THE EFFECT OF PROSTAGLANDIN F_{2a} ON LUTEINIZING HORMONE AND TESTOSTERONE SECRETION IN BULLS

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ABSTRACT

THE EFFECT OF PROSTAGLANDIN $F_{2\alpha}$ ON LUTEINIZING HORMONE AND TESTOSTERONE SECRETION IN BULLS

By

Terry E. Kiser

The objective of these experiments was to determine the role of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on LH and testosterone secretion in bulls.

In the first experiment, each of five Holstein and three Guernsey bulls (average weight 301 $\pm$ 21 kg) was given (sc) saline or 20 mg $PGF_{2\alpha}$ Tham salt (14.9 mg free acid) in a two-period crossover design. On the first day four bulls were given $PGF_{2\alpha}$ and four bulls were given saline, then the treatments were reversed on the second day. Blood serum LH averaged 1.1 $\pm$ 0.1 ng/ml before $PGF_{2\alpha}$; LH increased ($P<.05$) to 3.5 $\pm$ 1.0 ng/ml at 30 minutes after $PGF_{2\alpha}$, peaked (3.9 $\pm$ 0.9 ng/ml) at 45 minutes and declined to pre-injection values by 4 to 5 hours. Blood serum testosterone averaged 4.5 $\pm$ 0.2 ng/ml before $PGF_{2\alpha}$; it increased ($P<.01$) synchronously in each of the eight bulls to 8.5 $\pm$ 0.9 by 60 minutes after $PGF_{2\alpha}$, peaked at 15 to 16 ng/ml between 90 and 120 minutes, and then declined toward pre-injection concentrations by 180 minutes. The increase in testosterone was preceded by increased serum LH in each of the eight bulls.

In contrast, an average of one episodic increase of blood serum LH (average peak 14.2; range 8 to 22.8 ng/ml) occurred apparently at random intervals during the 8-hour period after bulls were given

saline. An increase of blood serum LH (average peak 3.0; range 1.5 to 4.3 ng/ml) occurred about 30 minutes before each of these testosterone surges. On the average, however, neither LH nor testosterone changed significantly after saline.

The second experiment utilized four Holstein bulls (average weight 355 $\pm$ 19 kg) with cannulae inserted into both jugular veins, one for infusion of saline or $PGF_{2\alpha}$ and the other for blood collection. Prostaglandin $F_{2\alpha}$ Tham salt was infused (0.2 mg/min; 0.15 mg free acid) into two bulls and the other two bulls were given an equivalent quantity of saline vehicle for 20 hours. The infusion treatments were reversed beginning 28 hours after completion of the first infusion. Blood sampling was continued for 8 hours following the 20-hour treatment infusion. Blood plasma LH averaged 1.2 $\pm$ 0.1 ng/ml before $PGF_{2\alpha}$ infusion; it doubled ($P<.07$) within 1.5 hours after the infusion was started and peaked ($P<.01$) at 4.2 $\pm$ 0.8 ng/ml at 6.5 hours before declining to basal concentrations before the end of the infusion. Blood plasma testosterone averaged 7.0 $\pm$ 0.6 ng/ml during the 90 minutes before iv infusion of $PGF_{2\alpha}$; it increased ($P<.01$) to 16.0 $\pm$ 1.5 ng/ml by 2.5 hours after the start of the infusion, remained near this peak until 10 hours and then gradually returned toward pre-injection values by the end of the infusion. Luteinizing hormone started to decline at least 3 hours earlier than testosterone, preceding the decline of testosterone in each of the four bulls. Episodic surges of testosterone similar to those in untreated bulls resumed within 8 hours after the conclusion of the 20-hour infusion of $PGF_{2\alpha}$. Two or three episodic surges of testosterone occurred in each of the bulls during the 20-hour control infusion of saline; the peak (17.2 $\pm$ 0.2 ng/ml) of these surges was

equivalent to the peak concentration of testosterone during infusion of $PGF_{2\alpha}$ and 18 of 19 control surges of testosterone were preceded by increased blood LH (average peak 2.8 ± 0.3 ng/ml).

The third experiment consisted of a preliminary and a main experiment. In the preliminary experiment four Holstein bulls (average weight 355 ± 19 kg) were used during a 3-day period. On the first day, starting at 0800 hours, jugular blood was taken at frequent intervals for 4 hours. On the second and third days each bull was fed 1.01 kg of feed containing 0.5 mg melengestrol acetate (MGA) at 0700 and again at 1900 hours. Then on the third day jugular blood was collected at frequent intervals, starting at 0800 hours and continuing for 4 hours. The average testosterone concentration during the 4-hour period before MGA pre-treatment was 8.5 ± 1.1 ng/ml, significantly greater ($P<0.01$) than average testosterone (1.8 ± 0.1 ng/ml) during a 4-hour period after MGA pretreatment.

The main experiment was conducted as a two-period crossover design with repeat measurement on four bulls (average weight 307 ± 36 kg). Each bull was fed 1.0 mg of MGA daily (0.5 mg at 0700 and at 1900 hours) throughout the experiment. Starting 24 hours after the first feeding, two bulls were given 20 mg $PGF_{2\alpha}$ Tham salt (sc) and two bulls were given saline (sc). The treatments were reversed 10 hours later. Then 48 hours after the first feeding of MGA, each of the four bulls was given iv 200 µg NIH-LH-B8. The testosterone response from these bulls was compared with four control bulls (average weight 328 ± 42 kg) given 200 µg LH after the same sequence of $PGF_{2\alpha}$ treatment but without MGA pretreatment.

After the four MGA-treated bulls were given saline, serum LH concentrations did not change significantly, ranging from 0.30 ± 0.05

to 0.40 ± 0.05 ng/ml. Similarly, serum testosterone fluctuated very little (0.8 ± 0.3 to 1.2 ± 0.2 ng/ml) and did not change significantly. By comparison, serum LH averaged 0.40 ± 0.01 ng/ml before MGA-treated bulls were given (sc) 20 mg $PGF_{2\alpha}$; it increased ($P<.05$) 5-fold at 45 minutes after $PGF_{2\alpha}$, peaked at 2.3 ± 0.5 ng/ml at 60 minutes and declined to basal values between 4 and 5 hours. Serum testosterone averaged 0.8 ± 0.3 ng/ml before $PGF_{2\alpha}$, increased ($P<.05$) to 13.4 ± 4.1 ng/ml at 75 minutes after $PGF_{2\alpha}$ and reached a peak concentration of 21.3 ± 2.3 at 105 minutes. Serum testosterone then was maintained at a stable concentration until 3 hours after $PGF_{2\alpha}$, and declined to basal concentrations at 7 hours after $PGF_{2\alpha}$. Pretreatment of bulls with MGA did not influence testosterone response to exogenous LH treatment compared with control bulls not pre-treated with MGA.

In the final experiment, each of five bulls was given intracarotid infusion of 0 (saline vehicle), 20, 200 and 2,000 ng of $PGF_{2\alpha}$/min and intrajugular infusion of 2,000 ng $PGF_{2\alpha}$/min and 0.2 mg $PGF_{2\alpha}$/min during 3 days. The infusions were maintained for 3 hours at 10 ml/hour with 12-hour intervals between the start of consequentive treatments.

Intracarotid infusion of saline, 20 and 200 ng $PGF_{2\alpha}$/min or jugular infusion of 2,000 ng $PGF_{2\alpha}$/min was ineffective in causing increased blood LH. In contrast, blood LH increased ($P<.05$) from 0.8 ± 0.1 ng/ml to a peak of 2.6 ± 0.5 ng/ml within 1 hour after beginning jugular infusion of the largest dose of $PGF_{2\alpha}$ (0.2 mg/min), then declined to 1.4 ± 0.3 ng/ml at the end of the 3-hour infusion. A similar increase ($P<.05$) (peak of 3.6 ± 1.1 ng/ml) occurred during intracarotid infusion of 2,000 ng $PGF_{2\alpha}$/min, but LH remained elevated

throughout this 3-hour intracarotid infusion. In addition, the LH response during intracarotid infusion of 2,000 ng $PGF_{2\alpha}$/min was greater ($P<.001$) than the comparable response during intrajugular infusion of 0.2 mg $PGF_{2\alpha}$/min.

Testosterone remained below 3 ng/ml during jugular infusion of 2,000 ng $PGF_{2\alpha}$/min, but the same dose of $PGF_{2\alpha}$ infused into the carotid increased ($P<.05$) blood testosterone from 1.3 $\pm$ 0.4 ng/ml before infusion to 7.2 $\pm$ 2.2 ng/ml at 2 hours during infusion and testosterone remained elevated until the intracarotid infusion was stopped. Episodic increases of testosterone occurred in two bulls during intracarotid infusion of saline, but duration of these testosterone surges was significantly shorter than duration of elevated testosterone during intracarotid infusion of 2,000 ng $PGF_{2\alpha}$/min or jugular infusion of 0.2 mg $PGF_{2\alpha}$/min.

The results from these four experiments demonstrate conclusively that $PGF_{2\alpha}$ causes increased blood LH and testosterone secretion in bulls. Furthermore, increased LH preceded testosterone surges after administration of PGF_2 , suggesting that increased LH was the primary stimulus for causing increased testosterone secretion. A constant $PGF_{2\alpha}$ stimulus via a 20-hour intravenous infusion caused a prolonged increase in LH and testosterone, but both hormones decreased to basal concentrations toward the end of the infusion, suggesting that a constant $PGF_{2\alpha}$ stimulus results in hypothalamic or pituitary refractoriness. If a constant $PGF_{2\alpha}$ stimulus caused depletion of hypothalamic LHRH or pituitary LH, the effect was short-lived because episodic secretion of LH and testosterone resumed again within 8 hours after the end of the infusion. Pre-treatment of bulls with MGA abolished episodic secretion of LH and testosterone, but $PGF_{2\alpha}$

overcame this inhibition, suggesting that feedback inhibition of episodic LH secretion possibly may be mediated through or involved with $PGF_{2\alpha}$. Finally, the data provide evidence that $PGF_{2\alpha}$ was acting at the brain to cause release of LH.

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By

Terry E. Kiser

A DISSERTATION

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TO MY WIFE

BIOGRAPHICAL SKETCH

Terry E. Kiser was born on August 24, 1947, in Sumner, Illinois. He attended public schools in Lawrence County and graduated from Sumner High School in June 1965. Following two years in the agricultural curriculum at Vincennes University, he decided to follow his interest in animal agriculture at Southern Illinois University and received a Bachelor of Science degree in June 1969. After serving two years as an operations and intelligence specialist in the United States Army, he enrolled in the graduate school of the University of Wyoming. He received the Master of Science degree in December 1973, majoring in Animal Science.

He then entered a Ph.D. program in the area of reproductive endocrinology and physiology in the Department of Dairy Science at Michigan State University under the joint directorship of Drs. W. D. Oxender and H. D. Hafs. He received the Doctor of Philosophy degree in March 1977 and accepted a position as assistant professor in the area of Bovine Reproductive Physiology, Department of Animal and Dairy Science, University of Georgia, at Athens.

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This dissertation involved the help of many individuals in the Dairy Science Department who unselfishly gave their time. Their cooperation and conscientious work are gratefully appreciated.

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Finally, a most sincere appreciation is expressed to my wife, Gloria, and children, Sheri, Karen and Kevin, for tolerating and supporting my efforts as a graduate student. Also, a thanks to my parents, Mr. and Mrs. Merrill Kiser, and Gloria's parents, Mr. and Mrs. James Jones, for encouraging my educational efforts.

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INTRODUCTION

With successful estrus and ovulation control through the use of prostaglandin in cattle, more emphasis will be placed on utilization of artificial insemination. As a result, fewer and better bulls should be used to sire the next generations of cattle. The bull is one factor affecting fertility of cattle, but little is known about the control of libido, sperm production and sperm quality in bulls, aside from general information to show that they are under the control of the endocrine system (reviewed by Steinberger and Steinberger, 1973; Neaves, 1975).

The purpose of this dissertation was to study the relationship between luteinizing hormone and testosterone secretion in bulls, with special attention to the possibility that prostaglandin $F_{2\alpha}$ may modulate this system.

Specifically, the objectives of this dissertation research were to determine:

1. The temporal relationship between blood serum LH and testosterone in bulls given a single subcutaneous injection of $PGF_{2\alpha}$.

2. Whether a continuous iv infusion of $PGF_{2\alpha}$ can maintain elevated blood LH and testosterone in bulls.

3. Whether $PGF_{2\alpha}$ causes LH release in bulls pre-treated with a progestogen.

4. If $PGF_{2\alpha}$ acts centrally at the hypothalamo-pituitary axis to cause release of LH.

REVIEW OF LITERATURE

Hypothalamo-Hypophyseal-Testis Interactions

A. General

Green and Harris (1947) were among the first to support the idea that the anterior pituitary was regulated by substances originating from the hypothalamus. And in 1948, Harris concluded that the essential hypothalamo-adeno-hypophyseal linkage was vascular, not neural.

Subsequent studies verified the hypothesis that the anterior pituitary was influenced by the hypothalamus, that the influence may be both stimulatory and inhibitory, and that the influence is mediated by neurohumors which normally reach the anterior pituitary via the hypophyseal portal blood vessels. After this hypothesis was established, subsequent investigations were conducted to identify and isolate individual neurohumors, define their mechanism of action, and elucidate their physiological role (reviewed by Geschwind, 1970; Meites, 1970).

The evidence from the neuroendocrine literature suggests at least nine releasing or inhibiting hormones exist in the hypothalamus. Thus far three have been isolated and synthesized: thyrotropin releasing hormone (TRH), luteinizing hormone releasing hormone/follicle stimulating hormone releasing hormone (LHRH/FSHRH) and somatotropin releasing inhibiting factor (SRIF). For the purpose of this review, only LHRH/FSHRH or the synthetic material commonly referred to as

gonadotropin releasing hormone (GnRH) will be discussed. In the course of this discussion LHRH and GnRH will be used to denote the same substance.

B. Gonadotropin Releasing Hormone and Gonadotropin Secretion

Synthetic gonadotropin releasing hormone causes increased LH in cattle (Golter et al., 1973; Zolman et al., 1973), sheep (Galloway et al., 1974; Reeves et al., 1972), pigs (Chakraborty et al., 1973), rats (Arimura et al., 1972), hamsters (Arimura et al., 1971) and humans (Roth, Grumbach and Kaplan, 1973; Arimura et al., 1973).

Presumably, GnRH acts directly on the pituitary to cause release of LH, because Zolman et al. (1973) reported a dose-related increase in LH release when bovine pituitary explants were exposed to purified porcine GnRH during superfusion with Medium 199.

Zolman et al. (1973) also demonstrated that LH release after GnRH differed between male and female cattle. The peak of LH in heifers during the luteal phase of the estrous cycle increased linearly with increasing dose of GnRH up to 80 μg. In contrast, the peak LH response in bulls increased in a linear manner with doses of GnRH up to 160 μg, the highest dose used.

In heifers, the steroid environment appears to alter LH release in response to GnRH. Kaltenbach et al. (1974) suggested that the high concentration of progesterone in luteal phase of the estrous cycle in heifers may dampen LH release after administration of GnRH. In contrast, Zolman, Convey and Britt (1974) found no significant difference in LH release after GnRH on day 15 of the cycle when progesterone was high, or on day 20 of the estrous cycle when progesterone was low in heifers. However, in the same study the magnitude

of LH release in response to GnRH was significantly related to estradiol and estrone concentrations at the time GnRH was administered. Increased concentrations of estradiol and estrone accentuated the LH response after GnRH.

Similarly, Reeves et al. (1971) showed that LH response to GnRH varied during the estrous cycle and was maximal around the period of estrus in ewes. Furthermore, LH response to GnRH was increased by pre-treatment with estradiol benzoate in ewes (Reeves et al., 1971). Conversely, daily injections of 20 mg progesterone for 14 days or infusion of 500 µg progesterone/hr for 76 hours suppressed the LH response to GnRH in anestrous and cyclic ewes, respectively (Hooley et al., 1974; Pant and Ward, 1974).

In summary, LH response after GnRH in the female is influenced by ovarian steroids. Estradiol appears to facilitate and progesterone to inhibit LH release after GnRH.

After administration of GnRH to bulls, serum LH increased similarly in 2-, 4- and 6-month-old bulls (Mongkonpunya et al., 1975); however, a clear testosterone response was evident only in 6-month-old bulls. Thiber (1976) and Galloway et al. (1974) also reported an increase in serum testosterone following administration of GnRH to post-pubertal bulls and rams, respectively.

When GnRH was given to steers 14 days after castration, peak LH concentrations were higher than in bulls; however, the areas under the LH response curve did not differ significantly (Mongkonpunya et al., 1974). In Mongkonpunya's study, testosterone replacement in steers did not restore the magnitude of peak LH after GnRH to that characteristic of bulls.

A greater LH response occurred when purified porcine GnRH was given to wethers than in rams (Reeves et al., 1970), and the LH response was related to dose. More recently, Pelletier (1976) reported that peak magnitude of LH occurred sooner and was greater in wethers than in rams. These authors suggested that the enhanced LH response to GnRH in castrate animals was not due to higher pituitary content, but to endogenous testosterone inhibition at the level of the pituitary. Furthermore, Galloway et al. (1974) suggested that pre-injection concentrations of endogenous testosterone could influence the amount of LH released after GnRH in rams; the lower basal testosterone led to greater LH response to a given dose of GnRH.

Exogenous testosterone treatment reversed the post-castration increase in basal LH concentrations in bulls (McCarthy and Swanson, 1976; Mongkonpunya et al., 1974) and in rams (Galloway and Pelletier, 1975).

Thus, in steers and wethers, exogenous testosterone lowers basal LH. In rams basal testosterone and the physiological state of the animal (castrate or intact) influences the LH response to GnRH. The published data suggest that exogenous testosterone does not influence the LH response to GnRH in steers, but further research will be necessary to delineate the complete steroid feedback relationship to gonadotropin secretion in the bovine male.

C. Relationship Between LH and Testosterone Secretion

Studies concerning the relationship between LH and testosterone secretion in the male are complicated by short term episodic fluctuations in the serum concentrations of these hormones. This necessitates

frequent sampling to fully characterize the profiles of LH and testosterone and their temporal relationship.

For example, Mongkonpunya et al. (1974) reported an average of 3.7 LH spikes daily in bulls. The magnitude of each episodic LH release was 2.7 $\pm$ 0.6 ng/ml, and each LH release was followed by an increase in blood testosterone. Thus, the testis appears to be sensitive to small changes in serum LH concentrations. Similar temporal relationships between LH and testosterone exist in all species studied, including rams (Katongole, Naftolin and Short, 1974; Sanford, Winter, Palmer and Howland, 1974), rabbits (Moor and Younglai, 1975) and humans (Naftolin, Judd and Yen, 1973), and similar episodes of testosterone increases were observed in male rats (Bartke et al., 1973) and mice (Bartke and Dalterio, 1975).

Other stimuli are known to alter testosterone secretion, but in general the alteration is mediated by LH. For example, pronounced seasonal changes of testosterone occur in rams (Gomes and Joyce, 1975; Katongole, Naftolin and Short, 1974; Purvis, Illius and Haynes, 1974; Schanbacher and Ford, 1976). In the report by Schanbacher and Ford (1976), baseline and peak concentrations of testosterone were higher in September than in May. Although season (May or September) had no effect on the number and magnitude of LH peaks, basal concentrations were higher in September than in May. In addition, a temporal relationship existed between LH and testosterone, indicating a cause and effect relationship, during both seasons.

Sanwal, Sundley and Edqvist (1974) reported synchronous variation of plasma concentrations of testosterone in bulls. In their study testosterone peaked synchronously at 0600, 1200 and 2000 hours

in four bulls. Presumably, the increase in testosterone was caused by release of LH, but the apparent synchrony of release remains open to question. Katongole et al. (1971) suggested that rhythms of testosterone were most likely due to some inherent central rhythm. In contrast, Smith et al. (1973) found no apparent synchrony of LH and testosterone secretion in bulls; rather, the episodic burst of LH occurred at random intervals during periods of light and darkness.

Other stimuli, such as exposure of bulls to estrous cows or ejaculation, cause increased testosterone (Smith et al., 1973). Similar changes occur in rats (Purvis and Haynes, 1974) and rabbits (Saginor and Horton, 1968; Haltmeyer and Eik-Nes, 1969) and rhesus monkeys (Rose, Gordon and Bernstein, 1972). But whether the increase in testosterone is mediated via increased LH is questionable. Katongole, Naftolin and Short (1971) suggested that sexual stimuli caused release of LH and testosterone in bulls, but neither Convey et al. (1971) nor Gombe et al. (1973) were able to confirm the observation that sexual stimuli caused increased LH. On the other hand, testosterone increased in six mature bulls (3 to 6 years old) and in two of six younger bulls (1.5 to 2.5 years old) at 30 minutes after ejaculation.

The question remaining to be answered from these experiments is whether LH precedes the increased testosterone following sexual stimulation. The possibility exists that LH increased prior to the increase in testosterone but that the increase was of low peak magnitude and short duration, such that detection at hourly samplings was improbable. Alternatively, testosterone may have increased without a preceding increase in LH. For example, local regulation of testosterone may occur during sexual stimulation in bulls. Thus,

if the observation of Convey et al. (1971) is correct, that testosterone increases in the absence of increased LH after ejaculation, it represents the exception rather than the rule with regard to LH and testosterone relationships.

Further understanding of the dynamic relationship between the pituitary and the testis has resulted by studying the effects of exogenous gonadotropins and androgen secretion in many species, including rats (El Safoury and Bartke, 1974; Moger and Armstrong, 1974; Purvis and Haynes, 1974), rabbits (Johnson and Ewing, 1971; Smith and Hafs, 1973), humans (Maver, Volkwein and Tamm, 1973; Weinstein et al., 1974), rhesus monkeys (Bennett et al., 1973), rams (Falvo et al., 1975), bulls (Katongole et al., 1971; Smith et al., 1973; Sundby, Tallman and Velle, 1975), and pigs (Andresen, 1975). In all species studied, injections of LH or HCG resulted in a rapid increase in testosterone concentrations.

After iv infusion of LH into bulls, serum testosterone concentrations doubled within 1 hour (Smith et al., 1973), and the concentration remained elevated 4 hours when the last sample was collected. In contrast, prolactin did not cause a significant testosterone increase and LH and prolactin together resulted in no greater increase in testosterone than LH alone. Others have reported increases in testosterone after HCG in bulls (Katongole et al., 1971; Lindner, 1969; Sundby, Tallman and Velle, 1975).

Human chorionic gonadotropin concentrations remained elevated for 3 days after administration to bulls (Sundby, Tallman and Velle, 1975), but testosterone was elevated for 8 days after HCG. Thus, HCG appears to prolong the testosterone surge even when HCG was not detectable in the blood.

Luteinizing hormone appears to initiate a sequence of biochemical events in the testes by first binding to specific membrane receptors on Leydig cells. The hormone-receptor complex causes an increase in the activity of adenylate cyclase which results in changes in the concentration of intracellular concentrations of cyclic AMP. Cyclic AMP modulates several metabolic events within the cell which together account for the action of LH (reviewed by Catt and Dufau, 1976; Means, Fakunding and Tindall, 1976; Marsh, 1976).

Dorrington and Fritz (1974) reported that LH (not FSH) increased cyclic AMP production in isolated interstitial cells of Leydig. Conversely, in isolated seminiferous tubules freed of interstitial cells, FSH (not LH) stimulated adenyl cyclase activity. Furthermore, cyclic AMP was released by rat testis during LH stimulation (Catt, Watanabe, and Dufau, 1973) and addition of cyclic AMP to slices of rat (Catt et al., 1973) and rabbit testis (Eik-Nes, 1971) stimulated testosterone production *in vitro*.

D. Hormonal Regulation of Spermatogenesis

That pituitary hormones are required for spermatogenesis was demonstrated by the classic hypophysectomy experiments of Smith et al. (1927, 1930). Luteinizing hormone, follicle stimulating hormone (FSH) and testosterone are all required for spermatogenesis and sperm maturation (reviewed by Steinberger and Steinberger, 1973). As discussed above, LH controls testosterone secretion. Since androgens maintain spermatogenesis (Walsh et al., 1934), Nelson (1937) suggested that LH maintenance of spermatogenesis was mediated via androgen secretion.

Greep and Fevold (1937) were the first to suggest LH control of Leydig cell function and FSH control of spermatogenesis. Follicle stimulating hormone appears necessary for the complete maintenance of spermatogenesis, but the effects of FSH can be mimicked by androgens, indicating a functional relationship between FSH and androgens in the process of spermatogenesis.

The discovery of a specific androgen binding protein (ABP) (French and Ritzen, 1973; Hansson et al., 1973a) produced by the Sertoli cell and stimulated by FSH (Hansson et al., 1973b) has added new insight into the hormonal control of spermatogenesis. Hansson et al. (1975) proposed that androgen binding protein produced by the Sertoli cell served to concentrate active androgen in close proximity to target cells within the seminiferous tubule and epididymis.

