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ROY O. GREEP

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PREFACE

The 1973 Laurentian Hormone Conference was held at Mont Tremblant, Province of Quebec, Canada, August 26–31. The proceedings of that meeting constitute this volume of *Recent Progress in Hormone Research* and illustrate anew the continuous gathering of investigative momentum and sophistication in the field of endocrinology. In accordance with long-standing tradition of the Laurentian Hormone Conference, the papers were carefully prepared masterpieces and the discussions spirited and penetrating. The program opened with the presentation of the 1973 Gregory Pincus Memorial Lecture by Professor Ernst Knobil.

Topics covered by the program included regulation of the gonadotropins in primates, their neutralization by specific antibodies, and their role in oocyte maturation. Attention then turned to the enzymatic interconversion of estrogens followed by a novel concept of the mechanism of steroid hormone action. On exploration of other new territory the thermogenic action of thyroid hormone was related to active sodium transport. Rounding out an exciting week was an updating on the structure, heterogeneity, and activity of many of the lesser known hormones or hormone-like substances such as the somatomedins A, B, and C, the epidermal growth factor, the insect hormones, parathyroid hormone 1,25-dihydroxycholecalciferol, and prostaglandins. All in all, it was a memorable week of excellent scientific fare.

Personally and on behalf of the Committee on Arrangements, I want to thank Drs. Griff Ross, Samuel Solomon, Kenneth Savard, Maurice Raben, Paul Munson, Louis Sherwood, Peter Ramwell, and John Potts, Jr. for skillfully chairing the sessions and guiding the discussions. It is a pleasure, also, to acknowledge the heroic labors of Miss Joanne Sanford and her associates, Mrs. Mina Rano and Miss Lucy Passalapi, in transcribing the lengthy discussions immediately after each session. The always helpful cooperation of Academic Press in producing Volume 30 is acknowledged with gratitude.

Cambridge, Massachusetts
April 8, 1974

ROY O. GREEP

CONTENTS

On the Control of Gonadotropin Secretion in the Rhesus Monkey[1,2]

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I. Introduction

We undertook our studies of the regulation of gonadotropin secretion in the rhesus monkey, a representative primate, unburdened by preconceived notions or firmly espoused hypotheses, if only because we were innocent of significant experience or insight regarding this aspect of adenohypophysial physiology in any species. I like to think that this deplorable ingenuousness may have facilitated our labors, and it is for this reason that our approaches to the problem, at least for a time, were deliberately unguided by comparative considerations. I apologize at the outset for this cowardly strategy and for the glaring omissions attributable to it.

The time courses of the circulating gonadotropic hormones during the menstrual cycle of the rhesus monkey (Fig. 1), which were ascertained after long and arduous methodological struggles (Neill *et al.*, 1967; Monroe *et al.*, 1970; Karsch *et al.*, 1973c; Yamaji *et al.*, 1973), are essentially identical to those described earlier in the human female. We perceived them as being the resultant of relatively continuous or tonic secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) interrupted once every 28 days, on the average, by an abrupt, massive discharge of these hormones which we viewed as representing "cyclic" secretion by analogy to the basic schema postulated for the rat (Schwartz, 1969). In the rhesus monkey, the peak of this midcycle gonadotropin surge precedes ovulation by 37 hours, on the average (Weick *et al.*, 1973).

II. Ovarian Control of Tonic Gonadotropin Secretion

The tonic secretion of LH and of FSH, as reflected by their concentrations in peripheral plasma at times other than during the preovulatory

[1] The Gregory Pincus Memorial Lecture.

[2] The studies from the author's laboratory have been generously supported by grants from the Ford Foundation and from the National Institutes of Health.

