Evaluation of Dopaminergic and Antidopaminergic Agents for Use in Equine Metabolic Physiology

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EVALUATION OF DOPAMINERGIC AND ANTIDOPAMINERGIC AGENTS FOR USE IN EQUINE METABOLIC PHYSIOLOGY

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The School of Animal Sciences

by

Nicole Arana Valencia
B.S., Louisiana State University, 2012
M.S., Louisiana State University, 2013
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# TABLE OF CONTENTS

ACKNOWLEDGEMENTS ........................................................................................................ ii

ABBREVIATIONS ............................................................................................................... v

ABSTRACT ......................................................................................................................... vi

CHAPTER

1 INTRODUCTION ............................................................................................................. 1

2 REVIEW OF LITERATURE .......................................................................................... 3

3 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 ...................................................... 19

4 LONG-TERM AND SHORT-TERM DOPAMINERGIC (CABERGOLINE) AND ANTIDOPAMINERGIC (SULPIRIDE) EFFECTS ON INSULIN RESPONSE TO GLUCOSE, GLUCOSE RESPONSE TO INSULIN, OR BOTH, IN HORSES ........................................................................... 39

5 EFFECTS OF VARIOUS METHODS OF SULPIRIDE ADMINISTRATION ON PROLACTIN RELEASE IN HORSES ...................................................................................... 62

6 DOPAMINERGIC AND ANTIDOPAMINERGIC EFFECTS ON HEART RATE AFTER BRIEF EXERCISE IN HORSES ...................................................................................... 83

7 OVERALL SUMMARY AND CONCLUSIONS ............................................................ 107

APPENDIX 1: PERMISSION STATEMENT FOR USE OF MATERIAL COVERED IN CHAPTER 3 ................................................................................................................................. 109

APPENDIX 2: PERMISSION STATEMENT FOR USE OF MATERIAL COVERED IN CHAPTER 4 ................................................................................................................................. 110

APPENDIX 3: PERMISSION STATEMENT FOR USE OF MATERIAL COVERED IN CHAPTER 5 ................................................................................................................................. 111

VITA ................................................................................................................................. 112
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH</td>
<td>adrenocorticotropic hormone</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>BCS</td>
<td>body condition score</td>
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<tr>
<td>BW</td>
<td>body weight</td>
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<tr>
<td>CLIP</td>
<td>corticotropin-like intermediate peptide</td>
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<tr>
<td>D2</td>
<td>dopamine 2 receptor</td>
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<tr>
<td>ECP</td>
<td>estradiol cypionate</td>
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<td>EMS</td>
<td>equine metabolic syndrome</td>
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<tr>
<td>FSH</td>
<td>follicle stimulating hormone</td>
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<tr>
<td>GR2I</td>
<td>glucose response to insulin</td>
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<tr>
<td>GH</td>
<td>growth hormone</td>
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<td>HR</td>
<td>heart rate</td>
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<tr>
<td>IM</td>
<td>intramuscular</td>
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<tr>
<td>IR</td>
<td>insulin resistance</td>
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<td>IR2G</td>
<td>insulin response to glucose</td>
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<tr>
<td>IV</td>
<td>intravenous</td>
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<tr>
<td>LSD</td>
<td>least significant difference</td>
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<tr>
<td>LH</td>
<td>luteinizing hormone</td>
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<tr>
<td>MSH</td>
<td>melanocyte stimulating hormone</td>
</tr>
<tr>
<td>PHDA</td>
<td>periventricular hypothalamic dopaminergic neurons</td>
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<tr>
<td>PPID</td>
<td>pituitary pars intermedia dysfunction</td>
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<tr>
<td>POMC</td>
<td>pro-opiomelanocortin</td>
</tr>
<tr>
<td>SQ</td>
<td>subcutaneous</td>
</tr>
<tr>
<td>SAIB</td>
<td>sucrose acetate isobutyrate</td>
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<tr>
<td>TRH</td>
<td>thyrotropin releasing hormone</td>
</tr>
<tr>
<td>THDA</td>
<td>tuberohypophysial dopaminergic neurons</td>
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<td>TIDA</td>
<td>tuberoinfundibular dopaminergic neurons</td>
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ABSTRACT

A series of experiments studied the effects and practical applications of dopaminergic and antidopaminergic compounds in equine metabolic physiology. The first experiment was performed to assess the long-term effects of repeated cabergoline injections (every 10 days for a total of seven injections) on prolactin and α-melanocyte stimulating hormone concentrations in insulin insensitive mares. Additionally, the experiment also evaluated the use of cabergoline for improving insulin sensitivity. Plasma prolactin and α-melanocyte stimulating hormone concentrations were suppressed throughout the duration of the experiment even when the mares were challenged with a low-dose dopamine antagonist, sulpiride, the day previous to their subsequent cabergoline injection. Insulin sensitivity was unaffected by cabergoline administration. To further investigate the effect of dopaminergic activity on metabolism, a second set of experiments compared the long-term and short-term effects of cabergoline and sulpiride in both insulin resistant and insulin sensitive horses during the spring and summer months. The results confirmed that neither dopaminergic or antidopaminergic drugs have any effect on insulin sensitivity in horses. The third series of experiments studied the prolactin response to different modes of administration and concentrations of sulpiride. Prolactin response to sulpiride in horses was found to be enhanced and extended by using a higher dosage (1g compared to 3g) and hydrophobic vehicles (vegetable shortening or sucrose acetate isobutyrate. The final series of experiments evaluated the effects of dopaminergic (bromocriptine, cabergoline and pergolide) and antidopaminergic (sulpiride) drugs on heart rate after a brief period of exercise (two minutes at a trot). A noticeable reduction in heart rate was found in horses that received bromocriptine or cabergoline treatment but not when the horses were treated with pergolide or sulpiride. Over all experiments, it was concluded that changes in dopaminergic
activity, whether stimulatory or inhibitory, has no effect on insulin sensitivity in horses. In addition, strong dopamine agonists were found to alter the heart rate in lightly exercised healthy horses.
CHAPTER 1: INTRODUCTION

Research into finding an effective treatment for common metabolic disorders in horses, such as equine metabolic syndrome (EMS) or pituitary pars intermedia dysfunction (PPID) and their related clinical signs, has gathered much attention in recent years. Both of these chronic disorders, although having different etiologies and pathogeneses, can lead to life-threatening conditions if not properly managed early in their progression. In the first case, the main cause leading to death is laminitis due insulin dysregulation, which is a collective term used to describe hyperinsulinemia and insulin resistance. In PPID, affected horses progressively become lethargic and immunosuppressed and develop hirsutism and muscle atrophy. Left untreated, horses can typically fall to secondary complications such as laminitis, pneumonia, and even neurological disorders.

Dopaminergic drugs, such as pergolide and cabergoline, have been studied and are currently applied for the treatment of equine pituitary diseases. The use of dopaminergic agents to improve metabolic efficiency has been described in humans and mice, but little is known of its effects in horses. The first experiment described herein (Chapter 3) assessed the efficacy of the dopaminergic agonist, cabergoline, as a source of long term dopaminergic activity, and evaluated its effects on insulin sensitivity in both normal and insulin resistant horses. Based on the findings in that first experiment, questions arose as to the overall effect of dopaminergic and antidopaminergic drugs on pancreatic function in horses, and that concept was studied in Chapter 4. Given the parallel application of antidopaminergic drugs in the equine industry for the induction of ovulation in seasonally anovulatory mares, a series of experiments was then conducted to study the factors that affected the efficacy of the dopaminergic antagonist, sulpiride, using prolactin secretion as the indicator of antidopaminergic activity (Chapter 5). Finally, one possible side-effect of the use of various dopaminergic drugs in equine medicine
was studied in Chapter 6, after it was observed in an allied experiment that bromocriptine altered the heart rate response of horses to brief exercise.
CHAPTER 2: REVIEW OF LITERATURE

2.1 Insulin Resistance in Horses

Insulin resistance (IR) is a metabolic condition in which normal concentrations of insulin produce a subnormal physiologic response [2.1]. In horses, long term exposure to feeds and grasses high in sugars and carbohydrates, in addition to reduced activity levels, are associated with higher incidences of obesity and higher than normal insulin production to reduce blood glucose levels [2.2]. Chronic hyperinsulinemia can lead to tissues becoming insensitive or lead to a down regulation of insulin receptors on target tissues [2.3]. Horses differ from humans in the sense that they are able to maintain high insulin output from pancreatic beta cells without succumbing to pancreatic exhaustion. In horses, this phenomenon is referred to as compensated insulin resistance [2.4, 2.5]. In humans, pancreatic exhaustion is imminent in chronic hyperinsulinemia, often leading to Type I diabetes.

The idea of the cause and effect cycle of diet and IR is made possible through experimental induction of IR. Treiber et al. [2.6] showed a metabolic shift towards insulin insensitivity when Thoroughbred yearlings were adapted to a high glycemic diet. The authors concluded that, despite appearing healthy, these yearlings exhibited increased insulin resistance and a compensatory increase in insulin secretion. In addition to diet as a culprit in IR pathogenesis, exogenous administration of glucocorticoids, such as dexamethasone [2.7], or of growth hormone [2.8, 2.9], can be used to experimentally induce IR in horses.

For many years the two “gold standards” for measuring insulin sensitivity in horses have been the euglycemic-hyperinsulinemic clamp technique [2.10, 2.11] and the minimal model analysis of glucose tolerance test data [2.5, 2.11, 2.12]. Although these tests can provide reliable estimates of tissue sensitivity to insulin, they are labor intensive and expensive to perform. This limits practicality of performing these tests in a smaller research facility or farm setting.
Caltabilota et al. [2.13] demonstrated, and Arana Valencia et al. [2.14–2.16] confirmed, the use of a single intravenous injection of human recombinant insulin in distinguishing horses with normal insulin sensitivity from those with compensated IR.

2.2 Metabolic Syndrome in Horses

Metabolic syndrome is a group of risk factors (obesity, insulin resistance, hyperinsulinemia, hyperglycemia, hypertension, hypertriglyceremia, dyslipidemia) that are associated with the risk of developing cardiovascular disease and type II diabetes in humans [2.17]. Early studies into metabolic syndrome in horses described obesity, IR, and laminitis to be all components of a clinical condition. Therefore, in 2002, the term equine metabolic syndrome (EMS) was adopted due to its similarities with the metabolic syndrome described in humans [2.18]. Affected animals share phenotypic characteristics such as obesity, with regional adiposities in the neck, shoulder, and rump region [2.18]. The syndrome in horses differs from human metabolic syndrome in that laminitis is the primary disease of interest resulting from increased adiposity, hyperinsulinemia, and IR [2.19]. Moreover, horses do not present fasting hyperglycemia or develop atherosclerosis, hypertension, coronary heart disease, or type I diabetes [2.4]. These abnormalities of insulin metabolism in horses are now collectively referred to as insulin dysregulation [2.20].

2.3 Pituitary Pars Intermedia Dysfunction

Equine pituitary pars intermedia dysfunction (PPID) is a spontaneous and progressive dopaminergic neurodegenerative disease that is generally seen in horses of any gender and breed over 15 years of age, although it is more commonly observed in ponies and Morgan horses [2.21, 2.22]. Pituitary pars intermedia dysfunction is due to a degeneration of the periventricular hypothalamic dopaminergic neurons (PHDA) innervating the melanotropes of the pars intermedia of the equine pituitary [2.23]. Under normal conditions, the release of the
neurotransmitter dopamine from these neurons would cause tonic inhibition of the release of melanotropic hormones [2.24]. Endocrinologically, affected horses present an overproduction and over secretion of pro-opiomelanocortin (POMC) peptides, such as α-melanocyte stimulating hormone (α-MSH, hereafter referred to as MSH), β-endorphin, corticotropin-like intermediate peptide (CLIP), and adrenocorticotropic [2.25]. In addition, pars intermedia adenomas are often observed histologically [2.26]. McFarlane et al. [2.23] determined neurodegeneration of the PHDA neurons results from chronic oxidative stress damage of the nerve terminal ends. Since the pars intermedia does not respond to negative feedback, only to tonic inhibition, the reduction of dopamine production allows the cells to become hyperplastic and hypertrophic, thereby over producing POMC derived peptides and propagating the formation of pituitary adenomas.

2.4 Dopaminergic Regulation of Lactotropes and Melanotropes

Dopamine is a catecholamine neurotransmitter and a neurohormone that is produced in limited areas of the central nervous system, and in other non-neuronal tissues such as the heart, intestines, kidneys, and adrenal medulla [2.27]. Within the mammalian brain there are three specific neuronal bodies within the hypothalamus that produce dopamine and innervate the lactotropes and melanotropes in the pars distalis and PI, respectively: the short-axon tuberoinfundibular dopaminergic neurons (TIDA), the tuberohypophysial dopaminergic neurons (THDA), and the PHDA [2.28].

