HIRSUTISM, VIRILISM, POLYCYSTIC OVARIAN DISEASE AND STEROID-GONADOTROPIN-FEEDBACK SYSTEM: A CAREER RETROSPECTIVE

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Running title: Steroid-gonadotropin-feedback system: A career retrospective

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ABSTRACT

This career retrospective describes how the initial work on the mechanism of hormone action provided the tools for the study of hirsutism, virilism and polycystic ovarian disease. After establishing excessive ovarian and or adrenal androgen secretion in polycystic ovarian disease, the question whether the disease was genetic or acquired, methods to manage hirsutism and methods for the induction of ovulation were addressed. Recognizing that steroid gonadotropin feedback was an important regulatory factor, initial studies were done on the secretion of LH and FSH in the ovulatory cycle. This was followed by the study of basic mechanisms of steroid-gonadotropin feedback system using castration and steroid replacement and the events surrounding the natural onset of puberty. Studies in ovariectomized rats showed that progesterone was a pivotal enhancer of the estrogen-induced gonadotropin release thus accounting for the preovulatory gonadotropin surge. The effects of progesterone were manifested by depletion of the occupied estrogen receptors of the anterior pituitary, release of hypothalamic LHRH, and inhibition of enzymes that degrade LHRH. Progesterone also promoted the synthesis of FSH in the pituitary. The 3α, 5α-reduced metabolite of progesterone brought about selective LH release and acted using the GABA A receptor system. The 5α-reduced metabolite of progesterone brought about selective FSH release; the ability of progesterone to bring about FSH release was dependent on its 5α-reduction. The GnRH neuron does not have steroid receptors; the steroid effect was shown to be mediated through the excitatory amino acid, glutamate, which in turn stimulated nitric oxide. These observations led to the replacement of the long accepted belief that ovarian steroids acted directly on the GnRH neuron by the novel concept that the
steroid-feedback effect was exerted at the glutamatergic neuron which in turn regulated
the GnRH neuron. The neuroprotective effects of estrogens on brain neurons are of
considerable interest.

Key words: Hirsutism, Polycystic ovarian disease, Estrogens, Androgens, Progesterone,
Steroid-gonadotropin-feedback
INTRODUCTION:

In this career retrospective article the author plans to describe briefly how he started working in the field of steroid hormones which led to work in the area of hirsutism, virilism and polycystic ovarian disease. These initial studies led to a number of interlocking questions resulting in a detailed study of steroid-gonadotropin feedback system leading into a lifelong carrier of scientific investigation from 1956 to 2010. The approach for solving the questions generated was inspired from the literature at the time the research was done as well as the incorporation of newer techniques as they developed. This article covers an extensive area of reproductive biology and it is not possible to refer to the literature surrounding the research due to the breadth of the area covered. However, it is fully referenced in the articles cited and the periodic reviews on the subject. The author also wishes to express his gratitude to the numerous graduate students, post doctoral fellows and faculty colleagues for their untiring collaboration and help in introducing new techniques in the laboratory from time to time..

In the mid 1950’s there was no clear understanding as to how steroid hormones exerted their biological action. In the area of glucocorticoids, based on the metabolism of cortisol (11β, 17α, 21-trihydroxy-4-pregnen-3, 20-dione) as well as the effectiveness of cortisol as compared to cortisone (17α, 21-dihydroxy-4-pregnen-3, 11, 20-trione) in tissues in which the conversion of cortisone to cortisol did not take place, it was postulated that cortisol may be the active hormone (51, 82,183). On the other hand, working with placental isocitric dehydrogenase in vitro, Talalay and Williams-Ashman in 1958 (212) explained the action of estradiol as a coenzyme in a redox system. To
investigate whether a hormone was required to participate in an oxidation-reduction cascade to exert biological action, Bush and Mahesh (52) studied the factors that governed the reduction of the 11-keto group to the 11β-hydroxyl group in corticosteroids. It was postulated that since the alpha side of the molecule is flat in the 3-keto-4-ene and 3α, 5α-reduced steroids, the enzyme involved in reducing the 11-keto group could approach the steroid from the alpha side with ease and reduce it to the 11β-hydroxyl group. On the contrary, in the 3α, 5β-steroids, the Ring A of the steroid is at right angle to the rest of the molecule and thus would provide steric hindrance in reduction at the 11-position. Thus the synthesis 3α, 11β-dihydroxy-5α-androstan-17-one; 3α-hydroxy-5α-androstan-11, 17-dione; 3α, 11β-dihydroxy-5β-androstan-17-one; 3α-hydroxy-5β-androstan-11, 17-dione and 4-androsten-3, 11, 17-trione was carried out and their metabolism studied in the human. The results clearly showed the reduction of the 11-ketone to the 11-hydroxyl compounds in 3-keto-4-ene and 3α, 5α-steroids but not in the 3α, 5β-steroids. In order to slow down the metabolism of cortisol in Ring A of the steroid, 2α-methyl cortisol and 2α-methyl cortisone were synthesized and tested for biological activity (70). The 2α-methyl cortisol was found to be very active biologically and the 2α-methyl cortisol had very little biological activity. Based on the structural requirements for reduction of the 11-ketone to the 11-hydroxyl group as determined by Bush and Mahesh (52), the 2α-methyl group would provide enough steric hindrance to prevent reduction of the 11-ketone to the 11β-hydroxyl group. This was verified experimentally by Bush and Mahesh (53) leading to the conclusion that the 11β-hydroxyl group of cortisol did not have to undergo any metabolism for the exertion of biological activity. The higher biological activity of 9α-fluoro steroids was also explained in part due to a greater
persistence of the 11\(\beta\)-hydroxyl group during metabolism (55). Jensen also provided proof that estradiol exerted its biological activity without being converted to estrone (102). Further examination of the metabolism of cortisol in various rat tissues identified the kidney as the major organ for the oxidation of cortisol to cortisone (139,140), a finding that proved in later years to be of great importance in the action of mineralocorticoids.

These initial studies in the mechanism of steroid hormone action required the development of methods for the separation, characterization and measurement of steroid hormone metabolites in human biological fluids and made studies in hirsutism, virilism and polycystic ovarian disease possible.

**HIRSUTISM, VIRILISM AND POLYCYSTIC OVARIAN DISEASE**

**STEROID SECRETION PATTERNS:**

The earliest case studied by the author was a case of twin sisters, one of whom had undergone severe psychological stress and had a sudden onset of hirsutism (54). The sister with hirsutism had a very high excretion of androgen metabolites in her urine as compared to her normal sister and was hyper responsive to Adrenocorticotropic Hormone (ACTH) stimulation in androgen production. That the excessive androgens were coming from the adrenal gland was demonstrated by large quantities of dehydroepiandrosterone (3\(\beta\)-hydroxy-5-androsten-17-one) and androstenedione (4-androsten-3, 17-dione) in the adrenal vein blood. In the mid 1950’s, wedge-resection of the polycystic ovaries was the standard way to treat polycystic ovarian disease. The ovarian wedges obtained from such patients, after obtaining informed consent, were extracted and the steroids obtained were
characterized. Several ovaries contained large quantities of dehydroepiandrosterone that was characterized by chromatographic properties and infra red spectroscopy along with 17α-hydroxy-5-pregnenolone (3β, 17α-dihydroxy-5-pregnen-20-one). Some ovaries contained large quantities of androstenedione as compared to normal ovaries (119,120,125). This was the first demonstration of androgen secretion by the polycystic ovary and the background references are provided in two reviews (122,125). In vitro incubation studies showed that the ovaries containing large quantities of dehydroepiandrosterone converted less substrate to androstenedione as compared to normal ovaries suggesting diminished 3β-hydroxy steroid dehydrogenase activity and those containing large quantities of androstenedione showed lower aromatase activity. Since androstenedione and dehydroepiandrosterone are weak androgens and we were not able to extract significant amount of testosterone from the polycystic ovaries studied, it was of interest to determine if these weak androgens could be converted peripherally into testosterone. Oral administration of dehydroepiandrosterone and androstenedione to women resulted in elevation of plasma testosterone levels (121).

