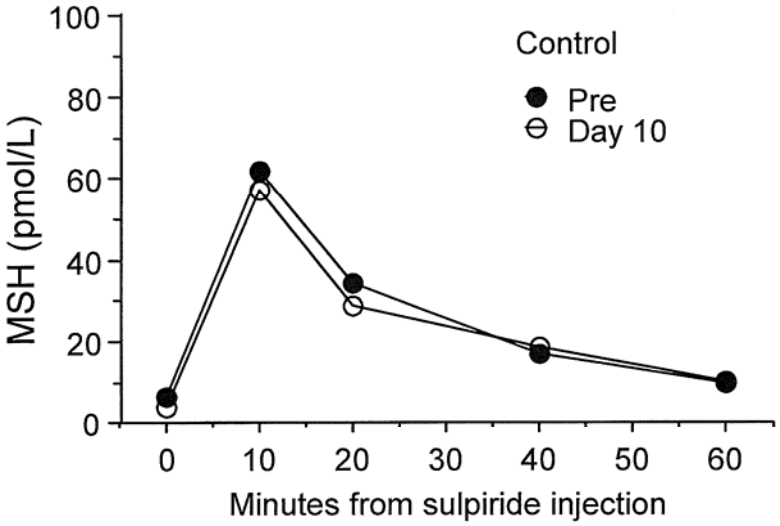
Fig. 2. Mean prolactin concentrations (panel A) in response to intravenous sulpiride injections (.01 mg/kg of body weight) in mares treated every 10 days with vehicle (controls; n = 5) or 5 mg of cabergoline in slow release vehicle (+cabergoline; n = 4). The first sulpiride injection was 5 days before the first treatment injection (vehicle or cabergoline), and successive sulpiride injections were administered 24 hours before the next treatment injection. The means in panel B are the prolactin areas under the curve for each group expressed as a percentage of the pre-treatment means. Pooled standard errors of the means were 10 ng/mL for prolactin concentrations and 13% for percentages. Means for the treated and control groups differed ($P < 0.01$) at each 10-day interval. There was also a general linear downward trend ($P < 0.08$) in the means for the control mares over time.

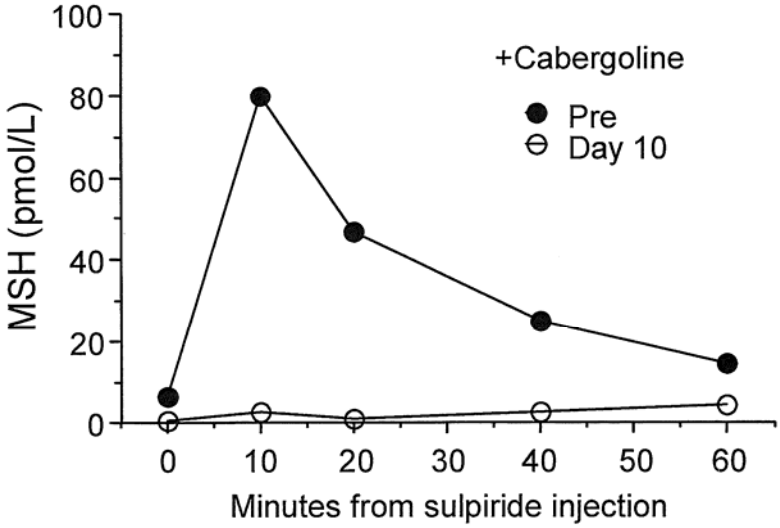
Fig. 3. Mean plasma concentrations of melanocyte stimulating hormone (MSH) in response to intravenous sulpiride injections (.01 mg/kg of body weight) in mares treated every 10 days with vehicle (controls; n = 5) or 5 mg of cabergoline in slow release vehicle (+cabergoline; n = 4).