The TIDA neurons originate in the arcuate nucleus of the hypothalamus and terminate in the median eminence of the adenohypophysis. When stimulated, these neurons release dopamine into hypothalomo-hypophysial portal blood vessels, where it reaches its target cells, the prolactin-producing lactotropes in the pars distalis of the adenohypophysis [2.29]. The THDA neurons also originate from the rostral area of the arcuate nucleus and terminate in both the pars intermedia and pars nervosa of the hypophysis [2.30], whereas the PHDA neurons arise from the
periventricular nucleus and terminate exclusively in the pars intermedia [2.24, 2.31]. Small, short portal vessels connect the capillary beds of the pars nervosa and pars intermedia with the pars distalis, thereby allowing dopamine of TIDA, THDA, and PHDA origin to reach the lactotropes [2.28].

Dopamine is directly involved in the regulation of lactotropes and melanotropes through tonic inhibition on their hormone secretion by direct action on dopamine 2 receptors located on cell surface [2.22]. There are five known dopamine receptors subtypes (D1 through D5) which can be subdivided into two groups; those that are D1-like (D1 and D5) and those that are D2-like (D2, D3, and D4) [2.32]. The most abundant form of dopamine receptor found on lactotropes and melanotropes are D2-like receptors [2.32]. These receptors are well established targets in clinical pharmacology for the treatment of numerous disorders such as schizophrenia, Parkinson’s disease, hypertension, pituitary tumors, and hyperprolactinemia [2.33] in humans and PPID, early induction of ovulation [2.34], and treatment for fescue toxicosis in horses [2.35,2.36].

For both lactotropes and melanotropes, activation of the D2 receptor by dopamine or dopamine agonists elicits an immediate hyperpolarization of the cell membrane, thereby preventing exocytosis of secretory vesicles [2.28]. In contrast, a decreased presence of dopamine or inability of dopamine binding to its receptor, due to pharmacological compounds such as dopamine antagonists, will cause the cell to remain in a depolarized state. This results in an increased expression of prolactin or POMC messenger ribonucleic acid, increased post-translational processing and secretion of prolactin or POMC-derived peptides, and hyperplasia and/or hypertrophy of the lactotropes or melanotropes themselves [2.37]. Intravenous injection of sulpiride acts almost immediately to cause an increase in measurable plasma prolactin and MSH concentrations in horses [2.14].
2.5 The Use of Dopaminergic Agonists in Horses

2.5.1 Pergolide

Pergolide is a short acting dopamine receptor agonist that was originally used for treatment of idiopathic Parkinson’s disease in humans but was later withdrawn in the United States due to its association with mitral valve regurgitation [2.38]. With the recognition of PPID as a dopaminergic neurodegenerative disorder in horses, pergolide was assessed to restore pituitary dopaminergic inhibition in PPID horses [2.39]. Unlike in humans, no detrimental side effects have been reported with extensive use in horses and, in 2012, pergolide was approved by the United States Food and Drug Administration for the control of clinical signs associated with PPID (Equine Cushing’s Disease) under the trade name Prascend® [2.40].

2.5.2 Cabergoline

Cabergoline is a potent synthetic ergoline derivative with a strong and selective agonist activity towards D2 receptors [2.41]. It has been used historically for the treatment of micro- and macroprolactinomas and other hyperprolactinemic disorders in humans as well as for the treatment of Parkinson’s disease [2.42]. When compared to other dopaminergic agonists, cabergoline is distinguished by its very long half-life (65 to 110 hours [2.41, 2.42]). Indeed, Hebert et al. [2.43] demonstrated the superior efficacy and duration of action of cabergoline compared to pergolide on plasma prolactin concentrations in horses. Only one intramuscular injection of 5 mg of cabergoline in 1 ml of slow-release vehicle was needed for complete suppression of prolactin even when the mares where challenged with sulpiride 10 days after treatment. In contrast, daily injections of 2 mg of pergolide had to be given in order to achieve similar results. The authors [2.43] go on to recommend the use of an injectable form of cabergoline in a slow-release vehicle as a possible alternative treatment for PPID, given that it
was shown to have more efficacious and longer lasting effects than orally administrated pergolide.

### 2.5.3 Bromocriptine

Bromocriptine is a presynaptic D2 dopamine receptor and α-adrenergic receptor agonist in central and peripheral neurons [2.44]. It was once a commonly used drug in human medicine for reducing prolactin concentrations and hence inhibiting lactogenesis in women after childbirth [2.45], however it is rarely used today in that capacity and with preference shifted for the use in treatment of Type II diabetes [2.46, 2.47] and Parkinson’s disease [2.48]. Johnson and Becker [2.49] were the first to confirm that administration of bromocriptine reduced serum prolactin concentrations in horses in May. Like pergolide, its effects are not long lasting like cabergoline. Although it is not commonly used in equine medicine, there is one report [2.50] in which bromocriptine was fed to a mare with inappropriate lactation, with allegedly positive results.

### 2.6 The Use of Dopaminergic Antagonists in Horses

Antidopaminergic drugs, such as sulpiride and domperidone, are commonly used in the equine industry for use in inducing early cyclicity and ovulation in seasonally anovulatory mares (sulpiride [2.34, 2.51, 2.52]; domperidone [2.53, 2.54]; or both [2.55]), treatment of fescue toxicosis in pregnant mares (domperidone [2.35, 2.36, 2.56]), and as a possible tool for diagnosing horses with PPID (domperidone [2.57–2.59] and sulpiride [2.14]). These agents are D2 receptor selective compounds that release the adenohypophysis from dopamine inhibition by competitively blocking the D2 receptors on lactotropes and melanotropes, thus inducing an over-secretion of prolactin and POMC peptides [2.60].

### 2.7 Other Physiological Effects of Dopaminergic and Antidopaminergic Drugs

As previously mentioned, dopamine can be synthesized in different areas of the peripheral and central nervous systems and therefore play an important role in modulating the
central nervous, renal, hormonal and cardiovascular systems [2.37] through stimulation of dopaminergic (D1 and D2-like receptors) and α- and β- adrenergic receptors [2.61]. Here, only the effects on due to D2 receptor activation or inhibition will be discussed.

Effects of dopamine agonists on different physiological systems is well characterized. Within the kidney, renal-produced dopamine is crucial for maintaining normal fluid and electrolyte balance as well as blood pressure. Activation of D2 receptors located proximal and distal convoluted tubules can induce natriuresis, diuresis, increase in renal blood flow, and glomerular filtration [2.62].

Metabolic changes have also been elucidated through regulation of dopamine receptors. Dopamine agonists can influence central circadian neuroendocrine activities that regulate metabolism to reduce insulin sensitivity in hibernating animals. In wild conditions, many seasonal animals develop obesity, insulin resistance, and hyperinsulinemia as they prepare to hibernate for winter conditions. Bromocriptine treatment in adult female Siberian hamsters acclimated to experimental winter conditions showed reduced hyperinsulinemia and lipogenesis [2.63]. In another study, Liang and others [2.64] demonstrated improved hyperglycemia and hyperlipidemia in diabetic and obese mice due to dopamine agonist administration. Treatment of diabetic db/db mice with combined D1 and D2 receptor agonists, bromocriptine, and SKF 3893, resulted in reduced hyperglycemia and hyperlipidemia, and improved pancreatic β-cell function, which stimulated insulin secretion. However, this metabolic effect was not evident in hyperinsulinemic PPID horses, when they were treated long-term with pergolide [2.65].

Cardiovascular changes are also common with dopamine receptor agonist administration. Bromocriptine has been demonstrated to produce hypotension and bradycardia in humans [2.66, 2.67] and in certain laboratory animals [2.68–2.70]. Activation of the presynaptic D2 receptors reduces sympathetic tone by reducing norepinephrine release [2.67,2.71]. This causes
vasodilation and subsequent decrease in heart rate, blood pressure, and peripheral resistance; however, cardiac output is unaffected [2.68]. In contrast, at high doses, dopamine can stimulate β-adrenergic receptors to cause inotropic and chronotropic effects which can result in increased heart rate and vasoconstriction [2.72]. Fescue toxicosis, resulting from the consumption of ergot-alkaloids from endophyte-infected fescue, has been associated with vasoconstriction and lameness in both cattle [2.73, 2.74] and horses [2.75]. Using Doppler ultrasonography, McDowell and others [2.76] demonstrated vasoconstriction of the distal palmar artery when horses were fed ground endophyte-infected tall fescue seeds. Specific ergot derivatives were later compared in vitro on medial palmar arteries and veins to confirm the vasoconstrictive action of ergot [2.75].

2.8 Vehicle Formulations for Intramuscular and Subcutaneous Injections

One problem faced by the equine industry is a lack of commercial availability of dopamine agonist and antagonist. There are only two drugs that are commercially available for horses, domperidone and pergolide. Domperidone, a D2 receptor antagonist under the trade name Equidone®Gel, is commercially available as an oral gel approved for prevention and treatment of fescue toxicosis in periparturient mares [2.77]. Pergolide, or Prascend®, is available as 1 mg tablets that need to be orally administered to PPID horses daily for the remainder of a horse’s life. However, other commonly used drugs in equine research, such as cabergoline and sulpiride, are only available through chemical companies, such as Sigma, or through compounding pharmacies. This can present problems in research as drug pharmacodynamics can heavily vary due to differences in drug concentrations and vehicle interactions from one researcher to another. Therefore, a safe, well-tolerated and readily available vehicle is required to obtain consistent results in research.
In addition to the properties of a vehicle, the route of administration of a particular substance affects the longevity of the compound. Intravenous (IV; varying doses [2.78]) or intramuscular (IM; 25 or 100 mg [2.49]) administration of a racemic mixture of sulpiride dissolved in saline produces a significant rise in serum prolactin within 15 minutes of injection irrespective of route of administration. However, the effect is short lived and serum prolactin level return to baseline within 2 (IV) or 7 (IM) hours post treatment. Colborn and others [2.79] administered 500 mg of sulpiride subcutaneously (SQ) every day for 14 days in the neck to stallions in winter in 2 mL of vegetable shortening. Vegetable shortening and the SQ route were chosen to delay the absorption of sulpiride as long as possible. Maximal prolactin secretion was achieved within 3 days and the effects lasted until the end of the sampling period (day 14). Administration of 100 mg of sulpiride in 2 mL oil SQ to geldings resulted in similar peaks in prolactin concentration within 60 min, however the effect did not appear to be as long lasting as with vegetable shortening [2.80].

Similar vehicle studies have been conducted for cabergoline. Herbert and others [2.43] initially evaluated the suppressive effect of cabergoline on prolactin secretion when dissolved in a proprietary mixture of oily liquids (BETpharm.com) to produce a sustained release of the drug over time. A single injection of 5 mg of cabergoline dissolved in 1 mL of vehicle given IM was able to suppress prolactin secretion for 10 days even when horses were challenged with a low dose of sulpiride (0.05 mg/kg BW; IV in saline). Further studies [2.15, 2.81] confirmed the long term suppressive effects of cabergoline in this vehicle. Due to difficulties obtaining the proprietary vehicle for further research, Oberhaus and others [2.82] compared the duration of suppression of prolactin secretion by 5 mg of cabergoline in proprietary vehicle and 5 mg of cabergoline in vegetable oil. Both treatments suppressed prolactin concentrations for 5 days with virtually identical patterns of suppression. Even though the natural half-life of cabergoline is
long (65 – 110 hours [2.42]), the addition of an oil-based or biodegradable polymer vehicle, such as sucrose acetate isobutyrate, might greatly prolong the duration of action for a given dose, and thereby prove a more desirable alternative to daily oral administration of pergolide for PPID, for example.

2.9 Rationale for Present Experiments

There is a current need for a pharmacological agent to assist in increasing metabolism or improving glucose utilization when diet and exercise are not sufficient to improve insulin sensitivity or when diet and exercise is not recommended in acute cases of laminitis. Studies investigating the use of dopamine agonists for the treatment of insulin sensitivity have shown mixed results in humans and mice, and only Donaldson et al. [2.65] has studied the effect of pergolide on insulin dynamics in horses concurrently afflicted with PPID. Additionally, other physiological effects of dopaminergic and antidopaminergic treatment in horses is lacking.

The purpose of the studies described herein were 1) to determine if insulin dynamics could be altered as a result of long-term and short-term administration of dopaminergic agonists (cabergoline) and antagonists (sulpiride) in horses, 2) to assess physiological factors that affect prolactin response to sulpiride administration, and 3) to assess whether or not dopaminergic and antidopaminergic agents have additional physiological effects not described in equine literature. It was hypothesized that 1) cabergoline nor sulpiride treatment would affect insulin sensitivity, 2) prolactin response to sulpiride would increase and be extended depending on location, vehicle and dose of sulpiride administration, and 3) dopamine agonists would decrease heart rate, but not sulpiride.

2.10 References


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3.1 Summary

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.

1Published in the Journal of Equine Veterinary Science; used with permission (see Appendix 1).
3.2 Introduction

Recent research by Hebert et al. [3.1] 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 [3.1], which, in the absence of cabergoline, caused relatively consistent elevations in prolactin secretion in both mares and estrogen-treated geldings [3.1,3.2]. Similarly, injections of pergolide in slow-release vehicle suppressed prolactin secretion, but for a much shorter period of time [3.1]. Because only one injection of cabergoline was tested in the experiment of Hebert et al. [3.1], the possibility of long-term detrimental effects or refractoriness could not be assessed.

