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Regulation of Gonadotropins by Steroids in Isolated Rat Pituitary Cells

Lenette L. Renier
Loyola University Chicago

REGULATION OF GONADOTROPINS BY STEROIDS IN ISOLATED RAT PITUITARY CELLS

by

Lenette L. Renier

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VITA

The author, Lenette L. Renier, is the daughter of Arthur H. Renier Jr. and Lenore M. Renier. She was born on November 30, 1964, in Chicago, Illinois.

Her elementary education was obtained in the public schools of Chicago, Illinois through grade 5 and completed at St. Edwards Grammar School in Chicago. Her Secondary education was completed in 1982 at Good Counsel High School, Chicago, Illinois.

In September, 1982, Miss Renier entered Loyola University of Chicago, receiving the degree of Bachelor of Science in Biology in May, 1986.

In September, 1987, Miss Renier was granted an assistantship in biology at Loyola University of Chicago, enabling her to complete the Masters of Science in 1990. In May, 1988, she was awarded first place in the poster presentations by the Loyola Chapter of Sigma XI. In August, 1988, she presented an abstract at the 21st annual meeting of The Society for the Study of Reproduction in Seattle, Washington. She was accepted into the 1994 veterinary class at University of Illinois.

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INTRODUCTION

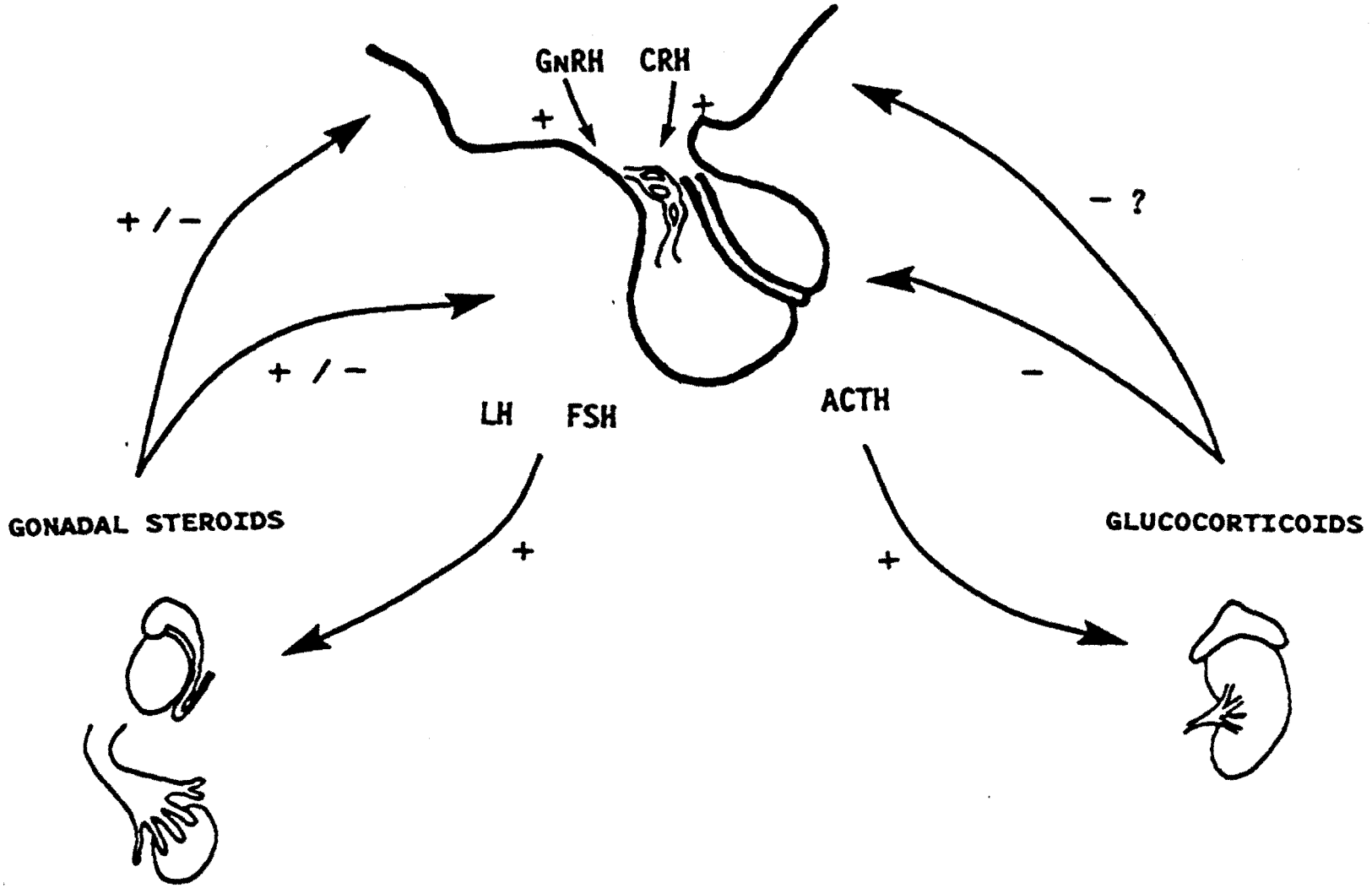
The information obtained through these studies will permit us to understand further the regulation of the reproductive system and infertility due to stress. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are important because they regulate secretion of the sex steroids by the gonads and growth and development of ova and sperm. In this thesis we will concentrate on two gonadotropins, LH and FSH, and their part in the regulation of the reproductive feedback loops (Fig. 1). LH and FSH are not always affected in the same way by a particular treatment although they are secreted by the same gland and their structures and functions are similar.

LH and FSH are glycoproteins and both are secreted by the anterior pituitary in response to gonadotropin-releasing hormone (GnRH), which is secreted by the hypothalamus. LH and FSH stimulate secretion of other steroid hormones by the gonads, including estradiol, progesterone, and testosterone. These hormones act in a negative feedback loop and inhibit secretion of LH and FSH, and possibly GnRH. Gonadal hormones and GnRH are not, however, the only factors that regulate the gonadotropins. Other hormones in the body, released

Figure 1: Hypothalamic-pituitary-gonadal-adrenal axis

GnRH from the hypothalamus stimulates (+) secretion of LH and FSH from the pituitary. These gonadotropins stimulate the gonads, either testes or ovaries, to secrete testosterone, estradiol and progesterone. These gonadal steroids stimulate or inhibit (-) secretion of LH and FSH by a direct effect at the anterior pituitary, or indirectly by inhibiting GnRH from the hypothalamus, or possibly a combination of the two.

Corticotropin-releasing hormone (CRH) stimulates secretion of adrenal cortical stimulating hormone (ACTH) from the anterior pituitary. ACTH stimulates secretion of glucocorticoids from the adrenal cortex. Glucocorticoids inhibit ACTH by a direct effect upon the pituitary and possibly indirectly by inhibiting CRH from the hypothalamus.


GnRH
CRH
+
+
+ / −
+ / −
− ?
−
LH
FSH
ACTH
GONADAL STEROIDS
GLUCOCORTICOIDS
+
+

during stress, inhibit reproductive function at least in part, by decreasing secretion of gonadotropins (Selye, 1939). Glucocorticoids are secreted by the adrenal gland in response to stress.

We will be using the anterior pituitary of the rat (Rattus norvegicus) as a model. The rat is a good model because it is easy to breed and house in the city, there are data from previous studies using rats, and the results obtained may be comparable to those for the primate in this particular area. For example, Frawley and Neill (1984) found that cultured pituitary cells from monkeys and rats are comparable when examining the effects of estradiol on GnRH-induced LH secretion.

Cell cultures of the pituitary gland can be used to determine if steroids have a direct effect on the pituitary itself in its ability to store and secrete LH and FSH. By using a cell culture system we will be able to determine if the steroids act directly on the anterior pituitary. If the steroids do not act at the pituitary, we will know they act at another place in the body, possibly the hypothalamus, if they exert any effect at all.

By understanding where and how the feedback mechanisms work, we will be able to understand further sexual dysfunction and infertility due to stress. We must understand this system to be able to alleviate these

problems in breeding most effectively. Another consideration may be control of the rodent populations by altering reproductive behavior.

Due to the extensive amount of literature in this area, we will limit this literature review to the *in vivo* and static cell culture *in vitro* studies in female rats.

REVIEW OF LITERATURE

Steroidal modulation of secretion of gonadotropins is an important part of the regulatory processes underlying normal reproductive function. Androgens, estrogens, and progestins, which are all sex steroids, and adrenal glucocorticoids all directly affect secretion of the gonadotropins in vitro, suggesting that direct effects of steroids on the anterior pituitary gland are physiologically relevant events in the regulatory process (Schally et al.,1973; Labrie et al. 1978; Suter and Schwartz, 1985; Tibolt and Childs 1985; Kamel and Kubajak 1987). Furthermore, accumulating evidence that activation of the hypothalamic-pituitary-adrenal axis inhibits normal reproductive function (Smith et al., 1971; Baldwin and Sawyer, 1974) suggests that other steroid hormones may be capable of altering the effects of glucocorticoid hormones on pituitary function (Campbell et al., 1977). (For an excellent review in other vertebrate species see Moberg, 1987.)

Hormones of the Anterior Pituitary

The anterior pituitary gland (adenohypophysis) produces at least ten known peptide hormones [follicle-stimulating hormone (FSH), luteinizing hormone (LH),

thyroid-stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), growth hormone (GH), prolactin (PRL), beta-endorphin, beta-lipotropin (beta-LPH), Met-enkephalin, and melanocyte-stimulating hormone (MSH)]. These hormones are released into the bloodstream where they circulate and regulate such target organs as the gonads, the adrenals, and the thyroid gland. We will be examining the effects of the gonadal steroids estradiol and progesterone and the adrenal steroid corticosterone on two gonadotropins of the anterior pituitary (LH and FSH). LH and FSH are important because they control secretion of the gonadal hormones and also regulate the growth and development of gametes.

In females LH stimulates luteal formation and secretion of estradiol (E_2) and progesterone (P_4). LH combines with receptors on the ovarian thecal cells and stimulates production of androgens, which are later taken up by the granulosa cells to serve as estrogen precursors. In males, LH stimulates secretion of testosterone, which regulates development of primary and secondary sexual characteristics.

In females FSH binds to receptors on the granulosa cell. This binding of FSH promotes follicular growth and proliferation in the ovaries. FSH also causes an augmented rate of secretion of estradiol. In males FSH cannot act alone to stimulate measurable androgen

production, but it enhances responses to LH. FSH additionally accelerates uptake of testosterone by Sertoli cells.

LH and FSH are interdependent in both sexes. LH can not exert its effects alone: it is dependent on FSH in both sexes to increase the population of receptors for LH. FSH also interacts with LH to increase spermatogenic activity in the testes.

Effects of Sex Steroids on LH

The sex steroids play a role in regulation of LH and FSH. Researchers have observed several effects of gonadal steroids on gonadotropin regulation. In vivo, estradiol exerted both positive and negative feedback effects on gonadotropin release, and the effects appeared to be dose- and time-dependent (Arimura and Schally, 1971; Kalra et al., 1973; Yen et al., 1974). In vitro, however, estradiol usually resulted in stimulatory effects on the secretion of gonadotropins (Drouin et al., 1976; Hsueh et al., 1979; Lagace' et al., 1980; Kamel and Krey, 1982). Estradiol caused an increase in pituitary responsiveness to GnRH, and thus an increase in secretion of LH (Drouin et al., 1976; Hsueh et al., 1979; Lagace' et al., 1980), without a decrease in cell content of LH (Kamel and Krey, 1982). This finding suggested that

positive feedback occurs directly at the level of the pituitary, at least in part. The failure to detect antagonism by estradiol of GnRH-stimulated secretion of LH and FSH in static cultures of pituitary cells could reflect 1) the transient nature of the inhibition, 2) desensitization of gonadotropes to continuous GnRH exposure (Drouin et al, 1976 b), or 3) the requirement of tissue integrity for manifestation of the inhibitory response.

In some studies, however, where the time of exposure to estradiol was relatively short, suppression of secretion of LH was observed (Frawley and Neill, 1984; Emons et al., 1986; Ortmann et al., 1988). This negative feedback at the level of the pituitary has also been observed in sheep (Alexander and Miller, 1982; Phillips et al., 1988), chickens (King et al., 1989) and monkeys (Frawley and Neill, 1984). Frawley and Neill have demonstrated that estradiol exerted a potent, but transient, inhibition of GnRH-stimulated LH secretion from rat pituitary cells by using a perfused monolayer culture (Frawley and Neill, 1984). Since the rat has an estrous cycle of only 4 to 5 days, the inhibitory effects of estradiol on secretion of LH may also be more short-lived than in the ewe, with an estrous cycle of 16 days, or a primate, with a menstrual cycle of 28 days. This finding suggested that negative feedback also occurs

directly at the level of the pituitary, at least in part.

In vivo, progesterone inhibited the release of LH in rats (Arimura and Schally, 1970; Caligaris et al, 1971) and monkeys (Spies and Niswender, 1972). In vitro, in the absence of GnRH (basal), progesterone stimulated secretion of LH (Lagace' et al., 1980), as also observed with estradiol (Drouin et al., 1976). Progesterone did not significantly change GnRH-stimulated production of LH except in high concentrations (10^{-6} M), when it decreased LH secretion (Hsueh et. al, 1979). We found no studies where varying lengths of incubation with progesterone altered the resulting concentration of LH.

Effects of Sex Steroids on FSH

In vitro, estradiol showed significant stimulatory effects on basal secretion of FSH (Lagace' et. al, 1980). At some doses of GnRH estradiol also enhanced secretion of GnRH-stimulated FSH (Lagace' et al., 1980; Kamel and Kubajak, 1987). No scientists have reported negative feedback on FSH by estradiol, as observed for LH, by using another time course of exposure of the cells to steroids. Progesterone caused a concentration-dependent increase in basal FSH release in cultures from female rats (Leveque and Grotjan, 1982). Progesterone also enhanced GnRH-induced secretion of FSH in cultured cells

(Lagace' et al., 1980) and in vivo (Caligaris et al., 1971). Neither testosterone nor dihydrotestosterone significantly affected release of FSH induced by GnRH. Like progesterone, testosterone and dihydrotestosterone caused a concentration-dependent increase in basal FSH release in cultures from female rats (Leveque and Grotjan, 1982). No scientists have reported negative feedback on FSH by progesterone by using altered (short-term and long-term) lengths of exposure of progesterone to rat anterior pituitary cells.

We could not find reports of negative feedback by gonadal hormones on FSH either because 1) negative feedback does not occur or 2) studies were not done with FSH as they were with LH. Many investigators do not measure levels of FSH because the responsiveness of FSH to any known regulatory signal is of small magnitude compared to the responsiveness of LH. For example, the response of LH to GnRH may be on the order of 50-fold, whereas the response of FSH may be on the order of 5-fold (Labrie et al., 1978).

Effects of Glucocorticoids on Reproductive Function

In addition to the sex steroids, the stress hormones also play a role in regulation of LH and FSH, but little is known about their sites of action. In

rats, stress caused by housing large numbers of rats per cage increased secretion of corticosterone (B) as measured in plasma (Eechaute et al., 1962; Barrett and Stockham, 1963). Adrenocortical function, measured by weight of the adrenal, increased, and reproductive function, measured by prevalence of pregnancy, decreased, with increasing density as a result of increasing social pressure. The increase in social pressure was determined by size of population and aggressive behavior, in studies using both Baltimore city wild rat populations and laboratory rat populations (Christian et al., 1965). Thus, pituitary-adrenocortical function is positively, and reproductive function negatively, correlated with the amount of social pressure in a population (Christian et al., 1965).

Several observations have suggested that the glucocorticoids are responsible for reproductive dysfunction. Secretion of glucocorticoids, which can be induced by stress, has been observed to cause reproductive dysfunction (Christian et al., 1965; Moberg, 1987). Implantation of glucocorticoids pellets in the medial basal hypothalamus inhibited development of the reproductive system of immature female and male rats (Smith et al., 1971). Implantation of natural or synthetic glucocorticoids also inhibited normal female sexual behavior in rats (DeCatanzaro and Gorzalka, 1979).

This reproductive dysfunction may be partially caused by the glucocorticoids' direct inhibitory effects exerted upon secretion or synthesis of gonadotropins by part of the hypothalamic-pituitary-adrenal axis (Fig. 1). Treatment with glucocorticoids, either *in vivo* or *in vitro*, interfered with testicular function in males (Desjardins and Ewing, 1971; Saez et al., 1977; Bambino and Hsueh, 1981; Welsh et al., 1982), and function of granulosa cells in females (Hsueh and Erickson, 1978; Schoonmaker and Erickson, 1983). *In vivo* treatment of rats with glucocorticoids blocked ovulation (Hagino et al., 1969; Smith et al., 1971; Baldwin and Sawyer, 1974). Further studies suggested that this blockade is due to prevention of the preovulatory surges of LH and FSH (Hagino et al., 1969; Baldwin, 1979). These studies suggest that, glucocorticoids alter reproductive function in both sexes by affecting secretion of gonadotropins as well as other physiological processes.

Effects of Glucocorticoids on LH and FSH

Some of the effects of glucocorticoids, mentioned in the previous section, could be due to direct effects on the anterior pituitary. *In vivo*, glucocorticoids inhibited GnRH-stimulated secretion of LH, but did not suppress FSH release (Ringstrom and Schwartz, 1985).

pituitary content of LH was not affected by treatment with glucocorticoids, suggesting that perhaps secretion rather than synthesis was affected (Ringstrom and Schwartz, 1987). Lack of responsiveness to exogenous GnRH suggested that the glucocorticoids had a direct inhibitory effect on the gonadotropes themselves.

In vitro, treatment of pituitary cells from female rats with glucocorticoids resulted in divergent effects on the gonadotropins: basal secretion of LH was inhibited (Suter and Schwartz, 1985; Tibolt and Childs, 1985; Kamel and Kubajak, 1987) while that of FSH was stimulated (Suter and Schwartz, 1985; Kamel and Kubajak, 1987). Secretion of LH maximally stimulated by GnRH was not affected, whereas maximally stimulated secretion of FSH was enhanced by glucocorticoids, as indicated by an increase in the slope of the GnRH dose-response curve (Suter and Schwartz, 1985). These findings are controversial, however, because in some studies GnRH-stimulated secretion of LH was suppressed by corticosterone, as demonstrated by a consistently increased ED_{50} for GnRH (Tibolt and Childs, 1985; Kamel and Kubajak, 1987). Variations were also observed for GnRH-stimulated secretion of FSH. One team observed an increase of the ED_{50}, indicating an inhibition of GnRH-stimulated secretion of FSH (Tibolt and Childs, 1985).

There is some evidence from in vivo studies that

the pituitary effects of glucocorticoids are stimulatory rather than inhibitory. When male rats were implanted with cortisol (F) *in vivo* four days before the pituitaries were removed and treated *in vitro* with F, a stimulatory effect was observed for basal secreted LH and FSH. GnRH-stimulated secretions of LH and FSH were also enhanced, as observed by a shift to the left of the GnRH dose-response curve (Suter and Orosz, 1987). These results suggested that glucocorticoids did not inhibit secretion of the gonadotropins by a direct negative effect on the pituitary.

Glucocorticoids may also be stimulatory by exerting indirect effects on other hormones that affect secretion of gonadotropins. Glucocorticoids may inhibit secretion of corticotropin-releasing hormone (CRH) and ACTH. Rivier et. al (1986) suggested that CRH inhibits secretion of LH. Moberg (1987) observed that ACTH inhibited secretion of both LH and FSH. If B blocks the factors that inhibit secretion of gonadotropins, then it could stimulate secretion of gonadotropins. The glucocorticoids may also indirectly inhibit secretion of gonadotropins by stimulating secretions of other hormones, possibly from the hypothalamus. Glucocorticoids, however, may also exert direct stimulatory effects when pituitary cells are treated with glucocorticoids alone and other effector hormones are not

present.

To determine the effect of glucocorticoids on the complete cellular system, cellular content and total concentrations of gonadotropins must be observed in addition to secretion. Glucocorticoids had no significant effect on cell content of LH or on the total amount per plate, under either basal or maximally stimulated conditions (Suter and Schwartz, 1985; Kamel and Kubajak, 1987). In contrast, B increased basal cellular and total FSH as well as maximally stimulated total FSH (Suter and Schwartz, 1985; Kamel and Kubajak, 1987). These studies demonstrate that B can alter concentrations of gonadotropins. We know, however, that regulated concentrations of gonadotropins are important for sexual function. If secretion of FSH is stimulated by B, then the follicular maturation may be untimely, resulting in sexual dysfunction. B may, therefore, disrupt sexual function by direct stimulatory effects on FSH, at least in part, at the pituitary.

Interactions of Sex Steroids and Glucocorticoids in Regulating LH and FSH

In the body gonadal and adrenal steroids are not isolated from each other; more than one steroid is present at any one time. Most previous investigators,

however, have studied the effects of gonadal hormones alone or glucocorticoids alone. It is possible that the glucocorticoids may interact with gonadal steroids. This idea is new and virtually unexplored. In vivo injections of synthetic glucocorticoids blocked the estrogen-induced LH surge in ovariectomized female rats (Baldwin and Sawyer, 1974). Treatment with estradiol in vitro increased GnRH-stimulated secretion of LH (Hsueh et al., 1979; Kamel and Krey, 1982) by rat pituitary cells, but addition of corticosterone blocked the stimulatory effect of estradiol (Kamel and Kubajak, 1987). Possible interactions between estradiol or progesterone and glucocorticoids in regulating FSH have not been explored.

Time Course of Steroid Exposure to Pituitary Cells

To determine the site of negative and positive feedback of E_2 on gonadotropins in rats, an in vitro model will be used. Negative feedback of E_2 on gonadotropins, however, has been difficult to demonstrate in monolayer cultures of rat pituitary cells. In vivo these negative feedback effects are seen. If these feedback effects are at the site of the pituitary, it is important to establish experimental conditions where both negative and positive feedback loops can be observed so that pituitary cell cultures can be successfully used as

a model. Previous studies using cell cultures employed a minimum preincubation time of 24-48 h of steroids with cells. Drouin stated that a period of 10 h is needed before any effect of E_2 could be measured (Drouin et al., 1976). Some scientists, however, observed inhibition of LH when the time of exposure to estradiol was relatively short (4-6 h) (Frawley and Neill, 1984; Emons et al., 1986; Ortmann et al., 1988). This negative feedback at the level of the pituitary has also been observed in sheep (Alexander and Miller, 1982) and monkeys (Frawley and Neill, 1984). Negative feedback in vitro has not been shown for FSH in rats. Negative feedback on FSH by estradiol and progesterone has been demonstrated in ovine pituitary cells (Phillips et al., 1988).

Investigator Variation

There is some controversy in the results discussed above. Not every investigator observes the same effect of a particular steroid on secretion of gonadotropins. Some of this variation observed may be due to experimental technique. For example, some investigators coated their plates with poly-lysine before plating the cells to achieve a greater percent of attachment. Conn observed, however, that poly-lysine mimics GnRH and that cells incubated on coated plates secreted LH as if they

were responding to GnRH when no GnRH was present (Conn et al., 1984). (Studies that employed poly-lysine to coat the plates were not included in this literature review and, therefore, will not be a factor of controversy in this thesis.)

Other variation in incubation procedures involves methodology of incubation of steroids with cells. Some investigators (Kamel and Krey, 1982; Kamel and Kubajak, 1987) removed the sex steroids and glucocorticoids after preincubation and did not reintroduce them in the second incubation period, so there were no steroids present when GnRH was being tested in the second incubation. (This procedure was omitted in their paper, but brought out in personal communication.) The levels of gonadotropins measured may have resulted either from the steroids or from removal of the steroids. In the present studies, we have included the ovarian and adrenal steroids when incubating with GnRH to avoid this variable.

The variable effects of B on LH observed <u>in vitro</u> may also be due to other factors. Neither Kamel and Kubajak nor Tibolt and Childs described charcoal-extraction of their serum. Charcoal-extraction removes steroids from serum which is conventionally added to cell culture medium. This serum is usually obtained from a horse or bovine fetus and it contains steroidal hormones normally found in these animals. Charcoal-extraction is

therefore necessary to remove these natural steroidal hormones from the serum before it can be used. Steroids present in the serum could effect secretion of gonadotropins and confound interpretation of the results. For example, it has been demonstrated that ovariectomy resulted in an approximately 2-fold increase in glucocorticoid receptor mRNA concentrations in rat anterior pituitary glands, which was reversible by administration of estradiol (Peiffer and Barden, 1987). If estradiol was present in the serum, a decrease in glucocorticoid receptor mRNA concentrations could have resulted and B would not have been able to bind to pituitary cells and affect gonadotropin secretion. A change in secretion of gonadotropins may, therefore, have been caused by steroids other than B due to a decrease in the concentration of glucocorticoid receptors. Glucocorticoids or estradiol may have affected the genome to cause increased secretion of gonadotropins. To avoid this possible variable, our serum in these experiments was charcoal-extracted prior to incubation with cells. Another variable may be the source of the serum. Some investigators use charcoal-extracted horse serum and others use charcoal-extracted fetal bovine serum in their culture medium (defined in Materials and Methods) among other cell culture variations.

Another variable is gender of the rat. Both Suter

and Schwartz (1985) and Kamel and Kubajak (1987) used females while Tibolt and Childs (1985) used males. Effects due to gender may not be completely ruled out in this case because the studies by Kamel and Tibolt for the effects on LH were comparable, but not the effects on FSH. Studies compared in this thesis will focus on experimentation in female rats.

Another variable may be time of the year. Most species breed only at specific times in the year. This seasonal breeding predisposes maximum reproductive function at a particular season. Our rats will be housed in conditions of consistent light and dark cycles, which assists in maintaining constant conditions in the room, to prevent any false seasonal cues.

A combination of variations could be causing the conflicting results. If small variations cause great changes then cell culture may not be the perfect model for this system. It has not been determined, however, that small variations are responsible for conflicting results. It is important for investigators to publish their exact incubation procedures to determine if the cause for variation may be in the procedure.

Experimental Design

We designed these experiments to study the

interactions of estradiol or progesterone with corticosterone in regulating secretion and storage of gonadotropins by the anterior pituitary of female rats. First, we examined whether estradiol had direct negative feedback effects on FSH as well as LH. Second, we studied the effect that duration of exposure of pituitary cells to steroidal hormones has on positive and negative regulation of LH and FSH. Third, we examined the effects of corticosterone on LH and FSH. Finally, we investigated whether corticosterone affects LH and FSH in a manner additive, antagonistic to, or synergistic with estradiol or progesterone.

MATERIALS AND METHODS

Reagents

We obtained powdered Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS) and trypsin (porcine pancreatic, type II) from Sigma (St Louis, MO). We dissolved corticosterone (B; U.S. Biochemical Corp. [Cleveland, OH]), estradiol (E_2; Sigma [St. Louis, MO]) and progesterone (P_4; Sigma [St. Louis, MO]) in 95% ethanol to a concentration of 10^{-2} M (3.5 mg B, 2.7 mg E_2, or 3.1 mg P_4 per ml). We then diluted the E_2 (10^{-2} M) to 10^{-5} M in ethanol. All of our subsequent dilutions employed DMEM as the diluent. Control plates not receiving any steroids received the same volume of ethanol as plates that received steroidal treatments to control for its possible effects. We silenized all glassware that contacted cells with Sigmacote (Sigma [St. Louis, MO]). We sterilized reagents and glassware used for cell cultures by passage through 0.2 um filters or by autoclaving.

Collection of Pituitaries

Our department maintains an Animal and Plant Health

Inspection Service-accredited animal care facility that houses adult (9-14 weeks) female Sprague-Dawley rats (Charles Rivers, Wilmington, MA) in conditions of controlled light (12 h light: 12 h dark) and temperature, with food and water provided ad libitum. For each replicate of an experiment, we collected 28-50 pituitaries, placed them in HEPES buffer (25 mM HEPES, 137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, and 10 mM glucose), and cut them into fragments. We then dispersed the pituitaries into single cells by incubation in HEPES buffer containing 0.1% (wt/vol) trypsin, 0.1% (wt/vol) bovine serum albumin, 100 U/ml penicillin, 100 U/ml streptomycin, and 0.5 mg/ml gentamycin for 90-95 min at 35 C in a 50-ml siliconized Bellco spinner flask. When dispersion was complete, we centrifuged the cells at 270 g for 5 min at room temperature. We resuspended cell pellets in culture medium (DMEM with 10% [vol/vol] charcoal-extracted FBS [Drouin and Labrie, 1976], 100 U/ml penicillin, 100 U/ml streptomycin, 40 U/ml mycostatin) and recentrifuged 4 times at 225 g. This procedure yielded an average of 2.24×10^6 cells per pituitary. After the final centrifugation, we counted the cells by hemocytometry (coefficient of variation = 9.3%) and resuspended the cells in the culture medium to a concentration of 2.7×10^5 cells/ml. We pipetted the cell suspension into plastic 16-mm culture plates at 1.5

ml/plate (4.0×10^5 cells per plate), which yielded a subconfluent culture, and incubated at 37 C in a water-saturated atmosphere of 95% air-5% CO_2. The techniques we used for the pituitary cell culture are standard (Hymer et al., 1973; Goodman, 1984).

We determined cell viability by performing the trypan blue-dye exclusion test. We added 0.1 ml trypan blue (4 mg/ml trypan blue, 0.14 M NaCl, 3.44 mM K_2HPO_4, and 3.29 mM methyl-para-hydroxybenzoate, pH 7.2) and 0.9 ml DMEM to the cells, incubated for 5 minutes, and then counted the cells to determine the percentage of non-viable (blue-stained) cells (Colowick and Kaplan, 1979). We determined that the cells were, on average, 99.6% viable.

We determined cell attachment to the plate by counting, with a hemocytometer, the number of cells discarded with the medium and wash the first time we changed the medium. This count allowed us to determine the percent attachment to the plates. We determined that an average of 93.0 to 99.6% of the cells were attached to the plates.

Experimental Procedure I - Short-Term Incubation

Since both negative and positive feedback of LH and FSH were observed in vivo it would be beneficial to

develop a parallel in vitro model in rats. Both negative and positive feedback may be occurring at the level of the pituitary with duration of the pituitary's exposure to the steroids determining which effect is manifest (Emons et al, 1986). In our short-term incubation, we used a 6 h incubation period of steroids with cells as opposed to longer, 24 to 48 h, incubation periods conventionally used in past studies.

We incubated the cells for 48 h after pipetting the cell suspension. Then we discarded this medium and rinsed each plate with DMEM before addition of 960 ul medium [DMEM with 2% (vol/vol) charcoal-extracted FBS, 40 U/ml mycostatin, 100 U/ml penicillin, 100 U/ml streptomycin, 90 U/ml bacitracin] (Savoy-Moore et al., 1980a). We added E_2 (0, 10^{-10} or 10^{-8} M) and B (0, 10^{-8} or 10^{-6} M) plus GnRH (10^{-11} to 10^{-7} M) to duplicate plates (Fig. 2). All of these concentrations are within the physiological range (Sarkar et al., 1976; Baldwin, 1979; Barraclough et al., 1981; Cohen and Mann, 1981; Ringstrom and Schwartz, 1984). Our four major groups of steroid treatments included: 1) control, no steroids, 2) E_2 doses alone, 3) B doses alone, or 4) combinations of E_2 and B doses incubated together with the cells. We incubated each of these groups in both the presence and absence of GnRH using a 3 X 3 X 6 factorial as a model. Control plates received medium with 0.1% ethanol alone.

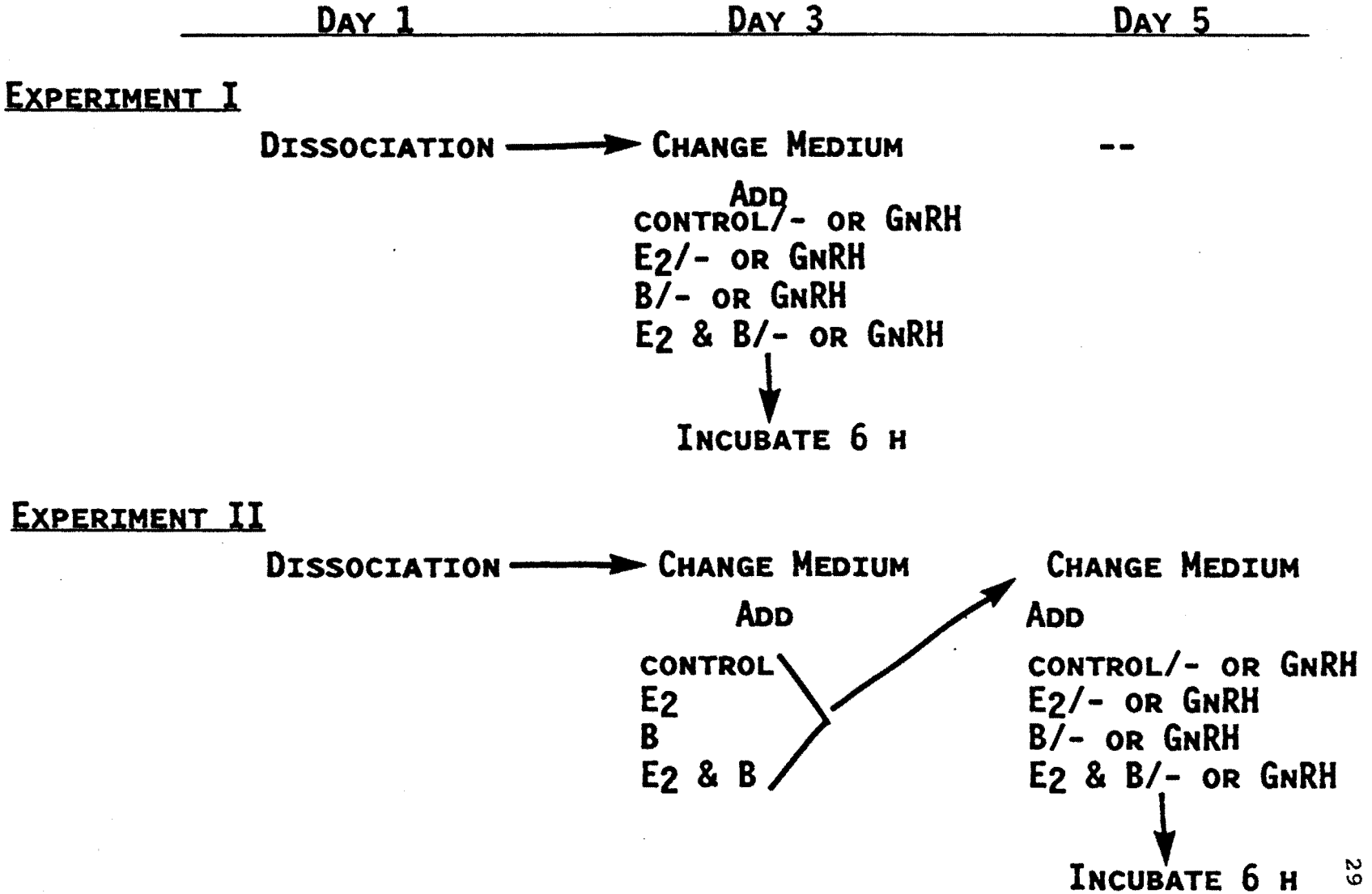
After 6 h, we saved the media at -20 C in separate vials. We then added 1 ml carbonate buffer (0.05 M Na_2CO_3, 2 M EDTA, 45,000 U/g bacitracin, pH 8.5) to each plate of cells and then froze and thawed them twice to disrupt the cells (Kamel and Kubajak, 1987). We then saved the cell contents separately for radioimmunoassay (RIA).