Prostaglandins and Reproductive Processes

A. Background

The early history of prostaglandins focused almost exclusively on the male. As early as 1930, Kurzrok and Lieb (1930) demonstrated that fresh human semen caused contractions of human uterine strips *in vitro*. Goldblatt (1933, 1935) and von Euler (1935) reported vasodepressor and smooth muscle stimulatory properties of alcoholic extracts of human seminal plasma. Furthermore, the active substance was soluble in organic solvents at acid pH (von Euler, 1936) and the substance was present in the semen and vesicular glands of humans and sheep. Von Euler (1935, 1939) named the substance "prostaglandin" apparently to distinguish it from other agents associated with the male reproductive tract, such as "vesiglandin" (von Euler, 1936), although prostaglandin is a misnomer since the greatest source of

the prostaglandins is the seminal vesicular glands, not the prostate gland as the name implies.

Initially von Euler (1936, 1939) defined prostaglandins as a nitrogen-free unsaturated carboxylic acid containing hydroxyl groups, but it was not until the 1950's and 1960's that the structure and identity of prostaglandins were determined (reviewed by Bergstrom, Carlson and Weeks, 1968).

The prostaglandins constitute some of the most biologically active substances ever discovered and undoubtedly participate in a wide variety of endocrine and metabolic systems. The natural prostaglandins are unsaturated hydroxy acids with 20 carbon atoms and containing a cyclopentane ring with two carbon side chains, one of which has a terminal carboxyl group.

Four main groups of naturally occurring prostaglandins are distinguished as E, F, A and B, denoting differences in the cyclopentane ring. A trans double bond between carbons 13-14 and a hydroxyl group at carbon 15 are common to the four naturally occurring prostaglandins. Prostaglandin E and F both contain a hydroxyl at carbon 11, but the E prostaglandins have a ketone, whereas F prostaglandins have a hydroxyl at carbon 9. Subscript numbers indicate the number of double bonds in the side chains. The terms α and β refer to the spatial configuration of the carbon 9 hydroxyl group. For example, the structure of $PGF_{2\alpha}$ is shown in Figure 1. Prostaglandins A and B are dehydration products of PGE.

The relationship between the structure and the biological activity of the prostaglandins was complicated by diverse species differences and different physiological states of animals. For example, PGE causes relaxation of non-pregnant human uterus while

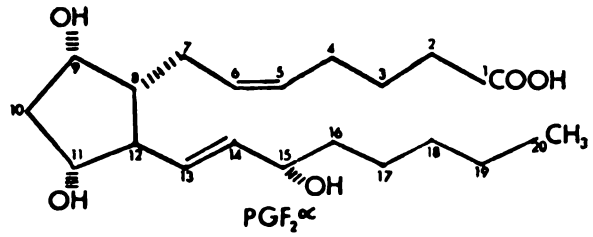

Figure 1. The structure of prostaglandin $F_{2\alpha}$.

PGF causes contraction, and both PGE and PGF cause contractions of pregnant myometrial tissue (Bygdeman, 1967). As an example of species difference, $PGF_{2\alpha}$ acts to decrease blood pressure in the rabbit and cat, similar to the action generally attributed to PGE (Horton and Main, 1965),but $PGF_{2\alpha}$ acts as a pressor in the dog and rat (DuCharme and Weeks, 1967).

In the intact animal, the structural activity relationship is difficult to assess, because prostaglandins are metabolized and inactivated very rapidly by the lungs (Nakano, 1973). Studies on structural activity suggest that E and F prostaglandins were the most biologically potent of the naturally occurring prostaglandins; that metabolism generally results in loss of activity (Kloeze, 1969); and altering the carboxyl side chain, the cyclopentane ring or the alkyl side chain changes the biological activity of a particular prostaglandin.

One of the more important structural features of prostaglandins is the hydroxyl group at carbon 15. The metabolite 15-Keto-PGE, which lacks a hydroxyl group at carbon 15 position, exhibits no biological activity in dogs and rats (Pike, Kupiecki and Weeks, 1967).

B. Effect of Prostaglandins on Gonadotropin and Steroid Secretion

Although the prostaglandins appear ubiquitous in mammalian tissue, the concentration present in most tissue is miniscule compared with the amounts found in the seminal vesicles.

However, prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ have been detected in brain tissue (Ambache, 1966; Coceani, Pace-Asciak and Wolfe, 1968; Coceani and Wolfe, 1965; Holmes and Horton, 1967; Horton and Main, 1966, 1967), cerebellum (Coceani and Wolfe, 1965; Ramwell and Shaw, 1966), spinal cord (Coceani et al., 1968; Ramwell, Shaw and Jessup, 1966) and spinal fluid (Feldberg and Myers, 1966). In addition, the prostaglandins were released spontaneously (Coceani and Wolfe, 1965; Ramwell and Shaw, 1966) after electrical stimulation, or after central nervous stimulation with picrotoxin, pentylenetetrazol and strychnine (Coceani and Wolfe, 1965; Ramwell and Shaw, 1966). Evidence for a specific prostaglandin synthetase in the brain (Van Dorp, 1966), taken together with the fact that prostaglandins are released after electrical stimulation, suggests that they perhaps have a role as a transmitter substance.

Other evidence demonstrates that prostaglandins injected into the cerebral ventricles (Horton, 1964) or intravenously (Duda, Horton and McPherson, 1968) have a long duration of action at central neurons, a finding incompatible with rapid metabolism of transmitter substances. Coceani, Puglisi and Lavers (1971) suggested that the prostaglandins were unlikely to mediate synaptic transmission, but possibly may act as a modulator substance. For example, the prostaglandins may regulate the release of transmitters or possibly act within the effector membrane to decrease or enhance the effectiveness

of a transmitter. In the latter cases, the prostaglandins may act as intracellular messengers by directly affecting an enzyme or by affecting the concentration of substances such as cyclic nucleotides or calcium ions. To my knowledge no reports are available linking prostaglandins to such systems in the brain; however, in other types of tissue the prostaglandins may directly influence the activity of enzymes such as adenylate kinase (Abdulla and McFarlane, 1971), adenylate cyclase (Ramwell and Shaw, 1970), phosphodiesterase (Amer and Marquis, 1972), and Na^+/K^+ activated ATPase (Johnson, Jessup and Ramwell, 1973).

The prostaglandins may increase or decrease the synthesis of cyclic AMP by adenyl cyclase or the breakdown of cyclic AMP by phosphodiesterase (Hinman, 1972). Westermann and Stock (1970) suggested that prostaglandins interfere with binding of ATP to adenylate cyclase to decrease cAMP concentrations, and Blecher et al. (1969) presented evidence that there are two agonist binding sites on adenylate cyclase and that PGE_1 inhibits only one. For reviews on this subject, see Daly (1976), Hittelman and Butcher (1973) and Kuehl (1974).

That the prostaglandins may be involved in the hypothalamo-hypophyseal control of gonadotropin secretion remains to be determined. Harms, Ojeda and McCann (1973), Spies and Norman (1973) and Tsafriri et al. (1973) were among the first to suggest a role for prostaglandins in control of pituitary hormone secretion. Harms et al. (1973) reported that PGE_2 injected into the third ventricle caused a 4- to 5-fold increase in LH, 15 minutes after treatment. Prostaglandin E_1 caused a significant increase in prolactin, and $PGF_{1\alpha}$ or $PGF_{2\alpha}$ had no effect on either LH or prolactin. In contrast to

intraventricular injection, PGE_1 or PGE_2 failed to alter LH or prolactin when injected directly into the anterior pituitary. Thus, the results from these data suggest a hypothalamic site of action for the prostaglandins.

Spies and Norman (1973) also reported increased LH after intraventricular injection of prostaglandin; however, in contrast to Harms et al. (1973), PGE_1 elicited LH release and was more effective than either PGE_2 or $PGF_{2\alpha}$ in inducing ovulations. Similar to the results of Harms et al. (1973), intrapituitary infusion of PGE_1 was less effective than intraventricular infusion in causing LH release, suggesting that the primary site of action was in the central nervous system. Tsafriri and co-workers (1973) also concluded that PGE_2 was effective in causing LH release.

Several papers have confirmed that PGE_1 and PGE_2 cause release of pituitary hormones (Batta, Zanisi and Martini, 1974; Harms, Ojeda and McCann, 1974). Whereas Harms et al. (1973) reported no increase in LH after $PGF_{2\alpha}$, recently Warberg, Eskay and Porter (1976) reported a marked increase in LH after infusion of $PGF_{2\alpha}$ into a lateral ventricle. Furthermore, $PGF_{2\alpha}$ caused an increase in serum LH in hamsters (Saksena, Lau and Chang, 1974), in sheep (Carlson, Barcikowski and McCracken, 1973) and in cattle (Hafs, 1975; Louis et al., 1974).

In heifers, blood plasma LH, prolactin, growth hormone (GH) and glucocorticoids increased either after a single im injection or a constant iv infusion of $PGF_{2\alpha}$. In bulls as in cows, increases in blood prolactin and glucocorticoids were related to the dose of $PGF_{2\alpha}$ (Hafs, 1975). However, Haynes et al. (1975) failed to prove that administration of $PGF_{2\alpha}$ caused LH release.

Recently, infusion of PGE_2 into a lateral ventricle resulted in a 2- to 3-fold increase in LHRH in portal blood plasma (Eskay et al., 1975), suggesting that LH release was mediated at least in part by an increased secretion of LHRH after PGE_2. In agreement, Ojeda, Wheaton and McCann (1975) demonstrated that PGE_2 stimulated release of LHRH. They suggested, therefore, that the hypothalamus was the principal site of action of PGE_2 to cause LH release. Indirect support for this suggestion was provided by Chobsieng et al. (1975) when they reported that antiserum to LHRH blocked PGE_2-induced release of LH in rats.

In a recent report, Harms, Ojeda and McCann (1976) indicated that the effects of PGE_2 on LH release were not mediated through adrenergic, dopaminergic, serotoninergic or cholinergic receptors. Antagonists which were previously shown to inhibit or block these receptors were unable to block PGE_2-induced release of LH. These observations suggested that PGE_2 acts directly on LHRH neurons or other LHRH-secreting elements to stimulate release of LHRH into portal vessels.

In my opinion, the published data collectively indicate that the principal site of action of PGE_2 on LH release is on the central nervous system. As reported by Harms et al. (1973, 1974), prostaglandins may act indirectly at the pituitary, but the response was small relative to that after intraventricular administration of PGE_2.

Treatment of rats with inhibitors of prostaglandin synthesis suppressed ovulation (Armstrong and Grinwich, 1972; Behrman, Orczyke and Greep, 1972). Indomethacin appeared to exert at least part of its effect at the ovary (Armstrong and Grinwich, 1972; Tsafriri, Koch and Lindner, 1973). However, aspirin interfered with pituitary

gonadotropin release (Behrman, Orczyke and Greep, 1972). In the latter study, aspirin blockade of ovulation was reversed by administration of LH or GnRH. In contrast, ovulation blockade by indomethacin was not reversed by either treatment. Similarly, indomethacin blocked the estradiol induced release of LH in anestrous sheep (Carlson et al., 1974).

More recently, Saksena et al. (1975) reported that treatment of male rats with indomethacin caused decreased blood LH and testosterone. They suggested that the decrease in testosterone was mediated by decreased plasma LH concentrations. Since indomethacin is known to block synthesis of prostaglandins and prostaglandins administered into the brain cause LH release, these results suggest evidence of a role for endogenous prostaglandin synthesis in release of LH. However, additional research will be necessary to determine the interrelationship of the prostaglandins with adrenergic, dopaminergic, serotoninergic and cholinergic neurotransmitters which are known to be involved with pituitary hormone secretion (reviewed by Wilson, 1974).

The presence of both E and F prostaglandins in the testis (Carpenter, 1974; Hargrove et al., 1973; Michael, 1973), the ability of the testis to synthesize prostaglandins (Carpenter et al., 1971) and the rapid metabolism of prostaglandins (Anggard, Larsson and Samuelsson, 1971; Nakano, Montague and Darrow, 1971; Nakano and Prancan, 1971) implicate these biologically active substances in a variety of testicular functions, including spermatozoal development and metabolism, the ability of sperm to fertilize, the ejaculatory process and steroidogenesis. These areas were recently reviewed by

Cenedella (1975). For purposes of this section, only the effects of prostaglandins on steroidogenesis will be discussed.

The majority of evidence in the literature supports the hypothesis that the prostaglandins inhibit testosterone secretion. Chronic administration of PGE_2 or $PGF_{2\alpha}$ (Bartke et al., 1973; Saksena, El Safoury and Bartke, 1973) or PGA_1 or A_2 (Saksena, Lau and Bartke, 1974) to male rats and mice resulted in a decrease in accessory gland weights and plasma testosterone concentration. More recently, Bartke, Kupfer and Dalterio (1976) reported that production of testosterone by decapsulated mouse testes *in vitro* was inhibited by adding PGA_1, PGA_2 or PGE_2 to the incubation medium.

Behrman et al. (1971) suggested that the inhibitory influence of prostaglandins in the rat ovary could be mediated via alterations in available esterified cholesterol. However, the decrease of ovarian cholesterol esters following administration of $PGF_{2\alpha}$ to female rats (Behrman et al., 1971) was in direct contrast to the increase in cholesterol ester concentrations in mouse testes after $PGF_{2\alpha}$ (Bartke et al., 1973), although steroidogenesis was inhibited in both cases after $PGF_{2\alpha}$. Bartke suggested that this increase in testicular concentration of esterified cholesterol was due to inhibition by $PGF_{2\alpha}$ of the utilization of esterified cholesterol. More research will be needed in this area to clarify the mechanisms involved.

Prostaglandins can have a dramatic effect on testicular blood flow. Free and Jaffe (1972) and Einer-Jensen and Soofi (1974) reported decreased testicular blood flow after administration of $PGF_{2\alpha}$ to rats. Free and Jaffe (1972) reported that intratesticular injections of $PGF_{2\alpha}$ into rats significantly increased testis venous

pressure and reduced blood flow. Prostaglandin E_1 and E_2 also decreased blood flow, but $PGF_{2\alpha}$ appeared to be the most potent. Thus, decreased blood flow could explain the decrease in testosterone after the prostaglandins in the studies of Bartke et al. (1973), Saksena, El Safoury and Bartke (1973) and Saksena, Lau and Bartke (1974).

On the other hand, testosterone secretion increased significantly when the dog testis was infused with PGE_2 via the spermatic artery (Eik-Nes, 1969). Eik-Nes suggested that PGE_2 might increase cyclic AMP concentrations in preparations of rat testes incubations *in vitro,* a suggestion that Keichline and Hagen (1973) verified. Keichline and Hagen (1973) also demonstrated that LH caused equivalent increases in cyclic AMP. When testicular tissue was incubated with 7-oxa-13-prostynoic acid, a competitive inhibitor of prostaglandins, neither LH nor PGE_1 stimulated increases in cyclic AMP, and the inhibition was not overcome by increasing the amount of LH added to the medium. Furthermore, addition of cyclic AMP to testicular explants, *in vitro,* resulted in increased testosterone concentrations (Rommerts et al., 1972). Thus, PGE_1, like LH, stimulated testosterone secretion in explants from rat testis. The finding that 7-oxa-13-prostynoic acid, a competitive inhibitor of prostaglandin action, blocked testosterone secretion in response to a LH stimulus suggests that PGE_1 possibly may act as an intermediate in the action of LH on testosterone secretion.

Finally, the work of Barcikowski, Saksena and Bartke (1973) suggested that androgens may be involved in the control of plasma prostaglandin concentrations in rats. In their study, plasma prostaglandins were significantly decreased 2 weeks after castration, and

administration of testosterone propionate restored plasma concentrations of $PGF_{2\alpha}$.

In summary, this review provides evidence that LH is the primary stimulator of testosterone secretion in the male. However, the complete physiological control of LH and testosterone secretion remains to be elucidated. The evidence is persuasive, at least in the rat, that the prostaglandins are implicated in LH release, but Haynes et al. (1975) were unable to prove that $PGF_{2\alpha}$ caused LH release in the bull. Furthermore, the majority of evidence in the literature supports the hypothesis that the prostaglandins inhibit testosterone secretion, but Haynes et al. (1975) reported increased testosterone concentrations after $PGF_{2\alpha}$ in bulls.

Therefore, the objectives of this dissertation were to determine:

1. The temporal relationship between blood serum LH and testosterone in bulls given a single subcutaneous injection of $PGF_{2\alpha}$.

2. Whether a continuous iv infusion of $PGF_{2\alpha}$ can maintain elevated blood LH and testosterone in bulls.

3. Whether $PGF_{2\alpha}$ causes LH release in bulls pre-treated with a progestogen.

4. If $PGF_{2\alpha}$ acts centrally at the hypothalamo-pituitary axis to cause release of LH.

Since four separate experiments are contained in this dissertation, the introduction, materials and methods and results and discussion will be given for each experiment separately.

EXPERIMENT 1

THE TEMPORAL RELATIONSHIP BETWEEN BLOOD SERUM LUTEINIZING HORMONE AND TESTOSTERONE AFTER $PGF_{2\alpha}$ OR SALINE IN BULLS

Introduction

As a prelude to determining the effect of $PGF_{2\alpha}$ on sperm output in bulls, Haynes et al. (1975) conducted an experiment to define some endocrine changes in bulls given $PGF_{2\alpha}$. They reported blood testosterone increased to peaks at 1 to 2 hours after treatment with $PGF_{2\alpha}$, and the testosterone peaks were proportional to the dose of $PGF_{2\alpha}$. However, Haynes et al. (1975) were unable to detect significant increases in serum LH after treatment with $PGF_{2\alpha}$. Since it is generally assumed that normally LH is the stimulator for testosterone secretion in bulls (Mongkonpunya et al., 1974; Smith et al., 1973), the following experiment was conducted to determine the temporal relationship between serum LH and testosterone in bulls after a single administration of $PGF_{2\alpha}$.

Materials and Methods

Each of five Holstein and three Guernsey bulls (7 to 12 months and weighing 301 $\pm$ 21 kg) was given (sc) 0.9 percent saline or 20 mg $PGF_{2\alpha}$ Tham salt (14.9 mg free acid) in a two-period crossover design, with repeated measurements on each bull. On the first day, four bulls were given $PGF_{2\alpha}$ and four were given saline, and the treatments were reversed on the following day.

One day before the experiment, a jugular vein was punctured with a 12-gauge thin wall needle (Becton-Dickinson, Rutherford, NJ, T462 LNR) and approximately 15 cm of intravenous polyvinyl tubing (Bo Lab, Derry, NH, BB317, Stock No. V10) was passed through the needle into the vein. Then the needle was removed and the exposed end of the tubing was fitted with a 16-gauge tubing adapter, flushed with 3.5 percent sodium citrate solution and sealed until use for blood collection. The cannula was held in a reclosable pouch made from adhesive tape and affixed to the neck with tag cement (Nasco, Fort Atkinson, WI, C2283 N-FO-617). To collect blood, approximately 3 to 5 ml of blood and sodium citrate solution were withdrawn and discarded. Then, 10 ml of blood was taken and transferred to a 15 x 85 mm test tube. The final step consisted of flushing the cannula with 4 ml of sodium citrate solution and resealing the cannula.

Blood was taken from each bull for 1 hour prior to treatment and for 8 hours after treatment to characterize hormonal changes. To maximize the possibility of detecting changes in LH after either $PGF_{2\alpha}$ or saline, blood was collected at 15-minute intervals for 1 hour prior and 2 hours after treatment, at 30-minute intervals for 2 hours and at hourly intervals for the remaining 4 hours.

Blood was allowed to clot at room temperature for 2 hours and stored at 4 C for approximately 48 hours prior to centrifugation at 2500 xg for 20 minutes. Serum was decanted and stored at -20 C until assayed.

Serum LH and testosterone were determined by specific radioimmunoassay previously described by Convey et al. (1976) and Mongkonpunya et al. (1975), respectively. The standards for the

assays reported were NIH-LH-B8 (National Institutes of Health, Bethesda, MD) and testosterone (Sigma Chemical Company, St. Louis, MO).

Serum hormone data from this two-period crossover experiment were analyzed by split-plot analysis of variance to account for correlation of errors arising out of repetitive measurement of individual animals (Gill and Hafs, 1971). Heterogeneous variance from one treatment to another was tested by Hartley's F_{max} test (Hartley, 1950) and if the departure from homogeneous variance was significant, then a conservative tabular value of F was used to test main effects and interactions (Gill and Hafs, 1971). Selected comparisons were made by Scheffé's procedure (Kirk, 1968).

Results and Discussion

In agreement with previously reported data (Mongkonpunya et al., 1974), basal concentrations of LH averaged 1.1 $\pm$ 0.1 ng/ml in bulls given saline or $PGF_{2\alpha}$ (Figure 2) and no significant baseline difference was observed between the two treatments. After $PGF_{2\alpha}$, LH increased ($P<.05$) to 3.5 $\pm$ 1.0 ng/ml at 30 minutes, peaked (3.9 $\pm$ 0.9 ng/ml) at 45 minutes and declined to pre-injection values by 4 to 5 hours. An unexplained increase in LH in one of the eight bulls caused a secondary increase in mean LH approximately 2 hours after $PGF_{2\alpha}$ treatment. If the data from this bull were excluded, mean serum LH would have decreased to baseline within 2.5 to 3 hours. In contrast, average serum LH was not significantly increased during the 8 hours after saline (Figure 2); the average ranged between 0.6 $\pm$ 0.2 and 1.4 $\pm$ 0.4 ng/ml for the eight bulls. The difference between LH released after $PGF_{2\alpha}$ and that released after saline declined with

Figure 2. Blood serum LH in eight bulls given saline or 20 mg $PGF_{2\alpha}$ (sc).

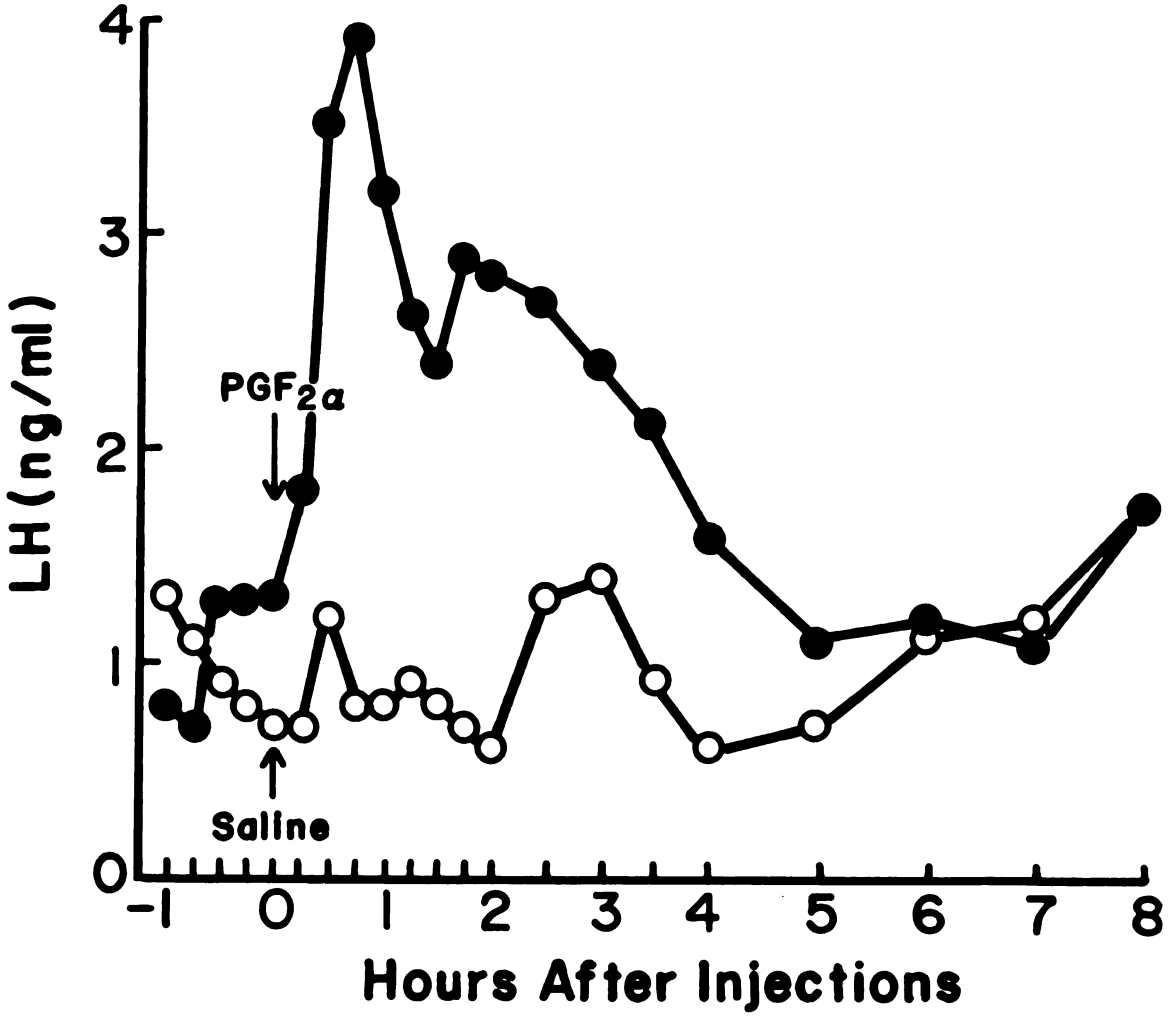
LH (ng/ml)
4
3
2
1
0
PGF2α
Saline
-1
0
1
2
3
4
5
6
7
8
Hours After Injections

Figure 2

time, resulting in a significant ($P<.005$) interaction of treatment and time.