Hebert et al. [3.1] 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 adrenocorticotropic hormone (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 [3.3,3.4]). Hebert et al. [3.1] 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 [3.5], similar to, but not as great a magnitude of, horses displaying symptoms of PPID [3.6,3.7]. 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. [3.8].
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 [3.9]. In addition, given the similarity in MSH response to secretagogue [3.5] between the insulin insensitive horses first described by Gentry et al. [3.10] and subsequently characterized by Cartmill et al. [3.11] and Caltabilota et al. [3.12], and horses either displaying or testing positive for PPID, we also evaluated whether cabergoline injections would improve 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.

3.3 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.

3.3.1 Preliminary Experiment

3.3.1.1 Mares and Treatments. Selected plasma samples collected from two groups (of three) in the experiment of Hebert et al. [3.1] 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 [3.13] 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, USA) 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.

3.3.1.2 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, USA). 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, USA). They were analyzed as a double-split-plot design, with treatment as the main effect, repetitive challenges (day -1 and 10) 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. Differences between groups within time periods were assessed by the least significant difference test [3.14].
3.3.2 Main Experiment

3.3.2.1 Mares and Treatments. Ten light horse mares between the ages of 11 and 22 yr, weighing between 486 and 584 kg, and with body condition scores [3.13] of 6 to 8 were selected from the resident herd due to their continual testing as insulin insensitive, based on the technique described by Caltabilota et al. [3.12], over at least three different trials; the latest assessment was completed in early August 2011. Such mares are also hyperleptinemic and hyperinsulinemic and display an exaggerated MSH response to sulpiride and TRH stimulation [3.5]. All mares were housed on pasture consisting of primarily alicia bermudagrass intermixed with common bermudagrass, bahiagrass and Dallis grass, and white clover. Hay prepared in summer from the same pasture grasses was supplemented as the availability of pasture grass diminished. The experiment was started on September 9, 2012 and concluded on November 18, 2012.

The ten mares were allotted to two groups of five such that ages, body conditions, leptin concentrations, and insulin sensitivities (based on an insulin challenge [3.12] described below) were similar between groups. Three pre-treatment assessments were done prior to cabergoline treatment (day 0): a sulpiride challenge (day -5) to assess baseline prolactin response of each mare, an insulin challenge (day -3), and a glucose infusion test (day -1). The day before each assessment, the mares were brought up from pasture and were held in small pens with minimal grass but with free access to water. No effort was made to rid the area of grass due to its paucity in the pens. At approximately 08:00 the next morning, the mares were walked to an outdoor chute and were loosely tethered at intervals to minimize stress and contact with each other. Upon completion of each assessment, the mares were returned to pasture.
3.3.2.2 Assessments of Treatment Effects. Sulpiride in saline was administered intravenously at a dose of 2 µg/kg of BW to each mare in the morning, and jugular blood samples were drawn via 21-gauge needles into evacuated tubes containing sodium heparin immediately before injection and then at 5, 10, and 20 min after injection. Plasma was harvested by centrifugation at 1200 x g and was stored at -15°C for later measurement of prolactin.

An insulin challenge was conducted on the morning of day -3, in which each mare was administered 50 mU/kg BW of recombinant human insulin (Sigma Chem. Co.) in sterile saline intravenously after a pre-injection (-10 and 0 min) determination of resting blood glucose concentration by use of a hand held glucometer (Precision Xtra, Abbot Laboratories, Abbot Park, IL, USA). The percentage decrease in blood glucose concentrations was determined at 40 and 60 min post-injection as described by Caltabilota et al. [3.12]. The greatest percentage (either at 40 or 60 min, whichever was greater) decrease in blood glucose concentration was used as an index of insulin sensitivity.

On the morning of day -1, all mares were administered glucose (50% aqueous solution; Durvet Inc., Blue Springs, MO, USA) 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 [3.1]. 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 [3.1]. 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.

3.3.2.3 Sample and Data Analyses. Pretreatment concentrations of leptin were measured by radioimmunoassay as described by Cartmill et al. [3.11]. 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 [3.15]. 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, USA). 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 3.3.1.2. 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, USA). The percentage decreases in glucose concentrations in pre- and post-insulin challenges and hair weights were analyzed by one-way ANOVA with repeated sampling [3.14], 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 [3.15] 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 [3.14].

3.4 Results

3.4.1 Preliminary Experiment

Mean concentrations of MSH in control mares and in mares treated with cabergoline are presented in Fig. 3.1. 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).
Fig. 3.1. 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. [3.1]. 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.

3.4.2 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 Fig. 3.2. 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.

![Graph A](image)

**Fig. 3.2.** 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.
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 Fig. 3.2. 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 Fig. 3.3. 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 Fig. 3.4. 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. 3.4).

Fig. 3.3. 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).
Mean weights of the hair samples shaved on the day of first treatment (day 0) and days 30 and 61 are presented in Fig. 3.5. 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 ($P = 0.064$).

**Fig. 3.4.** 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.
3.5 Discussion

Hebert et al. [3.1] 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.
Dopaminergic agonists have been tested in the past as appetite depressants, with moderate success. However, one problem often encountered was gradual resistance to the drug, or tolerance to its effects, such that increasing dosages were required the longer the drug was used (weeks to months; 3.16,3.17) to achieve the same effects. Thus, we incorporated the standard sulpiride challenges into this experiment, one day before each successive cabergoline injection, to assess the ability of cabergoline to keep prolactin secretion suppressed. The prolactin response to sulpiride in cabergoline treated mares was essentially zero in all challenges, including the post-treatment challenge on day 65. Given that this experiment was conducted during the autumn, prolactin secretion would be tending to decrease in conjunction with the decreasing day lengths [3.18]. This was in fact reflected in the downward trend in the prolactin areas for control mares in Fig. 3.2. Although the cabergoline injections used herein were suppressive under the conditions of this experiment, the efficacy of injections needs to be tested during the spring and summer, when prolactin production and secretion are the highest. Moreover, the administration of dopaminergic agonists for the treatment of PPID would basically be needed year around, given that the cause of the disease is likely permanent changes in the dopaminergic neural input to the intermediate lobe of the pituitary [3.4]. The efficacy of these cabergoline injections would therefore need testing under those conditions.

The MSH response to sulpiride injection in control mares was similar in magnitude to the responses we previously observed for insulin insensitive mares [3.5]. Treatment with cabergoline in the present experiment abolished the MSH response to sulpiride injection on days 39 and 65. Thus, the assumption that the suppressive effects of cabergoline on prolactin secretion and response to sulpiride injection should be similar for MSH secretion, as suggested by Hebert et al. [3.1], has been confirmed both for those samples [3.1], shown in Fig. 3.1, and for the longer-term
sampling in the present experiment. Both experiments were performed in the fall, when plasma MSH concentrations are highest [3.19,3.20]. However, the possible year-round suppression of MSH, and perhaps other products from the intermediate lobe of horses with PPID [3.4], 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 [3.9], and a lack of prolactin at that time results in a failure to shed [3.9,3.21]. Moreover, reduction of prolactin secretion in summer hastens the onset of winter pelage growth in mink [3.22], whereas prolactin treatment of voles subjected to short days prevents the onset of growth of the winter hair coat [3.23]. 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 [3.18], 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.

3.6 References


CHAPTER 4: LONG-TERM AND SHORT-TERM DOPAMINERGIC (CABERGOLINE) AND ANTIDOPAMINERGIC (SULPIRIDE) EFFECTS ON INSULIN RESPONSE TO GLUCOSE, GLUCOSE RESPONSE TO INSULIN, OR BOTH, IN HORSES

4.1 Summary

Dopaminergic drugs, such as those used to treat pituitary pars intermedia dysfunction (PPID) in horses, have been shown to improve insulin sensitivity in other species. It has been suggested that pergolide, used to treat PPID, may enhance insulin sensitivity in resistant horses, although evidence for that remains unclear. Four experiments were conducted herein to determine possible effects of dopaminergic inhibition or stimulation on two indices of insulin sensitivity in horses: the glucose response to insulin (GR2I), administered intravenously (IV) using a fixed dose of recombinant human insulin, and the insulin response to an acute IV infusion of glucose (IR2G). The first experiment tested the short-term effects of sulpiride (in saline; IV) 5 minutes prior to IR2G in insulin-sensitive and insensitive geldings. Experiment 2 tested the effects of a long-term sulpiride protocol (1.5 g intramuscularly [IM]) every 5 days for 45 days) on GR2I and IR2G in insulin-sensitive and insensitive geldings. Experiment 3 tested the short-term effects of 5 mg cabergoline IM on GR2I and IR2G in insulin-sensitive and insensitive mares. The fourth experiment tested the long-term effects of cabergoline IM on GR2I in insulin-sensitive mares. Results from these experiments revealed that neither increased nor decreased dopaminergic activity, in the long or short-term, had any impact on GR2I or IR2G in horses (P > 0.1), regardless of starting insulin sensitivity status. We conclude that dopaminergic agents have no benefit for treating insulin insensitivity in horses, in spite of a perception of such benefits permeating the industry.

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2Published in the Journal of Equine Veterinary Science; used with permission (see Appendix 2).
4.2 Introduction

There is a perception in the horse industry that pergolide, a short-acting dopaminergic agonist, might be useful in treating insulin resistance in horses [4.1]. This presumption may have arisen from the fact that horses with pituitary pars intermedia dysfunction (PPID), concurrently afflicted with insulin dysregulation, showed improvement in outward symptoms (e.g. shedding of retained curly coat, improved muscle mass [4.2]) when treated with pergolide. However, there are currently no studies supporting the use of dopamine agonists for the treatment of insulin resistance. In a study conducted by Donaldson et al [4.3], neither insulin nor glucose concentrations changed when horses with PPID were treated with pergolide mesylate for an average of two months. Additionally, Arana Valencia et al. [4.4] reported no change in insulin response to glucose infusion (IR2G) or glucose response to insulin administration (GR2I) when insulin resistant mares were given 5 mg of cabergoline every 10 days for 60 days in the fall. There are in fact reports in other species indicating a dopaminergic-pancreatic interaction [4.5]. For this reason, the experiments reported herein were designed to further explore the effects of dopaminergic antagonism with sulpiride and increased dopaminergic activity (by cabergoline treatment) on indices of insulin sensitivity in horses. Due to its strong antagonistic effects on the dopaminergic receptors of the pituitary, sulpiride was used to mimic the pathological conditions of a horse in late-stage PPID. Cabergoline, on the other hand, was used to study the effects on insulin sensitivity under high dopaminergic activity.

We specifically evaluated the 1) short-term (basically 1 day) and long-term (45 days or more) administration of sulpiride on insulin resistant and insulin-sensitive geldings, respectively, and 2) the short-term and long-term treatment of cabergoline on insulin-sensitive and insulin-resistant mares. Because both cabergoline and sulpiride have the expected biological effects in
the horse on lactotropes of the adenohypophysis [4.4,6-4.8], plasma prolactin concentrations were used in all experiments as a reflection of drug efficacy.

4.3 Materials and Methods

All procedures described in these experiments were approved by the Institutional Animal Care and Use Committee of the Louisiana State University Agricultural Center. Horses used in the described experiments were long-term residents of the LSU AgCenter Horse Farm in Baton Rouge, Louisiana, and were routinely maintained outdoors on native grass pastures during the warm seasons and on winter ryegrass when available. Alicia bermudagrass hay was supplemented as the availability of pasture grass diminished during the fall and winter months. All horses had long-term histories as being either insulin resistant or insulin-sensitive [4.9]. However, unless otherwise stated, insulin and glucose challenges were conducted prior to each experiment to confirm insulin sensitivity status to ensure proper inclusion into appropriate treatment groups.

4.3.1 Insulin Challenge (GR2I) Protocol

The insulin challenge protocol used herein was based on a validated technique described by Caltabilota et al [4.9]. The night before an insulin challenge, horses were brought from pasture and feed-deprived overnight in stalls with free access to water. The following morning, they were loosely tethered in stalls in a barn and two samples of jugular blood were collected 10 minutes apart using a tuberculin syringe fitted with a 22-gauge needle (-10 and 0 minute samples). Blood glucose concentration was determined in each sample immediately after withdrawal using a hand-held glucometer (OneTouch UltraMini, http://www.shoponetouch.com/product/35579). If blood glucose concentrations agreed to within 10% for the two samples, the horse was administered 50 mIU/kg of body weight (BW) of
recombinant human insulin (Sigma Chem Co., St. Louis, MO, USA) in sterile saline intravenously (IV). If the samples varied more than 10%, subsequent samples were drawn to establish a stable baseline before proceeding. Blood samples collected at 45 and 60 minutes after insulin injection were assessed for glucose concentration in the same manner, and the reduction in blood glucose (expressed as a percent decrease from mean pre-insulin samples) was calculated [4.9]. The greatest percent decrease (either at 45 or 60 minutes) in blood glucose concentration was recorded and used as an index of insulin sensitivity. Horses were deemed as sensitive if the percent decrease in blood glucose was 50% or higher, while insensitive animals had decreases of <30%. Horses whose percent decrease in blood glucose was between 30 and 50% were deemed intermediate and were not included in these experiments.