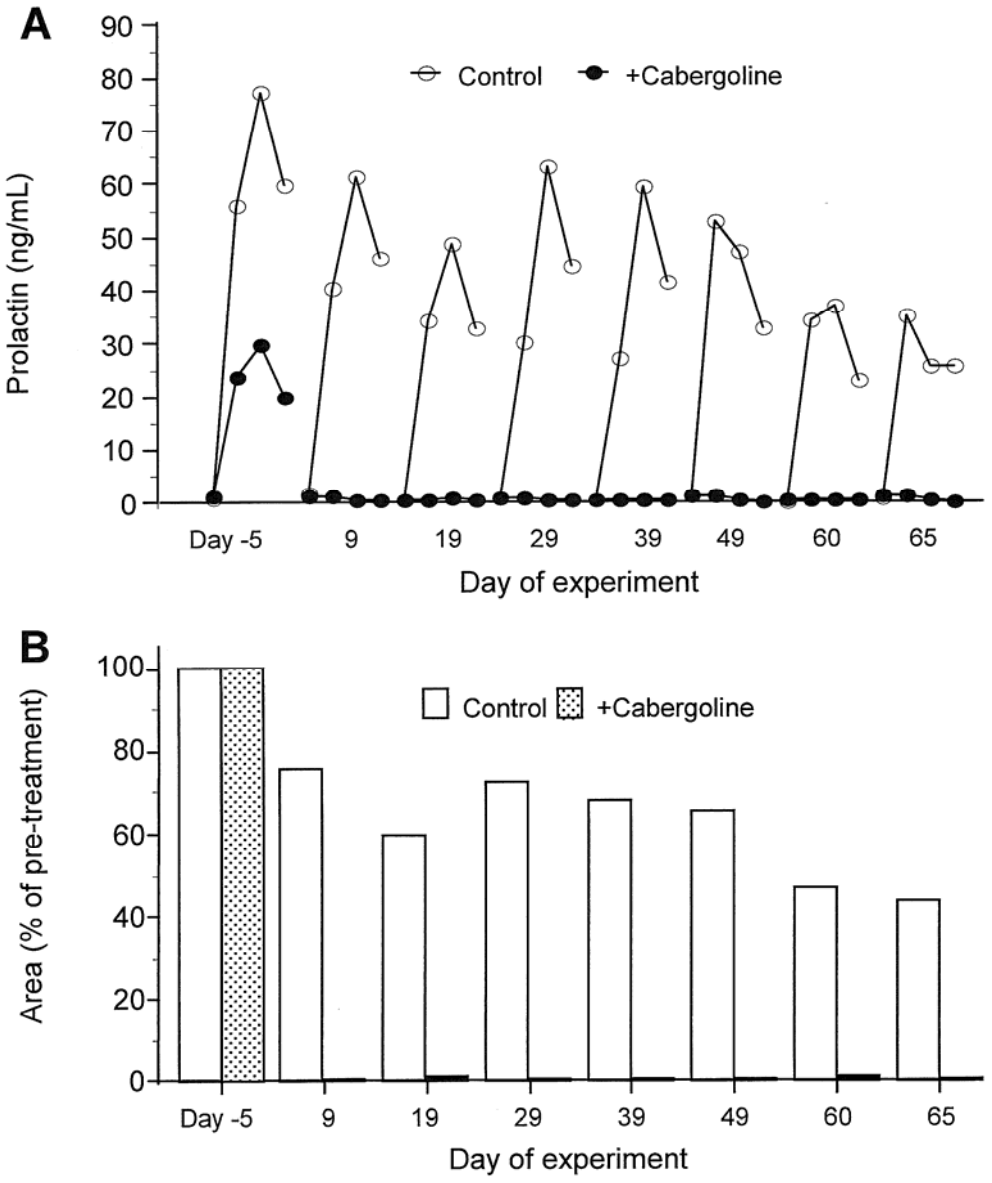
Plasma MSH was measured only in samples collected at the pre-treatment sulpiride injection (day 0), again on days 39 and 65. Pooled standard error of the means was 23 pmol/L. Means at 5 min after sulpiride for cabergoline-treated mares differed from controls on day 39 ($P = 0.011$) and at the end of the experiment (day 65; $P = 0.064$).