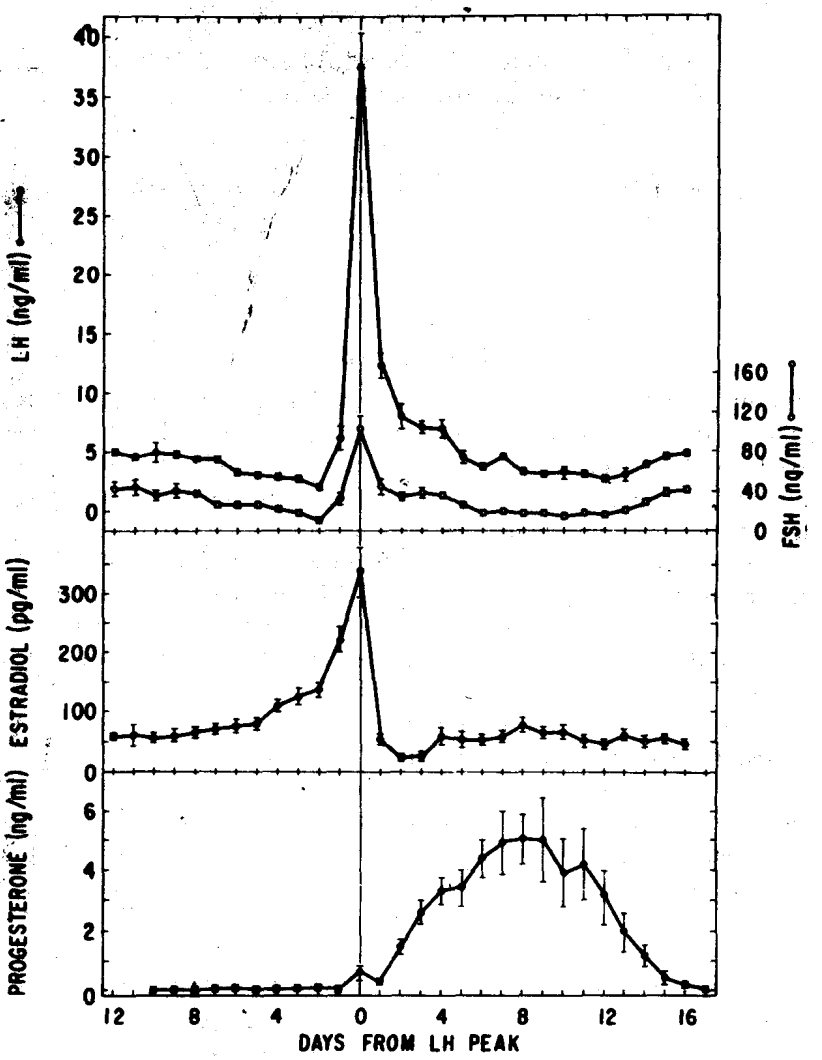

FIG. 1. Plasma concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, and progesterone throughout the normal rhesus monkey menstrual cycle normalized to the day of the mid-cycle LH peak (day 0). Each point represents the mean ±SE of 7 observations for FSH, 19 for LH, 11 for estradiol, and 7 for progesterone. Redrawn from Krey *et al.* (1973).

surge, appears to be controlled by a classical negative feedback loop involving, among other components, the ovary and the gonadotrophs of the adenohypophysis. The interruption of this negative feedback loop by

gonadectomy leads to prompt increases in plasma gonadotropin levels[3] which, in 2 or 3 weeks, achieve a relative plateau representing mean concentrations approximately 10 times greater than those observed preoperatively (Atkinson *et al.*, 1970). These elevated gonadotropin concentrations, as revealed by analysis of daily blood samples, are the resultant of striking, rhythmic, pulsatile discharges of the hormones at a frequency of approximately one every hour (Fig. 2), which for this reason and for want of a better term we have designated as being "circhoral" (Dierschke *et al.*, 1970). These pulsatile injections of the gonadotropins into the circulation appear to be superimposed on a background of continuous secretion which contributes but little to the gonadotropin level found in ovariectomized animals. A similar discontinuity in gonadotropin secretion has also been described in several other species, including man (Yen *et al.*, 1972), but the monkey differs from the latter in that the oscillations in circulating gonadotropins cannot be observed in intact animals, not even during the preovulatory surge (Weick *et al.*, 1973).