4.3.2 Glucose Challenge (IR2G) Protocol

Similar to the insulin challenge, the night before a glucose challenge, horses were brought in from pasture and feed-deprived overnight in stalls; water was available at all times. Early the next morning, a 14-gauge catheter was aseptically placed into the left jugular vein 1 hour before glucose administration. Baseline venous blood samples were obtained at -10 and 0 minutes prior to injecting a bolus of glucose (50% aqueous solution; Durvet Inc., Blue Springs, MO, USA). Glucose was infused at a dose of 100 mg/kg BW over a period of approximately 1 minute. Subsequent blood samples were drawn at 5, 10, 15, 20, 25, 30, 45 and 60 minutes relative to the start of glucose infusion; the catheter was flushed with sodium citrate solution (6% in saline) between each sample. A small aliquot of blood was aspirated and discarded prior to obtaining each blood sample to avoid possible dilution with the citrate solution. Approximately 6 mL of blood was drawn and placed into evacuated tubes containing 12.15 mg of EDTA as an anticoagulant (Vacutainer, B-D, Franklin Lakes, NJ, USA). Tubes were placed immediately on
ice and centrifuged within 20 minutes of blood collection in a refrigerated centrifuge at 4°C for 15 minutes at 1200 x g. Plasma was transferred to polypropylene tubes and frozen at -20°C until later analysis.

4.3.3 Experiments

4.3.3.1 Experiment 1. Short-term Sulpiride Treatment. Ten quarter horse mares were used. Five were known to be insulin resistant and five were insulin-sensitive. They ranged in age from 9 to 21 years, weighed 422 to 612 kg, and had body condition scores (BCS; [4.10]) from 5 to 8. Mares were chosen so that each mare within each insulin sensitivity group was matched by another mare of similar age, weight and BCS. Afterwards, matched mares were randomly assigned as treatment or control. The experiment was conducted on June 16 and 25, 2014. In order to assess the immediate effects of sulpiride on insulin secretion, a modified version of the glucose challenge was done. As previously described, the mares were deprived of feed overnight. The following morning, they were fitted with a 14-gauge jugular catheter and allowed to rest for 1 hour before the experiment was started. Baseline blood samples were collected (at -10 and 0 minutes) followed immediately by administration of sulpiride (.01 mg/kg BW dissolved in physiological saline) or vehicle (saline). Five minutes after treatment, a blood sample was collected followed immediately by onset of the IR2G protocol (glucose infusion). Blood samples were collected as previously mentioned for glucose challenges.

One week later, treatment groups were switched, and the process was repeated so that each mare served as her own control.

For hormonal analysis at a later date, all frozen samples were thawed and analyzed for prolactin and insulin. Prolactin concentration was assessed by radioimmunoassay previously validated for horse plasma [4.11]. Insulin concentrations in blood samples from the glucose
challenges were measured by radioimmunoassay using commercially available kit reagents (MP BioMedicals, Santa Ana, CA, USA) modified for use with horse samples [4.12]. Estimates of the limit of detection of the assays and the intra-assay coefficient of variation were 0.2 ng/mL and 8% for prolactin and 2.5 mIU/L and 5% for insulin. Multiple assays were needed for all prolactin samples, and the interassay coefficient of variation averaged 9%.

Plasma prolactin and insulin data were analyzed by analysis of variance (ANOVA) using the general linear model in SAS (SAS 9.4, SAS Institute, Cary, NC, USA). Treatment effects and the treatment x sampling time interaction were assessed in a switch-back design with repeated measures (repeated sampling times) [4.13]. Main factors in the analyses included insulin status, treatment, and horse; day was ignored because all horses were treated on the same days. These latter effects were tested with the horse x status x treatment interaction (equivalent to a replicated 2 x 2 Latin square). Sampling time and its interaction with treatment were tested with residual error. The significance of differences between insulin sensitivity groups over time was assessed by the least significant difference test [4.13].

4.3.3.2 Experiment 2. Long-term Sulpiride Treatment. The assessments of GR2I and IR2G were superimposed on a long-term experiment originally testing whether continuous sulpiride treatment would mimic the underlying cause of PPID in horses (reduced dopaminergic input to the intermediate lobe of the adenohypophysis [4.14,4.15]). Twelve light horse geldings ranging in age from 8 to 21 years, weighing 422 to 569 kg, and with BCS from 5 to 7 were used. The experiment was conducted from April 15 to June 4, 2014. An insulin challenge was conducted prior to initiation of treatment (March 16-17, 2014) in order to select six insulin-sensitive and six insulin-insensitive geldings from the main herd. Three geldings from each sensitivity group were selected as treated and three as control and were allotted (four groups total.
in a 2 x 2 factorial) so that age, weight, BCS, and glucose response to insulin were as equally distributed among groups as possible. Assignment of the groups to treatments was then performed by a random drawing.

Treated animals received intramuscular (IM) injections of 1.5 g of sulpiride (racemic mixture; provided by R.M. Gilley, Birmingham, AL, USA) in a slow-release vehicle (a proprietary mixture of oily liquids) every 5 days for 45 days starting on day 0 for a total of 10 injections; controls received just the vehicle. Five challenges with thyrotropin releasing hormone (TRH; Sigma) of all geldings were performed regularly over the 45-day experimental period to monitor the antidopaminergic activity of the sulpiride injections. In each case, geldings were calmly brought in from pasture in the morning, lightly tethered to an outdoor chute area and administered TRH at .002 mg/kg BW (in saline intravenously, IV); jugular blood samples were collected immediately before (time 0) TRH injections, and then at 5, 10, and 20 minutes after injection. Post treatment IR2G and GR2I were performed on days 49 and 50, respectively.

Plasma preparation and storage as well as hormonal assays were performed as described for Experiment 1. Plasma prolactin and insulin data were analyzed by repeated measures ANOVA with a 2 x 2 factorial arrangement of the main effects. Factors in the main analyses included insulin status, treatment, and the interaction, all tested by the horse within (status x treatment) term. Times of sampling and all its interactions were tested with residual error. Decreases in blood glucose concentrations were analyzed by ANOVA with a 2 x 2 factorial arrangement of treatments [4.13] with repeated sampling (pre- versus post-treatment). The significance of differences between groups over time was assessed by the least significant difference test [4.13].
4.3.3.3 Experiment 3. Short-term Cabergoline Treatment. Sixteen adult, light horse breed mares with long-term histories of being either insulin-sensitive (n = 8) or insulin-insensitive (n = 8) were used. Their ages ranged from 4 to 26 years, weights from 418 to 598 kg, and BCS from 5 to 7. The experiment was conducted in May of 2016.

Mares from each sensitivity group were assigned to either treatment or control, such that age, weight, and BCS were equally distributed among groups. The groups were then randomly assigned as treated or controls. Due to lack of stalls for overnight feed restriction, mares were evenly separated into two groups of 8, so that both treatments were equally represented in each group of 8, and the experiment was staggered by one day for each group. Due to weather and personnel scheduling complications, pretreatment GR2I and IR2G were performed one month prior to onset of treatment. Although this gap was longer than ideal, both tests confirmed the previous assessments for these mares.

On the first day of treatment (day 0), mares were brought in from the pasture, given a 1 mL injection IM of either 5 mg of cabergoline in vegetable oil (Crisco®) or oil alone. They were returned to pasture until the following day (day 1), when the GR2I was performed. They again returned to pasture until day 3 when the IR2G was performed. Two time points, 45 and 60 minutes post glucose infusion, were omitted from the protocol of this experiment, because, in our experience, the insulin concentration remains relatively stable and does not decrease significantly from 30 to 45 or to 60 minutes after glucose infusion.

Plasma preparation and storage as well as hormonal assays were performed as described for Experiments 1 and 2. Statistical analyses were performed as described for Experiment 2.

4.3.3.4 Experiment 4. Long-term Cabergoline Treatment. Like in Experiment 2, the assessment of IR2G in Experiment 4 was superimposed on a long-term experiment originally
testing the effects of cabergoline treatment on reproductive characteristics in seasonally transitional mares [4.16]. To minimize perturbation of that experiment, only the IR2G (and not a GR2I) was performed.

In the second year of that experiment (it was performed over 2 years), eight insulin-sensitive, light horse breed mares were randomly allotted to either treatment or control. Treated mares received 5 mg injections of cabergoline (dissolved in 1 mL of slow-release proprietary vehicle) and control mares just vehicle IM every 10 days for 60 days starting on day 0 (April 6, 2014) for a total of 7 injections. On day 49 (May 24), the mares were brought in from pasture and feed deprived overnight. The following morning, 10 minutes after the 6th injection of cabergoline or vehicle was given, the IR2G began. Blood samples were collected as previously described, plus samples at 45 and 60 min post infusion.

4.4 Results

4.4.1 Experiment 1. Short-term Sulpiride Treatment

Mean plasma prolactin concentrations in all mares treated with sulpiride or vehicle five minutes before an infusion of glucose are presented in Fig. 4.1. Sulpiride treatment increased (P < .0001) prolactin concentrations within five minutes, and they remained elevated for the entire sampling period. There was no effect of insulin sensitivity status on prolactin concentrations.

Glucose infusion increased (P < .001) plasma insulin concentrations in all horses (Fig. 4.2). Insulin concentrations in insulin insensitive mares were higher (P = .0037) over all sampling periods, and there was an interaction between sensitivity status and sampling times (P < .0001).
Fig. 4.1. Mean plasma prolactin concentrations in mares after vehicle injection (control) or sulpiride (0.1 mg/kg of body weight) injection five minutes before infusion of glucose in Experiment 1. The experiment was performed as a single switchback, and half (n = 5) of the mares were insulin-sensitive and half were insulin-insensitive. There was no effect (P > .1) of insulin sensitivity status nor interaction of status and treatment on prolactin concentrations. Asterisks joined by bar indicate differences (P < .05) between sulpiride-treated and control means. Pooled standard error of the means was 14.6 ng/mL.

Fig. 4.2. Mean plasma insulin concentrations in mares after vehicle injection (control) or sulpiride (0.1 mg/kg BW) injection five minutes before infusion of glucose. The experiment was performed as a single switchback, and half (n = 5) of the mares were insulin-sensitive and half (n = 5) were insulin-insensitive. Glucose was infused intravenously at 100 mg/kg of BW starting at the 5 minute time point. There was an effect of time (P < .001) in the analysis of variance as well as an effect of insulin sensitivity status (P = .0037). There was no effect (P > .1) of sulpiride treatment nor any interaction with other factors. Pooled standard of the means was 7.1 mIU/mL.
Both pre-glucose concentrations as well as the insulin response to infused glucose were greater in insensitive mares. There was no effect (P > .1) of sulpiride treatment or any interaction with other factors on insulin concentrations.

4.4.2 Experiment 2. Long-term Sulpiride Treatment

During the course of Experiment 2, two horses in the insulin insensitive treated group had to be euthanized on days 16 and 30 of treatment due to sudden onset of severe laminitis. Their data are not included in these analyses.

Mean plasma prolactin concentrations in control and sulpiride-treated geldings are presented in Fig. 4.3. On the day of IR2G, prolactin concentrations were higher (P < .001) in treated versus control geldings. In addition, averaged over the five TRH injections throughout the experiment (to provide an indication as to the long-term effects of treatment), the prolactin response to TRH was greater (P = .0033) in sulpiride-treated than in control geldings. Insulin sensitivity status did not affect prolactin concentrations in either case.

The percent decrease in blood glucose concentrations in response to 50 mIU/kg BW insulin (Fig. 4.4) was affected (P < .0001) by previously determined insulin sensitivity status. The percent decrease was greatest for insulin-sensitive geldings (60% versus 19% for insensitive geldings). There was also an interaction (P = .028) between status and the pre- versus post challenges: insulin-sensitive geldings had a decrease in response from pre- to post-treatment challenges (66 to 54%), whereas insulin-insensitive geldings had a slight increase (16 to 23%). There was no effect (P > .1) of sulpiride treatment or interaction of treatment with the other factors in the ANOVA.

Similar results were observed for the insulin response to glucose infusion (Fig. 4.5). Insulin insensitive geldings had greater (P = .003) overall insulin concentrations than sensitive
Fig. 4.3. **A:** Mean plasma prolactin concentrations in vehicle-treated (control) and long-term sulpiride-treated (sulpiride) geldings on day 50 of treatment in Experiment 2. Concentrations were higher ($P < .001$) in sulpiride-treated geldings relative to controls. Pooled standard error of the means (SEM) was 2.9 ng/mL. **B:** Mean plasma prolactin concentrations in vehicle-treated (Control) and long-term sulpiride-treated (Sulpiride) geldings after intravenous injection of thyrotropin releasing hormone (TRH) at time 0. Five TRH challenges were performed over the 45-day treatment period; the overall means are presented here to confirm the antidopaminergic activity of the sulpiride treatment. There was a treatment x minute interaction ($P = .0033$) in the analysis of variance. Asterisks indicate differences between groups for the designated sampling times. Pooled SEM was 10.1 ng/mL.

geldings, and a greater insulin response to infused glucose. The insulin response to glucose was unaltered ($P > .1$) by sulpiride treatment, and there was no interaction of treatment with other factors.

### 4.4.3 Experiment 3. Short-term Cabergoline Treatment

Mean plasma prolactin concentrations in control and cabergoline-treated mares before treatment and again during the glucose infusions on day 3 are presented in Fig. 4.6. Cabergoline suppressed ($P < .001$) prolactin concentrations from pretreatment concentrations by approximately 82%. Insulin sensitivity status did not affect prolactin concentrations.

