Hirsutism, virilism, polycystic ovarian disease, and the steroid-gonadotropin-feedback system: a career retrospective

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Submitted 19 September 2011; accepted in final form 20 October 2011

Mahesh VB. Hirsutism, virilism, polycystic ovarian disease, and the steroid-gonadotropin-feedback system: a career retrospective. Am J Physiol Endocrinol Metab 302: E4–E18, 2012. First published October 25, 2011; doi:10.1152/ajpendo.00488.2011.—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 excessive ovarian and or adrenal androgen secretion in polycystic ovarian disease had been established, 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 ovariecctomized rats showed that progesterone was a pivotal enhancer of 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.

estrogens; androgens; progesterone

IN THIS CAREER RETROSPECTIVE ARTICLE, the author describes 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 the steroid-gonadotropin feedback system, leading into a lifelong career of scientific investigation from 1956 to 2010. The approach for solving the questions generated was inspired by 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 all 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.

In the mid 1950s, there was no clear understanding of 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 compared with 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 might 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 α-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 α-side with ease and reduce it to the 11β-hydroxyl group. On the contrary, in the 3α,5β-stereoids, Ring A of the steroid is at a 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-androstane-17-one; 3α-hydroxy-5α-androstan-11, 17-dione; 3α,11β-dihydroxy-5β-androstane-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-stereoids but not in the 3α,5β-stereoids. 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-cortisone 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-flouro steroids was also explained, in part, due to a greater persistence of the 11β-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 compared with her normal sister and was hyperresponsive 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 (3β-hydroxy-5-androsten-17-one) and androstenedione (4-androsten-3,17-dione) in the adrenal vein blood. In the mid 1950s, 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 infrared spectroscopy along with 17α-hydroxy-5-pregnenolone (3β,17α-dihydroxy-5-pregnen-20-one). Some ovaries contained large quantities of androstenedione compared with 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 compared with normal ovaries, suggesting diminished 3β-hydroxysteroid 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 amounts of testosterone from the polycystic ovaries studied, it was of interest to determine whether 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), it was important to determine whether, 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 oversecretion of androgens, ovarian oversecretion of excessive androgens, or both. Human FSH enhanced ovarian hypersecretion 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 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 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 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 long-term ovarioctomized 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 polycystic ovary syndrome who underwent wedge resection of the ovary; the high pulsatile levels of LH persisted only until 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), arrenoblastoma (93, 132), adrenal rest tumor of the ovary (181), virilizing adrenal tumors (124, 127), and delayed onset of congenital adrenal hyperplasia (128). 11β-Hydroxyestrone was also isolated in a case of feminizing adrenal carcinoma (130). Three overall questions emerged from the aforementioned studies. The first question was whether there was a treatment to induce ovulation in patients with 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 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 polycystic ovary syndrome patients. No chromosomal abnormalities were found in patients with polycystic ovary syndrome (56); therefore, animal models were constructed in an attempt to answer that question.

**Induction of Ovulation in Patients with Polycystic Ovary Syndrome**

In attempts to search for agents other than human pituitary FSH or human postmenopausal 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-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, whereas it inhibited them at high doses (200). In the absence of estrogens in the ovariec-tomized rat, Clomiphene acted as a weak estrogen. It acted as an antiestrogen 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 antiestrogenic 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 polycystic ovary syndrome (94, 199). It was also able to stimulate spermatogenesis in men (103). Clomiphene was also able to induce ovulation in Chiari-Frommel syndrome, in which 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 five 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 Antiandrogens for the Management of Hirsutism**

Management of hirsutism in patients with polycystic ovary syndrome has been difficult, as the suggested approaches were ovarian suppression by 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. Establishment of an 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 antiandrogen, 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 antigonadotropic effect. The antigonadotropic 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 antiandrogen might become accidentally pregnant, causing fetal abnormalities, has prevented the use of antiandrogens in the management of hirsutism.

**Animal Models for Polycystic Ovarian Disease in Humans**

The question whether 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 diestrous vaginal smears with either polycystic ovaries or ovaries containing corpus luteum-like structures. With time, all ovaries became polycystic. Serum FSH levels were elevated compared with 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 one, with the exception that prolactin rather than LH was elevated. Further studies with precocious ovulation showed that the gonadotropin surge resulting in ovulation could be blocked with central nervous system blocking agents such as phenobarbital and reserpine (106). Precocious ovulation appeared to be mediated by the conversion of dehydroepiandrosterone to estrogens, because the nonaromatizable androgen 5α-dihydrotestosterone did not cause such an ovulation (107). Furthermore, cyanoketone, an inhibitor of 3β-hydroxysteroid dehydrogenase, which blocks the conversion of dehydroepiandrosterone to estrogen, prevented vaginal patency, and dehydroepiandrosterone-induced precocious ovulation (107). A study of the steroid profile after dehydroepiandrosterone administration showed an elevation in blood estradiol followed by 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 occurred only when the
high levels of dehydroepiandrosterone in blood were reduced (178). Because the earlier 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 polycystic ovary syndrome in women is often associated with obesity and insulin resistance and insulin type growth factors hyperstimulate 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 nonaromatizable 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 was 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 autoregulatory effect of androgens on ovarian theca cell androgen production (207, 208).

