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Regulation of Cortisol and Prolactin in Horses.

Donald R. Colborn

Louisiana State University and Agricultural & Mechanical College

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Regulation of cortisol and prolactin in horses

Colborn, Donald R., Ph.D.
The Louisiana State University and Agricultural and Mechanical Col., 1990
REGULATION OF CORTISOL AND PROLACTIN IN HORSES

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
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in
The Department of Animal Science

by
Donald R. Colborn
A.S., Hannibal-LaGrange College, 1983
B.S., M.S., University of Missouri-Columbia 1985, 1987
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ABSTRACT

In Experiment I, stallion exposure to an estrous mare on d 2 caused cortisol and PRL concentrations to increase (P < .05) following treatment on d 2, but did not vary (P > .05) on d 1, 3, 4, 5 or 6 when no exposure occurred. In Experiment II, cortisol concentrations increased (P < .05) within 10 min after sexual stimulation (SS), physical exercise (PE), restraint via a twitch (RT) and epinephrine administration (EA) but not during control bleedings. PRL concentrations increased (P < .05) following SS, PE and RT but not after EA or during control bleedings. In Experiment III cortisol concentrations in geldings increased (P < .05) following EA, PE and RT but not following SS or control bleedings. PRL concentrations increased (P < .05) following PE and SS. In Experiment IV sulpiride injection was used in stallions in an attempt to increase PRL concentrations. Prior to sulpiride treatment, concentrations of both cortisol and PRL increased (P < .05) by 40 to 80% in response to exercise; concentrations of LH and FSH also increased (P < .05) by approximately 10%. Sulpiride treatment stimulated (P < .05) daily PRL secretion by approximately 5-fold, and caused a decrease (P < .05) in daily FSH secretion whereas only isolated differences (P < .05) occurred between treatment groups for daily LH and cortisol secretion. At the end of treatment, there was no PRL response to exercise for stallions treated with sulpiride, but there was (P < .05) for control stallions; only control stallions exhibited a marginal cortisol response (P < .05) to exercise. The PRL response to TRH was increased (P < .05) 4-fold in stallions treated with sulpiride but was unchanged in control stallions. Sulpiride treatment
did not affect (P > .05) the LH or FSH response to exogenous GnRH. It is concluded that 1) returning stallions to the site of a previous period of sexual excitement does not cause a memory-mediated rise in cortisol or prolactin, 2) cortisol and PRL are secreted rapidly in response to some but not all forms of physical and chemical stress in stallions and geldings, 3) treatment with sulpiride during winter increases daily secretion and perhaps production of PRL in stallions and 4) the increase in PRL characteristics due to sulpiride does not stimulate the PRL response to exercise, but does increase the PRL response to TRH.
CHAPTER I

INTRODUCTION

The physiological response known as stress has been widely studied in animals for many years. In the horse, as in other species, a stimulus is considered stressful if it results in an increase in cortisol secretion from the adrenal glands. More recently, it has been found that the anterior pituitary hormone prolactin (PRL) is also secreted in horses following certain types of stress. However, this response is less consistent and is not widely understood.

The horse is a seasonal breeder with maximal sexual activity occurring during the summer when daylength is long. Both males and females undergo a period of reproductive quiescence during the winter. There is a desire in the horse industry to alter or prevent this quiescent period. This would allow producers to begin breeding their mares earlier in the year allowing them to take maximum advantage of the January 1st birthday that exists in some breeds.

Research in laboratory species has indicated that PRL might play a role in the seasonal effects of reproduction in animals. Injection of PRL into hamsters can help to restore gonadal function during the nonbreeding season. However, in the horse this is not possible due to the size of the animal and the limited amount of PRL available for injection. Therefore, a more logical approach would be to stimulate the horse to produce more of its own PRL in hopes of returning it to a functional breeding season state.
The first three experiments described herein were designed to determine how various types of stress affect plasma cortisol and PRL concentrations in stallions and geldings. Four different stimuli were assessed, including sexual stimulation, to determine if this stimulus differed from the more classical stressful stimuli with regards to PRL and cortisol secretion.

The fourth experiment was designed to determine first if the PRL and cortisol responses to stress that are observed in summer would occur in winter when PRL secretion is normally low. Second, an attempt was made to stimulate the stallion's own PRL secretion during the winter by administration of a dopamine receptor blocker, sulpiride, for a period of 14 days. It was of interest to determine whether an increase in PRL would alter the typical winter endocrine profiles of these stallions. For this reason, plasma concentrations of luteinizing hormone (LH) and follicle stimulating hormone (FSH) were also measured. Finally, the effects of sulpiride treatment on the responses of PRL and cortisol to stress were evaluated.
CHAPTER II
LITERATURE REVIEW

Early History of Prolactin

The anterior pituitary hormone PRL is involved in many different physiological systems in animals. Nicoll (1974) listed 85 different actions of this hormone and suggested that it should be renamed "versatilin" because of its diversity. It is currently believed that PRL is involved in such physiological processes as electrolyte balance, pregnancy and lactation and it also seems to be an important regulator of the seasonal cycle of many animals.

The actions of PRL were first reported in 1928 by Stricker and Grueter (referenced by Niall, 1981). These investigators reported that milk secretion could be induced in ovariectomized rabbits by injecting them with an extract of bovine pituitary. Riddle and Braucher (1931) reported that the crop sac of the pigeon, which produced 'crop milk', was under the regulation of a hormone of anterior pituitary origin. The subsequent development of their pigeon crop-sac assay led to the first method of measuring this hormone in animals. This lactogenic factor was then detected in pituitary extracts of many different species (Li, 1978).

Regulation of Prolactin

Studies into the regulation of PRL resulted in the discovery that the hypothalamus had some inhibitory effect on the secretion of this hormone. Everett (1954) reported that the rat could be placed in a state of pseudopregnancy by removing the pituitary from the influence of
the hypothalamus and transplanting it to the renal capsule. Talwalker et al. (1963) found that PRL concentrations decreased by 36 to 75% following incubation of the anterior pituitary with hypothalamus homogenate or extract. The hypothalamic substance was termed the Prolactin Inhibitory Factor (PIF). These revelations began the search for the identity of the hormone that regulated PRL release. It had been known for some time that the neurotransmitter dopamine was present in high concentrations in the median eminence, but it was believed to serve merely as a precursor to norepinephrine (Weiner and Bethea, 1981).

MacLeod et al. (1970) used rat pituitaries to show that dopamine would suppress PRL concentrations in a dose-related manner by inhibiting the release of PRL from the pituitary. Furthermore, they also showed that this phenomenon could be reversed by the addition of reserpine or perphenazine which deplete dopamine stores in the brain.

Danon and Sulman (1970) used perphenazine injections to show that PIF concentrations in the hypothalamus increased 1 h after treatment. It was postulated that this was due to perphenazine blocking the release of PIF from the hypothalamus. This data was supported by Ben-David et al. (1970) who showed that perphenazine treatment would increase PIF concentrations in the hypothalamus while also causing a decrease in PRL concentrations in the pituitary and a subsequent increase in plasma PRL concentrations.

Other dopaminergic drugs have been studied regarding their effects on concentrations of PIF and PRL. These drugs include bromocriptine (Rodway et al., 1983; Eisemann et al., 1984; Ireland et al., 1989), haloperidol (Langer et al., 1977), sulpiride (Collu and Bouvier, 1987;

In addition to the role of dopamine as the PRL inhibiting factor, much evidence exists to support the theory of a stimulatory mechanism as well. This unknown factor has been termed the PRL releasing hormone (PRH). Garthwaite and Hagen (1979) reportedly extracted a substance from male rat plasma that has some PRH activity. In addition, hypothalamic extracts have been shown to have PRH activity (Milmore and Reece, 1975). It has been speculated that thyrotropin releasing hormone (TRH) may act as the PRH since an injection of TRH will cause an immediate increase of PRL concentrations in many species including the horse (Thompson et al., 1986b). However, an exact definition of any PRH is still open to speculation and continued research is necessary in this area to further clarify this system.