After RIA, we added the amount of gonadotropin secreted into the medium to the amount remaining in the cells (cellular) to determine total LH and FSH in the system. The resulting curve could be statistically compared to other totals which received different steroid treatments. This comparison could be used to determine the effect of steroids on the system as a whole. The total value is an important tool used to determine if the cells were just secreting more or less hormone or if a treatment also affects the stored amount of gonadotropin present. If cells secrete less gonadotropin it is possible that they are storing the amount inside the cell that would have normally been secreted. By examination of total we could determine if secretion was the only factor affected or if a treatment also affected gonadotropin synthesis. We replicated the entire experiment three times (Fig. 2).

Figure 2: Incubation procedure for Experiments I and II

The top line represents Experiment I, the short-term (6 h) incubation. On day 1 we dissociated cells and incubated them for 48 h with no steroids. On day 3 we changed the medium. Then we added steroids in four major groups: 1) control, (no steroids), 2) E_2 doses alone, 3) B doses alone, or 4) E_2 and B, either in the presence or absence of GnRH and incubated for 6 h before collecting medium and cells for RIA.

The bottom line represents Experiment II, the long-term (48 h) incubation. On day 1 we dissociated cells and incubated them for 48 h with no steroids. On day 3 we changed the medium and added steroids (E_2 and B) in each of the 4 groups described in Experiment I, but no GnRH. On day 5 we again changed medium, added steroids in each of the four groups and GnRH. We then incubated the cells for 6 h before collecting medium and cells for RIA.


DAY 1
DAY 3
DAY 5
EXPERIMENT I
DISSOCIATION
CHANGE MEDIUM
--
ADD
CONTROL/- OR GnRH
E2/- OR GnRH
B/- OR GnRH
E2 & B/- OR GnRH
INCUBATE 6 H
EXPERIMENT II
DISSOCIATION
CHANGE MEDIUM
ADD
CONTROL
E2
B
E2 & B
CHANGE MEDIUM
ADD
CONTROL/- OR GnRH
E2/- OR GnRH
B/- OR GnRH
E2 & B/- OR GnRH
INCUBATE 6 H

Experimental Procedure II - Long-Term Incubation

Negative and positive feedback are both important factors in regulation of LH and FSH. To test if length of incubation of cells with steroids determines the manifest effect, we performed an experiment with an incubation time that was 48 h longer than the previous 6 h (short-term) incubation of Experiment I.

We incubated the cells for 48 h after pipetting the suspension. We then discarded the medium as in Experiment I. We added E_2 and B in the same concentrations as in the previous experiment, but did not add GnRH in the first incubation. The reasons we did not add GnRH during the first incubation were 2-fold: 1) to prevent desensitization of the cells to GnRH and 2) because our primary interest was not on effects of GnRH, but on the effects of E_2 and B. We incubated 48 h longer, then discarded the medium (in all but 2 plates, which were separated into cells and medium and frozen for RIA to determine net amounts of cellular LH and FSH at time 0) and rinsed the plates with DMEM before the addition of fresh medium containing E_2, B, and GnRH in the same concentrations as in Experiment I (Fig. 2). Concentrations of E_2 and B in the fresh medium were always the same as those with which the plate was

pretreated. We incubated cells with steroids and GnRH for 6 h before removal of medium and cells for RIA.

We removed and saved the medium and cell contents in separate containers for radioimmunoassay as in Experiment I. We replicated the entire experiment three times.

Experimental Procedures III & IV

We followed the procedure for (I) short-term incubation and (II) long-term incubation with one exception: we used progesterone (P_4) (2×10^{-8} or 1×10^{-7} M) (Barraclough et al., 1979; Savoy-Moore et al., 1980 b) in place of E_2. All other aspects remained constant for both incubation times (Fig. 3). We added 0.2% ethanol to the control plates that did not receive the steroid treatment.

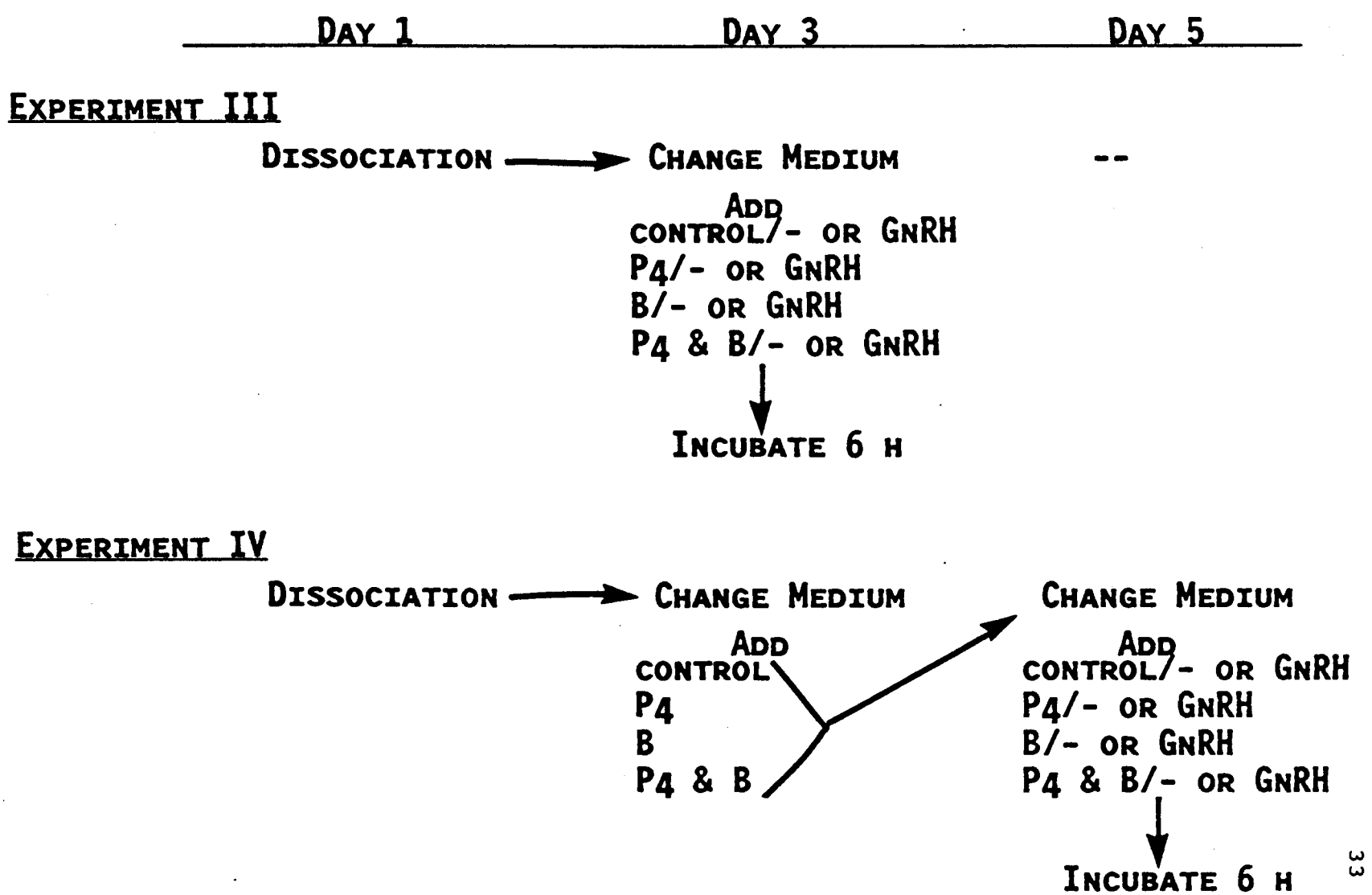
Radioimmunoassay

We assayed concentrations of LH and FSH in media and cell lysates according to standard procedures using reagents obtained from the National Hormone and Pituitary Program, with the exception of iodination-grade LH, with which Dr. Leo E. Reichert, Jr., of Albany Medical College, generously provided us. We used NIH-rLH-RP-2

Figure 3: Incubation procedure for Experiments III and IV

The top line represents Experiment III, the short-term (6 h) incubation. On day 1 we dissociated cells and incubated them for 48 h with no steroids. On day 3 we changed the medium. Then we added steroids in four major groups: 1) control, (no steroids), 2) P_4 doses alone, 3) B doses alone, or 4) P_4 and B, either in the presence or absence of GnRH and incubated for 6 h before collecting medium and cells for RIA.

The bottom line represents Experiment IV, the long-term (48 h) incubation. On day 1 we dissociated cells and incubated them for 48 h with no steroids. On day 3 we changed the medium and added steroids (P_4 and B) describe in Experiment III, but no GnRH. On day 5 we again changed medium, added the four groups of steroids and GNRH. We then incubated the cells for 6 h before collecting medium and cells for RIA.


DAY 1
DAY 3
DAY 5
EXPERIMENT III
DISSOCIATION
CHANGE MEDIUM
--
ADD
CONTROL/- OR GNRH
P4/- OR GNRH
B/- OR GNRH
P4 & B/- OR GNRH
INCUBATE 6 H
EXPERIMENT IV
DISSOCIATION
CHANGE MEDIUM
ADD
CONTROL
P4
B
P4 & B
CHANGE MEDIUM
ADD
CONTROL/- OR GNRH
P4/- OR GNRH
B/- OR GNRH
P4 & B/- OR GNRH
INCUBATE 6 H

and NIH-rFSH-RP-2 as standards. We radioiodinated LH and FSH by the chloramine T method. In our hands the rLH assay had a sensitivity of 0.06 ng RP-2/tube and the rFSH assay had a sensitivity of 0.62 ng RP-2/tube [at that point on the standard curve where the bound radioactive hormone in the tube containing a known standard amount of hormone is 85% of the bound radioactive hormone in the buffer control tube (B/B_0)]. The intra- and interassay coefficients of variation were 6.0% and 34.4% for LH and 5.8% and 25.4% for FSH. Because incubation medium alone caused a slight suppression of binding in both assays, all tubes within an assay received the same volume of medium. We diluted standards in unincubated medium. This procedure yielded standard curves identical to standards incubated without medium. The techniques we used for radioimmunoassay were standard.

We measured basal and GnRH-stimulated levels of LH and FSH as the amount secreted into the medium and the amount remaining in the cells. By adding the amount of each gonadotropin secreted into the medium to the amount remaining in the cells we determined the total amount of hormone for each sample. We could then determine if the total amount of each gonadotropin in the system was increased or decreased or if secretion was the only factor affected.

Statistics

We used Systat to perform a four-way analysis of variance on basal levels of gonadotropin and analysis of co-variance on GnRH dose-response curves for each treatment group. We also performed a one-way analysis of variance on total amounts of gonadotropins at the beginning of incubation and after 6 h of incubation. We calculated the standard error of the mean (S.E.M.) from the error mean square of the analysis of variance for basal values and from the error mean squared of the analysis of co-variance for the GnRH dose-response curves.

RATIONAL

These experiments allowed us to determine if corticosterone interacted with gonadal steroids and to elucidate the time frame needed for this interaction. The steroids may enhance, inhibit, or have no effect on the others' actions on the pituitary. The interactions between the steroids should have one of the following effects: an additive effect, where the effect of the interaction of the two is equal to the sum of each alone; a synergism, where the action of the two steroids together is greater than the sum of each steroid alone; an antagonism, where the action of each steroid is opposite; or neither of these, because there may be no interaction between the two steroids. If the two together act the same as either one alone, then this may indicate that the two steroids act through a common pathway.

The results of this work should provide additional understanding of the role of stress in reproductive dysfunction through clarification of the interaction of the steroids on the pituitary's ability to synthesize and secrete the gonadotropins luteinizing hormone (LH) and

follicle-stimulating hormone (FSH).

RESULTS OF EXPERIMENTS EMPLOYING ESTRADIOL

EXPERIMENT I [TREATMENT OF CELLS WITH E_2 AND B FOR 6 h]

For clarity, only the effects of the high dose of each steroid are shown on most graphs. Unless otherwise stated, the effects of the low dose of steroids was not different from control values. Values for all data can be found in the Appendix.

Comparison Between Amounts of Gonadotropin in the System Before and After 6 h of Incubation - Experiment I

To determine if 6 h of incubation of cells with steroids affected gonadotropin levels in the system, we measured the amount of gonadotropins present in the system before and after 6 h of incubation. Before 6 h of incubation, the cells (cellular) contained the only gonadotropins present in the system. After 6 h of incubation the medium contained secreted gonadotropins and the cells themselves also contained gonadotropins. To compare net amounts of gonadotropin we used cellular levels before 6 h of incubation (time 0) and secreted + cellular levels after 6 h of incubation to determine net

levels of gonadotropin. In Experiment I, 6 h of incubation did not alter the net amount of LH or FSH present in the system in the absence of GnRH relative to the amount of LH or FSH present before incubation, 6 h earlier (Fig. 4).

Effects of Steroids on Basal Levels of LH and FSH

After treating cells for 6 h with steroids with no GnRH present (basal), neither E_2 nor B alone had any effect on secreted, cellular, or total LH. Although neither steroid altered levels of LH relative to control when we incubated these two steroids separately, they did have an inhibitory effect when presented together. When we incubated E_2 and B together they decreased secreted LH by 27.2% ($P = 0.046$) (Fig. 5).

Both concentrations of E_2 decreased basal levels of cellular and total FSH ($P = 0.005$), but had no effect on secreted FSH (Fig. 6). The low dose of E_2 (10^{-10} M) decreased basal cellular FSH by 33.0% and total FSH by 29.2% (Fig. 6). The high dose of E_2 (10^{-8} M) decreased cellular FSH by 46.1% and total FSH by 39.1% ($P = 0.005$) (Figs. 5 & 6). B, conversely, had a stimulatory effect on secreted FSH. The high dose of B alone (10^{-6} M) increased basal secretion of FSH by 34.1% ($P = 0.024$), but had no effect on cellular or total FSH (Fig. 7).

Figure 4: Experiment I Net Basal Time 0 vs after 6 h

We measured total amounts of LH (top) and FSH (bottom) present in the system both before and after the first 6 h of the 48 h pre-incubation of Experiment II in the absence of GnRH. This incubation corresponded to Experiment I. Time 0 represents the period before the first 6 h of the 48 h incubation. We graphed the amount of total gonadotropin present in the system at time 0 next to the amount of gonadotropin present after 6 h for each dose of E_2 incubated in the presence or absence of B. We did not introduce steroids into the system until after time 0. We calculated error bars from the analysis of variance. Each bar represents the mean $\pm$ SEM of 3 cell cultures.

E_2: 0 = no steroids; 1 = 10^{-10} M; 2 = 10^{-8} M

B: 0 = no steroids; 1 = 10^{-8} M; 2 = 10^{-6} M

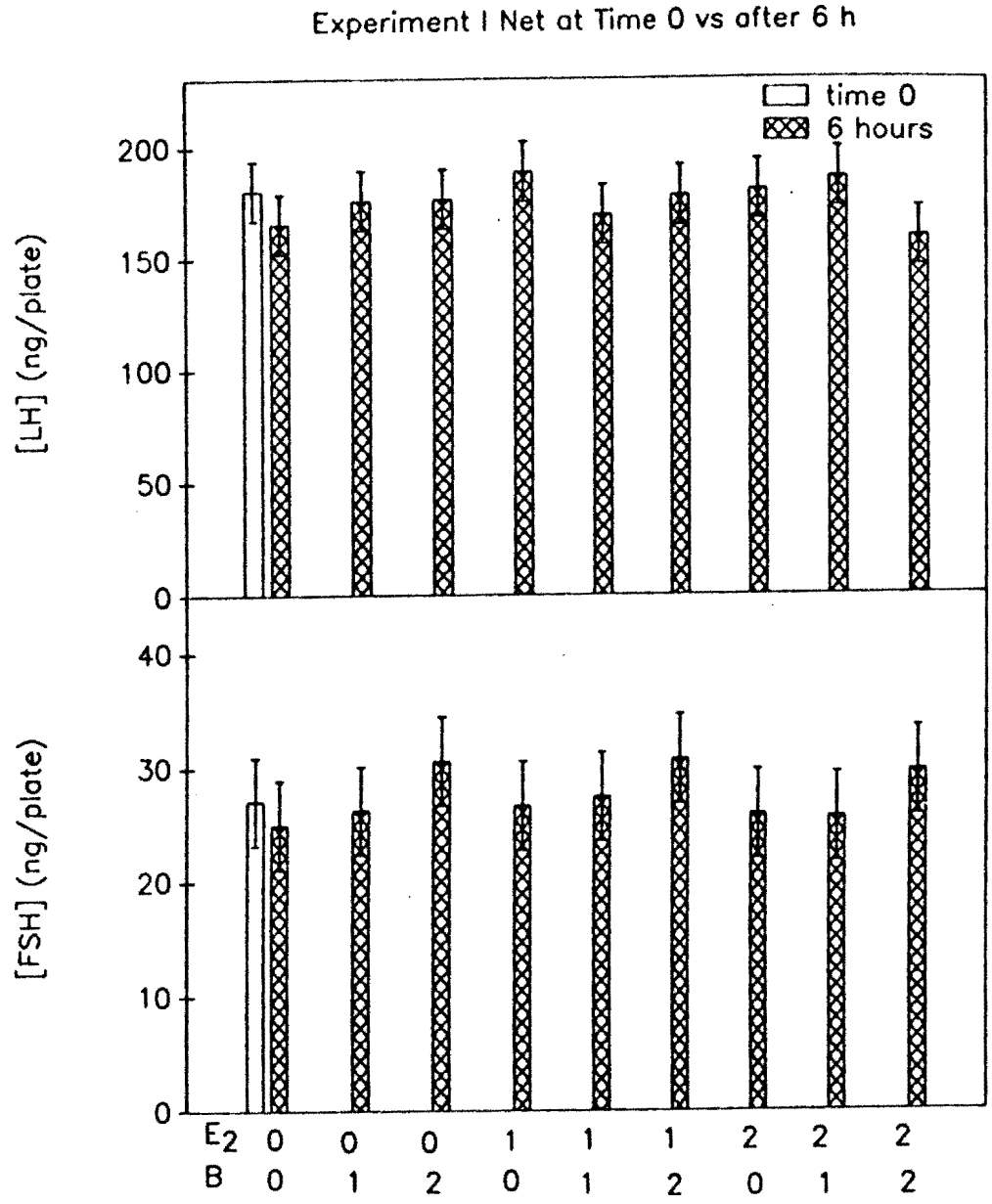

Experiment I Net at Time 0 vs after 6 h
time 0
6 hours
[LH] (ng/plate)
200
150
100
50
0
[FSH] (ng/plate)
40
30
20
10
0
E2 0 0 0 1 1 1 2 2 2
B 0 1 2 0 1 2 0 1 2

Figure 5: Experiment I Basal 6 h incubation

Effects of E_2 and B on basal secreted, cellular and total LH (top) and FSH (bottom) after 6 h of incubation in Experiment I. Control contains ethanol only. E_2 (10^{-8} M) and B (10^{-6} M) shown are high concentrations. E_2 + B represents the high concentration of each steroid incubated together. Bars represent mean $\pm$ SEM of 3 cell cultures. The * symbolizes significant differences relative to control.

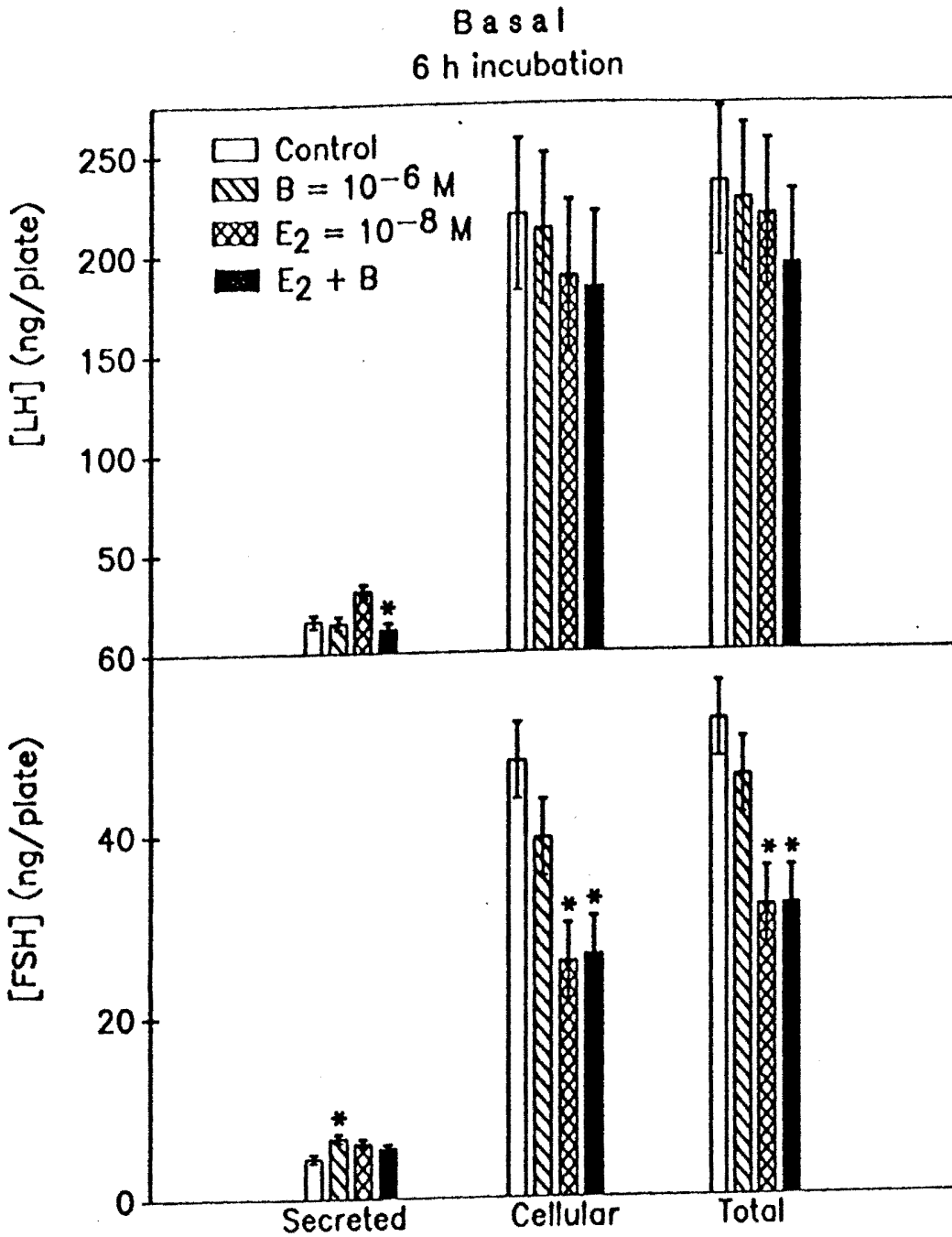

Basal
6 h incubation
Control
B = 10⁻⁶ M
E2 = 10⁻⁸ M
E2 + B
[LH] (ng/plate)
250
200
150
100
50
60
[FSH] (ng/plate)
40
20
0
Secreted
Cellular
Total

Figure 6: Experiment I. Basal FSH resulting from incubation with E_2.

Effects of E_2 on basal secreted, cellular and total levels of FSH after 6 h of incubation with E_2 in Experiment I. E_2 = 0 contains ethanol, but no estradiol. E_2 = 10^{-10} M represents the low dose and 10^{-8} M represents the high dose of E_2. The bars represent mean $\pm$ SEM of 3 cell cultures. The * symbolizes significant differences relative to control.

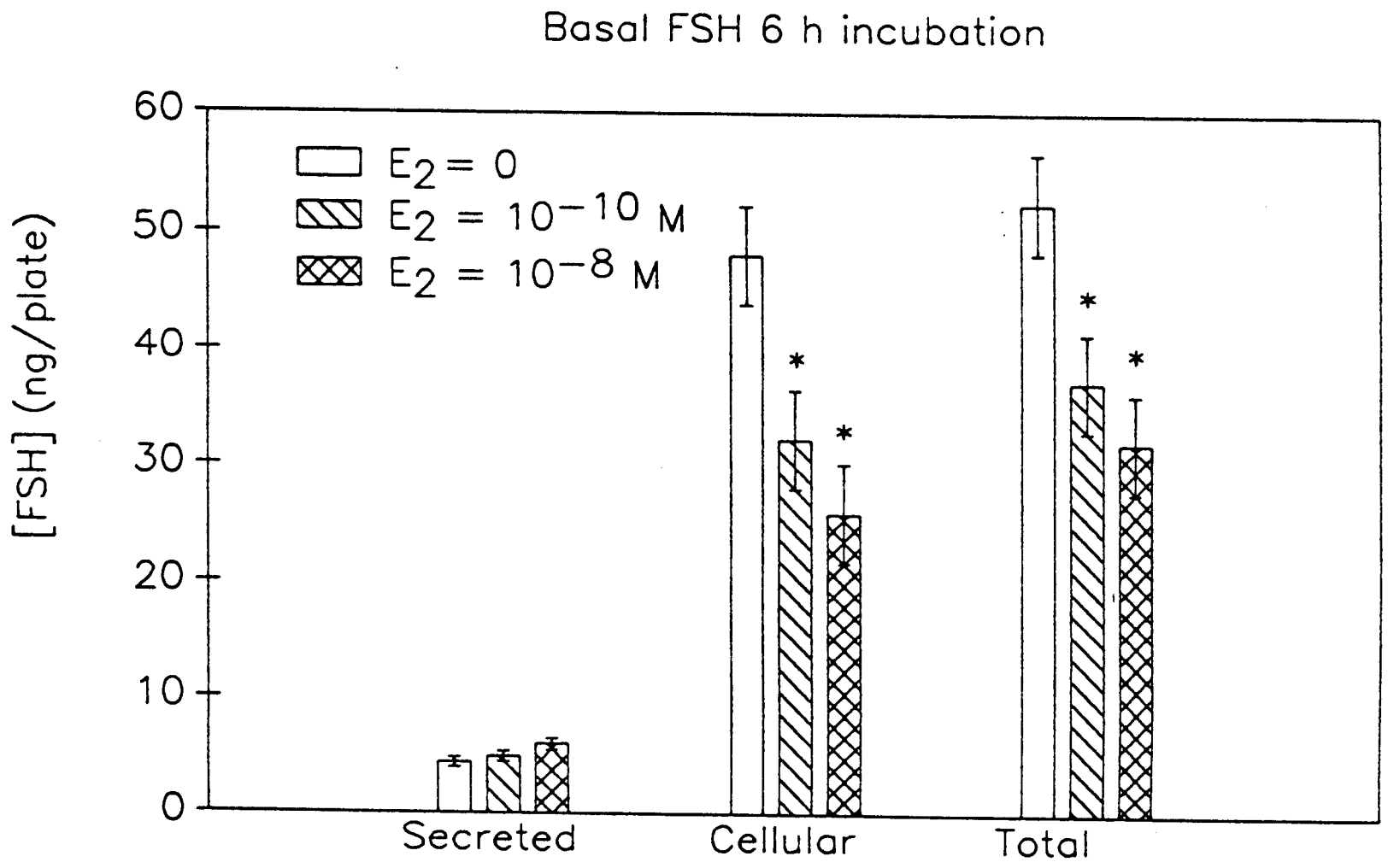
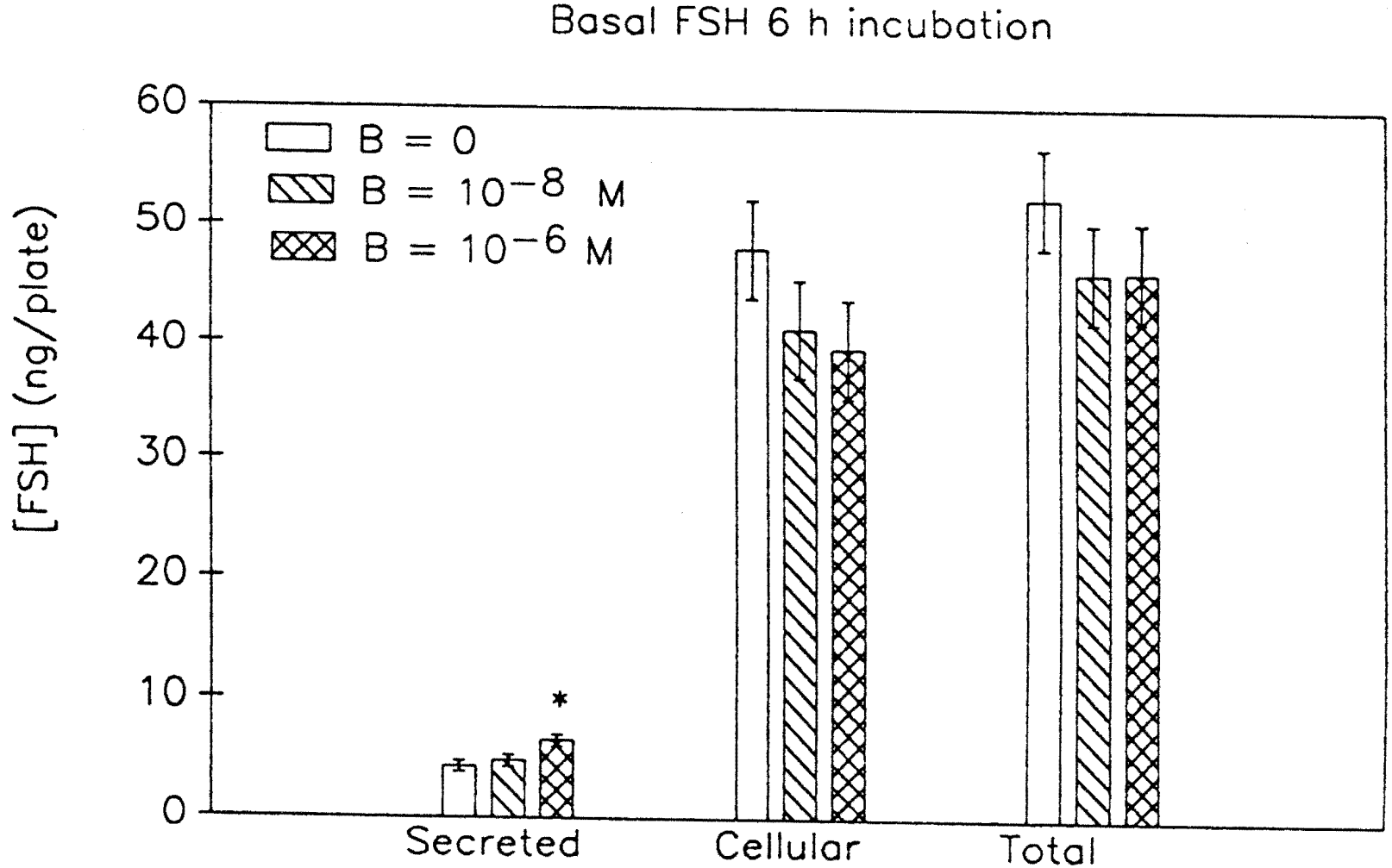

Basal FSH 6 h incubation
[FSH] (ng/plate)
60
50
40
30
20
10
0
$E_2 = 0$
$E_2 = 10^{-10}$ M
$E_2 = 10^{-8}$ M
*
*
*
*
Secreted
Cellular
Total

Figure 7: Experiment I. Basal FSH resulting from incubation with B.

Effects of B on basal secreted, cellular and total levels of FSH after 6 h of incubation with B in Experiment I. B = 0 contains ethanol, but no corticosterone. B = 10^{-8} M represents the low dose and 10^{-6} M represents the high dose of B. Bars represent mean $\pm$ SEM of 3 cell cultures. The * symbolizes significant differences relative to control.


Basal FSH 6 h incubation
[FSH] (ng/plate)
60
50
40
30
20
10
0
B = 0
B = 10⁻⁸ M
B = 10⁻⁶ M
*
Secreted
Cellular
Total

Unlike their action on LH, when we incubated E_2 and B together, E_2 blocked the stimulatory effect that B exerted on secreted FSH when incubated with cells in the absence of other steroids (Fig. 5).