Since in our earlier study (54) the adrenal was the source of excessive androgens, a finding that had also been reported by others (122,125 for references), it was important to determine if in a particular patient, the adrenal or the ovary or both were the source of excessive androgens. Thus urinary androgen metabolites were measured before and after adrenal and ovarian suppression (126). The steroids were also measured after ovarian stimulation with human pituitary Follicle Stimulating Hormone (FSH) (123). The results showed that different patients could have adrenal over secretion of androgens, ovarian over secretion of excessive androgens or both. Human FSH enhanced ovarian hyper
secretion of androgens and also caused multiple ovulations. In a subsequent study,
peripheral, adrenal vein and ovarian vein steroids were measured before and after ACTH
and Human Chorionic Gonadotropin (HCG) to further establish the adrenal or ovarian
source of excessive androgens (176).

Even before the availability of radioimmunoassay (RIA) for the determination of
serum gonadotropins, levels of Luteinizing hormone (LH) secretion in urine were
measured throughout the menstrual cycle using an anti-HCG antiserum. Levels of LH
were found to show a peak either coinciding with or preceding a rise in basal body
temperature which was then considered to be an indication of ovulation (203). With the
availability of RIA for measuring serum LH our group as well as others demonstrated a
high pulsatile level of LH in patients with the polycystic ovary syndrome (80). Initially
there was no explanation for this high pulsatile secretion of LH and a hypothalamic
defect was postulated. Most patients with the polycystic ovary syndrome show good
estrogenic vaginal smears due to ovarian secretion of estrogens and the peripheral
conversion of androgens to estrogens. Using pituitary stalk-sectioned rats in which an
aluminum foil was placed after stalk-resection to prevent regeneration of the
hypothalamic blood supply, Greeley et al (87) showed that the rats still responded to
Luteinizing Hormone Releasing Hormone (LHRH; also referred to as Gonadotropin
Releasing Hormone; GnRH) in the secretion of LH and FSH. Furthermore if such rats
were treated with estradiol, the pituitary showed enhanced sensitivity to the release of LH
(88). Studies of Legan and Karach (110) showed that in the long term ovariectomized
rats, a single injection of estradiol brought about daily LH surges whereas a single
injection of progesterone initially enhanced the estrogen triggered surge of gonadotropins
and then promptly brought about the extinction of the estrogen induced LH surge. There
were no multiple surges of gonadotropins on subsequent days of progesterone
administration after the first surge took place. Thus it appeared that persistent estrogen
stimulation to the hypothalamic–pituitary axis could cause persistent LH surges in the
human which could only be dampened by luteal levels of progesterone. This was shown
to occur in one patient with the polycystic ovary syndrome who under went wedge-
resection of the ovary; the high pulsatile levels of LH persisted only till the luteal rise of
progesterone occurred after the first ovulation (138). This change in hormonal profile
tends to explain the beneficial effects of wedge-resection on the ovulatory process. The
pivotal role of progesterone in initiating the surge of LH and FSH secretion and also the
termination of the surge will be discussed in detail, later in this article.

Other studies on hirsutism, virilism and steroid secretion carried out were in cases
with ovarian stromal hyperplasia (131,133), arrhenoblastoma (93,132), adrenal rest tumor
of the ovary (181), virilizing adrenal tumors (124,127) and delayed onset of congenital
adrenal hyperplasia (128). 11β-hydroxy estrone was also isolated in a case of feminizing
adrenal carcinoma (130).

Three overall questions emerged from the above studies. The first question was
whether there was a treatment to induce ovulation in patients with the polycystic ovarian
syndrome without undergoing surgical procedures such as the wedge-resection of the
ovary. The second question concerned the methods that could be employed for the
management of hirsutism. The third question was whether the polycystic ovary syndrome
was a genetic or an acquired disorder. Initial attempts to answer the third question were to
determine whether there was a chromosomal abnormality in the polycystic ovary
syndrome patients. No chromosomal abnormalities were found in patients with the
polycystic ovary syndrome (56). Therefore animal models were constructed in an attempt
to answer that question.

INDUCTION OF OVULATION IN PATIENTS WITH THE POLYCYSTIC OVARY
SYNDROME:

In attempts to search for agents other than human pituitary FSH or human postomenopausal gonadotropins that were very expensive and difficult to obtain at that time, a
variety of compounds were tested for their ability to induce ovulation. Clomiphene (1-
[p(β-diethylaminoethoxy)phenyl]-1,2-diphenyl-2-chloroethylene) was sent to us to be
tested as a contraceptive agent by a drug company. In immature female rats this
compound was found to stimulate uterine weight and in male rats the seminal vesicle and
ventral prostate weights in very low doses, while it inhibited them at high doses (200). In
the absence of estrogens in the ovariectomized rat, Clomiphene acted as a weak estrogen.
It acted as an anti-estrogen in the presence of estrogens. In unilateral ovariectomized rats,
Clomiphene in low doses increased ovarian weight thus indicating antagonism of
estrogen suppression on the hypothalamic-pituitary axis and the stimulation of
gonadotropin secretion (198). The anti-estrogenic activity of Clomiphene was further
demonstrated by its ability to inhibit the uptake of tritiated estradiol in the rat uterus and
pituitary gland (201). In the human, Clomiphene was able to induce ovulation in a variety
of unovulatory patients including patients with the polycystic ovary syndrome (94, 199).
It was also able to stimulate spermatogenesis in men (103). Clomiphene was also able to
induce ovulation in the Chiari-Frommel syndrome in which the estrogen levels are very
low (92). Thus Clomiphene became the accepted first treatment of choice for the
induction of ovulation, prior to the use of human menopausal gonadotropins followed by HCG (95). With the availability of RIA for gonadotropin secretion, Clomiphene treatment was shown to result in an ovulatory type of LH and FSH secretion in a variety of anovulatory patients (78, 79). It was also demonstrated that in patients that were resistant to Clomiphene in the induction of ovulation, 11 of the 13 patients treated ovulated and there were 5 pregnancies if the Clomiphene treatment was preceded by the synthetic glucocorticoid dexamethasone (213). It was shown previously in experimental animals that dexamethasone was able to bring about FSH release and this perhaps aided in the ovulation (36).

