INFORMATION TO USERS

This material was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.

2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again — beginning below the first row and continuing on until complete.

4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.

5. PLEASE NOTE: Some pages may have indistinct print. Filmed as received.

Xerox University Microfilms

300 North Zeeb Road
Ann Arbor, Michigan 48106

74-25,249

LEGAN, Sandra Jean, 1946-

THE POSITIVE FEEDBACK ACTION OF ESTRADIOL ON LUTEINIZING HORMONE SECRETION IN THE RAT.

The University of Michigan, Ph.D., 1974
Physiology

University Microfilms, A XEROX Company, Ann Arbor, Michigan

THIS DISSERTATION HAS BEEN MICROFILMED EXACTLY AS RECEIVED.

THE POSITIVE FEEDBACK ACTION OF ESTRADIOL ON LUTEINIZING HORMONE SECRETION IN THE RAT

by

Sandra Jean Legan

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Physiology)
in The University of Michigan
1974

Doctoral Committee:

Assistant Professor Fred J. Karsch, Co-Chairman
Professor Richard L. Malvin, Co-Chairman
Assistant Professor P. Landis Keyes
Professor A. Rees Midgley, Jr.
Assistant Professor David R. Mouw
Professor Arthur J. Vander

ACKNOWLEDGEMENTS

I would like to express my gratitude to the members of my doctoral committee, especially to Dr. Fred J. Karsch for his patience, encouragement and invaluable suggestions and criticisms, and to Dr. A. Rees Midgley, Jr., for his enthusiasm, inspiration and guidance throughout these studies.

In addition, I would like to express my appreciation to Dr. Arthur J. Vander for his continued interest and advice throughout my graduate training.

Particular thanks are expressed to Gary Coon and Cathie Stepien for their technical assistance and for those invaluable contributions which only students can give and teachers can recognize.

Special thanks are extended to the staff, fellows and technicians of the Reproductive Endocrinology Program for their innumerable contributions.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS.....ii

LIST OF ILLUSTRATIONS.....iv

LIST OF ABBREVIATIONS.....v

CHAPTER

I. INTRODUCTION.....1

II. GENERAL MATERIALS AND METHODS.....4

III. CHARACTERISTICS OF POSITIVE FEEDBACK ACTION OF ESTROGEN ON LH SECRETION IN LONG-TERM OVARIECTOMIZED RATS.....9

24-Hour Periodicity
Estrogen Dependence
Neural Control
Nature of Stimulus Required to Induce LH Release
Duration of Stimulus Required to Induce LH Release

IV. POSITIVE FEEDBACK OF ESTRADIOL-17β IN RATS OVARIECTOMIZED ON DIESTRUS OR PROESTRUS.....20

Endogenous versus Exogenous Stimuli
Effect of Pentobarbital Administration
Daily LH Surges in Pseudo-Intact Rats

V. MODULATION OF PITUITARY RESPONSIVENESS TO LRF BY ESTROGEN.....28

Pituitary Sensitivity to LRF During the Estrous Cycle
Effect of Ovariectomy on the Day Before Proestrus
Dependence on Additional Ovarian Hormones

VI. EVIDENCE FOR THE FEEDBACK OF OTHER OVARIAN HORMONES ON LH RELEASE.....34

Time Course of LH Release in Pseudo-Intact Rats
Rats made Pseudo-Intact Throughout the Cycle
Rats Receiving Estradiol Implants on Proestrus

VII. SUMMARY AND CONCLUSIONS.....41

LITERATURE CITED.....44

LIST OF ILLUSTRATIONS

Figure — Page

1. Plasma LH in Spartan Rats Treated with EB 2 Weeks after Ovariectomy....14
2. Plasma LH in Holtzman Rats Treated with EB 2 Weeks after Ovariectomy....14
3. Plasma LH in Spartan Rats Treated with 0.1 ml Sesame Oil 2 Weeks after Ovariectomy....16
4. Effect of Pentobarbital on Plasma LH in Spartan Rats Treated with 50 µg EB 2 Weeks after Ovariectomy....16
5. Plasma LH in Rats Receiving E_2 Implants for 4 Days 2 Weeks after Ovariectomy....17
6. Plasma LH in Rats Receiving E_2 Implants for 29½ Hours 2 Weeks after Ovariectomy....17
7. Plasma LH in Rats Ovariectomized on Proestrus....22
8. Plasma LH in Pseudo-Intact Rats....22
9. Effect of Pentobarbital on Plasma LH in Rats Ovariectomized on Proestrus and in Pseudo-Intact Rats....25
10. Serum LH in Rats Treated with Pentobarbital on Proestrus....25
11. Serum LH in Pseudo-Intact Rats....27
12. Serum LH on Days 1-12 in Pseudo-Intact Rats Receiving E_2 Implants after Ovariectomy at 10:00 a.m. on Day 0....27
13. Serum LH During 5-Day Estrous Cycle in Response to Gn-RH in Pentothal-Treated Holtzman Rats....30
14. Response to Gn-RH in Pentothal-Treated Holtzman Rats Ovariectomized One Day Before Proestrus....31
15. Pituitary Response to Gn-RH in Holtzman Pentothal-Treated Pseudo-Intact Rats....31
16. Time Course of LH Release in Proestrous Rats and in Pseudo-Intact Rats Receiving E_2 Implants after Ovariectomy at 10:00 a.m. on Day 0....37
17. Serum LH in Rats Receiving E_2 Implants Immediately Following Ovariectomy on L_1, L_2, N, C_1 and C_2....37
18. Serum LH on Days 1-15 in Intact Rats Receiving E_2 Implants at 11:30 a.m. on Proestrus (day 1)....39

LIST OF ABBREVIATIONS

a.m.	ante meridiem
C	degree(s) Centigrade
cc	cubic centimeter(s)
E_2	estradiol-17β
EB	estradiol benzoate
Fig.	figure
g	gram(s)
Gn-RH	gonadotrophin releasing hormone
hr	hour(s)
i.d.	inner diameter
ip	intraperitoneal(ly)
kg	kilogram(s)
LH	luteinizing hormone
LRF	luteinizing hormone releasing factor
M	molar
mg	milligram(s)
min	minute(s)
ml	milliliter(s)
mm	millimeter(s)
NaCl	sodium chloride
ng	nanogram(s)
NIAMDD-LH-RP-1	National Institute of Arthritis, Metabolism and Digestive Diseases Luteinizing Hormone Reference Preparation 1
o.d.	outer diameter
PBS	phosphate-buffered saline
p.m.	post meridiem

SE	standard error
sc	subcutaneous
µg	microgram(s)
µl	microliter(s)

CHAPTER I

INTRODUCTION

One of the major hormonal events in the mammalian reproductive cycle is the pre-ovulatory increase in serum luteinizing hormone (LH) concentration. Precise control of this massive (>100-fold) increase in serum LH is achieved by complex interaction of both neural and hormonal mechanisms. In the rat, the neural mechanism controlling LH secretion is localized in specific brain centers whose activity facilitates release of luteinizing hormone-releasing factor (LRF) from the hypothalamus. In addition, a neural "clock" mechanism which is "set" by the environmental light/dark cycle triggers the LH-release mechanism such that the LH surge in rats always occurs at a specific time of day. The hormonal control of LH release consists of the feedback of ovarian and possibly adrenal steroids on the pituitary, hypothalamus and other brain centers.

The ovarian hormone most directly involved in the control of LH release is estradiol-17β, which will hereafter be referred to as estradiol. Numerous observations made in several species indicate that estradiol is an important steroidal component of two feedback systems which govern the secretion of LH, the inhibitory or negative feedback loop, and the stimulatory or positive feedback system (Caligaris et al., 1971; Ferin et al., 1969; Goding et al., 1969; Knobil et al., 1972; Monroe et al., 1972; Neill, 1972; Scaramuzzi et al., 1971; Yen and Tsai, 1971; Yen and Tsai, 1972). One hypothesis which accounts for the phenomenon that a single steroid hormone can exert two completely diametric actions on LH secretion is that there are two separate mechanisms, the tonic and phasic, which function independently and concurrently

(Barraclough, 1966; Karsch et al., 1973a; Schwartz, 1969). Evidence obtained in rhesus monkeys (Karsch et al., 1973b; Knobil et al., 1972) indicates that the tonic mechanism, which regulates basal LH concentrations throughout the cycle, is responsive to relatively low sustained levels of estradiol, whereas the phasic mechanism, which controls the pre-ovulatory LH discharge, is activated by an increase in circulating estradiol to a level maintained for approximately 36 hr. Furthermore, in the rat, each mode of LH secretion appears to be controlled by an anatomically distinct region of the hypothalamus. The mechanism controlling tonic LH secretion appears to be localized in the ventromedial-arcuate area whereas the signals which initiate the LH surge originate in, or pass through, the anterior hypothalamic-preoptic area. (Barraclough, 1966; Gorski, 1971; Halasz, 1969). Thus, the bimodal action of estradiol on LH release in the rat may be explained by its differential feedback action on these two centers, an inhibition of the tonic center and stimulation of the phasic center. The identification of two anatomic centers controlling LH release has not yet been established in non-rodent species.

Although some evidence suggests a direct action of estradiol on the hypothalamus (Davidson, 1969; Neill, 1972), other evidence has shown an effect on the anterior pituitary as well (Arimura and Schally, 1971; Bogdanove, 1963; Piacsek and Meites, 1966; Schneider and McCann, 1970; Weick and Davidson, 1970). In addition, little is known concerning the mechanism of action of estradiol at either or both of these sites, or the nature of the estradiol stimulus.

In the studies to be described, the role played by estradiol in the induction of LH release was investigated. Our findings are dis-

cussed in the next six chapters. The general techniques and methods employed in these studies are described in Chapter 2. The next chapter contains a description of the 24-hr periodicity and other characteristics of positive feedback action of estradiol on LH release in long-term (2 weeks) ovariectomized rats. The development of a new experimental model for the intact rat and the characteristics of the estradiol-induced LH release mechanism in this model, including that of 24-hr periodicity, are described in Chapter 4. Following this, an investigation of a possible mechanism of action of estradiol on the pituitary in intact rats is presented. Evidence which indicates that other ovarian hormones in addition to estradiol may be required for the induction of a proestrous-like LH surge is discussed in Chapter 6. Finally, the last chapter contains a summation, analysis and conclusion of the findings.