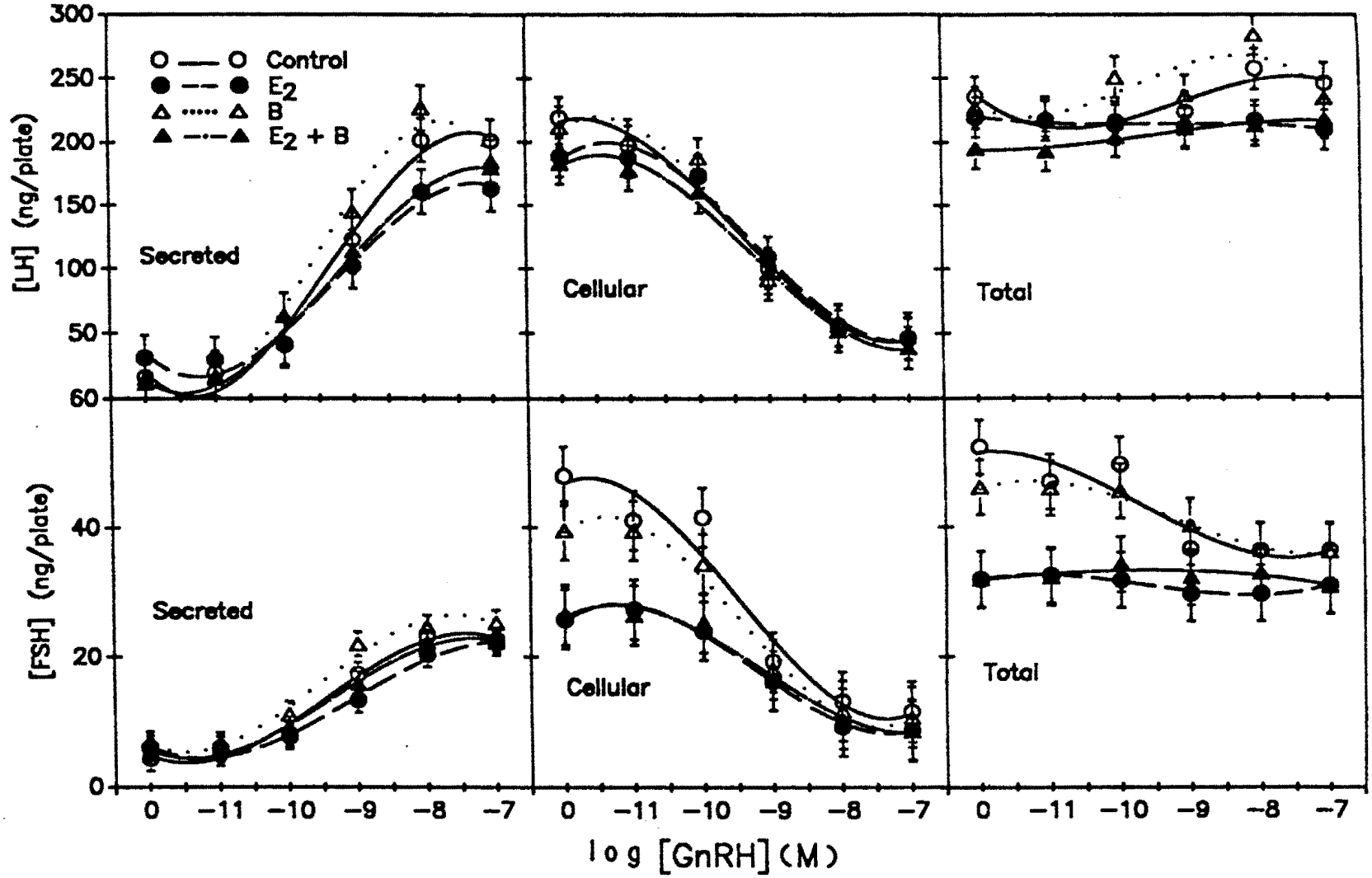
Effects of Steroids on GnRH-stimulated Levels of LH and FSH

After incubation for 6 h, GnRH enhanced secreted LH and decreased cellular LH ($P < 0.001$). As the concentration of GnRH increased, secreted LH increased and cellular LH decreased proportionally ($P < 0.001$). GnRH did not affect total LH (Fig. 8).

When we presented steroids with GnRH for 6 h we found that: 1) E_2 inhibited both LH and FSH, and 2) B stimulated FSH, but it did not affect LH. Both concentrations of E_2 reduced total LH by 12%, cellular FSH by 31%, and total FSH by 24% ($P < 0.001$). E_2 alone did not significantly change secreted LH or FSH or cellular LH from control. B had no effect on LH, but B (10^{-6} M) increased secreted FSH by 13% ($P < 0.001$) (Fig. 8). The actions of one steroid had no effect on the other during the 6 h incubation.

Figure 8: Experiment I (6 h incubation)

Quantities of LH (top) and FSH (bottom) in medium (left panel), in cells (middle panel), and of total content (cells + medium; right panel) in response to treatment with GnRH and steroids for 6 h. E_2 represents the high dose only (1×10^{-8} M), B represents the high dose only (1×10^{-6} M), E_2 + B represents the high dose of E_2 and B together. For simplicity only values obtained from incubation with high doses of steroids are shown on this graph. (Data for low doses of steroids can be found in the Appendix.) We calculated error bars from the analysis of co-variance. Points represent mean $\pm$ SEM of 3 cell cultures after the 6 h incubation only.


Control
E2
B
E2 + B
Secreted
Cellular
Total
[LH] (ng/plate)
[FSH] (ng/plate)
300
250
200
150
100
50
60
40
20
0
0 −11 −10 −9 −8 −7
log [GnRH] (M)

EXPERIMENT II [TREATMENT OF CELLS WITH E_2 AND B FOR 48 + 6 h]

Comparison Between Amounts of Gonadotropin in the System Before and After 6 h of Incubation - Experiment II

To determine if long-term incubation of cells with steroids affected net gonadotropin levels in the system, we again measured the net amount of gonadotropins present in the system before and after the final 6 h of incubation as described in Experiment I. To compare net amounts of gonadotropin we again used cellular levels at time 0 and secreted + cellular to determine net levels of gonadotropin after 6 h of incubation. In Experiment II, time 0 was on day 5, immediately following the second 48 h pre-incubation.

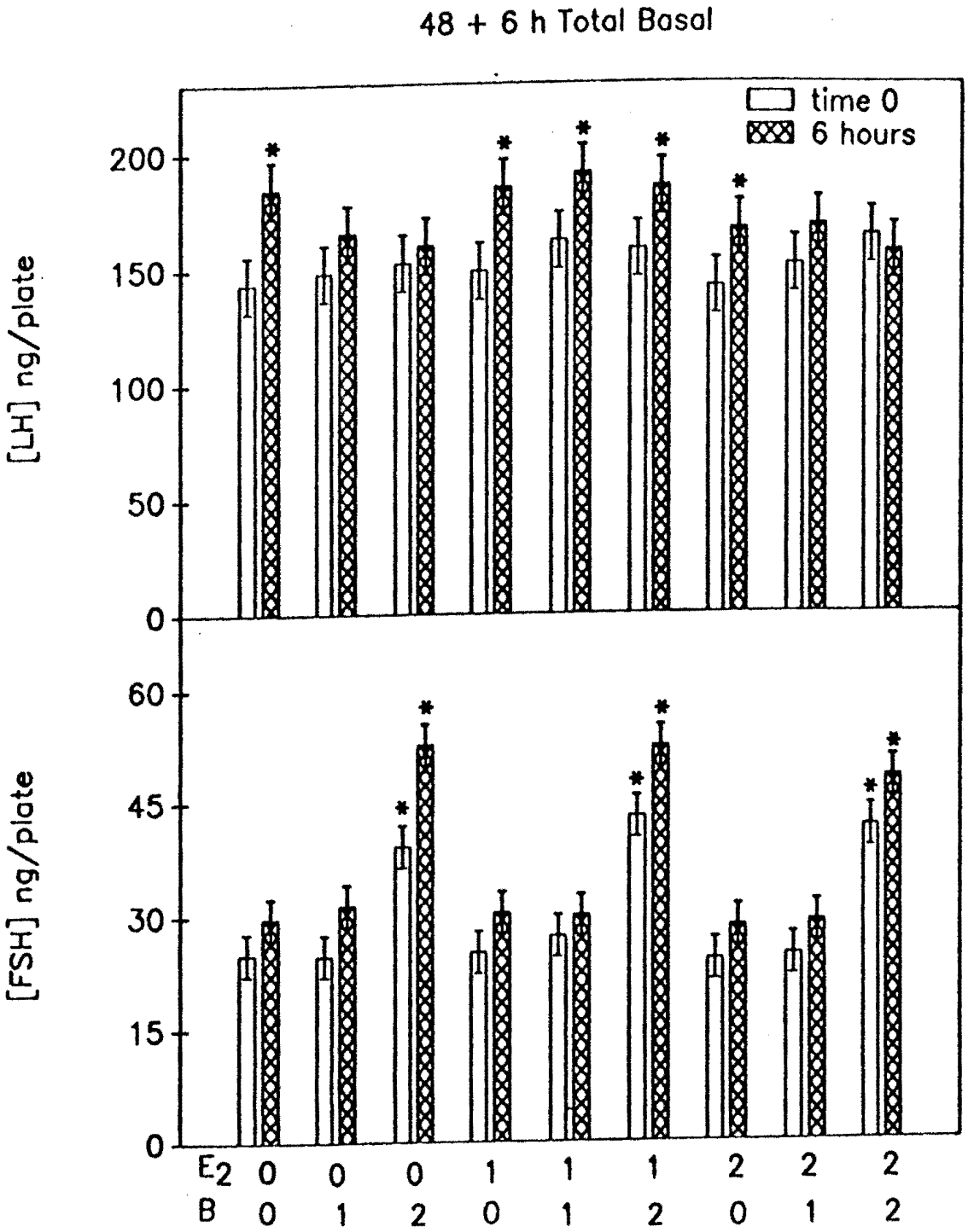
In Experiment II, net LH in the system increased after 6 h of incubation ($P < 0.001$) (Fig. 9). Steroidal treatment did not significantly alter this increase observed over time according to an analysis of variance. When we compared net FSH present in the system at time 0 and after 6 h of incubation we also found an increase in the amount of net FSH after 6 h ($P < 0.001$). Some effects on FSH were different from LH, however. We observed an effect of B for FSH not present for LH. When

Figure 9: Experiment II. Net Basal FSH from Time 0 and after 6 h.

We measured total amounts of LH (top) and FSH (bottom) present in the system both before and after the final 6 h incubation period of Experiment II in the absence of GnRH. Time 0 represents the period after the initial 48 h incubation, but before the 6 h incubation. We graphed the amount of total gonadotropin present in the system after 6 h next to the amount of gonadotropin present at time 0 for each dose of E_2 incubated in the presence or absence of B. We calculated error bars from the error mean squared from the analysis of variance. Each bar represents the mean $\pm$ SEM of 3 cell cultures. The * symbolizes significant differences between the treatment group and control (E_2 = 0 and B = 0 at time 0).

E: 0 = no steroids; 1 = 10^{-10} M; 2 = 10^{-8} M

B: 0 = no steroids; 1 = 10^{-8} M; 2 = 10^{-6} M


48 + 6 h Total Basal
time 0
6 hours
[LH] ng/plate
200
150
100
50
0
[FSH] ng/plate
60
45
30
15
0
E2 0 0 0 1 1 1 2 2 2
B 0 1 2 0 1 2 0 1 2

we incubated cells with a high dose of B (10^{-6} M) net levels of FSH in the system increased after 6 h ($P < 0.001$) relative to control at time 0, due to the effects of B. E_2 did not block the increase caused by B (Fig. 9).

Effects on Basal LH

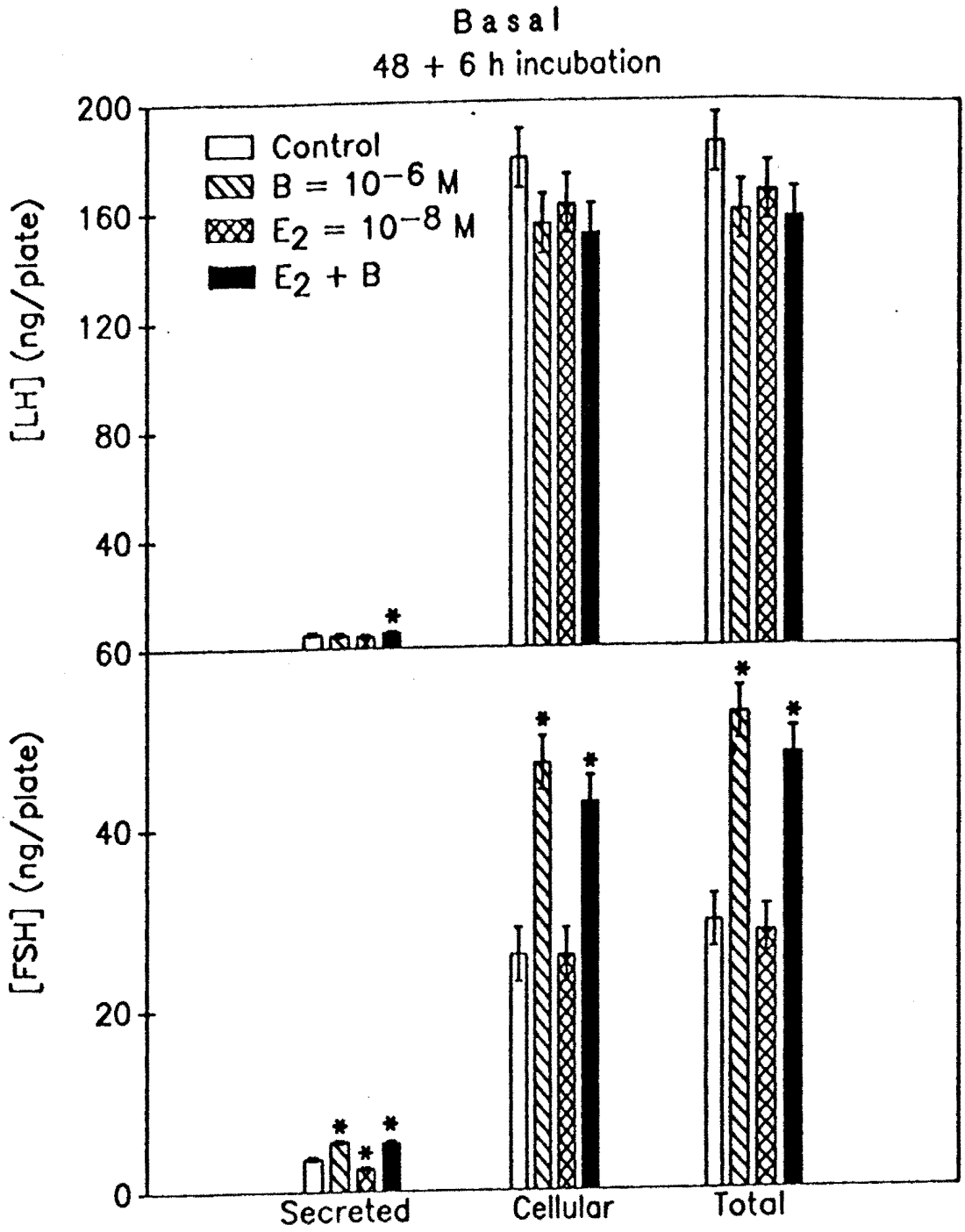
After incubating cells with steroids for 48 h, and then for another 6 h with steroids (hereafter referred to as 48 h) in the absence of GnRH, neither E_2 nor B alone affected basal secretion of LH. Together E_2 and B decreased basal secreted LH, but only by 5% ($P = 0.016$) (Fig. 10). We also observed that this suppression of secreted LH occurred only when we incubated E_2 and B together as in Experiment I.

Effects on Basal FSH

As in Experiment I (6 h incubation), E_2 inhibited and B stimulated basal secretion of FSH. After 48 h of incubation, E_2 (10^{-8} M) alone decreased by 30.5% and B (10^{-6} M) alone increased by 50.6% basal secretion of FSH ($P < 0.001$). E_2 partially blocked the stimulatory effect of B on basal secretion. When we presented E_2 and B together, B increased basal secretion of FSH by only

Figure 10: Experiment II. Basal incubation

Effects of E_2 and B on basal secreted, cellular and total levels of LH (top) and FSH (bottom) after long-term (48 h) incubation in Experiment II. Control contained ethanol only. E_2 (10^{-8} M) and B (10^{-6} M) shown are high concentrations. E_2 + B represent high concentrations of each steroid. Bars represent mean $\pm$ SEM of 3 cell cultures. The * symbolizes significant differences relative to control.


Basal
48 + 6 h incubation
Control
B = 10⁻⁶ M
E2 = 10⁻⁸ M
E2 + B
[LH] (ng/plate)
[FSH] (ng/plate)
200
160
120
80
40
60
40
20
0
Secreted
Cellular
Total

47.5% when incubated with E_2 ($P = 0.003$ for the interaction between E_2 and B). B also increased basal cellular FSH by 81.5% and total FSH by 77.7% ($P < 0.001$), but E_2 did not block these stimulatory effects as it did with secreted FSH (Fig. 10).

Effects on GnRH-stimulated LH

GnRH alone affected secreted, cellular, and total concentrations of LH as in Experiment I. GnRH increased secreted LH, decreased cellular LH, and did not affect total levels of LH.

B alone decreased GnRH-stimulated secretion of LH by 8.7% with 10^{-8} M B (data not shown) and by 17.9% with 10^{-6} M B ($P = 0.004$) (Fig. 11). When we incubated cells with E_2 no significant effect resulted for secreted LH relative to control. Treatment with steroids did not affect cellular or total LH.

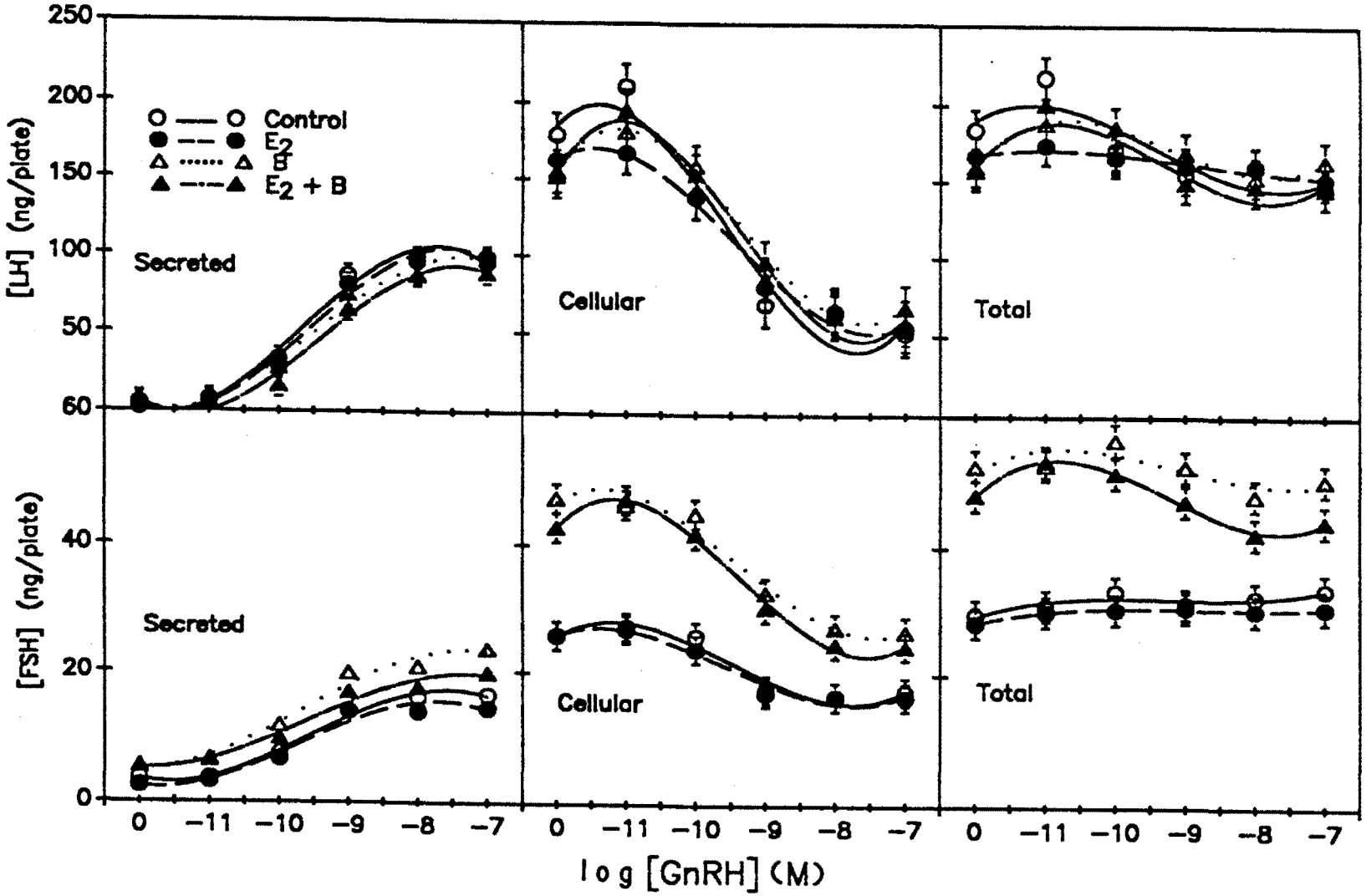
Effects on GnRH-stimulated FSH

Increasing concentrations of GnRH alone increased secreted and decreased cellular FSH ($P < 0.001$), but had no effect on total amounts of FSH. GnRH affected FSH as it affected LH (Fig. 11).

E_2 alone had no effect on responsiveness of FSH to

Figure 11 Experiment II (48 h incubation)

Quantities of LH (top) and FSH (bottom) in medium (left panel), in cells (middle panel), and of total content (cells + medium; right panel) in response to treatment with steroids for 48 h, then steroids + GnRH for 6 h. E_2 (10^{-8} M) represents the high dose only, B (10^{-6} M) represents the high dose only, and E_2 + B represents the high dose of E_2 and B together. Points represent mean $\pm$ SEM of 3 cell cultures after the final 6 h incubation only. We calculated error bars from the analysis of co-variance.


250
200
150
100
50
60
[LH] (ng/plate)
Control
E2
B
E2 + B
Secreted
Cellular
Total
40
20
0
[FSH] (ng/plate)
Secreted
Cellular
Total
0 −11 −10 −9 −8 −7
log [GnRH] (M)

GnRH, and B alone increased the slope of the GnRH dose-response curve for secreted, cellular and total FSH ($P < 0.02$) (Fig. 11). Since B increased the slope of the GnRH dose-response curve, neither the effect of B as a main effect nor the interaction between E_2 and B could be determined by the analysis of covariance.

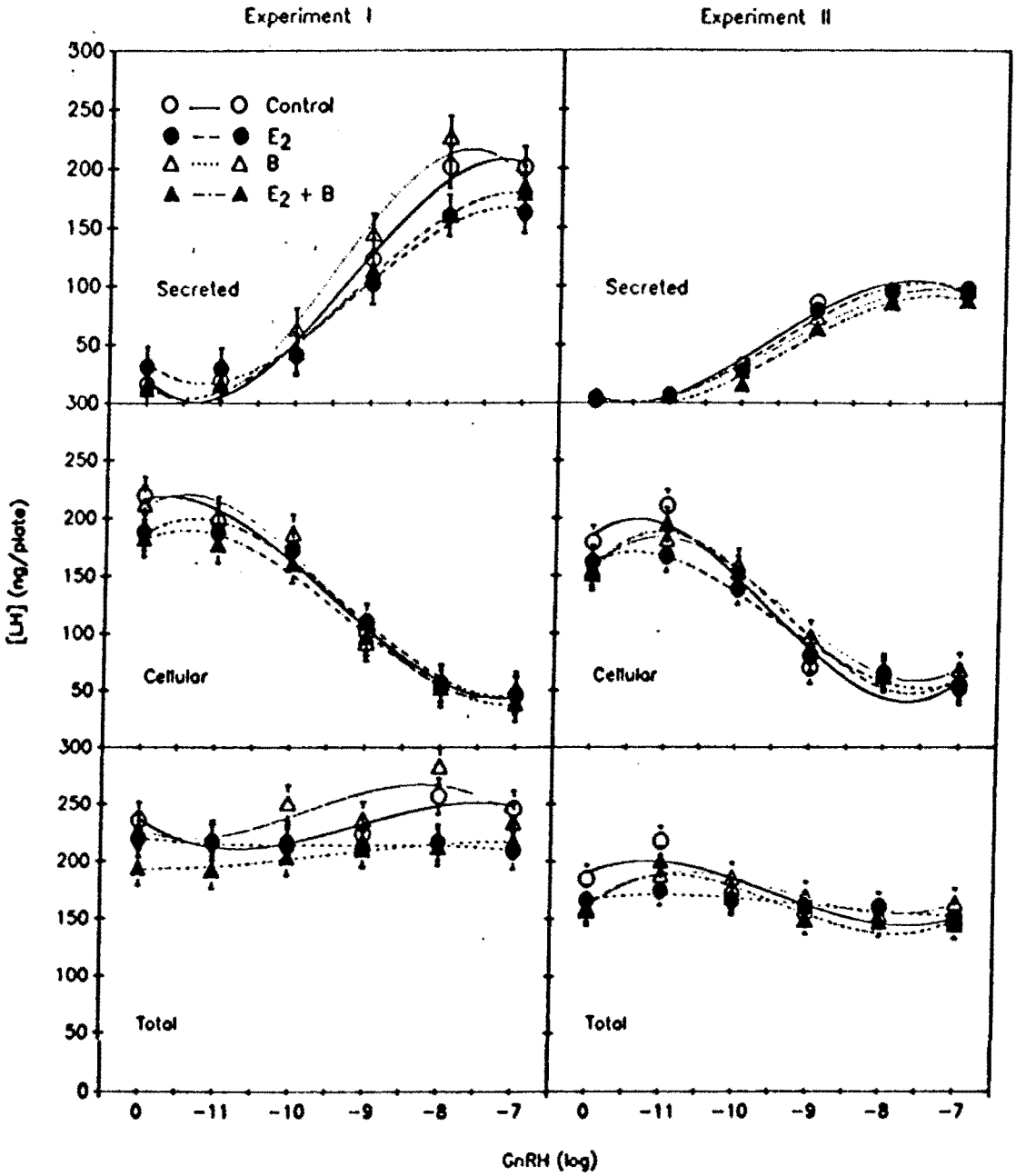
Comparison between Experiment I (6 h) and Experiment II (48 h)

We wanted to determine if a 48 h incubation of steroids with cells affected secretion, cell content or total gonadotropins differently from a shorter incubation. We compared the levels of gonadotropin resulting from each treatment in Experiment I (6 h) to those of Experiment II (48 + 6 h). After an incubation of 48 + 6 h in Experiment II, the amount of maximally secreted GnRH-stimulated LH decreased by 41.8% when compared to the 6 h incubation in Experiment I ($P < 0.001$). Total GnRH-stimulated LH increased by 22.3% in Experiment II relative to Experiment I ($P < 0.001$). The amount of GnRH-stimulated secreted LH ($P = 0.031$) and total LH ($P = 0.012$) increased in Experiment I compared to Experiment II (Fig. 12).

After a 48 h longer incubation used in Experiment II, GnRH-stimulated secreted FSH was decreased by 17.1%

Figure 12: GnRH-stimulated [LH] Experiment I vs Experiment II

Quantities of LH from Experiment I (left) and from Experiment II (right) in medium (top panels), in cells (middle panels), and of total content (cells + medium; bottom panels) in response to treatment with GnRH and steroids for 6 h. (We incubated cells in Experiment II with steroids for 48 h before the final 6 h incubation shown on this graph.) E_2 represents the high dose only (1 X 10^{-8} M), B represents the high dose only (1 X 10^{-6} M), E_2 + B represents the high dose of E_2 and B together. (Data for low doses of steroids can be found in the Appendix.) We calculated error bars from the analysis of co-variance. Points represent mean $\pm$ SEM of 3 cell cultures after the 6 h incubation only.

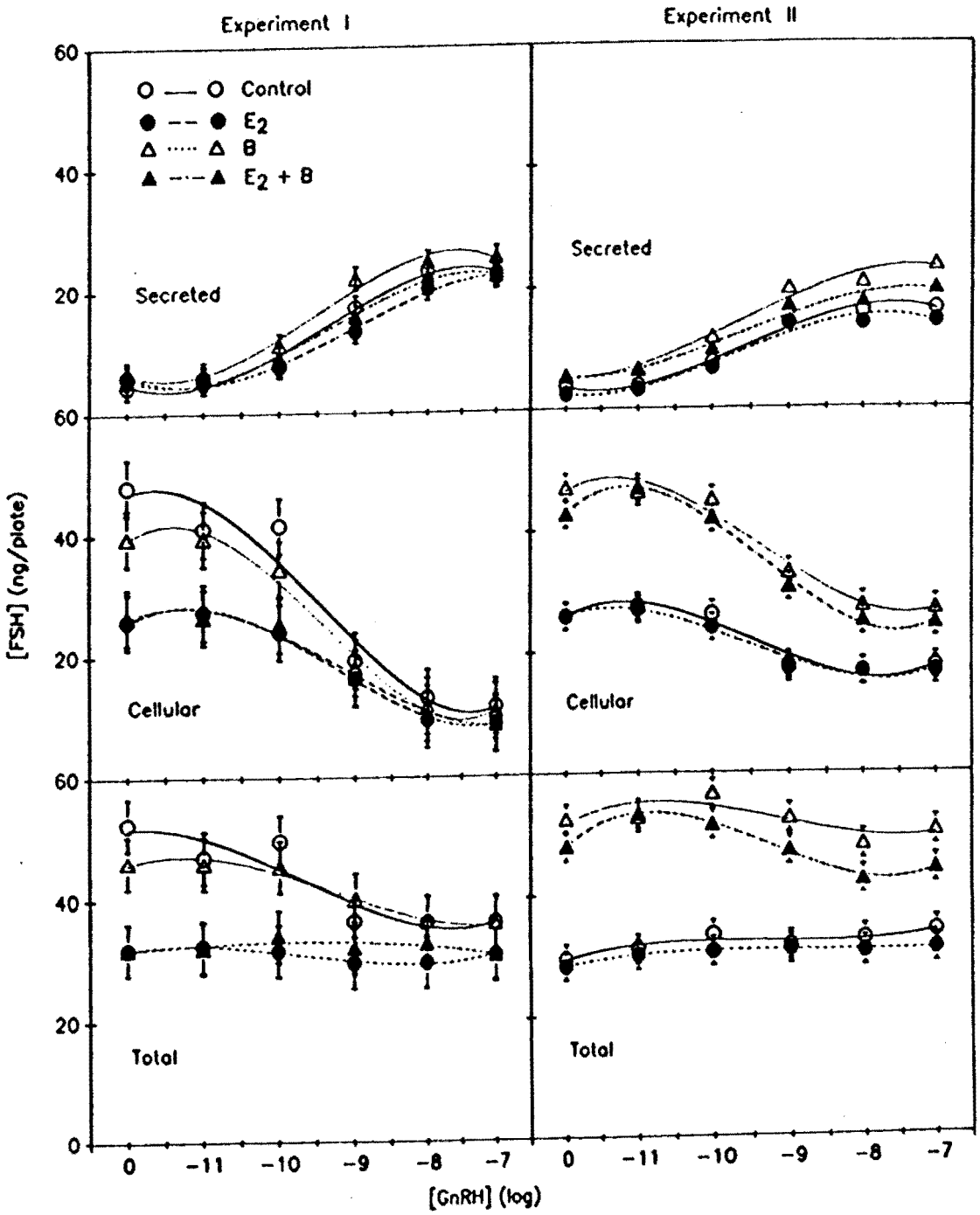

Experiment I
Experiment II
Control
E_2
B
E_2 + B
Secreted
Cellular
Total
[LH] (ng/plate)
GnRH (log)
300
250
200
150
100
50
0
-11
-10
-9
-8
-7

relative to a shorter incubation used in Experiment I as did LH. Cellular GnRH-stimulated FSH, however, increased when incubated 48 h longer in Experiment II. Cellular FSH increased by 18.0% relative to Experiment I ($P = 0.037$). After a 48 h longer incubation, B increased the amount of cellular and total GnRH-stimulated FSH ($P < 0.001$) in Experiment II when compared to Experiment I (Fig. 13).

B elevated secreted FSH in both time courses. In Experiment II (48 h incubation), E_2 blocked the stimulatory effects of B on total FSH. In the 6 h incubation, however, B continued to increase cellular and total FSH with no block by E_2 (Fig. 13). Since B changed the slope of the GnRH-dose response curve in the 48 h incubation, the effect of B as a main effect in the analysis of covariance could not be determined.

Figure 13 GnRH-stimulated [FSH] Experiment I vs Experiment II

Quantities of FSH from Experiment I (left) and from Experiment II (right) in medium (top panels), in cells (middle panels), and of total content (cells + medium; bottom panels) in response to treatment with GnRH and steroids for 6 h. (We incubated cells in Experiment II with steroids for 48 h before the final 6 h incubation shown on this graph.) E_2 represents the high dose only (1 X 10^{-8} M), B represents the high dose only (1 X 10^{-6} M), E_2 + B represents the high dose of E_2 and B together. (Data for low doses of steroids can be found in the Appendix.) Points represent mean $\pm$ SEM of 3 cell cultures after the 6 h incubation only. We calculated error bars from the analysis of co-variance.