STUDIES ON THE USE OF ANTI-ANDROGENS FOR THE MANAGEMENT OF HIRSUTISM:

The management of hirsutism in patients with the polycystic ovary syndrome has been difficult as the suggested approaches were ovarian suppression by the use of contraceptive steroids when the source of excessive androgens was the ovary, or adrenal suppression when the source of androgens was the adrenal or a combination of both treatments when the source of excessive androgens were the ovary and the adrenal. The establishment of ovarian or adrenal source required extensive diagnostic tests and long term suppression of the adrenal or the ovary was not an easily acceptable choice. Thus another approach was the use of an anti-androgen, 17α-methyl-B-nor-testosterone. In male rats 17α-methyl-B-nor-testosterone reduced seminal vesicle and ventral prostate weights and counteracted the growth promoting effects of testosterone on these organs (142). However in castrated male rats, it stimulated the growth of seminal vesicles and ventral prostate indicating weak androgenic effects. 17α methyl-B-nor-testosterone also...
enhanced the effect of testosterone in suppressing testicular weight indicating a weak anti-gonadotropic effect. The anti-gonadotropic effect was confirmed in female rats in which it decreased ovarian weight and the number of ovulations (214). In the human, it brought about a significant decrease in sebum production rate in the forehead, as well as a decrease in facial hair growth, as determined by the weight of hair shaved, starting at 30 days after it was administered (65,221). However, the concern that a patient on an anti-androgen may become accidentally pregnant causing fetal abnormalities has prevented the use of anti-androgens in the management of hirsutism.

ANIMAL MODELS FOR THE POLYCYSTIC OVARIAN DISEASE IN HUMANS:

The question whether the human polycystic ovary syndrome was a genetic disorder or whether it could be caused by excessive androgen secretion is a difficult one and it is still being debated. The possibility of an androgen related disorder was indicated by our early experiments that showed that the administration of large quantities of dehydroepiandrosterone or androstenedione in immature rats resulted in ovulatory failure and the presence of polycystic ovaries (202). A detailed examination of the effects of dehydroepiandrosterone on ovulatory failure was thus carried out (106). Administration of dehydroepiandrosterone to 27 day old female rats resulted in ovulatory type serum FSH and LH surge on Day 30 of life and the animals exhibited either constant estrous or constant diestrus vaginal smears with either polycystic ovaries or ovaries containing corpus-luteum like structures. With time all ovaries became polycystic. Serum FSH levels were elevated as compared to control rats, serum LH levels were similar to those in control rats and serum prolactin was elevated. The ovary was responsive to gonadotropin treatment and
the pituitary was responsive to LHRH stimulation. Discontinuation of the
dehydroepiandrosterone treatment resulted in a few irregular ovulatory cycles
followed by normal cyclicity. Thus this animal model was very similar to the
human with the exception that prolactin rather than LH was elevated. Further
studies with the precocious ovulation showed that the gonadotropin surge resulting
in the ovulation could be blocked with the central nervous system blocking agents
such as phenobarbital and reserpine (106). The precocious ovulation appeared to be
mediated by the conversion of dehydroepiandrosterone to estrogens because the
non-aromatizable androgen $5\alpha$-dihydrotestosterone did not cause such an ovulation
(107). Furthermore, cyanoketone, an inhibitor of 3$\beta$-hydroxysteroid dehydrogenase
that blocks the conversion of dehydroepiandrosterone to estrogen, prevented
vaginal patency and the dehydroepiandrosterone induced precocious ovulation
(107). A study of the steroid profile after dehydroepiandrosterone administration
showed an elevation in blood estradiol, followed by the depletion of the
cytoplasmic estradiol receptors in the hypothalamus and the pituitary and the
gonadotropin surge leading to ovulation (180). That these events occur in the
natural process of ovulation will be discussed later in this article. The restoration of
normal cyclicity in dehydroepiandrosterone treated rats only occurred when the
high levels of dehydroepiandrosterone in blood were reduced (178). Because the
erlier studies were done with immature rats, it was important to establish that the
administration of dehydroepiandrosterone caused polycystic ovaries and ovulatory
failure in adult rats as well. The effect of dehydroepiandrosterone in causing
polycystic ovaries in the adult rat was shown by Ward et al (217).
Since the polycystic ovary syndrome in women is often associated with obesity and insulin resistance and insulin type growth factors hyper stimulate theca cells to produce androgens, the direct effect of androgens on the ovary were examined. Hypophysectomized immature rats when ovulated with pregnant mare’s serum gonadotropin (PMSG) and HCG, showed follicular atresia and decreased ovulation rate when treated with the non aromatizable androgen 5α-dihydrotestosterone (2). That the effect of the androgen was on the ovary and not gonadotropin secretion was demonstrated by the fact that in the PMSG treated rat the preovulatory gonadotropin surge was unaffected even though the number of ovulations were reduced (62). The effect of the androgen could be reduced by pretreatment with estrogens. Thus androgens could cause abnormal follicular development, altered ovarian steroidogenesis and ovulatory failure. Evidence was also found for an auto regulatory effect of androgens on ovarian theca cell androgen production (207,208).

THE STEROID-GONADOTROPIN FEED BACK SYSTEM

The above mentioned experimental studies showed that in animals with normal ovaries and a normal hypothalamic-pituitary axis, the administration of androgens resulted in the formation of polycystic ovaries and an altered gonadotropin secretion pattern. Therefore a detailed examination of the steroid-gonadotropin feed-back system was undertaken. These studies included examining the role of FSH and LH in the ovulatory cycle, steroid-gonadotropin feedback before and after puberty, steroid and
gonadotropin changes during puberty and the ovarian signals for the preovulatory

gonadotropin surge.

EARLY WORK ON THE ROLE OF FSH AND LH IN THE OVULATORY PROCESS:

Before the advent of RIA for pituitary FSH and LH in serum, bioassays were used
for their determination. In the cycling rat there was an approximate 50% decrease in
pituitary LH and FSH content from the morning to the late evening on the day of
proestrus (84). Such a decrease was also observed in pubertal rats preceding their first
ovulation. In immature rats in which follicular development was initiated with a small
dose of PMSG and the endogenous gonadotropin surge blocked by phenobarbital,
avolation could be induced with purified pituitary FSH that did not contain enough LH
contaminant to cause ovulation. In the hamster, antiserum prepared against a preparation
of LH with ample FSH contaminant, blocked ovulation (85). However when the anti-FSH
component was adsorbed out, the ovulation blocking potency of the antiserum was
greatly reduced. In addition ovulation blocked by anti-LH could be reinstituted by
injection of an FSH preparation that contained very little or no LH contamination. (85).
These results showed a prominent role of FSH in ovulating the mature ovarian follicle. In
view of a common alpha subunit in FSH, LH and TSH, the ability to ovulate a mature
ovarian follicle by TSH, prolactin and the two LH subunits was also tried but they did not
induce ovulation (129). Although in the rodent, the need for the secretion of FSH as a
part of the preovulatory gonadotropin surge could be explained on the basis of the growth
of the ovarian follicles for the next cycle, the physiological role of FSH at the time of the
preovulatory gonadotropin surge, if any, in the human is still unclear. In this regard,
Strott et al (211) have reported several cases of short luteal phase in women in whom the only abnormality was a dulled or misplaced FSH peak during the ovulatory surge.

