MECHANISMS BY WHICH STRESS INFLUENCE SECRETION OF LUTEINIZING HORMONE IN CATTLE

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CHAPTER 1

INTRODUCTION

Stress may alter secretion of luteinizing hormone in cattle (Harms et al.,1983; Echternkamp, 1984). The effect of stress may alter the effectiveness of experiments designed to evaluate the influence of various factors on the control of secretion of LH and may also reduce reproductive efficiency in both males and females (Moberg, 1985b).

Stressors activate the hypothalamic-pituitary-adrenal (HPA) system (Dantzer and Mormede., 1983; Moberg, 1985a, 1991; Knol, 1991). The HPA responds to stress by initiating secretion of corticotropin-releasing hormone (CRH) from neurosecretory neurons in the hypothalamus. This peptide passes through the hypothalamo-hypophysial portal vessels to act upon the pituitary corticotropes to stimulate the secretion of adrenocorticotropic hormone (ACTH). The adrenal cortex respond to stimulation by ACTH and synthesized and secretes glucocorticoids, progesterone, and androgens.

During stress, circulating concentrations of adrenal glucocorticoids increase, and this alteration in corticoids is often used as an indicator of stress (Dantzer and Mormede., 1983; Stephens., 1980). Thus, in most studies of stress in animals, the focus has been on the response in secretion of glucocorticoids.

Excessive secretion of glucocorticoids may impair reproductive function in domestic animals. Treatment of cows with ACTH caused decreased basal concentrations of LH in plasma, and cortisol prevented the ovulatory surge of LH but did not affect basal LH concentration in cows (Stoebel and Moberg., 1982a). Similarly, Cortisol decreased GnRH-induced LH release from bovine pituitary cells in culture (Padmanabhan et al.,1983). Injection of 20 mg of dexamethasone, a synthetic glucocorticoid, into bulls decreased systemic LH concentrations for more than 6 h (Thibier and Rolland., 1976). Dubey and Plant, (1985) found that prolonged treatment of castrated monkeys with glucocorticoids depressed the circulating concentration of gonadotropins without altering the ability of the animals to secrete additional gonadotropins in response to exogenous GnRH stimulation. The glucocorticoid treatment affected gonadotropin secretion only after several weeks of exposure to steroids. Prolonged elevations of glucocorticoids are unlikely to occur during most stresses. During most stresses, circulating concentrations of glucocorticoids are elevated for only a

few hours. The long-term treatment with natural or synthetic glucocorticoids, which lasts for several days, is more indicative of the effects seen during pharmacological treatment or from adrenal disease (Moberg., 1991).

Recent studies suggest that other hormones of the HPA, rather than glucocorticoids, may have important roles in the mediation of the effect of stress on reproduction. Exogenous ACTH is more effective in suppressing the LH response to GnRH in cows than cortisol alone (Matteri and Moberg., 1982). Injections of 40 and 80 i.u. ACTH, but not 10 or 20 i.u., resulted in statistically significant reductions in LH release in rams when administered 3 h before GnRH administration (Matteri et al., 1984). Animals that did not exhibit LH suppression after ACTH treatment, had corticosteroid concentrations greater than in controls, suggesting a glucocorticoid-independent mechanism. Administration of ACTH to adrenalectomized rams will prevent exogenous GnRH from stimulating the secretion of LH in the absence of any adrenal steroids (Fuquay and Moberg., 1983).

Infusion of CRH inhibits gonadotropin release from the pituitary in the absence of circulating steroids of either adrenal or gonadal origin in rats (Rivier and Vale., 1984) and monkeys (Xiao et al., 1989). This effect of CRH on gonadotropin secretion is, at least in part, mediated by endogenous opioid peptides (EOP) in both

monkeys (Gindoff and Ferin, 1987) and rats (Petraglia et al., 1986).

During stress pro-opiomelanocortin-derived peptides (ACTH, b-endorphins and several enkephalins) are synthesized in the anterior lobe of the pituitary gland and ın the hypothalamus. These EOP have inhibitory effects on gonadotropin secretion in cows and this effect is reversed by naloxone administration (Whisnant et al., 1986; Malven and Hudgens, 1987; Rund et al., 1989). Thus, it is possible that the EOP are involved in the effect of stress on reproduction in cattle. Schoenemann et al., (1990) suggested that EOP may be involved in the mediation of the effect of stress on LH secretion in beef cattle.

The objectives of these experiments were to determine if stress alters secretion of LH in beef cattle and to examine if EOP are involved in the suppression of LH secretion during exposure of cattle to stress.

CHAPTER II

REVIEW OF LITERATURE

Stress can be defined in many ways and viewed from different perspectives. Broadly defined, stress can be considered as the response resulting from stimuli (stressors) of internal or external origin that tend to affect homeostasis or the tendency of the organism to maintain a uniform and beneficial physiological stability. Any stimulus that challenges homeostasis can be viewed as a stressor, and the challenge in biological function that occurs as the animal attempts to maintain homeostasis is the stress response. More specifically, from an animal scientist's point of view, the homeostatic, physiological, and behavioral responses detectable in the animal resulting from its interactions with stressors may be defined as stress responses (Stephen, 1980).

Stress response

Types of stress. Stressors can be viewed as stimuli that challenge the homeostasis or induce a stress response. There is not a specific

response that characterizes all stressors (Moberg, 1987). Depending on the stressor several types of stress responses occur. Stressors can be divided into two main groups, physical and psychological. Exposure of animal to novel or adverse environmental conditions are considered psychological (Dantzer and Mormede, 1983) or behavioral (Moberg, 1987) stressors and heat, cold, pain, surgery and exercise are considered to be physical stressors (Dantzer and Mormede, 1985).Moberg (1987) defined behavioral stress as any event or stimuli that is perceived by the animal as a threat to its well-being, not taking into account the physical stressors. This is the most common type of stress related to normal management practices. Thus, this type of behavioral stress will be assumed when the term "stress" is used in this thesis and the specific nature of stressor is not identified.

Stress response. Early stress research identified two modes of stress response. The emergency reaction, (Cannon, 1935) is characterized by release of adrenaline and noradrenaline which enables the subject to make quick physiological adjustments in response to acute stress. Both heart rate and cardiac output are increased. Glucose is released from the liver and it is redistributed from the skin and viscera to the central nervous system and muscles. Blood coagulating factors are released and the bronchi become dilated. If the stressor persists, the

general adaptation syndrome (Selye, 1950), occurs. It is characterized by the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland which in turn induces release of corticosteroids from the adrenal gland. Recent studies have revealed evidences for integrated action of the two modes of response (Knol, 1991). Selye, (1950) considered the general adaptation syndrome as a nonspecific response to all types of stress regardless of the nature of stressor. Later studies demonstrated that no one biological response is characteristic of all types of stressors (Mitchell et al., 1988; Hattingh et al., 1989) and that different animals may manifest different patterns of responses to the same stressor (Moberg, 1987).

The entire neuroendocrine system is involved in the stress response (Mason 1968). In addition to its effect on the adrenal gland, stress can influence the secretion of other pituitary hormones such as growth hormone, prolactin, thyroid-stimulating hormone and the gonadotropins (Moberg, 1985a). As with the adrenal response, the responses of these hormones to stress are variable.

In general, stressors activate the hypothalamic-pituitary-adrenal system. The stress response includes secretion of corticotropin releasing hormone (CRH) from the hypothalamus. CRH stimulates the anterior pituitary to release adrenocorticotropin hormone (ACTH), which in

turn, induces the secretion of corticosteroids from adrenal gland.

Model of stress response. Although it is not possible to present a model that incorporates all aspects of the biological response of animals to stress, Moberg (1985a, 1987) proposed a model in order to organize current concepts about stress and to provide direction for studies in animal stress. He divided the response of animals to a stressful event into three major components: recognition of a threat to homeostasis, the stress response, and the consequences of stress. It is at the level of the central nervous system where the recognition of a potential threat to homeostasis occurs and the organization of a biological response is initiated. The biological response is influenced by behavioral, autonomic nervous and neuroendocrine activity. The result of the biological response will be a change in biological function. When the change in biological function occurs over a prolonged period of time, this threatens the animal's well-being by placing it into a pre-pathological state which may eventually develop into a pathology. Some examples of pre-pathological states are a suppressed immune system, absence of a reproductive event that is essential for normal reproduction or excessive aggressive behavior.

Measurement of stress in domestic animals.

Monitoring adrenal cortex activity along with behavior changes have been the classical measurements of response to stress. Increased circulating corticosteroid concentrations are considered an indicator of stress (Dantzer and Mormede, 1983; Phillips et al., 1982; Echternkamp, 1984; Heuwieser et al., 1987;Dobson, 1988). Due to the lack of a nonspecific response that characterizes all stressors and the inter-animal variability in the response, the measurement of a single biological response such as the secretion of corticosteroids can be misleading. In the opinion of Moberg (1987), behavioral changes and adrenal cortical responses can only serve as indicators of significant stress if they result in changes in biological function that threaten the animal's well-being. Moberg proposed that the development of the pre-pathological state is the best indicator of behavioral stress. Others approaches have been made to define stress in animals. Wettemann et al. (1977) developed a method to evaluate adrenal function in steers. This method involved determining the maximum corticoid secretion following treatment with ACTH. This test or index indicated the ability of the adrenal to secrete corticoids in response to stress.

Stress and reproduction

Effect of stress on female reproductive function. Stress has deleterious effects on both male and female reproductive efficiency; however the female is more sensitive (Moberg, 1985b). In the female, successful reproduction depends on the proper sequence of endocrine events that occurs during the estrous cycle. Any disruption of the sequence will endanger the reproductive process. Some detrimental effects of stress on fertility are the inhibition of the expression of estrous behavior and the delay or blockade of the preovulatory LH release. When young cows were placed into an established herd of mature cows, they had shorter estrous cycles (Wagnon et al., 1972). Heat stress can suppress anterior pituitary release of LH (Wise et al., 1988), inhibit estrous behavior (Madan and Johnson, 1973) and may increase embryonic mortality during early pregnancy in cattle (Biggers et al., 1987). In ewes, heat stress reduces the length of estrous cycles, suppresses estrus and has adverse effects on ovulation (Casu et al., 1991).