The LH released in response to $PGF_{2\alpha}$ is clear in the present experiment, because LH increased in each of the eight bulls after $PGF_{2\alpha}$, but in only one bull after saline. In retrospect, this result was dependent upon the good fortune that all of the bulls were in a nadir of LH at the time of $PGF_{2\alpha}$ treatment; otherwise, interpretation would be complicated by the normal episodic release of LH. Furthermore, if the releases of LH are influenced by external stimuli or a conditioned stimulus, then the treatment effects would be confounded with the external stimuli. Assuming that the normal release in LH occurs at random intervals, then one may increase the opportunity to reveal significant treatment differences by increasing the number of bulls used in the experiment, by reducing the number of episodic spikes of LH with exogenous steroids or inhibitors of LH release, or by prolonging the treatment response so that the episodic spikes are masked.

The LH results of the present experiment are in contrast to those of Haynes et al. (1975). Although they observed increased serum LH concentrations after $PGF_{2\alpha}$, episodic secretion of LH in control bulls made interpretation of the data unclear. Possibly, differences between results from the present experiment and those from the experiment of Haynes et al. (1975) reflect differences in age of bulls. They used mature bulls (5 to 9 years) which were routinely used in a semen collection center, whereas bulls used in the present experiment were relatively young (7 to 12 months) and sexually inexperienced. Smith et al. (1973) reported that testosterone increased consistently in mature bulls, but not in young bulls

following ejaculation. Katongole et al. (1971) reported that stimuli such as the sight of a cow may elicit release of LH. Perhaps, in the experiment of Haynes, the presence of semen collection personnel or the subcutaneous sham injection could have caused a release of LH mediated by conditioned neuroreflex mechanism.

Increased LH after $PGF_{2\alpha}$ treatment has been reported previously. Carlson et al. (1973) observed an increase in serum LH after intracarotid infusion of $PGF_{2\alpha}$ into diestrous but not anestrous ewes. In addition, a single injection of $PGF_{2\alpha}$ into estrogen pre-treated, ovariectomized hamsters (Saksena et al., 1974) or into male rabbits (Agmo, 1975) and intraventricular administration of $PGF_{2\alpha}$ in rats (Warberg et al., 1976) were followed by increased blood LH. Furthermore, treatment of male rats with indomethacin, an inhibitor of prostaglandin synthesis, caused a significant decrease in the concentration of LH and testosterone (Saksena et al., 1975). Indomethacin also blocked the estradiol-induced release of LH in sheep (Carlson et al., 1974). Both of the latter studies suggest an obligatory role for prostaglandin synthesis in release of LH, but they do not provide definitive proof for $PGF_{2\alpha}$ involvement in LH release because indomethacin blocks synthesis of PGE as well as PGF. However, effects of indomethacin may be secondary because indomethacin may not only have a central effect at the head, but may affect physiological systems anywhere in the body.

Serum testosterone concentrations did not change significantly during the 8 hours after saline treatment (Figure 3). Average testosterone concentrations ranged between 4.3 ± 1.4 to 8.3 ± 2.7 ng/ml. Episodic secretion of LH and testosterone occurred in tandem in each bull, but on the average neither LH nor testosterone changed

significantly after saline (Figures 2 and 3). Before $PGF_{2\alpha}$, blood serum testosterone averaged 4.5 $\pm$ 0.2 ng/ml (Figure 3); it increased ($P<.01$) synchronously in each of the eight bulls to 8.5 $\pm$ 0.9 ng/ml by 60 minutes after $PGF_{2\alpha}$, peaked at 15 to 16 ng/ml between 90 and 120 minutes, and then declined toward pre-injection concentrations by 180 minutes. The overall analysis of variance for the testosterone response indicated a significant ($P<.06$) treatment effect, a significant ($P<.01$) time effect and a significant ($P<.001$) treatment by time interaction. Bull effects and period effects were nonsignificant.

The increase in blood serum testosterone after $PGF_{2\alpha}$ reported in this study agrees well with that described by Haynes et al. (1975) for mature bulls. In both studies synchronized peaks of testosterone occurred with maximal concentrations about 2 hours after treatment.

As another means of evaluating the testosterone response, the interval to and duration of the first detectable increase in testosterone after $PGF_{2\alpha}$ or saline were determined. An increment of 2 standard deviations over pre-injection values was considered a surge. Injection of $PGF_{2\alpha}$ reduced ($P<.01$) the interval to first increase in testosterone and prolonged ($P<.05$) the duration of the testosterone surge relative to those after saline (Table 1). Similar results were reported by Haynes et al. (1975). Interpolating from their data, 20 mg $PGF_{2\alpha}$ Tham salt administered to the bulls in the present study would be a dose sufficient to elicit a maximal response, based on body weight of the bulls.

The increase in testosterone which we report is in contrast to reduced testosterone at 3 and 12 hours after the last of a series of $PGF_{2\alpha}$ injections in rats and mice (Bartke et al., 1973; Saksena et al., 1973). Aside from the possible species differences, it is

Figure 3. Blood serum testosterone in eight bulls given saline or 20 mg $PGF_{2\alpha}$ (sc).

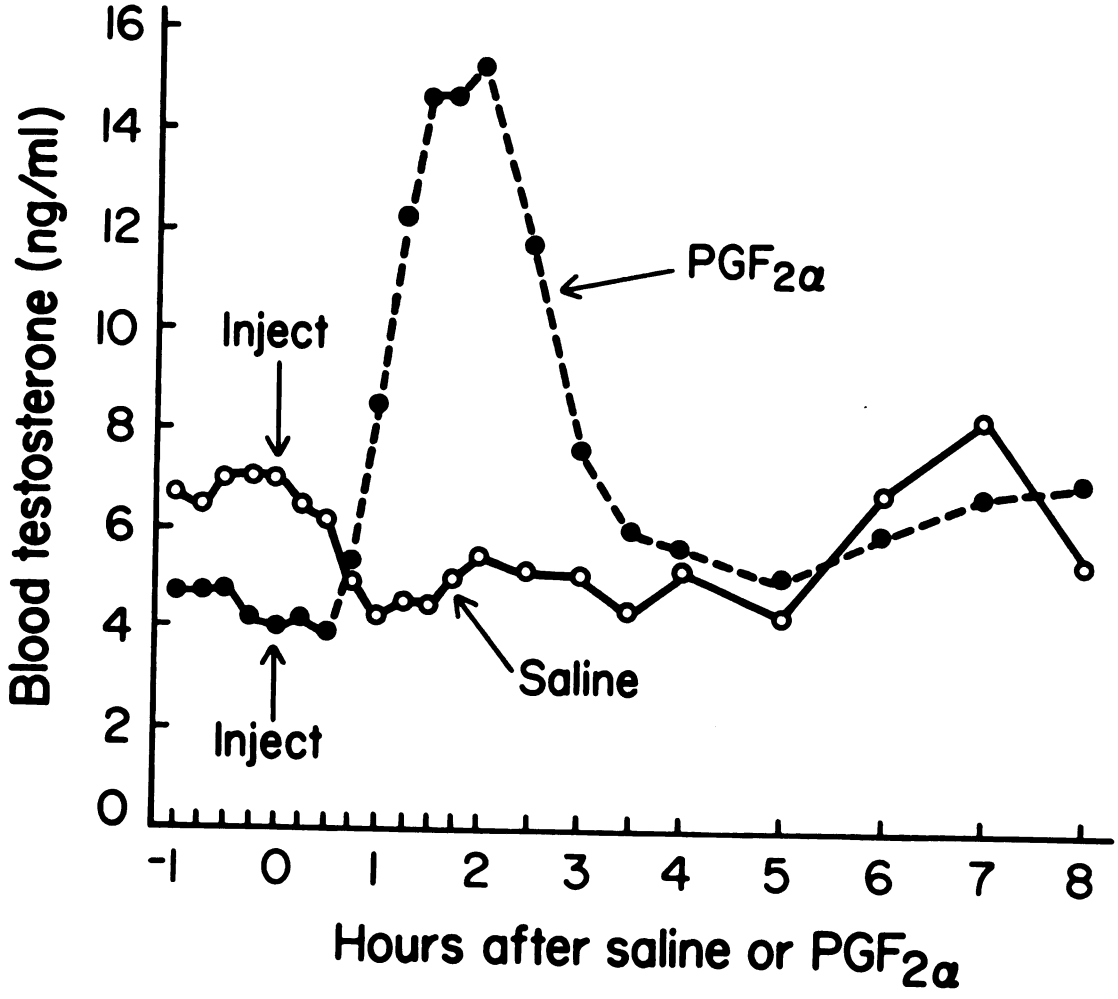
Blood testosterone (ng/ml)
16
14
12
10
8
6
4
2
0
-1
0
1
2
3
4
5
6
7
8
Hours after saline or PGF2α
PGF2α
Inject
Saline
Inject

Figure 3

Table 1. Interval to and duration of first testosterone surge after $PGF_{2\alpha}$ (20 mg, sc) or saline in eight bulls[a]

Criteria	Treatment	
	Saline	$PGF_{2\alpha}$
Interval to surge	274 ± 70	50 ± 10
Duration of surge	56 ± 14	173 ± 86

[a]An increment of 2 standard deviations over pre-injection values was considered a surge. Values are means ± standard errors.

possible that the reduced testosterone in rats and mice may have been associated with the chronic administration of $PGF_{2\alpha}$ in amounts which were much greater (∿40-fold) on a body weight basis than I gave to bulls. In the experiments of Bartke et al. (1973) and Saksena et al. (1973), chronic repeated injections may have depleted the hypothalamic stores of LHRH or possibly the pituitary releasable stores of LH. Previously Ojeda et al. (1976) presented evidence that PGE_2 acts on the LHRH neuron to induce discharge of LHRH into the hypophyseal portal vessels; then LHRH evokes release of LH. Thus, after chronic exposure to a prostaglandin stimulus, the ability of the hypothalamus to release LHRH or the capacity of the pituitary to release LH may be diminished. The net effect as shown by Bartke et al. (1973) would be decreased plasma testosterone concentration and reduced accessory gland weights. Perhaps if LH is released by acute treatment with $PGF_{2\alpha}$, $PGF_{2\alpha}$ may cause increased plasma concentrations of testosterone in rats as in bulls. Rippel, Johnson and White (1974) reported that the LH response was reduced following consecutive injections of GnRH at 24-hour intervals to anestrous and ovariectomized ewes. These

data support the hypothesis that the pituitary becomes refractory to repeated GnRH stimuli.

In the present study, the temporal relationship of changes in blood LH and testosterone (Figure 4) after an injection of $PGF_{2\alpha}$ resembles that in untreated bulls (Mongkonpunya et al., 1974) and that in bulls given synthetic GnRH (Mongkonpunya et al., 1975). For example, Mongkonpunya et al. (1974) reported an average of 3.7 $\pm$ 0.3 testosterone surges per 24 hours with an average magnitude of change of 2.7 $\pm$ 0.6 ng/ml. In agreement with the present study, each increase in LH was followed by an increase in testosterone concentration which averaged 12.0 $\pm$ 0.7 ng/ml. Prostaglandin $F_{2\alpha}$ caused a synchronous increase in serum LH in the present study; the first significant increase occurred at 45 minutes and preceded the first significant increase in testosterone which occurred at 60 minutes post-injection (Figure 4).

Furthermore, the average peak of LH after $PGF_{2\alpha}$ was 3.9 $\pm$ 0.9 ng/ml, comparable to peak episodic LH in Monkonpunya's experiment. Similarly, 20 mg $PGF_{2\alpha}$ administered to bulls in the present experiment (a dose sufficient to elicit a maximal response based on Haynes et al., 1975, data) resulted in increases of LH within physiological limits when compared with control bulls.

Although no significant temporal relationship exists between average LH and testosterone concentrations in bulls given saline (Figure 5), an average of one episodic surge of testosterone (average peak 14.2; range 8.0 to 22.8 ng/ml) occurred apparently at random intervals during the 8-hour period after bulls were given saline. Increased blood LH (average peak 3.0; range 1.5 to 4.3 ng/ml) occurred before each of these testosterone surges. However, because the

Figure 4. Blood serum LH and testosterone in eight bulls given 20 mg $PGF_{2\alpha}$ (sc).

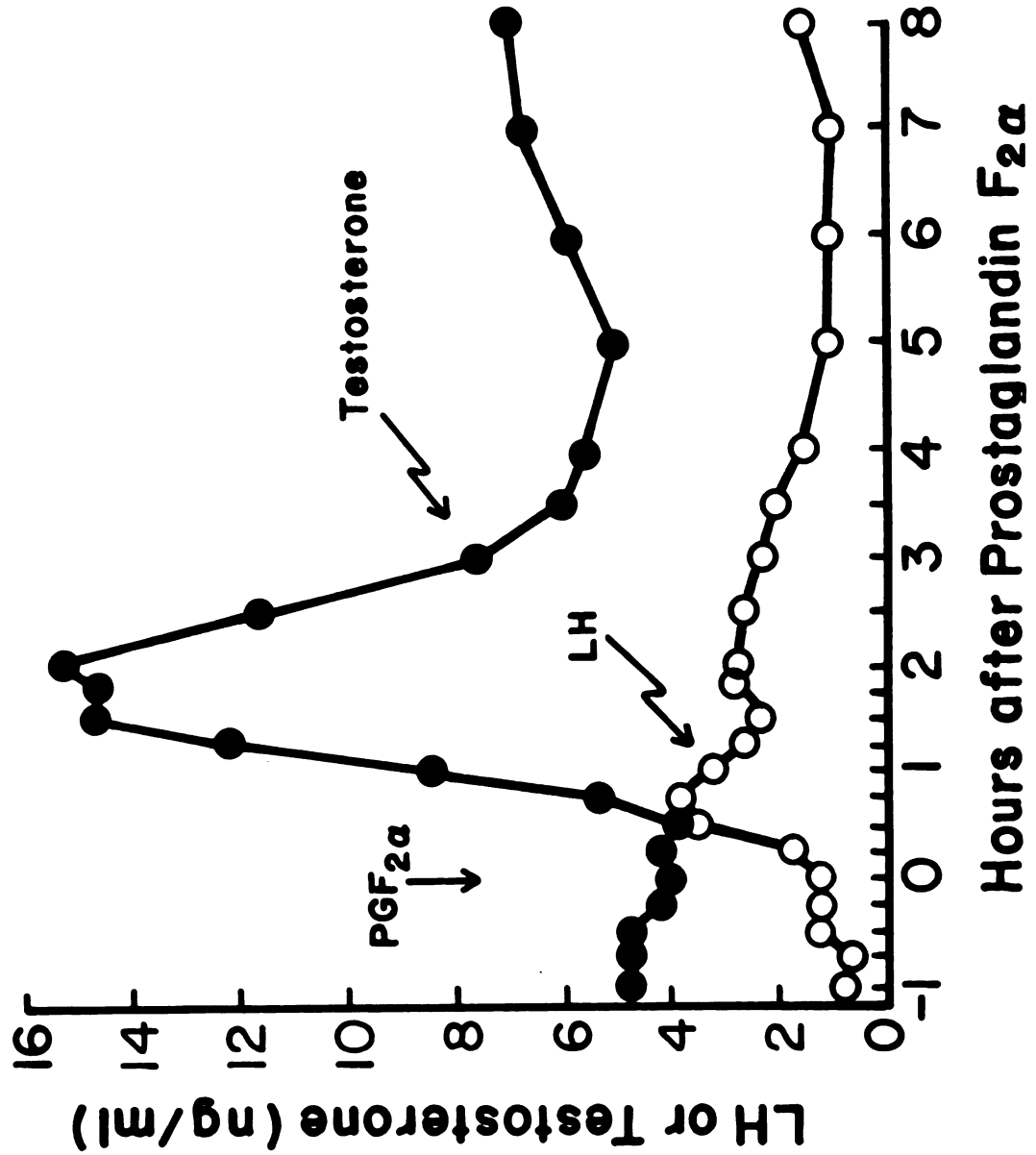
LH or Testosterone (ng/ml)
16
14
12
10
8
6
4
2
0
-1
0
1
2
3
4
5
6
7
8
Hours after Prostaglandin F2α
Testosterone
LH
PGF2α

Figure 4

Figure 4. Blood serum LH and testosterone in eight bulls given 20 mg $PGF_{2\alpha}$ (sc).

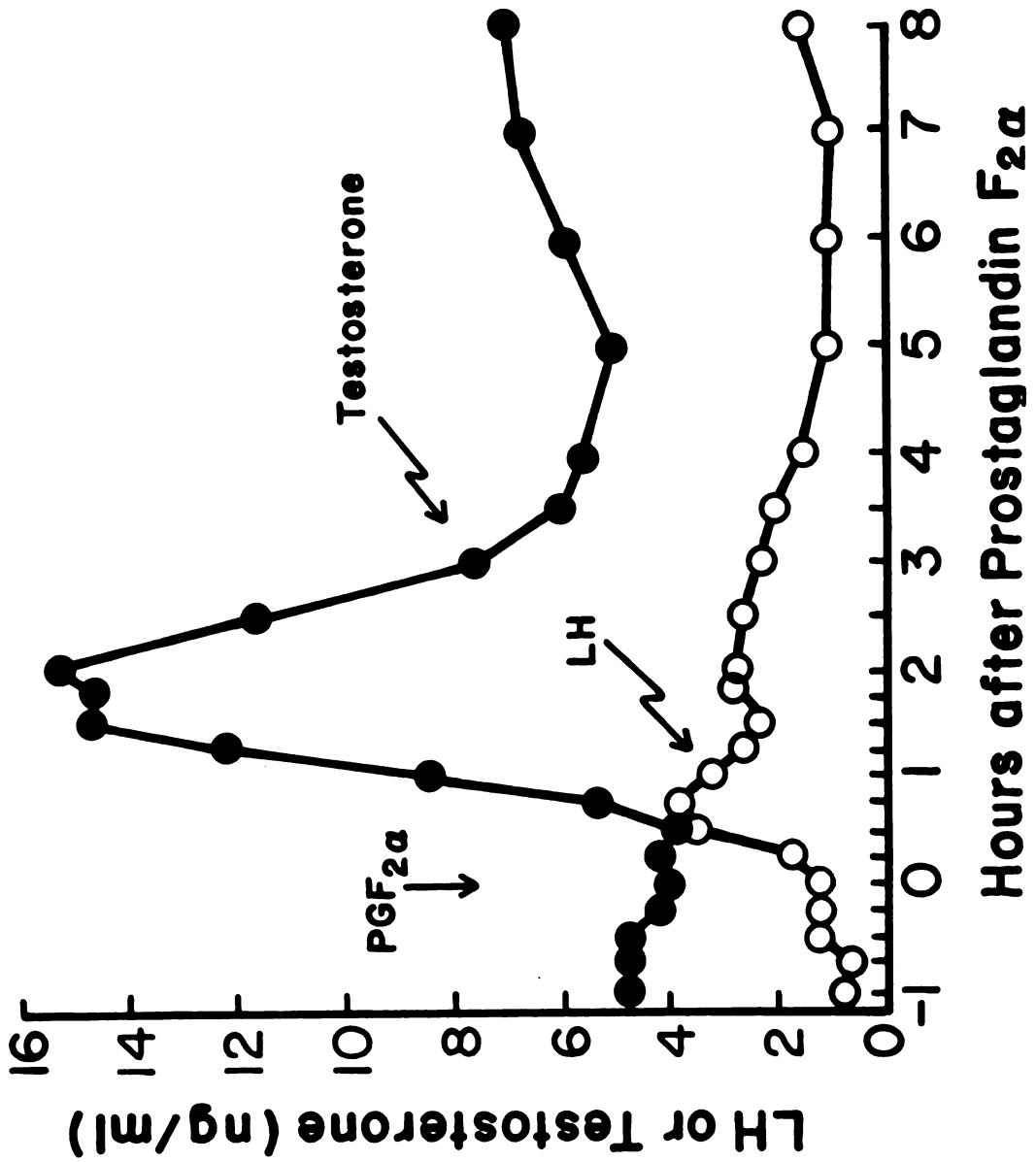
LH or Testosterone (ng/ml)
16
14
12
10
8
6
4
2
0
-1
0
1
2
3
4
5
6
7
8
Hours after Prostaglandin F2α
PGF2α
LH
Testosterone

Figure 4

Figure 5. Blood serum LH and testosterone in eight bulls given saline.

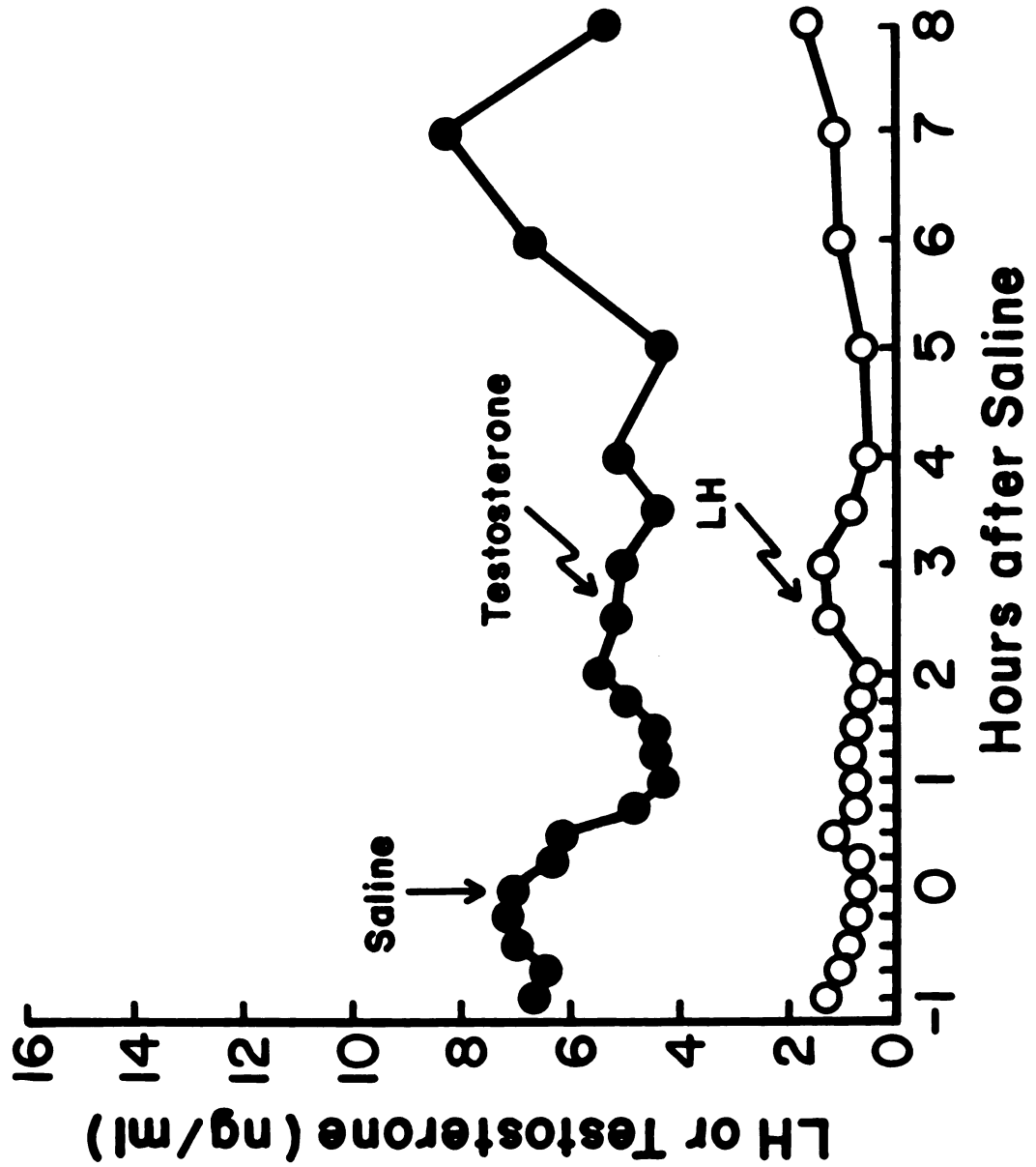
LH or Testosterone (ng/ml)
16
14
12
10
8
6
4
2
0
-1
0
1
2
3
4
5
6
7
8
Hours after Saline
Saline
Testosterone
LH

Figure 5

testosterone and LH surges after saline occurred at random, the average LH and testosterone concentrations did not change significantly during this 8-hour period.

