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Dopaminergic input to the equine pituitary: seasonal and estradiol effects

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DOPAMINERGIC INPUT TO THE EQUINE PITUITARY:
SEASONAL AND ESTRADIOL EFFECTS

A Thesis

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ABSTRACT

Two experiments were conducted to study the effects of season and estradiol administration on dopaminergic input to the equine adenohypophysis. Experiment 1 began in the spring with 16 horses (8 mares, 8 stallions) and was repeated again in the summer, fall, and winter. Horses were given sulpiride injections of eight incremental concentrations (0.25 to 32 μg/kg BW). Within each group of 8 horses, half received the sulpiride in an increasing manner, the other half in a decreasing manner. Prolactin concentrations peaked in the first 15 to 30 min in all horses in all seasons. Prolactin areas under the curve increased ($P < 0.001$) with increasing doses of sulpiride, and were highest ($P < 0.05$) in March for stallions, but in June for mares. The calculated half-maximum values, which should be proportional to the dopaminergic input to the pituitary, were lowest ($P < 0.05$) in June and greatest in September. The variation in half-maximum values with season indicated a change in dopaminergic input to the pituitary, with lowest input occurring in June in both genders. The lack of gender effect for half-maximal values indicated that mares and stallions respond similarly to the seasonal signals that result in changes in hypothalamic dopamine input to the pituitary. Experiment 2 was designed to determine if the degree of dopaminergic input to the adenohypophysis is altered by estradiol administration. Twelve geldings were used. On day 0, all geldings received an i.m. injection of either estradiol cypionate (ECP) or vegetable oil. Of the 6 geldings receiving ECP, half received 2 mL of a solution of 50 mg/mL (100 mg) and half received 10 mL of a solution of 10 mg/mL (100 mg) with control oil injection volumes matching those of treatment injections. On day 6, all geldings received an injection of sulpiride at 0.082 μg/kg BW in saline. This regimen was repeated every other day with the dose increasing each day, from 0.164 to 100 μg/kg BW. Estradiol pretreatment increased ($P < 0.05$) the prolactin response to sulpiride at the 1.025 μg/kg

dose and higher. However, the half-maximum values for ECP-treated and control geldings did not differ, indicating that the amount of sulpiride needed to counterbalance the amount of dopamine reaching the pituitary was unaltered by estradiol treatment. It was concluded that estradiol likely stimulates prolactin production and secretion after sulpiride directly at the lactotrope level, rather than by decreasing hypothalamic dopamine input to the lactotropes.

INTRODUCTION

The horse is a seasonal breeder. Mares experience periods of estrus activity during the spring and summer months and a period of inactivity during the winter months (Ginther, 1992; Daels and Hughes, 1993). Due to a long-standing practice by many breed associations of using January 1st as the official birth date of all foals born within a calendar year, most breeders consider it economically important to have foals born as close to that date as possible. Thus, the goal of most producers is to have mares pregnant in mid-February of any given year, so that the resulting foals will be born soon after, but not before, the January 1st birth date. Such foals would be expected to have a size and growth advantage on foals born later in the season, thus countless time, money, and effort have been put into research of methods to stimulate early ovarian activity in the otherwise anovulatory mare.

As the natural period of seasonal breeding activity approaches, several hormones display increased concentration levels in the mare (Thompson et al., 1986). The adenohypophyseal hormone prolactin, which is associated with lactation in most mammalian species, also displays an increase in the mare during this time. This increase in prolactin is accompanied by an increase in the number of viable follicles for ovulation. In 1993, Nequin et al. showed that increase in prolactin levels, both by administration of dopamine antagonist or bovine prolactin, stimulated follicular growth during the anovulatory period. However, it was later reported that administration of sulpiride, a dopamine antagonist, did not stimulate follicular development enough during the winter months to facilitate early breeding (Donadeu and Thompson, 2002). One possible explanation for an insufficient increase in prolactin in response to dopamine antagonist administration is estrogen. In 1991, Thompson et al. showed that during the breeding season (summer months), estrogen administration increased the production and also secretion of

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prolactin. Estrogen is lacking during the anovulatory period of the winter months. In 2006,

Kelley et al. reported that prolactin concentrations were dramatically increased in response to

sulpiride after estradiol treatment, with the date of first ovulation being advanced by an average

of 45 days. As in other species, prolactin production and secretion are most likely under a tonic

inhibitory control in the horse, based on the positive secretory response to administration of

dopamine antagonists. If dopamine input is the only regulation of prolactin production and

secretion, then prolactin increases with increasing day length, or increases after estradiol

stimulation, would be expected to be a result of reduced dopaminergic input to the

adenohypophysis. If such changes were not accompanied by changes in dopaminergic input,

then some other stimulatory factor(s) would be inferred. Therefore, the purpose of the first

experiment was to test the hypothesis that changes in dopaminergic input to the adenohypophysis

would explain the naturally occurring changes in prolactin secretion throughout the four seasons

of the year. The second experiment was designed to determine whether the degree of

dopaminergic input to the adenohypophysis is altered by the administration of estradiol.

CHAPTER I

REVIEW OF LITERATURE

Hypothalamic-pituitary-gonadal axis

Extensive research has shown a link between the hypothalamic-pituitary-gonadal axis and reproductive function. Environmental stimuli, such as photoperiod, cause the hypothalamus to send signals to other parts of the brain by way of chemical neurotransmitters (Guyton and Hall, 1996). A major hormone produced by the hypothalamus to regulate reproductive function is gonodotropin-releasing hormone (GnRH), a decapeptide hormone that affects the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the adenohypophysis (Alexander and Irvine, 1993). Throughout the seasons of the year, circulating blood levels of these hormones fluctuate in accordance with the reproductive status of the mare (Irvine and Alexander, 1993). When stimulated by external signals, such as photoperiod, stored GnRH from secretory granules within the median eminence of the hypothalamus is released and travels to the adenohypophysis via the capillary system of the primary plexus (Alexander and Irvine, 1993). Upon reaching the adenohypophysis, GnRH binds to receptors located on gonadotropes found in the pars distalis (Alexander and Irvine, 1993). The release of GnRH, and thereby LH and FSH, is pulsatile in fashion (Alexander and Irvine, 1993). A high frequency of GnRH pulses results in a greater rise in LH concentrations than FSH concentrations (Alexander and Irvine, 1993); alternately, a low frequency of GnRH pulses results in low LH and higher FSH secretion.