The mechanisms which eventuate in the circhoral, pulsatile discharges of the gonadotropic hormones from the pituitary continue to intrigue and to elude us. At the very least, they cannot be attributed to nonspecific periodic release of all the adenohypophysial hormones, such as might be occasioned by intermittent alterations in blood flow, since fluctuations in plasma growth hormone concentration, when these occur at all in ovariectomized monkeys, are not synchronous with the rhythmic increments in gonadotropin levels (Fig. 3).

The rather attractive, and initially compelling, possibility that the intermittent discharges of LH may be the consequence of an autoregulatory mechanism mediated by a "short-loop" negative feedback system (Motta *et al.*, 1969) whereby circulating levels of the gonadotropin could control its own secretion was also explored (T. Yamaji and E. Knobil, unpublished observations). Ovine LH, human LH, and hCG were infused intravenously into ovariectomized monkeys in order to determine whether elevated plasma concentrations of exogenous gonadotropin could influence the pulsatile pattern of endogenous LH secretion (Fig. 4). Although a study of similar design permitted the unambiguous demonstration that growth hormone secretion is under autoregulatory negative feedback control in the rhesus monkey (Sakuma and Knobil, 1970), these experiments

[3] In these and all other studies performed before an appropriate RhFSH radioimmunoassay was available to us, only LH measurements were reported. Since then, however, FSH has also been measured in plasma samples obtained in these earlier experiments, with the finding that in most instances the time courses of FSH and LH were markedly similar. The generic term "gonadotropin" will not be used when this was not the case or when FSH was not measured.

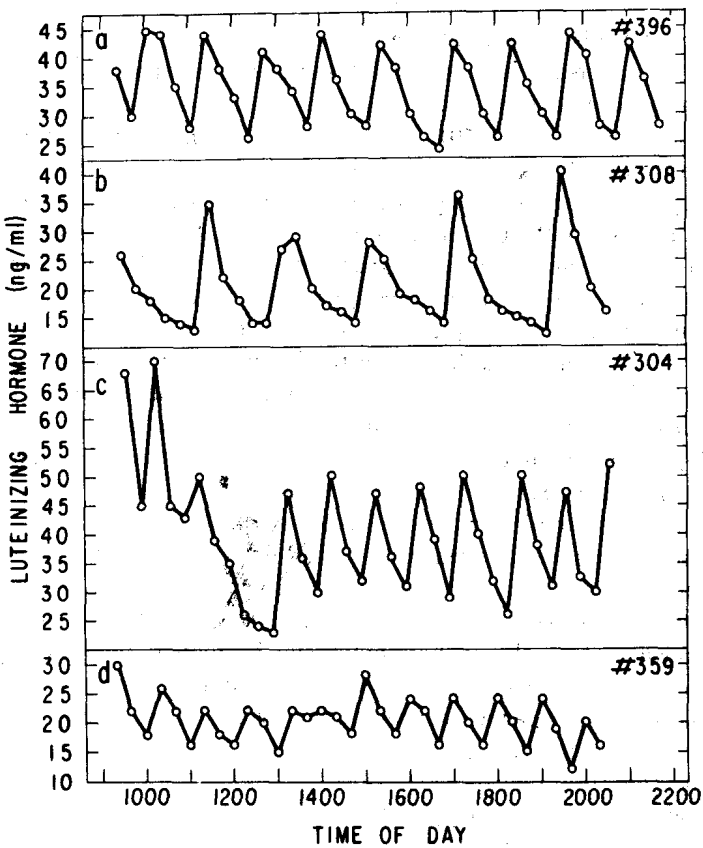

FIG. 2. Circhoral, pulsatile patterns of plasma luteinizing hormone concentrations in ovariectomized rhesus monkeys. Blood samples were taken every 20 minutes. From Dierschke *et al.* (1970) with permission.