Experiment I
Experiment II
Control
E2
B
E2 + B
Secreted
Cellular
Total
[FSH] (ng/plate)
[GnRH] (log)
60
40
20
0
0
-11
-10
-9
-8
-7

DISCUSSION

We first wanted to examine if E_2 exerted negative feedback effects on gonadotropins directly at the pituitary. Second, we wanted to determine if the length of time that we exposed cells to steroidal hormones determined the manifest effect. These experiments have demonstrated negative feedback of LH and FSH by E_2 in rat pituitaries. Furthermore, the negative feedback by E_2 seen in the short-term study (Fig. 8) was eliminated in the long-term study (Fig. 11), indicating that the duration of the pituitary's exposure to the steroids was a controlling factor in determining whether or not this effect was manifest (Figs. 12 and 13). Other studies have demonstrated suppression of GnRH-stimulated secretion of LH by E_2 (2.72 ng/ml) after a 4-hour incubation in monolayer cultures (Tang and Spies, 1975; Emons et al., 1986). Scientists who superfused anterior pituitaries with GnRH and E_2 also observed negative feedback of LH (Turgeon and Waring, 1981; Frawley and Neill, 1984; Liu and Jackson, 1984). Furthermore, a 48 h longer incubation period, used in Experiment II, eliminated this inhibitory effect by E_2 on LH. Frawley and Neill (1984) also observed an elimination of the inhibitory effect, but in some studies these longer

incubations with E_2 resulted in a complete reversal to a stimulatory effect (Liu and Jackson, 1984; Emons et al., 1986). We have also observed negative feedback of E_2 on FSH after incubating cells with E_2 for 6 h (Fig. 8). This negative feedback on FSH has not been previously documented with in vitro studies in rats. When we pre-treated cells with E_2 for 48 h before incubating them another 6 h with E_2 and GnRH, we observed an elimination of the negative feedback effect (Fig. 11). This study has shown that negative feedback by E_2 on FSH as well as on LH takes place, at least in part, at the level of the anterior pituitary gland. The length of time that we incubated cells with steroids did determine which effect we observed. We have observed that E_2 exerts inhibitory effects after a short-term incubation (6 h) and elimination of those inhibitory effects after a long-term incubation (48 h) in vitro. Liu and Jackson (1984) and Emons et al., (1986) have observed stimulatory effects by E_2 after long-term incubation and then elimination of the stimulatory effect in vitro. This research seems to indicate that mechanisms studied in vitro may yield clues to the dual nature of negative and positive feedback effects by estradiol in vivo. E_2 may be inhibitory due to an increase in degradation or decrease of synthesis of gonadotropins when first exposed to pituitary cells. Cells may also become desensitized to E_2 after a long

period, diminishing the inhibitory effect. Heber and Odell (1979) suggested that loss of affinity for GnRH binding at the receptor caused by E_2 may be a partial reason for the decrease in secretion of LH and FSH. Inhibited secretion caused by decreased affinity of GnRH, however, would not cause a decrease in cellular or total content of gonadotropins as we observed in Experiment I. Furthermore, we also observed inhibition of basal secretions of gonadotropin. Since GnRH was not present, the affinity of the receptor for GnRH could not have been a factor. E_2 must be affecting another site. Perhaps E_2 may affect the rate of transcription of certain genes that alter the rate of synthesis or degradation of gonadotropin subunits. E_2 may also affect post-translational events. For example, the steroid may interfere with glycosylation of the glycoprotein, or possibly affect the conformation of the glycoprotein. The mechanism for mediation in these areas, however, is not well established. If conformational changes resulted, it is possible that the receptor would not recognize the hormone or that the antibody in our assay would not bind to that hormone. E_2 exerted negative feedback effects on pituitary common alpha- and LH beta-subunit mRNA concentrations (Gharib et al., 1986; Gharib et al., 1987) and FSH beta-subunit mRNA concentrations in rats (Gharib et al., 1987). Furthermore, Phillips et al.

(1988) observed a decrease in transcription of FSH mRNA levels in ovine pituitary cell cultures, indicating that the mechanism for negative feedback of E_2 on gonadotropins may be transcriptional regulation. We cannot differentiate between degradation and synthesis using our data, but these would be excellent future experiments.

Previous failures to detect antagonism by estradiol of GnRH-stimulated secretion of LH and especially FSH [since the responsiveness of FSH to any known regulatory signal was of small magnitude (Labrie et al., 1978)] in static cultures of pituitary cells could reflect: 1) the transient nature of the inhibition, 2) desensitization of gonadotropes to continuous GnRH exposure (Drouin et al., 1976b; Strobl and Levine, 1988), or 3) the requirement of tissue integrity for manifestation of the inhibitory response. Our experimental design did not address the second or third issues, but they should be considered when drawing conclusions from <u>in vitro</u> data. Strobl and Levine (1988) have demonstrated that E_2 inhibited secretion of LH at the pituitary by using hypophysectomized rats as their model. Frawley and Neill (1984) have demonstrated that estradiol inhibited GnRH-stimulated LH secretion from rat pituitary cells by using a perfused monolayer culture. We have demonstrated that E_2 inhibited both GnRH-stimulated LH and FSH using static

monolayer cultures of rat pituitary cells. Since the rat has an estrous cycle of only 4 to 5 days, the inhibitory effects may also be more short-lived than in the ewe, with an estrous cycle of 16 days, or a primate, with a menstrual cycle of 28 days. The length of the cycle may possibly correlate to the length of the inhibitory effect observed.

In vitro studies are useful for pin-pointing effector organs of an observed response. They also reduce total numbers of animals needed, because cells from multiple animals can be pooled, which reduces individual variation and the need for extensive replicates. If *in vitro* studies simulate *in vivo* situations, then *in vitro* studies can be used with confidence. *In vivo*, scientists observed both negative and positive feedback effects of E_2 on gonadotropins (Libertun et al., 1974; Vilchez-Martinez et al., 1974; Schuiling and Gnodde, 1977; Matt et al., 1984; Strobl et al., 1989). Our data have demonstrated negative feedback of E_2 on LH and FSH and then the elimination of that effect (Figs. 12 and 13) *in vitro*, which parallels *in vivo* studies. Our data, however, did not demonstrate the positive feedback of E_2 on LH and FSH observed *in vivo*. Studies must be done that exhibit both negative and positive feedback effects on both gonadotropins. Dierschke et al. (1973) have suggested that P_4 can block

the positive feedback action of estrogens on gonadotropin secretion in monkeys. Perhaps enough P_4 was present before we removed the pituitaries to block positive feedback effects of E_2. Possibly altered durations of incubation, ovariectomy before harvesting of pituitaries or altered concentrations of cells per plate would replicate in vivo studies more closely.

As our third objective we wanted to investigate the effects of B on gonadotropins. Our fourth objective flows from the first three, to determine if B affects LH and FSH in a manner additive, antagonistic to or synergistic with E_2. In Experiment I (6 h), B enhanced basal secreted FSH as previously observed by Suter and Schwartz (1985), but we also observed that B did not block the inhibitory effect of E_2, nor did E_2 block the stimulatory effect of B. This finding suggested that these two steroids may be working through separate mechanisms at the level of the pituitary to affect secretion of FSH. Our long-term study (48 h) revealed a different observation. In our 48 h incubation E_2 was antagonistic and slightly blocked the stimulatory effects of B on basal secreted FSH, but not on total FSH. This block indicated that these two steroids may be working through the same mechanism at the level of the pituitary to affect secretion of FSH, but that a longer incubation

period was required before the effect could be observed.

After 48 h, the high concentration of B increased basal secreted, cellular and total concentrations of FSH relative to control (Fig. 10). These results agreed with results from Suter and Schwartz (1985). E_2 partially blocked the stimulatory effect of B on basal secretion of FSH. This block may be due to the ability of E_2 to decrease glucocorticoid receptor mRNA concentrations at the level of gene transcription in rat pituitary cells (Peiffer and Barden, 1987). If interference at the genomic level reduced the number of glucocorticoid receptors, then B could not bind to the cells to promote increased secretion of FSH. This action by E_2 in the anterior pituitary gland may result in a decreased sensitivity of this tissue to circulating glucocorticoids and could help to protect against effects of stress.

In Experiment I (6 h), E_2 decreased basal cellular and total FSH and B did not block this decrease (Fig. 5). Since the resulting amount of FSH present was the same when we presented E_2 alone or with a combination of E_2 and B, we could conclude that either E_2 and B may be working through a common pathway or that the duration of the incubation was not sufficient to elicit an effect.

In both studies, if we incubated cells with either steroid alone, we observed no change relative to control for secreted, cellular or total basal levels of LH. When

we incubated E_2 and B together they decreased basal secreted LH (Figs. 5 and 10). This synergistic decrease also indicated that these two steroids may be operating through different mechanisms at the level of the pituitary to affect basal LH secretion.

As GnRH concentrations increased, GnRH-stimulated secretion increased, and cell content decreased proportionately for LH and FSH (Figs. 8 and 11). Our effects of GnRH corroborate those of other scientists (Kamel and Krey, 1982; Suter and Schwartz, 1985; van Rees and de Koning, 1985; Kamel and Kubajak, 1987). The addition of secreted plus cellular levels of gonadotropin resulted in a flat line for the GnRH-dose response curve. This flat line represented the total amount of LH in the system and could be statistically compared to totals from cells receiving different steroid treatments, to determine the effect of steroids on the system. Total value is an important tool that can be used to determine if a treatment affects secretion or storage of gonadotropins. If we measured only secretion, we could not speculate as to synthesis because we would not be able to determine if the cell was only secreting stored hormone. By examining total amounts we could determine if a steroid treatment affected only secretion of gonadotropins or if steroids also affected the cell content. If a treatment increased total amounts of

gonadotropins, then either an increase of synthesis or decrease of degradation of gonadotropin must have occurred. We observed a flat line because GnRH alone did not change the total amount of hormone present in the system. Any change resulting from GnRH was a dose-dependent change in secreted gonadotropins only.

In Experiment II (48 h), B slightly decreased secretion of LH in response to GnRH. Our results corroborated those of *in vitro* studies by Kamel and Kubajak (1987) and Tibolt and Childs (1985). Our work, however, conflicted with *in vitro* results obtained by Suter and Schwartz (1985) who observed no effect of B on GnRH-stimulated LH. Suter and Schwartz (1985) incubated cells with GnRH for 48 h instead of 6 h. During this 42 h longer incubation, inhibition may have occurred and then effects of prolonged exposure to GnRH may have obliterated the effect. Our *in vitro* results also corroborate studies performed *in vivo*. *In vivo*, Ringstrom and Schwartz (1985) also observed this inhibition of GnRH-stimulated secreted LH by glucocorticoids. Since B increased the slope of the GnRH dose-response curve we could not statistically determine if any interactive effects occurred between E_2 and B for GnRH-stimulated FSH. Suter and Schwartz (1985) and Kamel and Kubajak (1987) observed a stimulatory effect of B on secreted, cellular, and total FSH. *In vivo*,

glucocorticoids also inhibited secretion of GnRH-stimulated LH and increased pituitary content of FSH (Ringstrom and Schwartz, 1985; Suter et al., 1988). Since treatment with glucocorticoids did not affect receptors for GnRH (Suter et al., 1988), modulations caused by B must modify some post-receptor event. Furthermore, it appeared that E_2 slightly blocked the stimulatory effect of B on total FSH (Fig. 11), although statistical analysis could not be performed due to the change in slope caused by B. Since B inhibited secretion of LH and enhanced cell content of FSH, B altered gonadotropin concentrations necessary for normal reproduction.

Another possible interpretation for the lack of inhibition of gonadotropins after 48 h may be a decreased sensitivity or integrity of the cells in culture over the longer frame of time. Total LH and total FSH concentrations were lower in Experiment II than in Experiment I (Figs. 12 & 13). We observed that length of incubation affected the feedback effects of E_2 on gonadotropin secretion. After incubating cells for 48 h (Experiment II) without steroids (control), GnRH-stimulated secreted and total LH levels were lower when compared to Experiment I (6 h) (Fig. 12). A long-term incubation may damage the cells or the GnRH receptors and

we may, therefore, not be able to detect an inhibitory effect as different from control due to decreased concentrations of gonadotropin. Two possibilities for a decrease in secretion of gonadotropin after long-term incubation include 1) a decrease in secretion with an increase of stored gonadotropin, possibly due to a decrease in sensitivity to GnRH which stimulates hormone secretion, or 2) a decrease of both secretion and cell content, suggesting a decrease in synthesis. Since secretion was decreased, we examined if secretion of hormone was affected by depleting stores of gonadotropin or if the cells added to the pools and produced more hormone to secrete. The total value, therefore, was an important tool used to determine if the rate of secretion was the only factor affected by a long-term incubation. Total values could also indicate if the cells were synthesizing hormone. We observed a decreased concentration of total LH when we compared controls in Experiment II to Experiment I (Fig. 12). Since the total value decreased, the cells must not have been synthesizing as much hormone or they increased the rate of degradation during the longer incubation.

When comparing Experiment I to II, we also observed a decrease in GnRH-stimulated secreted FSH along with an increase of cellular FSH with no change in total FSH in Experiment II controls relative to Experiment I controls

(Fig. 13). These decreased secretions and increased cellular content with no change in total indicated that the longer incubation time of Experiment II affected secretion of FSH. After the 48 h longer incubation, the gonadotropins may remain inside the cell due to a block between synthesis and secretion when they are incubating for the final 6 h. A possible intracellular pathway may be along the rough endoplasmic reticulum or transport to or within the Golgi apparatus where final glycosylation and then packaging for secretion may take place. It is possible that the size of a specific releasable pool of FSH may have been altered. Damaged to the cell due to length of time it was removed from the body can be ruled out because a damaged cell would result in decreased synthesis and total FSH would have decreased, which we did not observe.

In Experiment II (48 h) we compared the net amount of LH and FSH present in the system at time 0 (that was the amount in the cells at the start of our final incubation) to the net amount present after 6 h (that was, cellular + secreted) to determine if there was a change from the initial amount of gonadotropin in the system. We observed an increase in total gonadotropin levels over time and can therefore conclude that cells were viable and synthesizing gonadotropins during the 6 h period. In addition to the general increase in

gonadotropin over time, we observed an increase in cellular FSH when we presented the high dose of B (10^{-6} M) (Fig. 9). Previous studies by Suter and Schwartz (1985) supported this increase. At time 0, levels of total FSH in the system pre-incubated with B were higher than those levels in other treatment groups. This increase by B indicated that B either stimulated synthesis or inhibited degradation of FSH. The 6 h incubation period did not affect the results of steroidal treatments.

In the future, studies may be done to measure the amount of time needed for gonadotropin synthesis to begin and the rate of synthesis when treated with steroids in each gender. This information could be used to determine if steroids affect synthesis or degradation of gonadotropins. The site in the genome that is affected by various steroidal treatments should also be determined. We observed negative feedback of gonadotropins by E_2 and the decline of this effect. We did not, however, observe positive feedback by E_2, which is known to occur <u>in vivo</u>. <u>In vitro</u> models are needed which simulate both negative and positive feedback by E_2. Other experiments may determine the integrity of cells incubated over prolonged periods.

RESULTS OF EXPERIMENTS EMPLOYING PROGESTERONE

For clarity, only the effects of the high dose of each steroid are shown on most graphs. Unless otherwise stated the effect of the low dose of steroid was not different from controls values. Values for all data can be found in the Appendix.

EXPERIMENT III [TREATMENT OF CELLS WITH PROGESTERONE FOR 6 h]

Comparison Between Amounts of Gonadotropin in the System Before and After 6 h of Incubation - Experiment III

We observed no difference between amounts of net LH in the system (cellular) at the beginning of 6 h of incubation (time 0) and net LH in the system (secreted + cellular) after 6 h of incubation (Fig. 14). Similar to LH, net FSH in the system before and after 6 h of incubation was not different (Fig. 14).

Effects on Basal LH

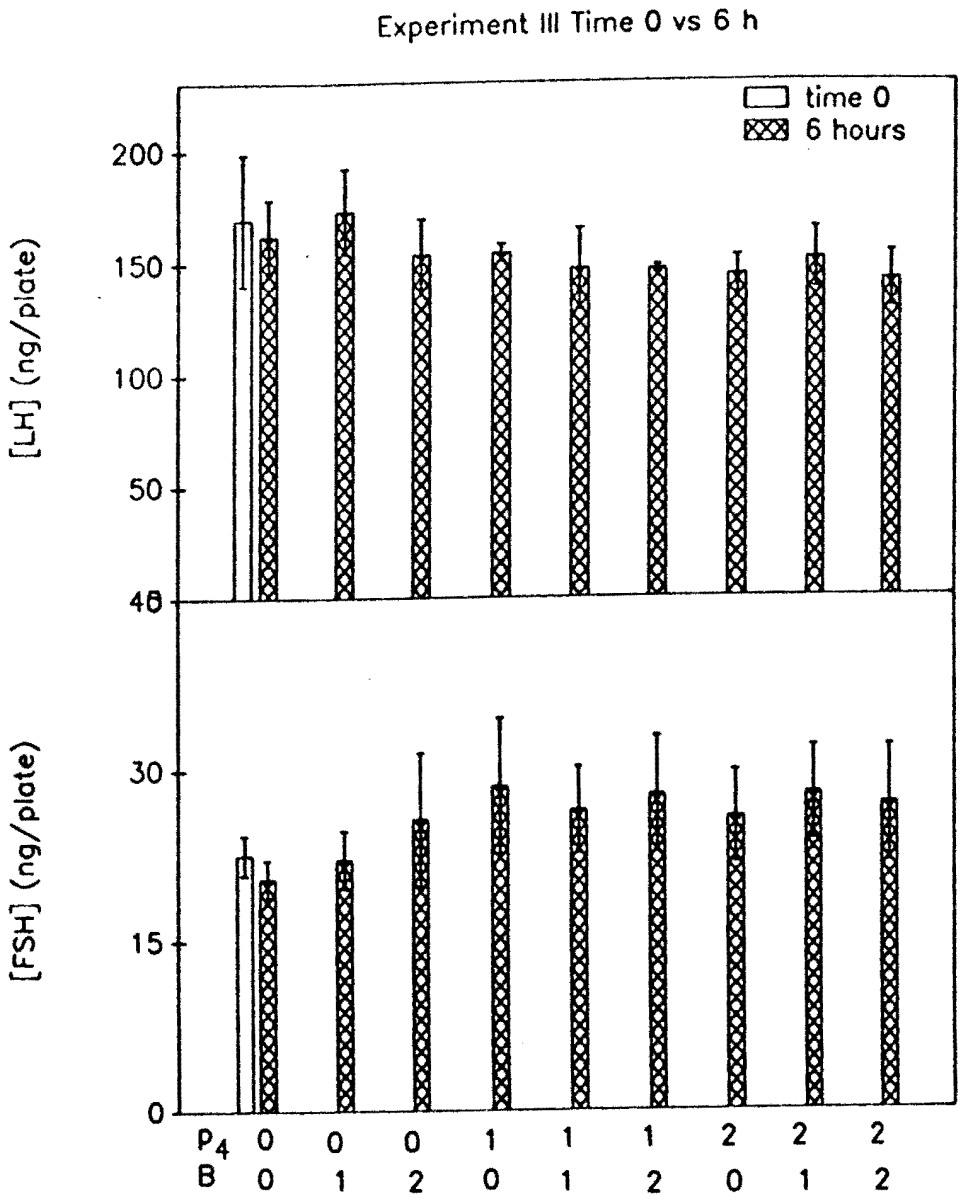
The only effect on basal LH was an inhibitory effect by P_4. After incubating the cells with the

Figure 14: Experiment III Basal Time 0 vs After 6 h

We measured net LH (top) and FSH (bottom) in the system both before (cellular) and after the final 6 h (cellular + secreted) incubation period in the absence of GnRH. Time 0 represents before the 6 h incubation. We graphed net gonadotropin present in the system after 6 h next to the amount present at time 0 for each dose of P_4 or B, or a combination of P_4 and B. Each bar represents the mean $\pm$ SEM of 3 cell cultures. We calculated error bars from the standard deviation of each mean determined from the one-way analysis of variance. There were no significant differences between the beginning and end of incubation.

P_4: 0 = no steroid 1 = 2 x 10^{-8} M 2 = 10^{-7} M

B: 0 = no steroid 1 = 10^{-8} M 2 = 10^{-6} M.


Experiment III Time 0 vs 6 h
time 0
6 hours
[LH] (ng/plate)
200
150
100
50
40
[FSH] (ng/plate)
30
15
0
P4 0 0 0 1 1 1 2 2 2
B 0 1 2 0 1 2 0 1 2

steroids for 6 h, P_4 decreased basal cellular LH by 11.8% ($P = 0.037$) and total LH by 11.2 % ($P = 0.034$) (Fig. 15). As in Experiment I, B alone had no effect on basal LH. Combined with P_4, B did not affect the ability of P_4 to decrease cellular or total basal LH (Fig. 16).

Effects on Basal FSH

Steroidal treatments affected FSH differently from LH. P_4 was stimulatory instead of inhibitory. P_4 increased secreted basal FSH by 98.2% ($P = 0.002$). P_4 had no effect on cellular ($P = 0.134$) or total FSH ($P = 0.376$). B alone did not change secreted FSH from control ($P = 0.452$). When we incubated cells with B and P_4, B neither enhanced nor suppressed the stimulatory effect of P_4 on secreted FSH. Neither B alone ($P > 0.780$) nor B combined with P_4 ($P > 0.124$) changed cellular or total FSH relative to control (Fig. 16).

Effects on GnRH-stimulated LH

GnRH increased secreted LH and decreased cellular LH ($P < 0.001$) with no effect on total LH (Fig. 17). This action of GnRH was the same as in Experiments I and II.

P_4 decreased the slope of the GnRH dose-response

Figure 15: Experiment III (6 h incubation)

Effects of P_4 on basal secreted, cellular and total levels of LH after 6 h of incubation. P_4 = 0 contains only ethanol, and no progesterone, $P_4 = 2 \times 10^{-8}$ M is the low concentration, and $P_4 = 1 \times 10^{-7}$ M is the high concentration. Bars represent mean $\pm$ SEM of 3 cell cultures. We calculated error bars from the analysis of variance. The * symbolizes significant differences relative to control P_4 = 0.

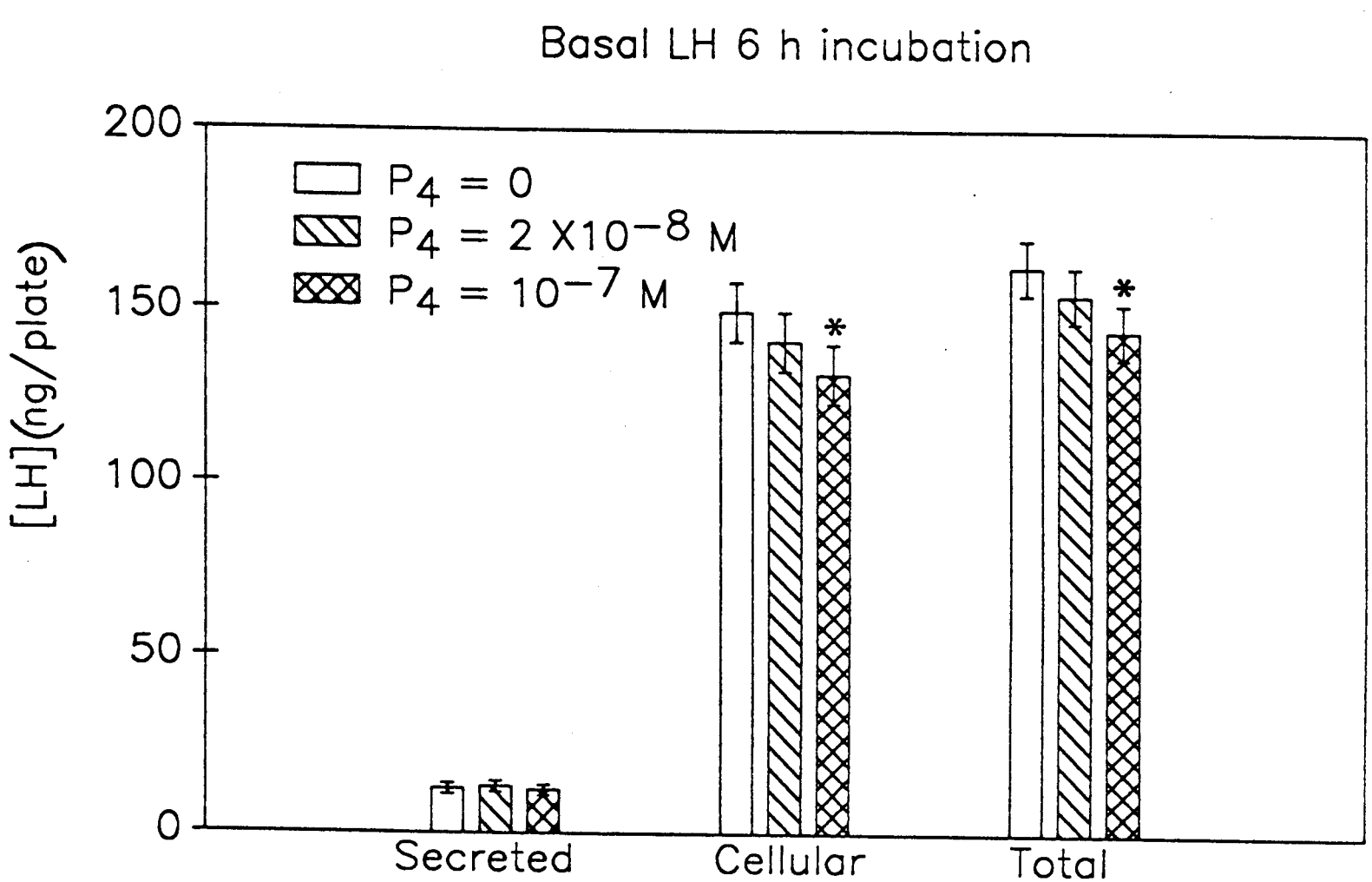

Basal LH 6 h incubation
200
150
100
50
0
[LH](ng/plate)
P4 = 0
P4 = 2 X10−8 M
P4 = 10−7 M
*
*
Secreted
Cellular
Total

Figure 16: Experiment III (6 h incubation)

Effects of P_4 and B on basal levels of LH (top) and FSH (bottom) after 6 h of incubation. Control contains ethanol only. P_4 (10^{-7} M) and B (10^{-6} M) shown are high concentrations. P_4 + B represents the high concentrations of P_4 and B together. Bars represent mean $\pm$ SEM of 3 cell cultures. We calculated error bars from the analysis of variance. The * symbolizes significant differences relative to control.

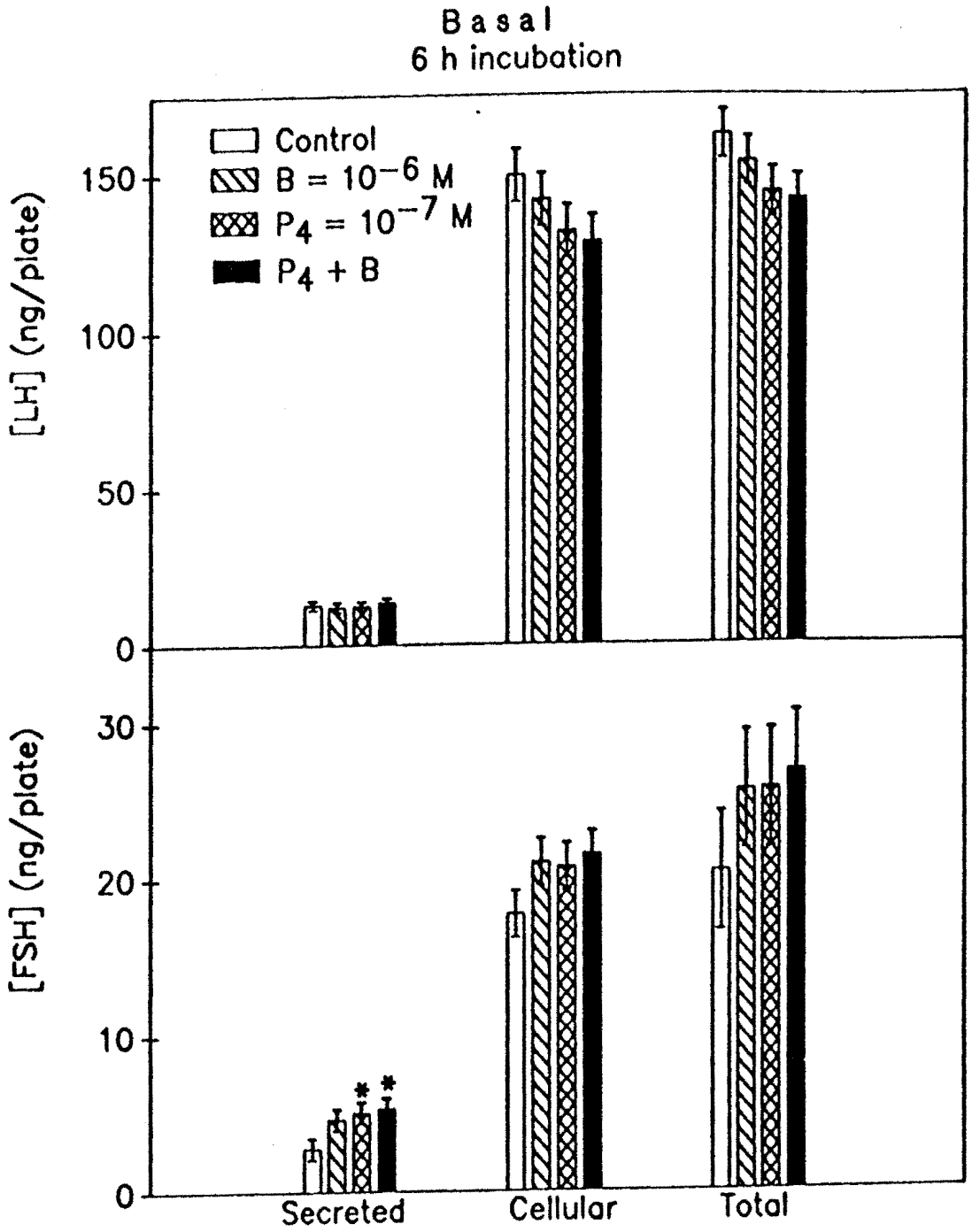
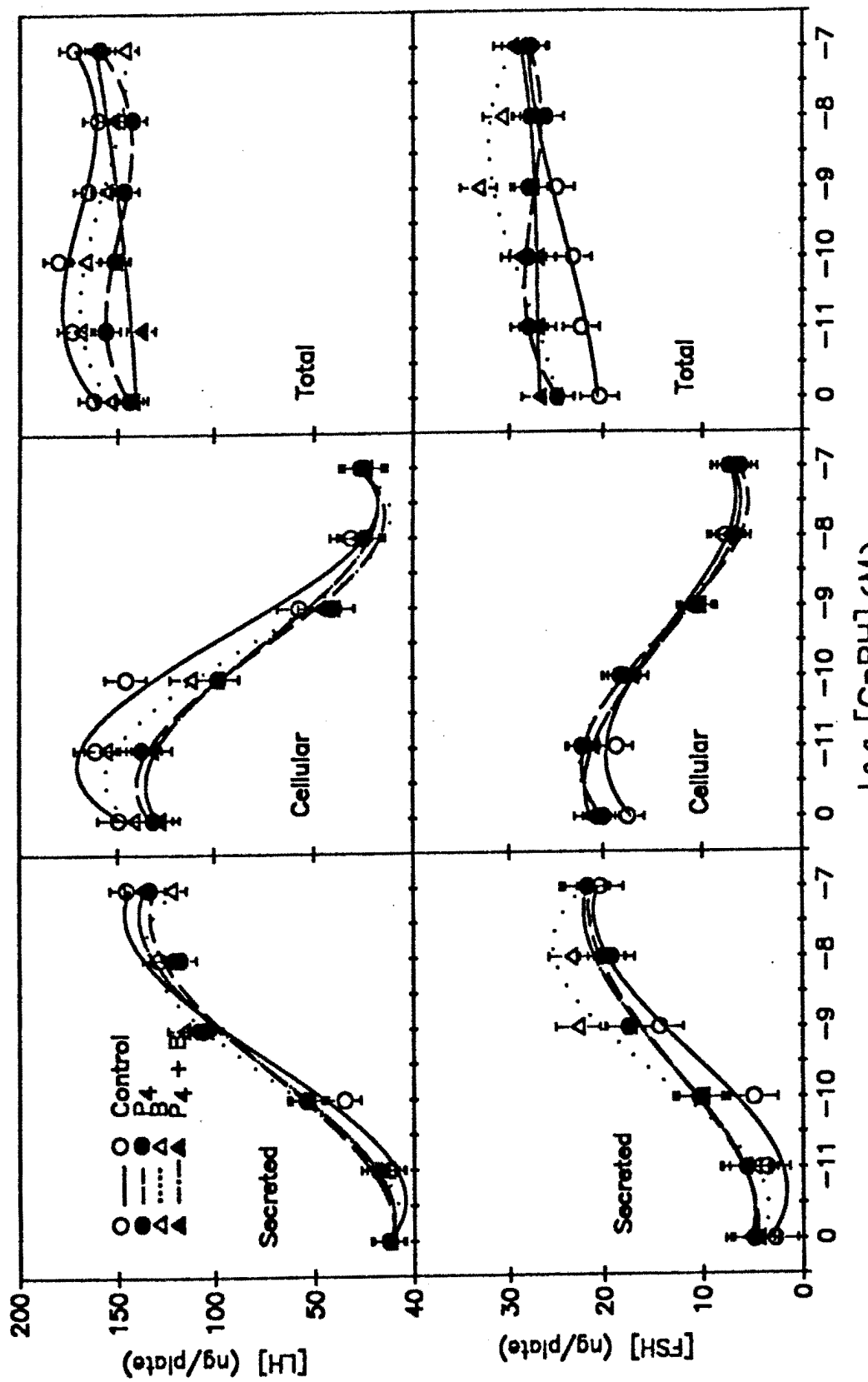

Basal
6 h incubation
Control
B = 10⁻⁶ M
P4 = 10⁻⁷ M
P4 + B
[LH] (ng/plate)
150
100
50
0
[FSH] (ng/plate)
30
20
10
0
*
*
Secreted
Cellular
Total

Figure 17: Experiment III (6 h incubation)

Quantities of LH (top) and FSH (bottom) in medium (left panel), in cells (middle panel), and of total content (cells + medium; right panel) in response to treatment with steroids and GnRH for 6 h. P_4 (10^{-7} M) represents the high dose only, B (10^{-6} M) represents the high dose only, and P_4 + B represents the high doses of P_4 and B together. Points represent mean $\pm$ SEM of 3 cell cultures after the 6 h incubation. We calculated error bars from the analysis of co-variance.


Control
P4
P4 + E2
Secreted
Cellular
Total
[LH] (ng/plate)
[FSH] (ng/plate)
log [GnRH] (M)
200
150
100
50
40
30
20
10
0
-7
-8
-9
-10
-11
0

curve for cellular GnRH-stimulated LH ($P = 0.038$) (Fig. 17). P_4 decreased cellular LH at low doses of GnRH. At higher doses of GnRH, however, very little LH remained inside the cells. High doses of GnRH, therefore, obliterated the inhibitory effects of P_4 on cellular LH (Fig. 17).