Ovarian function is highly dependent on the aforementioned gonadotropins (Alexander and Irvine, 1993). Hormone-specific receptors are located on different areas of the equine ovary. Most FSH receptors are found on the granulosa cells of ovarian follicles, while LH receptors are

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mainly located on thecal cells of the follicle (Alexander and Irvine, 1993). Along with follicular

maturation comes the production of estradiol by the follicle, through the cooperative actions of

both thecal and granulosa cells (Alexander and Irvine, 1993). This estradiol is responsible for

the increase in LH that ultimately triggers follicular rupture and ovulation (Nett, 1993). Thus, it

is likely that without the influence of the hypothalamic-pituitary axis on the gonads, there would

be no functional reproductive cycle in the mare.

Prolactin

Stricker and Grueter reported in 1928 (cited by Hadley and Levine, 2006) that milk

secretion was stimulated in rabbits given an extract of the adenohypophysis. However, when

injected directly into the ducts of the mammary gland, only the adjacent alveoli responded with

milk production. This indicated that prolactin must act in concert with other hormones to

stimulate generalized somatic effects (Hadley and Levine, 2006). Riddle, Bates and Dykshorn

concluded in 1933 (cited by Hadley and Levine, 2006) that there is a distinct part of bovine

pituitary extract that, when administered to pigeons, stimulated the growth of their crop sac.

They called this extract 'prolactin' (Hadley and Levine, 2006). Prolactin is produced in the

adenohypophysis and is a 199-amino acid, single-chain protein (Nett, 1993). While generally

thought of as a hormone associated primarily with mammary growth and lactation, prolactin is a

diverse hormone that seems to be tied to aspects of reproductive function in females as well as

males (Nett, 1993).

Seasonal effects on prolactin

Circulating levels of prolactin in mares are higher during the natural breeding season

(Johnson, 1986), commonly accepted to be April to September in the northern hemisphere

(Ginther, 1992). Photoperiod is not the only external factor that can affect prolactin levels,

however. Mares exposed to 16 hours of light in the fall and winter displayed an increase in prolactin secretion (Johnson, 1986; Nett, 1993). It was reported that exposure of anestrous mares to additional artificial light to simulate increased daylength of the spring and summer months could induce early onset of cyclicity (Sharp et al., 1975). This practice of extended light exposure is commonly and effectively used to induce early cyclicity in seasonally anovulatory mares (Sharp and Davis, 1993). However, using artificial light to induce early cyclicity in mares is expensive and labor intensive, and other methods of inducing ovarian stimulation are still being actively pursued.

In stallions, as well as geldings, circulating levels of prolactin display seasonal changes, indicating that in males, the gonads do not regulate prolactin secretion (Nett, 1993). Hypophysecotomy in adult male rats resulted in a decrease in the number of testicular LH receptors (Hadley and Levine, 2006). In mares, both circulating and stored prolactin levels are highest in the summer and lowest in the winter (Thompson et al., 1986). Another seasonal effect that seems to be controlled by prolactin is hair shedding in horses (Thompson and Depew, 1997) as well as in sheep (Lincoln and Tortonese, 1995). It should be recognized, however, that aside from the annual variations in prolactin levels due to season, there are no notable variations in accordance with the stage of the estrus cycle of the mare (Worthy et al., 1986; Nett, 1993). In smaller species, removal of the pituitary from its location under the hypothalamus and relocation to another part of the body results in a dramatic reduction in secretion rates of all adenohypophyseal hormones except for that of prolactin (Guyton and Hall, 1996), which actually increases. This indicates that prolactin is not stimulated by hormones from the hypothalamus but is under constant inhibitory control (Hadley and Levine, 2006).

Dopamine and Dopamine Antagonists

Dopamine is a neurohormone in the catecholamine class derived from the amino acid tyrosine (Guyton and Hall, 1996). When stimulated, dopamine is released into the bloodstream and reaches postsynaptic neuronal membranes, where it typically has an inhibitory effect (Hadley and Levine, 2006). It is believed that the normal regulation of prolactin production and secretion by the adenohypophysis is via tonic inhibition by dopamine (Guyton and Hall, 1996). As mentioned, disruption of the normal communication from the hypothalamus to the adenohypophysis results in increased prolactin secretion (Hadley and Levine, 2006). This idea of hypothalamic inhibition is confirmed by the observation that pituitary cells in culture also increase prolactin production and secretion (Oosterom et al., 1983), and dopamine added to the culture reduces prolactin production and secretion (Zhang et al., 1990). Like most other hypothalamic hormones, dopamine is secreted by the hypothalamus in response to neural signals transmitted from other parts of the brain (Guyton and Hall, 1996). The transport of neurosecretory chemicals, such as dopamine, occurs via the tuberoinfundibular tract. The neurons that make up the hypothalamus are divided into two groups – parvocellular and magnocellular, which are small and large cells, respectively (Hadley and Levine, 2006). The neurons of the parvocellular system meet at the pituitary stalk. This cluster is called the tuberoinfundibular tract and is so named for its connection from the tuber cinerum of the third ventricle to the infundibulum of the pituitary (Hadley and Levine, 2006). The neurons of this system connect to the primary plexus of the hypophyseal portal system of the median eminence (Hadley and Levine, 2006). This network of vascularization provides a restricted pathway between hypothalamic neurosecretory cells and those of the adenohypophysis (Hadley and Levine, 2006). Two different sub-types of dopamine receptors were discovered in 1979,

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referred to as D-1 and D-2 (Kebabian and Calne, 1979). The D-2 type receptors are found on the lactotropes in the adenohypophysis and mediate the effect of dopamine on prolactin production and secretion (Munemura et al., 1980; Creese et al., 1983).