THE STEROID-GONADOTROPIN FEED BACK SYSTEM

The abovementioned 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 feedback 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 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, ovulation could be induced with purified pituitary FSH that did not contain enough LH contaminant to cause ovulation. In the hamster, antiserum prepared against a prepituary FSH that did not contain enough LH contaminant blocked ovulation greatly reduced. In addition, ovulation blocked by anti-LH could be reinstated 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 α-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 can 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 in 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 6:00 PM on diestrus II or 9:00 AM 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 h in male rats and in 24 h for LH and 48 h 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 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 ovariec- tomized rat (144). Using 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 four- to sixfold higher in 45- and 95-day-old rats compared with 26-day-old rats, indicating that a maturation of the feedback system after puberty even though the increase in uterine weight caused by estrogen administration was comparable in the three groups (74). This change in sensitivity to the negative feedback 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. Measurement of the β-endorphin content in the hypothalamus of the immature ovariecrotomized rat treated with estradiol and progesterone or triamcinolone acetonide (9α-fluoro-11β,21- dihydroxy-1,4-pregnadiene-3,20-dione-16α,17α-acetonide) to induce the preovulatory type surge of gonadotropins resulted in an estradiol-induced decrease in β-endorphin preceding and during the LH surge, which was maintained the following morning (37). However, progesterone and triamcinolone acetonide brought back the β-endorphin levels.
Changes Occurring in Steroid Levels During the Preovulatory Gonadotropin Surge and 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 onward. The endometrial stroma, myometrium, and luminal epithelium started growing on day 32 of age along with an increase in uterine weight. These changes in 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. 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 2 μg 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 h after the estrogen injection in preparation for the estrogen receptor replenishment that started at 18 h. Thus, the initial decline in estrogen receptor content was not due to loss of estrogen receptor mRNA but to accelerated estrogen receptor processing (61). In the uterus, similar changes took place, and progesterone was shown to delay estrogen receptor replenishment (223, 224). Pituitary ultrastructure 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, 161–164). A detailed description, however, is outside 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, 17 113–115, 134). The results of these studies are also summarized in Figs. 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 ~10% of the preovulatory gonadotropin surge. Thus, experiments were done using doses of estrogens that were able to reduce the postcastration 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 either to stimulate the release of LH and FSH or to suppress them with the next higher dose. The pattern of the lower dose being stimulatory and the next higher dose being 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 article.

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 wt dose of estradiol in female rats was 2.0 μg/kg body wt (149). This was

Fig. 1. Stimulatory effects of progesterone on the hypothalamus in the release of LHRH and stimulation of 17β-hydroxysteroid dehydrogenase activity in the pituitary. It also had direct action on the pituitary in increasing the sensitivity of the pituitary to LHRH in release of LH and synthesis of new LH and FSH. Progesterone also inhibited peptidase activity in the hypothalamus and pituitary, slowing degradation of LHRH and increasing greater availability of LHRH for LH secretion. Enhanced 17β-hydroxysteroid 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. Stimulatory pathways are shown in green, inhibitory pathways in red.

AJP-Endocrinol Metab • doi:10.1152/ajpendo.00488.2011 • www.ajpendo.org
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 RU-486 (17β-hydroxy-11β-[4-dimethylaminoprogenyl]-17α-[prop-1-ynyl]-estra-4,9-diene-3-one) (193). RU-486 abolished the preovulatory gonadotropin surge in PMSG-treated 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 h before the initiation of the rise of LH (101) and 6 h 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 glucocorticoid triamcinilone acetonide were also able to induce a preovulatory type of gonadotropin surge, whereas 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 PMSG-primed rats, similar to progesterone, which served as a biological test for their effect (35). In the estrogen-primed ovariec-tomized rat, acute administration of ACTH also re-established estrogen receptor processing was also indicated.

Mechanism of action of progesterone on the release of gonadotropins. 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 occurred only 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 in the adult rats (75, 209).