CHAPTER II

GENERAL MATERIALS AND METHODS

Animals

Adult female Sprague-Dawley-derived rats (250-300 g) obtained from Holtzman Company (Madison, Wisconsin) or Spartan Farms (Haslett, Michigan) were housed in a temperature-controlled environment (21 C) with lights on from 5:00 a.m. to 7:00 p.m. Food and water were supplied ad libitum and vaginal smears were taken daily in intact rats. Only those rats having at least one normal estrous cycle prior to experimental manipulation were employed in these studies.

Surgical Procedures

Bilateral ovariectomy and cannulation of the left carotid artery (Gay et al., 1969) were performed employing ether, methoxyfluorane, or sodium pentothal anesthesia. In some cases it was necessary to supplement pentothal anesthesia with ether (<1 min) during the cannulation procedure.

Administration of Steroids

Estradiol benzoate (EB, 50 μg/rat) was injected sc in 0.1 ml sesame oil. Control animals received 0.1 ml vehicle alone. Estradiol-17β was administered by means of Silastic capsules implanted sc.

Silastic Implants

A Puritan wooden applicator was inserted into one end of a 15 mm length of Silastic tubing (0.062 in i.d., 0.125 in o.d.; Dow Corning) for a distance of approximately 5 mm and trimmed with a razor blade as close to the end of the tubing as possible. A 15 gauge, stainless steel, blunted hypodermic needle was inserted into the open end of the plugged tubing leaving a distance of exactly 5 mm between the inside

edge of the wooden plug and the tip of the needle. Crystalline estradiol-17β (Sigma) was placed in the open end of the needle and packed into the Silastic tubing using a straightened paper clip. When the 5 mm space between the plug and the needle was filled with estradiol, the needle was removed, and another wooden applicator was inserted into the open end of the tube. In some cases, a drop of methanol was used to rinse excess estradiol down the inner walls of the 5 mm open-ended segment of tubing before the second applicator was inserted. A distance of exactly 5 mm was measured between the inner ends of the wooden plugs by sliding the second wooden applicator in or out just before it was trimmed as close as possible to the Silastic tubing. Thus, it was possible to ensure that the dose of estradiol achieved by insertion of the capsules was the same in all cases, since diffusion of estradiol is dependent on the length of a capsule in which permeability, diameter, wall thickness and concentration gradient of estradiol remain constant (Dziuk and Cook, 1966; Karsch et al., 1973a; Kinc1 and Rudel, 1971). The ends of the capsule were coated with Silastic Medical Grade Elastomer (#382, Dow Corning). In some cases, within 24 hr after the Elastomer had hardened, the capsules were rinsed 3 times in methanol and then dried and stored until use. In order to prevent the transitory peak in circulating estradiol concentrations which occurs on the first day after insertion of implants which are not pre-incubated (Karsch et al., 1973a), all capsules were pre-incubated for at least 30 min and no longer than 18 hr in water at room temperature just before being inserted into the animals. To insert an implant, as small an incision as possible was made in the skin of the back. The implant was inserted into a pocket made by pushing a closed hemostat approximately 1 inch

through the incision and opening it to spread the sc fascia apart. The incision was closed with a wound clip.

To estimate the dosage of estradiol administered by this method, implants were incubated *in vitro* for 2 days in 30 ml of a medium containing 0.1% gelatin in 0.01 M phosphate-buffered 0.14 M NaCl, pH, 7.0 (PBS) with constant agitation at 37 C. The medium was changed every 4 hr. Under such conditions the capsules released approximately 1.5 µg estradiol per day as measured by radioimmunoassay (England *et al.*, in press). While this release rate provides an estimate of the daily dosage, it should be recognized that it may not replicate that occurring *in vivo*.

Synthetic LRF

The synthetic decapeptide employed in these studies was generously supplied by W.F. White, Abbott Laboratories, and will hereafter be referred to as Gn-RH (gonadotrophin-releasing hormone) to distinguish it from the natural releasing hormone, which will be designated as LRF. Stock solutions (100 ng/µl) were stored in 1 ml ampoules at 4 C. Gn-RH was diluted in PBS and volumes containing five 200 ng doses were dispensed into injection vials pre-rinsed in 0.1% gelatin in PBS. Vials were stored at 4 C until use. At the time of administration, 100 µl Gn-RH per rat were drawn into a 1 cc plastic syringe pre-rinsed in 0.1% gelatin in PBS, and injected into the carotid cannula. The cannula was immediately rinsed with 100 µl PBS.

Blood Collection

Blood samples in volumes of 300 µl, 500 µl, or 1 ml were withdrawn from unanesthetized rats via the left carotid cannula or by means of cardiac puncture. Results obtained were identical when both methods of

blood collection were employed in the same group of animals. A dilute solution of heparin (10 or 1000 units/ml) was employed to rinse the cannulae after each sample was withdrawn, and as needed throughout the course of the experiment. Approximately 20 µl of the heparin solution was left in the dead spaces of the cannulae between collection of samples to prevent clotting. In later experiments the heparin solution used was so dilute that blood samples clotted within 12 hr. Thus, problems of drawing off fibrin-containing plasma were avoided and serum LH concentrations were determined in these samples.

In the first two experiments, the results of which are depicted in Figures 1 and 2, samples of whole blood were dispensed into tubes containing twice the sample volume of 0.1% gelatin in PBS. The diluted samples were stored at 4 C for 12-36 hr after which they were centrifuged. The diluted plasma was drawn off and frozed until assayed. Following assay for LH, corrections for plasma dilutions were calculated assuming a hematocrit of 50%. Since normal rat hematocrits are slightly less than 50%, the calculated LH concentrations are slightly higher than actual plasma concentrations in these 2 experiments. However, the large relative changes in LH concentrations we observe at 5:00 p.m. remain valid. Moreover, identical results have been obtained when blood samples were not diluted (Legan, unpublished observations). In all other experiments, whole blood samples were stored at 4 C for 24 hr, centrifuged, and the undiluted plasmas or sera were drawn off and frozen until assayed.

Radioimmunoassay

LH concentrations were determined by radioimmunoassay as previously described (Niswender et al., 1968) with the following excep-

tions. The dilution of anti-ovine LH was 1:80,000 and 0.1% gelatin was used instead of bovine serum albumin (BSA). The standard employed was the rat pituitary reference preparation supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases, NIAMDD-LH-RP-1. In some assays, 12 hr instead of 24 hr incubations were carried out after addition of first antibody, labelled hormone and second antibody. There was no difference between the two methods in any of the following parameters: slope of standard curve, non-specific binding, binding of labelled hormone in the absence of unlabelled ligand, LH concentrations in two standard sera, and assay sensitivity. These parameters also remained the same whenever dilutions or stocks of second antibody or normal rabbit serum were changed.

Statistics

LH concentrations obtained from individual rats in the same group were pooled and means and standard errors (SE) were calculated. Statistical tests employed were Student's t-test and the paired t-test. A value of $p<0.05$ was considered significant. Hereafter, no statement concerning an increase or decrease in LH concentrations will be made unless the change is statistically significant ($p<0.05$).

CHAPTER III

CHARACTERISTICS OF POSITIVE FEEDBACK ACTION OF ESTROGEN ON LH SECRETION IN LONG-TERM OVARIECTOMIZED RATS

Several investigators have observed a critically-timed release of LH after administration of estradiol benzoate (EB) to ovariectomized rats. The time of release coincides with the initiation of the preovulatory LH surge in proestrous rats (4:00 p.m. to 6:00 p.m.) (Gay et al., 1970), which will subsequently be referred to as the "release period." Swelheim (1965) reported an increase in serum LH at 4:00 p.m. one day after injection of 50 μg EB in rats ovariectomized 2 weeks previously and pre-primed with 14 daily injections of 0.5 μg EB. Caligaris et al. (1971) observed an increase in plasma LH levels during the second and third release periods after injection of 20 μg EB in rats ovariectomized 1-6 months previously and primed 3 or 4 days earlier with 20 μg EB. Neill (1972) reported similar repetitive discharges of LH during the 2nd, 3rd, and 4th release periods following administration of 50 μg EB to rats ovariectomized 2 weeks previously and primed one day previously with 0.5 μg EB. In preliminary experiments of our own, an increase in plasma LH levels was observed during the second release period after a single injection of 50 μg EB with or without progesterone in Holtzman rats ovariectomized 8-20 weeks previously but not pre-primed with steroids (Legan et al., 1973b). In addition, in 2 out of 3 rats, an LH surge was seen during the 2nd and 3rd release periods after the single injection of EB. The first objective of the following studies was to examine in greater depth the phenomenon of repetitive estradiol-induced LH surges in ovariectomized rats not pre-primed with steroids.

The above data suggest that in the ovariectomized rat, administration of EB appears to trigger a repetitive mechanism regulating LH release, which, regardless of differences in dose and strain, occurs only during the release period. However, whether estradiol induces these surges by a positive feedback action on the phasic center, or whether it merely unmasks them by a negative feedback inhibition of the high rate of tonic LH secretion normally seen in ovariectomized rats has not yet been determined. The latter possibility is suggested by the recent observations of Beattie et al. (1973) that circulating LH concentrations increased 3-fold during the release period in ovariectomized rats which did not receive estradiol, and that administration of pentobarbital abolished these increases. The second objective of these studies was to investigate whether the daily LH surges observed in ovariectomized rats are actually induced by a positive feedback action of estradiol or merely unmasked by a negative feedback inhibition of the high level of tonic LH secretion.

It has not yet been determined whether the repetitive LH discharges observed in long-term ovariectomized rats are controlled by a neural mechanism. One way to examine this possibility is by administration of neural blocking agents, such as pentobarbital, which inhibit the preovulatory LH discharge in intact rats (Daane and Parlow, 1971; Schwartz, 1969). These and other observations (Barraclough, 1966; Davidson, 1969; Everett, 1964; Gorski, 1971; Halasz, 1969; McCann et al., 1968) provide compelling evidence that the proestrous LH surge is controlled by a neural pathway. The third objective of these studies was to determine whether the repetitive LH discharges observed in long-term ovariectomized rats are controlled, at least in part, by a neural mechanism.

In intact rats and in other species, the preovulatory LH surge is dependent upon an antecedent rise in circulating estradiol concentrations (Ferin et al., 1969; Knobil et al., 1972; Labhsetwar, 1970; Miyake, 1969; Spies et al., 1972). In the rhesus monkey, the increment in circulating estradiol levels has to be maintained for approximately 36 hr. When the estrogen stimulus is withdrawn prior to initiation of the LH discharge, the surge is abolished (Karsch et al., 1973b). In contrast, however, in the rat, withdrawal of the estrogen stimulus by means of ovariectomy several hr before the initiation of the LH surge (10:00 a.m. on proestrus) has no effect on the LH discharge (Schwartz, 1964). It remains to be determined whether the estrogen stimulus can be withdrawn prior to the initiation of daily LH surges in ovariectomized rats. The final objective of these studies was to examine the magnitude and duration of the estrogen stimulus required for the induction of repetitive LH discharges in ovariectomized rats.