The preovulatory LH surge in cattle can be delayed or suppressed due to transportation stress (Dobson, 1987; Nanda et al., 1989) or due to repeated acute stress (Stoebel and Moberg, 1982a) during the follicular phase of the estrous cycle.

Stoebel and Moberg (1982b) observed in cows, that

elevated plasma corticosteroids could inhibit the expression of estrous behavior and block LH surges even though titters of estrogen were typical for the follicular phase of the estrous cycle. Stress can affect the timing and the magnitude of the preovulatory peak of LH in cattle, consequently affecting ovulation (Harms et al., 1983). Management-related stress such as transportation or isolation can block estrogen-induced estrous behaviour in ewes (Ehnert and Moberg, 1991). Inhibition of the preovulatory release of LH and disturbances in the function of the ovaries (cystic or inactive ovaries) were observed in ewes (Przekop et al., 1989) when weak electric shocks were administered to the feet in different phases of the estrous cycle. On the other hand, Baucus et al. (1990) found that transportation induced hormonal and ascorbic acid responses which are indicative of stress, did not alter estrous behaviour, ovulation, duration of the estrus cycle, pregnancy rate or preovulatory surges of estradiol and LH when mares were transported for 792 km (12 h).

In addition to alter gonadotropin secretion, a direct effect of stress on the ovary may take place. Exposure of rat granulosa cells to high concentrations of corticoids in vitro, inhibits FSH-induced aromatase activity (Hsueh and Erickson, 1978; Adashi et al., 1981). Spicer and Zinn (1981), concluded that concentrations of cortisol in bovine follicular fluid do not have a major function in

the regulation of bovine follicles. In light of these experiments, I conclude that the primary mechanism by which stress disrupts estrous cycles is by altering gonadotropin secretion.

Effect of stress on male reproductive function. Once a male attains puberty, spermatogenesis is a continuous process. Any factor that blocks spermatogenesis for a time sufficient to affect the quantity or quality of the semen or to alter sexual behavior, will impair male fertility. Testosterone is secreted mainly by the Leydig cells in response to stimulation by LH. FSH is responsible for spermatogenesis up to the secondary spermatocytes, after which testosterone is responsible for the final stages of spermatogenesis (Hafez, 1987). Stress alters secretion of gonadotropin and male gonadal steroids, but there is little evidence of a decrease in fertility due to stress.

Heat stress reduces semen quality and fertility in most domestic animals (Casu et al., 1991). However, exposure of bulls to elevated ambient temperature for 15 days did not significantly alter serum testosterone concentrations (Minton et al., 1979). Probably the temperature effect is a physical effect occurring directly on the testis and not mediated by major alterations in endocrine control of spermatogenesis (Moberg, 1985b). Several stressors have inhibitory effect on LH release in

bulls (Welsh and Johnson, 1981), rams (Matteri et al., 1984), male rhesus monkeys (Norman and Smith, 1992) and male rats (Rivier et al., 1986; Petraglia et al., 1986; Briski and Sylvester, 1987). These stressors that inhibit LH secretion are rectal electroejaculation, restraint, intermittent electroshocks, and exposure to a novel environment. Activation of the hypothalamic-pituitary-adrenal (HPA) axis by stressors, causes a decrease in plasma testosterone concentrations in domestic and laboratory animals. Secretion of LH and testosterone decreased after rectal insertion of a electroejaculation probe in bulls (Welsh and Johnson, 1981). Prolonged elevations in concentrations of corticosteroids in bulls were coincident with basal concentrations of LH and testosterone (Welsh et al., 1981). A decrease in concentrations of testosterone in serum occurred after exercise in buffalo males (Agarwal et al., 1985). Restraint is a potent stimulus of the HPA axis that inhibits both LH and testosterone secretion in male rhesus macaques (Norman and Smith, 1992) and male rats (Armario et al., 1987;Demura et al., 1989; Lopez-Calderon et al., 1991). Testicular androgens decreased in men during prolonged physical strain combined with deficiencies in energy and sleep (Opstad, 1992).

In addition to stress-related decreases in plasma testosterone concentrations due to the inhibition of gonadotropin secretion, a direct inhibitory effect of

stress on testicular function has been suggested. Direct inhibitory effects of glucocorticoids on testicular LH-binding capacity and steroidogenesis occurs in vivo (Bambino and Hsueh, 1981;Welsh and Johnson, 1981) and in vitro (Bambino and Hsueh, 1981; Welsh et al., 1982). A stressor-induced increase in concentration of b-endorphin and ACTH in testicular interstitial fluid has been demonstrated (Mann and Wilson, 1988). These opioid peptides may inhibit testicular steroidogenesis.

Effect of stress on gonadotropin secretion: Mechanisms involved. Stressors activate the hypothalamic-pituitary- adrenal (HPA) system (Dantzer and Mormede, 1983; Moberg, 1985a, 1991; Knol, 1991). Hormones of the HPA axis have very important roles in modulating the effect of stress on reproductive functions.

Glucocorticoids. The effect of different stressors on reproduction is associated with a decrease in LH secretion and an increase in circulating concentrations of adrenal glucocorticoids. In bulls, endogenous concentrations of corticosteroids and progesterone are negatively correlated with concentrations of LH and testosterone (Welsh and Johnson, 1981). In heifers, repeated acute stress during the follicular phase at estrous cycle induces a brief elevation in corticosteroid concentrations and blocks the preovulatory surge of LH

(Stoebel and Moberg, 1982b). Corticosteroid secretion was increased and LH was suppressed due to restrain stress in rams (Matteri et al., 1984).Echternkamp (1984) found that 2- to 4-fold increases in systemic cortisol concentrations in beef cows did not affect LH secretion, whereas 10- to 20-fold increases in cortisol associated with intensive stress (handling), suppressed pulsatile LH release. He suggested that the influence of stress on gonadotropin secretion is dependent on the magnitude of the adrenal steroidogenic response.

To ascertain if the suppression of LH that occurs during stress is due to the increase in corticosteroid concentrations, Stoebel and Moberg (1982a) infused cortisol succinate into four cows during the follicular phase of the estrous cycle. They observed that the LH surge and estrous behavior were prevented in three of the four treated animals, but average basal plasma LH concentrations were elevated and estradiol concentrations were unaffected. When dairy heifers were given two doses of GnRH at an interval of 1.5 h during treatment with cortisol, basal LH concentrations were not altered due to cortisol treatment, but there was a slight depression in the LH response in cortisol treated animals after the first GnRH injection, but not after the second one (Matteri and Moberg, 1982). In adrenalectomized heifers infused with hydrocortisone succinate, mean LH concentrations were not altered (Li and Wagner, 1983a) but

the LH response to GnRH had a slower onset, lower maximum concentration and a longer response (Li and Wagner, 1983b). Injection of 20 mg of dexamethasone, a synthetic glucocorticoid, into bulls decreased systemic LH concentrations for more than 6 h (Thibier and Rolland, 1976) and the response of LH to GnRH was decreased (Chantaraprateep and Thibier, 1978). In contrast Wettemann et al., (1981) found that serum LH concentrations were unaffected by injection of 20 mg of dexamethasone in postpartum anestrous range cows that had minimal LH secretion.

Corticosteroids suppress gonadotropins in many species in addition to cattle. Treatment of gilts with hydrocortisone acetate (250 mg) twice daily for 12 d blocked the preovulatory LH surge (Barb et al., 1982). Long term treatment of orchidectomized rhesus monkeys with hydrocortisone acetate reduced circulating concentrations of LH and FSH (Dubey and Plant, 1985). Dexamethasone had a dose dependent effect on the suppression of basal gonadotropin secretion and blocked GnRH induced gonadotropin secretion in castrated rats (Rosen et al., 1988).Gaon and Liptrap (1989) observed an increase in pulse area and mean LH concentrations in boars treated with dexamethasone and GnRH when compared with boars treated with NaCl and GnRH. The reason for this stimulatory effect of dexamethasone in boars is unknown.

In addition to in vivo findings, there are some in

vitro studies that demonstrate an inhibitory action of glucocorticoids on gonadotropin secretion by cultured pituitary cells. Cortisol decreases basal LH release (Li and Wagner, 1983a) and GnRH induced LH release (Li and Wagner, 1983b; Padmanabhan et al., 1983) from bovine pituitary cells. Similarly, corticosterone inhibits LH response to GnRH challenge in cultured rat pituitary cells (Kamel and Kubajak, 1987). These findings suggest a direct action of glucocorticoids on the pituitary gland to depress both basal and GnRH induced LH release.

A direct action of glucocorticoid on GnRH secretion from hypothalamus has been suggested because adrenal steroids can penetrate the blood-brain barrier (Moberg, 1984). Dubey and Plant, (1985) found that long-term treatment of castrated male rhesus monkeys with hydrocortisone acetate depressed circulating concentration of gonadotropins but did not alter the ability of the pituitary to secrete gonadotropins in response to exogenous GnRH. The same effect was observed with prolonged treatment of male rats with dexametasone (Rosen et al., 1988). In both cases the glucocorticoid treatment affected gonadotropin secretion only after long exposure to steroids. Such a prolonged elevation of glucocorticoids is unlikely to occur during most stresses.

It can be concluded that glucocorticoids can at least partially mediate the effect of stress on reproduction. However, caution must be exercised in interpretating

results from exogenous hormone treatments because both the amount of hormone used and the time of treatment can be totally different from physiological responses to stress.