The role of the ovary on regulating the preovulatory surge of gonadotropins was shown by the fact that removing the ovary on the morning of diestrus II blocked the preovulatory LH surge (86). Ovariectomy at 6PM on diestrus II or 9AM on proestrus resulted in a lower LH surge at proestrus. Injection of progesterone on proestrus before the preovulatory surge of gonadotropins postponed the preovulatory surge for one day (86).

EXAMINATION OF THE STEROID-GONADOTROPIN FEEDBACK SYSTEM BY CASTRATION AND STEROID REPLACEMENT:

Immature rats castrated at day 26 of age showed a rise of LH and FSH by 8 hours in male rats and in 24 hours for LH and 48 hours for FSH in female rats (72). These levels could be suppressed by the administration of estradiol or testosterone. In the female rat, ovariectomy on day 26 of age and treatment with increasing doses of estradiol for 5 days showed a return to intact gonadotropin levels within the physiological dose range of estradiol as judged by the uterine weight (145). Increasing the dose of estrogens showed an increase in gonadotropin secretion due to the positive feedback effect, followed by suppression at higher dose levels. This method also provided a means of evaluating the biological activity of several synthetic estrogens. Progesterone by itself did not suppress gonadotropins in the ovariectomized rat (144). Using the seminal vesicle and ventral prostate weights, similar studies tested the steroid feedback system in male rats (73). Ovariectomy and estrogen replacement in 26, 45 and 95 day old rats showed that the dose of estradiol required to decrease serum gonadotropins was 4 to 6 fold higher in 45 and 95
day old rats as compared to 26 day old rats indicating a maturation of the feed-back system after puberty even though the increase in uterine weight caused by estrogen administration was comparable in the 3 groups (74). This change in sensitivity to the negative feed back effect of estradiol may in part be mediated by endogenous endorphins that are suppressive of gonadotropin secretion (188). An endorphin antagonist, naloxone, brings about a large increased release of LH at all times in the rat ovulatory cycle except at the time of the gonadotropin surge. A measurement of the $\beta$-endorphin content in the hypothalamus of the immature ovariectomized rat treated with estradiol and progesterone or triamcinilone acetonide ($9\alpha$-fluoro-11$\beta$, 21-dihydroxy-1, 4-pregnadiene-3, 20-dione $16\alpha$, $17\alpha$-acetonide) to induce the preovulatory type surge of gonadotropins resulted in an estradiol induced decrease in $\beta$-endorphin preceding and during the LH surge which was maintained the following morning (37). However, progesterone and triamcinilone acetonide brought back the $\beta$-endorphin levels to control levels on the morning after the gonadotropin surge thus appeared to reinstate the opioid inhibition.

**CHANGES OCCURRING IN STEROID LEVELS DURING THE PREOVULATORY GONADOTROPIN SURGE AND THE INITIATION OF PUBERTY:**

Ovarian and uterine histology and serum gonadotropins were measured in Holtzman rats from days 22 to 40 of age, in which ovulation occurred most consistently on day 38 of age (64). The percentage of large follicles showed a linear growth from day 22 onwards. The endometrial stroma, myometrium, and luminal epithelium started growing on day 32 of age along with an increase in uterine weight. These changes in the uterine histology were early indicators of ovarian estrogen secretion. An early small increase in serum LH occurred on day 34 with the preovulatory LH and FSH surge on
day 38. These observations suggested that ovarian estrogens may be the trigger for the
initiation of puberty. The measurement of steroids during the natural onset of puberty
showed an increase in blood estradiol, progesterone and testosterone preceding vaginal
opening and an increased sensitivity of the pituitary to LHRH (179). Correlated with the
gonadotropin surge was a fall in the cytoplasmic estrogen receptors of the pituitary and
the hypothalamus due to nuclear translocation by the secreted estradiol. The rise in
estradiol and the depletion of cytoplasmic estrogen receptors in the pituitary and
hypothalamus were similar to what occurred in normally ovulating rat (89) and immature
rats induced to ovulate by the administration of PMSG (177). Similar events also took
place in the precocious ovulation induced by the administration of
dehydroepiandrosterone (180). In the estrogen- primed ovariectomized rat, an injection of
2μgrams of estradiol brought about a decrease in the estrogen receptor content of the
pituitary without altering the estrogen receptor mRNA levels, which only increased 12
hours after the estrogen injection in preparation for the estrogen receptor replenishment
that started at 18 hours. Thus the initial decline in estrogen receptor content was not due
to loss of estrogen receptor mRNA but accelerated estrogen receptor processing (61). In
the uterus, similar changes took place and progesterone was shown to delay the estrogen
receptor replenishment (223,224). Pituitary ultra structure of the gonadotropes during the
preovulatory surge also showed degranulation of LH containing gonadotropes a few
hours prior to the degranulation of FSH containing gonadotropes, which was consistent
with the start of the LH surge a little earlier than the FSH surge. (63). Extensive work
was also done on the role of androgens and LHRH in the initiation of puberty in the male
rat (135,160,161,162,163,164). A detailed description however is out of the scope of this
article.

EFFECT OF PROGESTERONE AND CORTICOIDS IN INDUCING THE
PREOVULATORY TYPE OF SURGE OF GONADOTROPINS:

Extensive work done in this area showed that progesterone played an important part
in the preovulatory gonadotropin surge. The work consisted of defining the role of
progesterone, and its mechanism of action through depletion of occupied estrogen
receptors in the anterior pituitary; hypothalamic control of the secretion of LHRH;
regulation of peptidase activity that degrades LHRH; regulation of LH-β and FSH-β
mRNA levels and selective secretion of FSH and LH by progesterone metabolites. The
work has been the subject of periodic reviews on the subject (14, 16, 17 113,114,115
134). The results of these studies are also summarized in Figures 1 and 2.

Defining the role of progesterone in the preovulatory surge of gonadotropins:

Even though the administration of estrogens to ovariectomized rats was able to
induce an LH surge, the LH surge was only of a magnitude of about 10% of the
preovulatory gonadotropin surge. Thus experiments were done using doses of estrogens
that were able to reduce the post castration rise of gonadotropins but not prevent it, in
combination with various doses of progesterone (146). The dose of estradiol used was
enough to induce progesterone receptors but only cause a minimal if any estrogen
induced LH surge. The doses of progesterone used were able to either stimulate the
release of LH and FSH or suppress them with the next higher dose. The pattern that the
lower dose was stimulatory and the next higher dose was inhibitory was persistent in
several protocols and several experiments. Such a pattern has been shown to be important
in the human menstrual cycle where a low level of progesterone enhanced the preovulatory LH surge while the higher level helped terminate the surge (101). The stimulatory dose of progesterone was able to mount a preovulatory type gonadotropin surge of LH and FSH similar to what is seen at the time of proestrus. The sensitivity of the pituitary to LHRH was enhanced by the stimulatory dose of progesterone and suppressed by the inhibitory dose of progesterone (148). This pattern was also exhibited by the ability of progesterone to decrease occupied estrogen receptors of the anterior pituitary and the effect of progesterone and 5α-dihydroprogesterone to attenuate estrogen induced prolactin release which will be discussed later in this manuscript.