Regulation of Prolactin in the Horse

In the horse, Johnson and Becker (1987) reported that serum PRL concentrations could be increased by injection of the dopamine receptor antagonist metoclopramide. They also reported that the response occurred in a dose-dependent manner but was not affected by method of injection. Concurrently, it was reported that the D-2 dopamine receptor antagonist, sulpiride, also increased serum PRL concentrations in the mare, with maximum stimulation occurring at a dose of 25 mg. Finally, they stated that the dopamine agonist, bromocriptine, given at a dose of 10 mg, caused a significant reduction in serum PRL concentrations.
However, this effect was dependent on the time of the year, since PRL secretion decreased only when concentrations of PRL were normally high.

Ireland et al. (1989) utilized pregnant pony mares treated with bromocriptine or bromocriptine plus perphenazine to study the possible effects of fescue toxicosis in horses. Beginning on day 295 of gestation, mares in the bromocriptine and perphenazine groups were given bromocriptine twice daily at a dosage of .08 mg/kg of metabolic body weight (bw$^{.75}$). At day 305 the mares in the perphenazine group were given bromocriptine plus .375 mg/kg bw$^{.75}$ of perphenazine. They reported that the mares in the bromocriptine and perphenazine groups had lower concentrations of PRL than did the control mares. However, after day 305 of gestation, the mares in both the control and perphenazine groups had higher concentrations of PRL than did the bromocriptine mares.

Loch et al. (1990) used nonpregnant mares to study the effects of perphenazine on PRL secretion. They used levels of .5 and 1.0 mg/kg bw of perphenazine given orally. It was reported that PRL concentrations were increased in these mares at 3 and 6 h after administration but returned to normal levels within 11 h after administration.

Based on these and other studies, it appears that PRL secretion can be altered by dopamine agonists/antagonists in the horse as in other mammals. Therefore, it appears that the hypothalamus regulates PRL through the release of dopamine in the horse as in other species.

Role of Prolactin in Reproduction

Numerous studies have been conducted in an attempt to determine what role PRL plays in reproduction in animals. A wide range of
experimentation has occurred in both the male and the female of most livestock and laboratory species. While much is known about the effect of PRL on reproduction, some contradictory information is yet to be resolved.

Niswender (1974) used bromocriptine to lower serum concentrations of PRL in ewes and to study the effects of hypoprolactinemia on the estrous cycle. It was concluded that bromocriptine was effective in reducing PRL concentrations in cyclic ewes. However, there was no difference in concentrations of LH, FSH or progesterone in the control versus treated groups. He also reported that hypoprolactinemia did not cause any alteration in estrous cycle characteristics as evidenced by corpora lutea (CL) regression or estrous cycle lengths. Thus, it was concluded that in the ewe, either PRL was not important for cyclic ovarian function or extremely low levels of PRL were sufficient to maintain normal CL function.

Rodway et al. (1983) reported that treating anestrous ewes with bromocriptine caused a significant reduction in CL numbers following induced ovulation. It was reported that on d 8 following induced ovulation, ewes treated with bromocriptine averaged 1.6 CL compared to 2.8 for control animals. Also, the treatment did not prevent the ewes from returning to their anestrous state following the induced ovulation. Thus, it was concluded that there was a minimum level of PRL necessary during the preovulatory period in order to insure normal follicular development in ewes.

Studies in the pig have yielded conflicting results. Kraeling et al. (1982) studied the effects of bromocriptine injection on LH levels
in lactating sows and ovariectomized (OVX) gilts. While PRL concentrations were reduced following treatment in both groups, LH concentrations were only reduced in lactating sows. Among OVX gilts, the results were too inconsistent to determine what effect treatment had on circulating LH concentrations.

Additional work in this area was done by Bevers et al. (1983), who also used lactating sows to study the effects of bromocriptine administration on PRL and LH secretion. It was reported that treated sows had lower plasma concentrations of PRL and increased concentrations of LH relative to control animals. It was reported that this increase in LH secretion was even greater following weaning.

A further study was conducted in cyclic sows by Dusza et al. (1983). They found that sows treated with bromocriptine did not exhibit normal PRL peaks during the estrous cycle and treatment had no effect on LH concentrations, although progesterone levels in treated sows tended to fluctuate more and to decrease slowly.

Ireland et al. (1989) reported that progesterone concentrations in serum of bromocriptine treated pregnant pony mares were lower than control mares from 295 to 309 days of gestation. It was found that while the control mares exhibited a steady increase in progesterone, treated mares did not. At 305 days of gestation, half of the bromocriptine mares began receiving perphenazine in addition to the bromocriptine. Following this treatment regimen, progesterone concentrations were lower for the bromocriptine group than they were for the perphenazine or control animals. In addition, mares in the perphenazine group had lower concentrations than did the control
animals. Additionally, it was discovered that gestation length was greater for bromocriptide mares than either the perphenazine or control mares. Also, bromocriptide mares had 50% retained placentae, 75% dystocia rate and thickened placentae at a rate of 100%. The reported incidence rates for the other two groups were 0% for all characteristics.

The physiological involvement of PRL in reproduction in the male has also been widely studied in a variety of species. However, the bulk of the research has occurred in laboratory rodents and this model has served as the basis for comparison for other species.

In 1978, Zipf et al. reported that following hypophysectomy (HPX) in male rats, LH receptors in the testis declined by 80%. However, this loss of LH receptors could be minimized by treating the animals within 6 h of surgery with PRL. The combination of PRL, LH and growth hormone (GH) prevented any loss of LH receptors following HPX.

Pituitaries were transplanted to the kidney capsules in hamsters by Bartke et al. (1982) in order to initiate a state of hyperprolactinemia. They reported that the increase in PRL concentration in plasma was accompanied by an increase in FSH but not LH secretion. However, it was found that testicular LH receptors increased as did testicular weight and plasma testosterone concentrations in the hyperprolactinemic hamsters. It was concluded that PRL played a major role in stimulating spermatogenesis and steroidogenesis in the testes. This was further supported by work of Wahlstrom et al. (1983). In their study, they utilized immunocytochemistry to stain for receptors in the testes of rats and humans. According to their report, the staining was
positive for PRL in the Leydig cells of rats but was negative in humans.

Ohlson et al. (1981) used active immunization against PRL to study the effects of PRL on growth and reproduction in the ram. They were able to decrease free PRL by 91% after 10 mo of treatment and as a result they reported slower growth characteristics among the treated rams. When reproductive characteristics were evaluated, the authors found a nonsignificant reduction in testes weight of 32% in the treated rams and no effect on the weights of the accessory sex glands. In addition, there was no effect of immunization on LH, GH or thyroid stimulating hormone (TSH). Based on these results, they concluded that PRL might play a role in reproduction in rams.

In another study, Barenton (1981) infused PRL into the jugular vein of rams with seasonally regressed testes for a period of 3 d. Following this period, they reported no difference in LH or FSH concentrations in the plasma of the animals in the two groups. Also, they failed to detect any significant difference in numbers of LH or FSH receptors in the testes of these rams.

Estrogen has been shown to influence PRL concentrations in other species (Quadri et al., 1979; Shupnik et al., 1979). Therefore, Thompson and Johnson (1987) actively immunized stallions against estrogen to measure any effect this treatment would have on PRL. Immunization against estrogen resulted in increased testosterone secretion and spermatogenesis in these stallions (Thompson and Honey, 1984), yet there was no significant effect on PRL secretion. Therefore, it was concluded that further research was needed in the horse to determine what effect estrogen may have on PRL.
Seasonal Variations in Prolactin Characteristics

Concentrations of PRL have been shown to fluctuate throughout the year in many species. This annual variation appears to be mediated by photoperiod, because artificially prolonging or extending daylength alters PRL concentrations in numerous species.