ACTH, CRH. Administration of exogenous ACTH has been used to study the role of adrenal steroids on reproduction (Li and Wagner, 1983a, 1983b; Barb et al., 1982). ACTH may have an independent effect on gonadotropin secretion that does not involve adrenal steroids. In rodents, the administration of ACTH to adrenalectomized animals suppresses the secretion of LH (Ogle, 1977). In cows, exogenous ACTH administration is far more effective in suppressing the LH response to GnRH than infusion of cortisol (Matteri and Moberg, 1982). In rams, acute adrenocorticotropin treatment suppresses GnRH induced LH release (Matteri et al., 1984). Treatment of intact rams with ACTH increased plasma corticosteroids and significantly reduced the ability of GnRH to elicit the release of LH. However, infusion of cortisol at a rate which increased concentrations of cortisol in plasma to amounts greater than those observed after injection of ACTH, did not affect the ability of GnRH to induce the release of LH (Fuquay and Moberg, 1983). When the experiment was repeated in adrenalectomized rams, it was observed that ACTH treatment significantly reduced the LH response to exogenous GnRH administration. This suggests that the mechanism by which ACTH reduces the

responsiveness of the anterior pituitary gland to GnRH is independent of steroid production by the adrenal gland. Dobson et al., (1988) found that the suppressive effect of ACTH(1-24) on LH secretion, after treatment of anestrus ewes with GnRH or estradiol, was not dependent on increased plasma concentrations of cortisol. The release of LH was not suppressed within 30 min of ACTH(1-24) injection, in spite of increased cortisol concentration at the time of GnRH injection. However, by 3 hours after ACTH(1-24), when cortisol concentrations were still increased, there was a significant decrease in LH response. In contrast, Li and Wagner, (1983) observed that continuous infusion of ACTH to adrenalectomized heifers had no effect on LH, but neither did treatment of adrenalectomized heifers with hydrocortisone succinate, alter mean LH concentrations. In a study with bovine pituitary cells in vitro, Padmanabhan et al., (1983) found that ACTH had no effect on GnRH induced LH response, but cortisol at concentrations found normally in the blood of postpartum cows inhibited GnRH induced LH release. A similar response was observed by Li, (1987) in studies with pig pituitary cells in vitro.

Intracerebroventricular administration of corticotropin releasing hormone (CRH) to rats reduced concentrations of LH in plasma in the absence of circulating steroids of either adrenal or gonadal origin (Rivier and Vale, 1984). Administration of a CRH

antagonist (alpha-helical ovine CRH residues 9 to 41) to rats, abolished the stressor-induced inhibition of pituitary LH (Rivier et al., 1986). In ovariectomized and adrenalectomized rhesus monkeys, CRH inhibits LH and FSH secretion (Xiao et al., 1989) and this inhibitory effect is reversed by infusion of naloxone (opioid antagonist) (Gindoff and Ferin, 1987). These results indicate that the inhibitory effect of CRH is modulated by endogenous opioid peptides (EOP). In contrast to the response in rats and monkeys in which CRH caused a prolonged inhibition of gonadotropin secretion, the administration of CRH to sheep caused a dose-related stimulation of LH secretion from the anterior pituitary (Naylor et al., 1990). This suggests that methodological and species differences may exist.

Endogenous opioid peptides (EOP). CRH which is released during stress, induces the secretion of pro-opiomelanocortin (POMC) derived peptides such as ACTH, b-endorphin and several enkephalins (Rivier et al., 1982;Bruhn et al., 1984). EOP are involved in the mediation of the inhibitory effect of CRF on LH secretion in rats (Almeida et al., 1988;Petraglia et al., 1986) and primates (Gindoff and Ferin, 1987). Opioid peptides have inhibitory effects on gonadotropin secretion in rats (Almeida et al., 1988), primates (Van Vugt., et al 1984; Gindoff and Ferin, 1987), ewes (Malven and Hudgens, 1987;

Rawlings and Churchill, 1990) and cows (Whisnant et al., 1986;Myers et al., 1989; Rund et al., 1989) and this effect is reversed by administration of an opioid antagonist such as naloxone. Opioid inhibition of LH secretion has been demonstrated during estrous cycles in rhesus monkeys (Van Vugt et al., 1984) and in yearling heifers, but not in cows (Mahmoud et al., 1989). Concentrations of LH in serum and LH pulse frequencies were increased by naloxone in ewe lambs at 20, 25 and 30 weeks of age (Rawlings and Churchill, 1990), and at 3, 11, 13, 17 and 21 weeks of age in prepubertal Holstein bull calves (MacDonald et al., 1990). Based on these results, it is concluded that EOP inhibit the secretion of LH during infancy and before puberty. In steers, morphine (opioid agonist) had no significant effect on serum LH concentrations or LH pulse frequency, but it decreased pulse amplitude; in contrast, naloxone increased LH secretion, indicating a role of EOP in the modulation of LH secretion in steers (Peck et al., 1988).

Endogenous opioids are involved in stress-induced changes in plasma LH concentration in rats. Exposure of rats to ether for 15 minutes, immobilization for 8 h, subcutaneous gauze pad implantation, and complete food deprivation, all resulted in significant decreases in concentration of LH in the plasma of male rats (Briski et al., 1984). The inhibitory effects of stress were reversed in all cases by injections of naloxone. Petraglia

et al., (1986) exposed castrated male rats to inescapable intermittent footshock to study the effect of stress on LH secretion. They observed that exposure of rats to footshock induced a marked decrease in concentration of LH in plasma but intracerebroventricular administration of anti-b-endorphin and anti-dynorphin-A serum reversed electroshock induced decreases in LH concentrations. These results support the contention that endogenous opioid peptides are involved in the inhibition of LH secretion due to stress and the mechanism may involve decease release of GnRH. This mechanism for stress inhibition of LH secretion is also supported by Kalra, (1981), Wilkes and Yen, (1981)and Wiesner at al. (1984).

In addition to the effects of EOP on the hypothalamus, b-endorphin secreted from the pituitary during stress might directly influence gonadotropin regulation. This hypothesis was tested by Chao et al. (1986) using dispersed cells from bovine anterior pituitaries. They observed that naloxone had a stimulatory effect and physiological concentrations of methionine-enkephalin had an inhibitory effect on basal release of LH. These observations suggests that EOP may directly affect the pituitary to influence release of LH.

The influence of naloxone (N) and yohimbine (Y) on pulsatile LH secretion was evaluated in cyclic cattle (Schoenemann et al. 1990). Pulsatile LH secretion was unchanged in N, Y, and NY cows after treatment when

compared with secretion of LH before treatment. But basal and pulsatile LH secretion was inhibited in control cows after treatment. The investigators suggested that the cows were exposed to stress during the post treatment period and that naloxone and yohimbine may have attenuated the stress induced inhibition of pulsatile LH secretion. These results indicate that EOP may be involved in the mediation of the effect of stress on LH secretion in cattle.

In conclusion, stress has deleterious effects on both male and female reproductive activity. The primary mechanism by which stress impair reproductive efficiency is by altering gonadotropin secretion. Stressors activate the hypothalamic-pituitary-adrenal (HPA) axis. Hormones of the HPA axis (glucocorticoids, ACTH, CRH, EOP) have very important roles in modulating the effects of stress on reproductive functions.

CHAPTER III

MECHANISMS BY WHICH STRESS INFLUENCE SECRETION OF LUTEINIZING HORMONE IN CATTLE

Abstract

The influence of stress on LH secretion and the role that endogenous opioid peptides may have in the mediation of this effect were evaluated in 3 to 5 months old Hereford and Angus X Hereford steers. In experiment 1, five steers were previously tamed, whereas five other steers were stanchioned for the first time one day before the evaluation period. Blood samples (10 ml) were taken every 10 min for 12 h to quantify LH in serum and every hour to quantify cortisol in plasma. In experiment 2, ten steers were tamed, whereas ten other steers were weaned from dams on the day of sampling and cannulated one hour before sampling. Steers were given iv infusions of physiological saline (10 ml) or naloxone (1 mg/kg) 2 h after the initiation of the sampling period and every 2 h (.5 mg/kg) until the end of the sampling period (8 h). Blood samples were collected every 10 min to quantify LH

and every 30 min to quantify cortisol. In experiment 1, concentrations of cortisol were greater ($P < .03$) but concentrations of LH were less ($P < .02$) in plasma of stressed steers than in tamed steers. However, the frequency and amplitude of pulses of LH were similar for tamed and stressed steers. In experiment 2, pulsatile LH secretion was suppressed during the first 2 h of sampling in stressed compared with tamed steers. Suppression of pulsatile secretion of LH coincided with increased concentrations of cortisol in plasma. Concentrations of cortisol were greater in stressed steers than in tamed steers ($P <.001$) during the 8 h sampling period but the influence of stress on secretion of LH only existed for about 4 h. The regimen at wich naloxone was administered and the dose of naloxone used in this experiment did not influence concentrations of LH or the number and amplitude of pulses of LH in stressed or tamed steers. However, respiration rates were increased due to treatment. This suggest that the effect of stress on LH secretion in steers is not mediated by EOP. We conclude that taming steers reduces plasma concentrations of cortisol and increases concentration of LH ın serum.

Introduction

Stress has deleterious effects on both male and female reproductive functions. Secretion of LH in cattle

is altered by stress (Welsh et al., 1981; Stoebel and Moberg, 1982b; Harms et al., 1983; Echternkamp, 1984). Stress may alter the effectiveness of experiments designed to evaluate the influence of various factors on the control of secretion of LH.

The mechanisms by which stress alters LH secretion are not well understood. Stress is associated with an increase in circulating concentrations of corticosteroids in cattle (Matteri et al., 1984; Echternkamp, 1984; Moberg, 1991; Knol, 1991). Infusion of cortisol during the follicular phase of the bovine estrous cycle prevented the LH surge (Stoebel and Moberg, 1982a). GnRH induced LH release was decreased by cortisol infusion into heifers (Li and Wagner, 1983a). LH release after exposure of bovine pituitary cells to GnRH in culture, was reduced if cortisol was added to the media (Padmanabhan et al., 1983). Also, dexamethasone, a synthetic glucocorticoid, decreased systemic LH concentrations (Thibier and Roland, 1976) and the response of LH to GnRH in bulls (Chantaraprateep and Thibier, 1978).

In addition to glucocorticoids, other hormones involved in the hypothalamic-pituitary-adrenal axis, may have important roles in the mediation of the effects of stress on reproduction. In cows, exogenous administration of ACTH was more effective in suppressing the LH response to GnRH than was the infusion of cortisol (Matteri and Moberg, 1982). The suppressive effect of ACTH on LH

secretion induced by GnRH was not dependent on increased concentrations of cortisol in ewes (Dobson et al., 1988).