Sulpiride is a D-2 dopamine receptor blocker, and is therefore referred to as a dopamine antagonist. In 1978, Advis and Ojeda showed that in prepubertal female rats, the administration of sulpiride resulted in increased serum prolactin levels to the point of hyperprolactinemia as well as an early onset of puberty (Advis and Ojeda, 1978). In 1987, Johnson and Becker showed that sulpiride stimulated prolactin secretion in mares. Many studies followed to further explore this idea in mares as well as stallions (Colborn et al., 1991b; Thomson et al., 1996) and geldings (Thompson and Depew, 1997). In 1994, Redmond et al. found that domperidone (another dompaminergic antagonist) and sulpiride were both effective ways to increase prolactin levels and thereby treat fescue toxicosis in pregnant mares. Shortly thereafter, in 1997, Besognet et al. treated a group of seasonally anestrous mares with sulpiride in order to stimulate circulating prolactin concentrations and advance the mean date of their first ovulation. Prolactin concentrations were elevated at 2 and 9 hours after sulpiride injection in treated mares; there was no significant elevation in control mares. The treated group ovulated at a mean day of 77 (day of the year), whereas the control group ovulated at a mean of day 110.

Thyrotropin-releasing hormone (TRH)

Thyroid-stimulating hormone (TSH), also referred to as thyrotropin, is a glycoprotein hormone synthesized in the adenohypophysis in response to stimulation from TRH, a tripeptide produced in the hypothalamus (Hadley and Levine, 2006). The stimulation of TRH and subsequent release of TSH is accompanied by the release of prolactin in most mammals, an event mediated by specific receptors for TRH found on lactotropes (Gersch, 1979). In 1987, Johnson

and Becker showed that administration of TRH to both mares in estrus and diestrus stimulated serum prolactin concentrations, indicating that this model holds true in the horse as well. Colborn et al. (1991b) reported that administration of TRH to stallions in winter resulted in an increase in serum prolactin concentrations. Stallions previously treated with sulpiride for 10 days had a much greater prolactin response to TRH compared to vehicle-treated stallions, indicating that sulpiride stimulated both the production and secretion of prolactin at this time of the year. In contrast, daily treatment of mares with TRH in the winter resulted in a rapid (within 4 days) loss of the prolactin response, indicating that pituitary stores were being depleted, and prolactin production was not being stimulated (Gentry et al., 2002).

Exercise and Stress

In 1975, Euker et al. reported that acute stress stimulated serum prolactin concentrations in both intact and castrated male rats. Sexual stimulation has also been reported to increase serum concentrations of prolactin in male rats (Kamel, 1975). This response was shown to hold true for horses as well when, in 1989, Rabb et al. showed that sexual stimulation with and without ejaculation resulted in a rise in both serum prolactin and cortisol concentrations. To test the effects of exercise-related stress as well as stress related to sexual stimulation, Colborn et al. (1991a) executed a series of experiments and found that plasma prolactin and cortisol concentrations increased after both sexual stimulation and acute physical exercise in stallions; only cortisol concentrations increased after epinephrine injection. Those authors suggested that while the release of cortisol with regard to stress is likely due to a mass discharge of the sympathetic nervous system, the stress-related prolactin release is likely controlled by a neural pathway not mediated by catecholamines.

Estradiol effects on prolactin

In the mare, estradiol is produced and secreted by the growing ovarian follicle (Alexander and Irvine, 1993). In 1984, Brar and Fink reported that estradiol (estradiol-17β) and estrone administered to male rats increased plasma concentrations of prolactin. In 1991, Thompson et al. showed that treatment of ovariectomized pony mares with estradiol for 21 days briefly increased plasma prolactin levels about 15%, whereas the pituitary content at slaughter was increased 5-fold relative to vehicle-treated controls. Aurich et al. (1995) reported that estradiol benzoate, an estradiol analog, stimulated serum concentrations of both prolactin and LH, when administered in conjunction with naloxone, an opiod antagonist. In an attempt to stimulate prolactin concentrations in seasonally anovulatory mares and hasten ovulation, Kelley et al. (2006) treated mares every other day with estradiol benzoate for 10 days, followed by daily injections of sulpiride. Prolactin concentrations were dramatically increased in response to sulpiride after estradiol treatment relative to controls, and date of first ovulation was advanced by an average of 45 days.

Rationale for the present experiments

As mentioned earlier, prolactin production and secretion are assumed to be under tonic inhibitory control in the horse, as has been well described in other species, due to the positive secretory response to administration of dopamine antagonists. However, there have been reports for other species of possible stimulatory factor(s) from the hypothalamus (putative prolactin releasing factors), other than TRH (Tóth et al., 2001). If dopamine input were the only regulation of prolactin production and secretion, then prolactin increases with increasing daylength, or increases after estradiol stimulation, would be expected to be a result of reduced dopaminergic input to the adenohypophysis. If such changes were not accompanied by changes

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in dopaminergic input, then some other stimulatory factor(s) would be inferred. The purpose of the two experiments described herein was to test the hypothesis that changes in dopaminergic input to the adenohypophysis would explain 1) the naturally occurring changes in prolactin secretion over the four seasons of the year and 2) the stimulation of prolactin production and secretion in response to estradiol treatment. The approach was to use classic dose-response analysis (Tallarida, 1979) of the prolactin response to sulpiride to determine the half-maximum point (dose). The half-maximum dose should be an indication of the relative amount of dopamine reaching the adenohypophysis at the time of testing, and shifting of the half-maximum point left (towards smaller doses) would imply lesser dopaminergic input; conversely, shifting to the right (towards higher doses) would imply a greater dopaminergic input.