The major conclusion from this experiment is that administration of $PGF_{2\alpha}$ to bulls causes increased blood LH, followed by increased blood testosterone. Both peaks and the temporal relationship of these LH and testosterone surges resemble the normal episodic releases of these hormones in bulls.

EXPERIMENT 2

THE EFFECT OF A CONTINUOUS INTRAVENOUS INFUSION OF $PGF_{2\alpha}$ ON LUTEINIZING HORMONE AND TESTOSTERONE SECRETION IN BULLS

Introduction

The results of the first experiment clearly showed that $PGF_{2\alpha}$ administered to bulls as a single subcutaneous injection caused an increase in both LH and testosterone in bulls. Furthermore, after $PGF_{2\alpha}$ the first significant increase of LH preceded the first significant increase of testosterone, the same as the temporal relationship of LH and testosterone in control bulls. In addition, the peak and duration of elevated LH and testosterone were similar to those reported in control bulls.

In the first experiment, I advanced the hypothesis that the decrease in testosterone observed by Bartke et al. (1973) and Saksena et al. (1973) was the result of chronic treatment with $PGF_{2\alpha}$, possibly refractoriness or exhaustion of the hypothalamo-pituitary axis to a continuous prostaglandin stimulus.

In view of these considerations, the objectives of the second experiment were:

1. To determine if a continuous prostaglandin stimulus would maintain elevated LH and testosterone secretion in bulls.

2. To determine the temporal relationship between LH and testosterone following the conclusion of a continuous $PGF_{2\alpha}$ stimulus in bulls.

Materials and Methods

This experiment utilized four Holstein bulls (12 months old, weighing 355 ± 19 kg) with cannulae inserted in both jugular veins as previously described for the first experiment. One cannula was used for infusion of saline or $PGF_{2\alpha}$ Tham salt and the other for blood collection. A Harvard pump (Harvard Apparatus, Mills, MA) was used to infuse saline or $PGF_{2\alpha}$. Prostaglandin $F_{2\alpha}$ Tham salt was infused (0.2 mg/min) into two bulls and the other two bulls were given an equivalent quantity of saline vehicle for 20 hours. The infusion treatments were reversed beginning 28 hours after completion of the first infusion. Thus, each bull was given 240 mg $PGF_{2\alpha}$ during the 20-hour infusion. Blood was sampled at 30-minute intervals for 1 hour before infusion, during the 20-hour infusion, and for 8 hours afterward for determination of blood plasma LH and testosterone by radioimmunoassays described in Experiment 1.

The data were analyzed by a split-plot analysis of variance (Gill and Hafs, 1971) and selected comparisons were made by Scheffé's procedure (Kirk, 1968).

Results and Discussion

Blood plasma LH (Figure 6) averaged 1.2 ± 0.1 ng/ml before infusion of $PGF_{2\alpha}$; it doubled ($P<.07$) within 1.5 hours after the infusion of $PGF_{2\alpha}$ was started and peaked ($P<.01$) at 4.2 ± 0.8 ng/ml at 6.5 hours before declining to basal concentrations before the end of the infusion. By contrast, average LH concentration fluctuated between 0.6 ± 0.1 to 1.8 ± 1.1 ng/ml and did not change significantly during the infusion of saline. As shown in Figure 6, average LH concentrations following the end of the infusion did not differ in bulls

Figure 6. Blood plasma LH during iv infusion of saline or $PGF_{2\alpha}$ (0.2 mg/min).

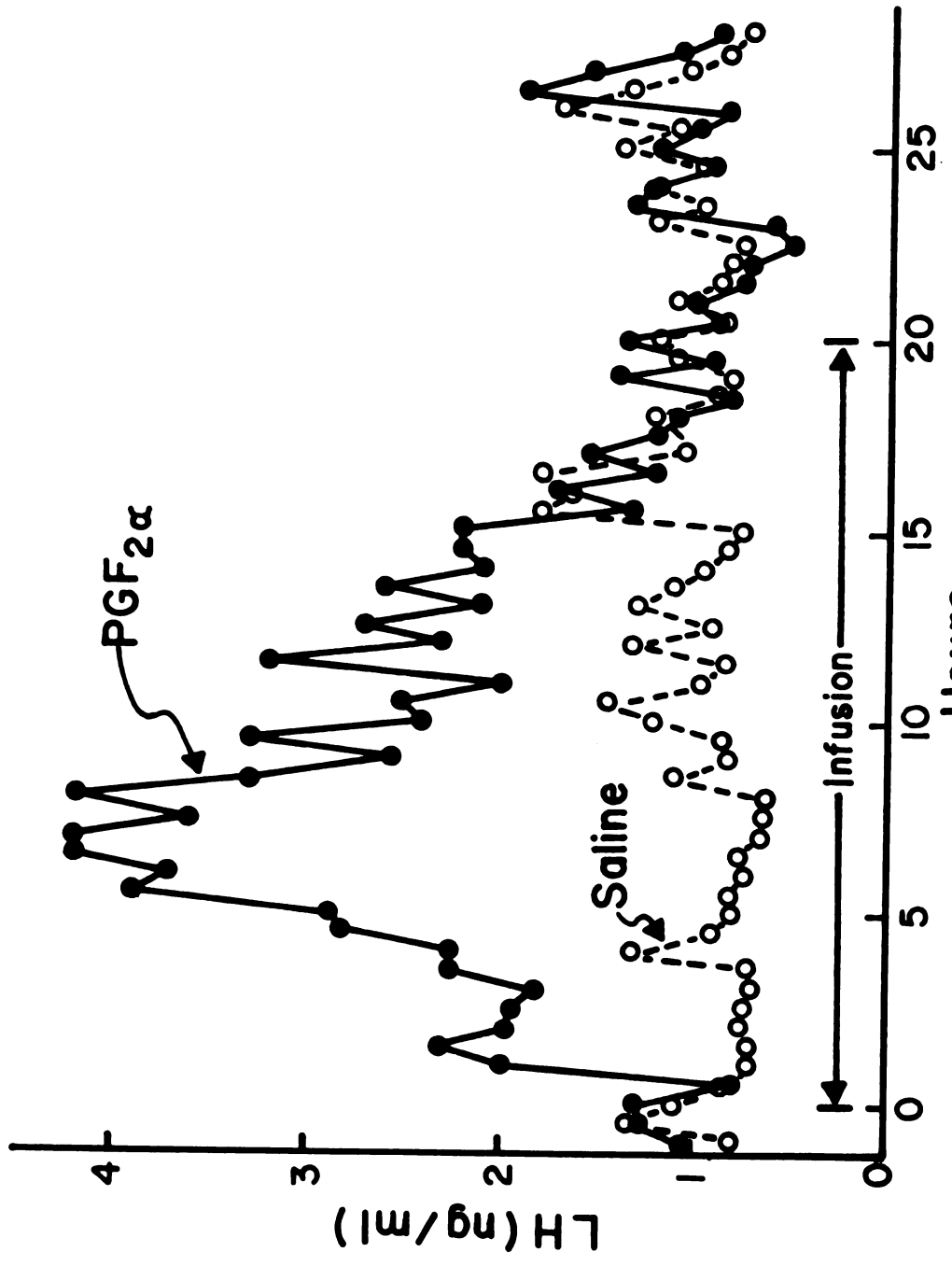

Figure 6

Figure 6. Blood plasma LH during iv infusion of saline or $PGF_{2\alpha}$ (0.2 mg/min).

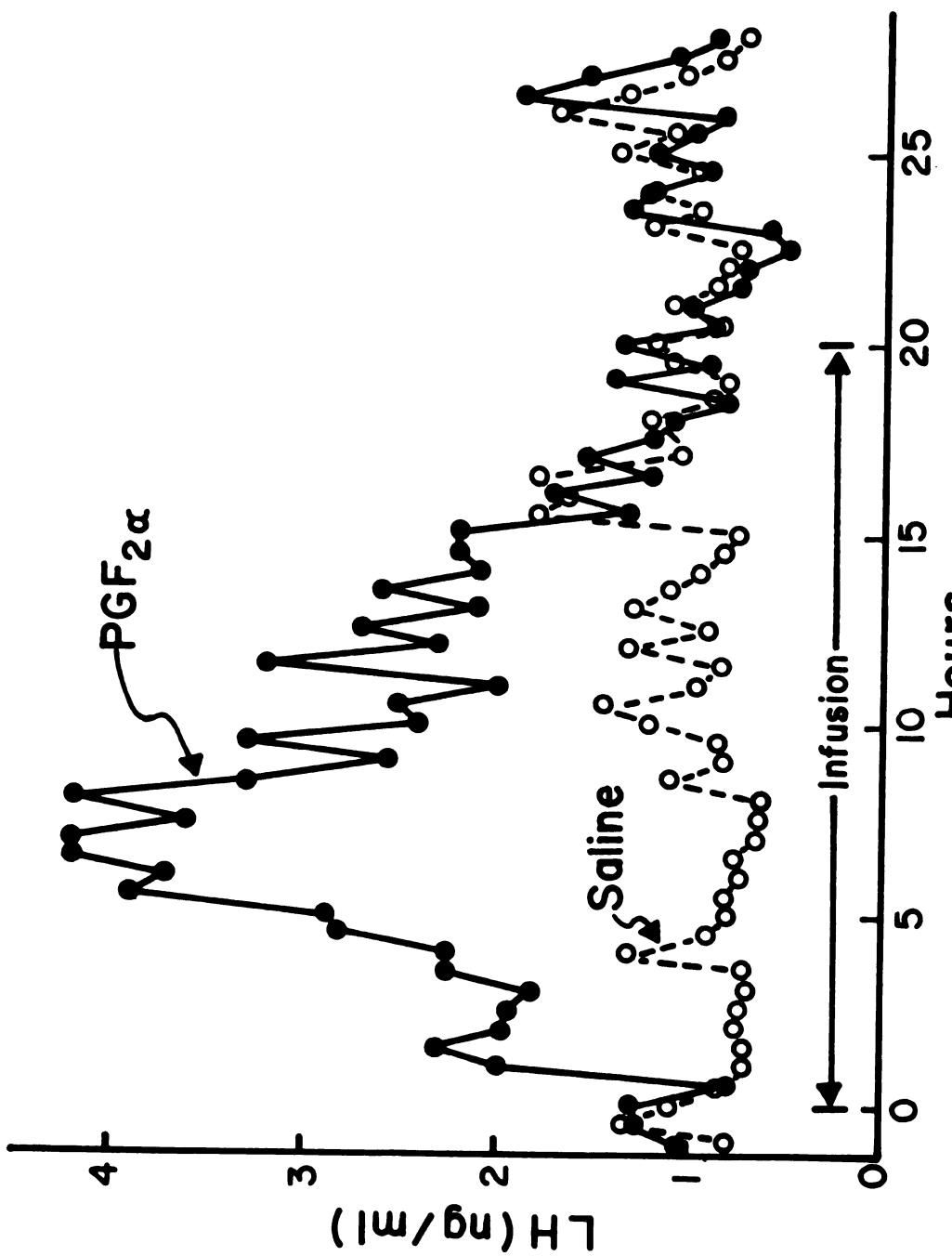

Figure 6

given saline or $PGF_{2\alpha}$. The overall pattern of LH during infusion of $PGF_{2\alpha}$ was similar to the pattern of LH during a continuous infusion of GnRH to bulls of equivalent age (Haynes, personal communication). Luteinizing hormone increased to a peak, and then declined before the end of GnRH infusion. The interval to peak LH response was shorter, occurring at about 2 hours, and the peak of LH was much greater, averaging greater than 40 ng/ml during infusion of GnRH. Presumably the pituitary becomes refractory to continuous GnRH stimulus. Similarly, the decline of LH before the end of the $PGF_{2\alpha}$ infusion may be refractoriness of the hypothalamo-hypophyseal axis to a continuous stimulus.

The testosterone profiles for each of the four bulls during infusion of saline or $PGF_{2\alpha}$ are illustrated in Figure 7. Testosterone increased to greater than 10 ng/ml in each bull within 2.5 hours after the start of the $PGF_{2\alpha}$ infusion, remained clearly elevated for 15 hours in three bulls and elevated for the entire infusion period in bull 2. Episodic surges of testosterone similar to those of control bulls resumed well within 8 hours after the conclusion of the 20-hour infusion of $PGF_{2\alpha}$.

Two or three episodic surges of testosterone occurred in each of the bulls during the 20-hour infusion of saline (Figure 7); the peak (17.2 ± 0.2 ng/ml) of these surges was equivalent to the peak concentration of testosterone during infusion of $PGF_{2\alpha}$.

The temporal relationship of LH and testosterone in bulls infused with saline (Figure 8) was in agreement with previously published data for untreated bulls (Mongkonpunya et al., 1974). In 18 of 19 cases, blood testosterone surges were preceded by an increase in LH (average peak 2.8 ± 0.3 ng/ml). Furthermore, increases in LH of as little as

Figure 7. Blood plasma testosterone in four bulls during infusion (iv) of saline or $PGF_{2\alpha}$. No samples were collected during a 20-hour period between the first (left) and second (right) experimental periods.

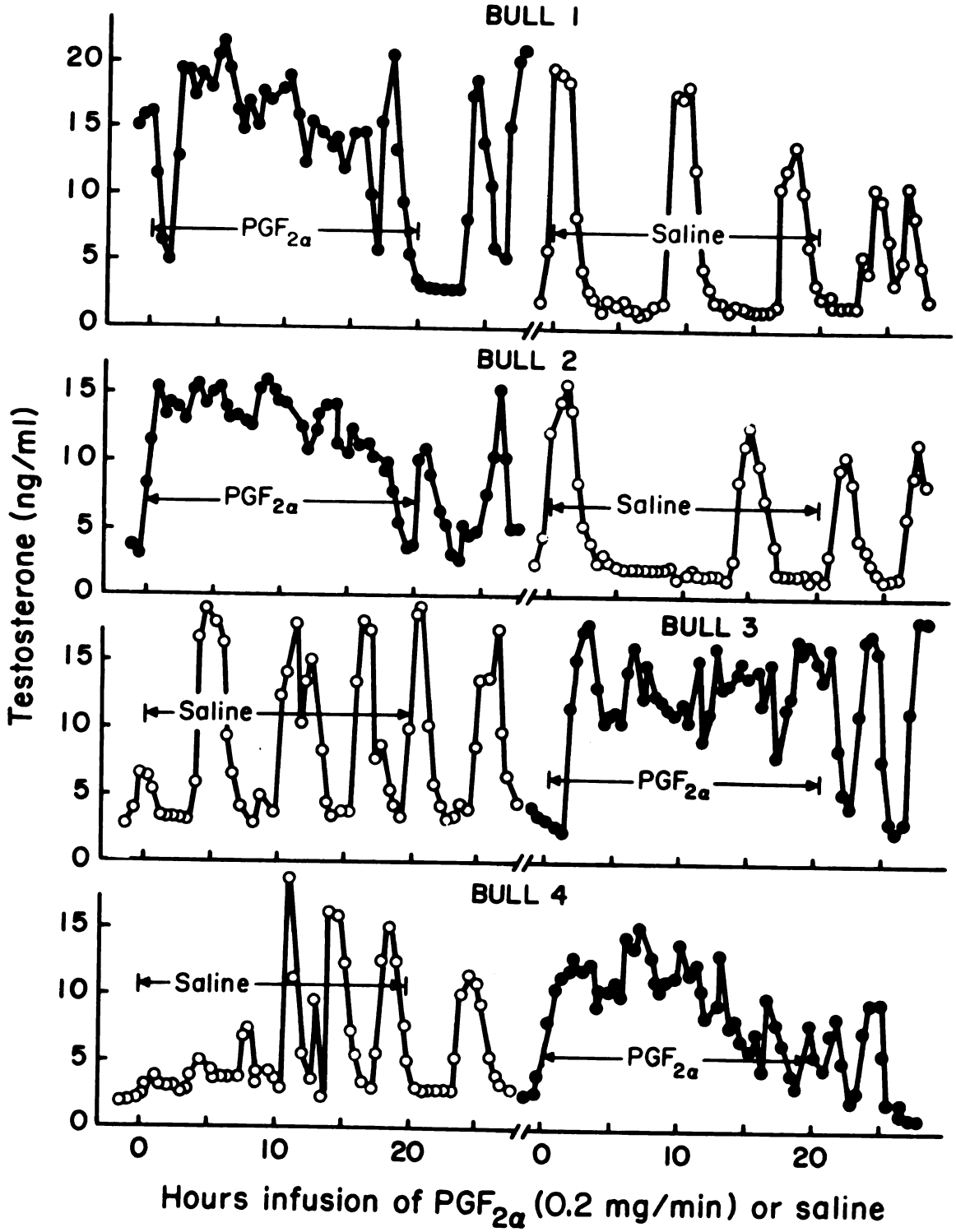
BULL 1
BULL 2
BULL 3
BULL 4
PGF2α
Saline
Testosterone (ng/ml)
Hours infusion of PGF2α (0.2 mg/min) or saline

Figure 7

Figure 7. Blood plasma testosterone in four bulls during infusion (iv) of saline or $PGF_{2\alpha}$. No samples were collected during a 20-hour period between the first (left) and second (right) experimental periods.

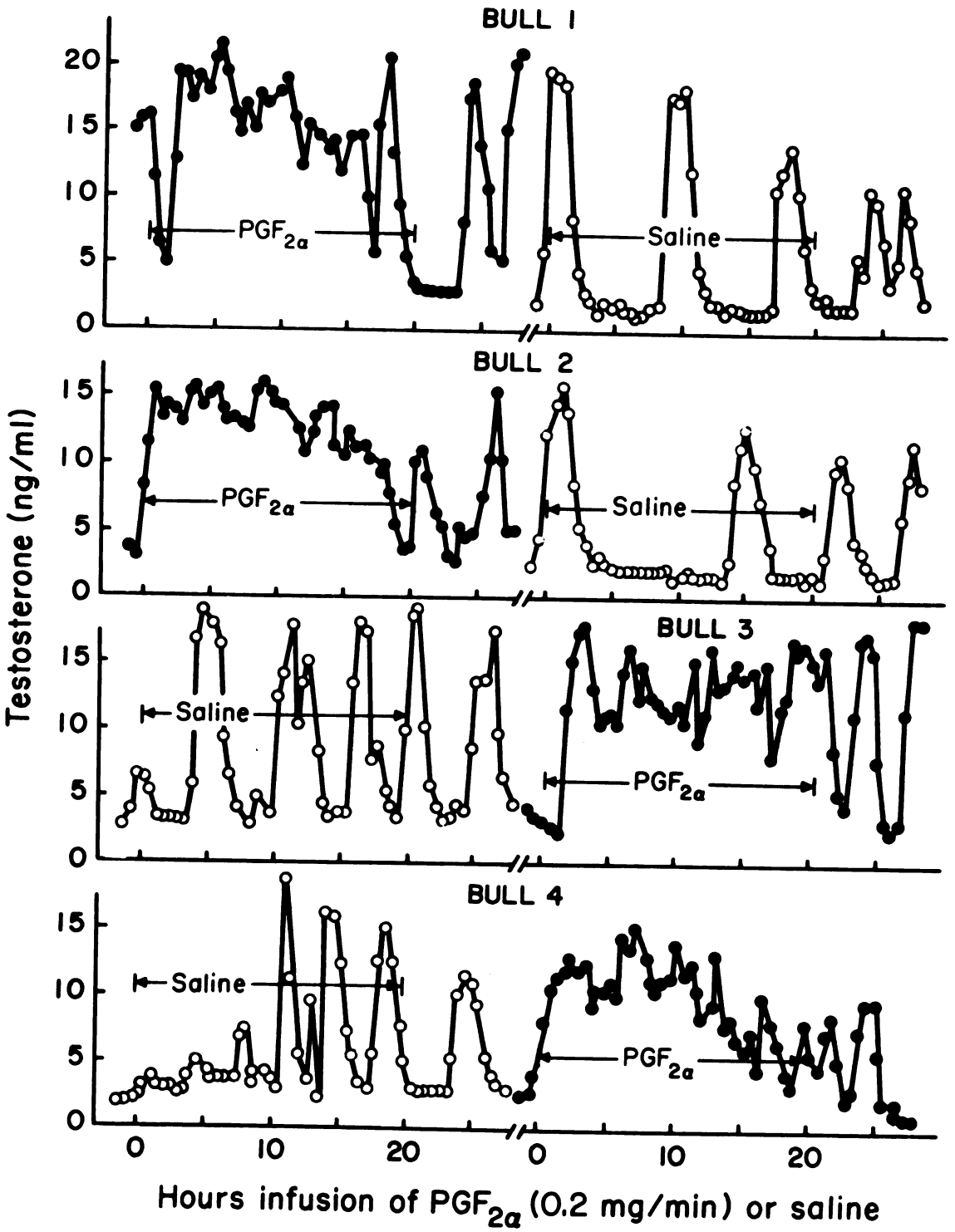
BULL 1
BULL 2
BULL 3
BULL 4
PGF2α
Saline
Testosterone (ng/ml)
Hours infusion of PGF2α (0.2 mg/min) or saline

Figure 7

Figure 8. Blood plasma LH and testosterone in four bulls during infusion of saline.

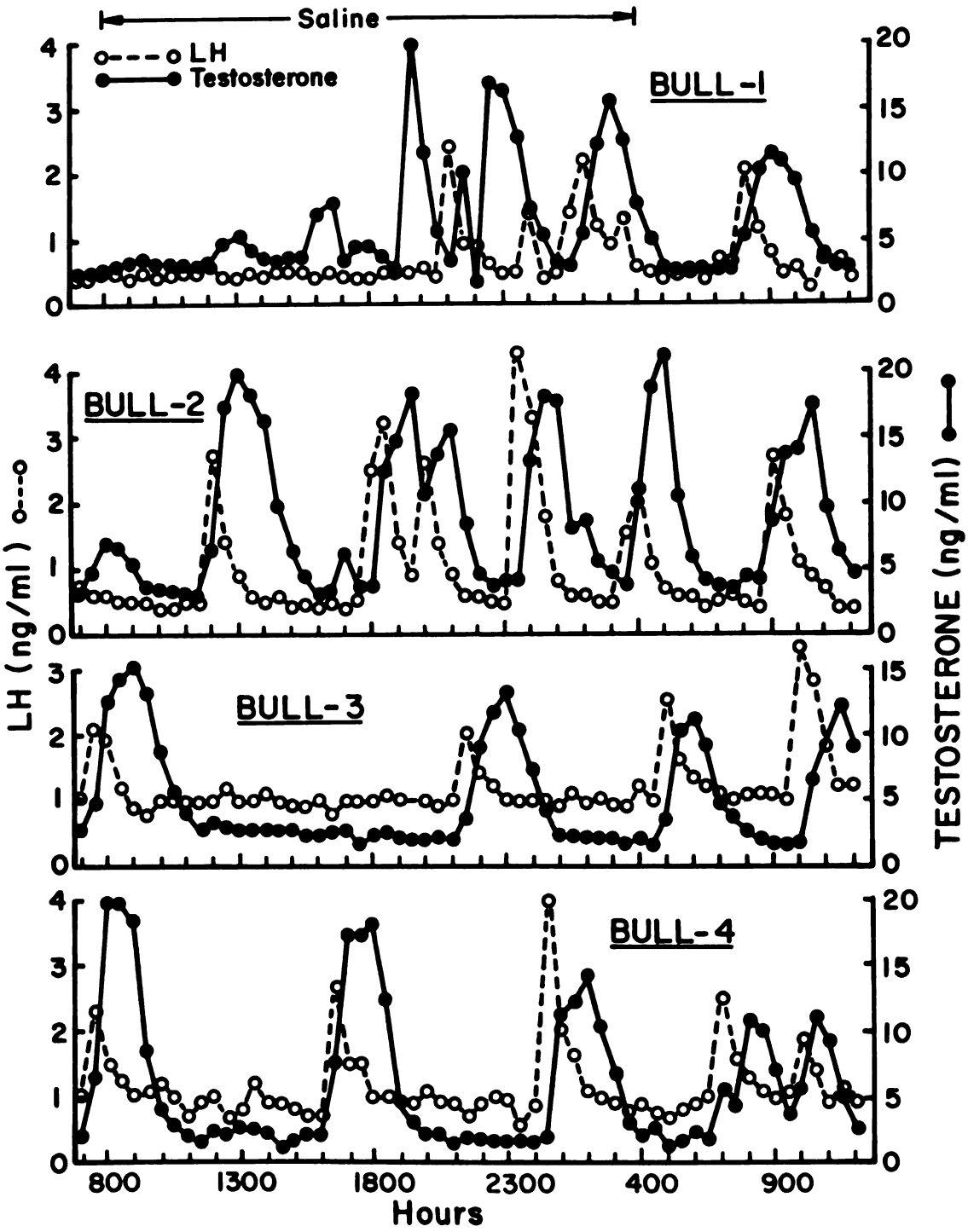
Saline
LH
Testosterone
BULL-1
BULL-2
BULL-3
BULL-4
LH (ng/ml)
TESTOSTERONE (ng/ml)
800
1300
1800
2300
400
900
Hours

Figure 8

Figure 8. Blood plasma LH and testosterone in four bulls during infusion of saline.