In the hamster, it has been shown that gonadal function is depressed by exposure of the male to periods of short daylength or total darkness (Hoffman and Reiter, 1965; Gaston and Menaker, 1967). Bex and Bartke (1977) reported that exposure of male hamsters to a lighting regimen of 5 h of light and 19 h of dark per day (5L:19D) for a period of 2 months caused gonadal atrophy when compared to animals maintained on a 14L:10D schedule. After the 2 month period on short days, hamsters were treated daily for 2.5 wk with saline, 250 μg PRL, 20 μg LH + 150 μg FSH or PRL + LH + FSH while remaining on the 5L:19D photoperiod. Following this time, all hamsters were sacrificed and LH binding in the testis was determined. It was reported that PRL alone or in combination with LH+FSH resulted in greater LH binding than was found in the control animals maintained on 14L:10D. Treatment with LH + FSH had no effect. This increase in LH binding was accompanied by an increase in testosterone concentrations and an increase in testicular weight.

Additional work in this area was conducted by Bex et al. (1978). Once again a photoperiod of 5L:19D was used to induce a decrease in secretion in PRL concentrations and gonadal atrophy in golden hamsters. It was reported that a single 250 μg injection of PRL was sufficient to increase testicular LH binding and testicular weight above those in control animals.
In addition to the effects of short photoperiod on LH receptors in the testis, Klemcke et al. (1986) reported that a 5L:19D photoperiod would result in a 94% reduction of PRL receptors in the testis of hamsters. This was accompanied by a decrease in plasma PRL concentrations but no effect was noted on LH or FSH secretion. The concentration of PRL receptors in testes and of PRL in plasma was reported to increase following the transplantation of the pituitary to the kidney capsule. Based on these results, it was concluded that PRL had the ability to increase the number of its own receptors in the testes of golden hamsters.

PRL concentrations follow similar annual patterns among livestock species regardless of whether they are seasonal or nonseasonal breeders. In nonseasonal breeders such as pigs, PRL levels are directly correlated to daylength. Ravault et al. (1982) reported that plasma PRL concentrations in wild boars and sows varied with season; the highest concentrations were observed in the month of July and lowest were observed in the winter. When domestic pigs were studied, no variation was found for boars while a direct correlation with photoperiod was discovered for gilts. Additionally, Trudeau et al. (1988) reported no annual variation in basal PRL concentrations in domestic boars. However, there was an effect of month on PRL following injection of TRH, with response to TRH increasing from February through August.

In the cow, Schams and Reinhardt (1974) reported that serum PRL concentrations were seasonal, with highest concentrations occurring in the summer and lowest values in the winter. Additionally, Petitclerc et al. (1983) changed the lighting scheme of prepubertal bulls from 8L:16D
to 16L:8D or 6L:8D:2L:8D and found that PRL concentrations increased by 418% within 6 weeks. However, it appears that this increase in PRL concentrations cannot be constantly maintained. Stanisiewski et al. (1987) measured PRL concentrations in calves under different lighting conditions. After 8 wk of 8L:16D, half of the calves were switched to a 16L:8D cycle. PRL concentrations increased among calves in the longer daylength group. Following wk 20, there was no difference among groups. This led to the conclusion that the PRL response to lighting had become refractory.

It is interesting to note that annual variations in PRL secretion among seasonal breeders are similar to those noted in nonseasonal breeders. Also, the patterns are similar regardless of whether the animal is sexually active during periods of long days or short days.

Ravault (1976) characterized the annual PRL changes in the ram. He reported that during the first December of an animal’s life, there was a brief rise in PRL secretion. However, in all additional years, the concentrations fell during the winter and increased 4- to 11-fold in the summer.

The horse has been shown to be sexually active during periods of long daylength. However, the annual pattern of PRL is similar to animals of nonseasonal or short-day breeding patterns. Thompson et al. (1986a) measured concentrations of PRL in the pituitary and serum of stallions, mares and geldings during the summer and winter. They reported that PRL concentrations in both the pituitary and serum were higher in the summer than in the winter, with stallions having greater serum concentrations than mares in summer.
In addition to annual variations of PRL in horses, Thompson et al. (1986b) also showed that the PRL response to an injection of TRH was dependent upon time of the year. In that study, mares in the estrous or anestrous period of the year were injected with TRH to stimulate PRL release. It was discovered that the PRL response to TRH was greater among estrous mares in the summer than in anestrous mares in the winter.

**Regulation of Cortisol**

Cortisol, a glucocorticoid hormone, is produced from cholesterol within the zona fasciculata of the adrenal gland. Cortisol has been shown to be regulated via direct hormonal stimulation through adrenocorticotropic hormone (ACTH) release from the anterior pituitary. Secretion of ACTH is regulated by corticotropin releasing factor (CRF) from the hypothalamus. Cortisol concentrations act as a negative feedback mechanism at both the hypothalamic level through CRF and at the pituitary level through a decrease in ACTH production and secretion (Baxter and Rousseau, 1979). Normal concentrations of glucocorticoids have been shown to fluctuate widely following various physical and psychological stimuli (Terjung, 1979). Following release of cortisol into the blood stream, it is found in either a bound or free state. The proportion of bound to free hormone has an impact on its biological activity. The major protein carrier of cortisol has been found to be cortisol binding globulin, which accounts for approximately 80% of the corticosteroid binding found in the blood. Most of the remaining binding of corticosteroid is accounted for by albumin (Ballard, 1979).
Diurnal Variations of Cortisol

Serum concentrations of glucocorticoids generally fluctuate during the day within a definite diurnal pattern. This fluctuation is accompanied by a diurnal pattern of ACTH release as well. It has been reported that concentrations of ACTH begin to increase during the night, reach peak concentrations in the early morning and then decrease throughout the day and into the evening (Baxter and Rousseau, 1979).

In general, cortisol concentrations follow similar patterns. Bottoms et al. (1972) reported diurnal rhythms of cortisol and corticosterone concentrations in mares, with the highest concentrations occurring during the morning. A similar response was noted for concentrations of cortisol, but not corticosterone, in pigs in that same study.

Flinsinska-Bojanowska et al. (1974) reported that peak concentrations of cortisol in Thoroughbred horses occurred around 0400, which was similar to the peak of 0600 reported by Larsson et al. (1979). In contrast, cortisol concentrations were reported to peak at around noon in horses by Evans et al. (1977), while Kirkpatrick et al. (1977) reported no peak in plasma corticosteroid concentrations of wild horses.

Effects of Stress in Animals

The physiological event defined as stress can be divided into two distinct areas. According to Selye (1980), the term stress can be defined as "the nonspecific result of any demand upon the body." Stress can be in the form of a physical response in which the animal seeks a more favorable environment or it can be a neuronal or endocrinological response which is mediated involuntarily. The term stressor is defined
as "whatever produces stress." The stressor can be any physical, environmental, nutritional or psychological stimulus which results in stress of the animal.

Many studies have been conducted in an attempt to understand the physiological mechanisms underlying stress in animals and humans. For the most part, these studies have centered around peripheral concentrations of corticosteroids in the animals studied. A significant and sudden increase in cortisol secretion was usually accepted as an indicator of stress.

In addition to cortisol, PRL has been shown to increase following certain types of stress.Neill (1970) reported that the stress associated with laparotomy and bleeding under ether anesthesia caused a 3- to 8-fold increase in PRL concentrations in female rats. This increase was also noted in OVX females but not in males. Likewise, PRL concentrations increased within 10 to 15 min in rats following removal from the cage and transportation to another room (Euker et al., 1975).