CHAPTER II

DOPAMINERGIC INPUT TO THE ADENOHYPOPHYSIS: REGULATION OF PROLACTIN SECRETION ACROSS FOUR SEASONS OF THE YEAR

Introduction

The current model of regulation of prolactin secretion in horses, similar to that of most mammals, is via dopamine input from the tuberoinfundibular dopaminergic (TIDA) system. Dopaminergic neurons that originate in the medialbasal hypothalamus project into the pars tuberalis of the adenohypophysis. Dopamine is then secreted into the hypothalamic hypophyseal portal system that feeds into the pars distalis. There, dopamine binds to the lactotropes and keeps prolactin secretion suppressed. Excessive prolactin secretion, or exogenous administration of prolactin, feeds back on the TIDA system to enhance dopaminergic activity, thereby completing a short-loop feedback and maintaining prolactin secretion. The TIDA neurons are also thought to be melatonin responsive, such that seasonal changes in photoperiod could be translated into increases or decreases in dopaminergic input to the pituitary. This seasonal change could result in low prolactin production and secretion levels in the winter and high levels in the summer. If seasonal variations in prolactin secretion in the horse are mediated by a variation in dopaminergic input from the TIDA system, then the dose of a dopaminergic antagonist to counterbalance this input should vary proportionally. Based on this approach, the present experiment was designed to test the hypothesis that the degree of dopaminergic input to the adenohypophysis in mares and stallions differs across seasons proportionally with the changes in prolactin secretion.

Materials and methods

   Animals and treatments. Sixteen horses were selected; all were either Quarter Horse or Thoroughbred type.   Both stallions and light horse mares were used, eight of each sex. Ages of the subjects ranged from 7 to 19 years old. Body condition scores (Henneke et al., 1983) of stallions ranged from 4.5 to 5.5 and that of mares ranged from 5.5 to 7.5. All animals were housed at the Louisiana State University Agricultural Center Equine Unit, Ben Hur Farm. Stallions were kept in individual paddocks with run-in sheds and mares were kept in adjacent pastures. Horses were maintained on native pasture grasses and supplemented with grass hay when necessary.

   Beginning in March 2008, the horses were administered sulpiride in saline (a racemic mixture was used; doses based on the L-isomer only) approximately every other day at the following doses: 0.25, 0.5, 1, 2, 4, 8, 16, 32 µg/kg of BW. Half the horses of each sex (n = 8) received the successive doses in an increasing manner and the remaining horses (n = 8) received the doses in a decreasing manner, in order to assess possible accumulative effects of the treatment. There was a minimum of 1or 2 days of rest with no injection between doses. Mares were treated on alternate days from stallions to avoid unnecessary excitement and perturbation of prolactin levels. The protocol and procedures were then repeated after the summer solstice in late June, 2008, after the autumnal equinox in late September, 2008, and after the winter solstice in late December into early January of 2009. The majority of horses were used in all four seasons, with a few exceptions of horses that were replaced for reasons unrelated to the experiment.

   Blood sample collection and analysis. Blood samples were collected by jugular venipuncture into evacuated, heparinized tubes at 0, 15, 30, 60, 90, and 120 min relative to i.v.

injection of sulpiride. Blood was centrifuged at an r.p.m. of 3000, plasma was harvested and

stored at -20°C. Prolactin concentrations were measured in plasma by radioimmunoassay (RIA)

as validated previously for equine samples (Colborn et al., 1991b) once all samples were

collected within a season.

       <u>Statistical analyses.</u> Plasma prolactin concentrations were analyzed for each season by

ANOVA procedure of SAS (SAS Institute Inc., Cary, NC), which took into account the

repetitive nature of the sampling. Factors in the analysis were gender, dose, sampling time, and

the appropriate interactions. Prolactin areas under the response curves were calculated by

subtracting the pre-injection prolactin concentrations, and then summing the net changes in

prolactin x time interval increments from 15 to 120 min for each horse. Areas were analyzed by

ANOVA that tested the effects of dose, gender, season, and the appropriate interactions. To

assess for possible shifting of the sensitivity to sulpiride across genders and seasons, logit-log

transformation was performed for the areas for each horse, and the half-maximum point

estimated from regression analysis. These data were analyzed by ANOVA for effects of gender,

season, and their interaction.

Results

       There was no effect ($P > 0.1$) of administering the sulpiride doses in an increasing vs.

decreasing manner. Prolactin concentrations peaked in the first 15 to 30 min in all horses in all

seasons (Figure 2.1). Prolactin areas under the curve increased ($P < 0.001$) with increasing doses

of sulpiride, and were highest ($P < 0.05$) in March for stallions, but in June for mares (Figure

2.2). The mean maximum prolactin response, expressed as area under the response curve

(Figure 2.3), differed between sexes in March ($P < 0.001$) and December ($P < 0.05$), with

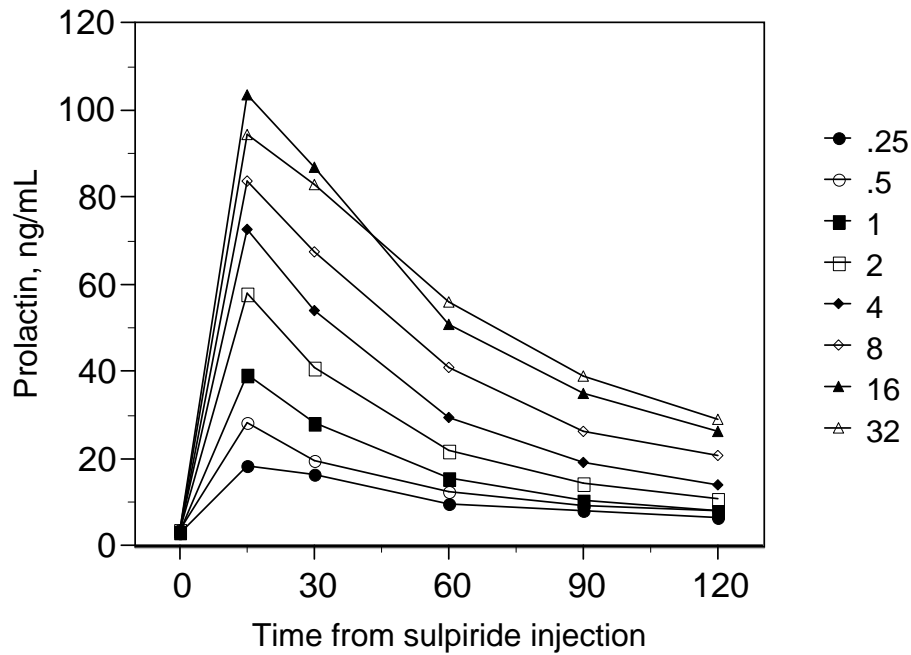stallions having greater responses than mares in both months. The calculated half-maximum

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Figure 2.1. Prolactin responses to the 8 doses (µg/kg BW) of sulpiride, averaged over both sexes and four seasons of the year. The maximum response generally occurred at 15 min, and occasionally at 30 min. There was a dose x time interaction for prolactin concentrations ($P < 0.0001$) in the ANOVA. The pooled SEM was 5.6 ng/mL.