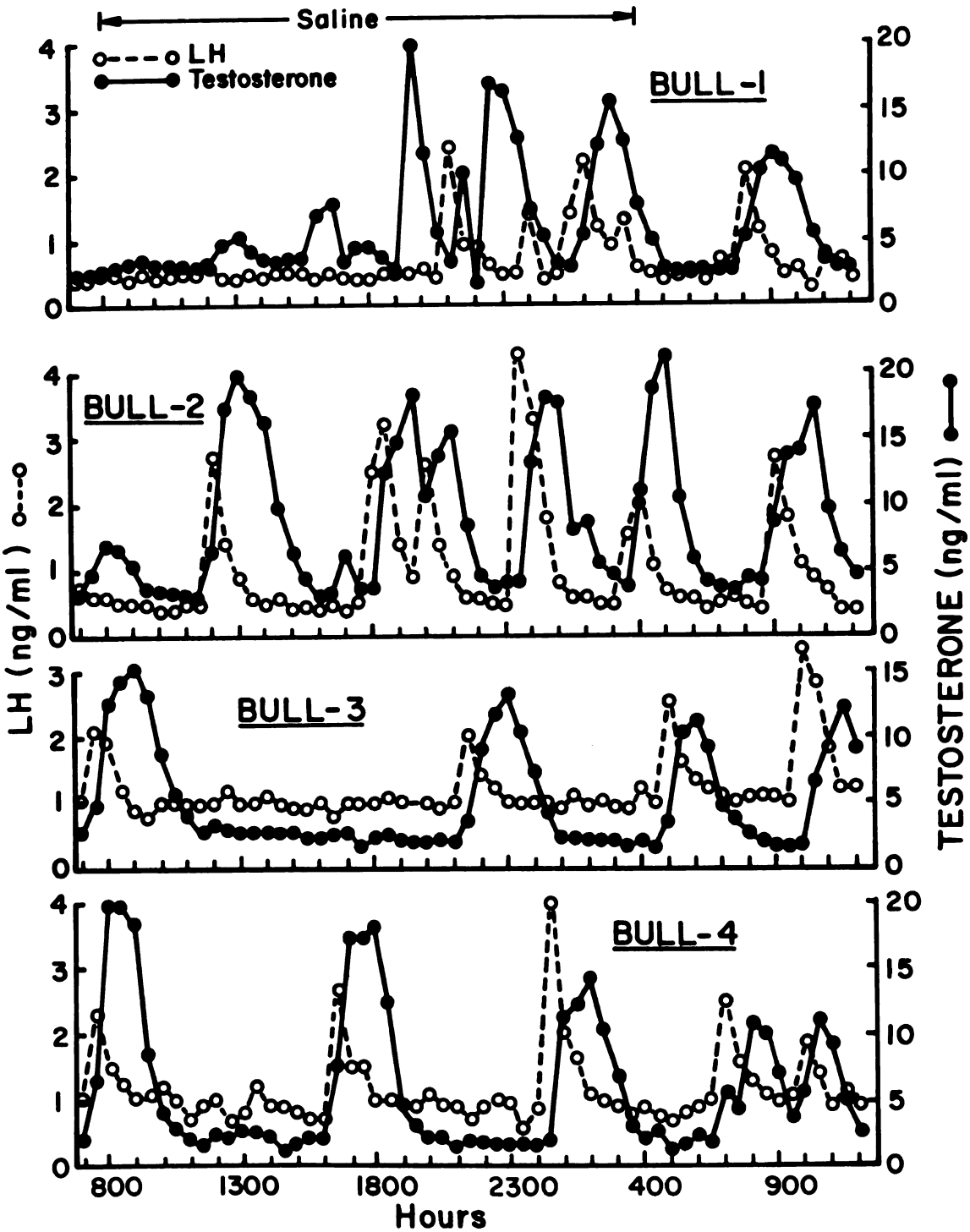
Saline
LH
Testosterone
BULL-1
BULL-2
BULL-3
BULL-4
LH (ng/ml)
TESTOSTERONE (ng/ml)
800
1300
1800
2300
400
900
Hours

Figure 8

1 ng/ml resulted in increased testosterone, but as illustrated for bull 4, the magnitude of change of LH was not proportionally related to the testosterone response.

The temporal relationship between LH and testosterone in bulls infused with $PGF_{2\alpha}$ was consistent with the hypothesis that LH is the primary stimulus of testosterone secretion in bulls given $PGF_{2\alpha}$ (Figure 9). For example, in all four bulls, blood LH increased prior to the increase in testosterone and, as blood LH declined, so did testosterone.

Frequent "bursts" of LH were observed in the bulls during infusion of $PGF_{2\alpha}$, in contrast to the less frequent "bursts" of LH in control bulls (Figure 7). The data suggest that, if $PGF_{2\alpha}$ released LHRH in these bulls as was shown for the rat, the release of LHRH occurred as frequent "bursts" which then caused "bursts" of LH.

In two bulls (1 and 3) there was a prolonged period of elevated LH, but the LH patterns during these elevations of LH suggested that LH was released in short ($\sim$15 minute) "bursts" followed by periods when circulating concentrations declined.

The frequent "bursts" of LH maintained what appears to be an elevation in baseline LH during infusion of $PGF_{2\alpha}$. Certainly as long as there were "bursts" of LH, there was also increased concentration of blood testosterone.

Thus, in overview, the increased concentration of LH during infusion of $PGF_{2\alpha}$ was not the result of an abrupt increase of LH, as was frequently observed after administration of GnRH to bulls (Mongkonpunya et al., 1975); rather, it resulted from an increase in the frequency of bursts of LH.

Figure 9. Blood plasma LH and testosterone in four bulls during infusion (iv) of $PGF_{2\alpha}$ (0.2 mg/min).

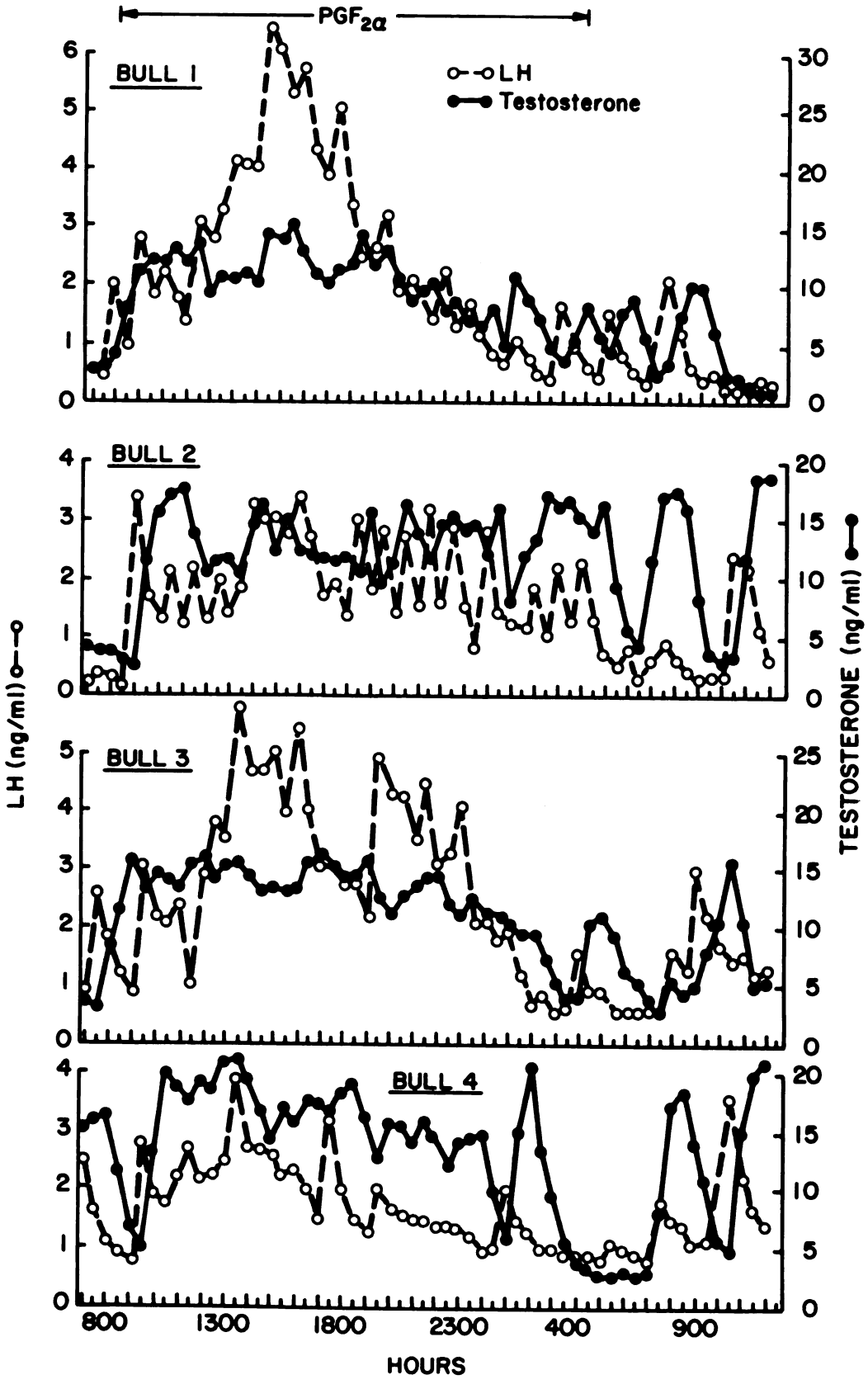
PGF2α
BULL 1
LH
Testosterone
BULL 2
BULL 3
BULL 4
LH (ng/ml)
TESTOSTERONE (ng/ml)
800
1300
1800
2300
400
900
HOURS

Figure 9

Figure 9. Blood plasma LH and testosterone in four bulls during infusion (iv) of $PGF_{2\alpha}$ (0.2 mg/min).

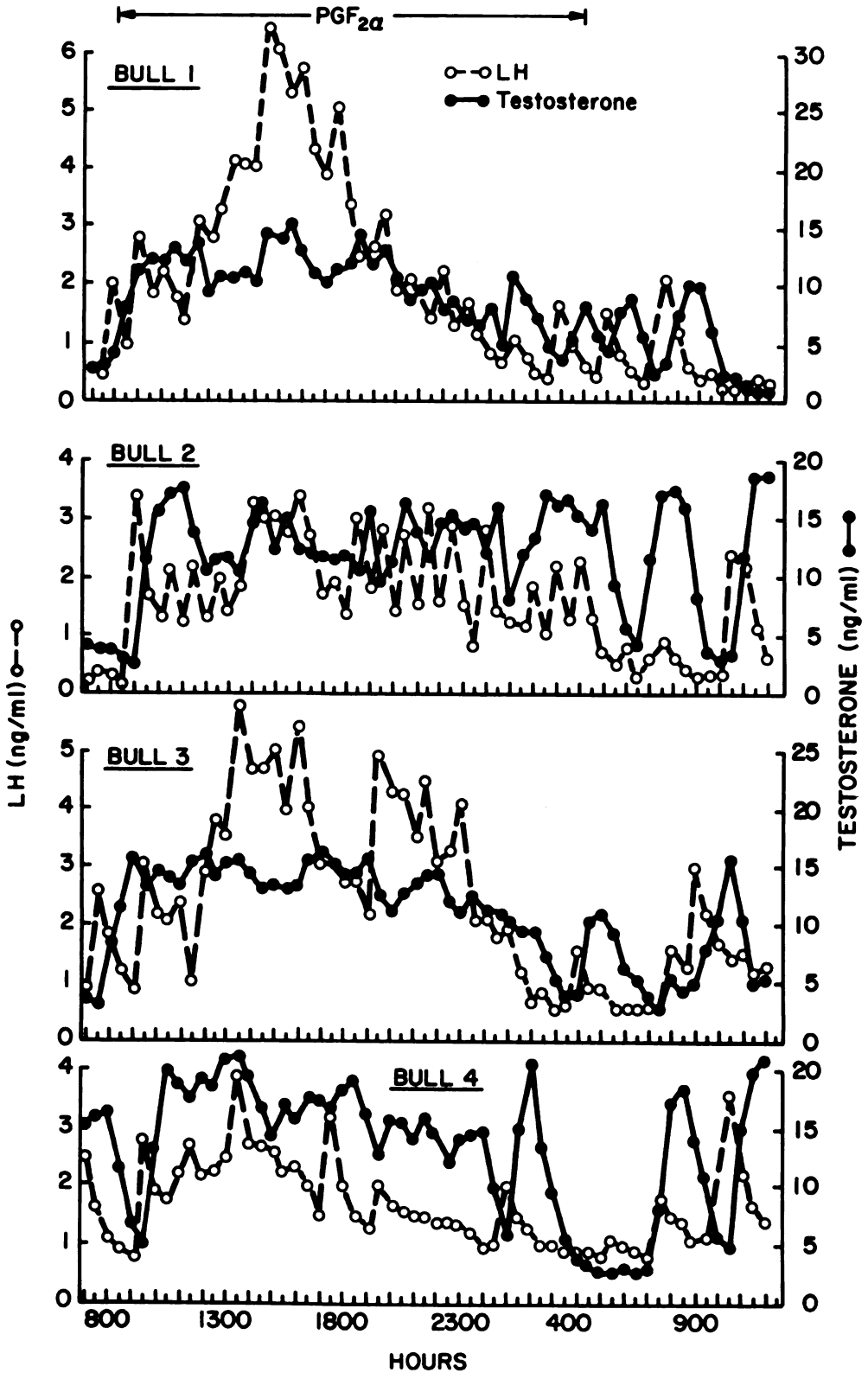
PGF2α
BULL 1
LH
Testosterone
BULL 2
BULL 3
BULL 4
LH (ng/ml)
TESTOSTERONE (ng/ml)
800
1300
1800
2300
400
900
HOURS

Figure 9

Figure 10. Average blood plasma LH and testosterone in four bulls during infusion (iv) of $PGF_{2\alpha}$ (0.2 mg/min).

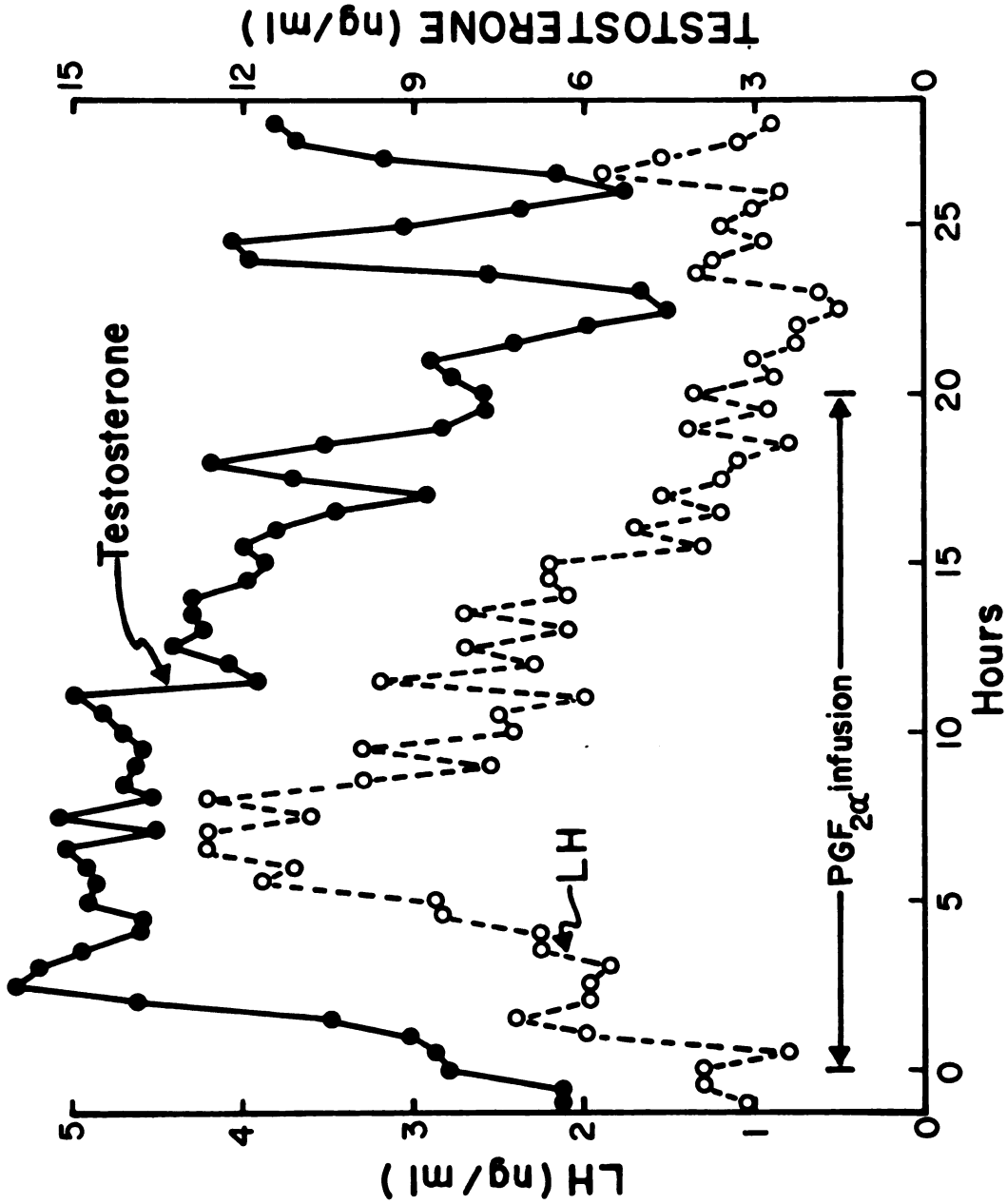
TESTOSTERONE (ng/ml)
15
12
9
6
3
0
Testosterone
LH
PGF2α infusion
LH (ng/ml)
5
4
3
2
1
0
0
5
10
15
20
25
Hours

Figure 10

When the LH and testosterone data for all four bulls were combined, as shown in Figure 10, average blood LH and testosterone increased and decreased in tandem. However, blood testosterone reached maximal concentrations sooner and the peak persisted longer than the comparable LH response. Perhaps the peak of testosterone at 2.5 hours after infusion of $PGF_{2\alpha}$ damped the increase in LH, because peak LH concentration was not apparent until approximately 6 hours after the infusion was started. Notwithstanding this observation, blood LH progressively increased for at least 4 hours during the $PGF_{2\alpha}$ infusion, in the face of high (about 15 ng/ml) testosterone (Figure 9). Then LH started to decline at least 3 hours earlier than testosterone, preceding the decline in testosterone in all four bulls.

This decline in LH beginning about 8 hours after the start of infusion of $PGF_{2\alpha}$ may be explained in several ways. If $PGF_{2\alpha}$ was acting directly on LHRH neurons to cause release of LHRH, perhaps the hypothalamic content of LHRH was exhausted. To my knowledge, there are no reports to support this hypothesis, but if in fact the hypothalamic content of LHRH was depleted, the effect was not long term because episodic secretion of LH and testosterone resumed again within 8 hours after the end of the infusion of $PGF_{2\alpha}$. Perhaps a more plausible explanation is depletion of releasable stores of LH from the pituitary. In sheep, the LH response to consecutive injections of GnRH was reduced even when GnRH was administered at 24-hour intervals (Rippel et al., 1974).

Alternatively, prolonged high concentrations of testosterone in the present experiment may begin to inhibit LH release at about 8 hours of $PGF_{2\alpha}$ infusion, similar to testosterone feedback on LH

release after successive injections of GnRH in rams (Galloway et al., 1974).

In summary, a constant infusion of $PGF_{2\alpha}$ caused a prolonged increase in LH and testosterone secretion in bulls, but neither LH nor testosterone remained elevated during the entire infusion period. The results of these data suggest that the hypothalamus or pituitary may become refractory to a continuous stimulus of $PGF_{2\alpha}$. I speculate that the mechanism(s) involved in causing decreased concentrations of LH and testosterone in this experiment could explain the decreased concentration of testosterone after chronic administration of $PGF_{2\alpha}$ to rats and mice (Bartke et al., 1973; Saksena et al., 1973).

EXPERIMENT 3

THE EFFECT OF EXOGENOUS LH OR $PGF_{2\alpha}$ ON BLOOD LH AND TESTOSTERONE IN BULLS GIVEN MELENGESTROL ACETATE

Introduction

The results of the first two experiments demonstrated that $PGF_{2\alpha}$ caused acute release of LH followed by increased testosterone secretion in bulls. The temporal relationship between changes in LH and testosterone as well as the magnitude of changes of these hormones were similar to changes observed in control bulls (Mongkonpunya et al., 1974). Similar surges of testosterone followed administration of LH (Smith et al., 1973) or GnRH (Mongkonpunya et al., 1975). Thus, LH normally induces testosterone secretion in bulls.

To the extent that $PGF_{2\alpha}$ stimulates acute release of LH, further experiments to determine if inhibitory effects of gonadal steroids or other compounds could be overcome by $PGF_{2\alpha}$ might add insight into control of LH and testosterone by $PGF_{2\alpha}$ in bulls.

In steers, basal LH was elevated by comparison to that in bulls (McCarthy and Swanson, 1976; Mongkonpunya et al., 1974) and testosterone or estradiol replacement caused a decline in baseline LH. Similarly, a synthetic progestogen, melengestrol acetate (MGA), suppressed the ovulatory surge of LH in cows (Hill et al., 1971). The high progestogenic potency and the ease of oral administration of MGA made this progestogen attractive in this preliminary experiment to

test whether $PGF_{2\alpha}$ could override possible progestogen inhibition of LH release in bulls.

Thus, the objectives of this experiment were to determine:

1. If MGA inhibited the episodic secretion of LH and testosterone in bulls.

2. If administration of $PGF_{2\alpha}$ can overcome the inhibitory effects of MGA on LH and testosterone secretion.

3. The effects of exogenous LH on testosterone secretion in bulls treated with MGA.

Materials and Methods

In a preliminary experiment four Holstein bulls (average weight 355 $\pm$ 19 kg) were used during a 3-day period. On the first day, jugular blood was taken at 0, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 210 and 240 minutes relative to the start of sampling at 0800 hours. On the second day each bull was fed 1.01 kg of concentrate feed containing 0.5 mg MGA at 0700 hours and again at 1900 hours. Blood samples were not taken on the second day. Starting at 0700 hours on the third day, each bull was fed 0.5 mg MGA, then jugular blood was collected at frequent intervals as on the first day, starting at 0800 hours. Thus, each bull was bled before oral ingestion of MGA and again starting 25 hours after beginning feeding of MGA. Blood serum testosterone was quantified by radioimmunoassay (Mongkonpunya et al., 1975). Differences in testosterone before and after MGA were compared using Student's *t*-test for paired observations.

The main experiment was conducted as a two-period crossover design with repeat measurements on four bulls (average weight 307 $\pm$ 36 kg). Each bull was fed 1.0 mg of MGA daily (0.5 mg at 0700 and

1900 hours) throughout this main experiment. Starting 24 hours after the first feeding, two bulls were given 20 mg $PGF_{2\alpha}$ Tham salt (sc) and two bulls were given saline (sc). The treatments were reversed 10 hours later. Blood was collected at 15-minute intervals for 1 hour prior to $PGF_{2\alpha}$ or saline, at 15-minute intervals for 2 hours after treatment, at 30-minute intervals for 2 hours, and then hourly until 8 hours. Then 48 hours after the first feeding of MGA, each of the four bulls was given 200 µg NIH-LH-B8 (iv). The testosterone response from these bulls was compared to four control bulls given 200 µg LH (average weight 328 $\pm$ 42 kg) after the same sequence of $PGF_{2\alpha}$ treatment, but without MGA.

Data from the main experiment were analyzed by split-plot analysis of variance with repeated measurements on each bull (Gill and Hafs, 1971).