Infusion of corticotropin releasing hormone (CRH) inhibits gonadotropin release from the pituitary in the absence of circulating gonadal and adrenal steroids in rats (Rivier and Vale, 1984) and monkeys (Xiao et al., 1989). This effect was at least partially mediated by endogenous opioid peptides (EOP) in both monkeys (Gindoff and Ferin., 1987) and rats (Petraglia et al., 1986). During stress, EOP are synthesized in the pituitary and hypothalamus. EOP have an inhibitory effect on gonadotropin secretion in cows and this effect is reversed by naloxone administration (Whisnant et al., 1986; Malven and Hudges., 1987; Rund et al., 1989). Therefore, the effects of stress on reproduction could be mediated by EOP in cattle.

Objectives of this study were to determine the effects of stress on LH secretion in cattle and to evaluate a possible role of EOP in the mediation of the stress effect.

Materials and Methods

Experiment 1. Ten Hereford and Angus x Hereford steers calves between 3 and 5 mo of age were used in this study to determine the effect of stress on secretion of LH. Calves were allotted by breed and age. Five steers

were weaned and placed in individual stalls in a barn and tamed. A tamed steer was defined as " an animal that could be led when a halter was placed on the head and was not nervous when in metabolism stalls and approached by people". The animals had halters placed on them and they were tied in pens for 2 h every day. After several days the steers were led. When the steers were adapted to the halter and could be led, they were placed in stanchions in a metabolism stall. The animals were brushed at least two times daily. After about 3 wk when the steers were tamed, the sampling of blood was initiated.

Five steers remained on pasture with their dams while the tamed steers were conditioned to the environment in the barn. Stressed steers were weaned two days before the start of blood sampling were transported to the barn. On the day prior to blood sampling, all steers were cannulated by inserting a cannula into the jugular vein. The steers were then placed in stanchions, keeping the stressed steers on one side of the barn and the tamed steers on the opposite side. During the taming period and during sampling, steers were fed a diet consisting of 50% concentrate and 50% cottonseed hulls. This diet permitted a weekly gain of about .9 kg per day.

Blood samples were collected every 10 min for 12 h and samples were stored for 12 h at 4^{o} C. Then samples were centrifuged (3000 x g for 30 min) and serum was decanted and stored at -20^{o} C until LH was quantified by

radioimmunoassay (Bishop and Wettemann, 1992). Each hour, a blood sample was taken to evaluate concentrations of cortisol. Oxalic acid (1.25 mg) was added to each 10 ml sample and samples were placed on ice. Within 1 h after collection, samples were centrifuged and plasma was decanted and stored at -20^{o} C until cortisol was quantified by radioimmunoassay (Dunlap et al., 1981).

Experiment 2. Twenty Hereford and Angus x Hereford steer calves between 4 and 5 mo of age were used in the second experiment to evaluate the effect of stress on secretion of LH and to investigate a possible role of opioids in the mediation of the stress effect. As in the first experiment, steers were allotted by breed and age, and half of the steer were weaned, transported to a barn, and maintained in pens. Ten steers were tamed as described for experiment 1. The other 10 steers remained on pasture with their dams until the tamed steers were conditioned to the environment (about three weeks).

The steers were divided into a group of 12 (replication 1) and a group of 8 animals (replication 2) to facilitate intensive blood collection. The tamed steers (replication one n=6; replication two n=4) were cannulated on the day prior to the initiation of the sampling. Stressed steers were weaned from dams on the day of sampling and cannulated one h before sampling. It was anticipated that this would cause maximum differences

in stress intensity between tamed and stressed steers. On the first day of sampling, blood samples (serum) were obtained at 10 min intervals for 8 h. Naloxone, an opioid antagonist, was used to determine if opioids were involved in the mediation of the effect of stress on LH secretion. In each replication, naloxone (1 mg/kg) was injected iv to half of the stressed and half of the tamed steers, 2 h after the beginning of sampling, and each 2 h (0.5 mg/kg) until the end of sampling. Since the sampling period lasted 8 h, 4 periods of 2 h were evaluated. Control steers were treated with saline. Respiration rates were monitored at 30 min after each naloxone infusions as a measure of the physiological response to treatment. Plasma samples were obtained every 30 min to evaluate concentrations of cortisol. Blood samples were processed as described for experiment one. After the end of the sampling period on day 1, the steers remained in the stanchions for an additional day. On the second day, serum samples were obtained at 10 min intervals and plasma was obtained at 30 min intervals for 4 h. LH and cortisol were quantified in serum and plasma, respectively.

A robust locally weighted regression method for smoothing scatterplots (Cleveland, 1979) was used to calculate base line concentrations of LH. A pulse of LH was defined as a point greater than 1 standard deviation above the base line. Pulse amplitude was the difference

between the greatest value during a pulse and the nadir within 30 min prior to the pulse.

Concentrations of cortisol and LH were analyzed as a 2 X 2 factorial, split plot analyses of variance (SAS, 1985) with taming and naloxone treatments as the main plot. Period (2 h interval) and time of sampling within period were the sub-plots. The influence of the treatment combination on concentrations of cortisol for each sampling time were compared using PDIFF option in the LSMEANS statement in SAS program (SAS, 1985).

Results and Discussion

Experiment 1. Concentrations of cortisol in plasma during the 12 h sampling period were influenced by treatment ($P < .03$). Steers that were stressed had greater concentrations of cortisol (16.6 ± 4.8 ng/ml) than the tamed steers (9.5 ± 4.8 ng/ml) that were adapted to the environment (Table 1).

Concentrations of cortisol in plasma are an acute response to stress, so concentrations were analyzed during the first and second 6 h of sampling. During the first 6 h sampling period, there was a treatment x time effect ($P < .03$) on concentrations of cortisol. However, during the second 6 h period, concentrations of cortisol were not significantly influenced by time, and tamed steers tended ($P < .09$) to have reduced concentrations of cortisol

compared with stressed steers (Figure 1). Concentrations of cortisol in plasma of stressed steers were greater ($P < .02$) during the first 6 h of sampling compared with concentrations during the second 6 h.

Stress is usually associated with increased concentrations of glucocorticoid in the plasma of both domestic (Stoebel and Moberg, 1982b; Matteri et al., 1984; Moberg, 1987) and laboratory animals (Rivier et al., 1986; Petraglia et al., 1986). Echternkamp, (1984) found that concentrations of cortisol were 11-fold greater in unacclimated than in acclimated ovariectomized cows. Such a large difference in cortisol concentrations between acclimated and unacclimated cows, when compared to the smaller differences between the tamed and stressed steers in our experiment, could be due to the fact that in the cow experiment the animals were cannulated 1 h before the evaluation period, which lasted for only 4 h. In our experiment, the steers on both treatments were cannulated the day before sampling and blood samples were taken hourly for a period of 12 h. Thus, the cows were probably subjected to more stress than the steers.

Taming of steers resulted in greater mean concentration of LH (7.5 ± 1.8 and 5.6 ± 1.8 ng/ml, for tamed and stressed steers, respectively, $P < .02$, Table 1). Concentrations of LH in serum increased with time of sampling in steers on both treatments ($P < .01$) but there was not a treatment X time interaction. These results are

in agreement with other experiments in which stress has also been associated with reduced concentrations of LH in cattle (Welsh et al., 1981; Harms et al., 1983). In rams, corticosteroid secretion was increased and LH was suppressed due to restrain stress (Matteri et al., 1984). Also, exposure of castrated male rats to inescapable intermittent footshock induced a marked decrease in concentrations of LH in serum (Petraglia et al., 1986).

Secretion of LH in steers on both treatments was pulsatile. The number of pulses per h and amplitude of pulses were not influenced by treatment and averaged 1.05 pulses/h and 6.02 ng/ml, respectively, for steers on both treatments (Table 1). Concentrations of LH in two representative steers from each treatment are depicted in Figure 2. McCarthy et al., (1979) found an average of .66 pulses/h in four and five months old steers. In their experiment, blood samples were taken at .5 h intervals, therefore some pulses could have been missed. Taming increased the incidence of the pulsatile secretion of LH during blood sampling of heifers (Harms et al., 1983). Restrain, intensive blood sampling stress (Schoenemann et al., 1990) and elevated cortisol concentrations (Echternkamp, 1984) were associated with ınhibition of pulsatile LH release. In heifers, repeated acute stress during the follicular phase of the estrous cycle induced a brief elevation in cortisol concentrations and blocked the preovulatory LH surge (Stoebel and Moberg, 1982b). The

lack of effect of stress on the number and amplitude of LH pulses in this experiment could be related to the experimental model. Pulsatile secretion of LH may not be influenced by stress in young castrated males, although mean LH is influenced.

Experiment 2. Respiratory rates of steers were influenced ($P < .001$) by taming and naloxone treatments and there were no taming X naloxone, taming X period or naloxone X period effects. Within 30 min of the naloxone treatments, respiration rates were greater ($P < .001$) in steers treated with naloxone compared with steers treated with saline (Table 2). Respiratory rates were also greater ($P < .001$) in stressed steers when compared with tamed steers (Table 2). The increase in respiratory rates after naloxone infusions indicates that an adequate dose of naloxone was given to cause a physiological response. The magnitude of the elevation in respiratory rates observed in our experiment is similar to that monitored by Schoenemann et al. (1990) after naloxone treatment of cows.

There was a taming X period X time effect ($P < .03$) on concentrations of cortisol (Figure 3). This interaction is associated with differences in magnitude in concentrations of cortisol in steers. Concentrations of cortisol in plasma of stressed steers were greater than concentrations in plasma of tamed steers at all sampling

times (Figure 4). The magnitude of the differences in cortisol concentrations was greater during the first 4 hours of sampling than during the last 4 hours.

There was a naloxone X time effect ($P < .08$) on concentrations of cortisol, but treatment with naloxone did not interact with the taming or period effects. This interaction is difficult to interpret and indicates that concentrations of cortisol at the four sampling times within each period were not the same for naloxone treated and control steers.