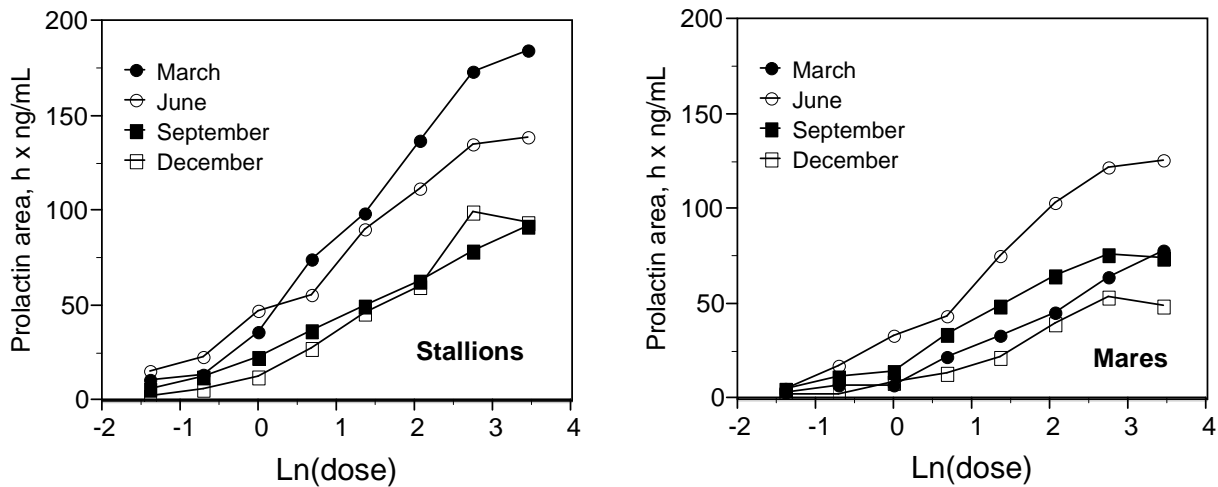Figure 2.2. Mean areas under the response curves for stallions and mares in the four seasons of the year. There was a sex x month x dose interaction ($P = 0.021$) in the ANOVA. The pooled SEM was 10.7 area units.
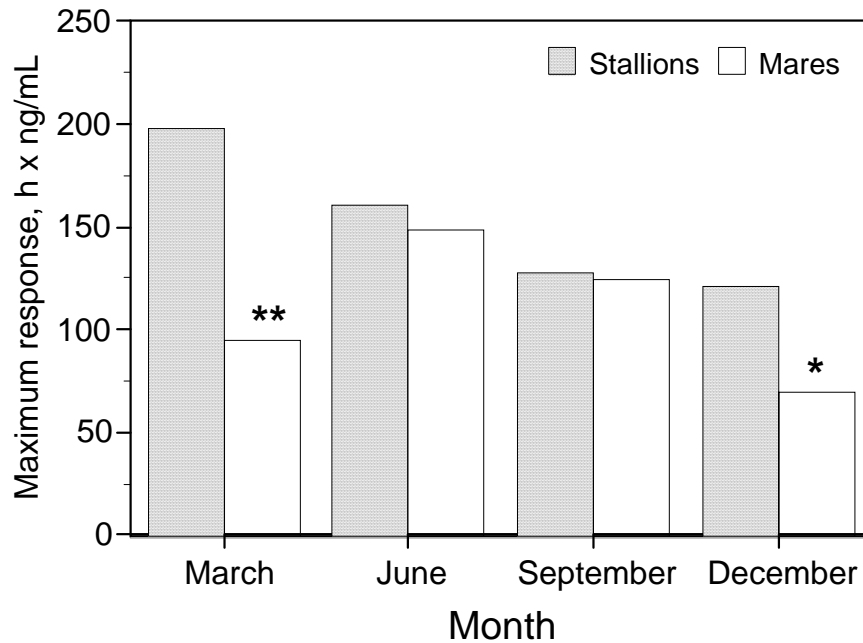
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Figure 2.3. Mean maximum prolactin responses (area under curve) for stallions and mares in the four seasons of the year. There was a sex x month interaction (P = 0.016) in the ANOVA. *Means differ between sexes (P < 0.05). **Means differ between sexes (P < 0.001). The pooled SEM was 15.2 area units.

values, which should reflect (be proportional to) the dopaminergic input to the pituitary, were lowest (P < 0.05) in June and greatest in September (Figure 2.4); there was no effect of gender on half-maximum values, nor any gender x month interaction.

Discussion

As has been reported previously (Johnson, 1986; Thompson et al., 1986a, b; Aurich et al., 2002), prolactin concentrations and response to sulpiride varied with season; however, the maximal responses differed between mares and stallions. The greatest response in stallions was in March, whereas it was in June for mares. Although cyclic activity of the mares was not monitored, it is likely that the mares were still anovulatory in March, and thus lacked the normal
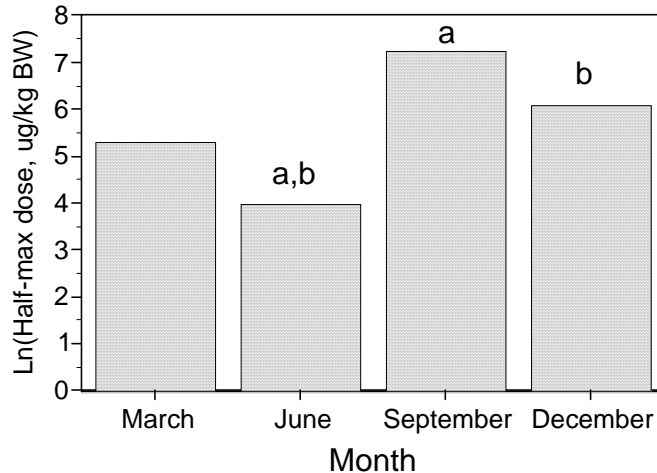
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Figure 2.4. Mean ln(ED50) averaged over stallions and mares for the four seasons across the year. [a,b]Mean for June differed from that for September (P = 0.006) and for December (P = 0.032). The pooled SEM was 0.13.