Results and Discussion

In the preliminary experiment, each bull exhibited one episode of increased serum testosterone (range 12.6 to 20.0 ng/ml) during the 4-hour blood sampling period before treatment with MGA. In contrast, surges of testosterone were abolished after the bulls were fed MGA; the highest concentration of testosterone was 2.5 ng/ml (Table 2). The average testosterone concentration before MGA was greater ($P<.001$) than that after feeding MGA (8.5 $\pm$ 1.1 vs 1.8 $\pm$ 0.1 ng/ml) (Table 2). Furthermore, variation in testosterone concentrations due principally to episodic surges of testosterone was greater ($P<.001$) before than after feeding MGA (SE = 1.1 vs 0.1 ng/ml).

Although serum LH was not quantified in this preliminary experiment, the results indicate that MGA suppressed episodic secretion of

Table 2. Average serum testosterone during a 4-hour interval before and after treatment with melengestrol acetate in four Holstein bulls

Bull	Before MGA		After MGA		Difference
	Mean ± SE	Range	Mean ± SE	Range	
	ng/ml				
69	11.5 ± 1.6	3.9-20.0	1.6 ± 0.1	1.0-2.1	9.9
70	8.8 ± 1.3	2.4-16.3	1.8 ± 0.1	1.3-2.0	7.0
71	6.4 ± 1.3	1.6-12.6	2.0 ± 0.1	1.5-2.5	4.4
72	7.3 ± 1.0	2.3-14.4	1.8 ± 0.1	1.3-2.2	5.5
Average	8.5 ± 1.1		1.8 ± 0.1		6.7 ±[a] 1.2

[a] $P<.001$.

LH, because in no instance in any of my experiments was testosterone increased without a preceding increase in LH. This conclusion assumes that exogenous MGA did not directly inhibit testosterone secretion at the testis. Hill et al. (1971) provided evidence for this assumption, because they reported that MGA did not influence either the life span or progesterone secretion of corpora lutea (CL) in cows, but MGA prevented the ovulatory surge of LH.

The objective of the main experiment was to determine if administration of $PGF_{2\alpha}$ stimulated release of LH and testosterone in bulls given MGA.

The analysis of variance for LH revealed that treatment effects approached significance ($P \simeq .08$); however, time effects and treatment

by time interaction were highly significant ($P<.001$). All other effects were nonsignificant.

After bulls were given saline during MGA treatment (Figure 11, top), serum LH concentrations did not change significantly ($P>.05$). The average LH concentrations ranged from 0.30 $\pm$ 0.05 to 0.40 $\pm$ 0.05 ng/ml. Pretreatment means were comparable between bulls given saline or $PGF_{2\alpha}$, averaging 0.6 $\pm$ 0.2 and 0.4 + 0.01 ng/ml, respectively, at the time of saline and $PGF_{2\alpha}$ injection. After administration of $PGF_{2\alpha}$, serum LH increased ($P<.05$) to 2.0 $\pm$ 0.5 ng/ml at 45 minutes, peaked at 2.3 $\pm$ 0.5 ng/ml at 60 minutes, and then declined to baseline between 4 and 5 hours (Figure 11, top).

Similarly to blood LH in bulls treated with MGA after saline, serum testosterone fluctuated between 0.8 $\pm$ 0.3 and 1.2 $\pm$ 0.2 ng/ml, and did not change significantly during the sampling period in bulls given saline (Figure 11, bottom). In contrast, serum testosterone averaged 0.8 $\pm$ 0.3 ng/ml before $PGF_{2\alpha}$, increased ($P<.05$) to 13.4 $\pm$ 4.1 ng/ml at 75 minutes after $PGF_{2\alpha}$, and reached a peak concentration of 21.3 $\pm$ 2.3 at 105 minutes. Serum testosterone then plateaued until 3 hours after $PGF_{2\alpha}$ and declined to baseline concentrations at 7 hours after $PGF_{2\alpha}$. The testosterone response appeared to be exaggerated compared to the response in Experiment 1. However, the prolonged LH response, illustrated in Figure 11 (top), after $PGF_{2\alpha}$ probably accounted for the prolonged testosterone response.

The results of this experiment establish that MGA inhibits episodic secretion of LH and testosterone in bulls. More recently, in a similar study, infusion of testosterone also completely abolished episodic LH release in bulls (Haynes, personal communication).

Figure 11. Blood serum LH (top) and testosterone (bottom) in four bulls pre-treated with melengestrol acetate and given saline (sc) or $PGF_{2\alpha}$ (20 mg, sc).

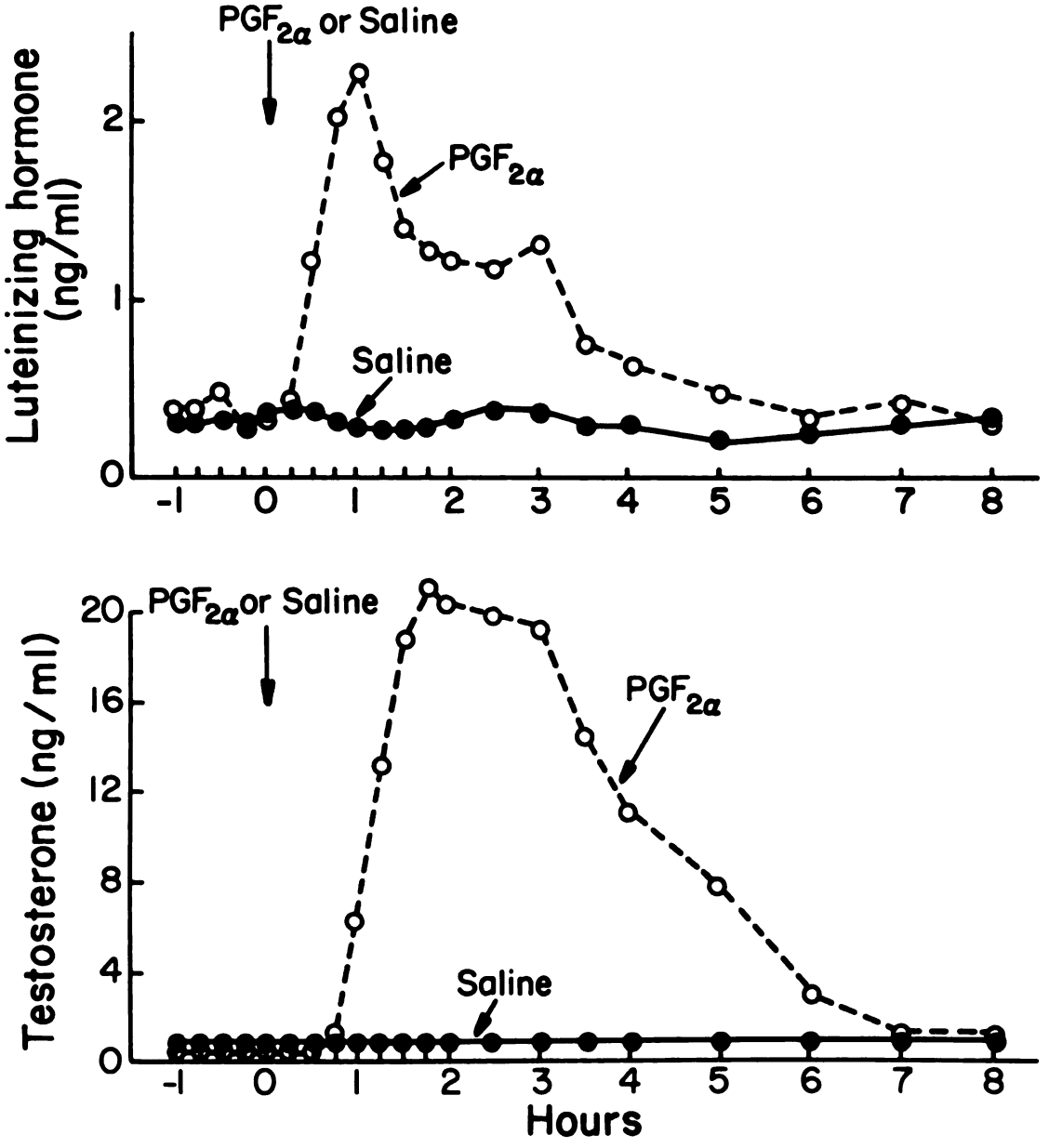
PGF2α or Saline
PGF2α
Saline
Luteinizing hormone (ng/ml)
0
1
2
-1
0
1
2
3
4
5
6
7
8
PGF2α or Saline
PGF2α
Saline
Testosterone (ng/ml)
0
4
8
12
16
20
Hours

Figure 11

Figure 11. Blood serum LH (top) and testosterone (bottom) in four bulls pre-treated with melengestrol acetate and given saline (sc) or $PGF_{2\alpha}$ (20 mg, sc).

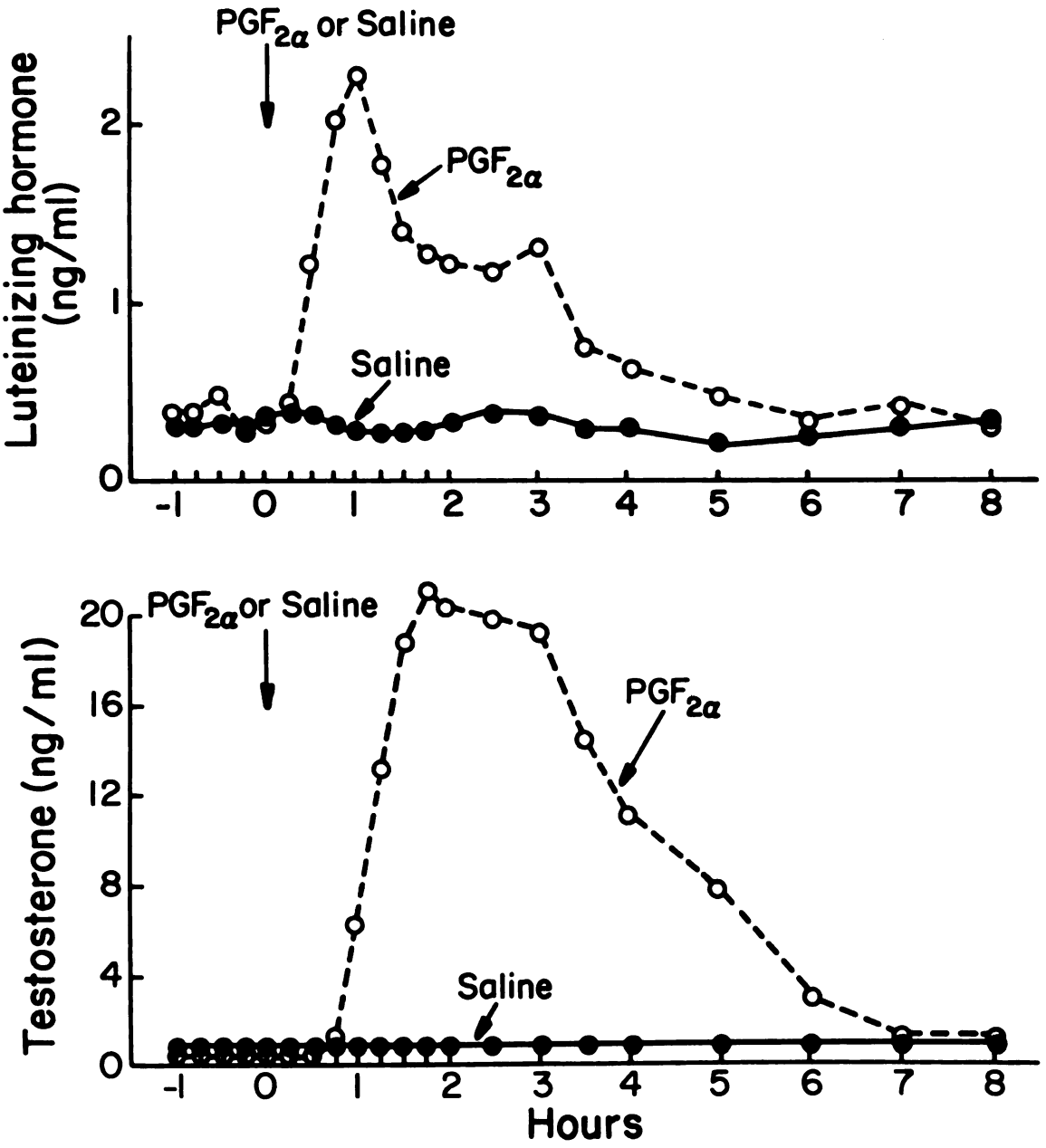
PGF2α or Saline
PGF2α
Saline
Luteinizing hormone (ng/ml)
0
1
2
-1
0
1
2
3
4
5
6
7
8
PGF2α or Saline
PGF2α
Saline
Testosterone (ng/ml)
0
4
8
12
16
20
Hours

Figure 11

Whether MGA acts directly at the pituitary or hypothalamus to inhibit release of LH remains to be determined.

In the present experiment, administration of $PGF_{2\alpha}$ to bulls pre-treated with MGA resulted in release of LH comparable to that after $PGF_{2\alpha}$ in untreated bulls or episodic release of LH in control bulls observed in Experiment 1. If MGA inhibited release of LH at the level of the pituitary, one might expect no LH release after $PGF_{2\alpha}$, even if LHRH was released by the prostaglandin stimulus, as has been demonstrated by Eskay et al. (1975) in rats.

McCarthy and Swanson (1976) concluded that testosterone feedback was not at the pituitary because the LH response to GnRH in steers pre-treated with testosterone was greater than in untreated steers. In support of this conclusion, intrahypothalamic implantation of crystalline testosterone in dogs (Davidson and Sawyer, 1961) and progesterone in rats (Smith, Weick and Davidson, 1969) exerted "negative feedback" on gonadotropic function. In the latter report, the effect of progesterone apparently was specific to the medial basal hypothalamic region, because implants placed in the anterior hypothalamic-medial preoptic region and in the anterior pituitary were ineffective. More recently, however, evidence was provided that progesterone can act on the pituitary to reduce but not block LH secretion after GnRH (Arimura and Schally, 1970).

In the present experiment, $PGF_{2\alpha}$ induced LH release in bulls in which episodic release of LH was suppressed by MGA.

Two explanations may be offered in this case, neither of which is definitive. Either $PGF_{2\alpha}$ is acting directly at the pituitary to cause release of LH or $PGF_{2\alpha}$ is overriding or superceding the inhibitory effects of MGA at a higher level (hypothalamus or higher centers).

The case for $PGF_{2\alpha}$ acting at the pituitary lacks support from the literature because, in most reports, intrapituitary injection of prostaglandin results in low or no release of LH (Harms et al., 1973; Norman and Spies, 1973), although in one report (Warberg, Eskay and Porter, 1976) intraventricular $PGF_{2\alpha}$ caused secretion of LH. I speculate that MGA inhibited hypothalamic prostaglandin secretion and thereby abolished LHRH secretion and the episodic release of LH. This explanation is compatible with the observation that $PGF_{2\alpha}$ caused release of LH in the face of MGA inhibition of LH in the present experiment.

Prior to initiation of Experiment 3, the possibility of MGA interacting directly at the level of the testis to modulate testosterone secretion was considered. However, as shown in Figure 12, MGA did not interfere with testosterone secretion after LH administration, in agreement with the data of Hill et al. (1971), who demonstrated that MGA did not alter progesterone secretion in cows.

In summary, this experiment demonstrated that MGA suppressed episodic release of LH and testosterone in bulls, but the inhibitory effect of MGA was overcome by $PGF_{2\alpha}$. The results suggest that MGA inhibition of episodic LH release was mediated by an action on prostaglandin secretion. Finally, the results indicate that MGA does not interact directly at the level of the testis to alter testosterone secretion in bulls.

Figure 12. Blood serum LH (top) and testosterone (bottom) in four control bulls and four melengestrol acetate treated bulls after 200 μg LH (iv).

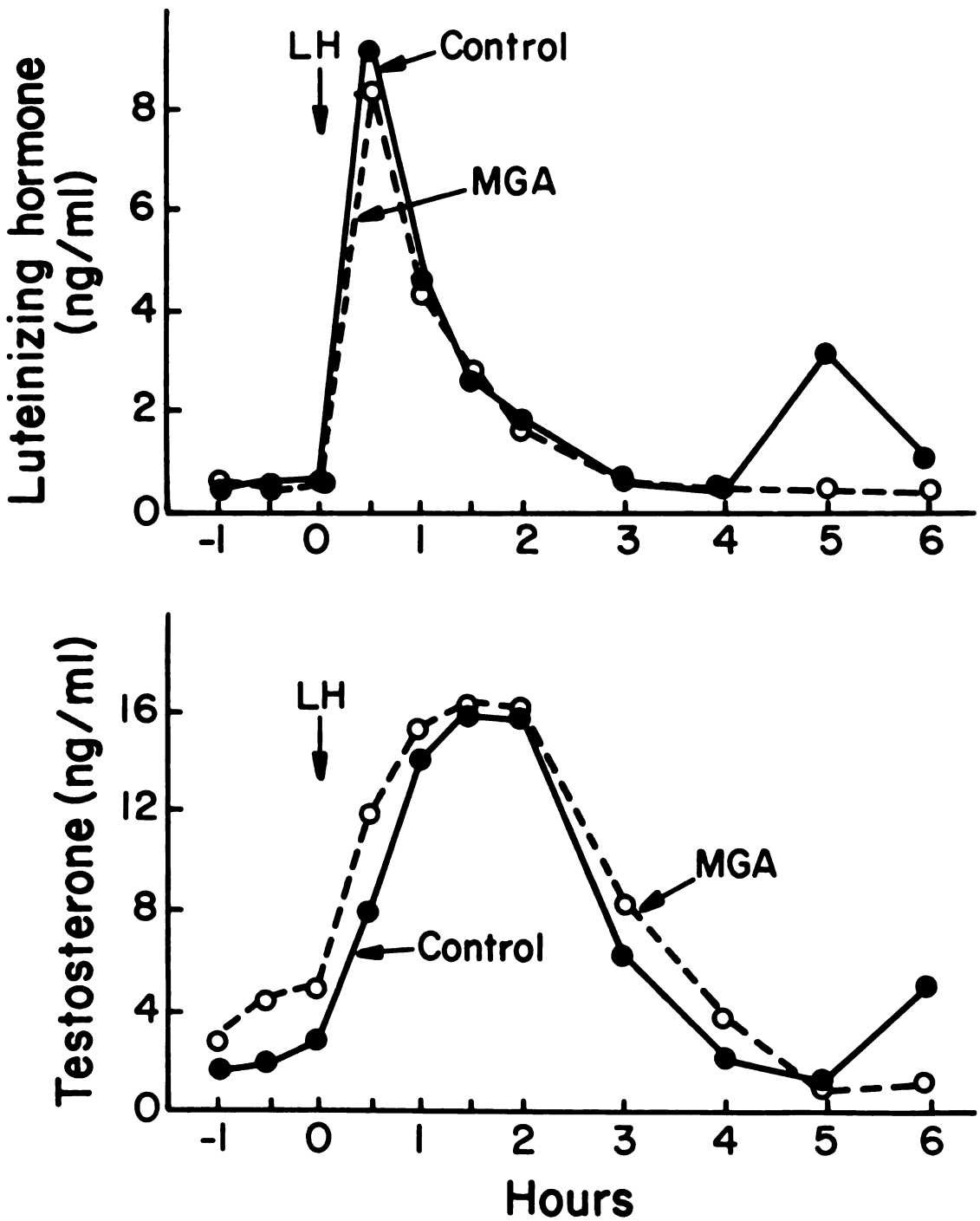
Luteinizing hormone (ng/ml)
LH
Control
MGA
0
2
4
6
8
-1
0
1
2
3
4
5
6
Testosterone (ng/ml)
LH
MGA
Control
0
4
8
12
16
-1
0
1
2
3
4
5
6
Hours

Figure 12

Figure 12. Blood serum LH (top) and testosterone (bottom) in four control bulls and four melengestrol acetate treated bulls after 200 µg LH (iv).

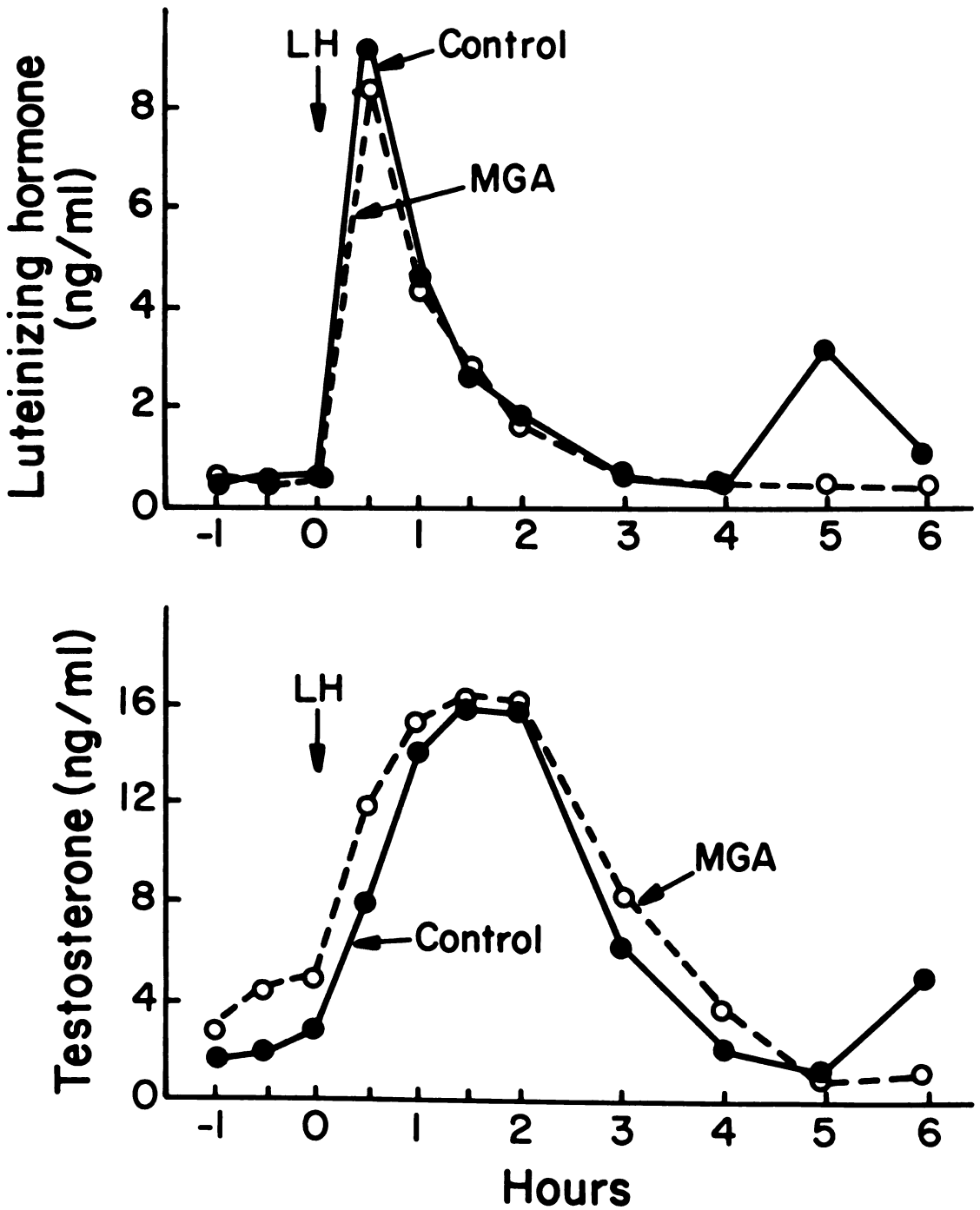
Luteinizing hormone (ng/ml)
LH
Control
MGA
0
2
4
6
8
-1
0
1
2
3
4
5
6
Testosterone (ng/ml)
LH
MGA
Control
0
4
8
12
16
-1
0
1
2
3
4
5
6
Hours

Figure 12

EXPERIMENT 4

THE EFFECT OF CAROTID ADMINISTRATION OF $PGF_{2\alpha}$ ON BLOOD LH AND TESTOSTERONE IN BULLS

Introduction

Although the first three experiments demonstrated that $PGF_{2\alpha}$ causes increased blood LH and testosterone in bulls, the locus of $PGF_{2\alpha}$ action remained to be ascertained. In previous experiments, administration of $PGF_{2\alpha}$ was by subcutaneous injection or intravenous infusion. Therefore, $PGF_{2\alpha}$ could act at one or more of several sites in the periphery to cause release of LH and testosterone. For example, Hafs et al. (1975) convincingly demonstrated that the transitory increase in blood serum LH within 2 hours of $PGF_{2\alpha}$ treatment was the result of a decrease in progesterone secretion, not a direct effect of $PGF_{2\alpha}$ at the hypothalamo-pituitary axis in cows. An analogous possibility could not be excluded on the basis of my first three experiments. In other words, the possibility existed that $PGF_{2\alpha}$ acted directly at the testis to modify testicular hormones, and the alteration in steroid feedback may have been responsible for the increase in LH and the subsequent testosterone surge.