failed to provide any evidence in support of the view that the circhoral discharges of LH are initiated by the decline in circulating LH below some critical, threshold concentration. Since nonsimian LH was utilized in this study, however, the possibility remains that experimentally imposed increments in circulating RhLH could inhibit endogenous LH secretion in the monkey, but this argument is not particularly compelling because nonsimian LH preparations possess striking gonadal stimulatory activity in hypophysectomized rhesus monkeys (Knobil and Josimovich, 1961). We were therefore led, by processes of exclusion, to consider the possibility that the signals which eventuate in the circhoral pulsatile release of gonadotropins from the pituitary gland originate in the central nervous system, being relayed to the gonadotropins by packets of

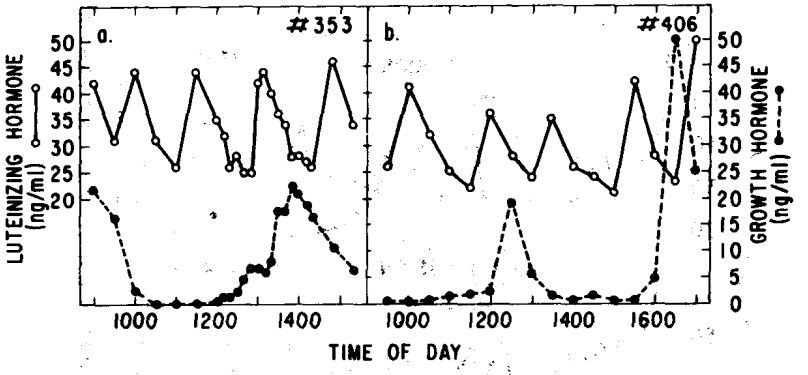

FIG. 3. Luteinizing hormone and growth hormone concentrations measured in the same plasma samples in ovariectomized rhesus monkeys. From Dierschke *et al.* (1970) with permission.

LH-releasing hormone (LRH) discharged into the pituitary portal circulation. This will be considered at greater length later in this discussion.

Closure of the negative feedback loop by the intravenous injection or infusion of estradiol-17β into ovariectomized monkeys, with resultant plasma concentrations of the steroid well within the physiological range (Yamaji *et al.*, 1972), leads to a prompt cessation of the pulsatile gonadotropin discharges and a resultant decline in the mean concentration of these hormones (Fig. 5). If plasma estradiol concentrations characteristic of those normally observed early in the follicular phase of the menstrual cycle (50–70 pg/ml) are maintained in ovariectomized animals for several days, mean gonadotropin levels which are consonant with this stage of the cycle are achieved (Karsch *et al.*, 1973b). These observations, along with the finding that progesterone alone, regardless of the plasma concentrations achieved or the duration of its administration, is without significant influence on the pulsatile mode of gonadotropin secretion or on their mean circulating levels in ovariectomized monkeys (Yamaji *et al.*, 1972), led us to the conclusion that estradiol is, in all likelihood, the primary ovarian component of the negative feedback loop which regulates tonic gonadotropin secretion (Karsch *et al.*, 1973b). In some experimental circumstances, however, a synergism between estrogen and progesterone in suppressing gonadotropin secretion is clearly demonstrable (Karsch *et al.*, 1973d). Although of considerable interest, the physiological significance of this interaction remains obscure since no inkling of its operation is evident in the course of the normal menstrual cycle (see Fig. 1).

A totally different dimension in the ovarian control of gonadotropin production must be introduced at this juncture, albeit parenthetically,