The percent decrease in blood glucose concentrations in response to insulin (Fig. 4.7) was affected ($P < .0001$) by previously determined insulin sensitivity status (sensitive vs. insensitive).
Fig. 4.4. Mean percent decrease in blood glucose concentrations after injection of recombinant human insulin at 50 mIU/kg BW in geldings before the start of Experiment 2 (Pre; March 16-17) and then 49 days after the onset of treatment (Post; June 3) with vehicle (Con) or sulpiride (Sulp). Treatments were administered every 5 days with vehicle or 1.5 g of sulpiride in a slow-release vehicle. Half of the geldings in each treatment group were insulin-sensitive at the beginning of the experiment, and half were insulin-insensitive. Percent decrease in blood glucose was calculated relative to average pre-injection concentration using either the 45 or 60 min samples (whichever was the greatest drop). There was an effect of starting insulin sensitivity status (P < .001) in the analysis of variance and an interaction between sensitivity status and date of challenge (P = .028). There was no effect of sulpiride treatment nor any interaction of treatment with sensitivity status (P > .1) in the analysis of variance. Pooled standard error of the means was 4.3%.

Sensitive mares displayed an approximate 60% decrease compared to 25% for insensitive mares. There was also an effect of pre- versus post challenges (P = .0062), in which all post-treatment decreases were consistently lower than pretreatment decreases. There was no effect (P > .1) of cabergoline treatment on glucose response to insulin injection, nor any interaction of cabergoline treatment with other factors.

Similarly, the insulin response to glucose infusion (Fig. 8) was affected by insulin sensitivity status (P = .0002), and there was an interaction of status with sampling time (P < .0001). Insulin insensitive mares had higher plasma insulin concentrations before glucose
Fig. 4.5. Mean plasma insulin concentrations in insulin-sensitive and insensitive geldings that had been treated every 5 days with vehicle (Con) or 1.5 g of sulpiride (Sulp) in a slow-release vehicle for 45 days. Half of the geldings in each treatment group were insulin-sensitive at the beginning of the experiment, and half were insulin-insensitive. Glucose was infused intravenously at 100 mg/kg BW starting at time 0. There was an effect of time (P < .001) in the analysis of variance as well as an effect of insulin sensitivity status (P = .003). There was no effect of sulpiride treatment nor any interaction with the other factors (P > .1). Pooled standard error of the means was 2.6 mIU/L.

infusion, and the insulin response after infusion was greater in insensitive mares than in sensitive mares. Again, there was no effect (P > .1) of cabergoline treatment nor any interaction of treatment with other factors.

4.4.4 Experiment 4. Long-term Cabergoline Treatment

Plasma prolactin concentrations were lower in cabergoline-treated mares in Experiment 4 on days 41, 43, and 45, but had recovered to control values by day 49 [4.16], immediately before the next cabergoline injection, which was 10 minutes before the glucose infusions started. This was the typical pattern of prolactin concentrations after each cabergoline injection in the spring of both years in that report: an immediate suppression for the first 4 or 5 days, followed by a gradual recovery until the subsequent injection. An indication of the long-term effects of the
The most common type of insulin dysregulation observed in horses is compensated insulin resistance [4.17,4.18]. It is referred to as "compensated" because the resistance to the
Fig. 4.7. Mean percent decrease in blood glucose concentrations after injection of recombinant human insulin at 50 mIU/kg BW in mares before the start of Experiment 3 (Pre) and 1 day after treatment with 5 mg of cabergoline or vehicle (Post). Half of the mares in each treatment group were insulin-sensitive at the beginning of the experiment, and half were insulin-insensitive. Percent decrease in blood glucose was calculated relative to average pre-injection concentration using either the 45 or 60 min samples (whichever was the greatest drop). There was an effect of starting insulin sensitivity status (P < .001) in the analysis of variance as well as an effect of day of glucose infusion (Pre versus Post; P = .006). There was no effect of cabergoline treatment nor any interaction of treatment with sensitivity status (P > .1). Pooled standard error of the means was 4.2%.

Action of insulin at the tissue level (primarily muscle) is made up for by hypersecretion of insulin from the pancreas, thereby keeping blood glucose concentrations at or near normal. Thus, horses with compensated insulin resistance typically have blood glucose concentrations within normal limits, have higher than normal resting plasma insulin concentrations, and have an exaggerated insulin response to glucose infusion [4.10,4.19]. These characteristics are exactly what we have observed in the four experiments presented herein before administration of any dopaminergic or antidopaminergic agent. The direct assessment of insulin sensitivity via recombinant human insulin injection at a standardized dose in Experiments 2 and 3 confirmed that the placement of horses into categories (insulin-sensitive versus insensitive) was appropriate. Moreover, the fact
Fig. 4.8. A: Mean plasma insulin concentrations before and after intravenous infusion of glucose (100 mg/kg of body weight) at time 0 in insulin-sensitive and insensitive mares in Experiment 3 before treatments were initiated. B: Mean plasma insulin concentrations before and after intravenous infusion of glucose (100 mg/kg of body weight) at time 0 in insulin-sensitive and insensitive mares in Experiment 3 on day 3 after treatment with 5 mg of cabergoline (Cab) or vehicle (Con). There was an effect of insulin sensitivity status (P < .001) in the analysis of variance, as well as an interaction (P < .001) between status and sampling times. There was no effect (P > .1) of cabergoline treatment and no interaction of treatment with other factors. Pooled standard error of the means was 6.4 mIU/L.

that horses treated with vehicle (controls) in each experiment displayed the same relative degree of insulin sensitivity before and after treatments supports the notion that the degree of decrease in blood glucose concentrations to injected insulin does indeed reflect a given horse's insulin sensitivity status (that is, the assessments are repeatable). As we have reported previously [4.9], this status has been found to be relatively consistent year after year in most horses.

Plasma prolactin concentrations were used in these experiments to confirm the dopaminergic and antidopaminergic activities of the cabergoline and sulpiride injections. We have reported on several occasions [4.4,4.8,4.16] that cabergoline is a potent dopaminergic agonist that suppresses prolactin secretion for at least 5 days, and we [4.7,4.8,4.20] and others [4.6,4.21] have reported the stimulatory, antidopaminergic activity of sulpiride in horses. In each experiment, the expected action of the selected agent on prolactin secretion was in fact observed.
Fig. 4.9. Mean insulin concentrations in control mares and in mares that had been treated every 10 days with 5 mg of cabergoline for 50 days (total of 6 injections); the 6th injection was administered 10 min before the start of infusion of glucose (time 0). Glucose was infused intravenously at 100 mg/kg BW. There was an effect of time (P < .05) in the analysis of variance but no effect of treatment nor any treatment by time interaction (P > .1). Pooled standard error of the means was 19.4 mIU/L.

The GR2I as described by Caltabilota et al. [4.9] is a direct assessment of the whole-body blood glucose response to a standard injection of insulin and seems to be applicable to detecting compensated insulin resistance, as in the horses in these experiments, as well as in short-term induced resistance to catecholamine, e.g., in response to epinephrine injection [4.22]. We have not tested the procedure after nonesterified fatty acid infusion, which Sessions et al. [4.23] reported causes immediate insulin resistance in mares, nor on the longer-term induced resistance displayed by horses treated with recombinant equine somatotropin [4.24,4.25]. We have applied the test to horses treated with dexamethasone [4.26], as dexamethasone is known to induce an acute state of compensated insulin resistance in horses [4.27]. In that experiment, the geldings became so resistant after 20 days of treatment that the highest dose of recombinant human insulin for the GR2I we were willing to use (125 mIU/kg of BW) had little effect on blood
glucose concentrations. Six of seven treated horses had negative-sloped dose-response curves that made calculation of an effective dose causing a 50% decrease in blood glucose (ED50) impossible; the seventh horse had an ED50 of 965 mU/kg of BW (sensitive horses average around 50 mU/kg BW or less). All the other dexamethasone-treated horses would have registered well into the thousands.

The majority of reports on the interaction of dopaminergic systems and glucose metabolism involve dopaminergic effects on insulin secretion from the pancreas. Evidence in rodents [4.5] as well as humans [4.28] indicate that dopamine suppresses, or moderates, insulin secretion from beta cells, in spite of a lack of innervation by dopaminergic fibers [4.26]. Although modest, the inhibition of insulin secretion from the pancreas in humans by bromocriptine has been shown to be consistent, and as a result, bromocriptine has been approved as a treatment for type 2 diabetes to improve glycemic control [4.29]. As reported herein as well as previously [4.3,4.4], the horse does not appear to respond to dopaminergic activity (cabergoline) in the same manner.

Disrupting the ability of beta cells to respond to dopamine (receptor knockout model) in rodents results in glucose intolerance [4.30], as does perturbation of the receptors with antidopaminergic drug treatment [4.5]. Based on those reports, treatment with sulpiride in Experiments 1 and 2 would be expected to result in an exaggerated insulin response to glucose infusion. Again, the lack of response to both short-term and long-term sulpiride treatment in the experiments herein indicate that the horse differs from those species in previous reports.

Although other factors (incretins) are gradually being recognized as influencing insulin action, particularly after a meal [4.31], their role in the procedures used herein are likely minimal. That is, incretins are thought to be released from the gut during absorption of digested
feedstuffs [4.32], whereas the IV infusion of glucose alone would not involve gut action per se 
(although glucose effects on the gut could be possible). Similarly, with direct action of 
exogenous insulin in feed deprived horses, as performed in Experiments 2 and 3, the 
involvement of incretins would be expected to be minimal or absent.

Although no direct evidence has been reported to show that dopaminergic drugs 
ameliorate insulin resistance in horses, there is a perception in the horse industry that such drugs 
can be used as such. In a survey of horse owners and veterinarians nationwide [4.1], it was 
apparent that many respondents confused insulin resistance, often involved with the metabolic 
syndrome in horses [4.17], with PPID. Moreover, 61% of respondents mentioned the 
dopaminergic agonist pergolide (sold commercially as Prascend®) as a treatment for insulin 
dysregulation. Unfortunately, correction of this fallacy throughout the industry will likely take 
much longer to occur than it took to be disseminated.

In conclusion, the four experiments reported herein confirm the observation of Donaldson 
et al [4.3] and Arana Valencia et al [4.4] that dopaminergic drugs do not alter the indices of 
insulin sensitivity in horses. We have extended those observations to include the fact that anti-
dopaminergic activity also has no effect on insulin response to glucose or the glucose response to 
insulin.

4.6 References

of equine insulin dysregulation and available treatments in southeastern United States. J Equine 

[4.2] Rohrbach BW, Stafford JR, Clermont RSW, Reed SM, Schott HC, Andrews FM. 
Diagnostic frequency, response to therapy, and long-term prognosis among horses and ponies 


5.1 Summary

The following four experiments assessed factors affecting prolactin responses to sulpiride administration in horses. Experiment 1 compared the efficacy of the (-) enantiomer of sulpiride to that of the commonly used (+/-) racemic mixture. Mares were used in an 8x8 Latin square to compare the prolactin responses to four doses of levosulpiride to four corresponding doses of the racemic mixture at twice the dose. Responses at each dose indicated equal and similar (P > .1) responses. Experiment 2 compared the efficacy of 1 gram of orally administered racemic sulpiride to 100 mg of intramuscularly injected sulpiride in oil in mares primed with 50 mg of estradiol cypionate (ECP). Prolactin responses in groups receiving sulpiride were robust but similar in magnitude with minor differences in timing. In Experiment 3, ECP-primed geldings received subcutaneous injections of 1.8 grams racemic sulpiride in vegetable shortening in one of three sites: the neck, the back below the withers, or the lower girth region; control geldings received no sulpiride. Prolactin responses to sulpiride lasted a minimum of 96 hours. In Experiment 4, prolactin responses to 3 g of racemic sulpiride in vegetable shortening were compared to similar injections (3 g) in 5 mL of sucrose acetate isobutyrate (SAIB; SucroMate) or just SAIB (control) in ECP-primed geldings. Controls had no prolactin response to SucroMate, whereas both treatment groups had extended prolactin responses lasting at least 10 days. It is concluded that prolactin responses to sulpiride in horses can be greatly extended by using hydrophobic vehicles like vegetable shortening or SAIB.

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5.2 Introduction

Antidopaminergic drugs are commonly used in the horse industry for problems such as fescue toxicosis (domperidone [5.1]) and induction of ovulation in seasonally anovulatory mares (sulpiride [5.2] and domperidone [5.3]). Some of these treatments have gone on to be commercially available for oral and injection administration, however, their vehicles are proprietary to the formulating company. Sulpiride, a dopamine antagonist, was first reported as a secretagogue for prolactin in horses by Johnson and Becker [5.4], who used the racemic mixture ([+/-]-sulpiride) dissolved in saline and administered intramuscularly (IM). Colborn et al [5.5] administered 500 mg of sulpiride subcutaneously (SC) to stallions in winter in 2 mL of vegetable shortening, which was described as soft but solid at body temperature. Prolactin concentrations peaked in treated animals within 4 days and remained elevated for the duration of the experiment (13 days). In addition, Arana Valencia et al [5.6] reported the use of a propriety mixture of oily liquids for the long-term administration of sulpiride to geldings. In that study, injections were given every five days as the vehicle was found to prolong the effect of sulpiride on prolactin for approximately five days. Although the changes in plasma prolactin concentrations over time to a single injection of sulpiride have been well documented for IM administration in saline [5.4] or oil [5.7] and IV injection in saline [5.8,5.9], the time course of prolactin secretion for the vehicles designed for slower release of sulpiride has not.