Therefore, the objective of my fourth experiment was to determine if $PGF_{2\alpha}$ acted directly on the brain to elicit release of LH in bulls.

Materials and Methods

In a 6 x 6 Latin square design, each of six yearling bulls (260 $\pm$ 27 kg) was given intracarotid infusion of 0 (saline vehicle), 20,

200 and 2000 ng of $PGF_{2\alpha}$/minute (min) and 2000 ng and 0.2 mg of $PGF_{2\alpha}$/min into a jugular vein. The infusion was maintained for 3 hours at 10 ml/hour by a Harvard pump, with 12-hour intervals between the start of consequentive treatments. In a previous experiment (Experiment 2 of this dissertation), significant increases in LH accompanied infusion of 0.2 mg $PGF_{2\alpha}$/min into a jugular vein. This dose (0.2 mg/min) was therefore used as a positive control. On the assumption that 95 percent of the $PGF_{2\alpha}$ is metabolized on one passage through the lungs (Piper and Vane, 1969) and 10 percent of the circulating blood passes through the head, then of the 0.2 mg $PGF_{2\alpha}$/min infusion into the jugular vein, only approximately 2000 ng $PGF_{2\alpha}$/min reaches the brain. If the major effect of $PGF_{2\alpha}$ in stimulating testosterone output is mediated through secretion of LH, then a dose of 2000 ng $PGF_{2\alpha}$/min given via the carotid should give a LH and testosterone response equivalent to 0.2 mg $PGF_{2\alpha}$/min administered by jugular infusion and a significantly greater response than 2000 ng $PGF_{2\alpha}$/min given via the jugular. Thus, the LH and testosterone response was compared after carotid and jugular infusion of 2000 ng $PGF_{2\alpha}$/min. Lower intracarotid doses of $PGF_{2\alpha}$ were included to investigate possible dose-response relationships.

Blood samples were collected from jugular cannulae at 30-minute intervals starting immediately prior to the start of the infusions and continuing for 12 hours.

To prepare the animals for carotid cannulation, each bull was given 1.5 g of Surital^{R} (Parke, Davis & Company, Allen Park, MI) and 30 g of Guaifenesin^{R} (Ganes Chemical Works, 535 5th Avenue, NY) intravenously and then maintained on halothane anesthesia (Fluothane^{R}, Ayerst Laboratory, Inc., New York, NY) for the duration of surgery.

To expose the carotid artery for cannulation, a 10 cm incision through the skin was made parallel and ventral to the jugular vein. Then the carotid was located by blunt dissection ventral to the jugular vein, between the cleidomastoid and sternomandibular muscle, the carotid artery was freed from surrounding tissue, and silk suture was passed under the anterior and posterior end of the carotid to lift the artery and control blood flow during insertion of the cannula. Prior to insertion of the cannula, a pursestring suture was placed in the wall of the carotid around the area of insertion. Then a small incision was made through the carotid sheath and silastic cannula was placed into the carotid and secured with the pursestring suture. The incision was closed with silk suture and the remaining end of the cannula was positioned under the skin dorsally to the carotid and exposed to the outside 10 cm from the incision. The entire cannulation procedure required about 1 hour.

The carotid cannula consisted of a 43 cm inner silastic cannula (.062" x .125", Dow Corning, Midland, MI) and an outer sheath of polyvinyl cannula (.156" x .25", Portex Limited, Hythe, Kent, England). The outer cannula was 30 cm in length and had a 1 cm x 1 cm x 0.5 cm silicone sponge (Bellco Glass, Inc., Vineland, NJ) attached around one end with silastic glue. The end with the silicone sponge was passed over the inner silastic cannula for 30 cm leaving 13 cm of the silastic cannula exposed. The silicone sponge surrounding the outer cannula was used to anchor the carotid cannula to the carotid sheath. A 48-hour interval was allowed after surgery before the start of the experiment.

Blood serum LH was quantified by specific radioimmunoassay (Convey et al., 1976). Serum testosterone was quantified by

radioimmunoassay using MSU antitestosterone #74 raised against testosterone-3-oxime-human serum albumin. In the initial validation (Kiser et al., 1977), the antiserum was used at a final dilution of 1:50,000. Percent binding in the zero tube was 30 percent and 10 pg of testosterone resulted in a reduction in binding from 30 to 20 percent. Dihydrotestosterone and androstenedione cross-reacted 60 and 1.7 percent, respectively, but no other steroid or sterol tested cross-reacted more than 0.14 percent (Table 3). Zero, 0.05, 1.0, 5 and 10 ng testosterone added per milliliter of estrous cow serum, bull serum and blood plasma from an adrenalectomized steer were assayed with and without isolation of testosterone by Sephadex LH-20 chromatography. Testosterone recovery averaged 89 and 92 percent before and after chromatographic isolation (Table 4). Serum testosterone averaged 0.04, 8.7 and 0.05 ng/ml for estrous cow, bull and steer, respectively, and the quantity of testosterone determined with and without chromatographic isolation did not differ significantly. Finally, testosterone was assayed in a single sample of bull blood serum in quadruplicate with the present antitestosterone assay and with the previous antitestosterone assay (Mongkonpunya et al., 1975) in five separate assays. Average testosterone concentration with the present assay (9.0 $\pm$ 0.9 ng/ml) was slightly less ($P \simeq .08$) than with the previous assay (10.1 $\pm$ 1.3 ng/ml), and the within and among assay coefficients of variation were 10.4 and 12.8 percent.

An additional modification of this assay (Haynes et al., 1977) utilized sheep antirabbit gamma globulin to separate free- from antibody-bound testosterone. Using this separational technique, the antitestosterone serum was diluted 1:10,000, but this alteration did

Table 3. Percent cross-reactions of rabbit antitestosterone (MSU #74)

Steroid or sterol	Percent cross-reaction[a]
Testosterone	100.00
Dihydrotestosterone	60.00
Androstenedione	1.70
Epiandrosterone	0.04
Dehydroepiandrosterone	0.02
Androstadiene-3,17-dione	0.14
Estradiol-17β	0.02
Estriol	0.02
Estrone	0.04
Progesterone	0.02
20α-hydroxyprogesterone	0.02
17α-hydroxyprogesterone	0.01
Cortisol	0.02
Corticosterone	<0.01
Cholesterol	<0.01
Cortisone	0.02
Aldosterone	<0.01

[a]Percent expressed as activity relative to testosterone.

Table 4. Percent recovery of added testosterone after direct extraction or column chromatographic isolation from sera from an estrous cow or bull, or from steer plasma

Added testosterone	Direct extraction			Chromatographic isolation		
	Cow	Bull	Steer	Cow	Bull	Steer
(ng)	%					
0.05	56	90	50	80	99	111
1.00	93	101	101	79	86	90
5.00	96	99	99	90	107	94
10.00	93	103	91	82	94	95

not change validity criteria estimates of the assay (Haynes et al., 1977).

Thus, quantification of serum testosterone in this experiment utilized a double antibody technique. Breifly, 50 µl of each unknown was placed in 2 disposable extraction tubes (15 x 85 mm). To account for procedural loss, 3,000 dpm ^{3}H-testosterone (85-105 Ci/mmol) was added to a third aliquot from a representative number (10 to 20 within each 300-tube assay) of unknowns. Each tube was vortexed with 2 ml of benzene-hexane (1:2) for 30 seconds, then stored at -20 C for at least 2 hours to freeze the aqueous phase. The organic solvent from each tube with ^{3}H-testosterone was decanted into "mini" scintillation vials (#125503, Research Products, Inc., Elk Grove, IL) for recovery (extraction efficiency) determination. The organic solvent from samples for quantification was decanted into 12 x 75 mm disposable culture tubes for radioimmunoassay. The solvent was evaporated and 200 µl of antitestosterone was added to each tube.

Sets of standard tubes containing 0.0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.40, 0.60, 0.80, and 1.00 ng testosterone were included in each assay and treated similarly to unknowns. After addition of antibody, each tube was vortexed 10 seconds and incubated at room temperature for 2 hours. Then approximately 5,000 cpm ^{3}H-testosterone (1,2,6,7-^{3}H-testosterone 85-105 Ci/mM) diluted in 200 µl of 0.1 percent gelatin in 0.1 M phosphate buffered saline (0.1 percent gel-PBS) was added to each tube and the tubes were vortexed 10 seconds and incubated at 4 C for approximately 24 hours. To separate free from antibody-bound testosterone, 400 µl of sheep antirabbit gamma globulin serum in an appropriate dilution to bind 80 percent of the ^{3}H-testosterone in the zero tube was added to each tube, vortexed and incubated for about 38 hours at 4 C. Finally, each tube was centrifuged for 30 minutes at 2,500 xg at 4 C. Free ^{3}H-testosterone in 0.5 ml of supernatant fluid was diluted to 5 ml with scintillation fluid (3a70B, Research Products International, Inc., Elk Grove Village, IL) and counted in a liquid scintillation spectrometer.

Statistical analyses were performed by split-plot analysis of variance (Gill and Hafs, 1971). Designed contrasts among treatments were made orthogonally and some non-orthogonal contrasts were analyzed using Bonferroni t (Miller, 1966). One-way analysis of variance with unequal numbers was used to test duration of LH and testosterone responses after treatments.

Results and Discussion

During the course of this experiment, it was determined that the carotid cannula of one bull was improperly placed; thus, the data

from this bull were excluded from statistical analyses. The data represent five bulls given six treatments.

Plasma LH averaged 1.3 $\pm$ 0.2 ng/ml for bulls in all 6 treatments before start of the infusions (Figure 13). After the start of infusions, both 0.2 mg $PGF_{2\alpha}$/min in the jugular and 2,000 ng $PGF_{2\alpha}$/min in the carotid caused an increase ($P<.05$) of plasma LH (Figure 13). However, release of LH after 2,000 ng $PGF_{2\alpha}$/min into the carotid was prolonged by comparison to the 0.2 mg $PGF_{2\alpha}$/min intrajugular treatment. In contrast, carotid administration of saline or jugular administration of 2,000 ng $PGF_{2\alpha}$/min treatments were ineffective in causing increased elevations in plasma LH. For clarity, since the patterns of LH after 20 and 200 ng/min of $PGF_{2\alpha}$ were similar to saline treatment, these data were not represented in Figure 13.

Closer inspection of the data reveals that plasma LH increased to a peak of 2.6 $\pm$ 0.5 ng/ml within 1 hour after beginning jugular infusion of 0.2 mg $PGF_{2\alpha}$/min, then declined to 1.4 $\pm$ 0.3 ng/ml at the end of the 3-hour infusion. A similar increase (3.6 $\pm$ 1.1 ng/ml) occurred during carotid infusion of 2,000 ng $PGF_{2\alpha}$/min, but LH remained clearly elevated throughout the 3-hour infusion period.

Statistical analysis of data for blood LH revealed a significant ($P<.005$) time effect and the different pattern of LH secretion following carotid infusion of 2,000 ng $PGF_{2\alpha}$/min and jugular infusion of 0.2 mg $PGF_{2\alpha}$/min, compared to the remaining treatments probably was responsible for the significant ($P<.003$) treatment by time interaction.

The "a priori" expectation was that 0.2 mg/min jugular infusion and 2,000 ng/min carotid infusion would result in increased plasma LH. Indeed, orthogonal contrasts of these two treatments versus

Figure 13. Blood plasma LH during a 3-hour infusion of $PGF_{2\alpha}$ (0 ng/min [C], 2,000 ng/min [C], 2,000 ng/min [J], and 0.2 mg/min [J]) into the carotid artery (C) or jugular vein (J).

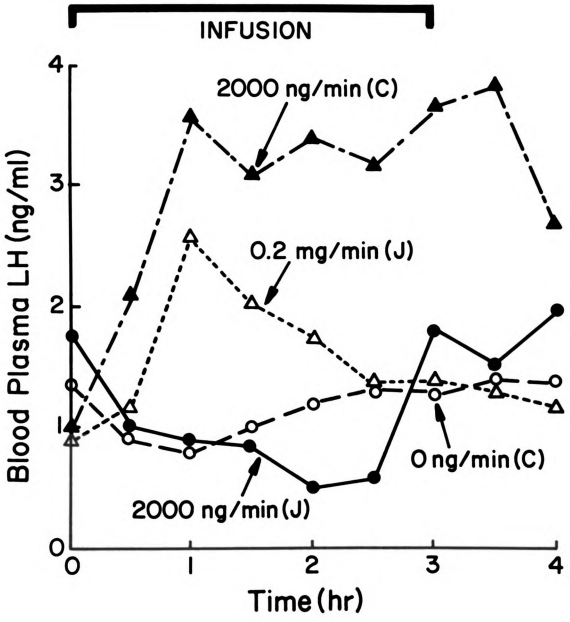
INFUSION
2000 ng/min (C)
0.2 mg/min (J)
0 ng/min (C)
2000 ng/min (J)
Blood Plasma LH (ng/ml)
Time (hr)
0
1
2
3
4

Figure 13

jugular infusion of 2,000 ng/min and carotid saline infusion indicated a greater response after 0.2 mg/min jugular and 2,000 ng/min carotid treatments ($P<.001$). These data suggest that $PGF_{2\alpha}$ acted at the brain to cause LH release, because the 2,000 ng $PGF_{2\alpha}$/min infused to the head via the carotid artery caused release of LH, while the same amount of $PGF_{2\alpha}$ infused away from the head via the jugular vein caused no significant elevation in serum LH. Furthermore, the LH response after carotid saline infusion did not differ from the 2,000 ng/min dose of $PGF_{2\alpha}$ infused into the jugular. However, the LH response after carotid infusion of 2,000 ng $PGF_{2\alpha}$/min was greater ($P<.001$) than after jugular infusion of 0.2 mg $PGF_{2\alpha}$/min. These data suggest that less than 1 percent of the 0.2 mg $PGF_{2\alpha}$ infused via the jugular vein actually reached the head.

Interpretation of the testosterone response was complicated first by the large variation in plasma testosterone (pooled SE = 16.1 ng/ml) and secondly by a nonsignificant increase in plasma testosterone in control bulls infused with saline (Figure 14). In two controls, episodic LH releases occurred immediately prior to the start of the saline infusion and caused increased testosterone secretion during the infusion period.

The overall analysis of variance revealed a significant time effect ($P<.005$) and a significant treatment by time interaction ($P<.001$). Orthogonal contrasts revealed no significant difference between treatments. However, the interaction between treatment and time indicated a different pattern of testosterone concentrations between treatments. For example, plasma testosterone remained below 3 ng/ml during jugular infusion of 2,000ng/min of $PGF_{2\alpha}$. In contrast, after infusion of the same dose of $PGF_{2\alpha}$ into the carotid, plasma

Figure 14. Blood plasma testosterone during a 3-hour infusion of $PGF_{2\alpha}$ (0 ng/min [C], 2,000 ng/min [C], 2,000 ng/min [J] and 0.2 mg/min [J]) into the carotid artery (C) or the jugular vein (J).

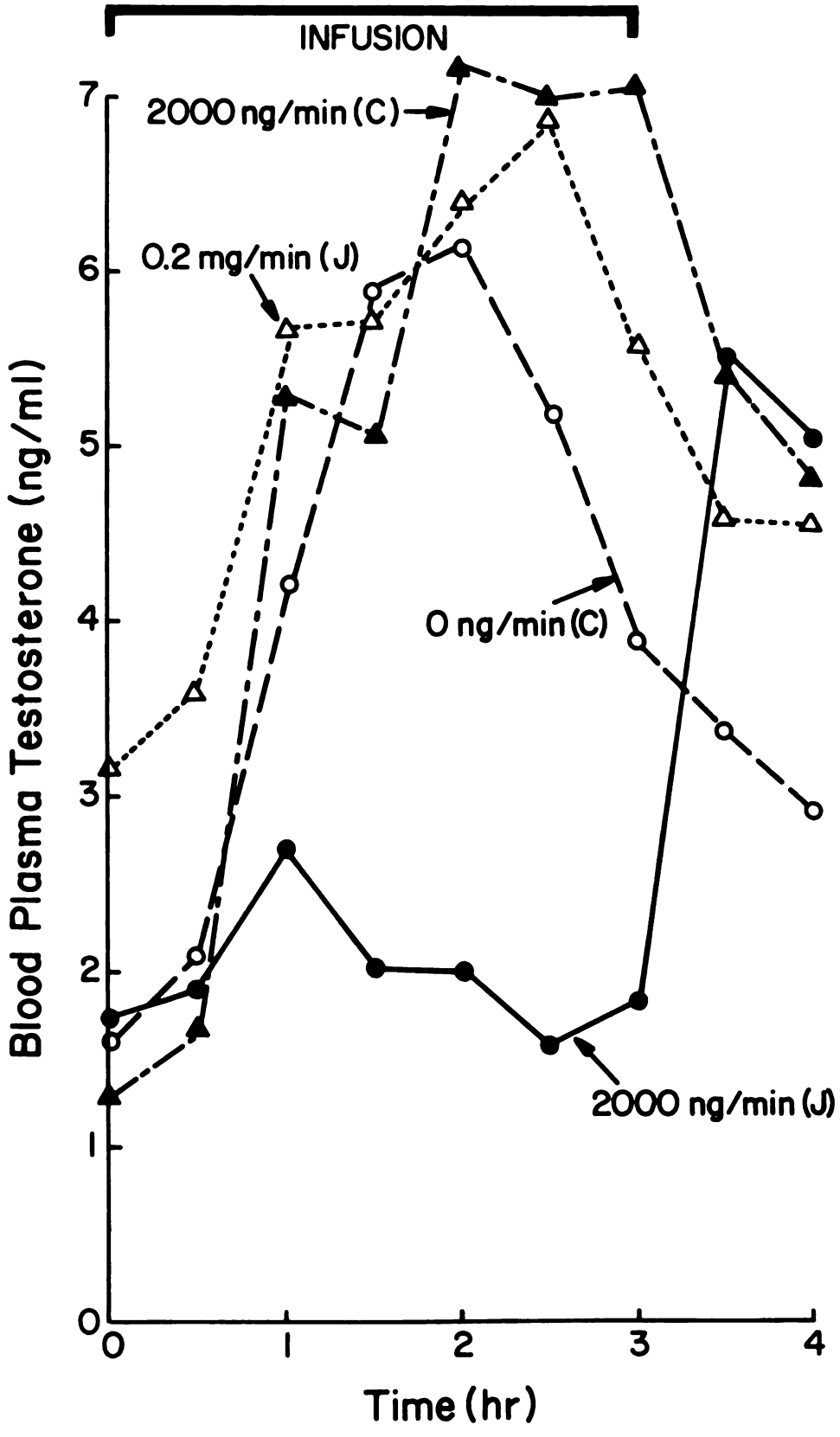
INFUSION
2000 ng/min (C)
0.2 mg/min (J)
0 ng/min (C)
2000 ng/min (J)
Blood Plasma Testosterone (ng/ml)
Time (hr)
0
1
2
3
4
5
6
7

Figure 14

Figure 14. Blood plasma testosterone during a 3-hour infusion of $PGF_{2\alpha}$ (0 ng/min [C], 2,000 ng/min [C], 2,000 ng/min [J] and 0.2 mg/min [J]) into the carotid artery (C) or the jugular vein (J).

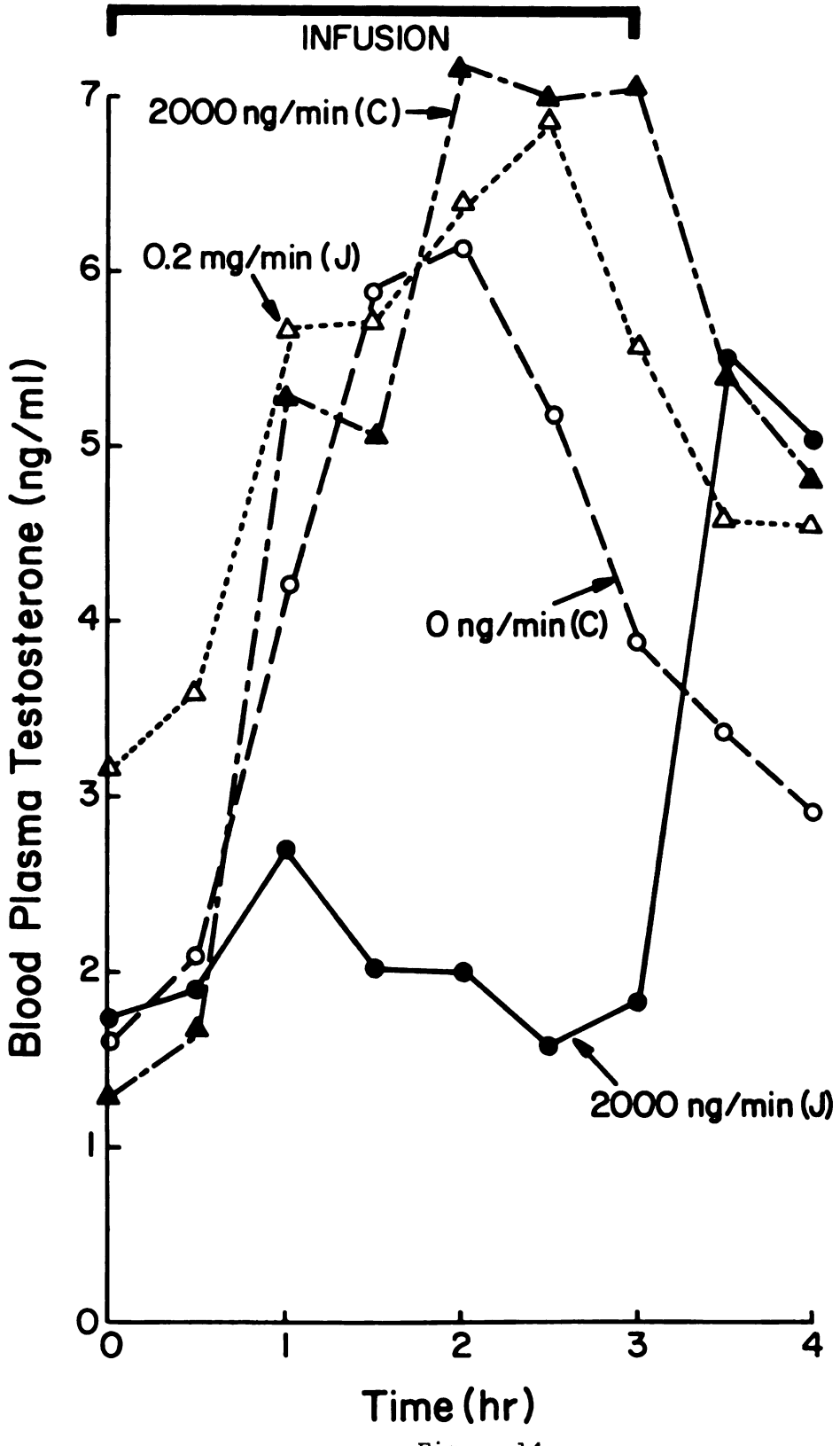
INFUSION
2000 ng/min (C)
0.2 mg/min (J)
0 ng/min (C)
2000 ng/min (J)
Blood Plasma Testosterone (ng/ml)
Time (hr)
0
1
2
3
4
5
6
7

Figure 14

testosterone increased ($P<.05$) from 1.3 $\pm$ .4 ng/ml before infusion to 7.2 $\pm$ 2.2 ng/ml after 2 hours of infusion. In addition, although no differences existed between plasma testosterone prior to infusion of treatments, average plasma testosterone was greater ($P<.05$) at 2.5 hours of infusion with 2,000 ng $PGF_{2\alpha}$/min into the carotid than at 2.5 hours of infusion with the same dose of $PGF_{2\alpha}$ into the jugular. This observation is consistent with the LH response during the same treatments.

Treatment effects also were evaluated by determination of the duration of LH and testosterone response. Two independent referees estimated the duration of elevated LH and testosterone after each treatment in each bull.

Prior to treatments, blood was sampled at frequent intervals for 24 hours to determine the duration of LH and testosterone surges. The average duration of LH and testosterone surges was 2.2 $\pm$ 0.2 and 3.2 $\pm$ 0.3 hours, respectively (Figure 15). By comparison, during carotid infusion of saline (0 ng [C]) only one bull exhibited increased LH (2.0 hour duration). In contrast, average duration of the LH surge (all five bulls) was 6.3 $\pm$ 0.7 and 5.2 $\pm$ 1.2 hours (Figure 15) during infusion of 2,000 ng [C] and 0.2 mg [J], respectively. Relative to bulls given saline, duration of LH was greater ($P<.05$) during the 2,000 ng [C] and 0.2 mg [J] treatments. In addition, average duration of LH response was greater after 2,000 ng [C] than that after 2,000 ng [J]. These observations were consistent with the view that $PGF_{2\alpha}$ acted at the brain to elicit LH release.