Methods

Two groups of rats (one obtained from Holtzman Company, the other from Spartan Farms) received EB (50 μg/rat sc) at 7:00 a.m. on the fifteenth day after bilateral ovariectomy (day 0). Blood samples were withdrawn at 6:00 a.m., prior to estradiol administration and at 12:30 and 5:00 p.m. daily on days 0 through 3 or 4. Additional blood samples were obtained at 8:00 a.m. on days 1 and 2 in the Spartan rats.

A third group of rats (Spartan) was treated with 0.1 ml sesame oil sc on the fifteenth day following ovariectomy (day 0). Blood samples were withdrawn as described for the first 2 groups. In addition, pentobarbital (30 mg/kg ip) was administered at 12:00 p.m. on day 3.

EB was injected sc in a fourth group of rats (Spartan) on the

fifteenth day after ovariectomy (day 0). Blood samples were withdrawn as described for the first 2 groups. Pentobarbital was administered on day 2 at 12:00 noon.

In subsequent groups, precise control of stimulus duration was achieved by administering estradiol-17β in Silastic capsules implanted sc. Thus, as described previously (Karsch et al., 1973b), it was possible to induce a rapid rise in serum estradiol concentrations (by insertion of the capsules) to a level which was maintained, and to terminate the stimulus abruptly (by removal of the capsules). Silastic capsules containing estradiol-17β were implanted into 2 groups of rats at 7:00 a.m. on the fifteenth day following ovariectomy (day 0). Blood samples were withdrawn as described for groups 1 and 2. The implants were removed from one group of rats at 12:30 p.m. on day 1, and from the remaining rats after the last blood sample was withdrawn at 5:00 p.m. on day 4.

An additional group of rats received Silastic capsules containing estradiol-17β on the fifteenth day following ovariectomy (day 0). Blood samples were withdrawn hourly from 3:00 p.m. to 9:00 p.m. on day 1.

Results and Discussion

Within one hour following injection of EB at 7:00 a.m. on the fifteenth day after ovariectomy, plasma LH concentrations decreased approximately 50% to levels 10- to 20-fold greater than those seen in diestrous rats, a result of negative feedback inhibition of LH release (Legan, unpublished observations). LH concentrations in samples obtained at 12:30 p.m. on days 1 to 3 remained low despite presumably decreasing circulating estradiol levels. On each of the 3 days following

estradiol injection, a large increase in plasma LH levels was observed at 5:00 p.m. (Fig. 1). These results indicate that the estradiol-induced LH release mechanism in ovariectomized rats has a 24-hr periodicity and is expressed during the release period. Similar results were obtained, with minor differences, when the experiment was repeated using Holtzman rats (Fig. 2 and Legan et al., 1973a).

It is important to note that there are variations in the repetitive LH discharges among rats from different suppliers. The largest and most reliable LH surges were observed in Spartan rats. A comparison of Figures 1 and 2 indicates that the daily LH surges were smaller and less reliable in Holtzman rats. In Charles River rats, the response was even smaller than that seen in Holtzman rats (Legan, unpublished observations). Therefore, in all subsequent experiments in which we investigated repetitive LH discharges, Spartan rats were employed.

The following observations clearly demonstrate that the repetitive LH surges observed at 5:00 p.m. require a positive feedback action of estradiol and that they are not merely unmasked by a negative feedback inhibition of the high rate of tonic LH secretion normally seen in long-term ovariectomized rats:

1) There was no consistent increase in plasma LH levels at 12:30 or 5:00 p.m. for 5 days following administration of 0.1 ml sesame oil sc to rats ovariectomized 2 weeks previously.
2) Even the highest levels of LH observed in oil-treated ovariectomized rats were well below those seen at 5:00 p.m. in estradiol treated rats (Fig. 1 and 3).

These results demonstrate that the daily LH surges in ovariectomized

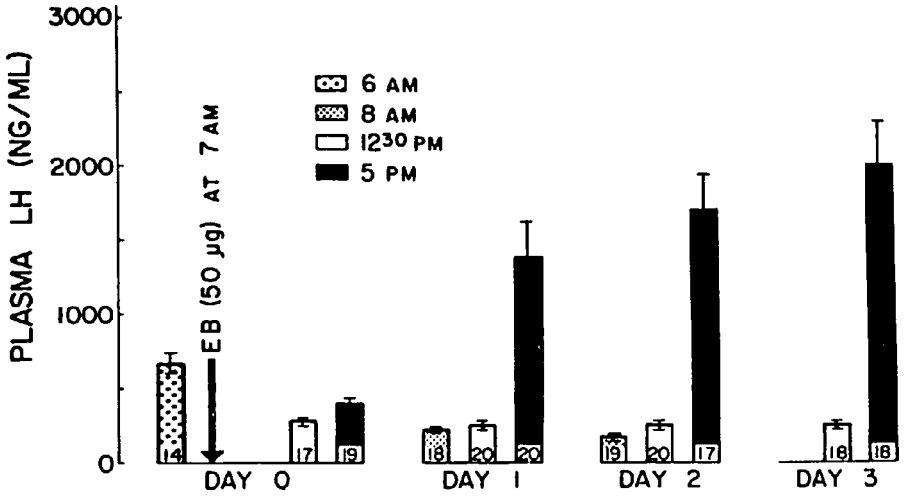


FIGURE 1. Plasma LH concentrations (mean ± SE) in Spartan rats treated with 50 µg estradiol benzoate (EB) sc in 0.1 ml sesame oil 2 weeks after ovariectomy. Numbers at the base of the bars indicate the number of animals in each group.

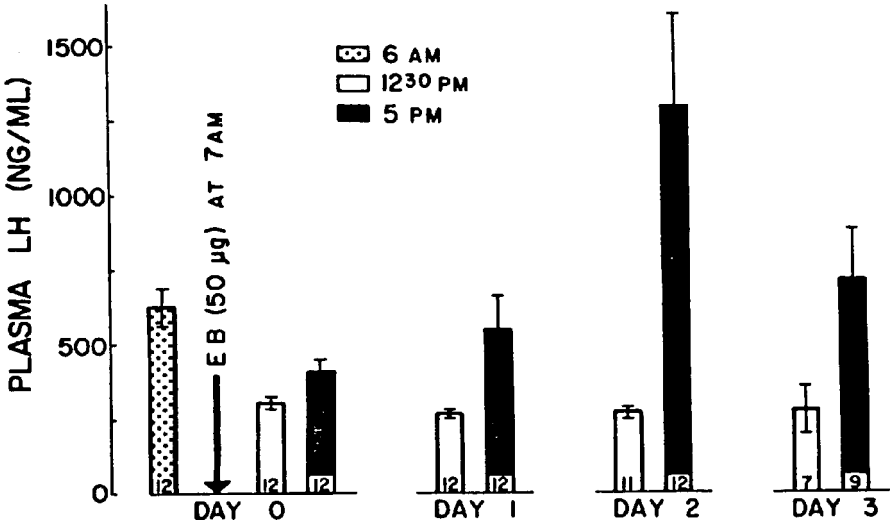


FIGURE 2. Plasma LH concentrations (mean ± SE) in Holtzman rats treated with 50 µg estradiol benzoate (EB) sc in 0.1 ml sesame oil 2 weeks after ovariectomy. Numbers at the base of the bars indicate the number of animals in each group.

rats are dependent upon the positive feedback action of estradiol, although our experimental design did not allow us to determine whether or not there is a daily spontaneous increment in circulating LH at another time during the release period in ovariectomized rats. The slight suppression of LH levels observed following pentobarbital administration on day 3 confirms previous observations (Ajika et al., 1972; Beattie et al., 1973) that treatment with pentobarbital can inhibit tonic LH secretion. The gradual change in plasma LH concentrations from days 0 to 4 reflects the increase in LH secretion normally seen during the first 4 weeks after ovariectomy (Blackwell and Amoss, 1971; Gay and Midgley, 1969; Yamamoto et al., 1970).

To determine whether a neural mechanism responsible for triggering the LH surge is operative in rats treated with estradiol 2 weeks after ovariectomy, a dose of pentobarbital required to block a proestrous LH surge was administered at 12:00 noon on the second day after injection of EB. As expected, an LH surge was observed at 5:00 p.m. on day 1. However, there was no surge on the day of pentobarbital administration (day 2) (Fig. 4) suggesting that estradiol-induced LH release in ovariectomized rats is controlled, at least in part, by a neural component. It is interesting to note that the surge recurred at 5:00 p.m. on day 3 and plasma LH levels were much greater than those usually seen at this time.

When Silastic implants were inserted on the fifteenth day following ovariectomy and left in place for 4 days, daily LH surges were observed which were similar to those seen after sc injection of 50 μg EB in oil (Fig. 5). Therefore, it appears that a large dose of estradiol is not required to elicit this response and that the relatively moderate

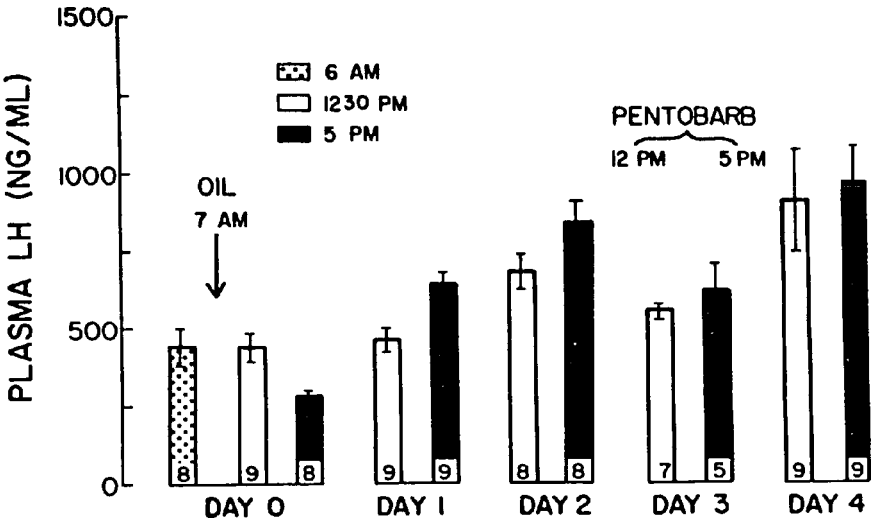


FIGURE 3. Plasma LH concentrations (mean ± SE) in Spartan rats treated with 0.1 ml sesame oil 2 weeks after ovariectomy. Numbers at the base of the bars indicate the number of animals in each group.