Although P_4 interacted with GnRH's effects on cellular LH, we did observe an inhibitory effect of P_4 on total LH. P_4 decreased total GnRH-stimulated LH in the system by 12.2% ($P < 0.001$). When we incubated B with P_4 an interactive effect resulted. B blocked the inhibitory effect of P_4 on total LH at high doses of GnRH ($P = 0.050$ for the interaction). By itself, however, B did not exert inhibitory or stimulatory effects. When we incubated cells with B and GnRH for 6 h secreted, cellular and total LH were not different from control, as in Experiment I.

Effects on GnRH-stimulated FSH

GnRH increased secreted FSH and decreased cellular FSH ($P < 0.001$) as in previous Experiments. Incubation with GnRH slightly enhanced total levels of FSH ($P = 0.003$).

P_4 decreased the slope of the GnRH dose-response curve for total FSH ($P = 0.030$). At low doses of GnRH P_4

enhanced total FSH relative to control. At high doses of GnRH, however, there was no difference between the effects of the control and P_4 on total FSH. B did not alter the inhibitory effect of P_4 on responsiveness to GnRH for total FSH ($P < 0.001$) (Fig. 17).

EXPERIMENT IV [TREATMENT OF CELLS WITH PROGESTERONE FOR 48 + 6 h]

Comparison Between the Amount of Gonadotropin in the System Before and After 6 h of Incubation - Experiment IV

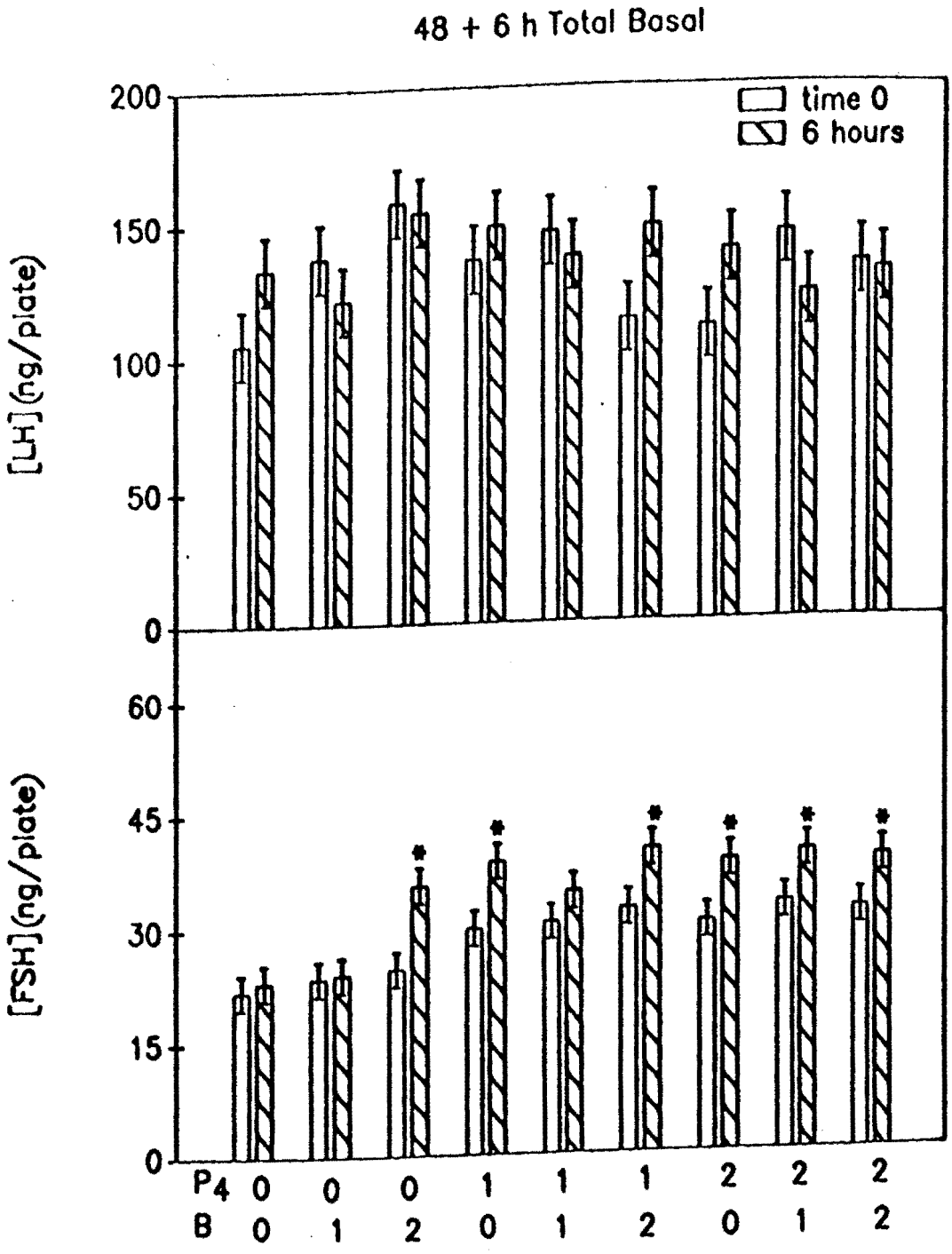
As in Experiment II, we measured amounts of net gonadotropin in the system before and after the final 6 h of incubation to determine if long-term incubation affected net gonadotropin levels in the system. We measured levels of cellular gonadotropin at time 0 which was after incubating cells in the presence or absence of steroids for 48 h, but before the final 6 h incubation. After the final 6 h of incubation we measured the amount of secreted plus cellular hormone (6 h). We observed no change in the amount of net LH in the system after 6 h when we compared the amount after 6 h to the control at time 0 when cells were incubated in the absence of steroids (Fig. 18). We also compared the amount of gonadotropin present with each treatment after 6 h to the

Figure 18: Experiment IV Basal incubation

We measured the amount of total LH (top) and FSH (bottom) in the system both before (cellular) and after (cellular + secreted) the final 6 h incubation period in the absence of GnRH. Time 0 represents the period after the initial 48 h incubation, but before the 6 h incubation. We graphed the amount of total gonadotropin present in the system after 6 h next to the amount present at time 0 for each dose of P_4 or B, or a combination of P_4 and B. We calculated the error bars from the analysis of variance. Each bar represents the mean $\pm$ SEM of 3 cell cultures. The * symbolizes significant differences between each treatment group and the control (P_4 = 0 and B = 0 at time 0.)

P_4: 0 = no steroid 1 = 2 x 10^{-8} M 2 = 10^{-7} M

B: 0 = no steroid 1 = 10^{-8} M 2 = 10^{-6} M.


48 + 6 h Total Basal
time 0
6 hours
[LH](ng/plate)
200
150
100
50
0
[FSH](ng/plate)
60
45
30
15
0
P4 0 0 0 1 1 1 2 2 2
B 0 1 2 0 1 2 0 1 2

amount present for that same treatment at time 0 (Fig. 18). As in the previous comparison, we observed no change in the amount of net LH for these second comparisons.

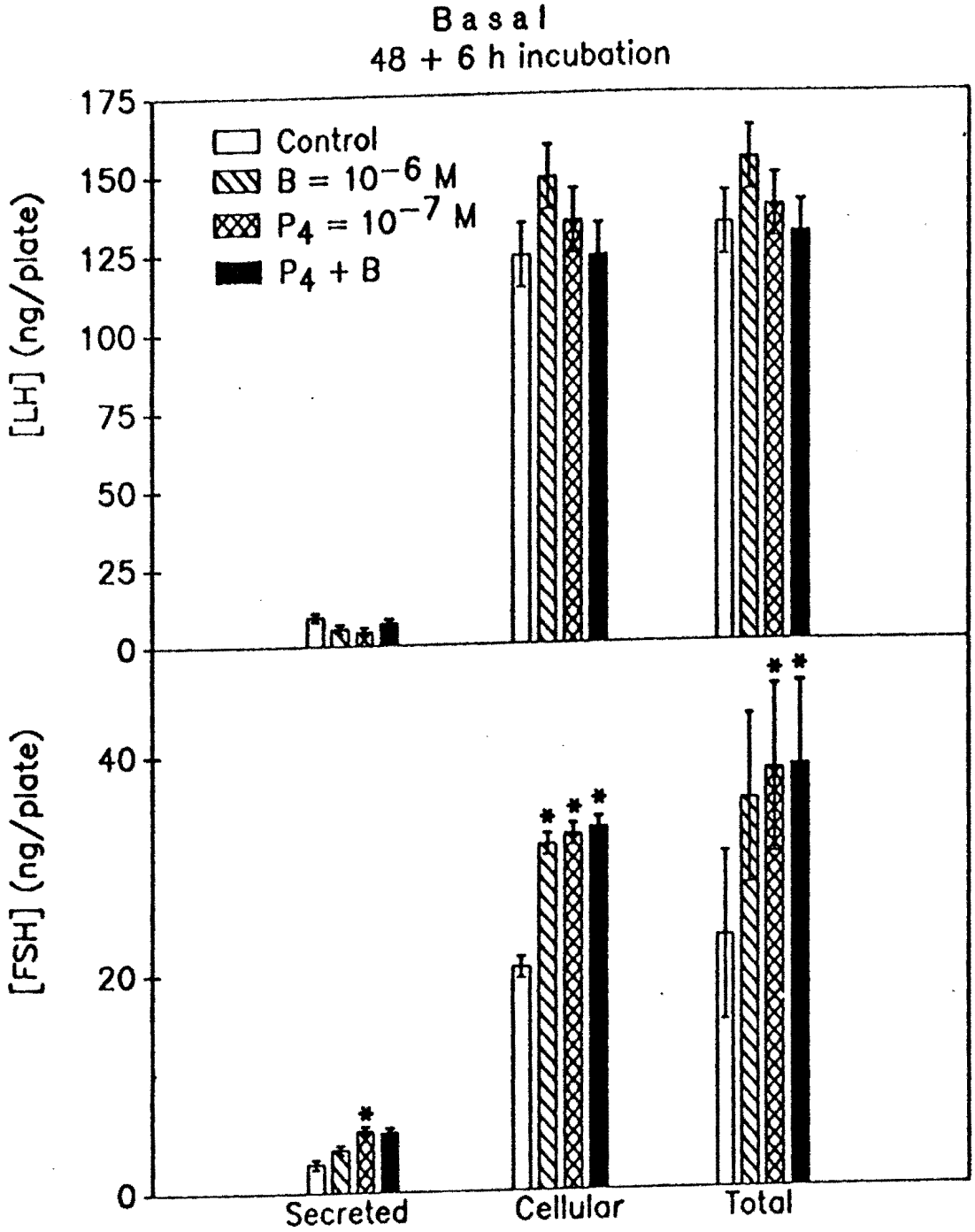
FSH was affected differently from LH when we compared the amount of net FSH present at time 0 with the amount present after 6 h. As in Experiment II, the amount of net FSH increased after 6 h of incubation ($P < 0.001$). Net FSH also increased when we incubated cells with either dose of P_4 ($P < 0.001$) or with a high dose of B (10^{-6} M) ($P = 0.028$) (Fig. 18) as in Experiment II. P_4 and B incubated together also increased the amount of net FSH in the system ($P < 0.001$). Furthermore, the effect of B and P_4 together was not different from B or P_4 alone ($P < 0.001$).

Effects on Basal LH

After 48 h of incubation of cells with steroids basal secreted, cellular, and total LH did not changed relative to control when we treated cells with P_4, B, or both P_4 and B (Fig. 19). B alone and B incubated with P_4 affected the gonadotropins as in Experiment III (6 h: short-term incubation), that is, no change relative to control. A longer incubation time (48 h), used in Experiment IV, eliminated the inhibitory effects observed

Figure 19: Experiment IV (48 h incubation)

Effects of P_4 and B on basal levels of LH (top) and FSH (bottom) after long-term (48 h) incubation. Control contains ethanol only. P_4 (10^{-7} M) and B (10^{-6} M) shown are high concentrations. P_4 + B represents the high concentration of P_4 and B together. Bars represent mean $\pm$ SEM of 3 cell cultures. We calculated error bars from the analysis of variance. The * symbolizes significant differences relative to control.


Basal
48 + 6 h incubation
Control
B = 10⁻⁶ M
P4 = 10⁻⁷ M
P4 + B
[LH] (ng/plate)
[FSH] (ng/plate)
175
150
125
100
75
50
25
0
40
20
0
Secreted
Cellular
Total

in Experiment III (6 h) on LH caused by incubation of cells with P_4 alone.

Effects on Basal FSH

Although the steroids did not affect basal LH, we did observe a stimulatory effect on FSH. Both doses of P_4 enhanced secreted FSH by 115.7%, cellular FSH by 59.5% ($P < 0.001$) and total FSH by 66.0% ($P = 0.028$) (Fig. 19). B enhanced cellular FSH by 55.0% ($P < 0.001$). Combined B and P_4 enhanced basal cellular FSH the same amount as either steroid alone ($P < 0.001$) (Fig. 19). B did not change secreted or total FSH relative to control (Fig. 19). B also did not block the stimulatory effect of P_4 on cellular and total FSH (Fig. 19).

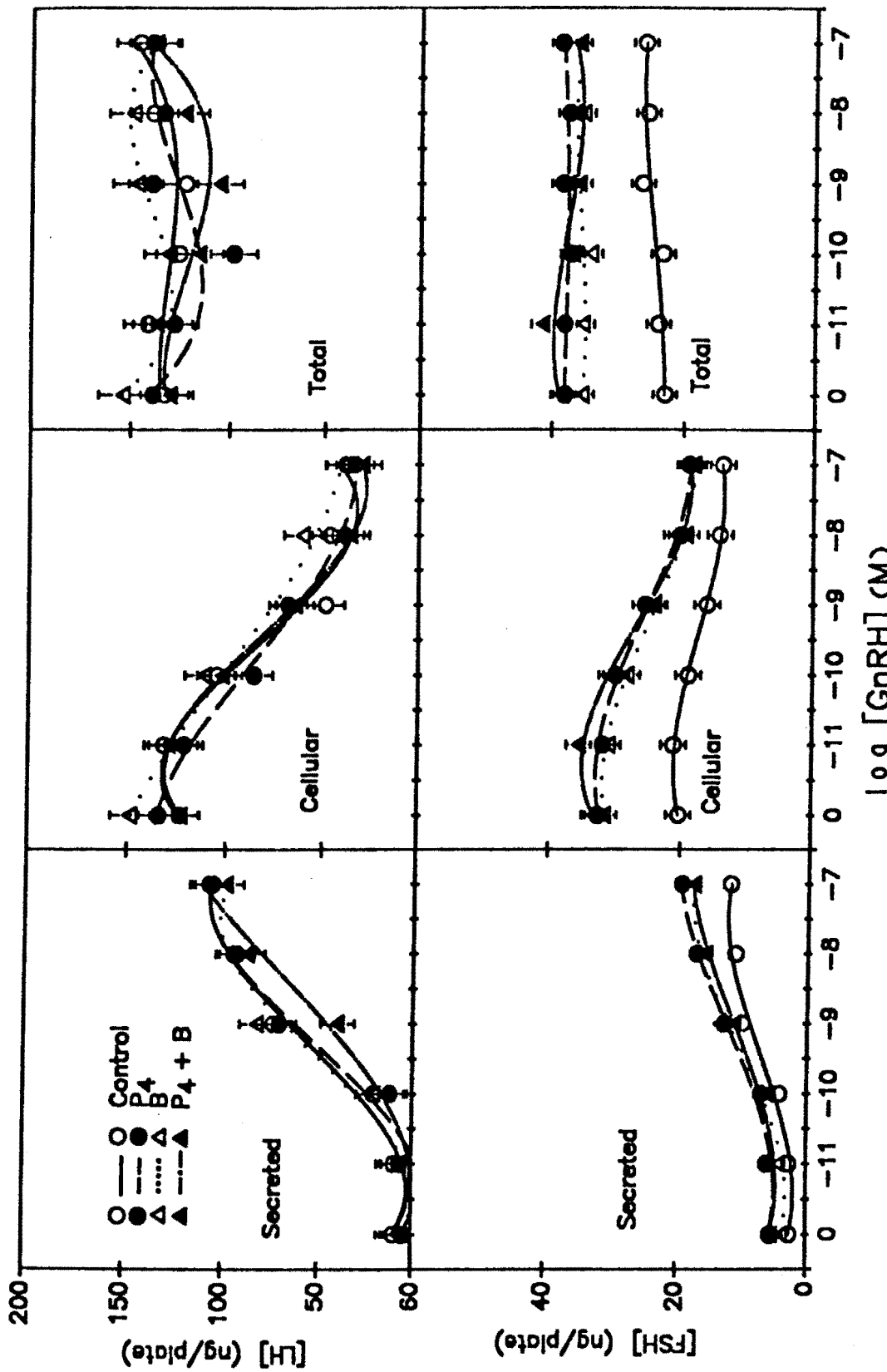
Effects on GnRH-stimulated LH

GnRH increased secreted LH and decreased cellular LH ($P < 0.001$) as in all previous experiments. GnRH had no effect on total LH in the system as in Experiment III (6 h).

As in Experiment III (6 h), P_4 inhibited GnRH-stimulated LH after 48 h. P_4 decreased cellular LH by 4.8% ($P < 0.005$) and total LH by 5.6% ($P < 0.001$) (Fig. 20). Neither B nor the combination of P_4 and B had an

Figure 20: Experiment IV 48 h incubation.

Quantities of LH (top) and FSH (bottom) in medium (left panel), in cells (middle panel), and of total content (cells + medium; right panel) in response to treatment with steroid for 48 h, then steroid + GnRH for 6 h. P_4 (10^{-7} M) represents the high dose only, B (10^{-6} M) represents the high dose only, and P_4 + B represents the high doses of P_4 and B together. Points represent mean $\pm$ SEM of 3 cell cultures after the final 6 h incubation. We calculated error bars from the analysis of co-variance.


Control
P_4
B
P_4 + B
Secreted
Cellular
Total
[LH] (ng/plate)
[FSH] (ng/plate)
log [GnRH] (M)
200
150
100
50
60
40
20
0
−11
−10
−9
−8
−7
0

effect on LH in the 48 h experiment (Fig. 20).

Effects on GnRH-stimulated FSH

GnRH increased secreted FSH and decreased cellular FSH ($P < 0.001$). GnRH affected FSH in this experiment as it did after 6 h of treatment in Experiment III.

As in Experiment III, P_4 enhanced GnRH-stimulated FSH, but did not affect the slope of the GnRH dose-response curve. After 48 h of incubation of cells with P_4, secreted FSH increased by 53.0%, cellular FSH by 51.7% and total FSH by 52.1% ($P < 0.001$) (Fig. 20).

As in Experiment II (48 h), B increased the slope of the GnRH dose-response curve for cellular FSH ($P = 0.044$) therefore no more statistical analysis could be performed on B with respect to cellular FSH. B increased total FSH by 43.6% ($P < 0.001$) (Fig. 20).

After 48 h of preincubation, P_4 alone and B alone both increased GnRH-stimulated FSH. When we incubated the two steroids together they also exerted a stimulatory affect. P_4 and B together increased the amount of secreted FSH by 39.3% ($P = 0.006$) and total FSH by 52.2% ($P < 0.001$). Together the two steroids did not increase secreted or total FSH any more than either steroid alone (Fig. 20).

DISCUSSION

Progesterone exerted effects similar to estradiol when using a short-term incubation (6 h) compared to a long-term incubation (48 h). We examined: 1) whether P_4 had direct feedback effects on gonadotropins, and 2) if length of incubation determined the observed effects. Feedback systems are important for understanding the controls of various steroids in reproductive cycles (Fig. 1). After 6 h of incubation, P_4 decreased basal cellular and total LH (Fig. 15). Since total LH decreased, P_4 may have either: 1) decreased synthesis or 2) increased degradation of LH or possibly 3) a combination of altered synthesis and degradation. Incubating for 48 h longer removed the inhibitory effects observed after 6 h, indicating that length of time determined the resulting effect. This alleviation of inhibitory effects by P_4 may be the result of a decreased sensitivity to P_4 after extended exposure. P_4 also caused an inhibition of GnRH-stimulated secretion of LH <u>in vivo</u> (Arimura and Schally, 1970; Caligaris et al., 1971). <u>In vitro</u>, however, neither Tang and Spies (1975) nor Drouin and Labrie (1981) observed inhibition of total LH by P_4, but they used a longer (48 h) incubation of cells with steroids. When using a 48 h incubation, we did not observe

inhibition of total LH either.

After both 6 h and 48 h incubations, P_4 also decreased total GnRH-stimulated LH in the system. This demonstrated negative feedback of P_4 on total LH at the level of the anterior pituitary. Lee et al. (1989) demonstrated that P_4 also appeared to suppress pulsatile GnRH secretion at the hypothalamus. P_4 may, therefore, exert negative feedback at both the pituitary and the hypothalamus, resulting in even lower concentrations of *in vivo* LH than can be demonstrated in this *in vitro* model.

The mechanism by which P_4 exerts negative feedback effects over a short period (6 h) and elimination of this inhibition during longer (48 h) incubations *in vitro* may yield clues to the biphasic action of P_4 on ovulation *in vivo* (Everett, 1948; Zeilmaker, 1966; Martin et al., 1974). These regulating mechanisms may have various effects on the rate of transcription of certain genes or they may affect post-translational events. For example, steroids may have effects on one or more of the following areas: 1) the number of receptors present for each particular steroid or for other substances that regulate the gonadotropins, 2) binding capabilities of regulatory factors (such as GnRH), 3) the conformation of the glycoprotein so that it was not recognized by the receptor or possibly by the assay we used or both, 4)

transduction of the protein, or possibly, 5) synthesis at the point of glycosylation. The site of positive feedback by P_4 may be at another site in the central nervous system.

Although P_4 had an inhibitory effect on LH, it had a stimulatory effect on FSH. P_4 increased secreted basal FSH when incubated for either time course (Figs. 16 and 18 bottom). P_4 also increased basal cellular and total FSH after a longer incubation (48 h), which corroborates the findings of Leveque and Grotjan (1982) (Fig. 19). Since P_4 increased total FSH, P_4 may have 1) increased synthesis, 2) decreased degradation of FSH, or 3) a combination of altered synthesis and altered degradation.

After 48 h of incubation, P_4 also increased GnRH-stimulated secreted, cellular and total concentrations of FSH (Fig. 20). Other scientists also observed stimulatory effects of P_4 on GnRH-stimulated FSH (Lagace' et al., 1980; Drouin and Labrie, 1981; Leveque and Grotjan, 1982). In vivo, P_4 also elicited an increase in serum FSH (Caligaris et al., 1971). These divergent effects of P_4 on the gonadotropins suggested that general cellular regulatory mechanisms for LH and FSH may be completely different. After 6 h, P_4 decreased the slope of the GnRH dose-response curve for total FSH, indicating that P_4 caused a decreased sensitivity of the cells to GnRH.

We did not observe negative feedback of P_4 on FSH in these studies at the site of the anterior pituitary. Possibly, we needed a shorter time course to observe negative feedback of P_4 if it is at the pituitary. The site of negative feedback of FSH by P_4 may be at another place, perhaps at the hypothalamus or another part of the central nervous system.

In our final objective, we investigated whether B affects LH and FSH in a manner additive, antagonistic or synergistic with P_4. P_4 and B increased the amount of GnRH-stimulated secreted and total FSH. Together the two steroids did not increase secreted or total FSH any more than either steroid alone (Fig. 20), which showed non-additivity of P_4 and B. Non-additivity indicated that these two hormones may be acting by some convergent mechanism. The two steroids may be 1) binding to the same receptor, or 2) binding to different receptors on the same cell, then binding to the same place to turn on gene transcription and therefore may be working through the same pathway to increase FSH. Studies by Strahle et al. (1989) supported the second hypothesis. When Strahle et al. (1989) incorporated mRNA for P_4 receptors into a hepatic cell line normally containing only B receptors, they observed that either P_4 or B could activate the same response in the cell by possibly

binding to the same region of the genome. This study by Strahle et al. (1989) indicated that differential expression of hormone receptors was at least one mechanism by which steroid-specific gene activation could be achieved. Glucocorticoid-regulated genes were rendered equally responsive to progestins when receptors for both steroid hormones were present (Strahle et al., 1989). This suggested that the common-pathway may be convergent binding of P_4 and B at the genomic level. These data suggest that pituitary cells may act in a similar fashion. In our study P_4 and B may be activating the same region of the genome to elicit an increase of FSH secretion by the pituitary cells. B, released in response to a stressor may, therefore, interfere with reproductive function by mimicking P_4.

In the future, investigators may explore the site of negative and positive feedback. The negative feedback of both estradiol and progesterone that we observed in the 6 h experiment may be due to some non-specific inhibition. To address this problem of non-specificity it would be important to perform a 6 h control of cells with steroids, perhaps cholesterol, expected to have little or no effect on gonadotropins. In conjunction with the problem of possible non-specificity, we need to determine if synthesis, degradation, or a combination of both are responsible for the resulting effects of

steroidal treatments. Divergent effects on LH and FSH may be the result of different gonadotropes for each or different intracellular mechanisms. If it is determined that LH and FSH are both secreted from the same cell at the same time, then intracellular mechanisms are responsible for these divergent effects which would be an important site for gonadotropin regulation.

This work may be used to facilitate research in reproductive dysfunction caused by stress in humans and animals. It would be economically important to farmers whose livestock do not breed, perhaps due to stress from drought or high temperatures. The studies could also be ecologically important for breeding endangered species that do not breed in the stress of captivity. Another future ecological perspective may be rodent control through altered reproductive function instead of poison.

CONCLUSION

In conclusion, negative feedback of E_2 on LH and FSH and of P_4 on LH occurred directly at the level of the pituitary when we incubated cells with steroids for 6 h. Furthermore, when we incubated the cells with steroids for 48 h longer, the inhibitory effects elicited by these steroidal hormones diminished, indicating that the duration of incubation determined the effect observed. B did change LH concentrations relative to control. B, however, stimulated secreted, cellular and total basal FSH and GnRH-stimulated total FSH. When we incubated cells with both E_2 and B, we observed an antagonistic decrease in FSH, with E_2 partially blocking the stimulatory effect of B. E_2 and B together decreased secreted basal LH in a synergistic manner. P_4 decreased basal cellular and GnRH-stimulated total LH and increased basal and GnRH-stimulated secreted, cellular, and total FSH. Together P_4 and B exhibited non-additivity and did not increase secreted or total LH any more than either alone. P_4 and B together increased basal cellular and GnRH-stimulated secreted and total FSH the same amount as either one alone, indicating a common pathway for the actions of P_4 and B.

REFERENCES

Alexander, D.C., and Miller, W.L., Regulation of ovine Follicle-stimulating hormone beta-chain mRNA by 17-beta-estradiol *in vivo* and *in vitro*. Journal of Biological Chemistry 257: 2282-2286, 1982.

Arimura, A., and Schally, A.V., Augmentation of pituitary responsiveness to LH-releasing hormone by estrogen. Proceedings of the Society of Experimental Biology of Medicine 136: 290-293, 1971.

Arimura, A., and Schally, A.V., Progesterone suppression of LH-releasing hormone-induced stimulation of LH release in rats. Endocrinology 87: 653-657, 1970.

Baldwin, D.M., and Sawyer, C.H., Effects of dexamethasone on LH release and ovulation in the cyclic rat. Endocrinology 94: 1397-1403, 1974.

Baldwin, D.M., The effect of glucocorticoids on estrogen-dependent luteinizing hormone release in the ovariectomized rat. Endocrinology 105: 120-128, 1979.

Bambino, T.H., and Hsueh, A.J.W., Direct inhibitory effect of glucocorticoids upon testicular luteinizing hormone receptor and steroidogenesis in vivo and in vitro. Endocrinology 108: 2142-2148, 1981.

Barraclough, C.A., Wise, P.M., Turgeon, J., Shander, D., DePaulo, L., and Rance, N., Recent studies on the regulation of pituitary LH and FSH secretion. Biology of Reproduction 20: 86-97, 1979.

Barrett, A.M., and Stockham, M.A., The effects of housing conditions and simple experimental procedures upon the corticosterone level in plasma of rats. Journal of Endocrinology, 26: 97-105, 1963.

Caligaris, L., Astrada, J.J., and Taleisnik, S., Biphasic effect of progesterone on the release of gonadotropins in rats. Endocrinology 89: 331-337, 1971.

Campbell, C.S., Schwartz, N.B., and Firlit, M.G., The role of adrenal and ovarian steroids in the control of serum LH and FSH. Endocrinology 101: 162-172, 1977.

Christian, J.J., Lloyd, J.A., and Davis, D.E., The role of Endocrines in the self-regulation of mammalian populations. Recent Progress in Hormone Research 21: 501-571, 1965.

Cohen, I.R., and Mann, D.R., Influence of corticosterone on the response to gonadotropin-releasing hormone in rats. Neuroendocrinology 32: 1-6, 1981.

Colowick, S.P. and Kaplan, N.O., Methods in Enzymology: Cell Culture vol. LVIII edited by: Jakoby, W.B. and Pastan, I.H., Academic Press New York p.150-152., 1979.

Conn P.M., Rogers, D.C., Seay, S.G., and Staley, D., Activation of luteinizing hormone release from pituitary cells by polycations. Endocrinology 115: 1913-1917, 1984.

DeCatanzaro, D., and Gorzalka, B.B., Effects of dexamethasone, corticosterone, and ACTH on lordosis in ovariectomized and adrenalectomized-ovariectomized rats. Pharmacology Biochemistry and Behavior 12: 201-206, 1979.

Desjardins, C., and Ewing L.L., Testicular metabolism in adrenalectomized and corticosterone treated rats. Proceedings of the Society for Experimental Biology of Medicine 137: 578-583, 1971.

Dierschke, D. J., Yamaji, T., Karsch, F.J., Weick R.F., Weiss, G., and Knobil, E., Blockade by progesterone of estrogen induced LH and FSH release in the rhesus monkey. Endocrinology 92: 1496-1501, 1973.

Drouin, J., Lagace', L., and Labrie, F., Estradiol-induced increase of the LH responsiveness to LH releasing hormone (LHRH) in rat anterior pituitary cells in culture. Endocrinology 99: 1477-1481, 1976.

Drouin, J., and Labrie, F., Interactions between 17-beta-estradiol and progesterone in the control of luteinizing hormone and follicle-stimulating hormone release in rat anterior pituitary cells in culture. Endocrinology 108: 52-57, 1981.

Drouin, J., and Labrie, F., Selective effect of androgens on LH and FSH release in anterior pituitary cells in culture. Endocrinology 98: 1528-1534, 1976.

Eechaute, W., Demeester, G., Lacroix, E., and Leusen, I., The adrenal cortex activity during experimental renal hypertension in rats. Arch. Intern. Pharmacodyn. 136: 161-173, 1962.

Emons, G., Ortmann, O., Fingscheidt, U., Ball, P., and Knuppen, R., Short-term effects of oestradiol and 4-hydroxyoestradiol on gonadotropin-releasing hormone induced luteinizing hormone secretion by rat pituitary cells in culture. Acta Endocrinologica 111: 312-320, 1986.

Everett, J.W., Progesterone and estrogen in the experimental control of ovulation time and other features of the estrous cycle in the rat. Endocrinology 43: 389-405, 1948.

Frawley, L.S., and Neill, J.D., Biphasic effects of estrogen on gonadotropin-releasing hormone induced luteinizing hormone release in monolayer cultures of rat and monkey pituitary cells. Endocrinology 114: 659-663, 1984.

Gharib, S.D., Bowers, S.M., Need, L.R., and Chin, W.W., Regulation of rat luteinizing hormone subunit messenger ribonucleic acids by gonadal steroid hormones. Journal of Clinical Investigations 77: 582-589, 1986.

Gharib, S.D., Wierman, M.E., Badger, T.M., and Chin, W.W., Sex steroid hormone regulation of follicle-stimulating hormone subunit messenger ribonucleic acid (mRNA) levels in the rat. Journal of Clinical Investigations 80: 294-299, 1987.

Goodman, A.L., In vitro evidence of a role for inhibin in female rabbits. American Journal of Physiology 246: E243-E248, 1984.

Hagino, N., Watanabe, M., and Goldzieher, J.W., Inhibition by adrenocorticotropin of gonadotropin-induced ovulation in immature female rats. Endocrinology 84: 308-314, 1969.

Heber, D., and Odell, W., Estrogen modulation of pituitary LHRH receptor in the rat: in vivo and in vitro studies. American Journal of Physiology 237: E136-E141, 1979.

Hsueh, A.J.W., and Erickson, G.F., Glucocorticoid inhibition of FSH-induced estrogen production in cultured rat granulosa cells. Steroids 32: 639-648, 1978.

Hsueh, A.J.W., Erickson, G.F., and Yen, S.S.C., The sensitizing effect of estrogens and catechol estrogen on cultured pituitary cells to luteinizing hormone-releasing hormone: Its antagonism by progestins. Endocrinology 104: 807-813, 1979.

Hymer, W.C., Evans, W.H., Kraicer, J., Mastro, A., Davis, J., and Griswold, E., Enrichment of cell types from the rat adenohypophysis by sedimentation at unit gravity. Endocrinology 92: 275-287, 1973.

Kalra, P.S., Fawcett, C.P., Krulich, L., and McCann, S.M., The effects of gonadal steroids on plasma gonadotropins and prolactin in the rat. Endocrinology 92: 1256-1268, 1973.

Kamel, F., and Krey, L.C., Gonadal steroid modulation of LHRH-stimulated LH secretion by pituitary cell cultures. Molecular and Cellular Endocrinology 26: 151-164, 1982.

Kamel, F., and Kubajak, C.L., Modulation of gonadotropin secretion by corticosterone: Interaction with gonadal steroids and mechanism of action. Endocrinology 121: 561-568, 1987.

King, J.A., Davidson, J.S., Mehl, A.E., Wakefield, I.K., Andersson, P.B., and Millar, R.P., Gonadal steroid modulation of signal transduction and luteinizing hormone release in cultured chicken pituitary cells. Endocrinology 124: 1830-1840, 1989.