The purpose of the experiments described herein was to compare various factors that affect the prolactin response to sulpiride, including enantiomer composition, oral administration, and various vehicle formulations, with the goal of a single-injection protocol with high efficacy and an extended period of stimulation on plasma prolactin concentrations to aid in equine reproductive research. Four experiments were performed to study (1) the relative activity of
racemic mixture of sulpiride ([+/-] sulpiride) to the (-) enantiomer (levosulpiride), (2) the relative efficacy of sulpiride administered orally to that injected IM in oil, (3) the effect of site of injection for sulpiride injected SQ in vegetable shortening, and (4) the efficacy of sulpiride injected IM in SucroMate (a commercially available suspension of deslorelin acetate in sucrose acetate isobutyrate [SAIB]) compared to injection in vegetable shortening. SucroMate was used for its SAIB vehicle because medical grade SAIB was not readily available for research at the time this research was performed.

5.3 Materials and Methods

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

5.3.1 Animals

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

5.3.2 Sample Collection and Hormone Analysis

Throughout all experiments, blood sampling was performed via jugular venipuncture with 21-gauge needles into 10-mL evacuated glass tubes containing 143 USP units of sodium
heparin (Becton, Dickinson & Co., Franklin Lakes, NJ, USA). Samples were routinely placed on ice until centrifugation at 1,200 x g for 10 minutes at 5°C. Plasma was harvested and stored frozen (-15°C) until the completion of a given experiment. Plasma prolactin concentrations were assayed in all samples; luteinizing hormone (LH) and follicle stimulating hormone (FSH) were also measured in Experiment 4. All hormones were measured by radioimmunoassay as described previously (prolactin [5.10], LH [5.11], and FSH [5.12]). Inter- and intra-assay coefficients of variation and limits of sensitivity were 7%, 12%, and 0.2 ng/mL for prolactin, 6%, 9%, and 0.2 ng/mL for LH, and 7%, 11%, and 1.4 ng/mL for FSH.

5.3.3 Experiments

5.3.3.1 Experiment 1. Comparison of Levosulpiride to the Racemic Mixture. Eight mature, light horse mares were used in a 8 x 8 Latin square design to test four doses each of levosulpiride (0.5, 1.25, 3.25, and 7.8 µg/kg of body weight) and the racemic mixture (1.0, 2.5, 6.25, and 15.6 µg/kg of BW) (both products purchased from Sigma Chemical Co., St. Louis, MO, USA). Doses for the racemic mixture should contain equal amount of both positive and negative enantiomers; thus, levosulpiride doses were exactly half of the racemic mixture. The experiment was carried out on 8 separate days starting on February 12, 2014 and ending March 2, 2014; there was at least 1 day of no treatment separating days of treatment. On each day of treatment, the mares were brought in from pasture in the morning and held in a dry lot for at least 1 hour; treatments were started around 1000 hours. For treatment, each mare was loosely tethered inside a large shed and a 5-mL sample of jugular blood (time 0) was drawn. Her assigned treatment for that day (in 3 mL of saline) was then administered IV via the left jugular vein, and post-injection blood samples were collected at 10, 20, and 30 minutes after injection.
5.3.3.2 Experiment 2. Oral Administration versus IM Administration. Fifteen light horse mares were used in the fall of 2010. On November 1, all mares were administered a single IM injection of 50 mg of estradiol cypionate (ECP; BET Pharm BioRelease Estradiol Cypionate, KY, USA; www.betpharm.com). On November 6, mares were brought in from pasture at 1600 hours and held overnight in an outdoor pen without access to feed but with *ad libitum* access to water. The following morning at 0700 hours, the mares were tethered loosely in sheltered pens for blood sampling and treatment. Blood samples were drawn at approximately 0800 hours and again 30 and 60 minutes later. Immediately after the last sample was drawn, 5 mares each were administered 1 of 3 treatments: (1) 100 mg of sulpiride (racemic mixture) in 5 mL of vegetable oil injected IM in the neck [5.7], (2) 1 g of sulpiride (racemic mixture; 10x dose of sulpiride in vegetable oil) in molasses fed as a top dressing on 0.5 kg of a commercially available sweet feed (Crossroads Feeds All Stock, Purina Animal Nutrition LLC, Shoreview, MN, USA), or (3) controls (no sulpiride). For each treatment, mares also received the appropriate placebo injection (2 mL of vegetable oil) and feeding (feed plus molasses but no sulpiride). Feed was offered in individual buckets, and all mares consumed the feed within the first 5 minutes. Post-treatment blood samples were drawn from each mare at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours.

5.3.3.3 Experiment 3. Effect of Site of Injection of 1.8 g Sulpiride in Vegetable Shortening. A preliminary trial comparing the prolactin responses to an injection of 1.5 g of sulpiride in canola oil (Crisco brand; J.M. Smucker Co., Orrville, OH, USA) either IM or SQ to a similar injection in vegetable shortening (Crisco; J.M. Smucker Co.) in ECP-treated geldings indicated that the shortening vehicle resulted in a longer period of elevated prolactin concentrations than did either oil injection. Experiment 3 was performed as a result of that preliminary data.
Fifteen light horse, long-term geldings were used in the fall of 2014. They were allotted to the treatment groups described below such that average age, weight, and BCS were similar in the groups. All geldings were administered 50 mg of ECP on November 4 as an IM injection in the neck. Six days later (November 10), 12 of the geldings were administered 1.8 g of sulpiride (saturating dose of racemic mixture) in vegetable shortening as a 5-mL SQ injection; three controls received shortening only in the same manner (in the neck area). Of the 12 geldings, three groups of four received their sulpiride injections in (1) the neck region (triangle area) usually used for injections, (2) in the back, behind the rear border of the withers, and about 6 inches down the side, and (3) in the thoracic area behind the elbow, about 6 inches up the side (Fig. 5.1).

Fig. 5.1. Sites of subcutaneous injection of sulpiride in vegetable shortening in Experiment 3.
For the three injection sites, the neck region served as the “usual” given that it is the site of most (at least many) IM or SQ injections given to horses. The withers and girth areas were chosen due to their potentially lower blood perfusion in the skin of those areas. This was determined from a generic thermograph of a resting horse, and it was assumed that cooler areas had less blood flow than warmer areas. Blood samples were collected at 10 and 0 minutes before the sulpiride/shortening injections, and again at 0.25, 0.5, 1, 3, 6, 12, 24, 36, 48, 60, 72, 84, and 96 hours after injections.

Given the results of the first phase of this experiment, a second phase was performed to compare two doses of sulpiride, 1.5 and 3.0 g, to determine if the greater dose would extend the prolactin response further. All injections were given in the girth area. Twelve of the 15 geldings used in the first phase were used; two control geldings were kept in that group, whereas 10 of the previously treated geldings were allotted to the two groups to receive sulpiride (n = 5 per group) such that previous prolactin responses (magnitudes) were approximately equalized in the groups.

All geldings received 50 mg of ECP on December 2, as described in the first phase. Sulpiride/vehicle injections were administered on December 8. Five geldings received 1.5 g of sulpiride in 5 mL of shortening SQ and five geldings received 3.0 g of sulpiride in the same manner; two controls received vehicle only. Blood sampling times were the same as in the first phase, except that additional samples were drawn at 108, 120, 132, 144, 156, and 168 hours after injection (through the morning of day 7).

5.3.3.4 Experiment 4. Comparison of SAIB to Vegetable Shortening as a Vehicle.

Twelve light horse geldings were used in a single switchback design in the late summer of 2016. On August 10, all geldings were administered 50 mg of ECP as an IM injection in the neck. Four days later (day 0), they were treated in three groups (n = 4 each): (1) controls, administered
5 mL of SAIB as SucroMate (Thorn BioScience, Louisville, KY, USA) IM, (2) 3 g of sulpiride mixed with 5 mL of SucroMate, administered IM, and 3) 3 g of sulpiride mixed with 5 mL of vegetable shortening, administered SQ. Samples of jugular blood were drawn from each gelding at -1, 0, 1, 3, 6, and 12 hours relative to injection, and then again daily in the mornings through day 14.

After a two-week washout period, the geldings were reassigned to treatments such that no gelding received the same treatment as in the previous phase. Administration of ECP and treatments were the same as for the first phase, as was the sample collection days and times. Due to the presence of deslorelin in the SucroMate formulation, samples were assayed for LH and FSH as well as prolactin in this experiment.

5.3.4 Statistical Analyses

Data from all experiments were analyzed by analysis of variance (ANOVA) using the general linear model of SAS (SAS Institute, Cary NC, USA). The design for each experiment is described in the following paragraphs. Subsequent preplanned comparisons of means were performed using the least significant difference (LSD) test [5.13] based on the residual mean square error from each ANOVA and a two-tailed t-critical value for $\alpha = 0.05$.

Prolactin data in Experiment 1 were used to calculate areas under each response curve, and the areas were then analyzed in an 8 x 8 Latin square design ANOVA with repeated measures. Area calculation involved first subtracting the pre-injection concentration from the subsequent values, and then summing the three residual increments and multiplying by 0.167 to get areas with the units ng $\cdot$ mL$^{-1} \cdot$ hour. Mean area for each dose of levosulpiride was compared to the corresponding dose of the racemic mixture (e.g., lowest versus lowest, highest versus highest, etc.) using the SLICE = dose option in the LSMEANS statement.
Prolactin data in Experiment 2 were analyzed in a completely random design ANOVA with repeated measures. Due to the large responses in prolactin concentrations in the two groups administered sulpiride relative to the controls, data from the treated groups were analyzed separately (without the controls) in a second ANOVA and the LSD value was applied across each time of sampling to determine differences in response.

Prolactin data in each of the two phases of Experiment 3 and the data from Experiment 4 were analyzed in the manner described previously for Experiment 2. In all three cases, the low, constant prolactin concentrations of controls were not included in the final ANOVA so that a more accurate comparison of the treated groups could be obtained.

5.4 Results

5.4.1 Experiment 1

Mean plasma prolactin concentrations and areas under the curve in response to the four doses of levosulpiride and the racemic mixture are presented in Fig. 5.2; only the areas were analyzed statistically. There was an increase (P < .05) in prolactin response for each increase in dosage for each treatment. Preplanned comparisons between the lowest doses (levosulpiride versus racemic mixture at twice the mass) and each of the other three paired comparisons indicated that there was no difference (P > .1) between the responses at any dose.

5.4.2 Experiment 2

Mean plasma prolactin concentrations in control mares and mares administered sulpiride orally as opposed to IM are presented in Fig. 5.3. The prolactin response to sham injection and feeding (without sulpiride; controls) resulted in little change in plasma prolactin concentrations relative to when sulpiride was administered. Injection of 100 mg of sulpiride resulted in a rapid (15 minute) rise in plasma prolactin concentrations that peaked at 30 minutes after injection.
Feeding of 1 g of sulpiride resulted in a slower rise in plasma prolactin, and the groups differed (P < .05) at 15 and 30 minutes after injection. Prolactin concentrations peaked in mares fed sulpiride at 60 minutes after feeding and were higher (P < .05) than those in injected mares at this time. The stimulated prolactin concentrations in both groups receiving sulpiride remained higher (P < .05) than those in control mares through 6 hours after administration.
Fig. 5.3. Mean plasma prolactin concentrations over time in control mares and in mares either fed 1.0 grams of sulpiride (racemic mixture) or injected intramuscularly with 100 mg of sulpiride in vegetable oil at time 0 in Experiment 2. The least significant difference value calculated from the ANOVA of the two treatment groups only (excluding controls) is shown; the pooled standard error of the means was 29 ng/mL.

5.4.3 Experiment 3

Prolactin concentrations in control geldings averaged 1.3 ng/mL and ranged between 0.9 and 2.3 ng/mL over the 96 hours (Fig. 5.4). In the first phase, there were immediate surges in prolactin concentrations in all sulpiride-treated geldings, with a primary (early) peak occurring at 0.5 to 3 hours after injection \( (P < .05) \). The response was biphasic, with concentrations falling at 6 to 12 hours, and then increasing again from 12 to 96 hours. There was an overall treatment effect \( (P = .076) \) in the ANOVA, with all groups being greater than the controls. A separate ANOVA without the controls indicated that differences due to site of injection occurred only at
the 1 (P = .026), 3 (P = .048), and 96-hour (P = .067) sampling periods. In general, prolactin concentrations were still on a linear trend upward at 96 hours in all treated groups.

Fig. 5.4. Mean plasma prolactin concentrations over time in control geldings and in geldings injected subcutaneously with 1.8 grams of sulpiride (racemic mixture) in vegetable shortening in the neck, withers, or girth region (see Fig. 5.3) in the first phase of Experiment 3. There was an effect of time (P < .001) in the ANOVA (performed with treatments only) but no effect (P > .1) of treatment or interaction of treatment with time. The least significant difference value calculated from the ANOVA is shown; the pooled standard error of the means was 9.6 ng/mL.