In the immature castrated male rat, the dose of estradiol needed to reduce the levels of gonadotropins comparable to what was achieved with the 0.1 μg/kg body weight dose of estradiol in female rats was 2.0 μg/kg body weight (149). This was perhaps due to the masculinization of the hypothalamus neonatally by estrogens, resulting in the change in sensitivity to estrogens. With this dose of estradiol, progesterone was able to induce a preovulatory type gonadotropin surge even in the male rat (149).

That progesterone played a pivotal role in ovulation was further demonstrated by the use of the progesterone and glucocorticoid antagonist RU486 (17β-hydroxy-11β-[4-dimethylaminopgenyl]-17α-[prop-1-ynyl]-estra-4, 9-diene-3-one) (193). RU486 abolished the preovulatory gonadotropin surge in PMSG treated rat immature rat and in the normally cycling adult rat. In this regard, it is of interest to note that Goldman et al (83) found an elevation of progesterone in ovarian vein blood at 1400 h on proestrus prior to the LH surge. Progesterone and LH levels also showed an increase at 1400 h in the study of Nequin et al (165) although several other investigators found no rise in
preovulatory progesterone possibly because they did not sample blood at 1400 h. A preovulatory increase in serum progesterone has been found in women 12 hours before the initiation of the rise of LH (101) and 6 hours before the rise of LH in rhesus monkeys (204).

In the estradiol-primed immature rat, deoxycorticosterone (21-hydroxy-4-pregnene-3, 20-dione) and the synthetic glucocorticoids triamcinilone acetonide were also able to induce a preovulatory type of gonadotropin surge where as cortisol was unable to do so (35, 36). The ability of deoxycorticosterone and triamcinilone acetonide to induce the surge was considered to be due to their interaction with the progesterone receptor. These steroids like progesterone were also very effective in stimulating fluid loss from the distended uterus caused by estrogen treatment. Low doses of the synthetic steroid dexamethasone were able to selectively bring about FSH release in the estrogen-primed rat (36). Deoxycorticosterone and triamcinilone acetonide were also able to induce ovulation in the PMSG-primed rats, similar to progesterone, which served as a biological test for their effect (35). In the estrogen-primed ovariectomized rat, the acute administration of ACTH also resulted in the preovulatory type of gonadotropin release. The surge required the presence of the adrenal glands and was antagonized by the progesterone and glucocorticoids antagonist RU486. ACTH increased both progesterone and deoxycorticosterone and the gonadotropin surge was considered to be mediated by these steroids (190).

Mechanism of action of progesterone on the release of gonadotropins:

(a) Antagonism of estrogen action on the pituitary: An examination of the effects of progesterone on the estrogen receptor of the anterior pituitary in ovariectomized
estrogen-primed rats showed that progesterone brought about a decrease in the estrogen receptors of the anterior pituitary (210). This created a controversy in the literature as several other investigators had failed to find an effect of progesterone on estrogen receptors of the anterior pituitary. A detailed examination of the progesterone effect showed that it only occurred during the period of the nuclear occupancy of the progesterone receptor and not before or after the event (57). Furthermore only during the period of nuclear occupancy of the progesterone receptor in the pituitary, did progesterone attenuate the effect of a second injection of estradiol on the increase in cytosolic progesterone receptors. The effect of progesterone on estrogen receptors was also confirmed in in vitro experiments and also in the adult rats (75,209).

In the adult rat, the measurement of both occupied and unoccupied estrogen receptor content was determined in the pituitary and the uterus after 0.8, 2.0 and 4.0 mg/kg body weight of progesterone. The 0.8 and 4.0 mg/kg body weight dose of progesterone was stimulatory to gonadotropin release while the 2.0 mg/kg body weight dose was inhibitory. The finding in the immature rat (146,148) and the adult rat ((57, 75) of a smaller dose of progesterone to be stimulatory to gonadotropin release and the next higher dose to be inhibitory was of great interest. It was shown that in the human menstrual cycle the initial increase in progesterone around the time of ovulation was stimulatory to gonadotropin release while a secondary rise coincided with the termination of the LH surge (101). High pulsatile levels of LH were also inhibited by rising levels of progesterone after ovulation in a patient with wedge-resection of the ovary (138).

In the pituitary and the uterus, the decrease in estrogen receptors occurred only in the occupied form and not the unoccupied estrogen receptors (75). However whereas the
pituitary showed a dose dependency of progesterone of the effect, the 0.8 and 4.0 mg/kg body weight doses being more effective than the 2.0mg/kg body weight dose in reducing occupied estrogen receptors, the uterus showed a systematic dose response. This was an interesting tissue difference. In the estrogen-primed progesterone treated group, 0.8 and 4.0 mg/kg body weight of progesterone increased estrogen receptor mRNA levels in the pituitary within 1 hour of administration whereas the 2.0 dose was ineffective. In spite of this early increase in estrogen receptor mRNA levels by the 0.8 and 4.0 mg/kg body weight of progesterone, these doses still depleted the occupied estrogen receptors of the pituitary. In the uterus, progesterone treatment in estrogen primed rats did not alter the estrogen receptor mRNA levels but depleted the occupied estrogen receptors (197). The mechanism for the decrease of occupied estrogen receptors of the pituitary and the uterus was by the stimulation of 17β-hydroxy dehydrogenase by progesterone (71, 76). This converted estradiol to estrone that had a lower affinity for the estrogen receptor than estradiol thereby reducing the suppressive effect of estrogens on gonadotropin secretion at the level of the pituitary. Accelerated estrogen receptor processing was also indicated. The antagonism of estrogen action by progesterone and 5α-dihydrotestosterone and their dose dependency was also shown in the inhibition of estrogen-induced prolactin release (30, 31, 38). Such dose dependence was also found in the inhibition of estrogen induced prolactin release by 5α-dihydroprogesterone (34). Surprisingly the anti androgen flutamide could not only block the action of 5α-dihydrotestosterone but progesterone as well (32). This was also true for RU486 which blocked progesterone as well as 5α-dihydrotestosterone effects.
(b) Hypothalamic regulation of LHRH secretion regulated by progesterone: It has already been stated that progesterone given to estrogen-primed ovariectomized rats increases the sensitivity of the pituitary to LHRH in the release of LH (148). Progesterone also stimulates the secretion of LHRH from the hypothalamus in the estrogen primed rat (182). The regulators of LHRH secretion, catecholamine as well as neuropeptide Y are also released by progesterone in the medial basal hypothalamus by progesterone and the glucocorticoid triamcinilone acetonide in the estrogen-primed ovariectomized rat (15, 26). The release of the above are more acutely related to LH secretion as compared to FSH secretion. The direct effect of neuropeptide Y on anterior pituitary cells in culture in sensitizing the pituitary to LHRH has also been demonstrated (168). It was also found that progesterone modulates the neuropeptide Y levels in the anterior pituitary during the progesterone-induced surge in the estrogen primed rat (169). Changes in galanin mRNA also correlate to the progesterone-induced gonadotropin surge (8). The above experiments clearly indicate that progesterone in estrogen-primed rats can bring about LHRH secretion that is responsible for the preovulatory surge of gonadotropins.