Sexual stimulation and mating have been shown to increase PRL concentrations in rats (Kamel et al., 1975; 1977; Kamel and Frankel, 1978). In the latter study, it was reported that PRL secretion increased within 5 to 15 min following mating. Testosterone concentrations did not increase until 30 to 60 min following mating.

Similar studies with the bull revealed that ejaculation caused a significant increase in PRL secretion within 5 min that abated within 30 min (Convey et al., 1971). Stearns et al. (1973) found no consistent effect of coitus on PRL concentrations in men and women although two out of six women had 8- to 10-fold increases in PRL within 10 min after
coitus. In contrast, significant increases in PRL secretion were seen in humans following general anesthesia and major surgery (Noel et al., 1972).

Effects of Stress in Horses

Several types of stress have been shown to increase concentrations of cortisol and/or PRL in horses. Physical exercise has been widely studied as a means of measuring the effects of stress on horses. Dybdal et al. (1980) measured plasma constituents in horses during and following completion of a 160-km endurance ride and found that corticosteroid concentrations were elevated during and after the ride. These findings were supported by Snow and Rose (1981) who reported a significant increase in cortisol concentrations following participation in an 80-km endurance ride. There were also elevated concentrations of epinephrine and norepinephrine in the animals following the ride.

Short-term periods of exercise have also been shown to be stressful in horses. Snow et al. (1982) collected blood samples from Thoroughbreds within 10 min of completion of races over distances of 1000 to 2402 m. They reported that following the race, there was increased levels of glucose, glycerol, lactate, pyruvate, uric acid and cortisol. Church et al. (1987) measured cortisol concentrations in blood before and after a 2-month training program involving short periods of moderate exercise and reported high increases of ACTH and cortisol following the exercise program. However, no significant differences were detected in these values when pre- and post-training were compared. Thus, it appeared that the training program did not have an effect on the release of these hormones in the horses.
Similar findings were reported by Hower and Wickler (1989), who studied the effects of training on cortisol and glucose concentrations in horses. It was reported that cortisol concentrations increased immediately after the exercise program in both trained and untrained horses. However, trained animals appeared to recover sooner than did untrained ones. Cortisol levels were not different from pre-treatment values in trained horses 30 min following exercise, but were still elevated in untrained horses.

Other types of stress have also been studied in horses. Thompson et al. (1988) utilized OVX pony mares to study the effects of restraint via a twitch, tranquilization with xylazine and anesthetization with xylazine plus ketamine on plasma concentrations of cortisol, LH, FSH and PRL. Twitching and anesthetization both caused an increase in cortisol secretions but no effect was seen following tranquilization. PRL concentrations increased only following anesthetization. Concentrations of LH and FSH did not change following any of the treatments.

Psychological stress was tested in horses via isolation of a horse from it's companion (Alexander et al., 1988). During the isolation period, all horses began to show signs of excitation in addition to hyperventilation and sweating. Plasma concentrations of epinephrine and norepinephrine increased in all animals as did packed red blood cell volume. While three of the five horses exhibited increased concentrations of ACTH, there was no overall effect on this hormone. Likewise, concentrations of cortisol were not effected by the isolation.

Baucus et al. (1990a) studied the effects of long distance transportation on early embryonic death in mares between d 16 to 22 or
32 to 38 of gestation. The mares were transported in a trailer a distance of 472 km over a period of 9 h. Blood samples were drawn before, during and after the journey and fetal condition was measured by ultrasonography. Early embryonic death occurred between d 3 and 13 and did not differ between the transported and nontransported mares. Cortisol and progesterone concentrations increased at mid-trip in the transported mares in comparison to pre-treatment and nontransported mares. It was concluded that transportation had no effect on early embryonic death, but it was suggested that transportation at a different stage of gestation or for longer periods of time might increase embryonic loss.

A similar study was conducted by Baucus et al. (1990b) to study the effects of transportation on estrous cycle characteristics in mares. Mares in this study were trailered for a distance of 792 km over a period of 12 h. Following treatment, there were no detectable differences in estrous characteristics including ovulation rate, estrous behavior, duration of estrus or pregnancy rates in the transported versus the nontransported mares. From an endocrine standpoint, there was a significant increase in cortisol values during and after transportation with values decreasing below baseline within 24 h after treatment.

Sexual activity has been shown to affect hormone concentrations in stallions. Tamanini et al. (1983) measured cortisol concentrations during mating or during exposure to an estrous female. It was concluded that cortisol concentrations increased significantly 7 to 30 min after
mating or female exposure. The values increased by approximately 2-fold and then decreased back to normal within 2 h after exposure.

A more comprehensive study was conducted with stallions by Rabb et al. (1989) in an attempt to determine the effects of sexual stimulation with or without ejaculation on secretion of various hormones. Stallions in the sexual stimulation group were given 5 min exposure to an estrous mare. Those in the ejaculation group were first exposed to the mare and then were allowed to mount and breed an artificial vagina. All stallions served in both of the treatment groups plus one control period. No difference was detected in LH or FSH concentrations in stallions in any group. However, both cortisol and PRL concentrations increased rapidly following sexual stimulation alone and following ejaculation. Also, it was noted that stallions that had previously been exposed to either sexual stimulation or ejaculation exhibited an increase in cortisol and PRL secretion during their control period. Stallions that had their control period prior to receiving either of the other two treatments did not show this tendency. It was suggested that the stallions associated the sights and sounds of the other stallions receiving their sexual stimulation with their own previous experience and this memory event mediated their own increase in hormonal secretion even in the absence of any direct stimulation.

**Rationale of Present Research**

The present research was designed in part to answer questions concerning the relationship between cortisol and PRL levels in horses following various types of stress. An attempt was made to determine if the memory-mediated increase in cortisol and PRL concentrations noted by
Rabb et al. (1989) could be initiated by returning the stallions to the site of a previous single episode of sexual stimulation and, if so, to determine how long this increase would continue to be exhibited. Additionally, various types of chemical and physical stimuli were administered to stallions 1) to determine what effect these might have on cortisol and PRL concentrations and 2) to attempt to determine how any hormonal variations were mediated. In a related study, these same stimuli were applied to geldings under similar circumstances to ascertain if any changes in hormonal concentrations were gonadally dependent.

A final experiment was designed to determine if the PRL response to physical stress observed in stallions in summer would be present in winter when PRL secretion is normally very low. Stallions were subsequently injected daily with the dopamine receptor blocker, sulpiride, and PRL response to physical stress was again determined. Pre- and post-treatment plasma samples following a GnRH/TRH injection were used to determine if PRL production-secretion were indeed stimulated.
CHAPTER III
CORTISOL AND PROLACTIN RESPONSES TO SEXUAL EXCITEMENT
AND STRESS IN STALLIONS

ABSTRACT

Sexual excitement induces rapid secretion of cortisol and PRL in stallions. Experiment I was conducted to determine if stallions associated location and/or procedure with previous sexual stimulation in that location. After a period of no sexual excitement on d 1, four lighthorse stallions were exposed to an estrous mare for 5 min on d 2. On d 3, 4, 5 and 6, stallions were returned to the same site and were allowed to stand for 5 min with no mare present. PRL and cortisol concentrations increased (P < .05) following treatment on d 2, but did not vary (P > .05) on d 1, 3, 4, 5 or 6. In Experiment II, six stallions were used to determine the short-term effects of 1) sexual stimulation (SS), 2) acute physical exercise (PE), 3) restraint via a twitch (RT), 4) epinephrine administration (EA) and 5) no stimulation (control) on plasma concentrations of PRL and cortisol. Stallions received one treatment per d separated by 2 d of no treatment. Cortisol concentrations increased (P < .05) within 10 min after SS, PE, RT and EA but not during control bleedings. PRL concentrations increased (P < .05) immediately following SS, PE and RT but not after EA or during control bleedings. In Experiment III, the same five treatments were administered to six geldings. Cortisol concentrations increased
(P < .05) following EA, PE and RT but not following SS or control bleedings. PRL concentrations increased (P < .05) following PE and SS. It is concluded that 1) returning stallions to the site of a previous single episode of sexual excitement does not cause a memory-mediated rise in either cortisol or PRL, 2) in stallions, cortisol and PRL are secreted in response to SS, PE and RT, while only cortisol is secreted in response to EA and 3) in geldings, PRL is secreted rapidly in response to PE and SS, while cortisol alone is released in response to EA, PE and RT.