Labrie, F., Drouin, J., Ferland, L., Lagace', L., Beaulieu, M., DeLean, A., Kelly, P.A., Caron, M.G., and Raymond, V., Mechanism of action of hypothalamic hormones in the anterior pituitary gland and specific modulation of their activity by sex steroids and thyroid hormones. Recent Progress in Hormone Research 34: 25-81, 1978.

Lagace', L., Massicotte, J., and Labrie, F., Acute stimulatory effects of progesterone on luteinizing hormone and follicle-stimulating hormone release in rat anterior pituitary cells in culture. Endocrinology 106: 684-689, 1980.

Lee, L.R., Haisenleder, D.J., Marshall, J.C., and Smith, M.S., Effects of progesterone on pulsatile luteinizing hormone (LH) secretion and LH subunit messenger ribonucleic acid during lactation in the rat. Endocrinology 124: 2128-2134, 1989.

Leveque, N.W., and Grotjan, H.E. Jr., Interaction of progesterone with testosterone and dihydrotestosterone on follicle-simulating hormone release by cultures of rat anterior pituitary cells. Biology of Reproduction 27: 110-117, 1982.

Libertun, C., Orias, R., and McCann, S.M., Biphasic effect of estrogen on the sensitivity of the pituitary to luteinizing hormone-releasing factor (LFR). Endocrinology 94: 1094-1100, 1974.

Liu, T.C., and Jackson G.L., Long-term superfusion of rat pituitary cells: Interaction of 17-beta-Estradiol with pulses of gonadotropin-releasing hormone on luteinizing hormone release. Proceedings of the Society for Experimental Biology and Medicine 177: 296-302, 1984.

Martin, J.E., Tyrey, L., Everett, J.W., and Fellows, R.E., Estrogen and progesterone modulation of the pituitary response to LRF in the cycling rat. Endocrinology 95: 1664-1673, 1974.

Matt, D.W., LaPolt, P.S., Judd, H.L., and Lu, J.K.H., Estrogen exposure affects the post-ovariectomy increases in both LH and FSH release in female rats. Neuroendocrinology 42: 21-27, 1986.

Moberg, G.P., Influence of the adrenal axis upon the gonads. Oxford Reviews of Reproductive Biology, 9: 456-496, 1987.

Ortmann, O., Emons, G., Knuppen, R., and Catt, K., Inhibitory actions of keoxifene on luteinizing hormone secretion by pituitary gonadotrophs. Endocrinology 123: 962-968, 1988.

Peiffer, A., and Barden, N., Estrogen-induced decrease of glucocorticoid receptor messenger ribonucleic acid concentration in rat anterior pituitary gland. Molecular Endocrinology 1: 435-440, 1987.

Phillips, C.L., Lin, L.W., Wu, J.C., Guzman, K., Milsted, A., and Miller, W.L., 17-beta estradiol and progesterone inhibit transcription of the genes encoding the subunits of ovine follicle-stimulating hormone. Molecular Endocrinology 2: 641-649, 1988.

Ringstrom, S.J., and Schwartz, N.B., Examination of prolactin and pituitary-adrenal axis components as intervening variables in the adrenalectomy-induced inhibition of gonadotropin response to castration. Endocrinology 114: 880-887, 1984.

Ringstrom, S.J., and Schwartz, N.B., Cortisol suppresses the LH, but not the FSH, response to gonadotropin-releasing hormone after orchidectomy. Endocrinology 116: 472-474, 1985.

Ringstrom, S.J., and Schwartz, N.B., Differential effect of glucocorticoids on synthesis and secretion on luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Journal of Steroid Biochemistry 27: 625-630, 1987.

Rivier, C., Rivier, J., and Vale W., Stress-induced inhibition of reproduction functions: Role of endogenous corticotropin-releasing factor. Science 231: 607-609, 1986.

Saez, J.M., Morera, A.M., Haour, F., and Evain, D., Effects of in vivo administration of dexamethasone, corticosterone and human chorionic gonadotropin on steroidogenesis and protein and DNA synthesis of testicular interstitial cells in prepuberal rats. Endocrinology 101: 1256-1263, 1977.

Sarkar, D.K., Chiappa, S.A., and Fink, G., Gonadotropin-releasing hormone surge in pro-oestrous rats. Nature 264: 461-463, 1976.

Savoy-Moore, R.T., Landefeld, T.D., and Marshall, J.C., Hormonal measurement in rat anterior pituitary cell culture: Loss of immunoreactive LH counteracted by fetal calf serum and bacitracin. Molecular and Cellular Endocrinology 18: 11-20, 1980a.

Savoy-Moore, R.T., Schwartz, N.B., Duncan, J.A., and Marshall, J.C., Pituitary gonadotropin-releasing hormone receptors during the rat estrous cycle. Science 209: 942-944, 1980b.

Schally, A.V., Redding, T.W., and Arimura, A., Effect of sex steroids on pituitary response to LH- and FSH-releasing hormone In vitro. Endocrinology 93: 893-902, 1973.

Schoonmaker, J.N., and Erickson, G.F., Glucocorticoid modulation of follicle-stimulating hormone-mediated granulosa cell differentiation. Endocrinology 113: 1356-1363, 1983.

Schuiling, G.A., and Gnodde, H.P., Oestrogen-induced changes in the secretion of luteinizing hormone releasing hormone in the long-term ovariectomized rat. Journal of Endocrinology 72: 121-126, 1977.

Smith, E.R., Johnson, J., Weick, RF., Levine, S., and Davidson, J.M., Inhibition of the reproductive system in immature rats by intracerebral implantation of cortisol. Neuroendocrinology 8: 94-106, 1971.

Spies, H.G., and Niswender, G.D., Effects of progesterone and estradiol on LH release and ovulation in rhesus monkeys. Endocrinology 90: 257-261, 1972.

Strahle, U., Boshart, M., Klock, G., Stewart, F., and Schutz, G., Glucocorticoid- and progesterone specific effects are determined by differential expression of the respective hormone receptors. Nature 339: 629-632, 1989.

Strobl, F.J., and Levine, J.E., Estrogen inhibits luteinizing hormone (LH), but not follicle-stimulating hormone secretion in hypophysectomized pituitary-grafted rats receiving pulsatile LH-releasing hormone infusions. Endocrinology 123: 622-630, 1988.

Strobl, F.J., Meredith, J.M., and Levine, J.E., Positive and negative feedback of estrogen on in vivo LH secretion by the isolated pituitary gland. Endocrinology 71 (Suppl 1): 73, (Abstract 202), 1989.

Suter, D.E., and Orosz, G., Inhibitory effects of cortisol an LH and FSH in vivo are not manifested in vitro. Biology of Reproduction **36 (Suppl 1)**: 167, (Abstract 363), 1987.

Suter, D.E., and Schwartz, N.B., Effects of glucocorticoids on secretion of luteinizing hormone and follicle-stimulating hormone by female rat pituitary cells in vitro. Endocrinology **117**: 849-854, 1985.

Suter, D.E., Schwartz, N.B., and Ringstrom, S.J., Dual role of glucocorticoids in regulation of pituitary content and secretion of gonadotropins. American Journal of Physiology **254**: E595-E600, 1988.

Tang, L.K.L. and Spies, H.G., Effects of gonadal steroids on the basal and LRF-induced gonadotropin secretion by cultures of rat pituitary. Endocrinology **96**: 349-356, 1975.

Tibolt, R.E., and Childs, G.V., Cytochemical and cytophysiological studies of gonadotropin-releasing hormone (GnRH) target cells in the male rat pituitary: Differential effects of androgens and corticosterone on GnRH binding and gonadotropin release. Endocrinology **117**: 396-404, 1985.

Turgeon, J.L. and Waring, D.W., Acute progesterone and 17-beta-estradiol modulation of luteinizing hormone secretion by pituitaries of cycling rats superfused in vitro. Endocrinology **108**:413-419, 1981.

van Rees, G.P. and de Koning, J., Regulation of the release of LH and FSH. Frontiers of Hormone Research **14**: 1-33, 1985.

Vilchez-Martinez, J.A., Arimura, A., Debeljuk, L., and Schally, A.V., Biphasic effects of estradiol benzoate on the pituitary responsiveness to LH-RH. Endocrinology **94**: 1300-1303, 1974.

Welsh, T.H., Jr., Bambino, T.H., and Hsueh, A.J.W. Mechanism of glucocorticoid-induced suppression of testicular androgen biosynthesis in vitro. Biology of Reproduction **27**: 1138-1146, 1982.

Yen, S.S.C., Vandenberg, G., and Siler, T.M., Modulation of pituitary responsiveness to LRF by estrogen. J. Clinical Endocrinology of Metabolism **39**: 170-177, 1974.

Zeilmaker, G.H., The biphasic effect of progesterone on ovulation in the rat. Acta Endocrinologica 51: 461-468.

APPENDIX

Numbers in this appendix represent concentrations of gonadotropin in ng/plate calculated from measurements of (ng/ml). Secretion measured from the media is abbreviated sec. Exp. # represents the experimental replicate. Each value is the mean of duplicate plates with duplicate RIA tubes for each. Following values for each experiment are Means of the 3 experiments.

EXPERIMENT I SHORT-TERM INCUBATION (6 h) WITH E_2 AND B

EB Exp #	log [GnRH]	log [E]	log [B]	[LH] sec (ng/plate) (ng/ml)1.2	[FSH] sec (ng/plate) (ng/ml)1.2	[LH] cell (ng/plate)	[LH] total (ng/plate)	[FSH] cell (ng/plate)	[FSH] total (ng/plate)
2	-7	-8	-8	217.9	26.88	85.4	303.3	11.2	38.08
2	0	-8	-6	4.3	6.76	303.0	307.3	40.0	46.76
2	-11	-8	-6	11.3	7.56	298.4	309.7	40.0	47.56
2	-10	-8	-6	66.2	12.48	256.2	322.4	39.0	51.48
2	-9	-8	-6	205.7	25.20	128.2	333.9	20.0	45.20
2	-8	-8	-6	245.5	33.00	84.1	329.6	12.6	45.60
2	-7	-8	-6	283.0	33.72	66.3	349.3	12.0	45.72
3	0	0	0	17.0	3.8	209.6	226.6	23.6	27.4
3	-11	0	0	13.2	5.4	191.5	204.7	25.5	30.9
3	-10	0	0	14.9	4.7	192.0	206.9	26.1	30.8
3	-9	0	0	36.6	6.8	159.9	196.5	21.2	28.0
3	-8	0	0	139.7	17.8	66.4	206.1	10.2	28.0
3	-7	0	0	175.4	21.4	39.4	214.8	9.5	30.9
3	0	0	-8	17.3	4.4	200.0	217.3	24.7	29.1
3	-11	0	-8	14.2	4.1	179.4	193.6	24.8	28.9
3	-10	0	-8	16.7	4.2	191.5	208.2	26.0	30.2
3	-9	0	-8	46.8	8.0	162.2	209.0	23.2	31.2
3	-8	0	-8	32.2	3.8	165.0	197.2	22.7	26.5
3	-7	0	-8	105.4	10.9	87.2	192.6	16.2	27.1
3	0	0	-6	15.4	5.8	205.9	221.3	30.4	36.2
3	-11	0	-6	15.4	5.6	179.8	195.2	28.0	33.6
3	-10	0	-6	19.4	5.9	180.4	199.8	26.8	32.7
3	-9	0	-6	58.9	10.4	141.2	200.1	21.4	31.8
3	-8	0	-6	133.9	17.9	78.1	212.0	13.6	31.5
3	-7	0	-6	186.5	20.4	35.0	221.5	10.7	31.1
3	0	-10	0	14.9	4.2	155.2	170.1	24.4	28.6
3	-11	-10	0	13.8	3.8	158.2	172.0	22.8	26.6
3	-10	-10	0	14.2	4.4	175.8	190.0	23.7	28.1
3	-9	-10	0	31.1	6.6	149.8	180.9	21.2	27.8
3	-8	-10	0	27.2	3.7	144.6	171.8	19.9	23.6
3	-7	-10	0	154.3	15.1	57.2	211.5	9.4	24.6
3	0	-10	-8	18.0	4.2	215.8	233.8	21.8	26.0
3	-11	-10	-8	14.4	3.9	202.0	216.4	23.6	27.5
3	-10	-10	-8	16.8	4.7	179.2	196.0	24.0	28.7
3	-9	-10	-8	37.1	6.9	140.2	177.3	19.6	26.5
3	-8	-10	-8	110.5	15.4	78.0	188.5	11.3	26.7
3	-7	-10	-8	151.4	17.3	44.6	196.0	8.9	26.2
3	0	-10	-6	15.5	5.8	197.0	212.5	27.4	33.2
3	-11	-10	-6	15.1	5.8	140.2	155.3	24.7	30.5
3	-10	-10	-6	18.5	6.3	130.6	149.1	24.6	30.9
3	-9	-10	-6	46.6	9.6	136.6	183.2	21.6	31.2
3	-8	-10	-6	133.0	16.6	65.5	198.5	12.1	28.7
3	-7	-10	-6	198.4	20.3	38.6	237.0	9.6	29.9
3	0	-8	0	16.3	4.4	151.3	167.6	24.0	28.4
3	-11	-8	0	11.0	3.9	148.4	159.4	23.6	27.5
3	-10	-8	0	13.9	4.2	144.9	158.8	22.5	26.7
3	-9	-8	0	29.0	5.7	140.2	169.2	20.7	26.4

EB Exp #	log [GnRH]	log [E]	log [B]	[LH] sec (ng/plate) (ng/ml)1.2	[FSH] sec (ng/plate) (ng/ml)1.2	[LH] cell (ng/plate)	[LH] total (ng/plate)	[FSH] cell (ng/plate)	[FSH] total (ng/plate)
2	0	0	0	15.1	6.74	281.5	296.6	101.4	108.14
2	-11	0	0	22.3	8.41	229.6	251.9	77.8	86.21
2	-10	0	0	44.6	11.52	199.2	243.8	82.8	94.32
2	-9	0	0	201.4	30.72	99.0	300.4	26.1	56.82
2	-8	0	0	310.8	36.96	59.4	370.2	21.0	57.96
2	-7	0	0	284.4	32.88	51.6	336.0	16.4	49.28
2	0	0	-8	3.5	6.43	595.0	598.5	81.3	87.73
2	-11	0	-8	5.1	6.97	244.1	249.2	57.6	64.57
2	-10	0	-8	42.2	11.56	203.4	245.6	95.0	106.56
2	-9	0	-8	243.1	25.68	124.6	367.7	27.7	53.38
2	-8	0	-8	247.2	28.80	50.6	297.8	12.2	41.00
2	-7	0	-8	274.1	36.36	58.4	332.5	13.2	49.56
2	0	0	-6	13.0	9.53	262.9	275.9	68.0	77.53
2	-11	0	-6	7.7	8.52	270.9	278.6	72.0	80.52
2	-10	0	-6	70.3	15.24	283.2	353.5	62.8	78.04
2	-9	0	-6	225.6	37.56	94.6	320.2	22.8	60.36
2	-8	0	-6	410.4	38.88	55.2	465.6	12.6	51.48
2	-7	0	-6	208.3	40.20	72.4	280.7	13.2	53.40
2	0	-10	0	7.3	6.94	254.1	261.4	54.6	61.54
2	-11	-10	0	6.5	7.67	264.1	270.6	52.7	60.37
2	-10	-10	0	35.2	11.33	231.6	266.8	44.9	56.23
2	-9	-10	0	176.0	31.20	122.6	298.6	22.1	53.30
2	-8	-10	0	197.0	40.08	62.7	259.7	11.3	51.38
2	-7	-10	0	205.3	36.00	61.6	266.9	10.8	46.80
2	0	-10	-8	8.4	7.01	239.8	248.2	39.8	46.81
2	-11	-10	-8	7.5	6.08	261.6	269.1	40.7	46.78
2	-10	-10	-8	36.6	11.11	246.2	282.8	44.3	55.41
2	-9	-10	-8	176.6	29.76	127.1	303.7	20.0	49.76
2	-8	-10	-8	226.1	40.80	70.4	296.5	12.4	53.20
2	-7	-10	-8	192.5	36.96	89.0	281.5	11.8	48.76
2	0	-10	-6	4.8	8.33	259.4	264.2	54.0	62.33
2	-11	-10	-6	4.1	8.47	294.0	298.1	45.8	54.27
2	-10	-10	-6	46.1	14.16	206.2	252.3	40.8	54.96
2	-9	-10	-6	195.1	36.48	160.8	355.9	21.4	57.88
2	-8	-10	-6	246.0	33.48	79.2	325.2	11.0	44.48
2	-7	-10	-6	235.2	30.72	80.0	315.2	11.9	42.62
2	0	-8	0	35.6	6.96	320.3	355.9	40.8	47.76
2	-11	-8	0	55.7	7.07	299.8	355.5	42.0	49.07
2	-10	-8	0	30.1	9.68	299.0	329.1	40.1	49.78
2	-9	-8	0	171.8	22.56	140.2	312.0	21.6	44.16
2	-8	-8	0	249.4	31.08	74.0	323.4	10.9	41.98
2	-7	-8	0	225.4	35.40	68.4	293.8	11.7	47.10
2	0	-8	-8	5.5	5.99	337.0	342.5	52.8	58.79
2	-11	-8	-8	5.9	6.13	287.3	293.2	41.0	47.13
2	-10	-8	-8	40.1	9.60	261.2	301.3	39.8	49.40
2	-9	-8	-8	205.9	22.80	136.8	342.7	24.2	47.00
2	-8	-8	-8	217.4	25.68	90.4	307.8	12.6	38.28

EB Exp #	log [GnRH]	log [E]	log [B]	[LH] sec (ng/plate) (ng/ml)1.2	[FSH] sec (ng/plate) (ng/ml)1.2	[LH] cell (ng/plate)	[LH] total (ng/plate)	[FSH] cell (ng/plate)	[FSH] total (ng/plate)
3	-8	-8	0	113.4	15.7	61.7	175.1	11.9	27.6
3	-7	-8	0	154.3	19.0	30.4	184.7	7.9	26.9
3	0	-8	-8	16.6	4.3	151.0	167.6	23.1	27.4
3	-11	-8	-8	11.0	3.7	138.0	149.0	23.0	26.7
3	-10	-8	-8	15.4	4.1	144.0	159.4	22.4	26.5
3	-9	-8	-8	28.2	6.0	138.8	167.0	20.2	26.2
3	-8	-8	-8	107.0	13.3	74.8	181.8	12.2	25.5
3	-7	-8	-8	159.4	17.5	28.2	187.6	7.6	25.1
3	0	-8	-6	16.8	5.4	144.4	161.2	23.2	28.6
3	-11	-8	-6	10.7	4.9	133.7	144.4	24.0	28.9
3	-10	-8	-6	9.7	5.5	157.6	167.3	25.2	30.7
3	-9	-8	-6	34.8	8.4	122.6	157.4	22.2	30.6
3	-8	-8	-6	136.3	19.6	49.9	186.2	12.7	32.3
3	-7	-8	-6	147.0	21.5	22.4	169.4	7.4	28.9
4	0	0	0	16.4	2.7	167.5	183.9	19.0	21.7
4	-11	0	0	22.1	4.0	170.2	192.3	19.9	23.9
4	-10	0	0	66.4	8.1	130.8	197.2	15.7	23.8
4	-9	0	0	131.0	14.3	42.9	173.9	10.5	24.8
4	-8	0	0	153.8	14.6	40.9	194.7	8.2	22.9
4	-7	0	0	142.8	13.9	44.2	187.0	8.7	22.7
4	0	0	-8	24.2	3.9	150.2	174.4	17.6	21.5
4	-11	0	-8	18.5	4.1	145.0	163.5	20.0	24.1
4	-10	0	-8	61.7	8.5	133.0	194.7	15.3	23.8
4	-9	0	-8	132.4	15.4	46.0	178.4	9.5	24.8
4	-8	0	-8	138.2	12.7	45.2	183.4	9.6	22.3
4	-7	0	-8	140.6	11.6	47.8	188.4	10.6	22.2
4	0	0	-6	17.5	4.6	167.8	185.3	20.2	24.8
4	-11	0	-6	29.5	5.5	156.4	185.9	18.4	23.9
4	-10	0	-6	100.8	12.4	98.0	198.8	13.4	25.8
4	-9	0	-6	149.9	18.1	39.5	189.4	10.1	28.2
4	-8	0	-6	136.9	17.0	35.8	172.7	9.0	26.1
4	-7	0	-6	158.5	15.4	41.7	200.2	8.4	23.7
4	0	-10	0	18.2	3.7	136.0	154.2	17.5	21.2
4	-11	-10	0	25.0	4.4	153.8	178.8	17.6	22.0
4	-10	-10	0	54.5	8.0	118.0	172.5	13.3	21.3
4	-9	-10	0	116.9	14.6	47.3	164.2	8.6	23.2
4	-8	-10	0	100.6	12.8	36.3	136.9	7.7	20.6
4	-7	-10	0	98.6	11.3	36.7	135.3	7.6	18.9
4	0	-10	-8	17.8	3.6	162.6	180.4	17.3	20.9
4	-11	-10	-8	24.7	4.3	122.2	146.9	17.2	21.5
4	-10	-10	-8	62.9	8.9	102.4	165.3	13.1	22.0
4	-9	-10	-8	111.6	14.2	41.2	152.8	8.9	23.1
4	-8	-10	-8	126.5	13.2	32.4	158.9	6.6	19.8
4	-7	-10	-8	107.3	11.7	48.6	155.9	7.6	19.3
4	0	-10	-6	19.9	3.6	156.4	176.3	17.6	21.2
4	-11	-10	-6	33.1	6.0	107.4	140.5	14.0	20.0
4	-10	-10	-6	61.4	9.4	68.2	129.6	12.8	22.2

EB Exp #	log [GnRH]	log [E]	log [B]	[LH] sec (ng/plate) (ng/ml)1.2	[FSH] sec (ng/plate) (ng/ml)1.2	[LH] cell (ng/plate)	[LH] total (ng/plate)	[FSH] cell (ng/plate)	[FSH] total (ng/plate)
4	-9	-10	-6	106.7	14.9	38.1	144.8	7.7	22.6
4	-8	-10	-6	98.6	12.7	35.4	134.0	5.5	18.2
4	-7	-10	-6	95.3	11.7	43.0	138.3	7.1	18.8
4	0	-8	0	41.4	6.7	93.8	135.2	12.8	19.5
4	-11	-8	0	21.6	4.6	115.1	136.7	16.4	21.0
4	-10	-8	0	78.8	9.4	73.4	152.2	9.4	18.8
4	-9	-8	0	105.7	11.9	48.0	153.7	6.7	18.5
4	-8	-8	0	119.0	14.2	32.0	151.0	5.4	19.5
4	-7	-8	0	109.3	12.0	40.0	149.3	6.9	18.9
4	0	-8	-8	21.1	3.5	132.2	153.3	15.4	18.9
4	-11	-8	-8	24.0	4.0	94.6	118.6	14.8	18.8
4	-10	-8	-8	58.3	7.4	86.3	144.6	11.8	19.2
4	-9	-8	-8	129.6	13.0	37.4	167.0	6.4	19.3
4	-8	-8	-8	113.9	12.5	34.0	147.9	5.6	18.1
4	-7	-8	-8	87.8	11.1	39.2	127.0	6.8	17.9
4	0	-8	-6	14.3	4.1	100.9	115.2	16.5	20.6
4	-11	-8	-6	22.1	4.9	100.9	123.0	15.4	20.3
4	-10	-8	-6	54.2	8.8	67.2	121.4	11.6	20.4
4	-9	-8	-6	100.1	13.7	39.2	139.3	7.0	20.7
4	-8	-8	-6	100.1	14.4	21.4	121.5	6.3	20.7
4	-7	-8	-6	108.0	11.5	27.1	135.1	6.5	18.0

log [GnRH]	log [E]	log [B]	MEAN LH sec (ng/plate)	MEAN LH cell (ng/plate)	MEAN LH total (ng/plate)	MEAN FSH sec (ng/plate)	MEAN FSH cell (ng/plate)	MEAN FSH total (ng/plate)
0	0	0	16.2	219.5	235.7	4.4	48.0	52.4
-11	0	0	19.2	197.1	216.3	5.9	41.1	47.0
-10	0	0	42.0	174.0	216.0	8.1	41.5	49.6
-9	0	0	123.0	100.6	223.6	17.3	19.3	36.5
-8	0	0	201.4	55.6	257.0	23.1	13.1	36.3
-7	0	0	200.9	45.1	245.9	22.7	11.6	34.3
0	0	-8	15.0	315.1	330.1	4.9	41.2	46.1
-11	0	-8	12.6	189.5	202.1	5.1	34.1	39.2
-10	0	-8	40.2	176.0	216.2	8.1	45.4	53.5
-9	0	-8	140.8	110.9	251.7	16.3	20.1	36.5
-8	0	-8	139.2	86.9	226.1	15.1	14.8	29.9
-7	0	-8	173.4	64.5	237.8	19.6	13.3	33.0
0	0	-6	15.3	212.2	227.5	6.6	39.5	46.1
-11	0	-6	17.5	202.4	219.9	6.5	39.5	46.0
-10	0	-6	63.5	187.2	250.7	11.2	34.3	45.5
-9	0	-6	144.8	91.8	236.6	22.0	18.1	40.1
-8	0	-6	227.1	56.4	283.4	24.6	11.7	36.3
-7	0	-6	184.4	49.7	234.1	25.3	10.8	36.1
0	-10	0	13.5	181.8	195.3	4.9	32.2	37.1
-11	-10	0	15.1	192.0	207.1	5.3	31.0	36.3
-10	-10	0	34.6	175.1	209.7	7.9	27.3	35.2
-9	-10	0	108.0	106.6	214.6	17.5	17.3	34.8
-8	-10	0	108.3	81.2	189.5	18.9	13.0	31.9
-7	-10	0	152.8	51.8	204.6	20.8	9.3	30.1
0	-10	-8	14.7	206.1	220.8	4.9	26.3	31.2
-11	-10	-8	15.6	195.3	210.8	4.8	27.2	31.9
-10	-10	-8	38.8	175.9	214.7	8.2	27.1	35.4
-9	-10	-8	108.4	102.8	211.3	16.9	16.2	33.1
-8	-10	-8	154.4	60.3	214.6	23.1	10.1	33.2
-7	-10	-8	150.4	60.7	211.1	22.0	9.4	31.4
0	-10	-6	13.4	204.3	217.7	5.9	33.0	38.9
-11	-10	-6	17.5	180.5	198.0	6.8	28.2	34.9
-10	-10	-6	42.0	135.0	177.0	10.0	26.1	36.0
-9	-10	-6	116.1	111.8	228.0	20.3	16.9	37.2
-8	-10	-6	159.2	60.0	219.2	20.9	9.5	30.5
-7	-10	-6	176.3	53.9	230.1	20.9	9.5	30.4
0	-8	0	31.1	188.5	219.6	6.0	25.9	31.9
-11	-8	0	29.4	187.8	217.2	5.2	27.3	32.5
-10	-8	0	41.0	172.4	213.4	7.8	24.0	31.8
-9	-8	0	102.2	109.5	211.7	13.4	16.3	29.7
-8	-8	0	160.6	55.9	216.5	20.3	9.4	29.7
-7	-8	0	163.0	46.3	209.3	22.1	8.8	30.9
0	-8	-8	14.4	206.7	221.1	4.6	30.4	35.0
-11	-8	-8	13.7	173.3	187.0	4.6	26.3	30.9
-10	-8	-8	37.9	163.8	201.8	7.0	24.7	31.7
-9	-8	-8	121.2	104.3	225.6	13.9	16.9	30.9
-8	-8	-8	146.1	66.4	212.5	17.2	10.1	27.3

log [GnRH]	log [E]	log [B]	MEAN LH sec (ng/plate)	MEAN LH cell (ng/plate)	MEAN LH total (ng/plate)	MEAN FSH sec (ng/plate)	MEAN FSH cell (ng/plate)	MEAN FSH total (ng/plate)
-7	-8	-8	155.0	50.9	206.0	18.5	8.6	27.0
0	-8	-6	11.8	182.8	194.6	5.4	26.6	32.0
-11	-8	-6	14.7	177.7	192.3	5.8	26.5	32.2
-10	-8	-6	43.4	160.3	203.7	8.9	25.3	34.2
-9	-8	-6	113.5	96.7	210.2	15.8	16.4	32.2
-8	-8	-6	160.6	51.8	212.4	22.3	10.5	32.9
-7	-8	-6	179.3	38.6	217.9	22.2	8.6	30.8

EXPERIMENT II LONG-TERM INCUBATION (48 h) WITH E_2 AND B

Time:

1 = plates removed after initial 48 h (before steroid treatment)

2 = plates removed after 6 h of steroid treatment

3 = plates removed after 48 h of treatment with steroids

4 = plates removed after 48 h of pretreatment with steroids and 6 h of treatment with steroids and GnRH

LEB Exp #	Time	log [GnRH]	log [E]	log [B]	[LH] sec (ng/plate) (ng/ml)1.2	[LH] cell (ng/plate) (ng/ml)1.5	total [LH] (ng/plate)	[FSH] sec (ng/plate) (ng/ml)1.2	[FSH] cell (ng/plate) (ng/ml)1.5	total [FSH] (ng/plate)
1	4	0	0	0	6.38	197.10	203.48	4.51	34.50	39.01
1	4	-11	0	0	8.98	303.60	312.58	4.25	35.85	40.10
1	4	-10	0	0	35.28	143.40	178.68	9.30	34.65	43.95
1	4	-9	0	0	101.40	75.30	176.70	16.08	23.10	39.18
1	4	-8	0	0	116.16	72.75	188.91	18.96	20.40	39.36
1	4	-7	0	0	111.12	64.50	175.62	19.08	23.55	42.63
1	4	0	0	-8	6.46	171.75	178.21	4.51	36.30	40.81
1	4	-11	0	-8	9.98	217.05	227.03	5.17	38.55	43.72
1	4	-10	0	-8	28.56	131.10	159.66	9.78	31.95	41.73
1	4	-9	0	-8	108.60	87.90	196.50	17.52	25.20	42.72
1	4	-8	0	-8	92.52	50.40	142.92	12.12	18.45	30.57
1	4	-7	0	-8	95.52	47.85	143.37	11.52	18.15	29.67
1	4	0	0	-6	5.11	172.95	178.06	5.93	72.30	78.23
1	4	-11	0	-6	8.66	235.20	243.86	7.67	66.30	73.97
1	4	-10	0	-6	36.72	203.40	240.12	14.88	64.20	79.08
1	4	-9	0	-6	89.16	123.45	212.61	23.52	46.95	70.47
1	4	-8	0	-6	98.40	70.80	169.20	25.68	36.15	61.83
1	4	-7	0	-6	116.16	63.75	179.91	28.08	36.30	64.38
1	4	0	-10	0	10.08	205.95	216.03	5.05	34.05	39.10
1	4	-11	-10	0	10.39	198.90	209.29	5.12	37.05	42.17
1	4	-10	-10	0	52.20	144.60	196.80	9.53	32.40	41.93
1	4	-9	-10	0	110.16	69.00	179.16	16.44	23.70	40.14
1	4	-8	-10	0	105.96	72.90	178.86	14.52	22.20	36.72
1	4	-7	-10	0	117.36	71.70	189.06	13.32	17.55	30.87
1	4	0	-10	-8	5.90	206.40	212.30	3.77	36.30	40.07
1	4	-11	-10	-8	8.88	204.15	213.03	4.90	40.50	45.40
1	4	-10	-10	-8	44.52	143.70	188.22	8.90	34.20	43.10
1	4	-9	-10	-8	93.36	76.80	170.16	15.60	23.70	39.30
1	4	-8	-10	-8	112.56	69.30	181.86	17.28	21.00	38.28
1	4	-7	-10	-8	117.60	68.10	185.70	18.00	21.90	39.90
1	4	0	-10	-6	6.84	222.90	229.74	8.23	60.15	68.38
1	4	-11	-10	-6	6.42	198.90	205.32	7.61	72.60	80.21
1	4	-10	-10	-6	24.60	171.90	196.50	11.93	62.85	74.78
1	4	-9	-10	-6	83.76	94.50	178.26	21.48	40.05	61.53
1	4	-8	-10	-6	81.60	76.80	158.40	20.16	32.40	52.56
1	4	-7	-10	-6	93.12	85.65	178.77	23.88	32.10	55.98
1	4	0	-8	0	4.88	175.50	180.38	2.50	33.30	35.80
1	4	-11	-8	0	8.64	178.80	187.44	3.60	34.80	38.40
1	4	-10	-8	0	33.72	141.90	175.62	8.78	30.15	38.93
1	4	-9	-8	0	97.20	83.70	180.90	16.56	20.40	36.96
1	4	-8	-8	0	106.56	79.20	185.76	15.84	21.30	37.14
1	4	-7	-8	0	108.48	59.55	168.03	16.08	22.35	38.43
1	4	0	-8	-8	8.66	172.20	180.86	3.90	33.90	37.80
1	4	-11	-8	-8	10.56	184.50	195.06	4.44	32.55	36.99
1	4	-10	-8	-8	30.72	165.00	195.72	7.70	31.05	38.75
1	4	-9	-8	-8	90.96	91.20	182.16	15.48	26.25	41.73
1	4	-8	-8	-8	72.00	60.00	132.00	11.04	19.20	30.24