steroidal (androgen or estrogen) stimulation of prolactin production and secretion. Thompson et al. (1994) reported that prolactin secretion was greater in mares and stallions in summer relative to geldings, which was attributed to the similar lack of gonadal steroids in the geldings.

The doses of sulpiride used produced prolactin responses (areas under the curve) from zero at the lowest doses to close to 200 hours x ng/mL for stallions in March. In general, the dose-response curves began to plateau at the highest dose, and in some cases, at lower doses. For instances when the curve was still increasing at the highest dose, a maximum response had to be estimated. These estimates were calculated as the response for the highest dose plus the increment between the highest and second highest doses.

Even though the maximal responses varied with month and between sexes, there was far less variation in the half-maximum values calculated from the dose-response curves. Theoretically, a shift in the dose-response curve to the left would indicate a decrease in

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dopaminergic input - that is, less sulpiride needed to counterbalance the dopamine affecting the pituitary. There were indeed differences in the half-maximum values averaged across both sexes, with the lowest occurring in June. This coincides with the maximum responses, at least in mares. The highest half-maximum values might have been expected to be in December, when prolactin secretion and responses to sulpiride were low. However, Steger and Bartke (1991), studying the seasonal golden hamster, concluded that short day lengths reduced prolactin secretion within 4 weeks, but that an increase in inhibitory input from tuberoinfundibular dopaminergic neurons was clearly not involved. It appears that dopaminergic input begins to increase in the fall, but it remains constant into the winter. The lack of gender effect for half-maximal values indicated that mares and stallions respond similarly to the seasonal signals that result in changes in hypothalamic dopamine input to the pituitary.

It is concluded that dopaminergic input to the adenohypophysis does vary with season in mares and stallions, with a general trend for the least input in the summer. There does not appear to be a close correlation between dopaminergic input and average seasonal prolactin concentrations as described by Johnson (1986). Whether other factors, such as a prolactin stimulatory factor, are involved in the long-term regulation of prolactin secretion needs to be determined.

DOPAMINERGIC INPUT TO THE ADENOHYPOPHYSIS:
THE STIMULATORY ROLE OF ESTRADIOL

Introduction

Regulation of prolactin secretion in mammals involves dopamine secretion from the hypothalamic tuberoinfundibular dopaminergic system, its release into the hypothalamic hypophyseal portal system, and its binding to lactotropes in the pars distalis (Hadley and Levine, 2000). This chain of events keeps prolactin secretion suppressed. The horse seems to fit this model, given the positive response in prolactin secretion after administration of sulpiride or domperidone, both dopamine receptor antagonists (Johnson and Becker, 1987; Colborn et al., 1991b; Redmond et al., 1994; Thompson and Depew, 1997; Besognet et al., 1997). Estradiol stimulates prolactin production and secretion by acting on adenohypophyseal lactotropes in various species. Estradiol treatment of seasonally anovulatory mares greatly enhanced prolactin secretion after administration of sulpiride, resulting in induction of ovulation in 8 of 9 treated mares within 21 days (Kelley et al., 2006). Whether or not the stimulatory effect of estradiol on prolactin secretion is via alteration of the dopaminergic input to the pituitary is not known. The present experiment was designed to test the hypothesis that the degree of dopaminergic input to the adenohypophysis is altered by estradiol administration. Gradually increasing doses of sulpiride were used to construct prolactin dose-response curves in geldings administered estradiol or injection vehicle alone; the dose producing half-maximum response was compared for the two groups. Geldings were used because they exhibit an estradiol-induced increase in prolactin secretion similar to that reported for mares and their lack of gonads ensured that the only source of estradiol would be treatment injections.

Materials and methods

  Animals and treatments. Twelve long-term geldings were used and were of Quarter

Horse and Thoroughbred types. Their ages ranged from 7 to 24 years old. Body condition

scores were within a range of 6 to 8. All horses were housed at Louisiana State University

Equine Unit, Ben Hur Farm. They were maintained on native grass pasture and supplemented

with grass hay as needed when pasture grasses were dormant. Treatments were initiated in early

November. On day 0, geldings (n = 6) received an i.m. injection of estradiol cypionate (ECP) or

vegetable oil (controls; n = 6). Of the 6 geldings receiving ECP, half (n = 3) received 2 mL of a

solution of 50 mg/mL (100 mg) and half (n = 3) received 10 mL of a solution of 10 mg/mL (100

mg). Both solutions were obtained from BET Labs (Lexington, KY). Control geldings received

either 2 or 10 mL of vegetable oil (n = 3 each).

  Beginning on day 6, all geldings received an i.v. injection of sulpiride at 0.082 µg/kg BW

in saline, followed by frequently collected blood samples. This regimen was repeated every

other day with the dose increasing each day. The doses administered after day 6 were 0.164,

0.4095, 1.025, 2.56, 6.4, 16, 40, and 100 µg/kg BW on day 8 through day 22.

  Blood sample collection and analyses. Blood samples were collected by jugular

venipuncture at 0, 15, 30, 60 and 120 min relative to injection into evacuated, heparinized tubes.

Blood was centrifuged at 3000 r.p.m., plasma was harvested in all blood samples and stored at -

20°C. Prolactin and LH concentrations were measured in plasma by RIA as validated previously

for equine samples (LH: Thompson et al., 1983; prolactin: Colborn et al., 1991b). Estradiol was

measured in dried acetone extracts of plasma by radioimmunoassay with commercially available

reagents (Diagnostic Laboratory Systems, Webster, TX).