Introduction

Cortisol concentrations in horses increase following exercise (Snow and Munro, 1975; Dybdal et al., 1980; Snow et al., 1982; Church et al., 1987; Hower and Wickler, 1989), during isolation stress (Alexander et al., 1988), after induction of hypoglycemia and after surgery (James et al., 1970), after restraint via a twitch (Thompson et al., 1988) and following sexual activity (Tamanini et al., 1983; Rabb et al., 1989).

PRL concentrations also increase following various stressful stimuli in rats (Euker et al., 1975; Dohler et al., 1977; Neill, 1970) and man (Noel et al., 1972). Thompson et al. (1988) found no effect of restraint via a twitch on PRL secretion in OVX pony mares although ketamine administration briefly increased PRL concentrations. Sexual stimulation increases PRL secretion in rats (Kamel et al., 1975, 1977; Kamel and Frankel, 1978) and bulls (Convey et al., 1971) as well as in stallions (Rabb et al., 1989).
Rabb et al. (1989) reported that stallions which had previously been exposed to sexual stimulation had increases in cortisol secretion and, to a lesser degree, PRL secretion in the absence of any visual or physical contact with mares. The authors suggested that these increases were due to the sounds of other stallions being stimulated at the same time.

The three experiments described herein were designed 1) to determine if stallions exhibited an increase in cortisol and/or PRL secretion when returned to the site of a previous sexual encounter and, if so, to determine how long the response lasted, 2) to compare the cortisol and PRL responses after sexual excitement to those occurring after various physical and chemical stimuli and 3) to determine how these same stimuli affected cortisol and PRL concentrations in geldings.

Materials and Methods

**Experiment I.** Four lighthorse stallions aged 6 to 14 yr were used. All stallions were sexually experienced and were given at least 7 d of sexual rest prior to the onset of the experiment. Stallions were housed in outdoor lots and were fed sufficient hay and grain concentrate to maintain good body condition. At approximately 0700 on the mornings of treatment, each stallion was fitted with a jugular catheter made from 14 cm of 18-gauge polyethylene tubing which was flushed with heparinized saline. Stallions were kept loosely tethered with ad libitum access to hay and water.

Beginning at approximately 0800 on d 1, 3, 4, 5 and 6, two 5-ml blood samples were drawn via the catheter 10 min apart (-10 and 0 min)
and were placed into heparinized tubes. Immediately following the 0 min sample, each stallion was led to a specified site along the fence of his lot and held there for 5 min. On d 2, the same procedure was followed except that an estrous mare was also brought to the site (across the fence) such that the stallion could make visual and limited physical contact for a total of 5 min. On all days, additional blood samples were drawn at 10, 20 and 30 min after time 0.

Experiment II. Six sexually experienced lighthorse stallions aged 6 to 14 yr were managed as described for Experiment I. After at least 7 d of sexual rest, all stallions were exposed to each of five treatments: 1) sexual stimulation (SS; 5 min exposure to an estrous mare); 2) acute physical exercise (PE; running for 5 min); 3) restraint via a twitch (RT; 5 min); 4) epinephrine administration (EA; 3 mg i.v.) and 5) control (no stimulation). Stallions received one treatment per day on five separate days, with 2 d of sexual rest between treatments (based on results of Experiment I). The order of treatments was unique for each stallion (Table 1). Also, stallions were started on treatment on a staggered schedule such that only two stallions were treated on any given day.

Blood sampling and other procedures were the same as described for Experiment I. Immediately following the 0 min blood sample, the appropriate treatment was administered to the stallion. Subsequent samples were taken at 10, 20 and 30 min after time 0. To minimize the amount of activity seen and heard by a stallion on his treatment day, the two treated stallions for each day were housed approximately 100 m apart.
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*Treatments were sexual stimulation (SS), acute physical exercise (PE), restraint via a twitch (RT), epinephrine administration (EA) and no treatment (control; C).*
Experiment III. Six long-term geldings were exposed to the same five treatments described for Experiment II. Geldings were managed in the same manner as the stallions. Geldings also received a unique sequence of treatments (Table 1) with each gelding receiving each treatment once with 2 d of rest between treatments. Sampling procedure and frequency were identical to those described for Experiment II.

RIA and statistical analyses. All blood samples were stored on ice and were centrifuged within 1 h after collection. Plasma was harvested and stored at -15°C. Plasma concentrations of PRL and cortisol were measured by RIA as described previously (Thompson et al., 1986b; 1988). For each hormone, all samples from a given stallion were included in a single assay.

In all experiments, data for each hormone were analyzed by split-plot ANOVA with stallions as the random effect (Steel and Torrie, 1980). Fixed main effects were days and sampling times in Experiment I and treatments and sampling times in Experiments II and III. Data were also analyzed following log transformation but this was found not to affect the overall results. Prior to analysis, data from each stallion were placed on a percent of pre-treatment basis (average of -10 and 0 min samples); however, only post-treatment samples (10, 20 and 30 min) were used in the analysis of variance. The least significant difference test was used to determine if treatment means differed from 100% (Steel and Torrie, 1980).
Results

Experiment I. Cortisol and PRL concentrations in stallions increased (P < .05) only on the day of exposure to an estrous mare (d 2; Figure 1). This increase occurred within the first 10 min for PRL, which remained elevated through the 20 and 30 min sampling periods. Cortisol concentrations increased only in the 30-min sample. On the pre-treatment (d 1) and all post-treatment days (d 3, 4, 5 and 6), none of the means for either cortisol or PRL differed from 100%.

Experiment II. Cortisol concentrations in stallions during the control period did not vary (P > .05) relative to pre-treatment (Figure 2). Cortisol concentrations for all other treatments increased (P < .05) rapidly after the onset of treatment. These concentrations remained elevated throughout 30 min for all treatments.

PRL concentrations increased (P < .05) in stallions in response to SS and PE (Figure 2) at 10 min and remained elevated throughout the remainder of the experimental period. PRL concentrations increased (P < .05) in response to RT only at 10 and 30 min. Means for control and EA treatments did not vary (P > .05) relative to time 0.

Experiment III. Cortisol concentrations in geldings for the control period and SS treatment did not vary (P > .05) relative to pre-treatment. Cortisol concentrations increased (P < .05) within 10 min after the onset of PE and within 20 min following EA and RT (Figure 3) and remained elevated through 30 min.
Figure 1. Concentrations of cortisol and prolactin in plasma of stallions exposed to an estrous mare for 5 min on d 2 and returned to the site of exposure on d 3, 4, 5 and 6. Day 1 was also a control period (no mare present). Data are expressed as percent of pre-treatment. Horizontal dashed lines bracketing 100% indicate ± the least significant difference (P < .05). Pooled standard errors for cortisol and prolactin were 8.57 and 7.43% respectively.
Figure 2. Concentrations of cortisol and prolactin in plasma of stallions exposed to sexual stimulation, physical exercise, restraint via a twitch, epinephrine administration or no stimulation (control) beginning immediately after time 0. Data are expressed as percent of pre-treatment. Horizontal dashed lines bracketing 100% indicate ± the least significant difference (P < .05). Pooled standard errors for cortisol and prolactin were 10.38 and 5.66% respectively.
Figure 3. Concentrations of cortisol and prolactin in plasma of geldings exposed to sexual stimulation, physical exercise, restraint via a twitch, epinephrine administration or no stimulation (control) beginning immediately after time 0. Data are expressed as percent of pre-treatment. Horizontal dashed lines bracketing 100% indicate ± the least significant difference (P < .05). Pooled standard errors for cortisol and prolactin were 7.7 and 16.37% respectively.
PRL concentrations in geldings did not vary (P < .05) relative to pre-treatment during the control period or following EA or RT (Figure 3). However, PRL concentrations increased rapidly (P < .05) following PE and SS and remained elevated through 30 min.