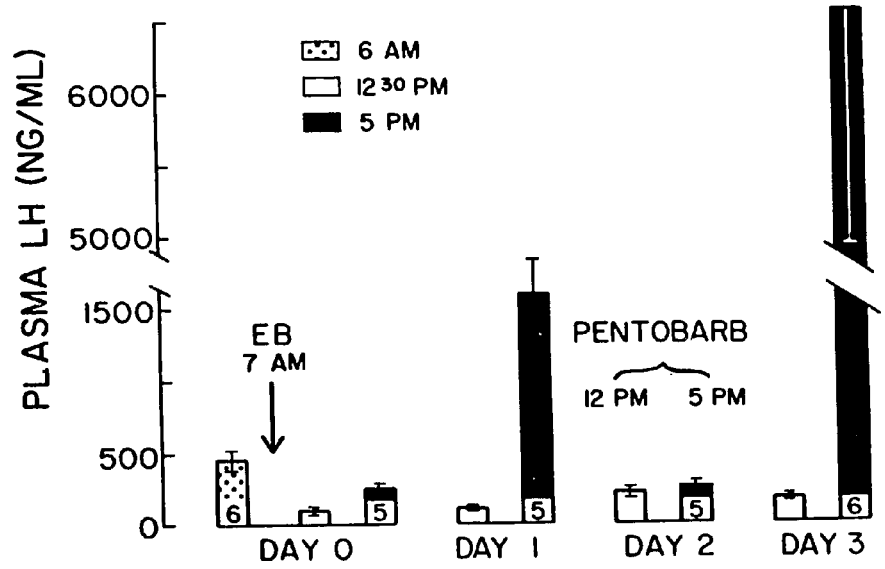


FIGURE 4. Effect of pentobarbital on plasma LH concentrations (mean ± SE) in Spartan rats treated with EB 2 weeks after ovariectomy. Numbers at the base of the bars indicate the number of animals in each group.

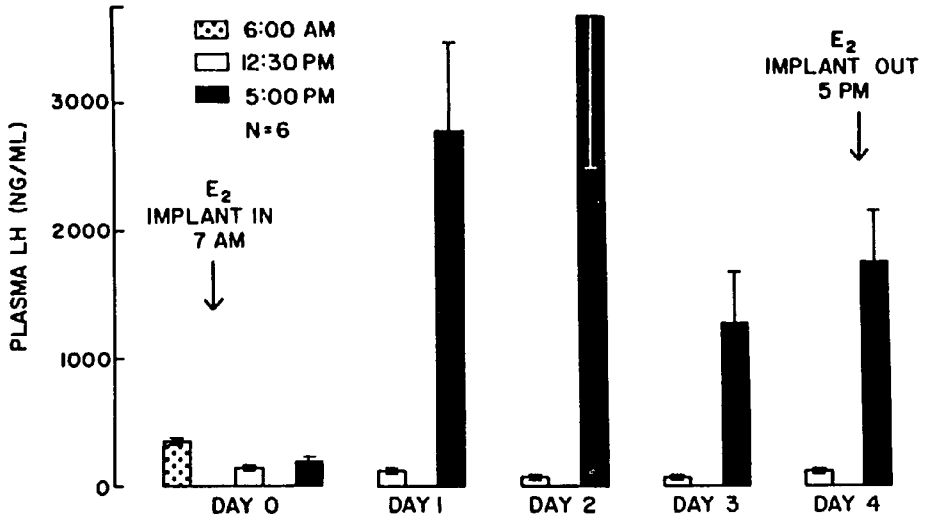


FIGURE 5. Plasma LH concentrations (mean ± SE of 6 observations) in Spartan rats receiving estradiol implants for 4 days 2 weeks after ovariectomy.

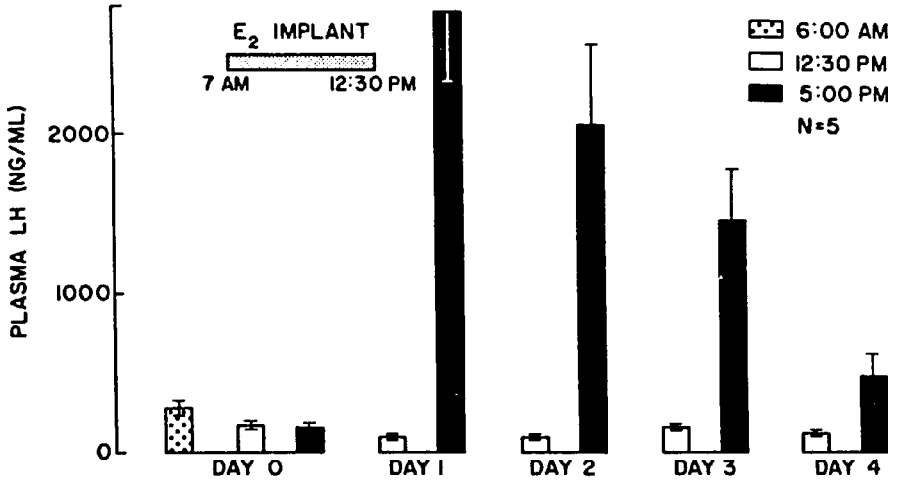


FIGURE 6. Plasma LH concentrations (mean ± SE of 5 observations) in Spartan rats receiving estradiol implants for 29½ hr 2 weeks after ovariectomy.

increase in plasma estradiol concentrations presumably achieved by these implants is sufficient for induction of LH release in ovariectomized rats.

The maximum LH concentrations attained during these estradiol-induced LH surges are similar to those observed in proestrous surges although basal LH levels are higher in estradiol-suppressed ovariectomized rats than in proestrous rats (Legan, unpublished observations). From hourly observations it was difficult to determine whether the large fluctuations in LH levels observed in some rats at the onset of the release period are due to initiation of the LH discharge or to variations in basal LH levels usually seen in ovariectomized rats. Thus, it remains to be determined whether the estradiol-induced LH surges in ovariectomized rats are similar in time course to proestrous surges.

To determine whether the repetitive LH surges in ovariectomized rats require continuously elevated plasma estradiol concentrations, Silastic capsules containing estradiol-17β were implanted sc at 7:00 a.m. and removed at 12:30 p.m. the next day, a stimulus duration similar to that occurring during the estrous cycle. An LH surge was observed during the release period on days 1-4 (Fig. 6), demonstrating that a rise and sustained elevation of estradiol for only 29½ hr triggers an LH surge on at least 4 consecutive days in ovariectomized rats. Not only do these observations strengthen earlier findings that the LH surge in the rat can be initiated following withdrawal of the estrogen stimulus (Schwartz, 1964), they introduce the concept of memory in an endocrine feedback system. Moreover, the observation that plasma LH levels had returned to baseline by 12:30 p.m. each day, and remained significantly less than those observed at 6:00 a.m., day 0, whether the

estradiol capsules were removed or left in place, provides evidence that both the negative and positive feedbacks of estradiol are maintained, or "remembered" in ovariectomized rats.

The mechanism whereby the estradiol stimulus is remembered in long-term ovariectomized rats remains to be determined. One hypothesis might be that the estradiol receptors in the hypothalamo-hypophyseal axis are hypersensitized following ovariectomy. Once the LH surge mechanism is "re-set" by a supra-threshold stimulus, sub-threshold concentrations of estradiol of adrenal origin may constitute a sufficient stimulus for the induction of daily LH surges. Another possibility is that the supra-threshold estradiol stimulus in ovariectomized animals may induce formation of new receptors with a much greater sensitivity. Elucidation of the mechanism of memory promises to be an interesting and challenging problem for further investigation. The experiments may also provide important information concerning the mechanism of action of estradiol.

CHAPTER IV

POSITIVE FEEDBACK OF ESTRADIOL-17β IN RATS OVARIECTOMIZED ON DIESTRUS OR PROESTRUS

The foregoing observations indicate that in ovariectomized rats a daily neural signal for LH discharge is given on at least 4 consecutive days following the estradiol stimulus. The observed "memory" in the repetitive surge mechanism in rats ovariectomized for 2 weeks, coupled with the earlier findings of Everett and Sawyer (1950) demonstrating a 24-hour periodicity in the surge mechanism of intact rats, suggest the intriguing possibility that the pre-ovulatory increase in circulating estradiol levels which occurs during the estrous cycle (Brown-Grant et al., 1970; Hori et al., 1968; Roser and Aron, 1973; Shaikh, 1971) might also trigger repetitive LH surges, if the processes of ovulation and corpus luteum formation were prevented. The following experiments were performed to investigate this possibility. The first attempt failed to demonstrate repetitive surges in rats ovariectomized on proestrus, and this was taken as a challenge to continue the investigation and thus determine whether daily signals for the LH surge occur at all in recently ovariectomized rats.

Methods

In all of the subsequent experiments, the day of initiation of the estrogen stimulus for LH release, whether endogenous or exogenous, will be designated as day 0.

Two groups of rats (one obtained from Holtzman Company and the other from Spartan Farms) were bilaterally ovariectomized at 10:00 a.m. on proestrus (day 1). Blood samples were withdrawn at 12:30 and 5:00 p.m. on days 1-3.

The remaining experiments described in this chapter employed Spartan rats only. Silastic capsules were implanted sc in a group of rats immediately following bilateral ovariectomy at 8:00 a.m. on diestrus 2 (day 0). The implants were removed at 12:00 noon the following day (expected proestrus) and blood samples were obtained at 12:30 and 5:00 p.m. on days 1-4.

Pentobarbital (30 mg/kg ip) was administered to 3 additional groups of rats at 12:30 p.m. on the day of proestrus, or expected proestrus (day 1). One of these groups was ovariectomized at 10:00 a.m. on proestrus. Another group received estradiol implants from 8:00 a.m. on diestrus 2 (day 0), immediately following bilateral ovariectomy, until 12:30 p.m., day 1. The third group was left intact. Blood samples were withdrawn at 12:30 and 5:00 p.m. for 4, 5 or 2 days in the three groups, respectively.

Two additional groups of rats received estradiol implants for either 2 or 12 days immediately following bilateral ovariectomy at 10:00 a.m. on diestrus 2 (day 0). Blood samples were obtained at the following times: in the first group, at 5:00 p.m. on day 0 and at 12:30 and 5:00 p.m. on days 1 and 2, and in the second group, at 12:30 and 5:00 p.m. on days 1-12.