(c) Regulation of the peptidase activity involved in the degradation of LHRH in the hypothalamus and the pituitary: The biological activity of LHRH in the release of LH and FSH is also dependent upon the amount of LHRH that survives degradation by the peptidase activity in the hypothalamus and the pituitary. The peptidase activity of the hypothalamus and the pituitary was lower in the hypothalamus and the pituitary on proestrus and diestrus1 as compared to other parts of the ovulatory cycle thus leaving the maximum amount of LHRH available during this time for biological action (166). To
examine the role of progesterone on changes in the peptidase activity in the hypothalamus, pituitary and serum, the activity was measured in estrogen- primed ovariectomized rats before and after progesterone administration. Estrogen administration increased the peptidase activity of the hypothalamus which was antagonized by progesterone (167). Progesterone similarly decreased the peptidase activity of serum but had no effect on the peptidase activity of the pituitary. These results indicate that progesterone administration brings about a suppression of hypothalamic peptidase activity permitting the availability more LHRH for mounting the preovulatory gonadotropin surge.

(d) Regulation of LH-β mRNA and FSH-β mRNA levels by progesterone during the preovulatory gonadotropin surge: Initial experiments did not show any consistent effect of progesterone on the pituitary LH-β mRNA and FSH-β mRNA levels in the ovariectomized estrogen- primed rat treated with progesterone whereas dexamethasone increased the level of FSH-β mRNA before the rise of serum FSH (27). This may be due to the fact that the gonadotropin subunit levels in ovariectomized estrogen- primed rats were several folds higher than those in intact estrogen- primed rats. In the estrogen-primed intact rat, progesterone administration brought about an elevation of LH-β mRNA and FSH-β mRNA in parallel with the preovulatory gonadotropin surge (28). This also occurred in the PMSG- primed immature rat. The changes in the mRNA levels were blocked by the anti progestin RU486 (28). Further work showed that a 361 base pair region of the FSH promoter gene contained several progesterone response elements and these mediated the progesterone effect on the FSH gene (170,171).
(e) Selective release of LH and FSH by progesterone metabolites: In the estrogen-primed immature ovariectomized rat the administration of the progesterone metabolite 5α-dihydroprogesterone brought about the selective release of FSH (155) and the progesterone metabolite 3α, 5α-tetrahydroprogesterone brought about a selective release of LH (156). The selective release of LH and FSH by the above mentioned progesterone metabolites also took place in the PMSG- treated immature rats exposed to constant light (157). Initially the effect of 3α, 5α-tetrahydroprogesterone was unexpected as the compound does not interact with the progesterone receptor. However, several steroidal anesthetics that have a 3α, 5α-reduced Ring A structure had been shown to use the Gamma Amino Butyric Acid A (GABA A) receptor system for their biological action. Further work showed that the action of 3α, 5α-tetrahydroprogesterone on LH release could be blocked by GABA A receptor antagonists but not by RU486 (33). Progesterone metabolites were also able to dampen the estrogen induced uterine contractions. Once again, similar to the FSH and LH release, the dampening effect of 5α-dihydroprogesterone was blocked by the progesterone receptor antagonist RU486, while the 3α, 5α-tetrahydroprogesterone effect was blocked by the GABA A antagonist (189). That the effect of progesterone in the release of FSH was mediated by its 5α-reduction to 5α-dihydroprogesterone was demonstrated by the use of a 5α-reductase inhibitor N,N-diethyl-4-methyl-3oxo-4-aza-5α-androstane- 17β-carboxamide. The use of this inhibitor blocked the reduction of progesterone in the 5α-position and the progesterone induced FSH surge without affecting the progesterone induced LH surge (Figure2) (191). 5α-dihydroprogesterone also brought about the depletion of occupied estrogen receptors of the anterior pituitary and the uterus similar to progesterone (77). A significant amount of
the stimulatory and inhibitory effect of LHRH on gonadotropin secretion by progesterone occurred at the level of the pituitary as shown in pituitary cell cultures (112). In estrogen-
primed pituitary cells, progesterone exposure for 1 to 6 hours brought about the potentiation of LHRH in the release of LH and FSH, while progesterone exposure of 12 hours or more were inhibitory. The $5\alpha$-reductase inhibitor blocked the effect of progesterone on FSH release. It was also of interest to note that in the estrogen-primed rat, the administration of the opioid antagonist naloxone brought about an increase in LH secretion. An inhibitor of GABA degradation aminoxyacetic acid and both GABA A and GABA B agonists blocked this action (44). In this regard, a decreased opioid response to naloxone was observed in patients with premenstrual tension (196). $3\alpha$, $5\alpha$-reduced steroids have an anesthetic and anxiety reducing effect. In women with premenstrual tension, the metabolism of progesterone to $3\alpha$, $5\alpha$-tetrahydroprogesterone was compromised (195).

EXCITATORY AMINOACIDS AS MEDIATORS OF THE STEROID-
GONADOTROPIN FEED BACK SYSTEM

ESTABLISHING THE POSSIBLE ROLE OF EXCITATORY AMINO ACIDS IN GONADOTROPIN SECRETION

Early work had suggested that glutamate may have a role in the release of LH (175). To determine whether excitatory amino acids mediated the steroid induced gonadotropin surge, estrogen-primed immature rats treated with progesterone or triamcinilone acetonide were injected with the N-methyl-D-aspartate (NMDA) antagonist MK801[ (+)-
5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine maleate\[(18) MK801
blocked the progesterone or triamcinilone acetonide induced LH and FSH surge. MK801
also reduced the number of ova ovulated in the PMSG- treated rat along with a decrease
in the LH and FSH surge (19). MK801 also decreased serum LH levels in the adult
cycling rat. The excitatory amino acid agonist NMDA given to estrogen –primed rats
brought about a prompt elevation of LH and FSH. The effect seemed to be at the
hypothalamus because medial basal hypothalamus/preoptic area fragments showed an
elevated release of LHRH in vitro 5 and 7 minutes after the administration of NMDA (19,
20, 21). These findings are of considerable importance as it was shown that the GnRH
neuron did not appear to have steroid receptors where there were steroid receptors in
 glutamate containing neurons (100, 111). Thus the GnRH neuron was regulated by other
neurons in the hypothalamus that had steroid receptors. The subject has been reviewed
extensively (22,23,24) To determine whether non-NMDA neurotransmission also
regulated the gonadotropin surge, the non NMDA receptor antagonist DNQX(6,7-
dinitroquinoxaline-2,3-dione) was administered via a third ventricular canula in estrogen-
primed adult rats treated with progesterone and PMSG- primed immature rats. DNQX
attenuated the LH and the prolactin surge without much effect on the FSH surge (29).
Both NMDA and non-NMDA receptors played a role in pulsatile LH release as shown by
the injection of the specific NMDA receptor antagonist AP5 (2-amino-5-phosphono-
pentanoic acid) and the non-NMDA antagonist, DNQX, in adult rats ovariectomized for 2
weeks via a third ventricular canula (185). Similar results were obtained in the male rat as
well although the NMDA receptor antagonist was more effective than the non-NMDA
receptor antagonist (186). AMPA( alpha-amino-3-hydroxy-5-methylisoxazole-4-
Propionic acid) also brought about LH release in the estrogen-primed rat indicating a role for AMPA receptors. The steroid-induced gonadotropin surge was blocked by the selective AMPA receptor antagonist NBQX (184).