In the second phase, prolactin patterns were similar to those in the first phase, with an immediate surge early (P < .05) followed by a later second rise (Fig. 5.5). Prolactin concentrations were still elevated at 168 hours after sulpiride injection (P < .1). There was no difference (P > .1) between the prolactin responses of the groups receiving 1.5 versus 3 g of sulpiride.
Fig. 5.5. Mean plasma prolactin concentrations over time in control geldings and in geldings injected subcutaneously with either 1.5 or 3.0 grams of sulpiride (racemic mixture) in vegetable shortening in the girth region (see Fig. 5.3) in the second phase of Experiment 3. There was an effect of time (P < .001) in the ANOVA (performed with treatments only) but no effect (P > .1) of treatment or interaction of treatment with time. The least significant difference value calculated from the ANOVA is shown; the pooled standard error of the means was 8.9 ng/mL. ANOVA, analysis of variance; LSD, least significant difference.

5.4.4 Experiment 4

Mean plasma prolactin concentrations in control geldings administered 5 mL of SucroMate and in those administered 3 g of sulpiride in either SucroMate or vegetable shortening are presented in Fig. 5.6. Prolactin concentrations were low and constant in geldings receiving SucroMate alone, whereas both groups of geldings receiving sulpiride had the typical biphasic rise (P < .001) in prolactin concentrations starting within 3 hours after injection. Prolactin concentrations remained elevated (P < .05) in the geldings receiving SucroMate through the blood sampling at 240 hours after injection. Prolactin concentrations in geldings receiving sulpiride in vegetable shortening were also higher (P < .05) than controls over most of
the 10-day period but were generally lower (P < .1) than those in geldings receiving sulpiride in SucroMate.

Fig. 5.6. Mean plasma prolactin concentrations over time in control geldings and in geldings injected with 3 grams of sulpiride (racemic mixture) either in vegetable shortening or in SucroMate (sucrose acetate isobutyrate vehicle with added deslorelin) at time 0 in Experiment 4. Injections were given subcutaneously in the girth area. The experiment was performed as a single switchback of the treated geldings. There was an effect of time (P < .001) in the ANOVA (performed with treatments only) but no effect (P > .1) of treatment or interaction of treatment with time. The least significant difference value calculated from the ANOVA is shown; the pooled standard error of the means was 6.8 ng/mL.

Plasma concentrations of LH and FSH in the three treatment groups (phase 1 only) are presented in Fig. 5.7. Plasma concentrations of both gonadotropins increased (P < .01) within one hour after injection of SucroMate and peaked at 3 hours. Concentrations of LH decreased thereafter to lowest concentrations at 4 to 6 days, whereas concentrations of FSH were still on a downward trend at 10 days. Mean concentrations of neither LH and FSH differed between the
two groups receiving SucroMate (with and without sulpiride). Concentrations of both LH and FSH in geldings receiving sulpiride in vegetable shortening were relatively constant across the 10-day period.

Fig. 5.7. Mean plasma concentration of lutenizing hormone (LH; graph A) and follicle stimulating hormone (FSH; graph B) over time in control geldings and in geldings injected with 3 grams of sulpiride (racemic mixture) either in vegetable shortening or in SucroMate (sucrose acetate isobutyrate vehicle with added deslorelin) at time 0 in Experiment 4. The experiment was performed as a single switchback of the treated geldings. There was an effect in time (P < .01) in the ANOVA (performed with treatments only) but not effect (P > .1) of treatment or interaction of treatment with time for both LH and FSH. Pooled SEM from the analyses of variance were 2.3 ng/mL for LH and 3.0 for FSH concentrations.
5.5 Discussion

Experiment 1 was performed basically to confirm the suspicion that the (-) enantiomer of sulpiride was likely the biologically active component of the (+/-)-racemic mixture. Studies in rats and humans have indicated that levosulpiride is indeed the most active form interacting with D2-dopaminergic receptors [5.14], but that the (+) enantiomer may have slight activity at the D4 receptor [5.15]. Due to a lack of availability of the (+)-enantiomer for testing, we compared levosulpiride doses to exactly twice the amount (based on weight) of the racemic mixture, which would theoretically contain an equivalent amount of the (-) enantiomer. By performing the experiment as a Latin square, we minimized the potential problem of large variation among mares in their prolactin responses. Plotting the prolactin areas for each sulpiride preparation (y-axis) against the natural logs of the doses (x-axis) resulted in two parallel linear dose-response curves separated along the x-axis by a factor of 0.62865 (natural log of 1.875). Whether this (1.875) is a true deviation from two or simply an imprecise measurement of the difference cannot be concluded from this one experiment. Testing of the (+)-enantiomer will be needed to be sure, given that some reports in humans indicate up to a 10% biological activity of the (+) enantiomer relative to levosulpiride.

Most usages of sulpiride in human medicine are either as an antipsychotic drug or an antiemetic and antidyspeptic drug [5.16]. Although the other major antidopaminergic drug used in equine medicine, domperidone [5.1], is orally active and typically administered via that route, sulpiride has traditionally been administered via IM or IV injection in horses [5.4,5.7-5.9]. We performed Experiment 2 to determine whether oral administration of sulpiride might be useful in slowing down, or extending, timewise, the effect of sulpiride in the horse. The prolactin responses to the IM injection of 100 mg of the racemic mixture and the feeding of ten times that
amount (1 gram) were similar both in magnitude as well as in timing of the response. We noticed no external signs of side-effects in any of the horses receiving sulpiride, although continued daily feeding of gram quantities of sulpiride could potentially disrupt gut function in the long term.

Both sulpiride and domperidone have proven useful in the combination estrogen-dopaminergic antagonist protocol that we have studied over the years for inducing ovulation in seasonally anovulatory mares. The pretreatment with estradiol was first reported by Kelley et al [5.2] to greatly enhance the prolactin response to either dopaminergic antagonist. Multiple studies over the past 10 years have shown that a positive response in prolactin concentrations up to a point is needed for success in that combination, whereas beyond a point (perhaps a threshold), further prolactin does not increase the success rate [5.17]. Mitcham [5.18] gave mares three IM injections of sulpiride (either 0.75 or 1.5 grams on days 1, 6, and 11 relative to ECP treatment) dissolved in a proprietary mixture of oily liquids to extend (slow) the release of the drug. The prolactin response to each injection was robust and similar across the three injections; however, prolactin concentrations had returned to baseline by the time of the next injection 5 days later. Switching to vegetable shortening as the vehicle for sulpiride [5.17] resulted in prolactin concentrations in seasonally anovulatory mares that stayed high longer (at least 6 days) and returned to baseline at 8 days. From our data in which SucroMate was used as a source of SAIB (Experiment 4) as a vehicle, it appears that sulpiride in SAIB may extend the prolactin response up to 10 days, if the results in geldings hold true for seasonally anovulatory mares. The biphasic responses of prolactin to the SQ injections of sulpiride in vegetable shortening in SAIB seen in Figs. 5.4-5.6 are typical of hydrophobic, depot-like injections, in which the readily available drug on the outer surface of the injection gets into the bloodstream.
quickly, creating a “burst” effects. The drug inside the depot is thereafter released slowly, producing the prolonged effects over several days.

It is possible that the presence of deslorelin in the SucroMate solution used as a vehicle for sulpiride could have altered the prolactin response to the overall protocol; however, we know of no evidence that would support that possibility. The LH and FSH responses to the injected deslorelin in geldings receiving either SucroMate alone or SucroMate with sulpiride were very similar to responses reported for deslorelin injection previously [5.19, 5.20]: an immediate rise in plasma LH and FSH concentrations was followed by a rapid decrease over the next several days, indicative of a downregulation of the gonadotropes in the adenohypophysis.

Although vegetable shortening per se is not defined sufficiently for commercial application, various vegetable oils have been used for decades as vehicles for steroids such as estradiol and progesterone. Highly purified versions of various oils are available commercially, albeit mainly for weightlifters wanting vehicles for steroid injection. Triolein, a triglyceride made from glycerol and three oleic acid residues can be produced in a highly purified form and has been used as the vehicle for steroidal treatment in research [5.21]. It is likely that partial reduction in the unsaturation level of the fatty acid sidechains of Triolein could lead to a product that would remain solid at body temperature, similar to the shortening used in Experiment 3. However, given that the basis of SucroMate, SAIB, is currently available as a pharmaceutical grade product (Eastman BioSustane, Eastman Chem. Co., Kingsport, TN, USA), its potential as a vehicle for sulpiride administration in seasonally anovulatory mares probably outweighs the potential of a highly purified lipid product.

In conclusion, it appears unlikely that oral administration of sulpiride would provide any advantage over parenteral administration for the applications used today in the horse industry.
Moreover, the extra cost of using levosulpiride (about twice as much) compared to using the racemic mixture of sulpiride may not be justified, unless required for some reason by regulatory factors. The use of hydrophobic vehicles like vegetable shortening or SAIB for sulpiride administration greatly lengthens the duration of its biological activity such that a single injection would be sufficient in the estradiol/sulpiride protocol for inducing ovulation in seasonally anovulatory mares.

5.6 References


CHAPTER 6: DOPAMINERGIC AND ANTIDOPAMINERGIC EFFECTS ON HEART RATE AFTER BRIEF EXERCISE IN HORSES

6.1 Summary

Bromocriptine is a dopamine receptor agonist which is 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 first 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. Experiment 1 tested changes in HR, adrenocorticotropin (ACTH), and growth hormone (GH) concentrations when geldings were pre-treated with 100 mg of bromocriptine 12 hours before exercise. Bromocriptine pretreatment reduced (P < .05) the exercise-induced rise in HR and reduced (P < .05) the ACTH and GH responses. Experiment 2 assessed the daily responses of HR to exercise after intramuscular administration of 5 mg of cabergoline in vegetable oil. As with bromocriptine, cabergoline treatment diminished the rise in HR due to exercise for the first 2 days of the 7 day experiment. In Experiment 3, daily feeding of 2 g 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 1 g of sulpiride in dissolved in vegetable oil, respectively. Taken together, bromocriptine and cabergoline, but not pergolide or sulpiride, dampened the cardiac sympathetic response to exercise thus lowering the HR.

6.2 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 [6.1, 6.2], in addition to the induction of lactation in mares [6.3]. 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 in the pars intermedia, thereby diminishing hormonal output by said cells [6.4]. Bromocriptine, another dopamine agonist, has been studied in horses [6.5–6.7], 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, however it is noted to have hypotensive and bradycardic effects. Hamed et al. [6.8] described a significant decrease in blood pressure, heart rate (HR) and total peripheral resistance after anesthetized dogs were intravenously infused with 1 μg/kg/min of bromocriptine for 20 minutes. Similar effects are reported in humans [6.9–6.11], rats [6.12], and cats [6.13]. Due to the presence of dopamine receptors in peripheral sympathetic neurons, activation of presynaptic dopamine receptors can cause an inhibition of sympathetic nerve function [6.14], thereby decreasing norepinephrine secretion [6.15]. Moreover, cardiac sympathetic function is inhibited, causing impairments of cardiac acceleration even when an external stimulus is applied [6.14].

Information on the sympatholytic effects of other dopamine agonists, such as cabergoline and pergolide, is limited, and none have been reported in the horse. In one report, McDowell et al. [6.16] demonstrated vasoconstriction in the distal palmar artery when horses were fed ground endophyte-infected fescue seeds.

The present experiments were conducted to compare the sympatholytic effects of bromocriptine administration in horses to that which is stated in the literature and to determine if any effects are shared with cabergoline and pergolide administration. This information would
useful for horse owners and veterinarians treating PPID horses that are concurrently in physical activities. An additional two experiments were conducted to determine if antidopaminergic activity had any effects on HR, given that in previous experiments conducted by our lab using sulpiride, we have observed sedative effects in mares (personal observation) and lessening of aggressive male behavior in stallions prior to seminal collection [6.17]. 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.

6.3 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, while the Experiments 4 and 5 assessed changes in HR due to sulpiride administration under short-term and long-term conditions. Given that the aforementioned agents have been extensively documented to affect prolactin secretion in horses [6.5, 6.19-6.23], plasma prolactin concentrations were used in all experiments as a measure of drug efficacy.

6.3.1 Experiment 1: Bromocriptine

A preliminary trial with four quarter horse mares (Fig. 6.1.) indicated that intravenous (IV) injection of 50 mg of bromocriptine maximally reduced (P < .01) prolactin concentrations
at 12 hours after injection. Based on those data, ten mature, quarter horse geldings were pretreated intravenously (IV) with either 50 mg of bromocriptine dissolved in 1 mL ethanol (200 proof, Pharmaco-Aaper, Brookfield, CT, USA; n = 5) or vehicle (1 mL ethanol; n = 5) 12 hours prior to a 2-minute exercise bout the following morning.

Fig. 6.1. 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.

Blood samples were collected via jugular venipuncture with a 21 G x 1” vacutainer needle into evacuated tubes with heparin at -15, 0, 5, 10, 20, and 30 min relative to the start of exercise (trotting in a round pen). Horses were allowed to rest for 7 days after which the experimental protocol was repeated with the treatments reversed (switchback design).