Results and Discussion

Plasma LH concentrations at 12:30 p.m. on day 1, in rats ovariectomized at 10:00 a.m. on proestrus (day 1), were characteristic of those observed prior to initiation of the proestrous LH surge (Fig. 7). The prominent increase in LH levels observed at 5:00 p.m. indicates that ovariectomy at 10:00 a.m. did not interfere with the pre-ovulatory LH surge, a finding which agrees with previous observations (Schwartz,

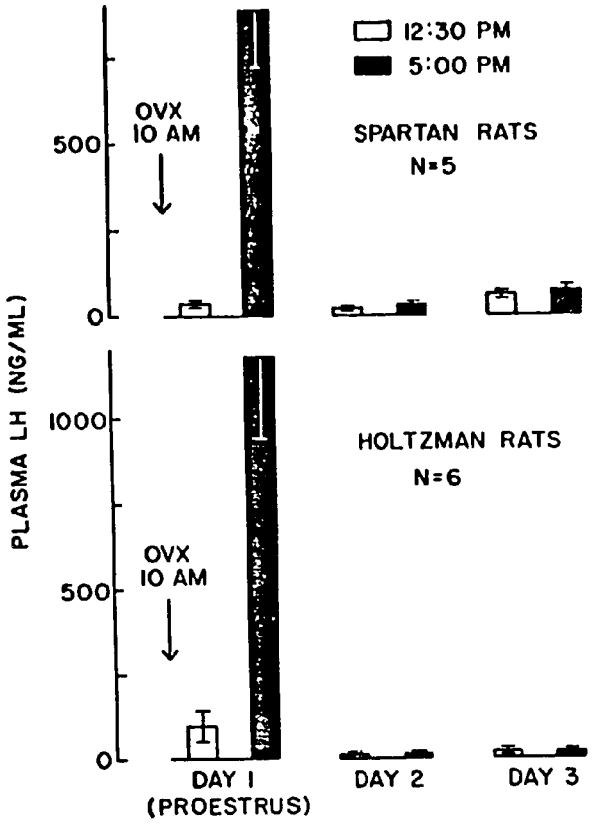


FIGURE 7. Plasma LH concentrations (mean ± SE of 5 or 6 observations) in rats ovariectomized on proestrus.

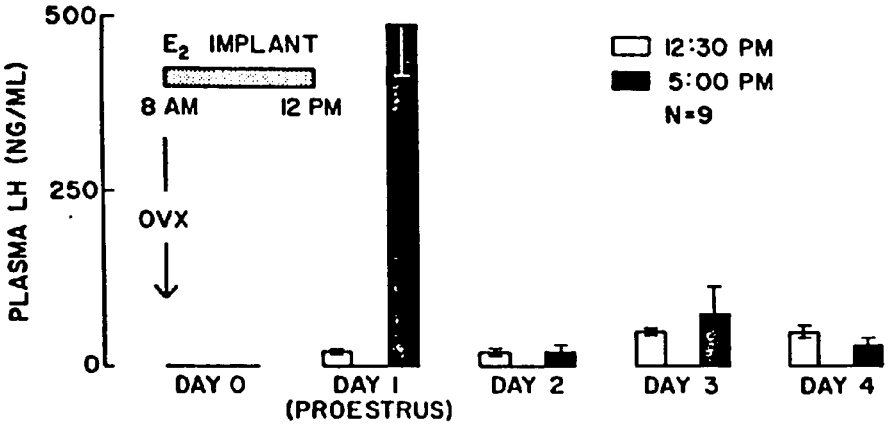


FIGURE 8. Plasma LH concentrations (mean ± SE of 9 observations) in pseudo-intact rats.

1964). On the next 2 days, however, no discharge of LH occurred (Fig. 7), indicating that repetitive surges do not occur if ovulation and corpus luteum formation are prevented by ovariectomy.

This absence of daily LH surges in rats ovariectomized on proestrus may be attributed to a difference between either the competency of the LH release mechanisms in long-term ovariectomized and recently ovariectomized rats, or the nature of the endogenous estrogen stimulus and that imposed by Silastic implants. That the latter hypothesis was not the case was demonstrated by inserting Silastic capsules containing estradiol-17β sc immediately following ovariectomy at 8:00 a.m. on the day before proestrus. This experimental model will subsequently be referred to as the "pseudo-intact" rat. On the following day (expected proestrus), the implants were removed at 12:00 noon; thus, the duration of the estrogen stimulus (28 hr) was similar to that which had induced daily surges in long-term ovariectomized rats, and similar to the endogenous estrogen stimulus of the estrous cycle. It is important to note that ovariectomy on the day before proestrus abolishes the preovulatory increment in circulating estradiol concentrations (Brown-Grant et al., 1970; Hori et al., 1968; Roser and Aron, 1973; Shaikh, 1971) and, unless estradiol is supplied soon after ovariectomy, the proestrous LH surge does not occur (Miyake, 1969). Although an LH surge occurred during the release period on the day of expected proestrus (day 1), no subsequent LH surge was observed (Fig. 8). This observation led to the conclusion that the failure to elicit daily LH surges in rats ovariectomized on proestrus or in pseudo-intact rats is a function of the competency of the LH release mechanism and not the type of estrogen stimulus.

The failure to observe a memory in the estradiol-induced LH release mechanism of pseudo-intact rats or rats ovariectomized on proestrus does not necessarily mean that daily signals for LH release are not given. Alternatively, it is possible that the occurrence of one LH surge renders the LH release mechanism in such rats unresponsive to these signals. That this was not the case, however, was shown in the 2 groups of rats in which pentobarbital was administered during the first release period following withdrawal of the estrogen stimulus (implants or endogenous). No LH surge was observed during the release period on the day of pentobarbital blockade or on any of the next 3 days (Fig. 9). The absence of an LH surge on the day following administration of pentobarbital was somewhat surprising in the light of Everett and Sawyer's (1950) observation that ovulation, and presumably the pre-ovulatory LH discharge, was delayed one day in intact rats treated with pentobarbital on proestrus. Therefore, we repeated Everett and Sawyer's experiment, and verified that an LH surge occurred during the first release period after pentobarbital administration (Fig. 10), a result which strengthens the argument for the existence of a neural signal for LH release on at least 2 consecutive days when the ovaries are left intact.

What accounts for the difference between intact rats and those ovariectomized on proestrus which determines whether or not an LH surge will occur on the day following pentobarbital blockade? The continuous presence of estradiol prior to the release period may be the distinguishing factor. As indicated by the biologic evidence of Everett and Sawyer (1950) and direct radioimmunoassay measurements made by Naftolin et al. (1972), circulating estradiol concentrations in intact rats re-

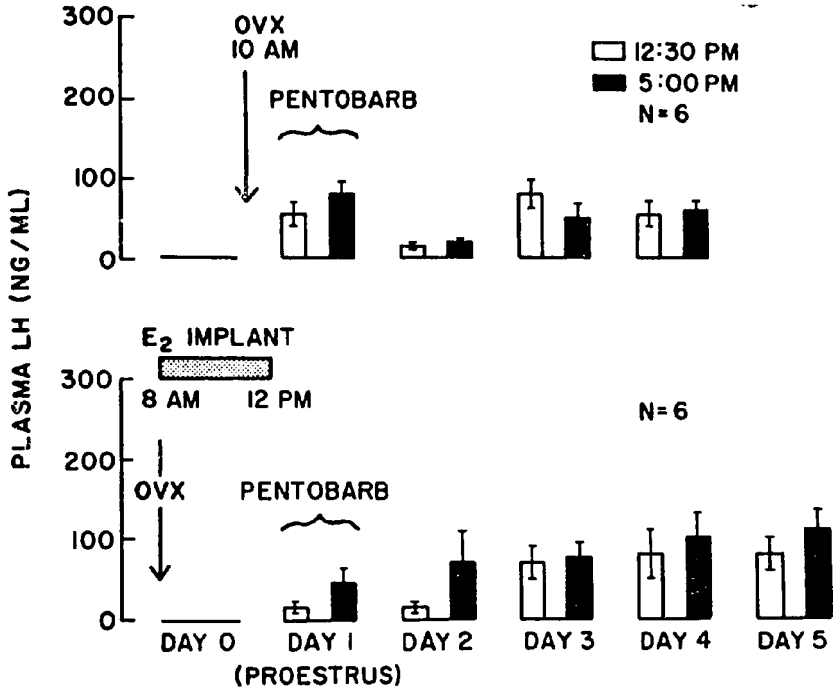


FIGURE 9. Effect of administration of pentobarbital on plasma LH concentrations (mean ± SE of 6 observations) in rats ovariectomized on proestrus and in pseudo-intact rats.

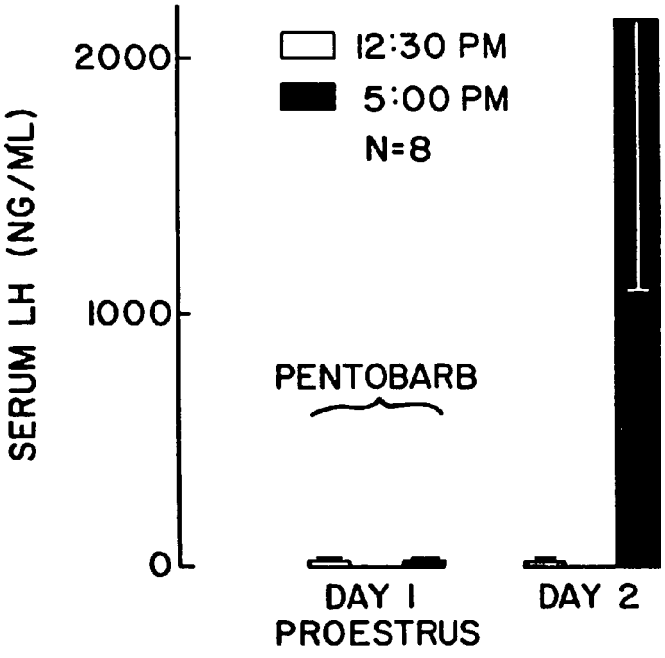


FIGURE 10. Serum LH concentrations (mean ± SE of 8 observations) in intact rats given pentobarbital on proestrus.

mained high for at least one day following administration of pentobarbital on proestrus. If this hypothesis is tenable, daily LH surges should be elicited in pseudo-intact rats merely by leaving the Silastic implants in place, rather than removing them after 28 hr. That this was indeed the case may be seen in Figure 11. This observation leads to the natural conclusion that daily signals for the LH surge do exist in pseudo-intact rats but that the memory in the mechanism which mediates the positive feedback action of estradiol in long-term ovariectomized rats does not exist in pseudo-intact rats. When the estradiol implants were left in pseudo-intact rats for 14 days, LH discharges occurred at 5:00 p.m. daily for 10 consecutive days (Fig. 12). These results show for the first time that in the pseudo-intact rat, LH discharges and their antecedent neural signals can occur on at least 10 consecutive days.