Since NMDA was more effective in gonadotropin release in the estrogen-primed female rat as compared to the ovariectomized rat, the NMDA receptor in the hypothalamus was characterized (141) and the NMDA receptor binding and NMDA receptor mRNA was measured in the hypothalamus on castration and after estrogen priming (41). No change was observed in castrated female rats after estrogen treatment or in castrated male rats with testosterone treatment. The effect of steroids on NMDA receptor mRNA levels in the pituitary was also examined. Estrogen treatment of the ovariectomized rat showed a decline in pituitary NMDA mRNA. However progesterone treatment to the estrogen primed rat resulted in an increase in pituitary NMDA mRNA which is in keeping with the direct effects of progesterone on the pituitary during the gonadotropin surge mentioned earlier in this article (9). During the rat ovulatory cycle NMDA Recept1 (NMDAR1) and Glutamate Receptor 1 (GluR1) did not change much but the levels of the kainite receptor GluR6 (Glutamate Receptor 6) decreased just preceding the surge. This decrease was demonstrated to be due to progesterone action (42).

The administration of NMDA advanced puberty in the rat by 2.5 days whereas the non-NMDA agonist kainite or the non-NMDA antagonist had no effect on puberty, indicating that NMDA was primarily involved in the process of puberty (43). The NMDA receptors did not change significantly in the pituitary during puberty while the AMPA receptors...
receptors showed an increase (219). This indicated selective excitatory amino acid receptor involvement during puberty.

DETERMINING WHETHER GLUTAMATE WAS ACTUALLY SECRETED DURING THE GONADOTROIPN SURGE

The next important question was to determine whether the role of glutamate which was indicated to be important in the gonadotropin surge by the study of various agonist and antagonist studies of various receptors of glutamate action, could be established by the actual secretion of glutamate during the ovulatory surge of gonadotropins. To achieve this, micro dialysis was done in vivo from the preoptic area through a canula implanted in the estrogen-primed rat given progesterone to induce ovulation. There was a significant increase in the preoptic area release of glutamate and aspartate immediately preceding the preovulatory gonadotropin surge (187).

Naloxone has been shown to bring about LH release by inhibiting the opioid inhibitory mechanism in several studies. In male rats, administration of naloxone brought about an increase in serum LH and an increase in nitric oxide syntase (NOS) in the preoptic area and medial basal hypothalamus within 20 minutes (5). This increase was blocked by the NMDA antagonist MK801. Micro dialysis experiments in vivo of the preoptic area also showed a significant increase in glutamate secretion within 15 minutes of naloxone administration. Thus the opioid block of gonadotropin secretion appeared by their suppression of glutamate release.

LOCALIZATION OF NMDA-R1 RECEPTORS IN THE HYPOTHALAMUS AND THE ROLE OF NITRIC OXIDE SYNTASE
Physiochemical localization of the NMDA-R1 containing neurons showed extensive distribution in the hypothalamus including the Organum Vasculosam of the Lamina Termanailis (OVLT), median preoptic nucleus and the median preoptic area (4). This NMDA-R1 staining area did not colocalize with the GnRH neuron but surrounded several of them. They colocalized with NOS 1 activity. Central administration of an NOS inhibitor abolished the steroid induced preovulatory surge indicating that NMDA acted through nitric oxide in the release of LHRH. NMDA-R1 was also found in almost all cell types of the anterior pituitary suggesting some pituitary action of NMDA (6). Further work showed that the hypothalamus contained all three isoforms of nitric oxide syntase namely brain NOS (NOS 1), microphage NOS (NOS 2) and endothelial NOS (NOS 3). Of these NOS 1 was the major isoforms and acted through the activation of c-GMP (3). The role of NOS 1 in the release of steroid induced gonadotropin surge was further confirmed by the administration of antisense nucleotides to NOS 1 which attenuated the steroid-induced gonadotropin surge (1). NOS levels were also shown to increase during the proestrus gonadotropin surge in the rat (108). A regulatory role of carbon monoxide on LH release has also been suggested (109). The role of gaseous neurotransmitters in the release of gonadotropins has been reviewed (7).

MECHANISM OF ACTION OF PROGESTERONE IN RELEASING GLUTAMATE AND SUPPRESSING GABA

The next question was as to how progesterone brought about the release of glutamate during the preovulatory gonadotropin surge. This was shown to be due to the suppression of Glutamic Acid Decarboxylase 67 (GAD67) by progesterone in the estrogen-primed rat, resulting in the decrease of glutamate converted to GABA, thus
permitting more glutamate and less GABA to be secreted (Figure3) (215). The suppression of GAD67 during the preovulatory surge of gonadotropins was also shown to occur in the adult rat (39). In addition, the synaptic terminals appositions to the GnRH neuron of the Vesicular GABA Transporter (VGAT) was decreased while the Vesicular Glutamate Transporter 2 (VGLUT2) were increased during the proestrus gonadotropin surge, thus further confirming the role of glutamate in the surge(104).

ROLE OF HYPOTHALAMIC ASTROCYTES IN STEROID-GONADOTROPIN FEEDBACK

In order to determine, whether astrocytes played any role in stimulating hypothalamic LHRH secretion, conditioned medium from hypothalamic astrocytes was incubated with either hypothalamic fragments or the immortalized GnRH neuronal cells, the GT1-7 cells (49). The hypothalamic conditioned media brought about the release of LHRH. Ultra filtration of the astrocytes conditioned medium to remove peptides greater than 10 kDa resulted in loss of activity. The peptide responsible for the release of LHRH was found to be Transforming Growth Factor-β (TGF-β). Antibodies to TGF-β abolished the ability of the conditioned media to release LHRH. Furthermore, the hypothalamus and the GT1-7 cells possessed TGF-β receptors. Estrogens stimulated astrocytes to release TGF-β and this action was attenuated with estrogen receptor antagonists. Astrocytes also possessed estrogen receptors. Astrocytes conditioned medium also protected GT1-7 cell cultured in serum free medium from death and this was attributed to TGF-β. The neuroprotective pathway appeared to be via the activation of c-Jun/AP-1 pathway (66). Similar to estradiol, tamoxifen was also able to reduce the extent of stroke.
in experimental animals in which the medial carotid artery was blocked (150). The
neuroprotective effects of estrogens were exerted in part by the stimulation of TGF-β
secretion using a non-genomic mechanism (68). This was another example of the non-
genomic effect of steroids (13). The role of astrocytes in reproduction and
neuroprotection has also been reviewed (116). Extensive work on the mechanisms
involved in the estrogen effects on neuroprotection has continued but is outside the scope
of this article to be described in detail (11, 45, 67, 69, 105, 216, 222).