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Statistical analyses. Estradiol data were analyzed as a 2 x 2 factorial arrangement of treatments with repeated measures ANOVA with the GLM Procedure of SAS (SAS Institute Inc., Cary, NC); estradiol treatment and injection volume were main effects. For each prolactin response to sulpiride injection, net area under the curve was calculated by subtracting the pre-injection prolactin concentration, and then summing the net changes in prolactin x time interval increments from 15 to 120 min. Areas were analyzed by ANOVA as described for estradiol concentrations. Individual data for each group were analyzed by regression analysis after calculation of the log of the doses (x-axis) and logit transformation of the area (y-axis); the dose of sulpiride resulting in half-maximum areas was calculated for each gelding and analyzed by one-way ANOVA.

Results

Estradiol concentrations were affected by estradiol treatment ($P < 0.026$) and day ($P < 0.001$), and there was a treatment x day interaction ($P < 0.001$; Figure 3.1). Estradiol concentrations in geldings receiving ECP rose to approximately 20 pg/mL by day 1, and were above 10 ng/mL through day 8. The volume of ECP administered did not affect estradiol concentrations ($P > 0.1$).

Mean LH concentrations were stimulated ($P = 0.038$) by ECP treatment (treatment effect and treatment x day interaction) and remained higher than those in control geldings for approximately 25 days (Figure 3.2). There was no effect of estradiol volume on LH concentrations ($P > 0.1$).

Estradiol pretreatment increased ($P = 0.0054$) the prolactin response to sulpiride at the 1.025 µg/kg BW dose and higher (Figure 3.3). There was a sulpiride dose x estradiol treatment
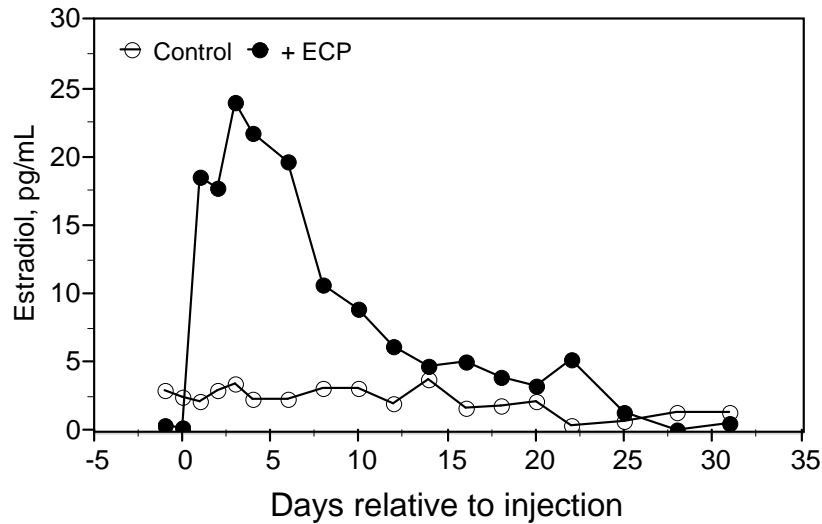
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Figure 3.1. Mean plasma concentrations of estradiol in geldings receiving an i.m. injection of 100 mg of ECP (+ECP) or vegetable oil (control). There was an effect of treatment (P < 0.026), day (P < 0.001), and treatment x day (P < 0.001) in the ANOVA. There was no difference due to ECP volume (2 mL vs. 10 mL; P > 0.1). The polled SEM was 2.4 pg/mL.
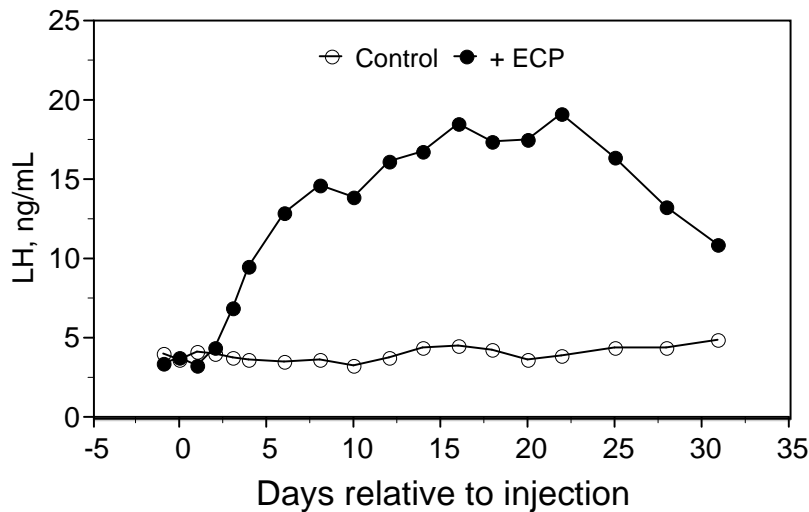