Discussion

The results of Experiments I and II were similar to those reported by Rabb et al. (1989) for cortisol and PRL concentrations in stallions following sexual stimulation with or without ejaculation. In all three experiments, PRL concentrations increased rapidly following sexual stimulation and then declined. Similar results have been reported for the rat (Kamel et al., 1975, 1977; Kamel and Frankel, 1978) and bull (Convey et al., 1971), in which PRL concentrations peaked rapidly following mating. However, it has been reported that PRL concentrations vary considerably in man following sexual intercourse (Noel et al., 1972; Stearns et al., 1973).

There was no increase in cortisol or PRL secretion on subsequent days following return of the stallions to the site of their previous sexual stimulation. These data confirm the contention of Rabb et al. (1989) that the cortisol and PRL responses during control periods in their stallions previously exposed to sexual stimulation were not induced by the location of the control treatments but rather by the sounds of other stallions being sexually stimulated at the same time. We also concluded from Experiment I that 2 days of rest between treatments would be more than sufficient to avoid carry-over effects.
from one treatment to the next, assuming that sounds and other potential stimuli were absent.

Cortisol concentrations in stallions following sexual stimulation in Experiment I increased at a slower rate than those in Experiment II or those reported previously (Tamanini et al., 1983; Rabb et al., 1989). In the latter three experiments, cortisol concentrations increased within 10 min or less relative to treatment. In Experiment I, cortisol concentrations did not rise until 30 min following treatment. The reason for this delayed response is not known.

In contrast to stallions, geldings exhibited no increase in cortisol secretion after exposure to an estrous mare. As expected, geldings showed only casual interest in mares during the exposure period. Because there was no sexual excitement, there was no associated physical excitement, and hence no apparent stress response. However, there was a PRL response to SS in the geldings. This response was observed in only 3 of the 6 geldings used in the study, but due to magnitude of the increase in these animals, a level of significance was achieved. Due to the apparent variation to this stimulus among geldings, it is uncertain as to the exact nature of this observed increase. The only other treatment in which geldings differed from stallions was RT, which itself was variable in stallions. We feel that the cortisol and PRL responses observed in these experiments are likely mediated through similar mechanisms in stallions and geldings, and are not gonadally dependent. However, it may be that stallions are generally more excitable due to the presence of testicular androgens and
estrogens in their blood, and therefore may respond to stimuli of intermediate intensity more readily than geldings.

In stallions as well as geldings, bolus administration of epinephrine resulted in a rapid increase in cortisol concentrations in all animals. James et al. (1970) injected similar amounts of epinephrine into two ponies over a 2 to 5 min period and found no significant increase in cortisol concentrations, even though lacrimation, profuse sweating, trembling and rapid respiration were observed. The slower rate of administration used by James et al. (1970) apparently accounted for the lack of cortisol rise in their ponies. The lack of a PRL response to epinephrine injection indicates that the PRL responses to other forms of stressful stimuli are via a generalized neural stimulation, rather than through catecholamine or cortisol stimulation.

Physical activity for 5 min caused a rapid increase in cortisol concentrations both in stallions and geldings. These results are in agreement with others concerning cortisol concentrations in horses following exercise of various intensities and durations (Snow and Munro, 1975; Dybdal et al., 1980; Snow et al., 1982; Church et al., 1987; Hower and Wickler, 1989). It was reported that physical training does not affect this increase in cortisol (Church et al., 1987), although trained horses do have a more rapid recovery rate of cortisol concentrations, decreasing back to normal within 30 min (Hower and Wickler, 1989). In Experiment II and III, all stallions and geldings were untrained, which likely accounts for the continued elevation of cortisol concentrations at 30 min after treatment in the stallions.
Restraint via a twitch caused a rapid increase in cortisol concentrations both in stallions and geldings. Thompson et al. (1988) reported a similar increase in cortisol secretion in ovariec-tomized pony mares following RT, with peak concentrations occurring within 20 min. In contrast to ovariec-tomized pony mares and geldings, stallions also exhibited a transient increase in plasma PRL concentrations following RT. Given the diversity in these three experiments and the variability in response within Experiment II, it is apparent that the PRL response to RT is not as consistent as the cortisol response.

The increased PRL secretion in stallions following sexual excitement has been speculated as being part of a generalized stress response rather than a result of any specific reproductive function (Rabb et al., 1989). The results of this study are consistent with this idea, given that similar PRL and cortisol responses occurred in stallions and geldings following various types of stressful stimuli. However, the fact that RT produced varied results indicates that all apparently stressful stimuli may not have the same impact on PRL secretion. Because the role of PRL in the metabolism of the horse has yet to be defined, the reason for its secretion in response to stress is unknown.
CHAPTER IV

PLASMA CONCENTRATIONS OF CORTISOL, PROLACTIN, LH AND FSH IN STALLIONS FOLLOWING PHYSICAL EXERCISE AND INJECTION OF SECRETAGOGUE BEFORE AND AFTER SULPIRIDE TREATMENT IN WINTER

ABSTRACT

Ten lighthorse stallions were used to determine 1) if the PRL and cortisol responses observed after acute exercise in summer would occur in winter when PRL secretion is normally low, 2) if subsequent sulpiride treatment for 14 d would increase PRL secretion and response to TRH and exercise and 3) if secretion of LH, FSH and cortisol would be affected by sulpiride treatment. On January 11, blood samples were drawn from all stallions prior to and after a 5-min period of strenuous running. On January 12, blood samples were drawn before and after an i.v. injection of GnRH plus TRH. From January 13 through 26, five stallions were treated with 500 mg of sulpiride s.c. daily; the remaining five stallions were given vehicle. Blood samples were drawn immediately before treatment each day. The exercise and secretagogue regimens were repeated on January 27 and 28, respectively. Prior to sulpiride treatment, concentrations of both cortisol and PRL increased (P < .05) by 40 to 80% in response to exercise; concentrations of LH and FSH also increased (P < .05) by approximately 10%. Sulpiride treatment
stimulated (P < .05) daily PRL secretion by approximately 5-fold, and caused a decrease (P < .05) in daily FSH secretion. However, only isolated differences (P < .05) occurred between treatment groups for daily LH and cortisol secretion. At the end of treatment, there was no PRL response to exercise for stallions treated with sulpiride, but there was (P < .05) for control stallions; only control stallions exhibited a marginal cortisol response (P < .05) to exercise. The PRL response to TRH was increased (P < .05) 4-fold in stallions treated with sulpiride but was unchanged in control stallions. Sulpiride treatment did not affect (P > .05) the LH or FSH response to exogenous GnRH. We conclude that 1) stallions do respond to acute exercise in winter with an increase in secretion of both cortisol and PRL, 2) treatment with sulpiride during winter increases daily secretion and perhaps production of PRL in stallions, indicating that the dopaminergic suppression of PRL is present in winter and 3) the increase in PRL characteristics due to sulpiride does not stimulate the PRL response to exercise, but does increase the PRL response to TRH.

Introduction

PRL secretion fluctuates annually in many species with highest concentrations occurring during summer (Ravault, 1976; Ravault et al., 1982; Petitclerc et al., 1983; Stanisiewski et al., 1987). Several studies have shown that a similar pattern of PRL secretion occurs in the horse (Thompson and Johnson, 1987; Thompson et al., 1987). In addition, Thompson et al. (1986a) found that pituitary PRL concentrations in horses were higher in summer than in winter, as was the PRL response to
exogenous TRH (Thompson et al., 1986b). PRL concentrations also increase in horses following treatment with perphenazine (Ireland et al., 1989; Loch et al., 1990), metoclopramide (Johnson and Becker, 1987) or sulpiride (Johnson and Becker, 1987).