The greater cortisol concentrations in the plasma of stressed steers in the second experiment compared to those in the first experiment were probably due to the management of the stressed steers. In the second experiment, the stressed steers were cannulated and confined in stalls for only one hour before the initiation of blood sampling, whereas in the first experiment steers were cannulated and confined in stalls about 18 h before blood sampling commenced.

Stress coincides with an increase in cortisol concentrations in domestic animals. The maximum increase in cortisol concentrations in unacclimated ovariectomized cows occurred during the first 2 h of the sampling period and then concentrations decreased until the end of the study (Echternkamp, 1984). Significant, brief increases in concentrations of cortisol were observed by Welsh et al, (1981) after a rectal electroejacualtion probe was

inserted into bulls, and Stoebel and Moberg (1982b) found increased concentrations of cortisol after 15 min of acute stress in dairy heifers. Cortisol concentrations were also greater in ewes immediately after shearing than at later sampling periods (Dobson, 1988).

Treatment with naloxone did not influence the number or amplitude of LH pulses. There was a naloxone treatment X period effect ($P < .07$) on concentrations of LH, but none of the others two or three way interactions with naloxone were significant. Steers treated with naloxone had reduced concentrations of LH during periods 3 and 4 when compared with concentrations of LH in serum of saline treated animals during the same periods (Figure 5). This interaction is difficult to explain. We hypothesized that treatment with naloxone should increase concentrations of LH if opioids inhibited LH secretion during stress, but the opposite occurred. This effect of naloxone to decrease LH secretion could be related to the mechanism of action of naloxone at axons. After inhibition of the effect of opioids at the axon during the large dose of naloxone given during period 2, the increased opioids at the axons may suppress LH secretion during periods 3 and 4. Using the dose of naloxone and the animal model in this experiment, our results suggests that the effect of stress on LH secretion in steers is not mediated by endogenous opioid peptides (EOP). Schoenemann et al., (1990) suggested that naloxone and yohimbine may attenuate

the stress induced inhibition of pulsatile LH secretion during the luteal phase of the estrous cycle in cow. In contrast, intracerebroventricular administration of opioid antagonists reversed the inhibitory effect of exposure of castrated male rats to inescapable intermittent footshock on LH secretion (Petraglia et al., 1986). In addition, corticotropin releasing hormone (CRH) inhibited gonadotropin release from the pituitary, and this effect was mediated by EOP and reversed by naloxone in both, monkeys (Gindoff and Ferin., 1987) and rats (Petraglia et al., 1986). The mechanism by which stress inhibits LH secretion may vary between species or between animal models within species.

There was a stress treatment X period effect on the frequency of pulses of LH. In tamed steers, LH pulse frequency was similar at each 2 h sampling period (Table 3). In stressed steers, LH pulse frequency averaged .3, 2.0, 2.2, 1.1 pulses per 2 h in periods 1, 2, 3 and 4, respectively. The maximum inhibitory effect on LH pulsatility was exerted during period 1, coincidentally when the cortisol concentrations were greatest. Amplitudes of LH pulses were not influenced by stress treatment or time.

There was a stress treatment X period effect ($P < .001$) on mean concentrations of LH. Tamed steers had greater mean concentrations of LH than stressed steers during periods 1 and 2, and steers on both treatments had

similar mean concentrations of LH during periods 3 and 4 (Figure 6). Mean concentrations of LH in serum of tamed steers were similar during each 2 h period on day 1. There was a time effect on concentration of LH in serum of steers ($P < .07$). This was related to increasing or decreasing concentrations of LH in steers on all treatment combinations within periods (Figure 7).

Stress inhibits pulsatile LH secretion in heifers (Harm et al., 1983) and cows (Schoenemann et al., 1990). Echternkamp, (1984) found that in beef cows, two- to four-fold increases in systemic concentrations of cortisol did not affect LH secretion, whereas 10 to 20 fold increases associated with intensive stress suppressed pulsatile LH release. He suggested that the influence of stress on gonadotropin secretion is dependent on the magnitude of the adrenal steroidogenic response. In our experiment, pulsatile LH release was suppressed during the first 2 h of sampling (period 1), when concentrations of cortisol were much greater than during the other periods (figure 7). A threshold concentration of cortisol may be required for cortisol to inhibit pulsatile LH secretion. Administration of cortisol succinate to cows during the follicular phase of the estrous cycle prevented or delayed the LH surge (Stoebel and Moberg, 1981a). Also, cortisol depressed or blocked LH response to GnRH in heifers (Matteri and Moberg, 1982) and in cultured bovine pituitary cells (Li and Wagner, 1983b; Padmanabhan et al.,

1983). Dexamethasone, a synthetic glucocorticoid, also suppressed secretion of LH in bulls (Thibier and Rolland, 1976) and in rats (Rosen et al., 1988).

Treatment of cows with ACTH was far more effective in suppressing the LH response to GnRH than was the infusion of cortisol (Matteri and Moberg, 1982). In intact and adrenalectomized rams, ACTH significantly reduced concentrations of LH after exogenous GnRH administration, but infusion of cortisol, at a rate which increased plasma corticosteroid concentration to above those observed after injection of ACTH, did not affect the ability of GnRH to induce the release of LH (Fuquay and Moberg, 1983). On the other hand, Li and Wagner, (1983a) observed that continuous infusion of ACTH to adrenalectomized heifers had no effect on LH, and treatment of heifers with hydrocortisone succinate did not appear to influence mean concentrations of LH. In a study with bovine pituitary cells, Padmanabhan et al., (1983) found that ACTH had no effect on GnRH induced LH response. However, cortisol, at concentrations normally found in blood of postpartum cows, inhibited GnRH induced LH release. Since LH secretion in the bovine is regulated by secretion of GnRH by the hypothalamus, and adrenal steroids can penetrate the blood-brain barrier, a direct action of glucocoticoids on GnRH secretion from hypothalamus could take place when concentrations of cortisol increase above some threshold value.

During the second day of the experiment, concentrations of cortisol were greater ($P < .001$) in stressed steers (14.4 ± 1.4 ng/ml) when compared with concentrations in tamed steers (7.8 ± 1.4 ng/ml). Concentrations of cortisol were influenced by treatment of steers with naloxone the previous day (Table 4). Steers that were treated with naloxone had greater ($P < .05$) plasma concentrations of cortisol than steers that were treated with saline. However, the stress treatment X naloxone was not significant.

The number of LH pulses, amplitude of LH pulses and mean concentrations of LH on day 2 were not influenced by treatments (Table 4). In agreement with the threshold theory, concentrations of cortisol may have been less than the required threshold and did not influence LH secretion.

In experiment 1, concentrations of cortisol in plasma were greater and concentrations of LH were reduced in stressed steers, when compared with concentrations in tamed steers. However, the number of pulses of LH per hour and amplitude of the pulses were not significantly different between the two groups of steers.

In the second experiment, as in the first experiment, concentrations of cortisol in plasma were greater and concentrations of LH were reduced in stressed steers when compared with tamed steers during the first hours of sampling. Maximum concentrations of cortisol in stressed steers occurred during the first 2 h of sampling and

pulsatile secretion of LH and mean concentrations of LH in serum were reduced. Treatment of steers with naloxone at the dose and the regimen used in our experiment did not affect the number or amplitude of pulses of LH. However, treatment with naloxone did significantly increase respiratory rates. Concentrations of cortisol were greater in stressed than in tamed steers throughout the experiment, but the effect of stress on secretion of LH only existed for about 4 h during the initial sampling period. Taken together, results from these experiments indicate that taming steers reduces plasma concentrations of cortisol and increases secretion of LH.

Implications

Many management practices are stressful for domestic animals. Stress interferes with pituitary secretion of LH. Thus stress may influence pulsatile secretion of LH which is required for preovulatory follicular growth and may regulate the ovulatory surge of LH. Altered reproductive endocrine functions due to stress may reduce reproductive efficiency and greatly decrease economic returns from livestock production. Stress may also alter the effectiveness of experiments designed to evaluate the influence of various factors on the control of LH secretion. Our results indicate that stress should be minimized when managing cattle.

Table 1. Concentrations of cortisol and LH in plasma of tamed and stressed steers

CRITERIA	TREATMENT	
	TAMED	STRESSED
Steers, no	5	5
Cortisol		
Concentration, ng/ml		
12 h	9.5 ± 4.8[a]	16.6 ± 4.8
1st 6 h	10.3 ± 5.1	19.4 ± 5.1
2nd 6 h	8.6 ± 3.1	13.4 ± 3.1
LH		
Mean, ng/ml	7.5 ± 1.8	5.6 ± 1.8
Pulse frequency, per h	1.1 ± .1	1.0 ± .1
Pulse amplitude, ng/ml	6.0 ± .5	6.1 ± 1.3

[a] mean ± SE .