Figure 3.2. Mean plasma concentrations of LH in geldings receiving an i.m. injection of 100 mg of ECP (+ECP) or vegetable oil (control). There was an effect of treatment (P = 0.038), day (P < 0.001), and treatment x day (P < 0.001) in the ANOVA. The pooled SEM was 1.7 ng/mL.
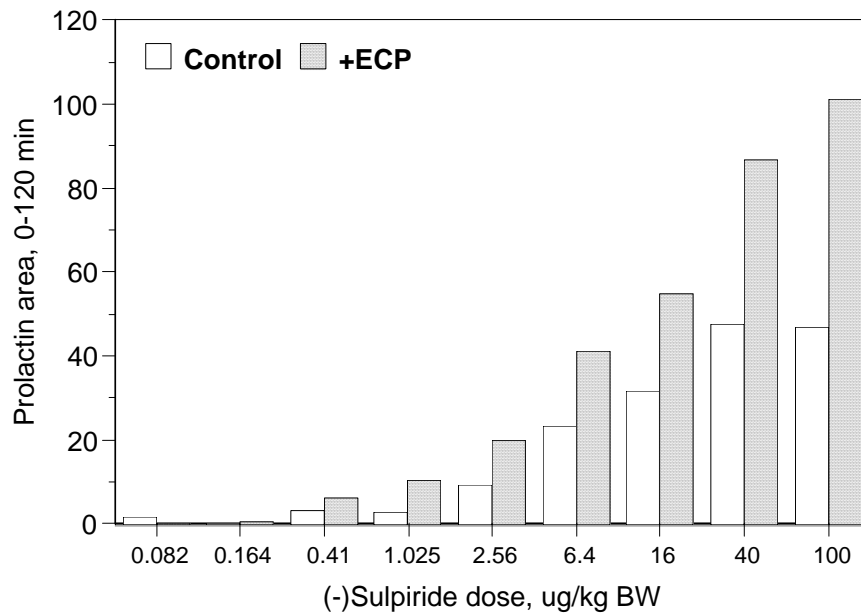
Figure 3.3. Mean prolactin responses, expressed as areas under the curve, in geldings administered 100 mg of ECP (+ECP) or vegetable oil (control). There was an effect of treatment (P = 0.0054) and dose (P < 0.001), as well as interaction of treatment x dose (P < 0.001) in the ANOVA. Areas differed between estradiol-treated and control geldings at the 1.025 μg/kg BW dose and higher (P < 0.05). The pooled SEM was 5.0 area units.

interaction (P < 0.001) in the ANOVA. Analysis of the half-maximum values from the logit-log regression analysis revealed that the natural log of the mean half-maximum dose of sulpiride in control geldings was not different (P > 0.1) from that of estradiol-treated geldings. (2.12 vs. 2.82; SEM = 0.38); nor did the mean half-maximum values differ (11.4 vs. 24.1 μg/kg BW; SEM = 6.8 μg/kg BW).

Discussion

Treatment of geldings with ECP increased both LH secretion and the prolactin response to sulpiride as reported previously (Thompson et al., 2008). Similar increases were reported for seasonally anovulatory mares (Aurich et al. 2002; Kelley et al., 2006; Mitcham et al., 2010). The stimulatory effect of ECP on LH secretion was used to confirm the bioactivity of the ECP

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injection, and it also indicates the duration after injection that the estradiol was active. As seen from Figure 3.2, the duration of stimulation for LH secretion was greater than 30 days, well beyond the last sulpiride injection in the series (day 22).

Although ECP administration stimulates LH secretion in the absence of sulpiride treatment (or any other dopamine antagonist) in horses (Garcia and Ginther, 1978; Thompson et al., 2008), the stimulatory effect of estradiol on prolactin secretion in the absence of a dopamine antagonist is minimal (Thompson et al., 1991). Kelley et al. (2006) was the first to combine estradiol pretreatment with sulpiride injections to produce prolactin concentrations in seasonally anovulatory mares well in excess of those produced by sulpiride alone (Donadeu and Thompson, 2002). In fact, in geldings treated daily with sulpiride in the winter, prolactin responses actually decreased over time, indicating a depletion of pituitary stores and a lack of stimulation of production (Thompson and Depew, 1997); only as the day length increased did the prolactin responses begin to rise again. Thus, the stimulatory effect of estradiol on prolactin secretion appears to be through some mechanism other than altering dopamine input from the hypothalamus to the adenohypophysis, but is maximally expressed only in conjunction with dopamine antagonist administration.

The half-maximum values obtained for control and ECP-treated geldings did not differ. This further indicates that the stimulatory effect of estradiol is via a mechanism other than altering the dopaminergic input to the adenohypophysis. Although many reports describe the "anti-dopaminergic" effects of estradiol in rats (i.e., a reduction in dopaminergic activity of the TIDA neurons; Raymond et al., 1978; Ferland et al., 1979; Morel et al., 2009), Stone et al. (1970) reported that 4 days of estradiol treatment of male rats increased the pituitary content mRNA for preprolactin, the precursor from which prolactin is eventually cleaved. In 1985, de

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Greef et al. reported that the estradiol-induced rise in prolactin secretion in female rats 3 days after injection was accompanied by a 50% reduction in dopamine concentrations in hypophyseal stalk blood and a 240% increase in TRH concentrations. Alternatively, Pasqualini et al. (1986) suggested that estradiol treatment of ovariectomized rats caused a reduction in the dopaminergic receptors on the lactotropes in the adenohypophysis, thereby reducing the responsiveness to hypothalamic dopamine reaching the gland (hence greater prolactin secretion). And finally, Boockfor et al. (1986) reported that estradiol treatment of dispersed pituitary cells in vitro shifted the proportions of cells that released growth hormone, prolactin, or both hormones, which indicated that estradiol may convert cells that release only growth hormone to those that release both growth hormone and prolactin. It is evident that more research needs to be conducted with horses to determine the exact mechanism by which estradiol stimulates prolactin production and secretion in this species.

SUMMARY AND CONCLUSIONS

Two experiments were conducted to examine whether the changes in dopaminergic input to the adenohypophysis would explain variations in prolactin secretion across the four seasons of the year as well as the stimulation of prolactin production and secretion in response to estradiol treatment. In the first experiment, both prolactin concentrations and response to sulpiride varied with season. However, maximal responses differed between mares and stallions. The greatest response was in the spring for stallions but in the summer for mares. The variation in half-maximum values indicated a seasonal change in dopaminergic input to the pituitary, with lowest input occurring in June in both sexes. This seasonal change is consistent with reports of variations in other seasonally breeding species in which dopaminergic control can be measured directly.

As estradiol treatment has been shown to increase prolactin production and secretion, the second experiment tested whether the stimulatory effect of estradiol was associated with an alteration of the dopaminergic input to the pituitary. Estradiol administration did not alter the dosage of sulpiride necessary to counterbalance the dopaminergic input to the pituitary despite the fact that the estradiol treatment did double the prolactin response in treated geldings. It was concluded that estradiol most likely stimulates prolactin production and secretion directly at the lactotrope level, rather than via decreasing hypothalamic dopamine input to the lactotropes. Further research is necessary to confirm this conclusion.

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