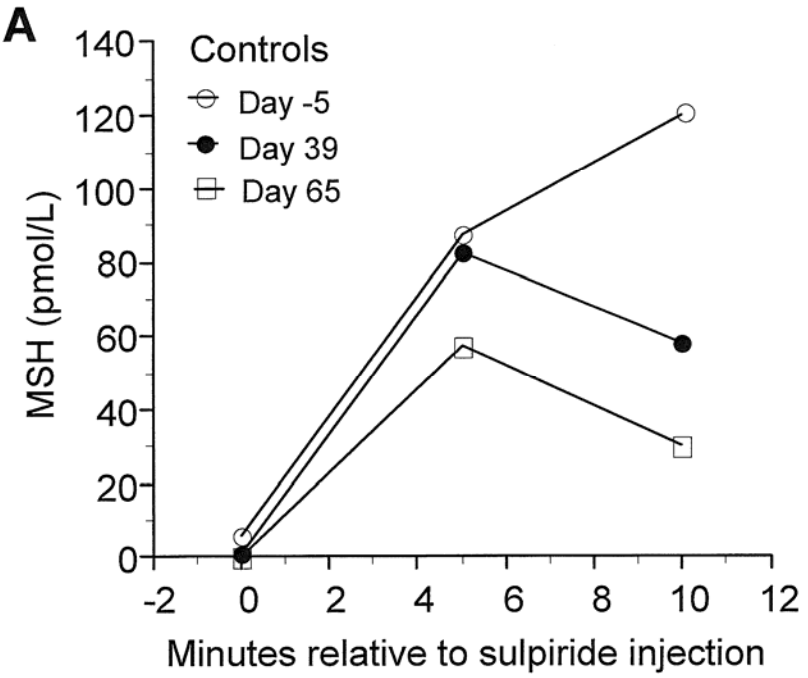
Fig. 4. Panel A: Mean plasma insulin concentrations after intravenous infusion of glucose (100 mg/kg of body weight) in mares treated every 10 days with vehicle (controls; n = 5) or 5 mg of cabergoline in slow release vehicle (+cabergoline; n = 4). Glucose was infused on day 64; there was no difference between groups at any time. Panel B: Mean percentage decrease in blood glucose concentrations in response to intravenous insulin injection (50 mIU/kg of body weight) before onset of treatment on day -3 (Pre) and on day 62 (Post), 24 hours after the last (7^{th}) treatment injection. There was no difference between groups for either insulin injection. Pooled standard errors of the means were 3.4 mIU/L for insulin concentrations and 10% for percentage decrease in blood glucose concentrations.

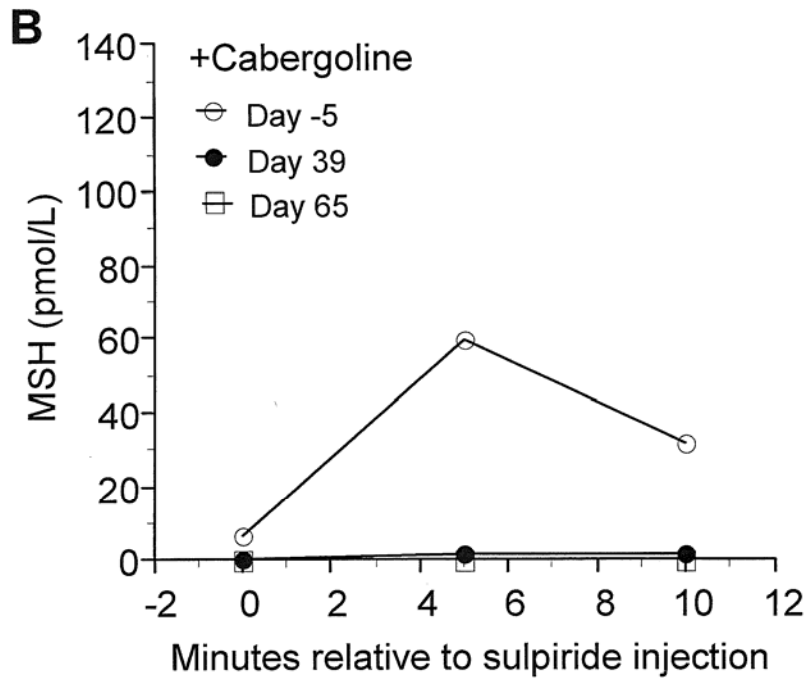
Fig. 5. Mean weight of hair shaved from the shoulder area (5 x 5 cm square) on days 0, 30 and 61 relative to the first treatment injections in mares treated every 10 days with vehicle (controls; n = 5) or 5 mg of cabergoline in slow release vehicle (+cabergoline; n = 4). P-values for comparisons of differences between means for the two groups are shown. Pooled standard error of the means was 0.06 mg.