Rabb et al. (1989) reported that exposing stallions to an estrous mare caused rapid surges in secretion of PRL and cortisol. More recently, it was shown that PRL concentrations also increase in stallions following various types of physical stress (Chapter III, Experiment II). These latter experiments were performed during the summer when PRL secretion is normally high (Johnson, 1986; Thompson et al., 1987). Thus, the objectives of the present experiment were to determine 1) if the PRL and cortisol responses observed after acute exercise in summer would occur in winter when PRL secretion is normally low, 2) if subsequent sulpiride treatment for 14 d would increase PRL secretion and response to TRH and exercise and 3) if secretion of LH, FSH and cortisol would be affected by sulpiride treatment.

Materials and Methods

Ten lighthorse stallions aged 4 to 14 yr were used. All stallions were sexually experienced and had received at least 60 d of sexual rest before the experiment. Stallions were kept in outdoor lots and were fed hay and a grain mixture to maintain good body condition.

At approximately 0700 on January 11 (d -2), a 14-gauge indwelling catheter was placed in one jugular vein of each stallion. All stallions were then allowed to rest for a period of 1 h. After two blood samples (5 ml) were taken 10 min apart (-10 and 0 min) via the catheter, each
stallion was lunged in a circle within its pen for a period of 5 min. Additional blood samples were drawn at 10, 20 and 30 min relative to time 0.

On d -1, stallions again received jugular catheters at approximately 0700 and three blood samples (5 ml) were taken 15 min apart (-30, -15 and 0 min). Then a mixture of GnRH and TRH (.5 and 2 mg, respectively) was injected i.v. via the catheter of each stallion. Subsequent blood samples were taken at 15, 30, 45, 60, 75, 90, 120, 150, 180, 210 and 240 min after the GnRH/TRH injection.

For treatment, stallions were randomly allotted into two groups of five. Blood samples were drawn from each stallion via jugular venipuncture at approximately 0700 each day from d 0 through 13. On these same days, five stallions were administered 500 mg of sulpiride s.c. in 2 ml of vegetable shortening. The five remaining stallions received daily injections of 2 ml of shortening.

On d 14, stallions were catheterized, bled and exercised as described for d -2. On d 15, stallions were catheterized, bled and administered GnRH and TRH as described for d -1. All blood samples collected in this experiment were placed into heparinized tubes, were stored on ice and were then centrifuged at 1200 x g within 1 h after collection. Plasma was harvested and stored at -15°C. Plasma concentrations of LH, FSH, PRL and cortisol were measured by RIA as described previously (Thompson et al., 1983a,b; 1986b; 1988). All samples from a given stallion were included in a single assay for each hormone.
Hormonal data collected at the first exercise period were first placed on a percent of pre-treatment basis and were then analyzed by ANOVA with stallions (n = 10) and times as main effects (Steel and Torrie, 1980). Only post-treatment samples were analyzed in the ANOVA. The least significant difference (LSD) value (Steel and Torrie, 1980) was used to identify means that differed from 100%. Data collected over time from the two treatment groups were analyzed by ANOVA that accounted for the repetitive nature of the sampling (split-plot design; Gill and Hafs, 1971); differences between groups for each period were determined by the LSD test. Net areas under the LH, FSH and PRL curves after the two GnRH/TRH injections were calculated as described by Thompson and Nett (1984) and were analyzed by split-plot ANOVA.

Results

Plasma concentrations of LH, FSH, cortisol and PRL following the first period of acute exercise are shown in Figure 4. Concentrations of cortisol and PRL increased (P < .05) 40 and 80%, respectively, within 20 min after onset of exercise. Concentrations of LH and FSH also increased (P < .05) after exercise, but to a much lesser extent (about 10%).

During the treatment period, two of the stallions in the treated group became lethargic and ceased eating. These stallions were treated with a single injection of Banamine and vitamin B complex. Since no other problems occurred and a normal appetite returned, these two stallions were allowed to continue on the project.

Concentrations of FSH in daily plasma samples of treated animals
Figure 4. Mean concentrations of LH, FSH, cortisol and prolactin (expressed as % of pre-treatment) in plasma of stallions (n = 10) before and after 5 min of strenuous exercise (initiated at time 0) on d -2. Dashed lines indicate ± one LSD value (P < .05) from 100%. Pooled standard errors for LH, FSH, cortisol and prolactin were 2.23, 1.48, 4.75 and 21.67% respectively.
began to decline (P < .05) on d 7 and remained suppressed relative to controls throughout the remainder of the study (Figure 5). LH concentrations in daily samples differed (P < .05) between groups on several days (Figure 5) but there was no consistent pattern evident.

Concentrations of cortisol in daily blood samples also differed on a few isolated days (Figure 5). However, values for both groups tended to decrease equally as treatment progressed.

Concentrations of PRL in daily blood samples increased (P < .05) 8-fold within 4 d of sulpiride treatment and remained elevated (P < .05) during treatment (Figure 5). Concentrations of PRL in control stallions remained low throughout the treatment period.

In the second exercise period (post-treatment), there was no FSH response (P > .05) to exercise in either group of stallions (Figure 6). However, a transient rise in LH occurred in the treated stallions. The cortisol response to exercise persisted (P < .05) only in the control group of stallions; in addition, only control stallions exhibited an increase (P < .05) in PRL concentrations in response to the second exercise period (Figure 6).

Relative to the first injection of GnRH/TRH, there was no change (P > .05) in the LH or FSH response to GnRH in either group of stallions after treatment (Figure 7). In contrast, treatment with sulpiride resulted in a 4-fold increase (P < .05) in the PRL response to TRH (Figure 7). Although the PRL response to TRH was initially greater (P < .05) in control stallions than in stallions treated with sulpiride, there was no change (P > .05) in response in control stallions from the first to the second administration of TRH.
Figure 5. Mean concentrations of LH, FSH, PRL and cortisol in daily plasma samples from stallions treated with 500 mg of sulpiride (Treated) or vehicle (Control) before (d -2, -1 and 0) and through 14 d of treatment. The vertical line within each panel indicates the LSD value (P < .05) for comparison between groups within each day. Pooled standard errors for LH, FSH, cortisol and prolactin were 15.53, 4.29, 2.28 and 53.2% respectively.
Figure 6. Mean concentrations of LH, FSH, cortisol and prolactin (expressed as % of pre-treatment) in plasma of stallions previously treated with sulpiride (Treated) or vehicle (Control) before and after 5 min of strenuous exercise (initiated at time 0) on d 14. Dashed lines indicate ± one LSD value (P < .05) from 100%. Pooled standard errors for LH, FSH, cortisol and prolactin were 14.55, 3.49, 10.12 and 11.49% respectively.
Figure 7. Mean concentrations of LH, FSH and prolactin in plasma of stallions before and after an injection of GnRH/TRH on d -1 (Pre-treatment) and d 15 (Post-treatment). Bar graphs (plus 1 standard error) indicate the net areas under the curves for each hormone. Means with different superscripts differ (P < .05).
Discussion

PRL concentrations increased rapidly in these stallions following exercise in winter. This is in agreement with previous data from stallions during the summer months (Experiment I). Thompson et al. (1986b) reported that the PRL response to TRH was greatest in summer and minimal in winter. Therefore, it was uncertain whether PRL would be secreted in stallions following physical exercise during winter. Although this response was variable among animals, it is concluded that acute exercise will cause an increase in PRL concentrations during the winter months.