Table 2. Respiration Rates of steers at 30 min after naloxone infusions

Treatment	N	Naloxone infusion 1	2	3	Mean	SE
Tamed[a]	10	49.6	58.8	47.2	51.8	3.1
Stressed[a]	10	69.4	81.3	68.1	72.9	3.1
Naloxone[b]	10	69.8	83.3	69.5	74.2	3.1
Saline[b]	10	49.2	56.8	45.8	50.6	3.1

[a] Includes naloxone and saline treated steers

[b] Includes tamed and stressed steers

Table 3. Concentrations of cortisol and secretion of LH in tamed and stressed steers during each 2 h period

	TREATMENT								
	TAMED				STRESSED				
Steers, No.	10				10				
PERIOD	I	II	III	IV	I	II	III	IV	SE
Cortisol, ng/ml	10.9	11.5	10.0	10.3	32.6	25.8	19	17.9	4.0
LH									
Mean, ng/ml	11.6	11.7	11.5	11.8	5.5	9.0	11.7	10.3	1.5
Pulse frequency, per period	2.4	2.3	2.0	2.1	.3	2.0	2.2	1.1	.2
Pulse amplitude, ng/ml	9.1	8.6	8.5	7.8	6.9	8.5	8.7	7.4	.8

Table 4. Concentrations of cortisol and secretion of LH in steers during day 2

	TREATMENTS			
	TAMED[a]	STRESSED[a]	NALOXONE[b]	SALINE[b]
Steers, No.	10	10	10	10
Cortisol, ng/ml	7.7 ± 1.4	14.3 ± 1.4	12.1 ± 1.4	9.9 ± 1.4
LH				
Mean, ng/ml	9.6 ± .2	8.9 ± .2	8.8 ± .2	9.7 ± 2.6
Pulse frequency, per 4 h	4 ± .3	3.3 ± .3	3.7 ± .3	3.6 ± .3
Pulse amplitude, ng/ml	7.6 ± .8	6.4 ± .8	7.5 ± .8	6.6 ± .8

[a] Includes naloxone and saline treated steers

[b] Includes tamed and stressed steers

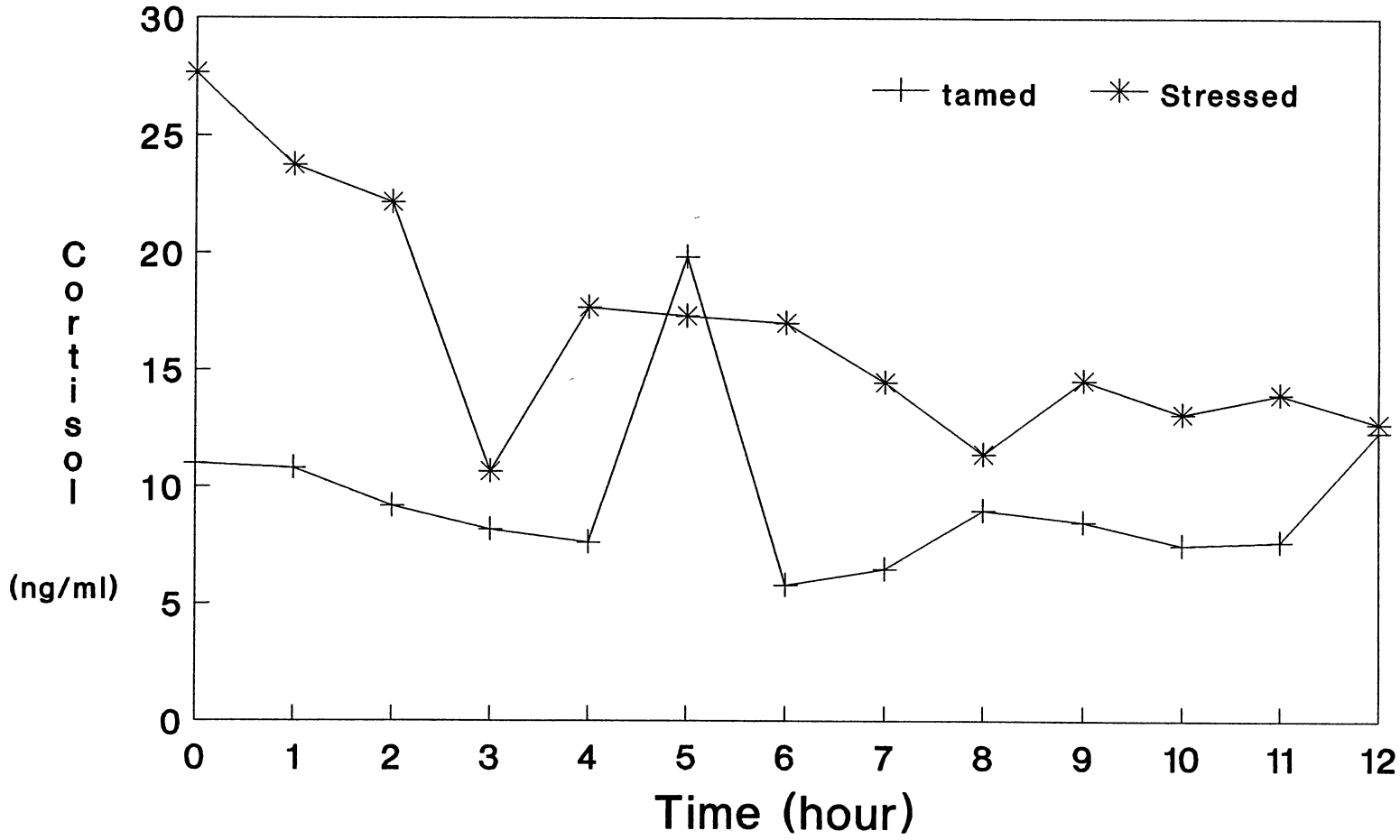

Figure 1. Concentrations of cortisol in plasma of tamed and stressed steers.

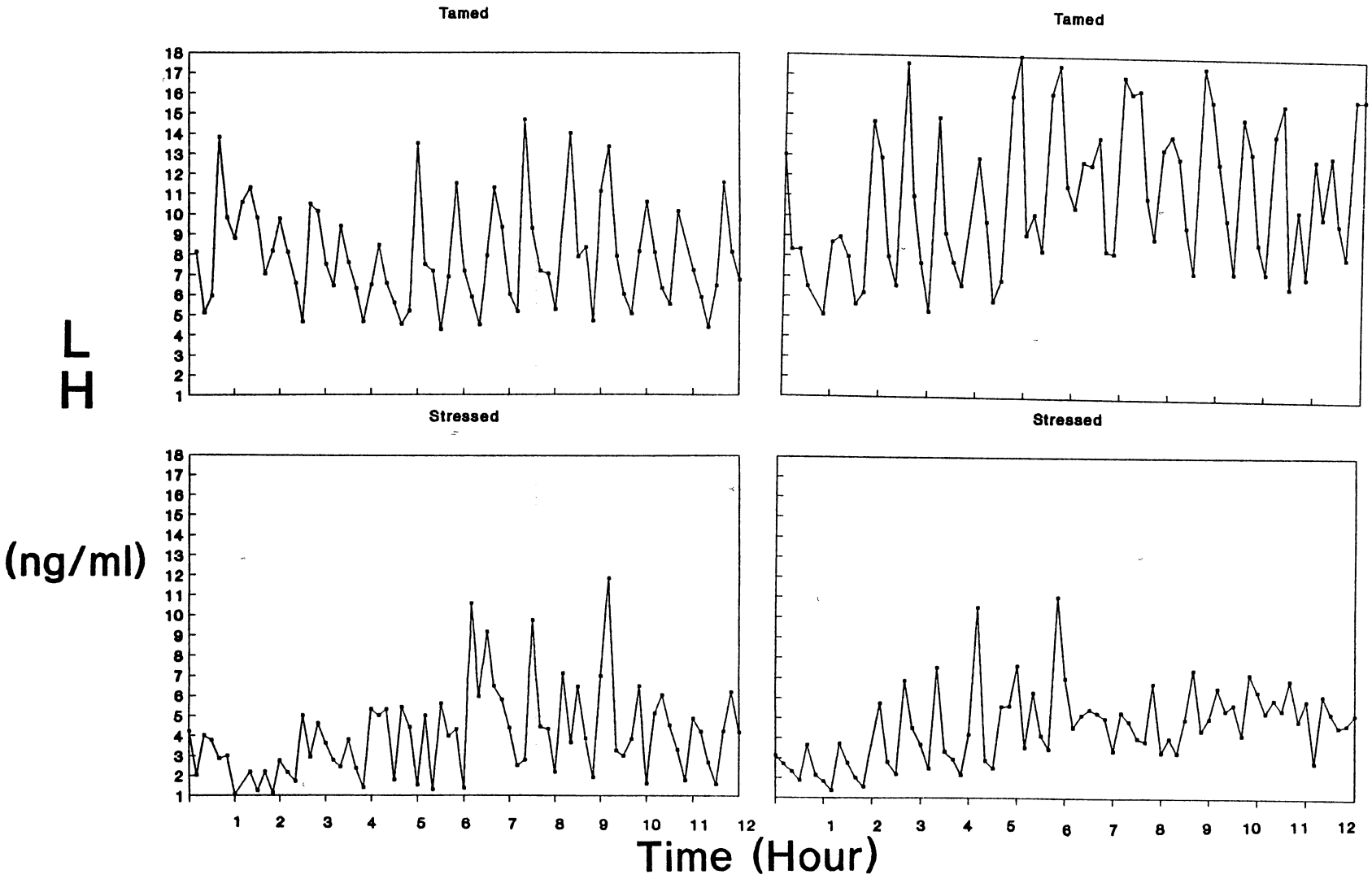

Figure 2. Concentrations of LH in 2 representative tamed and 2 representative stressed steers.