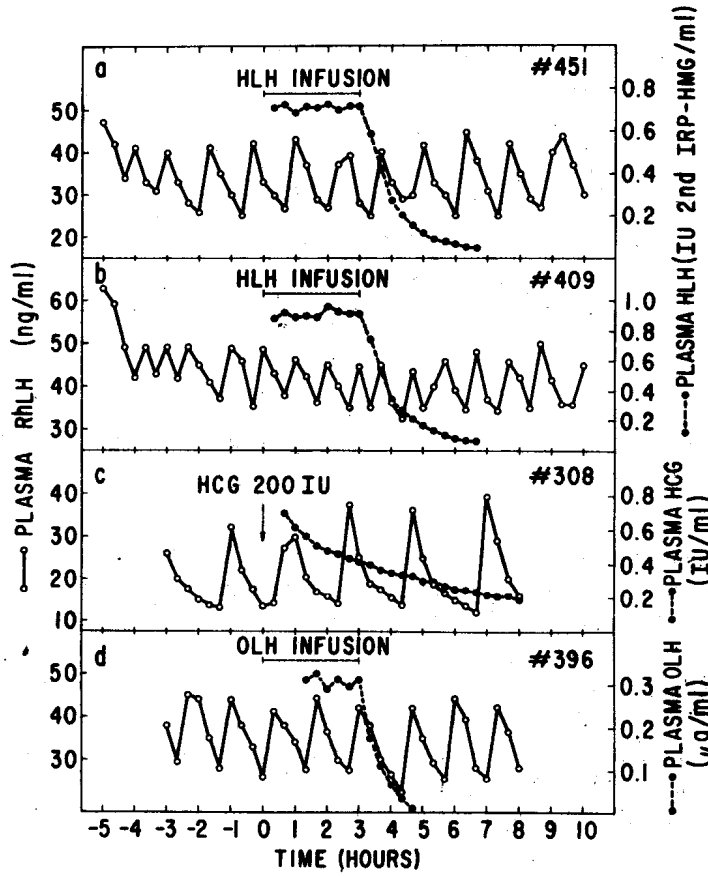

FIG. 4. Failure of exogenous human luteinizing hormone (a, b), human chorionic gonadotropin (c), and ovine luteinizing hormone (d) administration to inhibit endogenous luteinizing hormone (RhLH) secretion in ovariectomized rhesus monkeys. Horizontal bars (a, b, d) indicate the duration of intravenous infusion beginning at 0 time. The arrow shows the time of the single intravenous injection of hCG. Endogenous and exogenous LH concentrations were measured in the same plasma samples by the appropriate, specific radioimmunoassays.

because its physiological significance remains to be delineated. In the course of validating the radioimmunoassay for RhFSH now used in our laboratory (Yamaji *et al.*, 1973), it was found that preparations of this gonadotropin derived from pituitary glands of ovariectomized rhesus monkeys had more than twice the biological activity, relative to immunoreactive potency, as FSH preparations obtained from the pituitaries of intact animals (Peckham *et al.*, 1973). This striking increment in biological activity was related to a slower disappearance of the pituitary FSH