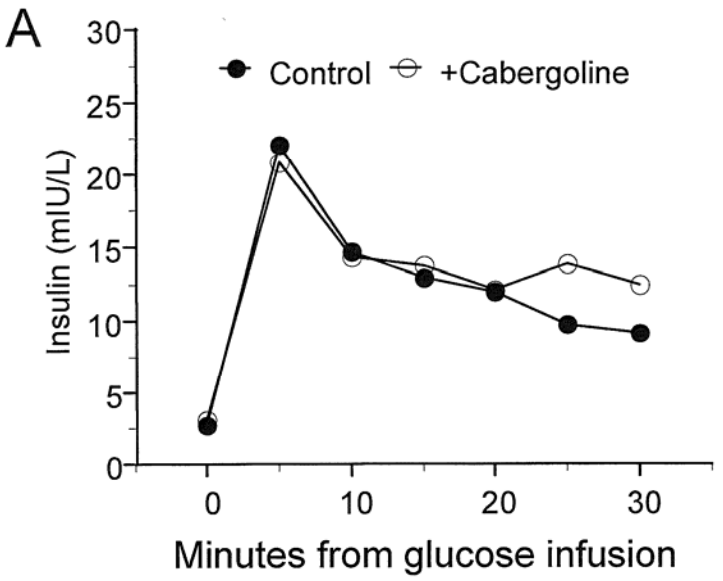


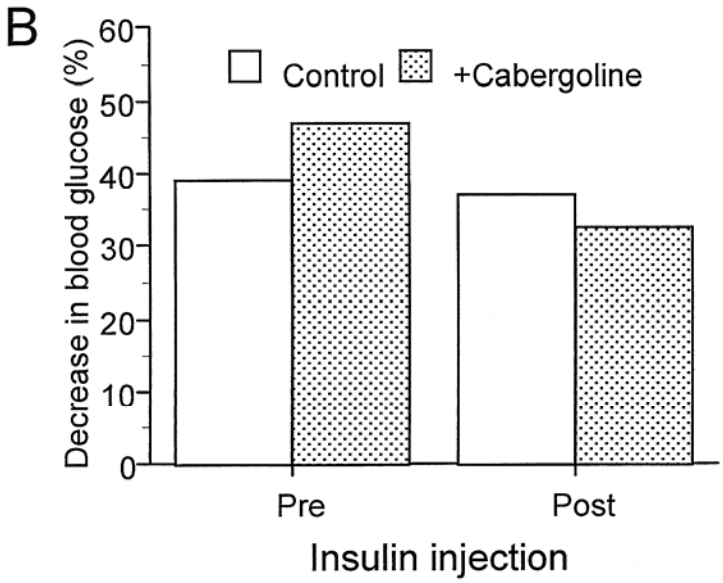



A
Prolactin (ng/mL)
90
80
70
60
50
40
30
20
10
0
Control
+Cabergoline
Day -5
9
19
29
39
49
60
65
Day of experiment
B
Area (% of pre-treatment)
100
80
60
40
20
0
Control
+Cabergoline
Day -5
9
19
29
39
49
60
65
Day of experiment


A
Controls
Day -5
Day 39
Day 65
MSH (pmol/L)
140
120
100
80
60
40
20
0
-2
0
2
4
6
8
10
12
Minutes relative to sulpiride injection


B
+Cabergoline
Day -5
Day 39
Day 65
MSH (pmol/L)
140
120
100
80
60
40
20
0
-2
0
2
4
6
8
10
12
Minutes relative to sulpiride injection


A
Control
+Cabergoline
Insulin (mIU/L)
30
25
20
15
10
5
0
0
10
20
30
Minutes from glucose infusion


B
Control
+Cabergoline
Decrease in blood glucose (%)
60
50
40
30
20
10
0
Pre
Post
Insulin injection