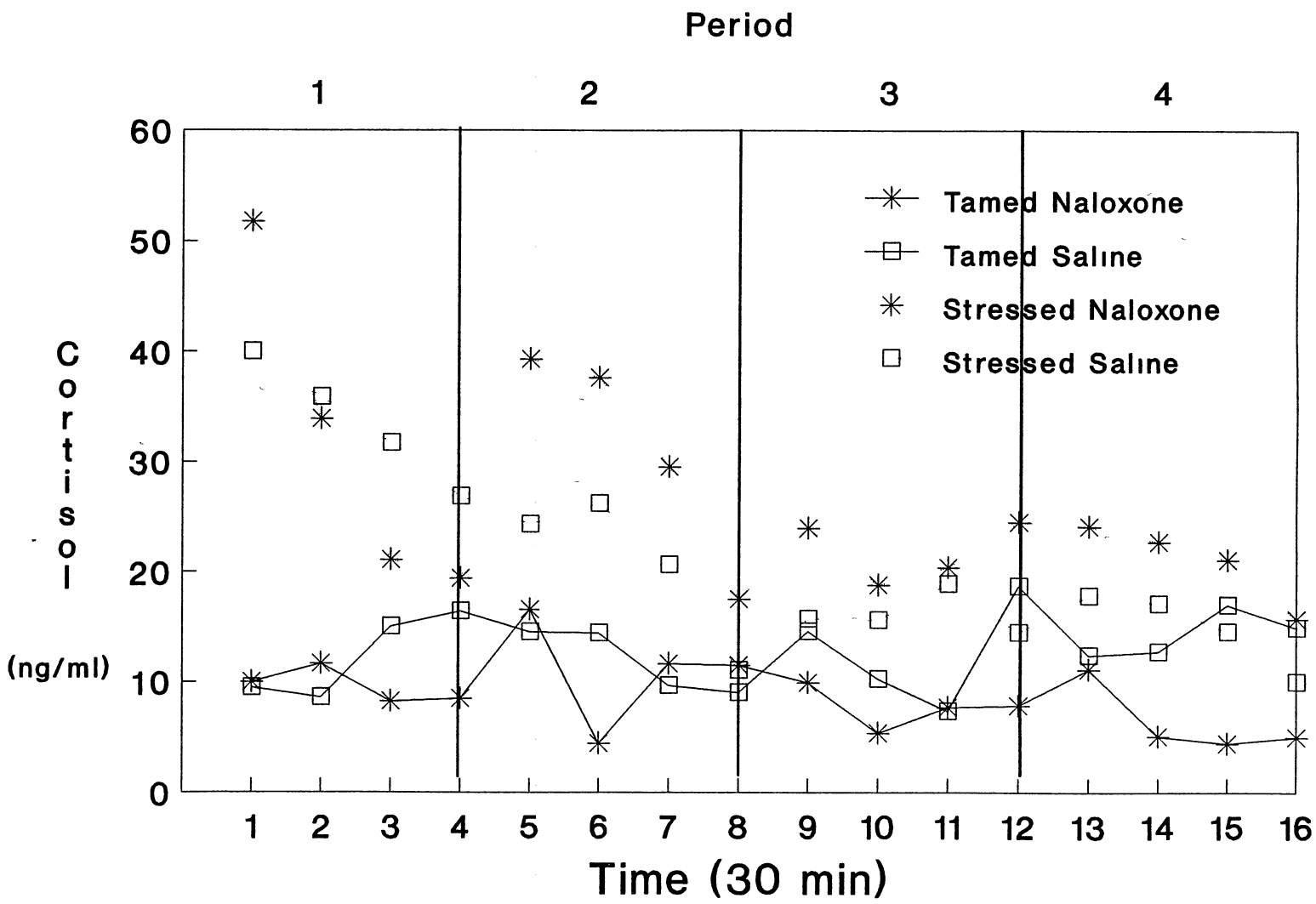

Figure 3. Concentrations of cortisol in plasma of tamed and stressed steers treated with Naloxone or Saline.

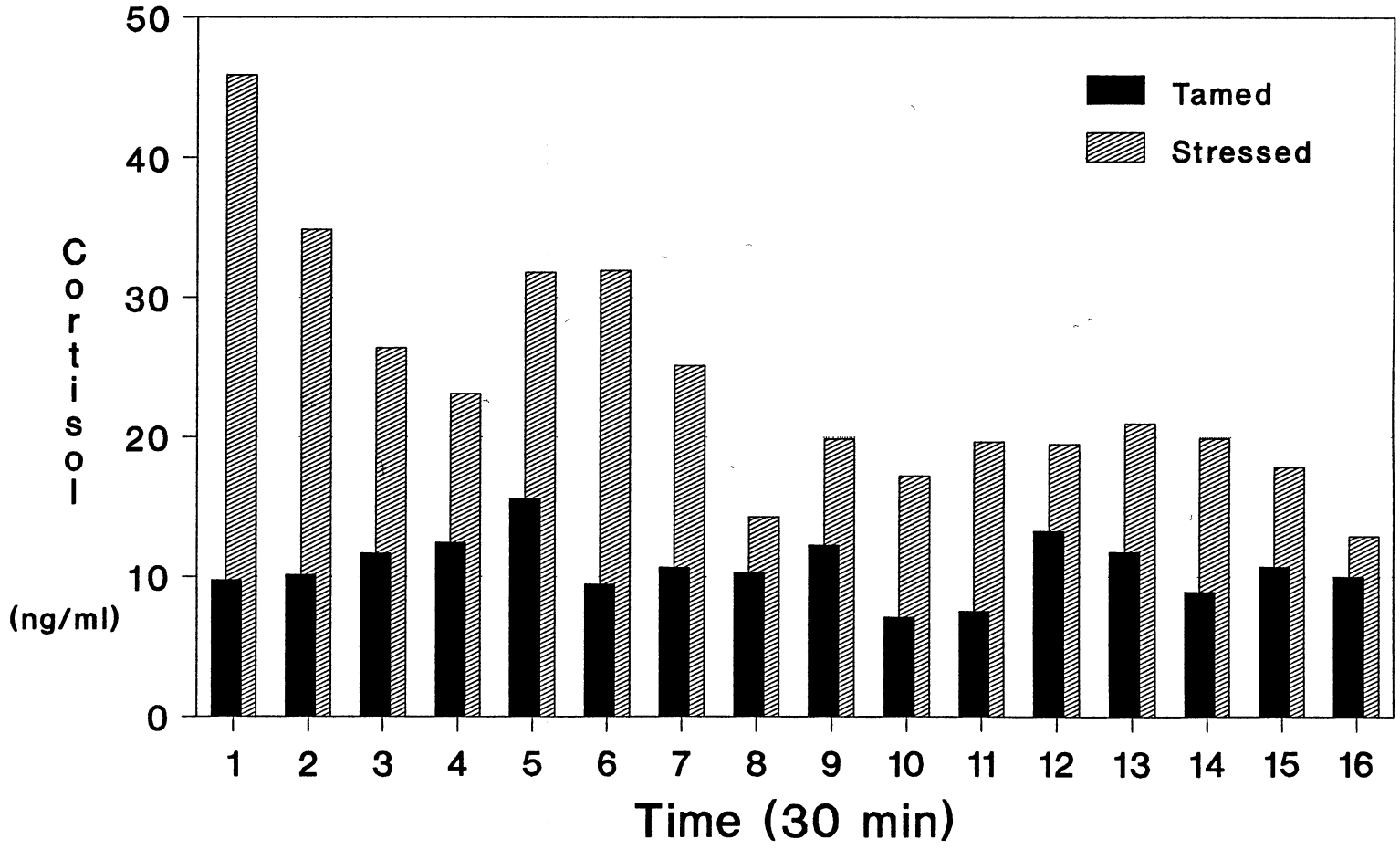

Figure 4. Concentrations of cortisol in plasma of tamed and stressed steers.

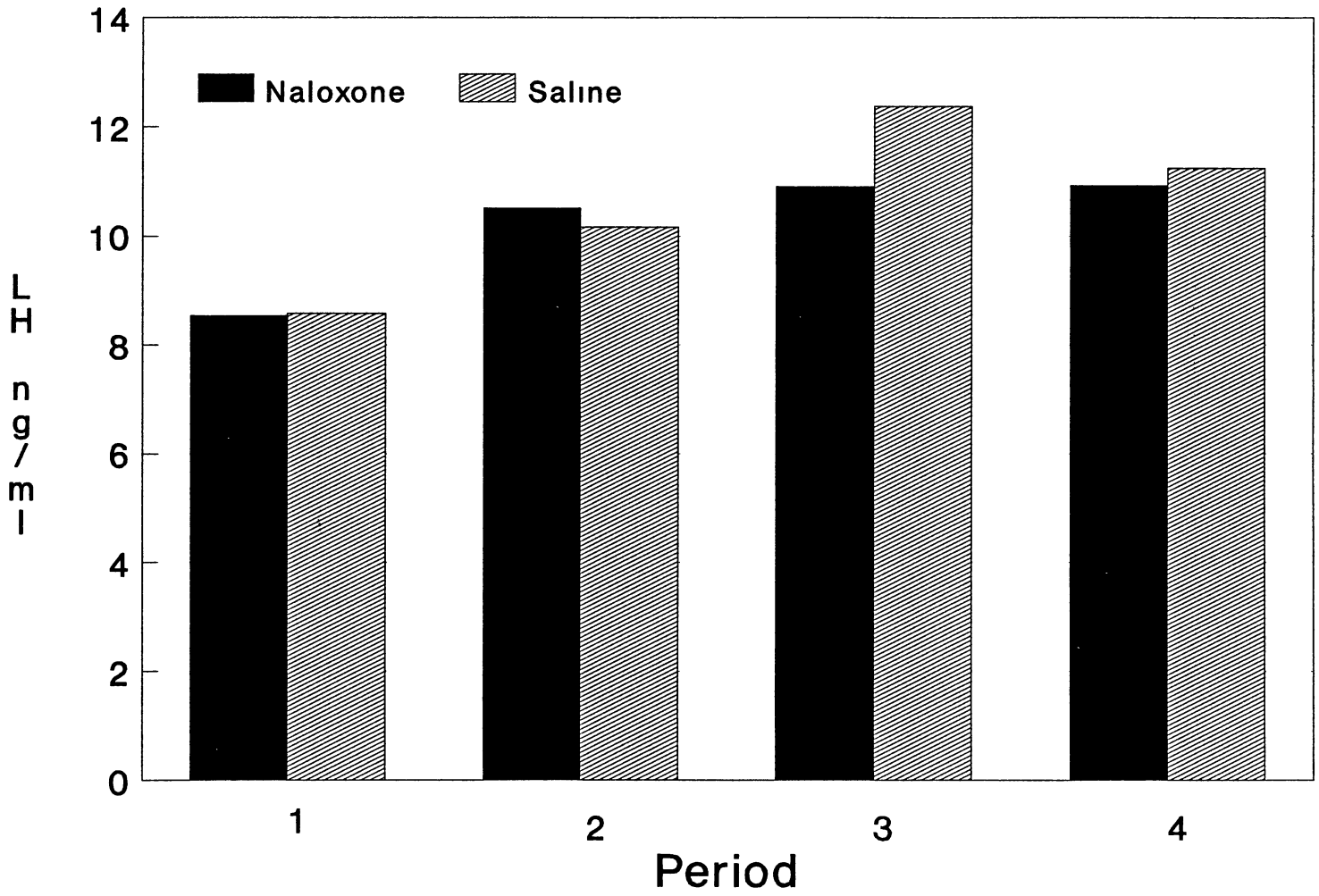

Figure 5. Concentrations of LH in serum of steers treated with naloxone or saline.