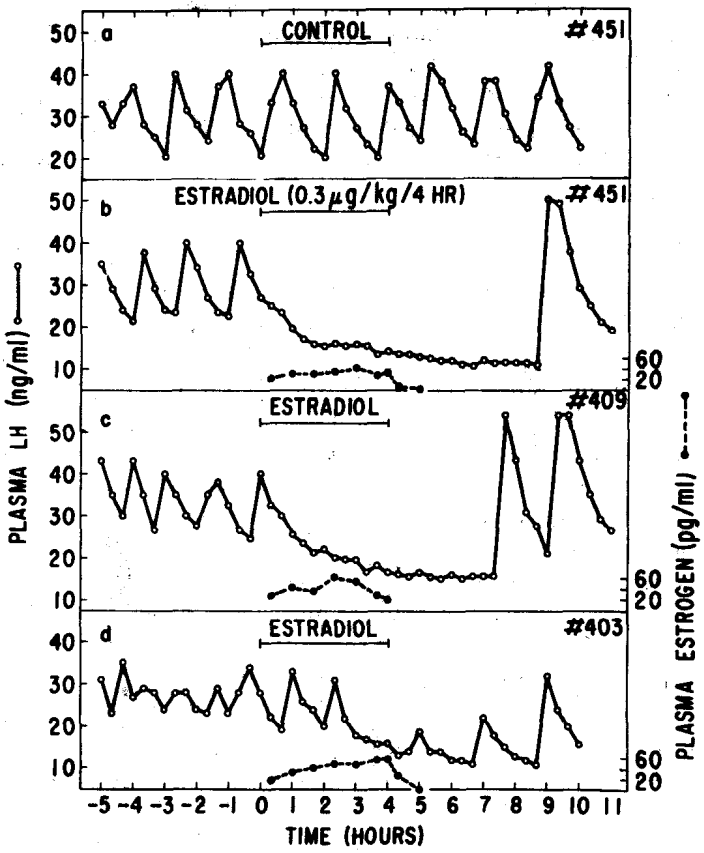

FIG. 5. Inhibition of tonic luteinizing hormone (LH) secretion in ovariectomized rhesus monkeys by low physiological concentrations of estradiol maintained by constant, intravenous infusion beginning at 0 time. Plasma estrogen concentrations prior to infusion were undetectable. From Yamaji *et al.* (1972) with permission.

preparations derived from ovariectomized monkeys from the circulation of assay rats. Exclusion chromatography revealed a preponderance of FSH components of larger molecular size in these pituitary extracts when compared to those of intact animals, suggesting that differences in the structure of FSH may account for the differences in biological activity observed. These qualitative differences in pituitary FSH were also found in the sera of intact and ovariectomized rhesus monkeys, indicating that ovarian activity may control not only the quantity but also the molecular size and consequent biological activity of the FSH discharged into the circulation (Peckham *et al.*, 1973). In view of the well-established relationship between the biological activity of glycoprotein hormones, their

half-time in the circulation and the degree of sialylation (Morel *et al.*, 1971; Van Hall *et al.*, 1971), it is tempting to suppose that the influence of ovariectomy on the structure and biological activity of FSH is attributable to increases in sialic acid content. Preliminary observations suggest that the physicochemical characteristics of RhLH are similarly under ovarian control.

III. Ovarian Control of the Preovulatory Gonadotropin Surge

The demonstration by Michel Ferin and his colleagues (Ferin *et al.*, 1969) that the administration of an antiserum to estrogen blocked ovulation in the rat firmly established the long-held view that estrogen may play an essential, albeit permissive, role in the control of ovulation in this species (Everett, 1961, 1972). This important observation, coupled with the nearly contemporaneous findings that small quantities of estrogen administered to sheep (Goding *et al.*, 1969; Scaramuzzi *et al.*, 1970) and rats (Caligaris *et al.*, 1971) led to an acute release of LH which resembled the spontaneous, preovulatory discharges of the hormone, lent credence to the notion that the unambiguous preovulatory rise in circulating estrogens during the primate menstrual cycle (see Fig. 1) may represent the critical stimulus for the initiation of the gonadotropin surge (Vande Wiele *et al.*, 1970; Hotchkiss *et al.*, 1971).

Our initial attempts to test this compelling hypothesis by the administration of estrogen to rhesus monkeys on the second or third day of the menstrual cycle in the hope of inducing premature LH surges were uniformly unsuccessful. In these early experiments estradiol-17β was given in single intramuscular or subcutaneous injections either in oil or as crystalline suspensions, resulting in rapid but short-lived elevations in plasma estrogen concentrations with peaks in excess of 2000 pg/ml. When, however, the increments in circulating estrogen were sustained for several days by substituting estradiol benzoate for the free alcohol and injecting it subcutaneously in oil, thus mimicking the time course of plasma estrogen concentrations normally observed during the late follicular phase of the cycle, LH surges (and FSH surges, as we were to find later; see Fig. 12a) indistinguishable from those occurring spontaneously were reproducibly elicited (Fig. 6). This stimulatory or positive feedback action of estrogen on LH and FSH secretion was invariably preceded by a decline in circulating gonadotropin levels attributable to the negative feedback action of the steroid. Clearly, the positive feedback effect of estrogen on gonadotropin release is not simply a function of some threshold plasma concentration of the steroid. It must also be critically dependent on a time component, but the precise duration of the effective stimulus, or its threshold for that matter, could not be ascertained from these experi-