OTHER RELATED STUDIES

The author has been involved in a number of related studies, a detailed description of
which is outside the scope of this article. They are: Studies of steroid gonadotropin
feedback in aging (25, 42, 104, 147, 225); testicular feminization (154); gonadal
dysgenesis (90, 91, 143,); metabolism of oral contraceptives (47, 48, 151, 152, 153);
heterogeneity in granulosa cells (192, 194); regulation of follicular development by
diethyl stilbesterol (59, 60); and estrogen and progesterone receptors in the human
endometrium (158, 159) and pregnancy in the rat (172, 173, 174). Other studies refer to the
role of kinins in the ovulatory cycle and pregnancy and the possible role of Bradykinin in
the preovulatory gonadotropin surge (12, 81, 205, and 206). The role of leptin in
reproduction was also of considerable interest (10, 40, 50, 58, 218, 220). Finally,
computer modeling of partially unwound DNA structures caused by receptor binding
show a stereochemical fit of estradiol in the cavity in the center of the hormone response
element. The fit in the DNA cavity is a much more accurate prediction of biological
activity as compared to estrogen binding to its receptor. The technique has led to the
development of search engines that can be used in drug design (46, 96, 97, 98, 99, 117, 118, 136, 137)

SUMMARY AND CONCLUSIONS

Early work on the polycystic ovary syndrome was a major breakthrough as it established the ovary with or without the involvement of the adrenal as a source of androgens. The reason for the occurrence of hirsutism and virilism in variety endocrine patients was also established. Experimental models for creating polycystic ovaries as well as other experiments established the detrimental effects of excessive androgens on follicular development that resulted in cystic ovaries. The studies also resulted in establishing the use of Clomiphene for the induction of ovulation in anovulatory women. Animal studies showing the ability of dexamethasone in releasing FSH resulted in the use of dexamethasone in combination with Clomiphene to induce ovulation, in women had not shown to have excessive androgen secretion, who had failed to ovulate on treatment with Clomiphene alone. A link between premenstrual tension and abnormal metabolism of progesterone was also established. The above are excellent examples of translational applications of basic science research.

There is no clear understanding of the importance of the FSH surge at the time of ovulation in the human. The finding that FSH can bring about the rupture of the mature ovarian follicle and that several women with short luteal phase have a decreased or displaced FSH surge is intriguing and further work needs to be done in this area.

The studies described in this article also provide the sequence of events starting with follicular growth and an increase in estrogens resulting in the occurrence of puberty in the
female and similar effects with androgens in the male rat. Although the studies provide a solid foundation of the events that take place during puberty, the mechanisms that trigger the initial increase in follicular growth and estrogen secretion in female rats and androgen secretion in male rats are still obscure and require further investigation.

The role of estradiol in triggering the preovulatory surge of gonadotropins has been well recognized in the literature. The studies described in this article clearly show the pivotal role of progesterone in the process of modulating the preovulatory surge of gonadotropins. The dependence of progesterone action on estrogens is due to the synthesis of progesterone receptors by estrogen action. Progesterone acts by stimulating hypothalamic release of LHRH; suppressing the peptidase activity thus making more LHRH available; increasing the pituitary sensitivity to LHRH along with synthesis of new LH and FSH; and decreasing the occupied nuclear estrogen receptors of the pituitary thus overriding the suppressive effects of estrogens on the pituitary in gonadotropin release. The differential regulation of FSH and LH has also been a subject of considerable interest. The demonstration of selective effects of progesterone metabolites on FSH and LH secretion and the use of not only the progesterone receptor but the GABA A receptor as well in this process is therefore of considerable importance.

The work described in this article has resulted in the replacement of the long held belief that gonadal steroids act directly on the GnRH neuron to regulate gonadotropin secretion, with the new and novel concept that estrogens and progesterone act on the glutamatergic neurons of the hypothalamus that contain steroid receptors and these neurons in turn regulate the GnRH neuron via NOS-1. Thus this new pathway adds on to
the multiple pathways known or currently under discovery of the regulation of reproduction which is essential for the preservation and continuation of the species. Finally the concept of the neuroprotective effect of estrogens on brain function is of considerable importance. This neuroprotective effect is lost during years of estrogen deprivation during menopause leading to increased incidence of stroke and dementia. Studies on the mechanism of loss of the neuroprotective effect of estrogens during estrogen deprivation and methods to circumvent it would be of considerable value for the health and well being of our aging population.

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Figure Legends

Figure 1: The figure shows the stimulatory effects of progesterone on the hypothalamus in the release of LHRH and the stimulation of 17β-Hydroxy Steroid Dehydrogenase activity in the pituitary. It also had direct action on the pituitary in increasing the sensitivity of the pituitary to LHRH in the release of LH and the synthesis of new LH and FSH. Progesterone also inhibited peptidase activity in the hypothalamus and pituitary slowing the degradation of LHRH and increasing greater availability of LHRH for LH secretion. The enhanced 17β-Hydroxy Steroid Dehydrogenase activity brings about increased conversion of estradiol to estrone in the pituitary resulting in decreased nuclear occupancy of the estrogen receptor. Progesterone thus decreased the inhibitory action of estrogens on the pituitary on LH release and decreased progesterone receptor synthesis. Decreased progesterone receptors prevent the stimulatory effect of progesterone on LH secretion. The stimulatory pathways are shown in green and the inhibitory pathways in red.

Figure 2 Progesterone is converted into two major metabolites 5α-Dihydro Progesterone and 3α,5α-Tetrahydro Progesterone. The former uses the progesterone receptor and brings about selective release of FSH. The latter uses the GABA A receptor and brings about selective release of LH. The administration of a 5α-reductase inhibitor in vivo or in vitro reduced FSH release without affecting LH release. Stimulatory pathways are shown in green while the inhibitory pathways are shown in red.

Figure 3 Progesterone inhibition of Glutamic Acid Dehydrogenase 67 (GAD67) permits
more glutamate release and less GABA release thus permitting an LH surge. Stimulatory events are shown in green and inhibitory events in red.

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FIGURE 1

Stimulatory

- Progesterone
  - Pituitary 17β-Hydroxy Steroid Dehydrogenase
  - Estradiol to Estrone
  - Decreased Occupied Pituitary Estrogen Nuclear Receptor
  - Decreases Estrogen Inhibition Of Pituitary
  - Decreased Progesterone Receptor Synthesis

Inhibitory

- Progesterone
  - Suppresses Peptidase Activity
  - Results in more LHRH

- LHRH
  - Enhanced Synthesis of LH and FSH
  - Increased Sensitivity To LHRH
  - Enhanced LH Secretion
FIGURE 2

Progesterone

5α-Reductase Inhibitor (Decreases)

5α-Dihydro Progesterone (Progesterone Receptor)

FSH Release

3α,5α- Tetrahydro Progesterone (GABA Receptor)

LH Release

5α-Reductase Inhibitor
Decreases FSH Release

LH Release not altered

Stimulatory

Inhibitory
FIGURE 3

Glutamate $\rightarrow$ GAD 67 $\rightarrow$ GABA

Progestosterone inhibits

More Glutamate $\rightarrow$

More NOS-1 $\rightarrow$

GnRH Neuron $\rightarrow$

LHRH Release

Stimulatory $\rightarrow$ Inhibitory

Less GABA