The cessation of the surges after 10 days, including their attenuation prior to day 10, may be due to an inability of the pituitary to continue such a high rate of synthesis and release of LH. Alternatively, the signal may weaken and eventually stop altogether due to the absence of other ovarian hormones. Another possibility is that the rigorous schedule of blood withdrawal interfered with the normal functioning of the hypothalamo-hypophyseal axis.

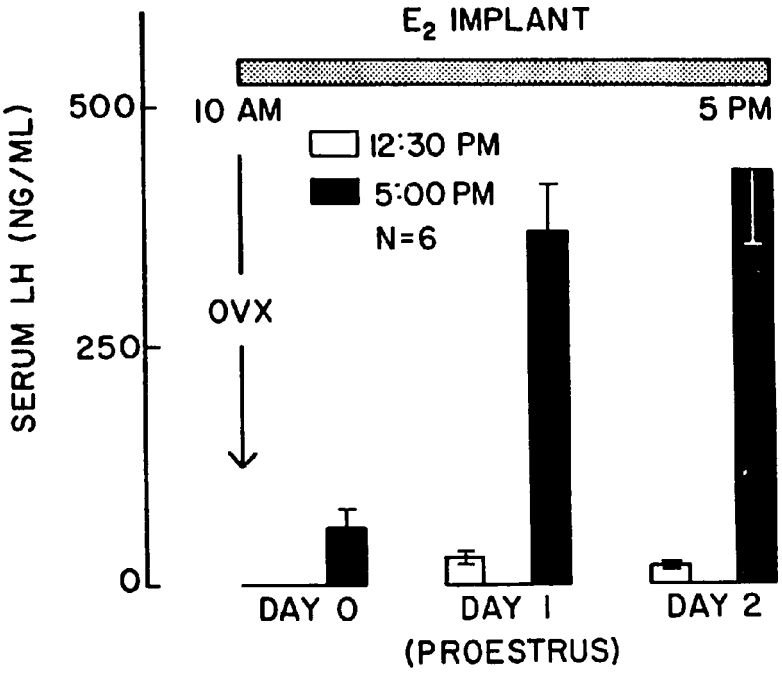


FIGURE 11. Serum LH concentrations (mean ± SE of 6 observations) in pseudo-intact rats.

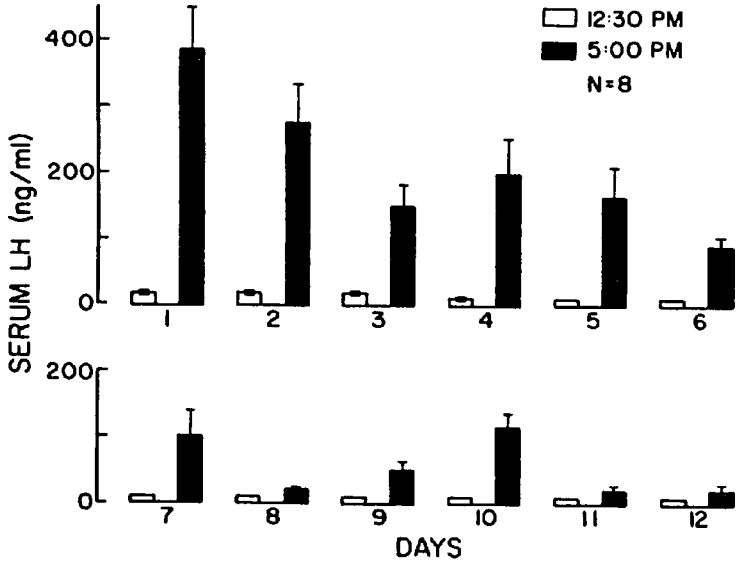


FIGURE 12. Serum LH (mean ± SE of 8 observations) on days 1-12 in pseudo-intact rats receiving estradiol implants after ovariectomy at 10:00 a.m. on day 0.

CHAPTER V

MODULATION OF PITUITARY RESPONSIVENESS TO LRF BY ESTROGEN

Estradiol may induce LH release by increasing hypothalamic LRF secretion (Chowers and McCann, 1965; Doecke and Doerner, 1965; Ramirez and Sawyer, 1965; Weick and Davidson, 1970), by changing pituitary responsiveness to LRF (Chowers and McCann, 1965; Doecke and Doerner, 1965; Ramirez and Sawyer, 1965; Weick and Davidson, 1970), or by both mechanisms. It has been demonstrated that estradiol acts directly on the pituitary *in vitro* to stimulate LH release (Piacsek and Meites, 1966; Schneider and McCann, 1970) and that it causes increased responsiveness to LRF in diestrous rats (Arimura and Schally, 1971; Debeljuk *et al.*, 1972) and anestrous ewes (Reeves *et al.*, 1971b). Recent observations in sheep (Reeves *et al.*, 1971a), hamsters (Arimura *et al.*, 1972), rats (Aiyer *et al.*, 1973; Cooper *et al.*, 1973; Kalra *et al.*, 1973b; Martin *et al.*, 1973; Rippel *et al.*, 1973) monkeys (Krey *et al.*, 1973b) and humans (Nillius and Wide, 1972; Yen *et al.*, 1972), indicate that there is an increase in pituitary responsiveness to exogenous GnRH during the pre-ovulatory phase of the cycle when circulating estradiol levels are high. The following experiments were performed to investigate the pituitary responsiveness to LRF throughout the estrous cycle of the rat and to examine whether the observed differences can be attributed to ovarian estradiol secretion.

Methods

Holtzman rats having 5-day estrous cycles were cannulated via the left carotid artery following administration of pentothal (35 mg/kg ip) at 12:00 noon on diestrus 2, diestrus 3, proestrus, or estrus. Additional doses of pentothal were administered as needed throughout the

afternoon to maintain anesthesia through 4:15 p.m., thus minimizing possible variations in endogenous LH and LRF secretion which occur throughout the estrous cycle. At 4:00 p.m. a blood sample was withdrawn and 200 ng Gn-RH were injected into the carotid cannula. Fifteen minutes later, a second blood sample was obtained by decapitation and exsanguination.

Two additional groups of rats were ovariectomized at 8:00 a.m. on diestrus 3 and treated with Gn-RH as described above at either 4:00 p.m. on diestrus 3 or 4:00 p.m. on the following day (expected proestrus). Two other groups of rats were ovariectomized at 8:00 a.m. on diestrus 3 and treated with Silastic capsules containing estradiol-17β implanted sc immediately following ovariectomy. In one of these groups, Gn-RH was administered as described above at 4:00 p.m. on diestrus 3 (i.e., after 8 hr of exposure to estradiol). In the remaining group, the estradiol implants were removed at 12:00 p.m. on the day of expected proestrus and Gn-RH was administered at 4:00 p.m. that same afternoon (i.e., after 28 hr of exposure to estradiol).

Results and Discussion

Pituitary responsiveness to Gn-RH, as measured by the difference between pre- and post-injection LH concentrations, remained relatively low with minor variations, except on proestrus, when it increased dramatically (Fig. 13). These results confirm previous observations of increased pituitary responsiveness to Gn-RH in proestrous rats (Aiyer et al., 1973; Cooper et al., 1973; Kalra et al., 1973b; Martin et al., 1973; Rippel et al., 1973). The increase in responsiveness on proestrus was abolished when the pre-ovulatory increase in estradiol levels was prevented by means of ovariectomy at 8:00 a.m. on the day before

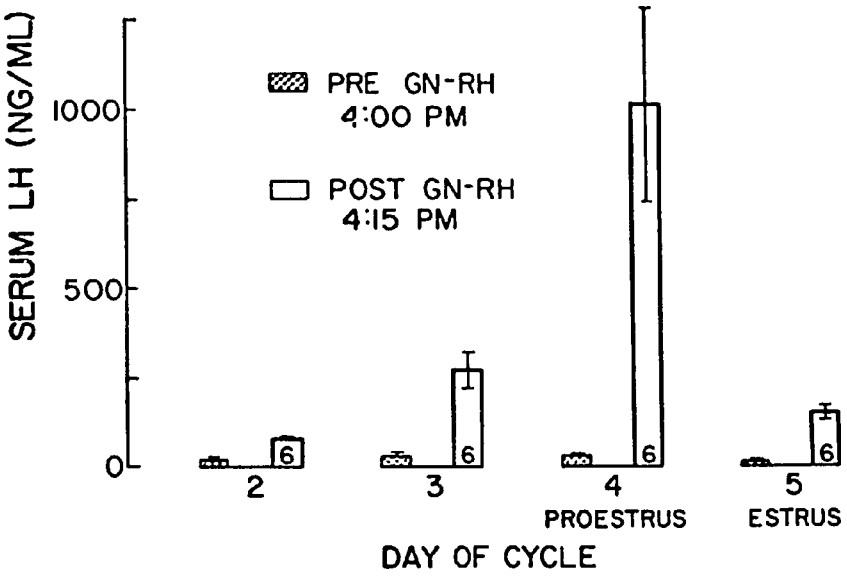


FIGURE 13: Serum LH concentrations (mean ± SE of 6 observations) during 5-day estrous cycle in response to 200 ng Gn-RH in pentothal-treated Holtzman rats.

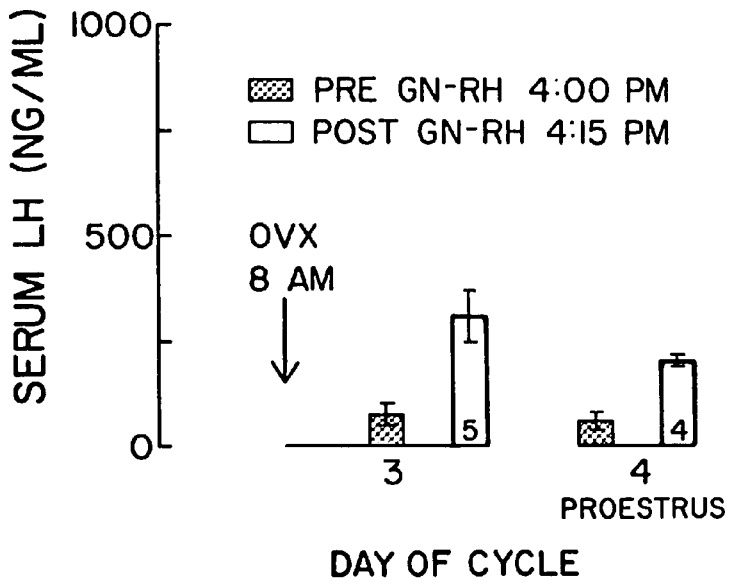


FIGURE 14: Pituitary response to 200 ng Gn-RH in pentothal-treated Holtzman rats ovariectomized one day before proestrus. Numbers at the base of the bars indicate the number of animals in each group.