In the adult rat, 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 wt progesterone. The 0.8 and 4.0 mg/kg body wt dose of progesterone was stimulatory to gonadotropin release, whereas the 2.0 mg/kg body wt 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, whereas 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 wt doses being more effective than the 2.0 mg/kg body wt 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 wt progesterone increased estrogen receptor mRNA levels in the pituitary within 1 h of administration, whereas the 2.0 dose was ineffective. Despite this early increase in estrogen receptor mRNA levels by the 0.8 and 4.0 mg/kg body wt 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-hydroxysteroid dehydrogenase by progesterone (71, 76). This converted estradiol to estrone, which 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 antiandrogen flutamide could not only block the action of 5α-dihydrotestosterone but that of progesterone as well (32). This was also true for RU-486, which blocked progesterone as well as 5α-dihydrotestosterone effects.

HYPOTHALAMIC REGULATION OF LH SECRETION REGULATED BY PROGESTERONE. It has already been stated that progesterone given to estrogen-primed ovariectomized rats increases the sensitivity of the pituitary to LH release in the release of LH (148). Progesterone also stimulates the secretion of LH from the hypothalamus in the estrogen-primed rat (182). The regulators of LH 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 than 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 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-mentioned experiments clearly indicate that progesterone in estrogen-primed rats can bring about LHRH secretion, which is responsible for the preovulatory surge of gonadotropins.

REGULATION OF PEPTIDASE ACTIVITY INVOLVED IN THE DEGRADATION OF LH AND 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 diestrus 1 compared with other parts of the ovariectomy cycle, thus leaving the maximum amount of LHRH available during that 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 of more LHRH for mounting the preovulatory gonadotropin surge.

REGULATION OF LH-ß MRNA AND FSH-ß MRNA LEVELS BY PROGESTERONE DURING THE PREOVULATORY GONADOTROPIN SURGE. Initial experiments did not show any consistent effect of progesterone on the pituitary LH-ß mRNA and FSH-ß mRNA levels in the ovariectomized estrogen-primed rat treated with progesterone, whereas dexamethasone increased the level of FSH-ß 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 severalfold higher than those in intact estrogen-primed rats. In the estrogen-primed intact rat, progesterone administration brought about an elevation of LH-ß mRNA and FSH-ß 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 antiprogestin RU-486 (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).

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 A ring structure had been shown to use the GABA receptor system for their biological action. Further work showed that the action of 3α,5α-tetrahydroprogesterone on LH release could be blocked by GABA receptor antagonists but not by RU-486 (33). Progesterone metabolites were also able to dampen the estrogen-induced uterine contractions. Once again, similar to the FSH and LH release, the dampening effects of 5α-dihydroprogesterone was blocked by the progesterone receptor antagonist RU-486, whereas the 3α,5α-tetrahydroprogesterone effect was blocked by the GABA receptor antagonist (189). That the effect of progesterone in the release of FSH was mediated by the 5α-reduction to 5α-dihydroprogesterone was demonstrated by the use of a 5α-reductase inhibitor N,N-diethyl-4-methyl-3-oxo-4-aza-5α-androstan-17β-carboxamide. The use of this inhibitor blocked the reduction of progesterone in the 5α position and the progesterone induced a FSH surge without affecting the progesterone-induced LH surge (Fig. 2) (191). 5α-Dihydroprogesterone also brought about the depletion of occupied estrogens receptors of the anterior pituitary and the uterus similar to progesterone (77). A significant amount of the stimulatory and inhibitory effects 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 1–6 h brought about the potentiation of LHRH in the release of LH and FSH, whereas progesterone exposure of 12 h or more was inhibitory. The 5α-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 naltrexone brought about an increase in LH secretion. An inhibitor of GABA degradation aminooxyacetamide and both GABA receptor antagonists blocked this action (44). In this regard, a decreased opioid response to naltrexone was observed in patients with premenstrual tension (196). 3α,5α-reduced steroids have an anesthet-
ic- and anxiety-reducing effect. In women with premenstrual tension, the metabolism of progesterone to 3α,5α-tetrahydroprogesterone was compromised (195).

EXCITATORY AMINO ACIDS 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 triamcinolone 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 triamcinolone 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 prompt elevations of LH and FSH. The effect seemed to be at the hypothalamus, because medial basal hypothalamus/preoptic area fragments showed an elevated release of LHRR in vitro 5 and 7 min after the administration of NMDA (19–21). These findings are of considerable importance, as it was shown that the GnRH neuron did not appear to have steroid receptors whereas 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 (22–24). To determine whether non-NMDA neurotransmission also regulated the gonadotropic surge, the non-NMDA receptor antagonist DNQX (6,7-dinitroquinoxaline-2,3-dione) was administered via a third ventricular cannula in estrogen-primed adult rats treated with progesterone and PMSG-primed immature rats. DNQX attenuated the LH and the prolactin surges without much effect on the FSH surge (29). Both NMDA and non-NMDA receptors played a role in pulsatile LH release, as shown by injection of the specific NMDA receptor antagonist AP5 (2-amino-5-phosphono-pentoic acid) and the non-NMDA antagonist DNQX in adult rats ovariectomized for 2 wk via a third ventricular cannula (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 (α-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 gonadotropic 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 compared with the ovariectomized rat, the NMDA receptor in the hypothalamus was characterized (141), and the NMDA receptor binding and NMDA receptor mRNA were measured in the hypothalamus upon 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 gonadotropic surge mentioned earlier in this article (9). During the rat ovulatory cycle, NMDA receptor 1 (NMDAR1) and glutamate receptor 1 (GluR1) did not change much, but the levels of the kinase receptor GluR6 decreased just preceding the surge. This decrease was demonstrated to be due to progesterone action (42).