LEB Exp #	Time	log [GnRH]	log [E]	log [B]	[LH] sec ng/plate (ng/ml)1.2	[LH] cell ng/plate (ng/ml)1.5	total [LH] ng/plate	[FSH] sec ng/plate (ng/ml)1.2	[FSH] cell ng/plate (ng/ml)1.5	total [FSH] ng/plate
1	4	-7	-8	-8	106.80	59.40	166.20	13.08	19.50	32.58
1	4	0	-8	-6	5.17	164.40	169.57	5.90	56.55	62.45
1	4	-11	-8	-6	6.14	173.40	179.54	7.44	60.45	67.89
1	4	-10	-8	-6	19.80	177.90	197.70	12.24	56.25	68.49
1	4	-9	-8	-6	77.28	89.40	166.68	20.04	39.30	59.34
1	4	-8	-8	-6	85.44	64.80	150.24	19.92	31.95	51.87
1	4	-7	-8	-6	93.60	62.40	156.00	24.48	33.30	57.78
1	1	0	0	0	29.55	195.30	224.85	10.20	32.40	42.60
1	2	0	0	0	7.68	174.60	182.28	1.80	28.35	30.15
1	2	0	0	-8	5.26	194.70	199.96	2.38	31.20	33.58
1	2	0	0	-6	7.34	192.75	200.09	5.74	32.40	38.14
1	2	0	-10	0	8.54	193.50	202.04	2.75	29.70	32.45
1	2	0	-10	-8	7.20	176.10	183.30	2.26	32.10	34.36
1	2	0	-10	-6	8.15	180.90	189.05	5.45	32.55	38.00
1	2	0	-8	0	12.24	177.30	189.54	2.62	30.60	33.22
1	2	0	-8	-8	9.36	205.50	214.86	2.95	27.30	30.25
1	2	0	-8	-6	9.19	178.50	187.69	5.95	31.50	37.45
1	3	0	0	0	15.96	155.40	171.36	19.20	31.05	50.25
1	3	0	0	-8	15.12	151.95	167.07	21.24	28.65	49.89
1	3	0	0	-6	13.92	172.20	186.12	41.88	50.55	92.43
1	3	0	-10	0	16.56	165.90	182.46	21.60	33.90	55.50
1	3	0	-10	-8	17.04	201.90	218.94	23.04	37.50	60.54
1	3	0	-10	-6	16.56	203.10	219.66	40.08	62.55	102.63
1	3	0	-8	0	18.24	156.30	174.54	19.20	33.00	52.20
1	3	0	-8	-8	15.12	156.60	171.72	19.44	35.40	54.84
1	3	0	-8	-6	15.12	196.65	211.77	40.08	62.55	102.63
2	4	0	0	0	4.66	129.90	134.56	3.00	20.25	23.25
2	4	-11	0	0	6.55	159.90	166.45	3.05	21.00	24.05
2	4	-10	0	0	38.40	115.95	154.35	6.02	19.65	25.67
2	4	-9	0	0	83.64	58.65	142.29	12.60	13.68	26.28
2	4	-8	0	0	93.84	50.70	144.54	13.68	13.59	27.27
2	4	-7	0	0	80.52	38.85	119.37	12.72	11.93	24.64
2	4	0	0	-8	4.97	137.25	142.22	3.24	19.35	22.59
2	4	-11	0	-8	6.00	147.60	153.60	3.67	20.55	24.22
2	4	-10	0	-8	34.32	109.50	143.82	6.40	18.60	25.00
2	4	-9	0	-8	74.64	49.80	124.44	11.93	13.77	25.70
2	4	-8	0	-8	71.28	47.40	118.68	10.06	11.18	21.23
2	4	-7	0	-8	78.60	46.20	124.80	9.06	12.06	21.12
2	4	0	0	-6	4.97	131.10	136.07	4.66	30.30	34.96
2	4	-11	0	-6	5.52	144.45	149.97	5.06	31.05	36.11
2	4	-10	0	-6	31.20	125.40	156.60	10.46	30.30	40.76
2	4	-9	0	-6	75.12	81.60	156.72	17.76	22.80	40.56
2	4	-8	0	-6	81.00	62.70	143.70	17.64	21.90	39.54
2	4	-7	0	-6	78.48	86.40	164.88	18.48	20.25	38.73
2	4	0	-10	0	6.67	176.85	183.52	3.14	20.25	23.39
2	4	-11	-10	0	5.18	229.80	234.98	3.07	21.15	24.22
2	4	-10	-10	0	21.84	159.30	181.14	4.69	19.20	23.89

LEB Exp #	Time	log [GnRH]	log [E]	log [B]	[LH] sec ng/plate (ng/ml)1.2	[LH] cell ng/plate (ng/ml)1.5	total [LH] ng/plate	[FSH] sec ng/plate (ng/ml)1.2	[FSH] cell ng/plate (ng/ml)1.5	total [FSH] ng/plate
2	4	-9	-10	0	73.20	70.50	143.70	11.38	13.71	25.09
2	4	-8	-10	0	78.12	55.50	133.62	9.84	11.79	21.63
2	4	-7	-10	0	84.72	63.30	148.02	10.03	11.52	21.55
2	4	0	-10	-8	5.16	180.00	185.16	3.16	19.50	22.66
2	4	-11	-10	-8	4.42	224.55	228.97	3.17	21.00	24.17
2	4	-10	-10	-8	33.84	161.40	195.24	5.12	19.80	24.92
2	4	-9	-10	-8	75.36	71.85	147.21	11.95	15.45	27.40
2	4	-8	-10	-8	78.00	54.00	132.00	11.86	12.23	24.08
2	4	-7	-10	-8	94.92	56.85	151.77	13.44	11.34	24.78
2	4	0	-10	-6	5.11	161.70	166.81	6.26	34.65	40.91
2	4	-11	-10	-6	6.12	181.20	187.32	5.86	34.80	40.66
2	4	-10	-10	-6	20.88	124.80	145.68	8.41	32.40	40.81
2	4	-9	-10	-6	58.80	79.65	138.45	15.36	22.80	38.16
2	4	-8	-10	-6	68.40	63.60	132.00	15.12	17.70	32.82
2	4	-7	-10	-6	61.68	69.90	131.58	13.80	16.95	30.75
2	4	0	-8	0	3.46	157.20	160.66	2.23	19.50	21.73
2	4	-11	-8	0	5.33	171.00	176.33	2.40	19.65	22.05
2	4	-10	-8	0	27.24	142.80	170.04	5.12	18.30	23.42
2	4	-9	-8	0	69.60	76.80	146.40	11.53	13.25	24.78
2	4	-8	-8	0	78.48	63.30	141.78	11.44	11.83	23.27
2	4	-7	-8	0	75.36	57.90	133.26	11.23	11.31	22.54
2	4	0	-8	-8	6.55	149.70	156.25	2.57	18.45	21.02
2	4	-11	-8	-8	5.02	180.00	185.02	2.82	18.60	21.42
2	4	-10	-8	-8	19.92	140.25	160.17	4.90	17.40	22.30
2	4	-9	-8	-8	64.32	71.40	135.72	11.64	12.45	24.09
2	4	-8	-8	-8	68.88	59.25	128.13	9.00	12.51	21.51
2	4	-7	-8	-8	70.80	60.90	131.70	8.59	10.92	19.51
2	4	0	-8	-6	4.15	150.90	155.05	4.37	30.75	35.12
2	4	-11	-8	-6	3.84	274.20	278.04	5.32	36.60	41.92
2	4	-10	-8	-6	12.84	146.70	159.54	8.40	27.00	35.40
2	4	-9	-8	-6	55.32	76.80	132.12	15.12	19.35	34.47
2	4	-8	-8	-6	76.56	65.70	142.26	16.08	17.40	33.48
2	4	-7	-8	-6	76.68	57.90	134.58	16.56	14.34	30.90
2	1	0	0	0	60.90	173.70	234.60	11.67	19.05	30.72
2	2	0	0	0	11.86	160.80	172.66	2.44	15.30	17.74
2	2	0	0	-8	10.60	162.90	173.50	2.69	14.89	17.58
2	2	0	0	-6	11.18	165.45	176.63	4.45	16.20	20.65
2	2	0	-10	0	12.48	183.30	195.78	3.24	15.90	19.14
2	2	0	-10	-8	14.28	172.20	186.48	2.62	16.95	19.57
2	2	0	-10	-6	17.76	184.05	201.81	4.63	17.10	21.73
2	2	0	-8	0	13.08	191.10	204.18	2.83	14.79	17.62
2	2	0	-8	-8	14.88	183.15	198.03	2.90	15.30	18.20
2	2	0	-8	-6	18.36	143.40	161.76	4.21	16.20	20.41
2	3	0	0	0	66.72	143.55	210.27	15.96	15.90	31.86
2	3	0	0	-8	77.04	153.45	230.49	15.72	15.30	31.02
2	3	0	0	-6	73.20	156.90	230.10	35.76	24.90	60.66
2	3	0	-10	0	94.32	157.20	251.52	15.00	14.76	29.76

LEB Exp #	Time	log [GnRH]	log [E]	log [B]	[LH] sec ng/plate (ng/ml)1.2	[LH] cell ng/plate (ng/ml)1.5	total [LH] ng/plate	[FSH] sec ng/plate (ng/ml)1.2	[FSH] cell ng/plate (ng/ml)1.5	total [FSH] ng/plate
2	3	0	-10	-8	41.76	158.10	199.86	15.12	15.45	30.57
2	3	0	-10	-6	37.92	153.60	191.52	34.08	25.20	59.28
2	3	0	-8	0	61.80	151.80	213.60	12.84	13.53	26.37
2	3	0	-8	-8	65.52	176.70	242.22	12.72	12.99	25.71
2	3	0	-8	-6	55.80	174.60	230.40	28.80	23.40	52.20
3	4	0	0	0	4.82	210.45	215.27	3.37	23.10	26.47
3	4	-11	0	0	6.11	168.00	174.11	3.84	25.35	29.19
3	4	-10	0	0	23.76	157.95	181.71	6.72	23.70	30.42
3	4	-9	0	0	72.72	74.40	147.12	13.08	16.05	29.13
3	4	-8	0	0	83.16	66.00	149.16	15.24	15.45	30.69
3	4	-7	0	0	92.04	50.70	142.74	17.28	16.50	33.78
3	4	0	0	-8	7.79	167.40	175.19	4.46	26.10	30.56
3	4	-11	0	-8	6.97	151.50	158.47	4.08	23.70	27.78
3	4	-10	0	-8	13.32	166.80	180.12	5.86	22.20	28.06
3	4	-9	0	-8	59.28	83.10	142.38	13.44	19.05	32.49
3	4	-8	0	-8	74.76	56.40	131.16	13.20	14.64	27.84
3	4	-7	0	-8	86.64	53.25	139.89	13.92	14.22	28.14
3	4	0	0	-6	4.56	161.10	165.66	5.81	38.70	44.51
3	4	-11	0	-6	5.96	165.30	171.26	6.82	41.40	48.22
3	4	-10	0	-6	13.44	148.50	161.94	9.67	40.35	50.02
3	4	-9	0	-6	55.92	84.30	140.22	18.00	28.80	46.80
3	4	-8	0	-6	81.36	67.35	148.71	19.20	24.00	43.20
3	4	-7	0	-6	95.28	52.95	148.23	24.48	24.00	48.48
3	4	0	-10	0	7.54	148.20	155.74	4.27	24.00	28.27
3	4	-11	-10	0	8.04	154.50	162.54	3.97	24.60	28.57
3	4	-10	-10	0	25.92	136.20	162.12	5.45	22.80	28.25
3	4	-9	-10	0	80.40	63.45	143.85	13.80	17.85	31.65
3	4	-8	-10	0	89.28	48.30	137.58	12.96	14.61	27.57
3	4	-7	-10	0	91.44	49.50	140.94	13.68	13.02	26.70
3	4	0	-10	-8	3.83	172.05	175.88	3.36	23.55	26.91
3	4	-11	-10	-8	5.42	149.25	154.67	3.54	24.00	27.54
3	4	-10	-10	-8	20.76	148.35	169.11	5.59	24.30	29.89
3	4	-9	-10	-8	77.40	67.50	144.90	13.32	15.90	29.22
3	4	-8	-10	-8	96.48	67.05	163.53	14.16	14.23	28.39
3	4	-7	-10	-8	96.96	57.15	154.11	16.20	14.37	30.57
3	4	0	-10	-6	9.82	149.25	159.07	7.43	40.20	47.63
3	4	-11	-10	-6	6.41	206.40	212.81	6.19	41.10	47.29
3	4	-10	-10	-6	19.44	136.35	155.79	7.72	35.70	43.42
3	4	-9	-10	-6	62.40	81.90	144.30	17.28	29.70	46.98
3	4	-8	-10	-6	74.40	78.75	153.15	19.08	24.90	43.98
3	4	-7	-10	-6	83.52	67.50	151.02	19.08	26.10	45.18
3	4	0	-8	0	5.18	154.05	159.23	2.83	24.90	27.73
3	4	-11	-8	0	6.70	152.70	159.40	3.67	26.40	30.07
3	4	-10	-8	0	23.40	130.50	153.90	6.17	23.40	29.57
3	4	-9	-8	0	73.68	81.15	154.83	13.44	17.70	31.14
3	4	-8	-8	0	100.68	52.65	153.33	14.40	16.50	30.90
3	4	-7	-8	0	110.16	45.30	155.46	15.72	15.90	31.62

LEB Exp #	Time	log [GnRH]	log [E]	log [B]	[LH] sec ng/plate (ng/ml)1.2	[LH] cell ng/plate (ng/ml)1.5	total [LH] ng/plate	[FSH] sec ng/plate (ng/ml)1.2	[FSH] cell ng/plate (ng/ml)1.5	total [FSH] ng/plate
3	4	0	-8	-8	9.12	158.85	167.97	3.77	24.45	28.22
3	4	-11	-8	-8	7.81	156.00	163.81	3.60	26.10	29.70
3	4	-10	-8	-8	21.12	148.20	169.32	4.90	23.70	28.60
3	4	-9	-8	-8	76.32	76.20	152.52	12.36	19.80	32.16
3	4	-8	-8	-8	91.92	50.70	142.62	12.48	17.40	29.88
3	4	-7	-8	-8	84.36	46.80	131.16	11.96	15.30	27.26
3	4	0	-8	-6	7.36	139.50	146.86	5.78	40.80	46.58
3	4	-11	-8	-6	6.62	137.10	143.72	6.35	43.80	50.15
3	4	-10	-8	-6	15.36	135.00	150.36	8.47	42.30	50.77
3	4	-9	-8	-6	58.92	91.65	150.57	15.36	32.70	48.06
3	4	-8	-8	-6	96.24	54.60	150.84	16.56	25.20	41.76
3	4	-7	-8	-6	93.12	54.90	148.02	18.24	25.80	44.04
3	1	0	0	0	53.40	172.20	225.60	13.23	29.70	42.93
3	2	0	0	0	9.38	132.30	141.68	2.75	24.30	27.05
3	2	0	0	-8	6.50	147.90	154.40	2.80	24.75	27.55
3	2	0	0	-6	9.60	143.10	152.70	4.92	27.90	32.82
3	2	0	-10	0	9.12	159.15	168.27	2.66	25.65	28.31
3	2	0	-10	-8	8.95	129.60	138.55	2.54	25.80	28.34
3	2	0	-10	-6	8.36	135.00	143.36	4.73	27.60	32.33
3	2	0	-8	0	10.56	137.70	148.26	3.00	23.70	26.70
3	2	0	-8	-8	10.94	135.00	145.94	3.05	25.20	28.25
3	2	0	-8	-6	11.29	118.50	129.79	4.36	26.70	31.06
3	3	0	0	0	16.80	131.55	148.35	16.80	27.75	44.55
3	3	0	0	-8	15.84	139.50	155.34	17.40	30.15	47.55
3	3	0	0	-6	15.84	128.40	144.24	30.72	42.00	72.72
3	3	0	-10	0	21.72	123.90	145.62	17.04	26.70	43.74
3	3	0	-10	-8	16.32	126.15	142.47	16.56	28.80	45.36
3	3	0	-10	-6	17.28	118.20	135.48	29.52	41.40	70.92
3	3	0	-8	0	18.12	118.20	136.32	15.36	25.80	41.16
3	3	0	-8	-8	18.24	120.60	138.84	15.24	25.80	41.04
3	3	0	-8	-6	18.24	120.60	138.84	28.32	38.70	67.02

Time	log [GnRH]	log [E]	log [B]	MEAN [LH] secreted ng/plate	MEAN [LH] cell ng/plate	MEAN [FSH] secreted ng/plate	MEAN [FSH] cell ng/plate	MEAN [LH] total ng/plate	MEAN [FSH] total ng/plate
4	0	0	0	5.29	179.15	3.63	25.95	184.44	29.58
4	-11	0	0	7.21	210.50	3.71	27.40	217.71	31.11
4	-10	0	0	32.48	139.10	7.35	26.00	171.58	33.35
4	-9	0	0	85.92	69.45	13.92	17.61	155.37	31.53
4	-8	0	0	97.72	63.15	15.96	16.48	160.87	32.44
4	-7	0	0	94.56	51.35	16.36	17.32	145.91	33.68
4	0	0	-8	6.40	158.80	4.07	27.25	165.20	31.32
4	-11	0	-8	7.65	172.05	4.31	27.60	179.70	31.91
4	-10	0	-8	25.40	135.80	7.34	24.25	161.20	31.59
4	-9	0	-8	80.84	73.60	14.30	19.34	154.44	33.64
4	-8	0	-8	79.52	51.40	11.79	14.76	130.92	26.55
4	-7	0	-8	86.92	49.10	11.50	14.81	136.02	26.31
4	0	0	-6	4.88	155.05	5.46	47.10	159.93	52.56
4	-11	0	-6	6.72	181.65	6.52	46.25	188.37	52.77
4	-10	0	-6	27.12	159.10	11.67	44.95	186.22	56.62
4	-9	0	-6	73.40	96.45	19.76	32.85	169.85	52.61
4	-8	0	-6	86.92	66.95	20.84	27.35	153.87	48.19
4	-7	0	-6	96.64	67.70	23.68	26.85	164.34	50.53
4	0	-10	0	8.10	177.00	4.16	26.10	185.10	30.26
4	-11	-10	0	7.87	194.40	4.06	27.60	202.27	31.66
4	-10	-10	0	33.32	146.70	6.56	24.80	180.02	31.36
4	-9	-10	0	87.92	67.65	13.87	18.42	155.57	32.29
4	-8	-10	0	91.12	58.90	12.44	16.20	150.02	28.64
4	-7	-10	0	97.84	61.50	12.34	14.03	159.34	26.37
4	0	-10	-8	4.96	186.15	3.43	26.45	191.11	29.88
4	-11	-10	-8	6.24	192.65	3.87	28.50	198.89	32.37
4	-10	-10	-8	33.04	151.15	6.54	26.10	184.19	32.64
4	-9	-10	-8	82.04	72.05	13.62	18.35	154.09	31.97
4	-8	-10	-8	95.68	63.45	14.43	15.82	159.13	30.25
4	-7	-10	-8	103.16	60.70	15.88	15.87	163.86	31.75
4	0	-10	-6	7.26	177.95	7.31	45.00	185.21	52.31
4	-11	-10	-6	6.32	195.50	6.55	49.50	201.82	56.05
4	-10	-10	-6	21.64	144.35	9.35	43.65	165.99	53.00
4	-9	-10	-6	68.32	85.35	18.04	30.85	153.67	48.89
4	-8	-10	-6	74.80	73.05	18.12	25.00	147.85	43.12
4	-7	-10	-6	79.44	74.35	18.92	25.05	153.79	43.97
4	0	-8	0	4.51	162.25	2.52	25.90	166.76	28.42
4	-11	-8	0	6.89	167.50	3.22	26.95	174.39	30.17
4	-10	-8	0	28.12	138.40	6.69	23.95	166.52	30.64
4	-9	-8	0	80.16	80.55	13.84	17.11	160.71	30.96
4	-8	-8	0	95.24	65.05	13.89	16.54	160.29	30.44
4	-7	-8	0	98.00	54.25	14.34	16.52	152.25	30.86
4	0	-8	-8	8.11	160.25	3.41	25.60	168.36	29.01
4	-11	-8	-8	7.80	173.50	3.62	25.75	181.30	29.37
4	-10	-8	-8	23.92	151.15	5.83	24.05	175.07	29.88
4	-9	-8	-8	77.20	79.60	13.16	19.50	156.80	32.66
4	-8	-8	-8	77.60	56.65	10.84	16.37	134.25	27.21

Time	log [GnRH]	log [E]	log [B]	MEAN [LH] secreted ng/plate	MEAN [LH] cell ng/plate	MEAN [FSH] secreted ng/plate	MEAN [FSH] cell ng/plate	MEAN [LH] total ng/plate	MEAN [FSH] total ng/plate
4	-7	-8	-8	87.32	55.70	11.21	15.24	143.02	26.45
4	0	-8	-6	5.56	151.60	5.35	42.70	157.16	48.05
4	-11	-8	-6	5.54	194.90	6.37	46.95	200.44	53.32
4	-10	-8	-6	16.00	153.20	9.70	41.85	169.20	51.55
4	-9	-8	-6	63.84	85.95	16.84	30.45	149.79	47.29
4	-8	-8	-6	86.08	61.70	17.52	24.85	147.78	42.37
4	-7	-8	-6	87.80	58.40	19.76	24.48	146.20	44.24
1	0	0	0	47.95	180.40	11.70	27.05	228.35	38.75
2	0	0	0	9.64	155.90	2.33	22.65	165.54	24.98
2	0	0	-8	7.45	168.50	2.62	23.61	175.95	26.23
2	0	0	-6	9.38	167.10	5.04	25.50	176.48	30.54
2	0	-10	0	10.05	178.65	2.88	23.75	188.70	26.63
2	0	-10	-8	10.14	159.30	2.47	24.95	169.44	27.42
2	0	-10	-6	11.42	166.65	4.94	25.75	178.07	30.69
2	0	-8	0	11.96	168.70	2.82	23.03	180.66	25.85
2	0	-8	-8	11.73	174.55	2.97	22.60	186.28	25.57
2	0	-8	-6	12.95	146.80	4.84	24.80	159.75	29.64
3	0	0	0	33.16	143.50	17.32	24.90	176.66	42.22
3	0	0	-8	36.00	148.30	18.12	24.70	184.30	42.82
3	0	0	-6	34.32	152.50	36.12	39.15	186.82	75.27
3	0	-10	0	44.20	149.00	17.88	25.12	193.20	43.00
3	0	-10	-8	25.04	162.05	18.24	27.25	187.09	45.49
3	0	-10	-6	23.92	158.30	34.56	43.05	182.22	77.61
3	0	-8	0	32.72	142.10	15.80	24.11	174.82	39.91
3	0	-8	-8	32.96	151.30	15.80	24.73	184.26	40.53
3	0	-8	-6	29.72	163.95	32.40	41.55	193.67	73.95

EXPERIMENT III SHORT-TERM INCUBATION (6 h) WITH P_4 AND B

Time: 1 = plates removed after initial 48 h (before steroid treatment)

4 = plates removed after 6 h of steroid and GnRH treatment

PB Exp #	log [GnRH]	log [P]	log [B]	time	LH sec (ng/plate) (ng/ml)1.2	LH cells (ng/plate)	LH total (ng/plate)	FSH sec (ng/plate) (ng/ml)1.2	FSH cells (ng/plate)	FSH total (ng/plate)
1	0	0	0	4	19.44	127.60	147.04	3.88	19.52	23.40
1	-11	0	0	4	16.32	166.20	182.52	4.86	23.07	27.93
1	-10	0	0	4	45.36	147.30	192.66	6.66	22.39	29.05
1	-9	0	0	4	114.96	60.70	175.66	20.95	10.71	31.66
1	-8	0	0	4	144.12	28.00	172.12	31.73	5.87	37.60
1	-7	0	0	4	157.68	26.10	183.78	33.40	6.06	39.46
1	0	0	-8	4	15.12	168.00	183.12	4.04	22.96	27.00
1	-11	0	-8	4	15.72	157.30	173.02	4.02	25.67	29.69
1	-10	0	-8	4	53.76	144.60	198.36	7.37	22.38	29.75
1	-9	0	-8	4	122.16	50.60	172.76	22.34	11.19	33.53
1	-8	0	-8	4	144.12	28.40	172.52	28.30	6.62	34.92
1	-7	0	-8	4	161.28	26.60	187.88	29.32	6.03	35.35
1	0	0	-6	4	14.76	125.20	139.96	6.98	30.12	37.10
1	-11	0	-6	4	15.12	152.90	168.02	6.90	29.32	36.22
1	-10	0	-6	4	60.48	87.90	148.38	15.86	22.81	38.67
1	-9	0	-6	4	120.60	36.80	157.40	39.95	12.11	52.06
1	-8	0	-6	4	133.68	22.40	156.08	39.92	6.92	46.84
1	-7	0	-6	4	138.72	22.60	161.32	34.42	6.30	40.72
1	0	-7.7	0	4	13.80	140.30	154.10	7.46	32.92	40.38
1	-11	-7.7	0	4	14.40	130.20	144.60	6.47	33.25	39.72
1	-10	-7.7	0	4	36.00	65.20	101.20	11.83	21.47	33.30
1	-9	-7.7	0	4	91.56	44.40	135.96	26.51	11.43	37.94
1	-8	-7.7	0	4	120.60	29.80	150.40	35.77	7.40	43.17
1	-7	-7.7	0	4	132.72	25.80	158.52	39.68	6.36	46.04
1	0	-7.7	-8	4	16.80	113.40	130.20	8.06	25.52	33.58
1	-11	-7.7	-8	4	12.72	150.40	163.12	7.07	26.85	33.92
1	-10	-7.7	-8	4	43.68	142.50	186.18	13.08	22.29	35.37
1	-9	-7.7	-8	4	98.64	48.20	146.84	35.05	11.05	46.10
1	-8	-7.7	-8	4	125.64	27.60	153.24	38.20	6.40	44.60
1	-7	-7.7	-8	4	121.44	20.40	141.84	40.62	5.86	46.48
1	0	-7.7	-6	4	13.68	129.40	143.08	8.51	28.43	36.94
1	-11	-7.7	-6	4	12.00	143.00	155.00	7.04	30.01	37.05
1	-10	-7.7	-6	4	24.00	105.40	129.40	8.87	22.39	31.26
1	-9	-7.7	-6	4	87.00	43.70	130.70	22.12	12.49	34.61
1	-8	-7.7	-6	4	120.00	27.00	147.00	25.32	7.16	32.48
1	-7	-7.7	-6	4	138.60	21.20	159.80	28.82	6.37	35.19
1	0	-7.0	0	4	12.60	129.50	142.10	5.59	27.88	33.47
1	-11	-7.0	0	4	17.52	144.90	162.42	6.01	33.42	39.43
1	-10	-7.0	0	4	55.56	101.60	157.16	13.90	25.90	39.80
1	-9	-7.0	0	4	96.00	42.00	138.00	24.30	12.53	36.83
1	-8	-7.0	0	4	112.80	25.40	138.20	27.78	7.61	35.39
1	-7	-7.0	0	4	145.80	26.20	172.00	31.31	5.85	37.16
1	0	-7.0	-8	4	14.88	142.20	157.08	5.56	30.46	36.02
1	-11	-7.0	-8	4	16.08	129.80	145.88	5.32	31.44	36.76
1	-10	-7.0	-8	4	52.56	82.70	135.26	12.04	22.29	34.33
1	-9	-7.0	-8	4	105.24	40.10	145.34	19.69	11.43	31.12
1	-8	-7.0	-8	4	127.56	25.30	152.86	27.10	7.78	34.88

PB Exp #	log [GnRH]	log [P]	log [B]	time	LH sec (ng/plate) (ng/ml)1.2	LH cells (ng/plate)	LH total (ng/plate)	FSH sec (ng/plate) (ng/ml)1.2	FSH cells (ng/plate)	FSH total (ng/plate)
1	-7	-7.0	-8	4	132.24	20.50	152.74	27.77	5.88	33.65
1	0	-7.0	-6	4	14.40	128.00	142.40	5.35	31.64	36.99
1	-11	-7.0	-6	4	15.84	136.60	152.44	5.59	30.18	35.77
1	-10	-7.0	-6	4	55.92	96.20	152.12	11.98	23.81	35.79
1	-9	-7.0	-6	4	107.64	46.80	154.44	20.22	12.79	33.01
1	-8	-7.0	-6	4	129.12	28.70	157.82	27.24	8.51	35.75
1	-7	-7.0	-6	4	133.80	25.80	159.60	31.16	7.17	38.33
2	0	0	0	4	7.90	137.20	145.10	1.44	16.20	17.64
2	-11	0	0	4	7.97	128.90	136.87	2.23	16.40	18.63
2	-10	0	0	4	27.36	130.60	157.96	3.23	16.50	19.73
2	-9	0	0	4	87.36	49.00	136.36	9.70	11.10	20.80
2	-8	0	0	4	96.24	28.40	124.64	14.16	8.14	22.30
2	-7	0	0	4	99.36	23.60	122.96	13.44	7.56	21.00
2	0	0	-8	4	8.51	128.00	136.51	1.93	16.60	18.53
2	-11	0	-8	4	8.95	118.40	127.35	1.46	16.60	18.06
2	-10	0	-8	4	22.92	118.10	141.02	3.04	15.20	18.24
2	-9	0	-8	4	80.88	42.20	123.08	11.06	10.50	21.56
2	-8	0	-8	4	101.04	27.30	128.34	13.32	8.13	21.45
2	-7	0	-8	4	106.32	25.60	131.92	13.56	7.84	21.40
2	0	0	-6	4	7.39	129.20	136.59	2.57	15.40	17.97
2	-11	0	-6	4	9.80	130.60	140.40	2.88	18.30	21.18
2	-10	0	-6	4	53.88	108.10	161.98	7.49	16.10	23.59
2	-9	0	-6	4	99.24	33.60	132.84	13.20	9.60	22.80
2	-8	0	-6	4	100.08	25.40	125.48	13.92	7.30	21.22
2	-7	0	-6	4	99.84	23.00	122.84	16.08	8.20	24.28
2	0	-7.7	0	4	10.37	137.60	147.97	4.31	18.80	23.11
2	-11	-7.7	0	4	9.95	109.70	119.65	4.27	16.20	20.47
2	-10	-7.7	0	4	42.72	93.40	136.12	7.01	14.30	21.31
2	-9	-7.7	0	4	85.92	36.40	122.32	14.64	10.80	25.44
2	-8	-7.7	0	4	97.44	24.00	121.44	13.92	8.33	22.25
2	-7	-7.7	0	4	112.32	22.00	134.32	15.00	7.46	22.46
2	0	-7.7	-8	4	10.88	117.40	128.28	3.96	16.80	20.76
2	-11	-7.7	-8	4	10.39	109.40	119.79	4.20	17.20	21.40
2	-10	-7.7	-8	4	43.44	108.20	151.64	6.88	16.00	22.88
2	-9	-7.7	-8	4	69.84	47.30	117.14	10.10	10.60	20.70
2	-8	-7.7	-8	4	100.32	21.50	121.82	15.60	7.44	23.04
2	-7	-7.7	-8	4	102.24	25.10	127.34	16.08	6.96	23.04
2	0	-7.7	-6	4	10.14	139.20	149.34	5.05	16.60	21.65
2	-11	-7.7	-6	4	11.04	124.90	135.94	4.79	14.60	19.39
2	-10	-7.7	-6	4	32.88	124.80	157.68	5.70	13.80	19.50
2	-9	-7.7	-6	4	94.32	37.80	132.12	12.48	9.49	21.97
2	-8	-7.7	-6	4	100.08	24.90	124.98	13.80	6.80	20.60
2	-7	-7.7	-6	4	102.00	21.40	123.40	13.80	5.90	19.70
2	0	-7.0	0	4	6.43	124.20	130.63	3.80	16.00	19.80
2	-11	-7.0	0	4	9.55	123.00	132.55	4.30	17.40	21.70
2	-10	-7.0	0	4	53.64	86.90	140.54	7.43	15.00	22.43
2	-9	-7.0	0	4	89.64	34.00	123.64	13.44	10.20	23.64

PB Exp #	log [GnRH]	log [P]	log [B]	time	LH sec (ng/plate) (ng/ml)1.2	LH cells (ng/plate)	LH total (ng/plate)	FSH sec (ng/plate) (ng/ml)1.2	FSH cells (ng/plate)	FSH total (ng/plate)
2	-8	-7.0	0	4	105.84	21.40	127.24	14.64	6.95	21.59
2	-7	-7.0	0	4	110.40	23.20	133.60	16.92	6.93	23.85
2	0	-7.0	-8	4	11.62	114.10	125.72	6.13	17.00	23.13
2	-11	-7.0	-8	4	11.02	111.20	122.22	5.74	16.80	22.54
2	-10	-7.0	-8	4	43.20	94.20	137.40	8.14	15.40	23.54
2	-9	-7.0	-8	4	85.44	36.00	121.44	15.60	10.50	26.10
2	-8	-7.0	-8	4	100.68	23.40	124.08	16.20	6.42	22.62
2	-7	-7.0	-8	4	108.24	22.60	130.84	16.08	5.89	21.97
2	0	-7.0	-6	4	8.88	111.30	120.18	5.02	16.60	21.62
2	-11	-7.0	-6	4	11.45	106.50	117.95	5.54	17.60	23.14
2	-10	-7.0	-6	4	53.28	85.70	138.98	9.19	13.50	22.69
2	-9	-7.0	-6	4	92.40	35.50	127.90	14.88	8.94	23.82
2	-8	-7.0	-6	4	107.04	21.00	128.04	16.80	7.01	23.81
2	-7	-7.0	-6	4	130.80	21.00	151.80	18.24	7.65	25.89
3	0	0	0	4	11.14	183.25	194.39	2.99	17.32	20.31
3	-11	0	0	4	12.10	188.25	200.35	3.50	16.66	20.16
3	-10	0	0	4	31.97	158.70	190.67	4.60	15.54	20.14
3	-9	0	0	4	120.73	61.75	182.48	11.68	10.07	21.74
3	-8	0	0	4	145.42	37.40	182.82	14.34	7.96	22.30
3	-7	0	0	4	179.15	30.30	209.45	13.57	7.08	20.65
3	0	0	-8	4	11.11	188.60	199.71	2.72	18.02	20.75
3	-11	0	-8	4	13.75	164.90	178.65	2.58	16.80	19.38
3	-10	0	-8	4	31.68	157.60	189.28	4.16	16.87	21.03
3	-9	0	-8	4	116.96	61.20	178.16	12.54	10.46	22.99
3	-8	0	-8	4	138.04	27.60	165.64	13.56	7.18	20.73
3	-7	0	-8	4	127.94	26.50	154.44	12.98	6.23	19.21
3	0	0	-6	4	14.21	170.60	184.81	4.31	17.39	21.70
3	-11	0	-6	4	14.74	184.00	198.74	4.43	19.00	23.43
3	-10	0	-6	4	49.99	139.30	189.29	7.50	16.75	24.25
3	-9	0	-6	4	127.20	50.10	177.30	14.45	10.10	24.55
3	-8	0	-6	4	135.77	31.10	166.87	16.75	7.91	24.66
3	-7	0	-6	4	128.56	26.10	154.66	16.24	7.53	23.77
3	0	-7.7	0	4	15.96	145.20	161.16	4.54	17.34	21.88
3	-11	-7.7	0	4	16.92	147.80	164.72	4.87	18.68	23.55
3	-10	-7.7	0	4	47.34	125.70	173.04	9.11	16.18	25.29
3	-9	-7.7	0	4	133.15	54.40	187.55	17.57	10.30	27.87
3	-8	-7.7	0	4	147.67	30.60	178.27	16.28	8.16	24.44
3	-7	-7.7	0	4	153.60	23.00	176.60	16.34	7.44	23.77
3	0	-7.7	-8	4	11.64	171.00	182.64	5.15	19.55	24.70
3	-11	-7.7	-8	4	16.61	156.20	172.81	6.26	18.36	24.61
3	-10	-7.7	-8	4	36.78	168.40	205.18	8.23	15.50	23.73
3	-9	-7.7	-8	4	117.38	58.20	175.58	16.03	11.50	27.53
3	-8	-7.7	-8	4	152.54	30.60	183.14	18.13	9.41	27.53
3	-7	-7.7	-8	4	172.69	25.10	197.79	19.08	9.05	28.13
3	0	-7.7	-6	4	15.59	131.80	147.39	5.69	18.85	24.54
3	-11	-7.7	-6	4	16.99	130.60	147.59	5.06	18.09	23.15
3	-10	-7.7	-6	4	40.45	111.20	151.65	8.06	16.37	24.43