Cortisol concentrations have also been shown to increase in horses following various stressful stimuli such as exercise (Snow and Munro, 1975; Dybdal et al., 1980; Snow et al., 1982; Church et al., 1987; Hower and Wickler, 1989) and following sexual activity (Tamanini et al., 1983; Rabb et al., 1989). The increase in cortisol following exercise in this study is consistent with those findings. However, the magnitude of the response was lower than that previously reported for stallions following exercise during the breeding season (Chapter III, Experiment I).

Acute exercise also caused a slight increase in LH and FSH secretion within 10 min. It was previously reported (Rabb et al., 1989) that sexual stimulation with or without accompanying ejaculation did not stimulate LH or FSH secretion in stallions during the breeding season. It is possible that the normally low secretion rate of LH and FSH in winter, which is thought to be due to reduced GnRH input to the pituitary (Hart et al., 1984), results in a relatively supersensitive state of the pituitary. Thus, a small release of GnRH from the
hypothalamus in response to exercise would be detectable in winter, but
would not be detectable in summer due to the normally higher rates of LH
and FSH secretion.

Various dopamine agonists and antagonists have been used to alter
PRL concentrations in horses, including perphenazine (Ireland et al.,
1989; Loch et al., 1990), metoclopramide (Johnson and Becker, 1987)
sulpiride (Johnson and Becker, 1987) and bromocriptine (Johnson and
Becker, 1987; Ireland et al., 1989). In those previous reports, PRL
concentrations in blood were altered rapidly in response to treatment.
Johnson and Becker (1987) reported that the D-2 dopamine receptor
blocker, sulpiride, caused a rapid increase in PRL secretion that lasted
approximately 5 h in mares in summer. Moreover, they found no
difference in PRL response to 25 or 100 mg of sulpiride. Thus, a daily
dose of 500 mg sulpiride was chosen for use in the present study to test
its ability to stimulate PRL production and secretion in stallions
during winter. Vegetable shortening was chosen as the vehicle because
it is known to slow the release of similar compounds into the blood
(e.g., steroids; McNeill-Wiest et al., 1988).

Treatment with sulpiride did in fact increase concentrations of
PRL in daily blood samples within 3 d. The increase persisted
throughout the remainder of the experimental period. Plasma
concentrations of PRL produced by sulpiride treatment were comparable to
those previously reported for stallions in summer (Thompson and Johnson,
1987; Thompson et al., 1987). This suggested that the pituitary was
released from dopaminergic suppression to a degree similar to that of
the normal pituitary in summer. Given that the stallion pituitary is
under dopaminergic suppression in both summer and winter, it is possible for the seasonal variations in PRL secretion to be due to a seasonal variation in dopaminergic input to the pituitary.

Concentrations of FSH in daily blood samples differed between treatment groups beginning on d 7. These data are in contrast to those reported for the hamster by Bartke et al. (1982), in which an increase in PRL secretion was accompanied by a dramatic rise in FSH secretion. However, because FSH concentrations in treated animals following physical exercise or administration of secretagogue did not differ following treatment, it is uncertain exactly how FSH might have been affected. Concentrations of LH in daily blood samples were greater in stallions treated with sulpiride than in control stallions on isolated days. Bartke et al. (1982) reported that plasma LH concentrations were unchanged in hyperprolactinemic hamsters that had undergone surgical transplantation of the pituitary to the renal capsules. From the data generated in the present experiment, it is concluded that neither sulpiride treatment nor an increase in PRL secretion had any stimulatory effect on LH, but possibly was inhibitory to FSH secretion.

Plasma concentrations of cortisol in daily blood samples did not consistently differ among groups. However, cortisol concentrations in animals from both groups tended to decrease throughout treatment. This was particularly evident among animals in the treated group. This may indicate some affect of sulpiride treatment on production and release of cortisol and/or ACTH.

Following sulpiride treatment, acute exercise did not increase FSH concentrations in either the treated or control stallions. There was a
transient rise in LH concentrations occurring within 20 min after exercise. This increase only occurred in the treated stallions and may be of a similar nature to that which occurred prior to treatment. That is, a small increase in GnRH is sufficient to cause a detectable increase in LH in winter when LH levels are normally low.

Acute physical exercise failed to cause an increase in cortisol response in treated stallions. However, the magnitude of the response for both groups did tend to be lower relative to the first period of exercise. Cortisol concentrations increased only at 20 min after onset of exercise in the control animals. This response was delayed in comparison to previously reported data (Experiment I), in which cortisol concentrations increased an average of 80% at 20 min and remained elevated through 30 min.

The PRL response to acute exercise was not altered in control stallions between the first and second exercise periods, whereas the response of stallions treated with sulpiride was diminished by treatment. This effect was opposite to that expected. It was thought that increasing the production and storage of PRL in the pituitary would result in a greater response to acute exercise. The fact that the response was diminished by sulpiride treatment may indicate that the pituitary was in fact less sensitive to whatever signal occurs with exercise to cause PRL release. This reduced sensitivity would be analogous to that discussed earlier for LH and FSH secretion in summer. That is, when production and secretion rates are high, a greater signal may be required to cause measurable release of hormone. An alternate explanation might involve the side-effects of sulpiride treatment. Two
of the stallions treated with sulpiride exhibited some lethargy and depression. Even though the remaining stallions in the group did not show clinical signs of depression, sulpiride treatment may have caused enough of a decrease in the activity of the central nervous system to negatively impact on the response to exercise.

The average responses of LH and FSH to exogenous GnRH were not affected by sulpiride treatment. Therefore, it is concluded that the hypothalamic-hypophyseal regulation of these gonadotropins does not involve dopaminergic neurons.

Administration of TRH caused a rapid release of PRL in all stallions. Although there was a difference between groups in PRL response to exogenous TRH before treatment with sulpiride, comparison of pre- to post-treatment responses revealed that there was no change in response for control stallions from the first to the second injection of TRH. In contrast, the PRL response to exogenous TRH was increased 4-fold in stallions treated with sulpiride. This fact, coupled with increased daily secretion rates an average of 6-fold, indicates that production rate of PRL must have been increased proportionally. Thus, antagonism of the dopaminergic system not only stimulates PRL secretion, but also stimulates PRL production. This fact may be useful from a practical standpoint once the role of PRL in the horse is better understood.

In conclusion, PRL and cortisol concentrations increased rapidly in response to acute exercise in stallions during the winter. The response was similar to that observed in stallions during the breeding season. Secretion of LH and FSH were also stimulated by acute exercise
In winter, but to a lesser degree than PRL and cortisol. Daily administration of sulpiride caused increased PRL secretion within 3 d after the onset of treatment. This increase was coupled with a significant increase in the PRL response to exogenous TRH, indicating a stimulation of PRL production. There was no apparent effect of sulpiride treatment on LH or cortisol characteristics, but treatment may have caused a decline in daily FSH concentrations.
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VITA

Donald Robert Colborn was born August 19, 1963 in Hannibal, Missouri to Robert and Betty Colborn. He graduated from Hannibal High School in 1981 and then began attending college at Hannibal-LaGrange College where he received the Associate of Science in Engineering in 1983. He then transferred to the University of Missouri-Columbia where he received the Bachelor of Science degree in Animal Science in 1985 and the Master of Science degree in Animal Science in 1987. He began work toward the Doctor of Philosophy at Louisiana State University in July of 1987. Following completion of this degree, the author plans to accept a post-doctoral fellowship to continue his current research in reproductive physiology, followed by an eventual faculty position at a land-grant university.

He and his wife, Lori, are parents of one son, James Robert, and a second child is due in November of 1990.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Donald R. Colborn

Major Field: Animal Science

Title of Dissertation: Regulation of Cortisol and Prolactin in Horses

Approved:

Donald L. Thompson Jr.
Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Ronald W. Kraus

Robert A. Stone

D.C. Frank

Dennis D. Frank

Lee Sauter

Keith C. White

Date of Examination:

June 22, 1990