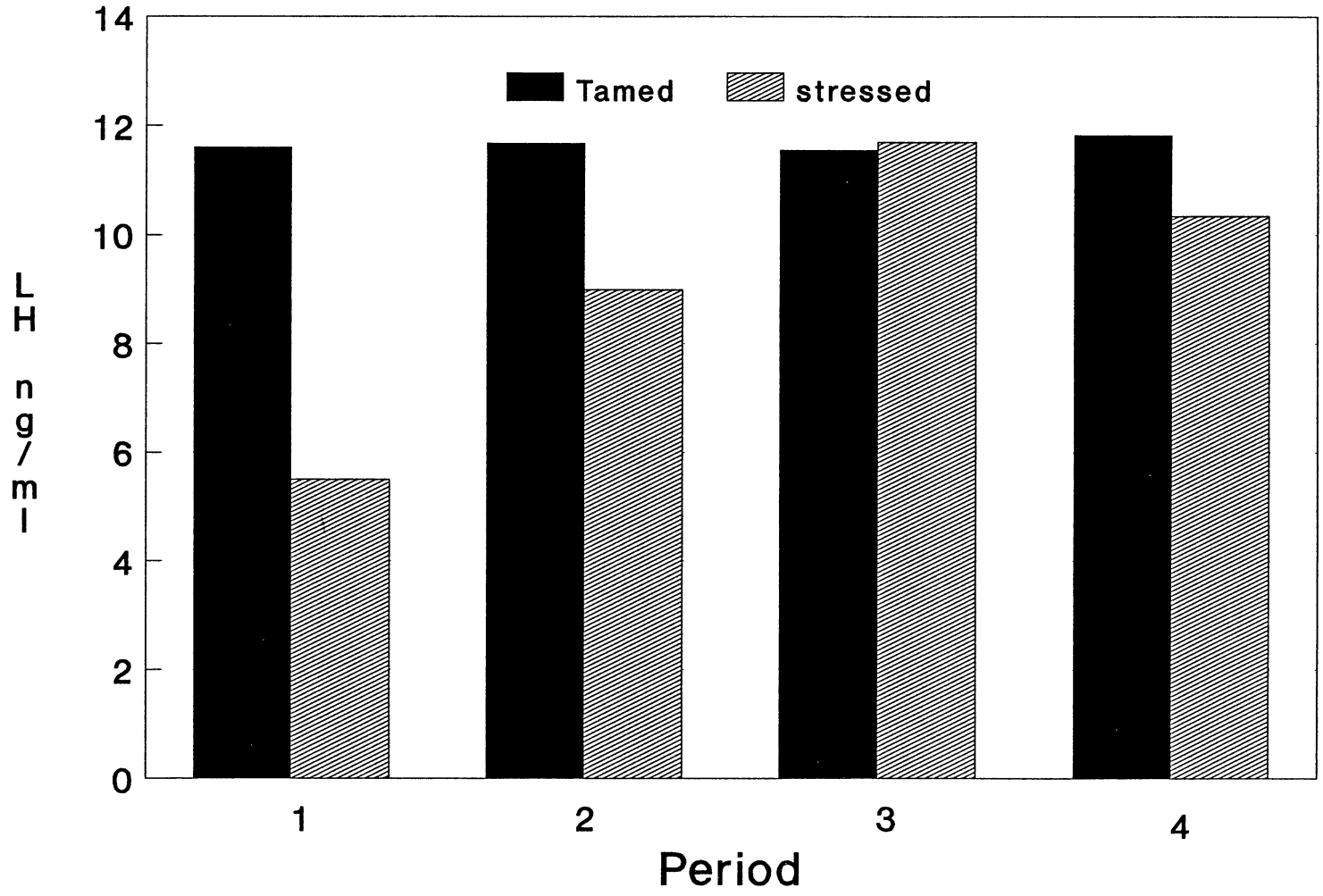

Figure 6. Concentrations of LH in serum of tamed and stressed steers.

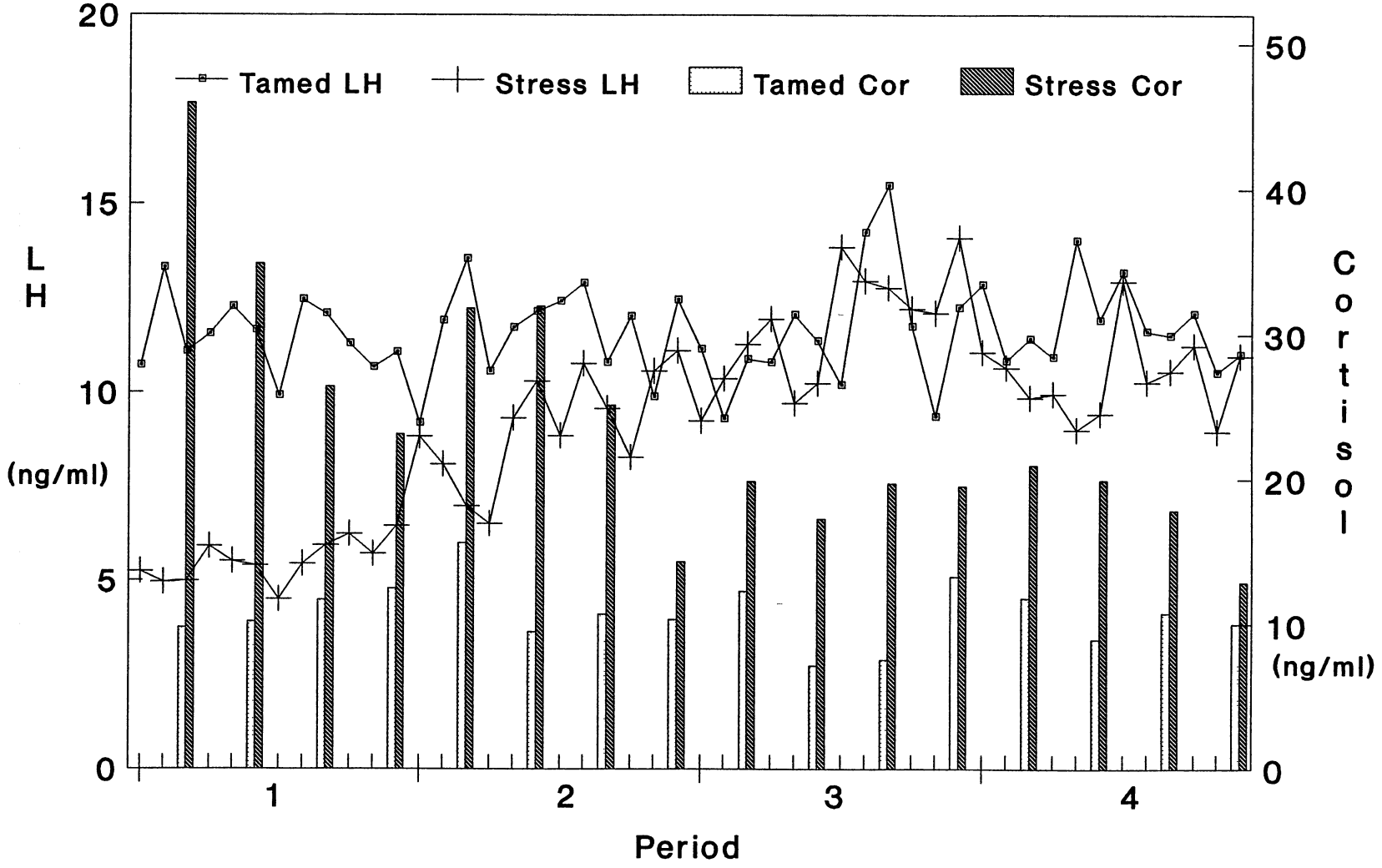

Figure 7. Concentrations of LH and Cortisol in plasma of tamed and stressed steers.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Stress has deleterious effects on both male and female reproductive efficiency. Stress may alter secretion of LH in cattle (Harms et al., 1983; Echternkamp, 1984). This effect of stress may alter the effectiveness of experiments designed to evaluate the influence of various factors on the control of secretion of LH and may also reduce reproductive efficiency in both males and females (Moberg, 1985). Reduced reproductive efficiency greatly reduces economic returns from livestock production.

The mechanisms by which stress alters LH secretion are not well understood. Recent studies suggest that the effects of stress on reproduction could be mediated by EOP in cattle. The objectives of these experiments were to determine if stress alters secretion of LH in beef cattle and to evaluate a possible role of EOP in the mediation of the stress effect.

Hereford and Angus x Hereford steer calves between 3 and 5 mo of age were used in these experiments. In each experiment, half of steers were tamed and the other half

remained on pasture with their dams. After about three weeks when the steers were tamed, sampling of blood was initiated. Blood samples were taken every 10 min to quantify LH and every 30 min (exp.2), or every hour (exp.1) to quantify cortisol. In experiment 2, half of the stressed and half of the tamed steers were given iv infusions of physiological saline or naloxone (1 mg/kg) at 2 h after the initiation of the sampling period and every 2 h (.5 mg/kg) until the end of the sampling period (8 h).

Concentrations of cortisol in plasma were greater and concentrations of LH were reduced in stressed steers when compared with concentrations in tamed steers in both experiments. In experiment 1, the number of pulses and amplitude of the pulses were not influenced by stress. However, in experiment 2, pulsatile LH release was suppressed during the first 2 h of sampling, when the concentrations of cortisol were much greater than during the rest of sampling. This suggest that a threshold concentration of cortisol may be required for cortisol to inhibit pulsatile LH secretion.

The influence of stress on secretion of LH only existed for about 4 h during the initial sampling period. This means that a few hours of conditioning animals to an environment can avoid loss of effectiveness of experiments designed to evaluate the influence of various factors on the control of LH secretion.

Treatment of steers with naloxone did not influence

the number and amplitude of pulses of LH. Steers treated with naloxone had reduced concentrations of LH during the last two periods when compared to control steers. We hypothesized that treatment with naloxone should increase concentrations of LH if opioids were mediating the effect of stress. Thus, based on the treatments used in this experiment, we suggest that the effect of stress on LH secretion in steers is not mediated by EOP.

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