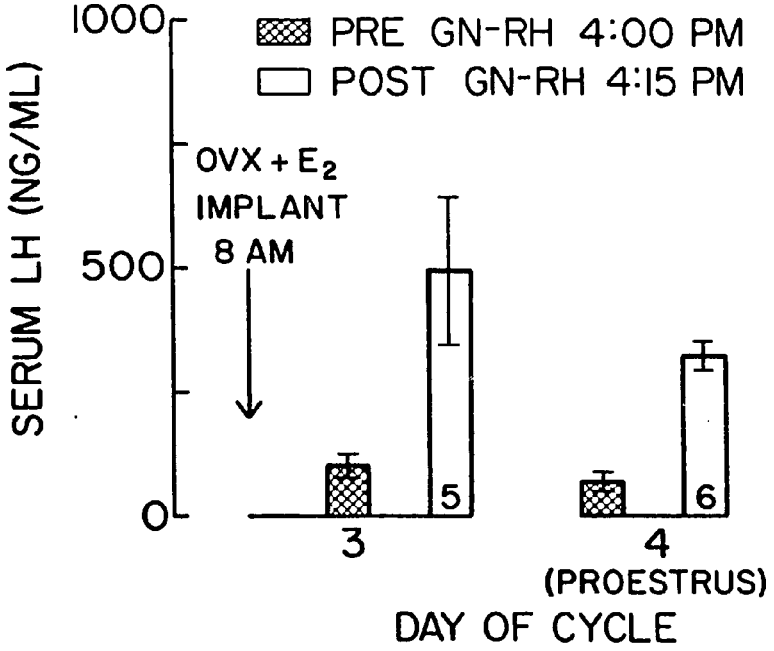


FIGURE 15: Pituitary response to 200 ng Gn-RH in Holtzman pentothal-treated pseudo-intact rats. Numbers at the base of the bars indicate the number of animals in each group.

proestrus (diestrus 3) (Fig. 14). It has already been demonstrated that when the pre-ovulatory increment in circulating estradiol concentrations is prevented by ovariectomy (Miyake, 1969) or its biological effect inhibited by administration of antibodies which specifically neutralize estradiol (Ferin et al., 1969), the pre-ovulatory LH discharge does not occur. In addition, estradiol has been shown to increase pituitary responsiveness to Gn-RH both in vivo and in vitro in a variety of experimental situations (Chowers and McCann, 1965; Doecke and Doerner, 1965; Piacsek and Meites, 1966; Ramirez and Sawyer, 1965; Schneider and McCann, 1970; Weick and Davidson, 1970). These observations, coupled with the above findings strongly suggest that, in the physiological setting of the normal estrous cycle, the pre-ovulatory increment in estradiol secretion causes an increase in pituitary responsiveness to LRF on proestrus.

To test this hypothesis, estradiol implants were inserted sc immediately following ovariectomy at 8:00 a.m. on diestrus 3. Pituitary responsiveness to Gn-RH was low on the afternoon of diestrus 3, eight hours after insertion of the implants (Fig. 15). On the afternoon of expected proestrus, there was no dramatic increase in pituitary responsiveness to Gn-RH, which remained at a level similar to that seen on diestrus 3 (Fig. 15). These results indicate that, under these experimental conditions, estradiol does not cause an increase in pituitary responsiveness to Gn-RH identical to that seen in intact rats on proestrus. However, pituitary response to LRF on expected proestrus was slightly greater in rats treated with estradiol implants for 28 hr than in untreated rats (Fig. 14 and 15), indicating that estradiol may act by increasing pituitary responsiveness to LRF, but that our experimental

stimulus was not identical to the endogenous stimulus which causes the massive increase in pituitary response on proestrus.

It is interesting to note that there was no difference in pituitary responsiveness to Gn-RH whether the estradiol stimulus was maintained for 8 hr or 28 hr, in spite of the fact that insertion of an estradiol implant for 28 hr in recently ovariectomized rats is an adequate stimulus for induction of an LH surge (Fig. 8). It may be that the estrogen stimulus achieved through the use of estradiol implants does not precisely replicate the physiologic changes in circulating estrogen required to bring about either a normal increase in pituitary responsiveness to Gn-RH or a normal proestrous LH surge. It remains to be determined whether the LH surges we observed in pseudo-intact rats after a stimulus duration of 28 hr are identical in time course and magnitude to proestrous surges. Alternatively, it might be postulated that other ovarian hormones in addition to estrogen play a role in causing the marked increase in pituitary responsiveness to Gn-RH seen on proestrus.

CHAPTER VI

EVIDENCE FOR THE FEEDBACK OF OTHER OVARIAN HORMONES ON LH RELEASE

In the preceding chapter, it was shown that administration of estradiol to rats ovariectomized on the day before proestrus does not restore pituitary responsiveness to exogenous Gn-RH to the degree seen in intact rats on proestrus. One interpretation of this finding is that other ovarian hormones may play an important role in the regulation of the pre-ovulatory LH surge. It is interesting to note, however, that a stimulus duration of 28 hr which was employed in those experiments reliably elicited a discharge of LH. In the light of these observations, it became necessary to determine whether or not these induced surges are identical in magnitude and time course to proestrous surges. In addition, if other ovarian hormones do, in fact, play a role in regulating LH release, it might be expected that the repetitive LH surges induced in rats made pseudo-intact on different days of the cycle would be altered according to the changing hormonal environment which occurs throughout the estrous cycle. Finally, the effect of other ovarian hormones on the repetitive LH surge mechanism was investigated by observing whether or not the pattern of daily repetitive LH surges observed in rats implanted with estradiol was changed when the ovaries were left intact and ovulation and corpus luteum formation occurred.

Methods

Blood samples (600 µl) were obtained at 1:00, 2:00, 4:00, 5:00, 6:00, 7:00, 8:00 and 10:00 p.m. from a group of intact rats on proestrus. The day of proestrus was verified by the presence of ova in the oviduct on the following morning. Another group of rats received

Silastic capsules containing estradiol-17β immediately following bilateral ovariectomy at 10:00 a.m. on diestrus 2 (day 0). Blood samples were withdrawn at the same times as in the proestrous rats on day 1, and at 1:00, 2:00, 5:00, 7:00 and 10:00 p.m. on days 2 and 3.

In 5 additional groups of Spartan rats, estradiol implants were inserted sc at 8:00 a.m. immediately following ovariectomy on each of the 5 days of the estrous cycle (day 0). The implants were left in place for 5 days. Blood samples were withdrawn at 5:00 p.m., day 0, and at 12:30 and 5:00 p.m. on days 1-5. At the time of ovariectomy, uteri and oviducts were examined to confirm the stage of the estrous cycle.

Finally, estradiol implants were inserted sc into a group of intact Spartan rats at 11:30 a.m. on proestrus (day 1) and were left in place for 14 days. Blood samples were withdrawn at 5:00 p.m. on day 1, and at 12:30 and 5:00 p.m. on days 2-14.

In the second experiment above, all groups except C_2 were composed of rats having either 4-day or 5-day estrous cycles. There was no difference in the data obtained from these 2 groups on days when the vaginal smears were identical. Therefore, the groups were pooled according to their vaginal smears and the data are presented in this manner. In rats having 4-day estrous cycles (L_1, L_2, N and C_1) the vaginal smears on diestrus 1 and 2 (L_1 and L_2) contain predominantly leukocytes. Nucleated epithelial cells (N) appear on the day of proestrus, and cornified cells (C) are present on estrus. In rats having 5-day cycles (L_1, L_2, N, C_1 and C_2), diestrus 1 and 2 are designated the same as in rats having 4-day cycles. However, in rats with 5-day cycles, vaginal smear patterns of N, C_1 and C_2 are referred to as dies-

trus 3, proestrus, and estrus, respectively.

Results and Discussion

As can be seen in Fig. 16, the LH discharges in pseudo-intact rats on days 1, 2 and 3 were similar in time course to proestrous surges; however, the maximum concentrations of plasma LH were less than ½ those observed on proestrus. Although the difference may be attributed to the differences between the estrogen stimulus achieved by the estradiol implants and that occurring as a result of endogenous estradiol secretion, this does not seem probable because the magnitude of the estradiol-induced LH surge is not dose-related once a threshold level is attained, either in monkeys (Karsch et al., 1973b) or in ovariectomized rats, as shown in Chapter 2. A much more likely hypothesis is that some other ovarian hormone(s) in addition to estradiol must be present in order to induce LH discharges identical to proestrous surges. It is noteworthy that, coincident with the initiation of the LH surge on proestrus, ovarian progesterone secretion increases (Piacsek et al., 1971). Furthermore, LH surges identical to those observed on proestrus can be induced in rats ovariectomized on diestrus 2 only when both progesterone and estrogen are administered (Krey et al., 1973a).

If ovarian hormones other than estradiol control the amount of LH released in a surge, then the magnitude of estrogen-induced LH discharges should be different in rats made pseudo-intact at varying stages throughout the estrous cycle, when the steroidal environment is changing. That this is the case is shown in Fig. 17. Although insertion of estradiol implants on each day of the cycle induced repetitive LH surges, the LH discharge on day 1 was maximal when the vaginal smear on the day of ovariectomy and insertion of implants (day 0) contained

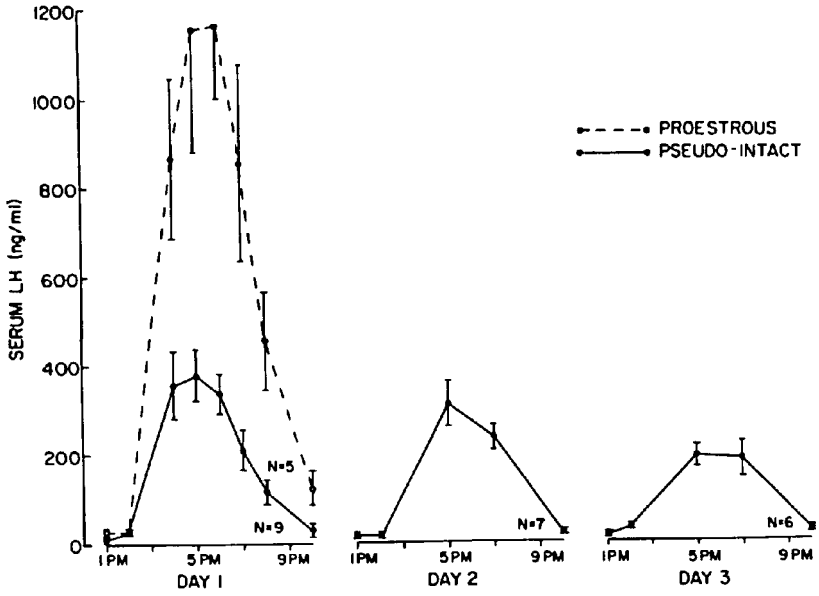


FIGURE 16. Time course of LH release in proestrous rats and in pseudo-intact rats receiving estradiol implants after ovariectomy at 10:00 a.m. on day 0.