PB Exp #	log [GnRH]	log [P]	log [B]	time	LH sec (ng/plate) (ng/ml)1.2	LH cells (ng/plate)	LH total (ng/plate)	FSH sec (ng/plate) (ng/ml)1.2	FSH cells (ng/plate)	FSH total (ng/plate)
3	-9	-7.7	-6	4	129.72	51.90	181.62	17.41	10.79	28.20
3	-8	-7.7	-6	4	154.56	26.00	180.56	16.00	7.67	23.67
3	-7	-7.7	-6	4	158.26	17.50	175.76	15.24	5.97	21.21
3	0	-7.0	0	4	18.24	141.40	159.64	5.71	18.12	23.83
3	-11	-7.0	0	4	27.61	145.10	172.71	6.26	16.91	23.18
3	-10	-7.0	0	4	51.86	104.90	156.76	9.19	13.66	22.85
3	-9	-7.0	0	4	131.47	46.00	177.47	15.17	9.73	24.90
3	-8	-7.0	0	4	133.13	27.80	160.93	16.10	7.16	23.26
3	-7	-7.0	0	4	144.49	26.20	170.69	17.06	6.69	23.75
3	0	-7.0	-8	4	18.34	153.30	171.64	5.71	18.47	24.18
3	-11	-7.0	-8	4	23.04	145.40	168.44	5.74	17.28	23.02
3	-10	-7.0	-8	4	52.34	115.80	168.14	9.28	15.42	24.70
3	-9	-7.0	-8	4	127.20	55.00	182.20	17.44	9.96	27.40
3	-8	-7.0	-8	4	133.92	31.40	165.32	17.47	7.39	24.86
3	-7	-7.0	-8	4	130.98	25.00	155.98	15.40	6.38	21.77
3	0	-7.0	-6	4	17.64	145.60	163.24	5.52	16.36	21.88
3	-11	-7.0	-6	4	20.04	153.40	173.44	6.07	16.27	22.34
3	-10	-7.0	-6	4	49.64	112.20	161.84	9.16	14.23	23.39
3	-9	-7.0	-6	4	111.84	58.20	170.04	16.99	9.70	26.69
3	-8	-7.0	-6	4	138.24	33.10	171.34	17.05	7.49	24.54
3	-7	-7.0	-6	4	149.76	24.90	174.66	18.06	6.81	24.88
1	0	0	0	1	41.16	163.40	204.56	13.68	27.70	41.38
2	0	0	0	1	55.68	121.80	177.48	10.32	20.10	30.42
3	0	0	0	1	61.44	222.60	284.04	10.56	19.70	30.26

log [GnRH]	log [P]	log [B]	time	Mean [LH] sec	Mean [LH] cells	Mean [LH] total	Mean [FSH] sec	Mean [FSH] cells	Mean [FSH] total
0	0	0	4	12.82	149.35	162.17	2.77	17.68	20.45
-11	0	0	4	12.13	161.12	173.24	3.53	18.71	22.24
-10	0	0	4	34.90	145.53	180.43	4.83	18.14	22.97
-9	0	0	4	107.68	57.15	164.83	14.11	10.63	24.73
-8	0	0	4	128.59	31.27	159.86	20.08	7.32	27.40
-7	0	0	4	145.40	26.67	172.06	20.14	6.90	27.04
0	0	-8	4	11.58	161.53	173.11	2.90	19.20	22.09
-11	0	-8	4	12.81	146.87	159.67	2.69	19.69	22.38
-10	0	-8	4	36.12	140.10	176.22	4.86	18.15	23.00
-9	0	-8	4	106.67	51.33	158.00	15.32	10.71	26.03
-8	0	-8	4	127.73	27.77	155.50	18.39	7.31	25.70
-7	0	-8	4	131.85	26.23	158.08	18.62	6.70	25.32
0	0	-6	4	12.12	141.67	153.79	4.62	20.97	25.59
-11	0	-6	4	13.22	155.83	169.05	4.74	22.21	26.94
-10	0	-6	4	54.78	111.77	166.55	10.28	18.55	28.84
-9	0	-6	4	115.68	40.17	155.85	22.53	10.60	33.14
-8	0	-6	4	123.18	26.30	149.48	23.53	7.38	30.91
-7	0	-6	4	122.37	23.90	146.27	22.25	7.34	29.59
0	-7.7	0	4	13.38	141.03	154.41	5.44	23.02	28.46
-11	-7.7	0	4	13.76	129.23	142.99	5.20	22.71	27.91
-10	-7.7	0	4	42.02	94.77	136.79	9.32	17.32	26.63
-9	-7.7	0	4	103.54	45.07	148.61	19.57	10.84	30.42
-8	-7.7	0	4	121.90	28.13	150.04	21.99	7.96	29.96
-7	-7.7	0	4	132.88	23.60	156.48	23.67	7.09	30.76
0	-7.7	-8	4	13.11	133.93	147.04	5.72	20.62	26.35
-11	-7.7	-8	4	13.24	138.67	151.91	5.84	20.80	26.64
-10	-7.7	-8	4	41.30	139.70	181.00	9.40	17.93	27.33
-9	-7.7	-8	4	95.29	51.23	146.52	20.40	11.05	31.45
-8	-7.7	-8	4	126.17	26.57	152.73	23.97	7.75	31.72
-7	-7.7	-8	4	132.12	23.53	155.66	25.26	7.29	32.55
0	-7.7	-6	4	13.14	133.47	146.60	6.42	21.29	27.71
-11	-7.7	-6	4	13.34	132.83	146.18	5.63	20.90	26.53
-10	-7.7	-6	4	32.44	113.80	146.24	7.54	17.52	25.06
-9	-7.7	-6	4	103.68	44.47	148.15	17.34	10.92	28.26
-8	-7.7	-6	4	124.88	25.97	150.85	18.37	7.21	25.58
-7	-7.7	-6	4	132.95	20.03	152.99	19.29	6.08	25.37
0	-7.0	0	4	12.42	131.70	144.12	5.04	20.67	25.70
-11	-7.0	0	4	18.23	137.67	155.89	5.52	22.58	28.10
-10	-7.0	0	4	53.69	97.80	151.49	10.17	18.19	28.36
-9	-7.0	0	4	105.70	40.67	146.37	17.64	10.82	28.46
-8	-7.0	0	4	117.26	24.87	142.12	19.51	7.24	26.75
-7	-7.0	0	4	133.56	25.20	158.76	21.76	6.49	28.25
0	-7.0	-8	4	14.94	136.53	151.48	5.80	21.98	27.78
-11	-7.0	-8	4	16.71	128.80	145.51	5.60	21.84	27.44
-10	-7.0	-8	4	49.37	97.57	146.93	9.82	17.70	27.52
-9	-7.0	-8	4	105.96	43.70	149.66	17.58	10.63	28.21
-8	-7.0	-8	4	120.72	26.70	147.42	20.26	7.20	27.45

log [GnRH]	log [P]	log [B]	time	Mean [LH] sec	Mean [LH] cells	Mean [LH] total	Mean [FSH] sec	Mean [FSH] cells	Mean [FSH] total
-7	-7.0	-8	4	123.82	22.70	146.52	19.75	6.05	25.80
0	-7.0	-6	4	13.64	128.30	141.94	5.30	21.53	26.83
-11	-7.0	-6	4	15.78	132.17	147.94	5.73	21.35	27.08
-10	-7.0	-6	4	52.95	98.03	150.98	10.11	17.18	27.29
-9	-7.0	-6	4	103.96	46.83	150.79	17.36	10.47	27.84
-8	-7.0	-6	4	124.80	27.60	152.40	20.36	7.67	28.03
-7	-7.0	-6	4	138.12	23.90	162.02	22.49	7.21	29.70
0	0	0	1	52.76	169.27	222.03	11.52	22.50	34.02

EXPERIMENT IV LONG-TERM INCUBATION (48 h) WITH P_4 AND B

Time: 3 = plates removed after 48 h of treatment with steroids

4 = plates removed after 48 h of pretreatment with steroids and 6 h of treatment with steroids and GnRH

LPB Exp #	log [GnRH]	log [P]	log [B]	time	LH sec (ng/plate) (ng/ml)1.2	LH cells (ng/plate)	LH total (ng/plate)	FSH sec (ng/plate) (ng/ml)1.2	FSH cells (ng/plate)	FSH total (ng/plate)
1	0	0	0	4	5.71	134.60	140.31	3.10	26.40	29.50
1	-11	0	0	4	6.17	152.90	159.07	3.58	28.60	32.18
1	-10	0	0	4	25.20	111.10	136.30	5.18	25.05	30.23
1	-9	0	0	4	77.76	64.10	141.86	12.72	19.50	32.22
1	-8	0	0	4	111.36	48.40	159.76	14.16	18.00	32.16
1	-7	0	0	4	112.20	45.20	157.40	14.64	16.40	31.04
1	0	0	-8	4	6.46	123.60	130.06	3.65	27.10	30.75
1	-11	0	-8	4	6.72	121.80	128.52	4.20	26.60	30.80
1	-10	0	-8	4	27.84	109.90	137.74	5.54	25.70	31.24
1	-9	0	-8	4	79.92	66.20	146.12	11.71	20.50	32.21
1	-8	0	-8	4	114.00	42.80	156.80	14.40	16.50	30.90
1	-7	0	-8	4	109.56	44.30	153.86	15.36	15.80	31.16
1	0	0	-6	4	4.66	134.80	139.46	4.92	36.80	41.72
1	-11	0	-6	4	4.28	130.60	134.88	4.87	37.10	41.97
1	-10	0	-6	4	19.20	107.50	126.70	5.71	33.05	38.76
1	-9	0	-6	4	67.44	90.00	157.44	14.16	27.30	41.46
1	-8	0	-6	4	94.08	64.40	158.48	18.72	23.90	42.62
1	-7	0	-6	4	108.12	54.60	162.72	21.84	22.25	44.09
1	0	-7.7	0	4	6.26	141.40	147.66	6.58	37.20	43.78
1	-11	-7.7	0	4	6.42	149.60	156.02	5.47	33.50	38.97
1	-10	-7.7	0	4	24.60	123.20	147.80	6.49	29.60	36.09
1	-9	-7.7	0	4	72.00	91.00	163.00	15.00	27.00	42.00
1	-8	-7.7	0	4	97.44	55.00	152.44	19.08	22.40	41.48
1	-7	-7.7	0	4	100.68	35.00	135.68	20.40	19.40	39.80
1	0	-7.7	-8	4	4.10	113.80	117.90	4.92	33.60	38.52
1	-11	-7.7	-8	4	4.18	117.80	121.98	4.94	34.35	39.29
1	-10	-7.7	-8	4	20.40	114.40	134.80	7.20	31.65	38.85
1	-9	-7.7	-8	4	6.24	79.50	85.74	13.92	26.85	40.77
1	-8	-7.7	-8	4	88.56	59.80	148.36	18.24	22.40	40.64
1	-7	-7.7	-8	4	86.40	41.40	127.80	19.44	20.10	39.54
1	0	-7.7	-6	4	5.40	145.20	150.60	6.11	38.90	45.01
1	-11	-7.7	-6	4	5.76	136.80	142.56	6.50	40.70	47.20
1	-10	-7.7	-6	4	22.08	110.40	132.48	8.30	39.45	47.75
1	-9	-7.7	-6	4	61.44	67.80	129.24	14.88	28.00	42.88
1	-8	-7.7	-6	4	86.16	43.40	129.56	18.24	22.60	40.84
1	-7	-7.7	-6	4	90.96	40.10	131.06	20.52	21.20	41.72
1	0	-7.0	0	4	3.50	122.30	125.80	5.80	38.05	43.85
1	-11	-7.0	0	4	4.20	121.00	125.20	6.32	38.05	44.37
1	-10	-7.0	0	4	12.12	103.60	115.72	7.72	35.60	43.32
1	-9	-7.0	0	4	48.48	82.30	130.78	13.20	28.60	41.80
1	-8	-7.0	0	4	86.52	50.50	137.02	18.60	23.20	41.80
1	-7	-7.0	0	4	94.56	43.60	138.16	20.40	21.40	41.80
1	0	-7.0	-8	4	5.35	127.00	132.35	6.56	38.40	44.96
1	-11	-7.0	-8	4	4.74	129.00	133.74	6.35	40.50	46.85
1	-10	-7.0	-8	4	16.80	110.20	127.00	7.30	36.15	43.45
1	-9	-7.0	-8	4	61.32	71.20	132.52	14.64	27.15	41.79
1	-8	-7.0	-8	4	87.12	42.60	129.72	18.48	22.75	41.23

LPB Exp #	log [GnRH]	log [P]	log [B]	time	LH sec (ng/plate) (ng/ml)1.2	LH cells (ng/plate)	LH total (ng/plate)	FSH sec (ng/plate) (ng/ml)1.2	FSH cells (ng/plate)	FSH total (ng/plate)
1	-7	-7.0	-8	4	98.52	31.40	129.92	20.76	19.40	40.16
1	0	-7.0	-6	4	5.45	107.60	113.05	6.41	36.20	42.61
1	-11	-7.0	-6	4	5.71	127.10	132.81	6.38	38.00	44.38
1	-10	-7.0	-6	4	20.76	118.00	138.76	7.44	35.90	43.34
1	-9	-7.0	-6	4	57.12	79.00	136.12	13.44	31.15	44.59
1	-8	-7.0	-6	4	88.56	47.20	135.76	18.60	23.90	42.50
1	-7	-7.0	-6	4	99.12	36.80	135.92	20.28	21.25	41.53
1	0	0	0	3	28.08	100.20	128.28	18.84	33.00	51.84
1	0	0	-8	3	27.36	122.60	149.96	18.72	33.20	51.92
1	0	0	-6	3	20.16	133.40	153.56	29.52	21.40	50.92
1	0	-7.7	0	3	21.96	127.30	149.26	27.36	33.30	60.66
1	0	-7.7	-8	3	23.40	123.40	146.80	26.88	34.60	61.48
1	0	-7.7	-6	3	18.72	124.50	143.22	29.52	38.20	67.72
1	0	-7.0	0	3	24.00	114.20	138.20	31.92	39.80	71.72
1	0	-7.0	-8	3	17.16	156.00	173.16	30.96	41.00	71.96
1	0	-7.0	-6	3	20.40	126.50	146.90	30.96	36.20	67.16
2	0	0	0	4	8.94	132.20	141.14	2.83	19.42	22.25
2	-11	0	0	4	10.43	131.60	142.03	3.00	18.40	21.40
2	-10	0	0	4	29.36	99.50	128.86	4.78	14.85	19.63
2	-9	0	0	4	94.97	42.10	137.07	9.79	15.92	25.71
2	-8	0	0	4	88.20	33.20	121.40	10.81	14.14	24.95
2	-7	0	0	4	98.58	25.00	123.58	13.46	14.42	27.88
2	0	0	-8	4	6.70	107.40	114.10	4.50	16.32	20.82
2	-11	0	-8	4	6.78	110.40	117.18	4.79	34.11	38.90
2	-10	0	-8	4	18.18	128.00	146.18	5.76	29.70	35.46
2	-9	0	-8	4	50.99	53.10	104.09	12.58	24.22	36.80
2	-8	0	-8	4	88.33	36.60	124.93	9.70	10.86	20.56
2	-7	0	-8	4	67.58	31.50	99.08	9.35	11.64	20.99
2	0	0	-6	4	6.52	170.60	177.12	2.95	30.43	33.38
2	-11	0	-6	4	10.19	131.50	141.69	4.08	27.68	31.76
2	-10	0	-6	4	32.35	124.30	156.65	7.63	29.43	37.06
2	-9	0	-6	4	83.89	44.50	128.39	15.02	24.12	39.14
2	-8	0	-6	4	75.72	48.90	124.62	15.46	18.20	33.66
2	-7	0	-6	4	81.24	28.60	109.84	17.21	16.64	33.85
2	0	-7.7	0	4	14.63	149.90	164.53	5.38	33.67	39.05
2	-11	-7.7	0	4	11.50	108.70	120.20	6.43	30.52	36.95
2	-10	-7.7	0	4	24.05	145.10	169.15	6.53	31.93	38.46
2	-9	-7.7	0	4	64.04	74.50	138.54	16.10	22.94	39.04
2	-8	-7.7	0	4	104.04	64.20	168.24	13.82	18.30	32.12
2	-7	-7.7	0	4	90.41	55.60	146.01	12.73	19.22	31.95
2	0	-7.7	-8	4	6.07	148.10	154.17	4.82	31.41	36.23
2	-11	-7.7	-8	4	6.70	148.50	155.20	6.58	28.55	35.13
2	-10	-7.7	-8	4	22.70	124.20	146.90	8.74	30.36	39.10
2	-9	-7.7	-8	4	73.51	87.90	161.41	15.26	25.45	40.71
2	-8	-7.7	-8	4	98.42	39.20	137.62	17.74	20.24	37.98
2	-7	-7.7	-8	4	68.34	25.80	94.14	17.78	17.66	35.44
2	0	-7.7	-6	4	11.40	108.00	119.40	6.82	34.36	41.18

LPB Exp #	log [GnRH]	log [P]	log [B]	time	LH sec (ng/plate) (ng/ml)1.2	LH cells (ng/plate)	LH total (ng/plate)	FSH sec (ng/plate) (ng/ml)1.2	FSH cells (ng/plate)	FSH total (ng/plate)
2	-11	-7.7	-6	4	13.32	168.10	181.42	7.38	36.90	44.28
2	-10	-7.7	-6	4	26.77	108.00	134.77	8.47	35.68	44.15
2	-9	-7.7	-6	4	79.54	52.10	131.64	15.26	24.28	39.54
2	-8	-7.7	-6	4	107.89	38.20	146.09	14.17	20.04	34.21
2	-7	-7.7	-6	4	117.80	31.00	148.80	17.02	20.96	37.98
2	0	-7.0	0	4	6.67	143.00	149.67	6.25	31.99	38.24
2	-11	-7.0	0	4	8.47	125.50	133.97	7.20	28.76	35.96
2	-10	-7.0	0	4	12.02	54.20	66.22	7.70	27.68	35.38
2	-9	-7.0	0	4	73.10	54.90	128.00	13.97	25.30	39.27
2	-8	-7.0	0	4	91.01	29.70	120.71	18.26	19.78	38.04
2	-7	-7.0	0	4	98.69	34.50	133.19	20.86	19.50	40.36
2	0	-7.0	-8	4	9.26	95.00	104.26	4.67	31.58	36.25
2	-11	-7.0	-8	4	10.15	102.50	112.65	5.23	33.10	38.33
2	-10	-7.0	-8	4	14.88	146.30	161.18	7.27	36.31	43.58
2	-9	-7.0	-8	4	84.53	59.70	144.23	17.56	27.48	45.04
2	-8	-7.0	-8	4	57.46	45.50	102.96	13.06	21.10	34.16
2	-7	-7.0	-8	4	85.90	40.90	126.80	13.75	20.78	34.53
2	0	-7.0	-6	4	10.19	135.60	145.79	4.78	35.22	40.00
2	-11	-7.0	-6	4	10.39	153.60	163.99	6.62	38.96	45.58
2	-10	-7.0	-6	4	18.61	90.40	109.01	6.47	30.94	37.41
2	-9	-7.0	-6	4	60.13	77.10	137.23	11.23	24.30	35.53
2	-8	-7.0	-6	4	84.46	38.00	122.46	14.08	21.05	35.13
2	-7	-7.0	-6	4	126.80	39.10	165.90	16.68	19.67	36.35
2	0	0	0	3	19.68	97.10	116.78	9.91	15.00	24.91
2	0	0	-8	3	21.48	154.60	176.08	8.66	19.00	27.66
2	0	0	-6	3	22.68	212.50	235.18	24.36	26.70	51.06
2	0	-7.7	0	3	20.64	153.50	174.14	25.20	30.00	55.20
2	0	-7.7	-8	3	26.64	182.40	209.04	25.44	28.00	53.44
2	0	-7.7	-6	3	21.24	98.20	119.44	27.00	30.30	57.30
2	0	-7.0	0	3	24.72	86.20	110.92	18.48	19.50	37.98
2	0	-7.0	-8	3	27.72	141.50	169.22	24.24	23.90	48.14
2	0	-7.0	-6	3	25.44	143.40	168.84	27.60	28.10	55.70
3	0	0	0	4	13.56	104.55	118.11	1.92	15.32	17.24
3	-11	0	0	4	11.71	111.60	123.31	1.57	16.76	18.33
3	-10	0	0	4	7.39	105.55	112.94	2.94	17.30	20.24
3	-9	0	0	4	49.50	39.95	89.45	7.85	13.40	21.25
3	-8	0	0	4	79.08	57.80	136.88	8.80	11.01	19.81
3	-7	0	0	4	111.84	45.50	157.34	8.21	11.17	19.38
3	0	0	-8	4	11.33	108.95	120.28	2.23	17.92	20.15
3	-11	0	-8	4	13.22	135.20	148.42	3.58	19.78	23.36
3	-10	0	-8	4	11.35	118.85	130.20	4.56	18.04	22.60
3	-9	0	-8	4	60.30	47.20	107.50	9.29	13.12	22.41
3	-8	0	-8	4	111.96	58.90	170.86	10.46	11.88	22.34
3	-7	0	-8	4	135.00	27.95	162.95	13.03	11.51	24.54
3	0	0	-6	4	5.14	139.95	145.09	3.86	27.52	31.38
3	-11	0	-6	4	5.18	111.90	117.08	4.08	28.31	32.39
3	-10	0	-6	4	8.86	103.50	112.36	4.25	22.44	26.69

LPB Exp #	log [GnRH]	log [P]	log [B]	time	LH sec (ng/plate) (ng/ml)1.2	LH cells (ng/plate)	LH total (ng/plate)	FSH sec (ng/plate) (ng/ml)1.2	FSH cells (ng/plate)	FSH total (ng/plate)
3	-9	0	-6	4	95.82	60.25	156.07	11.41	21.32	32.73
3	-8	0	-6	4	93.54	70.80	164.34	13.25	16.60	29.85
3	-7	0	-6	4	107.76	35.40	143.16	14.23	16.06	30.29
3	0	-7.7	0	4	4.30	128.25	132.55	3.94	28.70	32.64
3	-11	-7.7	0	4	11.64	150.65	162.29	6.38	30.52	36.90
3	-10	-7.7	0	4	14.94	135.75	150.69	7.64	25.84	33.48
3	-9	-7.7	0	4	69.12	57.45	126.57	12.37	20.31	32.68
3	-8	-7.7	0	4	111.96	31.45	143.41	15.14	18.12	33.26
3	-7	-7.7	0	4	132.60	29.15	161.75	18.41	16.19	34.60
3	0	-7.7	-8	4	8.48	129.95	138.43	4.66	23.90	28.56
3	-11	-7.7	-8	4	6.58	164.35	170.93	4.42	26.86	31.28
3	-10	-7.7	-8	4	16.74	134.50	151.24	6.10	24.62	30.72
3	-9	-7.7	-8	4	73.02	57.25	130.27	12.64	20.94	33.58
3	-8	-7.7	-8	4	109.68	36.10	145.78	15.84	17.08	32.92
3	-7	-7.7	-8	4	123.78	26.50	150.28	16.70	13.36	30.06
3	0	-7.7	-6	4	10.25	162.95	173.20	4.80	28.36	33.16
3	-11	-7.7	-6	4	9.77	158.05	167.82	6.05	29.11	35.16
3	-10	-7.7	-6	4	16.80	140.80	157.60	7.75	26.83	34.58
3	-9	-7.7	-6	4	69.66	69.35	139.01	12.31	21.04	33.35
3	-8	-7.7	-6	4	114.60	32.70	147.30	14.88	16.73	31.61
3	-7	-7.7	-6	4	147.48	33.40	180.88	18.46	16.30	34.76
3	0	-7.0	0	4	3.41	137.90	141.31	4.88	27.56	32.44
3	-11	-7.0	0	4	6.53	119.20	125.73	5.14	28.88	34.02
3	-10	-7.0	0	4	11.45	101.30	112.75	6.23	26.46	32.69
3	-9	-7.0	0	4	90.78	69.60	160.38	11.42	22.74	34.16
3	-8	-7.0	0	4	106.56	37.15	143.71	14.54	18.02	32.56
3	-7	-7.0	0	4	124.26	23.55	147.81	17.38	16.36	33.74
3	0	-7.0	-8	4	7.99	122.45	130.44	5.99	30.70	36.69
3	-11	-7.0	-8	4	7.68	127.55	135.23	5.64	31.44	37.08
3	-10	-7.0	-8	4	5.47	123.35	128.82	6.58	27.14	33.72
3	-9	-7.0	-8	4	51.12	79.75	130.87	12.31	22.89	35.20
3	-8	-7.0	-8	4	93.66	29.70	123.36	17.06	19.42	36.48
3	-7	-7.0	-8	4	125.28	27.05	152.33	19.01	17.50	36.51
3	0	-7.0	-6	4	5.90	126.85	132.75	5.21	27.78	32.99
3	-11	-7.0	-6	4	5.87	108.25	114.12	5.02	29.51	34.53
3	-10	-7.0	-6	4	0.46	99.05	99.51	4.99	25.05	30.04
3	-9	-7.0	-6	4	2.12	40.45	42.57	9.64	18.20	27.84
3	-8	-7.0	-6	4	89.34	22.00	111.34	14.78	18.04	32.82
3	-7	-7.0	-6	4	99.60	13.20	112.80	15.89	14.90	30.79
3	0	0	0	3	27.84	119.60	147.44	13.20	17.40	30.60
3	0	0	-8	3	31.68	135.40	167.08	15.72	18.30	34.02
3	0	0	-6	3	26.64	126.60	153.24	27.72	25.60	53.32
3	0	-7.7	0	3	30.96	126.80	157.76	26.64	26.10	52.74
3	0	-7.7	-8	3	26.64	132.20	158.84	25.92	28.60	54.52
3	0	-7.7	-6	3	24.48	116.20	140.68	26.88	27.80	54.68
3	0	-7.0	0	3	27.72	131.20	158.92	30.12	31.20	61.32
3	0	-7.0	-8	3	25.44	137.80	163.24	29.40	33.00	62.40

LPB Exp #	log [GnRH]	log [P]	log [B]	time	LH sec (ng/plate) (ng/ml)1.2	LH cells (ng/plate)	LH total (ng/plate)	FSH sec (ng/plate) (ng/ml)1.2	FSH cells (ng/plate)	FSH total (ng/plate)
3	0	-7.0	-6	3	28.32	129.80	158.12	27.36	30.80	58.16

log [GnRH]	log [P]	log [B]	time	Mean [LH] sec	Mean [LH] cells	Mean [LH] total	Mean [FSH] sec	Mean [FSH] cells	Mean [FSH] total
0	0	0	4	9.40	123.78	133.19	2.62	20.38	23.00
-11	0	0	4	9.44	132.03	141.47	2.72	21.25	23.97
-10	0	0	4	20.65	105.38	126.04	4.30	19.07	23.37
-9	0	0	4	74.08	48.72	122.79	10.12	16.27	26.39
-8	0	0	4	92.88	46.47	139.35	11.26	14.38	25.64
-7	0	0	4	107.54	38.57	146.11	12.10	14.00	26.10
0	0	-8	4	8.16	113.32	121.48	3.46	20.45	23.91
-11	0	-8	4	8.91	122.47	131.37	4.19	26.83	31.02
-10	0	-8	4	19.12	118.92	138.04	5.29	24.48	29.77
-9	0	-8	4	63.74	55.50	119.24	11.19	19.28	30.47
-8	0	-8	4	104.76	46.10	150.86	11.52	13.08	24.60
-7	0	-8	4	104.05	34.58	138.63	12.58	12.98	25.56
0	0	-6	4	5.44	148.45	153.89	3.91	31.58	35.50
-11	0	-6	4	6.55	124.67	131.22	4.34	31.03	35.37
-10	0	-6	4	20.14	111.77	131.90	5.86	28.31	34.17
-9	0	-6	4	82.38	64.92	147.30	13.53	24.25	37.78
-8	0	-6	4	87.78	61.37	149.15	15.81	19.57	35.37
-7	0	-6	4	99.04	39.53	138.57	17.76	18.32	36.08
0	-7.7	0	4	8.40	139.85	148.25	5.30	33.19	38.49
-11	-7.7	0	4	9.85	136.32	146.17	6.10	31.51	37.61
-10	-7.7	0	4	21.20	134.68	155.88	6.89	29.12	36.01
-9	-7.7	0	4	68.39	74.32	142.70	14.49	23.42	37.91
-8	-7.7	0	4	104.48	50.22	154.70	16.02	19.61	35.62
-7	-7.7	0	4	107.90	39.92	147.81	17.18	18.27	35.45
0	-7.7	-8	4	6.22	130.62	136.84	4.80	29.64	34.44
-11	-7.7	-8	4	5.82	143.55	149.37	5.31	29.92	35.23
-10	-7.7	-8	4	19.95	124.37	144.31	7.34	28.88	36.22
-9	-7.7	-8	4	50.92	74.88	125.81	13.94	24.41	38.35
-8	-7.7	-8	4	98.89	45.03	143.92	17.27	19.91	37.18
-7	-7.7	-8	4	92.84	31.23	124.07	17.98	17.04	35.02
0	-7.7	-6	4	9.02	138.72	147.73	5.91	33.87	39.78
-11	-7.7	-6	4	9.62	154.32	163.93	6.64	35.57	42.21
-10	-7.7	-6	4	21.88	119.73	141.62	8.18	33.99	42.16
-9	-7.7	-6	4	70.21	63.08	133.30	14.15	24.44	38.59
-8	-7.7	-6	4	102.88	38.10	140.98	15.76	19.79	35.55
-7	-7.7	-6	4	118.75	34.83	153.58	18.66	19.49	38.15
0	-7.0	0	4	4.53	134.40	138.93	5.64	32.53	38.18
-11	-7.0	0	4	6.40	121.90	128.30	6.22	31.90	38.12
-10	-7.0	0	4	11.86	86.37	98.23	7.22	29.91	37.13
-9	-7.0	0	4	70.79	68.93	139.72	12.86	25.55	38.41
-8	-7.0	0	4	94.70	39.12	133.81	17.14	20.33	37.47
-7	-7.0	0	4	105.84	33.88	139.72	19.54	19.09	38.63
0	-7.0	-8	4	7.54	114.82	122.35	5.74	33.56	39.30
-11	-7.0	-8	4	7.52	119.68	127.21	5.74	35.01	40.75
-10	-7.0	-8	4	12.38	126.62	139.00	7.05	33.20	40.25
-9	-7.0	-8	4	65.66	70.22	135.87	14.84	25.84	40.68
-8	-7.0	-8	4	79.41	39.27	118.68	16.20	21.09	37.29

log [GnRH]	log [P]	log [B]	time	Mean [LH] sec	Mean [LH] cells	Mean [LH] total	Mean [FSH] sec	Mean [FSH] cells	Mean [FSH] total
-7	-7.0	-8	4	103.23	33.12	136.35	17.84	19.23	37.07
0	-7.0	-6	4	7.18	123.35	130.53	5.46	33.07	38.53
-11	-7.0	-6	4	7.32	129.65	136.97	6.01	35.49	41.50
-10	-7.0	-6	4	13.28	102.48	115.76	6.30	30.63	36.93
-9	-7.0	-6	4	39.79	65.52	105.31	11.44	24.55	35.99
-8	-7.0	-6	4	87.45	35.73	123.19	15.82	21.00	36.82
-7	-7.0	-6	4	108.51	29.70	138.21	17.62	18.61	36.22
0	0	0	3	25.20	105.63	130.83	13.98	21.80	35.78
0	0	-8	3	26.84	137.53	164.37	14.37	23.50	37.87
0	0	-6	3	23.16	157.50	180.66	27.20	24.57	51.77
0	-7.7	0	3	24.52	135.87	160.39	26.40	29.80	56.20
0	-7.7	-8	3	25.56	146.00	171.56	26.08	30.40	56.48
0	-7.7	-6	3	21.48	112.97	134.45	27.80	32.10	59.90
0	-7.0	0	3	25.48	110.53	136.01	26.84	30.17	57.01
0	-7.0	-8	3	23.44	145.10	168.54	28.20	32.63	60.83
0	-7.0	-6	3	24.72	133.23	157.95	28.64	31.70	60.34

APPROVAL SHEET

The thesis submitted by Lenette L. Renier has been read and approved by the following committee:

Dr. Diane E. Suter, Director
Assistant Professor, Biology, Loyola

Dr. Sonia J. Ringstrom
Professor (Chairperson), Natural Science, Loyola

Dr. Frederick H. Wezeman
Professor (Chairman), Biology, Loyola

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

4/20/95 Diane E. Suter

Date Director's Signature