Heart rates were measured with Polar Equine M400 Heart Rate Monitors (Amazon.com). Plasma concentrations of prolactin [6.24] and GH [6.25] were measured by double-antibody radioimmunoassay as described previously. Plasma ACTH was measured by radioimmunoassay with commercially available kit reagents (MP Biomedicals Inc, Costa Mesa, CA, USA).
Data were analyzed as a replicated Latin square with repeated measures [6.26]. Differences between means for each sampling time were assessed by the pdiff option in SAS (least significant difference comparison [6.26]) where appropriate.

6.3.2 Experiment 2: Cabergoline

Ten quarter horses were used (five mares and five geldings). They ranged in age from 8 to 21 years, weighed 420 to 590 kg, and had a body condition scores (BCS; [6.27]) of 5 to 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 Chem. Co, St. Louis, MO, USA) dissolved in 1 ml of vegetable oil with a few drops of DMSO (Sigma) added in order for cabergoline to go into solution. Controls received 1 ml of DMSO/vegetable oil. All treatments were injected intramuscularly in the neck area.

The experiment began on April 17, 2017. The following exercise protocol was performed every day for 6 consecutive days; treatments were administered on the first day (day 0; pretreatment) after the exercise protocol was completed. Beginning at approximately 06:30, all the horses were quietly walked from the pasture into an outside chute. One by one, the horses were taken out of the chute and fitted with a heart rate monitor (Polar Equine M400) attached to a surcingle. When ready, the heart rate monitor was activated to continuously record HR. A jugular blood sample (denoted “shed”) was taken via a 21-gauge needle into a 7-mL evacuated glass tube containing 143 USP units of sodium EDTA (Becton, Dickinson & Co., Franklin Lakes, NJ, USA), and the horse was then walked to a nearby round pen where a “pre-exercise” blood sample was taken immediately before exercise. The horse was then lunged at the trot for two minutes. A “post-exercise” blood sample was collected as quickly as possible once the
horse stopped trotting and the heart monitor was turned off once outside the round pen. After all horses had been exercised, they were returned to pasture until the following morning.

All blood samples were immediately placed in an ice water bath upon collection and centrifuged at the end of the day’s experiment in a refrigerated centrifuge at 4°C for 15 minutes at 1200 x g. Plasma was transferred to polypropylene tubes and frozen at -20°C until later analysis. From the continuous heart monitor data, 5 data points were collected. Shed (resting HR), walking peak HR, pre-exercise HR, peak exercise HR, and post-exercise HR (immediately after stopping).

At the end of the experiment, all frozen blood samples were thawed and analyzed for prolactin concentrations by radioimmunoassay previously validated for horse plasma [6.24]. For prolactin, estimates of the limit of detection of the assay and the interassay coefficient of variation were 0.2 ng/mL and 7 %, respectively. Plasma preparation, storage, hormonal assay protocols for prolactin were the same for all experiments.

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 x 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 [6.26].
6.3.3 Experiment 3: Pergolide

Nine horses from Experiment 2 were used in this experiment except for one mare that was withdrawn due to issues unrelated to the previous experiment. One gelding and 2 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.

Due to time limitations, the experiment was carried out in two equal replicates, staggered by 1 day. For each replicate, on day 1, 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®) 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 08:00 until day 8. Horses were exercised starting at 07:00 only on days 1, 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 min before starting exercise protocol. Statistical analyses for prolactin and HR were performed as described for Experiment 2.

6.3.4 Experiment 4: Short-term Sulpiride Treatment

The same horses used in Experiment 3 were used in Experiment 4 after a one week wash out period. 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 2 with two exceptions. Due to the short half-life of
sulpiride 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 was 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 x 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 x 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. Replicates were ignored in the analyses.

6.3.5 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 [6.28]. 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 twelve horses were exercised as described in Experiment 2 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.
6.4 Results

6.4.1 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. 6.2. Prolactin increased (P < .05) in response to exercise in control geldings, while previous bromocriptine treatment reduced (P < .01) prolactin concentrations by 90%; no prolactin response to exercise was noted in this group.

Fig. 6.2. 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.

Heart rate increased (P < .001) in controls when they were walked to the exercise area (Fig. 6.3.). 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, their HR were lower than controls (P = .011). Trotting for 2 minutes increased (P < .001) HR in controls as well as in
 geldings treated with bromocriptine (Figure 6.3.); however, bromocriptine reduced \( P = .0002 \) the post-exercise HR relative to 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 adrenocorticotropicin concentrations observed when geldings were treated with vehicle (Figure 6.4.). Plasma GH concentrations were higher \( P < .05 \) before exercise and at 5 and 10 minutes after exercise when geldings were treated with bromocriptine (Figure 6.5.), but the magnitude of the exercise-induced responses \( P < .05 \) were similar for both control and treatment exercise bouts.

![Fig. 6.3. 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.](image-url)
Fig. 6.4. Mean plasma adrenocorticotropic hormone (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.

Fig. 6.5. 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.
6.4.2 Experiment 2: Cabergoline

Due to inclement weather, no data was collected on day 3 of the experiment. Additionally, one mare’s prolactin and HR data were 2 to 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 6.6. 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.

![Fig 6.6. 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.](image)

By chance, the mean initial HR in treated and control groups prior to treatment tended to differ (P = .13), therefore, to better detect differences between groups, the mean net change from day 0 for each treatment group at each time point was calculated and analyzed in the ANOVA
Overall, HR was reduced ($P < .05$) in response to a short bout of exercise 24 hours after cabergoline treatment. By day 2, overall HR tended to differ between groups ($P = .067$), though no more differences were noted on subsequent days. When the net change in HR as it related to each horse’s day 0 at each time point were analyzed, the changes in HR due to treatment became much more evident.

Fig. 6.7. 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.

95
### 6.4.3 Experiment 3: Pergolide

Daily pergolide treatment administered top dressed over sweet feed inhibited (P < .0001) prolactin secretion in treated horses compared to control horses thought the sampling period (Fig 6.8). Mean changes in HR after pergolide administration and in response to an acute bout of exercise are presented in Fig 6.9. 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 difference (P = .0002) between treatment 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 present significant differences when using the least significant difference test. There were no further differences in HR at each of the other time points.

![Graph showing prolactin concentrations](image)

**Fig 6.8** 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 two-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.
Fig 6.9. 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 towards 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.

6.4.4 Experiment 4: Short-term Sulpiride Treatment

Mean plasma prolactin concentrations and HR are presented in Fig. 6.10. Sulpiride treatment increased (P < .0001) prolactin concentrations within 10 minutes and remained elevated for the entire sampling period, thereby confirming antidopaminergic activity. However, mean HR at each site was unaffected due to sulpiride treatment (P > .1).

6.4.5 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 6.11. Three time points were measured by day; Shed (at rest), Pre, and Post exercise. Plasma prolactin concentrations
Fig. 6.10. **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 min), 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 x 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 2x2 Latin square. There was no treatment effect (P > .1) on HR at any given time point. Pooled standard error of the mean was 3.7 bpm.

...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.
Fig. 6.11. 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 the line and asterisk, with the effect lasting till the end of the sampling period. Pooled SEM was 1.11 ng/mL.

Mean HR in response to exercise after administration of sulpiride or vehicle are presented in Fig. 6.12. 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.
Fig. 6.12. 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.

6.5 Discussion

Previous experiments [6.17, 6.24, 6.25, 6.29-6.31] have reported rises in plasma prolactin and GH concentrations when horses are subjected to brief periods of stress, such as 2 to 5 minute exercise bouts, twitching, teasing, and seminal collections (that is, any activity that increases heart rate 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 receptors on prolactin secretion [6.5] and 2) for its relatively short half-life compared to cabergoline. The preliminary trial indicated that intravenous injection of 100 mg of BC
maximally reduced prolactin concentrations at 12 hours after injection. Based on this information, the main experiment was performed with bromocriptine administered 12 hours before exercise the following morning. It was found that bromocriptine not only reduced the exercise-induced rise in HR but blunted the rise in exercise-induced prolactin and ACTH secretions as well. Growth hormone tended to be higher in bromocriptine treated geldings than controls although the overall response was unaffected due to treatment.

This bradycardic effect of bromocriptine observed in Experiment 1 can be explained by its inhibitory action on peripheral sympathetic neurons [6.12]. Since D2 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 [6.15, 6.10]. As a consequence, peripheral blood vessels dilate which is followed by a decrease in HR, blood pressure, and peripheral resistance, though cardiac output generally remains unaffected [6.8].

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 heart rate due to acute or chronic treatment in horses.

Since Experiment 1 was done on one day, any possible effects on heart rate carried over to subsequent days could not be determined. Therefore, for Experiments 2 though 5, the acute and chronic effects of the selected agents were assessed. Additionally, in each experiment, the expected suppression of prolactin secretion by the selected agent was in fact observed, confirming the dopaminergic activity of the treatments.
Due to 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 stress, 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 due to its similar binding affinity to D2 dopaminergic receptors on presynaptic terminals [6.32]. 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 [6.22]. 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 antidyspeptic drug [6.33]. In past experiments, sedative effects have been noted in horses 5 to 10 minutes after sulpiride administration (N. Arana Valencia and D. L. Thompson, Jr., personal observations). In addition, Thomson et al. [6.17] noted reduced a slowing of male 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 in the literature 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 discernable 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 [6.8-6.13]. The implication of this effect may be of importance for horses engaged in competitive activities and certainly deserves further investigation.

6.6 References


Chapter 7: Overall Summary and Conclusions

A series of experiments were conducted to address the effects and practical applications of dopamine agonists and antagonists in equine metabolism. The first experiment (Chapter 3) evaluated the inhibitory effects of 5 mg of cabergoline in a proprietary vehicle on prolactin and MSH concentration for possible application in PPID treatment, in addition to studying its effect in improving insulin sensitivity in insulin resistant mares. Although cabergoline treatment was successful in inhibiting prolactin and MSH secretion, insulin sensitivity was unaffected due to treatment. Cabergoline treatment could prove to be an alternative treatment option for PPID horses, based on its long duration of action (~ 7 days) on dopamine regulated hormones after one intramuscular injection. It was not recommended, however, as a viable treatment option for improving insulin sensitivity. To further evaluate if dopaminergic regulation had a role in metabolic efficiency, changes in insulin sensitivity were compared after acute treatment of cabergoline or sulpiride and chronic treatment of sulpiride in insulin resistant and insulin sensitive horses (Chapter 4). The effects of acute administration of sulpiride or cabergoline on insulin sensitivity were also assessed. It was concluded that dopaminergic inhibition or stimulation had no effect on insulin sensitivity in horses.

The third set of experiments (Chapter 5) studied selected factors that affect the prolactin response to sulpiride in horses. The (-) enantiomer of sulpiride, levosulpiride, was compared to the (+/-) racemic mixture of sulpiride, in addition to different modes of administration and vehicles which could be used for practical application in research and industry. It was determined that the physiologic response of prolactin to sulpiride is dose-dependent and its duration of action is greatly extended days with a single injection using hydrophobic vehicles, such as vegetable shortening or sucrose acetate isobutyrate. This could be applied to existing
protocols to advance ovulation in seasonally anestrous mares by providing sustained increases in prolactin secretion.

Lastly, a final series of experiments (Chapter 6) compared differences in exercise-induced response in heart rate when horses were administered therapeutic doses of commonly used dopamine agonists (bromocriptine, cabergoline and pergolide) and the antidopaminergic drug sulpiride. By comparing heart rates in both control and treated groups, it was determined that bromocriptine and cabergoline, but not pergolide or sulpiride, blunted the rise in heart rate before and after a short 2-minute exercise bout.

Taken together, these experiments support the hypothesis that dopaminergic agonists, like those used to treat PPID, are not effective in improving insulin sensitivity in horses, as it has been observed in other species. In addition, PPID and insulin resistance are two different metabolic conditions with apparently different etiologies. Though preliminary, dopamine agonists were found to alter the sympathetic response to stressors and should be used with caution when equine athletes are undergoing treatment until more research is available to rule out any possible detrimental effects as a result of decreased sympathetic activity.
APPENDIX 1: PERMISSION STATEMENT FOR USE OF MATERIAL COVERED IN CHAPTER 3
APPENDIX 2: PERMISSION STATEMENT FOR USE OF MATERIAL COVERED IN CHAPTER 4
APPENDIX 3: PERMISSION STATEMENT FOR USE OF MATERIAL COVERED IN CHAPTER 5

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VITA

Nicole Arana Valencia, daughter of Norberto Arana Soto and Yolanda Valencia Lucena, was born in San Juan, Puerto Rico. Nicole attended Wesleyan Academy and Rosa Bell Academy, graduating with honors from Rosa Bell in Guaynabo, Puerto Rico, in May of 2005. She received her Bachelor of Science and Master of Science degrees in the area of animal sciences at Louisiana State University in May of 2012 and August of 2013, respectively. She began her doctoral degree at Louisiana State University under the direction of Dr. Donald Thompson, Jr. on August of 2013, with an interest in equine neuroendocrinology and metabolism. During her undergraduate time at LSU, Nicole met and married Allan James McIlwain in April of 2011 and, in October of 2015, the couple welcomed their first son, Killian, into the world. Upon completion of the doctoral degree, Nicole will be attending the LSU School of Veterinary Medicine to pursue a degree in veterinary medicine.