Similarly, the durations of the testosterone surges after 2,000 ng [C] and 0.2 mg [J] treatments were greater ($P<.05$) than those after 0 ng [C] or 2,000 ng [J]. Average duration of testosterone was

Figure 15. Mean duration of LH and testosterone surges occurring prior to (control) and during infusion of $PGF_{2\alpha}$ into five bulls. Numbers within the bars represent the number of LH and testosterone surges occurring during infusion of $PGF_{2\alpha}$.

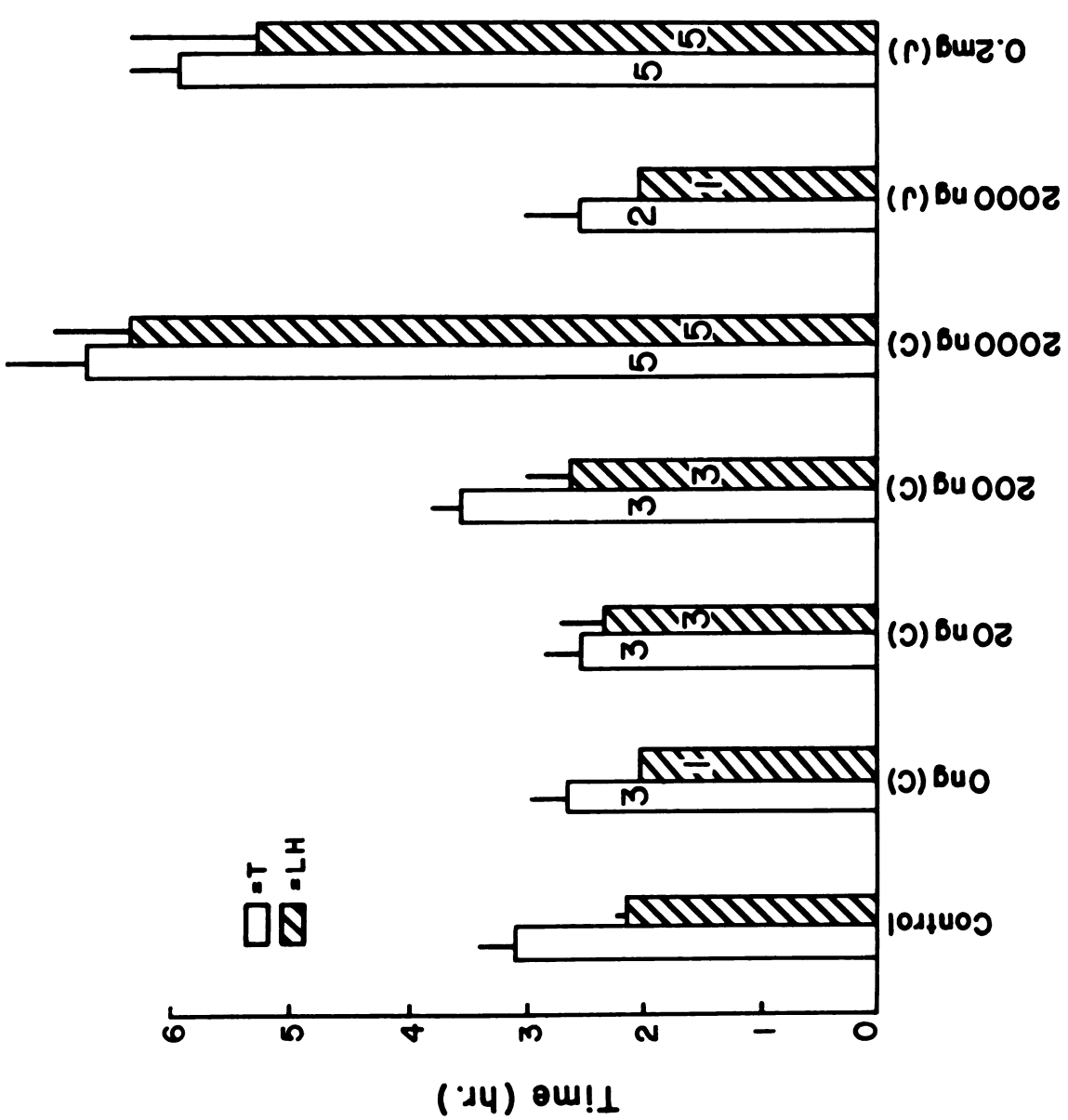
Time (hr.)
0
1
2
3
4
5
6
T
LH
Control
0ng (C)
20 ng (C)
200 ng (C)
2000 ng (C)
2000 ng (J)
0.2mg (J)

Figure 15

Figure 15. Mean duration of LH and testosterone surges occurring prior to (control) and during infusion of $PGF_{2\alpha}$ into five bulls. Numbers within the bars represent the number of LH and testosterone surges occurring during infusion of $PGF_{2\alpha}$.

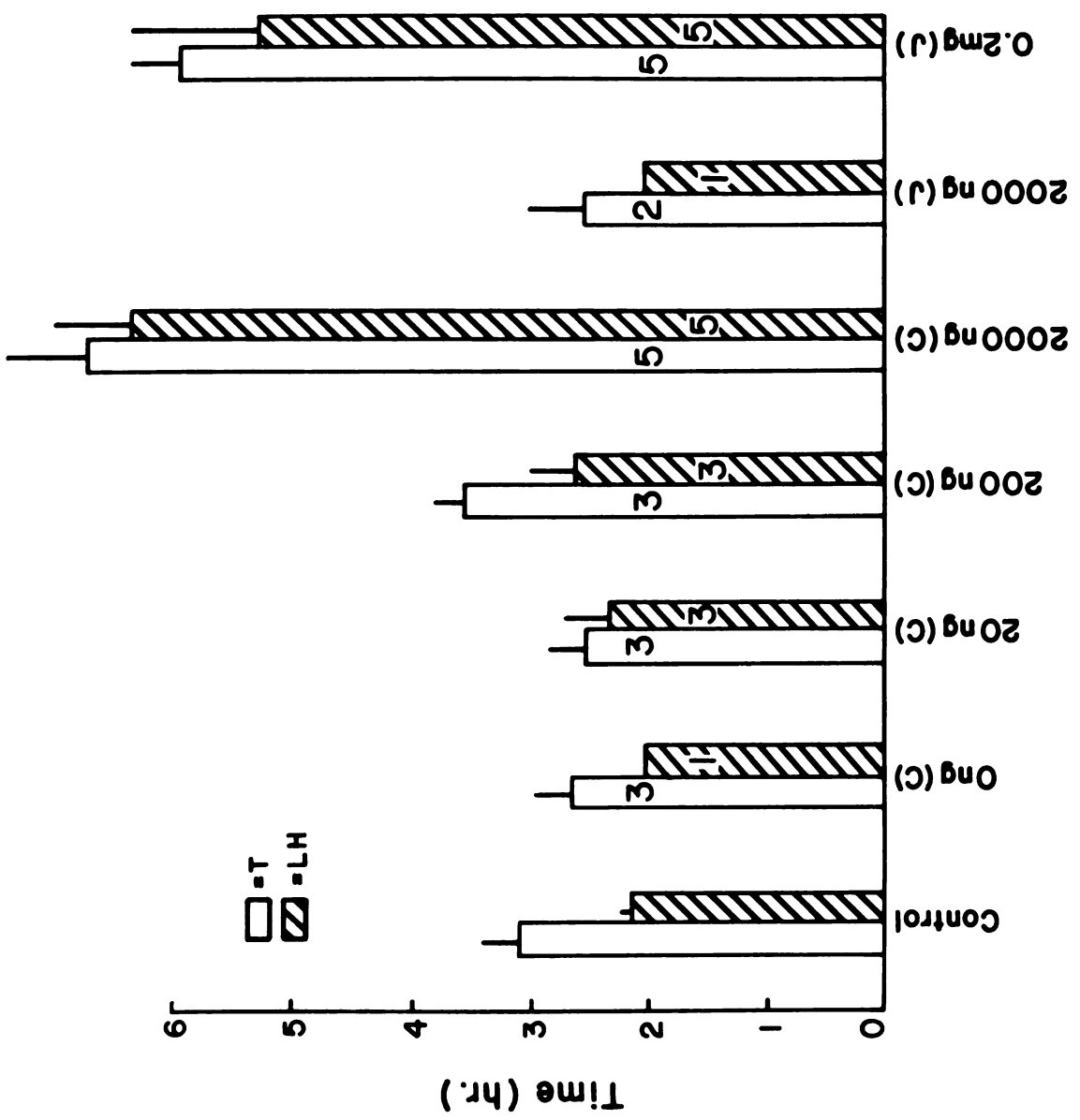
Time (hr.)
0
1
2
3
4
5
6
=T
=LH
Control
0ng (C)
20 ng (C)
200 ng (C)
2000 ng (C)
2000 ng (J)
0.2mg (J)

Figure 15

6.7 ± 0.7 and 5.8 ± 0.4 hours for 2,000 ng [C] and 0.2 mg [J] treatments, respectively, compared to 2.6 ± 0.3 and 2.5 ± 0.5 hours for 0 ng [C] and 2,000 ng [J] treatments.

In summary, infusion of $PGF_{2\alpha}$ into a carotid in an amount that was ineffective when infused into the jugular vein caused rapid and prolonged increase in plasma LH and testosterone in bulls. These results provide persuasive evidence that $PGF_{2\alpha}$ acted directly at the head to cause release of LH; presumably, the subsequent increase in plasma testosterone was in response to the elevated LH.

Whether $PGF_{2\alpha}$ acted directly at the level of the LHRH neurons to elicit release of LHRH which caused the increase in LH, as suggested by Eskay et al. (1976), awaits further investigation.

GENERAL DISCUSSION

Haynes et al. (1975) reported that $PGF_{2\alpha}$ caused increased testosterone secretion in bulls, but they were unable to prove that $PGF_{2\alpha}$ caused release of LH. Although these researchers acknowledged the possibility that $PGF_{2\alpha}$ may have caused release of LH, which then caused increased testosterone, they favored the hypothesis that $PGF_{2\alpha}$ acted directly at the testis to increase testosterone secretion. This assumption was based on the report of Eik-Nes (1969) that PGE_2 caused increased testosterone secretion in the perfused dog testis. Furthermore, Flack, Jessup and Ramwell (1969) found that prostaglandins stimulated corticosteroid synthesis in superfused rat adrenals. Haynes et al. (1975) also ruled out alteration in peripheral blood flow because the predominant action of $PGF_{2\alpha}$ is vasoconstriction, which would decrease blood flow and, presumably, testosterone secretion. In support of this idea, Einer-Jensen and Soofi (1974) reported that intratesticular administration of $PGF_{2\alpha}$ decreased blood flow in rats. Furthermore, Haynes et al. (1975) found no difference in heart rate or peripheral blood pressure after $PGF_{2\alpha}$ compared to controls.

The experiments discussed in this dissertation provide, for the first time, conclusive evidence that $PGF_{2\alpha}$ is a potent releaser of LH in bulls. Furthermore, the magnitude of LH release after $PGF_{2\alpha}$ was similar to the peak magnitude of LH observed during episodic

secretion in bulls. In other words, $PGF_{2\alpha}$ released LH in amounts that were in the physiological range for control bulls.

In my experiments, pharmacological doses of $PGF_{2\alpha}$ were administered, but these treatment doses were necessary in view of the rapid clearance of $PGF_{2\alpha}$ in the peripheral blood. A closer evaluation of the amounts of $PGF_{2\alpha}$ used in these experiments indicate that indeed $PGF_{2\alpha}$ was a potent stimulus for LH release. For example, in the final experiment, the highest dose of $PGF_{2\alpha}$ (2,000 ng/min) administered into the carotid interpolates to approximately 200 pg $PGF_{2\alpha}$/ml of blood actually reaching the brain area based on the approximate blood flow to the head (9L/min).

Other researchers have demonstrated that PGE_2 causes release of LH in rats, but until recently very little information was available implicating $PGF_{2\alpha}$ to release of LH. Warberg et al. (1976) reported increased LH after $PGF_{2\alpha}$ in rats, in contrast to results from Harms et al. (1973). The discrepancy between these reports remains to be resolved; however, Warberg et al. (1976) used about 5-fold more $PGF_{2\alpha}$ than did Harms et al. (1973).

In contrast to the rapid increase of testosterone that Haynes et al. (1975) observed after $PGF_{2\alpha}$ and the increase of testosterone in the present series of experiments, the majority of reports indicate that $PGF_{2\alpha}$ causes inhibition of testosterone, at least in rodents.

Aside from the possible species differences, I speculate that the reduced testosterone in rats and mice may have been associated with the chronic administration of $PGF_{2\alpha}$ in amounts which were much greater ($\sim$40-fold) on a body weight basis than I gave to bulls. In addition, these researchers measured testosterone at 3 and 12 hours after the last of a series of $PGF_{2\alpha}$ injections. Based on the research

reported in this dissertation, sampling of blood would have to occur immediately after administration of $PGF_{2\alpha}$ to maximize the possibility of detecting increased testosterone secretion. Furthermore, my research data would indicate that increased testosterone secretion occurs acutely after $PGF_{2\alpha}$ and possibly not after a chronic or constant $PGF_{2\alpha}$ stimulus.

Hafs, Louis and Stellflug (1974) demonstrated increased sperm output in rabbits and bulls. However, from a practical standpoint, it would appear contraindicated to administer $PGF_{2\alpha}$ to increase sperm output in bulls and at the same time cause a decrease in blood testosterone, an essential hormone for complete spermatogenesis and sperm maturation. The results of the present experiments do not rule out a detrimental effect of $PGF_{2\alpha}$ on testosterone secretion in bulls when used on a chronic basis. In fact, the evidence suggests that chronic administration of $PGF_{2\alpha}$ may cause a decrease in both LH and testosterone, because LH and testosterone were declining at the end of a 20-hour infusion of $PGF_{2\alpha}$ in Experiment 2.

From a physiological point of view, it is difficult to reconcile the fact that, in addition to release of LH, $PGF_{2\alpha}$ causes release of growth hormone (GH), prolactin and glucocorticoids in bulls (Hafs, 1975; Hafs et al., 1977). Prolactin, GH and glucocorticoids increased several-fold within 5 to 15 minutes after $PGF_{2\alpha}$ treatment in cows (Louis et al., 1974) and bulls (Hafs, 1975; Hafs et al., 1977) alike, much more rapidly than LH release. Moreover, the release of LH after $PGF_{2\alpha}$ in diestrous heifers is dependent upon rapid withdrawal of progesterone, which typifies luteolysis induced by $PGF_{2\alpha}$ (Hafs et al., 1976). These observations suggest that the action of $PGF_{2\alpha}$ to cause release of prolactin, GH and glucocorticoids may differ from the

action of $PGF_{2\alpha}$ to cause LH release. Prostaglandin $F_{2\alpha}$ may possibly act directly on the pituitary to cause the rapid release of prolactin, GH and glucocorticoids and at the same time act at the hypothalamus or other sites to elicit release of LH. Certainly a sex difference in $PGF_{2\alpha}$-induced release of LH is indicated from these observations. In bulls, feedback of testicular androgens may elevate the release threshold for LH compared with that for prolactin, a hormone for which feedback has not been demonstrated in cattle (Beck et al., 1976).

The evidence suggests that prostaglandins may be a common intermediate in the control of secretion of anterior pituitary hormones and that specificity to one hormone arises out of local production and local utilization of the prostaglandin. Perhaps one also could speculate that dual control of hormone secretion exists. In one case, catecholamines or other intermediates, which appear to be established as controllers of hormone secretion (Wilson, 1974), may control synthesis of releasing factors and prostaglandins may control release. Alternatively, there may be a dual control mechanism for both synthesis and release and each may operate independently.

That $PGF_{2\alpha}$ caused release of LH and testosterone in bulls is clear from the data in the present series of experiments. However, whether $PGF_{2\alpha}$ normally is a physiological mediator of LH release remains unanswered.

SUMMARY AND CONCLUSIONS

The purpose of these experiments was to determine the effect of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on secretion of LH and testosterone in bulls.

The first experiment was designed to determine the temporal relationship between blood serum LH and testosterone in bulls given a subcutaneous injection of $PGF_{2\alpha}$ or saline. Before $PGF_{2\alpha}$, blood serum LH averaged 1.1 $\pm$ 0.1 ng/ml; after $PGF_{2\alpha}$, LH increased 3-fold at 30 minutes, peaked (3.9 $\pm$ 0.9 ng/ml) at 45 minutes and declined to pre-injection values after 4 or 5 hours. Blood serum testosterone averaged 4.5 $\pm$ 0.2 ng/ml before $PGF_{2\alpha}$; it increased synchronously in each of the eight bulls to 8.5 $\pm$ 0.9 ng/ml by 60 minutes after $PGF_{2\alpha}$, peaked at 15 to 16 ng/ml between 90 and 120 minutes, and then declined toward pre-injection values by 180 minutes. Thus, the testosterone surge followed the $PGF_{2\alpha}$-induced LH surge by 30 to 60 minutes.

In contrast, an average of one episodic surge of testosterone (average peak 14.2; range 8.0 to 22.8 ng/ml) occurred apparently at random intervals during the 8 hours after bulls were given saline. An increase of blood serum LH (average peak 3.0; range 1.5 to 4.3 ng/ml) occurred about 30 minutes before each of these testosterone surges. On the average, neither LH nor testosterone changed significantly after saline. The major conclusion from this experiment is that administration of $PGF_{2\alpha}$ to bulls causes increased blood LH, followed by increased blood testosterone. Both peaks and the temporal

relationship of these changes in LH and testosterone were similar to the normal episodic releases of these hormones in bulls.

The second experiment was designed to test whether a continuous iv infusion of $PGF_{2\alpha}$ could maintain elevated blood LH and testosterone in bulls. Blood plasma LH averaged 1.2 $\pm$ 0.1 ng/ml before $PGF_{2\alpha}$ infusion; it doubled within 1.5 hours after the infusion was started and peaked approximately 4-fold higher than pre-infusion values at 6.5 hours before declining to basal concentrations before the end of the 20-hour infusion. Blood plasma testosterone averaged 7.0 $\pm$ 0.6 ng/ml during the 90 minutes before infusion of $PGF_{2\alpha}$; it increased 2-fold by 2.5 hours after the start of the infusion, remained near this peak until 10 hours and then gradually returned toward pre-infusion values by the end of the infusion. Thus, LH and testosterone increased together after the start of the infusion, but the peak testosterone concentration occurred sooner and the duration of the peak testosterone concentration persisted longer than the LH response. Luteinizing hormone started to decline at least 3 hours earlier than testosterone, preceding the decline of testosterone in all four bulls. Episodic release of LH and testosterone resumed within 8 hours after the end of the 20-hour $PGF_{2\alpha}$ infusion. Two or three episodic surges of testosterone occurred in each of the bulls during the 20-hour control infusion of saline; the peak (17.2 $\pm$ 0.2 ng/ml) of these surges was equivalent to the peak concentration of testosterone during infusion of $PGF_{2\alpha}$, and 18 of 19 control surges of testosterone were preceded by increased blood LH (average peak 2.8 $\pm$ 0.3 ng/ml). In summary, constant infusion of $PGF_{2\alpha}$ caused prolonged increases in LH and testosterone secretion in bulls, but both LH and testosterone

declined toward basal values before the end of the 20-hour infusion period.

In the third experiment, the major objective was to determine if the inhibitory effects of melengestrol acetate (MGA) on episodic release of LH and testosterone could be overcome by $PGF_{2\alpha}$. A preliminary experiment demonstrated that feeding 0.5 mg MGA twice daily (0700 and 1000 hours) abolished surges of testosterone. The average testosterone concentration during a 4-hour period before MGA was 8.5 $\pm$ 1.1 ng/ml. During the period 1 to 5 hours after the third feeding of MGA, testosterone averaged only 1.8 $\pm$ 0.1 ng/ml.

In the main experiment, each bull was fed 1.0 mg of MGA daily (0.5 mg at 0700 and 1900 hours). After four MGA-treated bulls were given saline, blood LH concentrations did not change significantly, ranging from 0.30 $\pm$ 0.05 to 0.40 $\pm$ 0.05 ng/ml. Similarly, serum testosterone fluctuated between 0.8 $\pm$ 0.3 to 1.2 $\pm$ 0.2 ng/ml and did not change significantly. By comparison, serum LH averaged 0.40 $\pm$ 0.01 ng/ml before MGA-treated bulls were given (sc) 20 mg PGF_2 ; it increased 5-fold at 45 minutes after $PGF_{2\alpha}$, peaked at 2.3 $\pm$ 0.5 ng/ml at 60 minutes and declined to basal values between 4 and 5 hours. Serum testosterone averaged 0.8 $\pm$ 0.3 ng/ml before $PGF_{2\alpha}$, increased 25-fold to peak at 105 minutes after $PGF_{2\alpha}$, plateaued until 3 hours after $PGF_{2\alpha}$ and declined to baseline concentrations by 7 hours. In conclusion, 1) treatment of bulls with MGA abolished LH and testosterone secretion in bulls and 2) $PGF_{2\alpha}$ caused release of LH and, subsequently, testosterone in the face of MGA inhibition.

Although the first three experiments demonstrated that $PGF_{2\alpha}$ caused increased blood LH and testosterone, the site of action of $PGF_{2\alpha}$ remained to be ascertained. Therefore, the objective of the

final experiment was to determine if $PGF_{2\alpha}$ acted directly on the brain to elicit release of LH in bulls. Prostaglandin $F_{2\alpha}$ was administered by a 3-hour intracarotid (to affect the brain) or intrajugular (systemic treatment) infusion in five bulls. Intracarotid infusion of saline or jugular infusion of 2,000 ng $PGF_{2\alpha}$/minute did not cause increased blood LH; LH averaged 1.1 and 1.0 ng/ml during the 3-hour infusion for intracarotid saline and jugular infusion of 2,000 ng $PGF_{2\alpha}$/min, respectively. Blood LH increased from 0.8 $\pm$ 0.1 ng/ml to a peak of 2.6 $\pm$ 0.5 ng/ml within 1 hour after beginning jugular infusion of a large dose of $PGF_{2\alpha}$ (0.2 mg/min), then declined to 1.4 $\pm$ 0.3 ng/ml at the end of the 3-hour infusion. A similar increase (to a peak of 3.6 $\pm$ 1.1 ng/ml) occurred during intracarotid infusion of 2,000 ng $PGF_{2\alpha}$/min, but LH clearly remained elevated throughout the 3-hour infusion period. The LH response during intracarotid infusion of 2,000 ng $PGF_{2\alpha}$/min was greater than that during intrajugular infusion of 0.2 mg $PGF_{2\alpha}$/min. The data indicate that $PGF_{2\alpha}$ acted at the brain to cause LH release because the 2,000 ng/min dose of $PGF_{2\alpha}$ infused to the head via the carotid caused release of LH, whereas the same amount of $PGF_{2\alpha}$ infused away from the head via the jugular caused no significant elevation in LH. Testosterone remained below 3 ng/ml during jugular infusion of 2,000 ng $PGF_{2\alpha}$/min, but the same dose of $PGF_{2\alpha}$ increased blood testosterone from 1.3 $\pm$ 0.4 ng/ml before intracarotid infusion of $PGF_{2\alpha}$ to 7.2 $\pm$ 2.2 ng/ml at 2 hours during infusion, and testosterone remained elevated until the intracarotid infusion of $PGF_{2\alpha}$ was stopped.

I conclude that $PGF_{2\alpha}$ acted directly at the brain to cause release of LH; then LH caused the subsequent increase in plasma testosterone.

The results from this series of experiments provide conclusive evidence, the first report in bulls, that $PGF_{2\alpha}$ causes increased blood LH. Luteinizing hormone increased when $PGF_{2\alpha}$ was given as a single subcutaneous injection. The temporal relationship of changes in LH and testosterone suggested that the increase in testosterone was caused by increased LH secretion. Secondly, a 20-hour intravenous infusion of $PGF_{2\alpha}$ resulted in a prolonged increase of LH and testosterone, but LH and testosterone decreased to basal concentrations toward the end of the 20-hour infusion. The results indicate that a continuous $PGF_{2\alpha}$ stimulus results in hypothalamic or pituitary refractoriness, but the refractoriness was short-lived because episodic secretion of LH and testosterone (similar to that observed in control bulls) resumed again within 8 hours after the end of the infusion. Thirdly, suppression of episodic secretion of LH and testosterone by MGA was overcome with a single sc injection of $PGF_{2\alpha}$. I speculate that MGA might possibly inhibit hypothalamic $PGF_{2\alpha}$ secretion and thereby inhibit LH release. Lastly, intracarotid, but not intrajugular, infusion of 2,000 ng $PGF_{2\alpha}$/min caused an increase in LH and testosterone. These results provide persuasive evidence that $PGF_{2\alpha}$ acts at the brain to cause release of LH.

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