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, whereas the AMPA receptors showed an increase (219). This indicated selective excitatory amino acid receptor involvement during puberty.

Determining Whether Glutamate Was Actually Secreted During the Gonadotropin Surge

The next important question was to determine whether the role of glutamate, which was indicated to be important in the gonadotropic 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, microdialysis was done in vivo from the preoptic area through a cannula 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 synthase (NOS) in the preoptic and media basal hypothalamus within 20 min (5). This increase was blocked by the NMDA antagonist MK801. Microdialysis experiments in vivo of the preoptic area also showed a significant increase in glutamate secretion within 15 min of naloxone administration. Thus, the opioid block of gonadotropin secretion appeared by their suppression of glutamate release.

Localization of NMDAR1 Receptors in the Hypothalamus and the Role of NOSs

Physicochemical localization of NMDAR1-containing neurons showed extensive distribution in the hypothalamus, including the organum vasculosum of the lamina terminalis (OVLT), median preoptic nucleus, and median preoptic area (4). This NMDAR1 staining area did not colocalize with the GnRH neuron but surrounded several of them; they colocalized with NOS-1 activity. Central administration of a NOS inhibitor abolished the steroid-induced preovulatory surge, indicating that NMDA acted through NO in the release of LHRR. NMDAR1 was also found in 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 NOS, namely brain NOS (NOS-1), microphage NOS (NOS-2), and endothelial NOS (NOS-3). Of
these, NOS-1 was the major isoform and acted through the activation of cGMP (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 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 (Fig. 3) (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) was 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

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 GT-1-7 cells (49). The hypothalamic conditioned medium 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 (TGFβ). Antibodies to TGFβ abolished the ability of the conditioned medium to release LHRH. Furthermore, the hypothalamus and the GT-1-7 cells possessed TGFβ receptors. Estrogens stimulated astrocytes to release TGFβ, and this action was attenuated with estrogen receptor antagonists. Astrocytes also possessed estrogen receptors. Astrocytes’ conditioned medium also protected GT-1-7 cell cultured in serum-free medium from death, and this was attributed to TGFβ. The neuroprotective pathway appeared to be via the activation of the 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 TGFβ secretion using a nongenomic mechanism (68). This was another example of the nongenomic effect of steroids (13). The role of astrocytes in reproduction and neuroprotection has also been reviewed (116). Extensive work on the mechanisms involved in estrogen effects on neuroprotection has continued but is outside the scope of this article but is described in detail elsewhere (11, 45, 67, 69, 105, 216, 222).

Other Related Studies

This 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), estrogen and progesterone receptors in the human endometrium (158, 159), and pregnancy in the rat (172–174). 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, 206). The role of leptin in reproduction is also of considerable interest (10, 40, 50, 58, 218, 220). Finally, computer modeling of partially unwound DNA structures caused by receptor binding shows 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 than estrogen binding to its receptor. The technique has led to the development of search engines that can be used in drug design (46, 96–99, 117, 118, 136, 137)

Summary and Conclusions

Early work on 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 reasons for the occurrence of hirsutism and virilism in a variety of 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 not shown to have excessive androgen secretion but who had failed to ovulate on treatment with Clomiphene alone. A link between premen-
strual 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 LH, suppressing the peptidase activity and thus making more LH available, increasing the pituitary sensitivity to LH 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 GABAA 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 novel concept that estrogens and progesterone act on the glutamatergic neurons of the hypothalamus that contain steroid receptors and that these neurons in turn regulate the GnRH neuron via NOS-1. Thus, this new pathway adds 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.

ACKNOWLEDGMENTS

I wish to express my gratitude to numerous graduate students, postdoctoral fellows, and faculty colleagues for their untiring collaboration and help in introducing new techniques in the laboratory from time to time.

GRANTS

This research was supported by Grants AM-04429, HD-04626, HD-10795, HD-16688, HD-14196 and HD-16396 from the National Institutes of Health.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: V.B.M. conception and design of research; V.B.M. performed experiments; V.B.M. analyzed data; V.B.M. interpreted results of experiments; V.B.M. drafted manuscript; V.B.M. edited and revised manuscript; V.B.M. approved final version of manuscript.

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