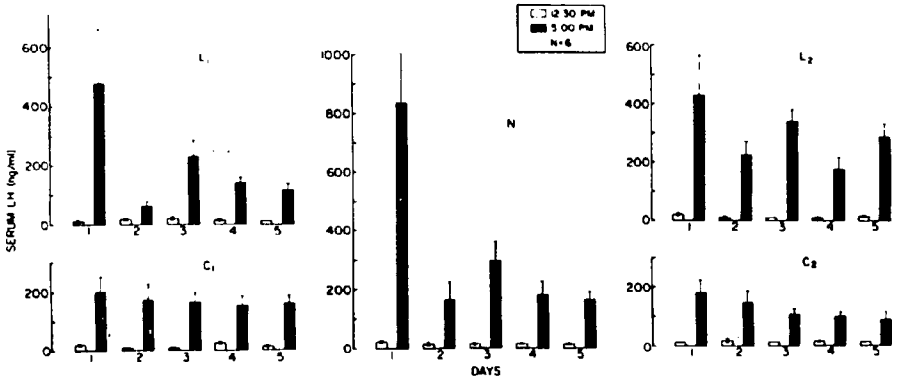


FIGURE 17. Serum LH concentrations (mean ± SE of 6 observations) in rats receiving estradiol implants immediately following ovariectomy on L_1, L_2, N, C_1 and C_2.

nucleated epithelial cells (N), intermediate when vaginal smears on day 0 contained leukocytes (L_1 and L_2) and lowest when the vaginal smears on day 0 contained cornified cells (C_1 and C_2). LH release was identical to that on proestrus only if rats were made pseudo-intact when their vaginal smears contained nucleated epithelial cells. These results indicate that the stage of the estrous cycle can influence the magnitude of the first estradiol-induced LH discharge, but without a thorough characterization of the time courses of all ovarian hormones throughout the cycle, it is not possible to correlate the differing responses during the cycle with the activity of any one steroid. In addition, these results provide further evidence for a daily signal for LH release in intact rats.

The insertion of estradiol implants into intact rats approximately 4½ hr prior to the release period on proestrus (day 1) did not interfere with the normal proestrous LH surge (Fig. 18). However, following ovulation, which was presumed to occur on the morning of day 2, no subsequent discharges of LH were observed except on day 3, when serum LH concentrations at 5:00 p.m. were similar to those observed in pseudo-intact rats. These results, along with the foregoing observations in pseudo-intact rats that LH discharges can occur for 10 consecutive days, suggest that a substance secreted by the ovaries following ovulation may inhibit LH release. It is interesting that no inhibition of the LH discharge occurred on day 3, two days after proestrus. Although the reason for the recurrence of an LH surge on day 3 is not known, these data correlate with the observation that rats receiving estrogen on proestrus to induce pseudopregnancy show mating behavior that night and again 2 nights later (Powers and Zucker, 1969). Histologic exami-

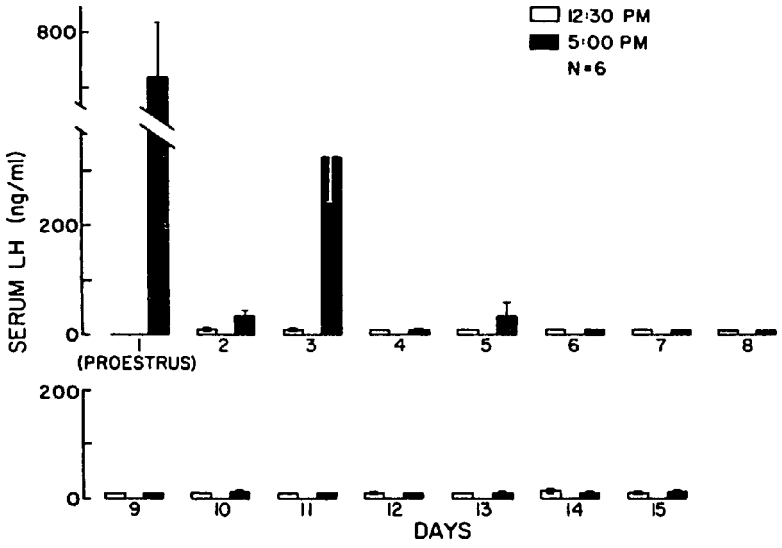


FIGURE 18. Serum LH concentrations (mean ± SE of 6 observations) on days 1-15 in intact rats receiving estradiol implants immediately following ovariectomy at 11:30 a.m. on proestrus (day 1).

nation of the ovaries on day 15 revealed numerous antral follicles and many well-formed corpora lutea. These observations agree with those of Alloiteau (1957) that the maintenance of high levels of estrogen in intact rats induces pseudopregnancy and they provide evidence, although speculative, that the progesterone which is secreted in increased quantities during pseudopregnancy inhibits repetitive estradiol-induced LH surges. It is noteworthy that the levels of progesterone present during the luteal phase of the menstrual cycle can inhibit estradiol-induced LH release in the rhesus monkey (Dierschke et al., 1973).

It would appear, therefore, that at least one ovarian hormone other than estradiol modulates, in both a stimulatory and inhibitory manner, the reponse of the hypothalamo-hypophyseal axis to estrogen. While the identity of this substance remains to be established, a likely candidate for both stimulatory and inhibitory actions is progesterone. It has been demonstrated by numerous investigators (Brown-Grant, 1967; Brown-Grant and Naftolin, 1972; Everett, 1948; Kalra et al., 1973a; Kobayashi et al., 1969; Uchida, 1972) that progesterone has both a facilitatory and inhibitory action on LH release.

CHAPTER VII

SUMMARY AND CONCLUSIONS

The phasic mode of LH secretion in the rat is triggered prior to the release period by an increase in circulating estradiol concentrations to a level which is maintained for a finite interval. This LH release mechanism functions with a 24-hr periodicity, is expressed only during the release period, and has a neural component. Furthermore, in long-term ovariectomized rats the estradiol stimulus can be "remembered" for at least 3 days after its withdrawal. In recently ovariectomized rats, however, a continuous estradiol stimulus is required for the appearance of daily LH surges. During the estrous cycle of the rat, a marked increase in pituitary responsiveness to exogenous Gn-RH occurs on proestrus. This increased responsiveness is dependent on the feedback action of one or more ovarian hormones. Although the foregoing experiments failed to identify this hormone to be estradiol, they did not exclude the possibility, suggested by many previous findings, that estradiol, alone or in combination with other steroids, modulates pituitary responsiveness to exogenous Gn-RH throughout the estrous cycle.

The daily LH discharges in recently ovariectomized rats continue for at least 10 consecutive days in rats made pseudo-intact on diestrus 2 and for at least 5 days in rats made pseudo-intact at other times of the estrous cycle, provided high levels of circulating estradiol are maintained and ovaries containing corpora lutea are not present. These observations show, for the first time, that signals for an LH surge occur every day in rats in which the pituitary has never been released from the negative feedback inhibition of ovarian steroids. Further, these observations are consistent with the hypothesis that daily neural

signals for an LH surge are given throughout the estrous cycle of the rat. In addition, it may be postulated that the LH discharge occurs only when estradiol, either alone or in combination with another ovarian hormone, has sensitized the pituitary to LRF, thus obviating the need for an action of estradiol on the hypothalamic surge center and localizing the positive feedback action of estradiol at the level of the pituitary. One might also postulate that the presence of a memory in the estradiol-induced LH release mechanism in long-term ovariectomized rats, and its absence in pseudo-intact rats or rats ovariectomized on proestrus, may be attributed to the duration of the estradiol-induced increase in pituitary responsiveness to LRF.

Several observations made in the course of the above experiments indicate that estradiol is a necessary but not sufficient stimulus for LH discharges identical to the proestrous surge. In addition, in spite of a continuous elevation of estradiol levels, a substance secreted by the ovaries following ovulation can inhibit repetitive LH discharges. As discussed in Chapter 6, the most likely candidate for both of these roles is progesterone. If progesterone can be shown to both facilitate and inhibit estrogen-induced LH release, it remains to be determined by what mechanism this single steroid hormone can exert 2 completely diametric actions on LH secretion.

In the course of these experiments, a new experimental model for examining the control of LH secretion, the pseudo-intact rat, was developed. The characteristics of precise timing of both stimulus and response, and the repetitiveness of an easily-measured event, the LH surge, render the pseudo-intact rat eminently useful for studying biological clock mechanisms, as well as for investigating the sites and

mechanisms of feedback action of steroids on gonadotropin release in the whole animal, and at the cellular or molecular level. The periodicity inherent in the model permits individual animals to serve as their own controls. In addition, non-specific short-term effects of surgical stress and/or anesthesia on the LH release mechanism can readily be distinguished from the specific effects of experimental manipulations. This model also provides an opportunity to investigate a unique phenomenon, that of memory in an endocrine feedback system. Finally, it should provide a wealth of information about the physiology of the estrous cycle, especially if physiologic concentrations of circulating steroids are replicated.

LITERATURE CITED

Aiyer, M.S., Grieg, F. and G. Fink. 1973. Changes in sensitivity of the anterior pituitary gland to synthetic luteinizing hormone releasing factor during the oestrous cycle of the rat. J. Endocr. 57:xxvi. (Abstract)

Ajika, K., Kalra, S.P., Fawcett, C.P., Krulich, L. and S. M. McCann. 1972. The effect of stress and Nembutal on plasma levels of gonadotropins and prolactin in ovariectomized rats. Endocrinology 90:707-715.

Alloiteau, J.J. 1957. A propos de la pseudo-gestation consecutive a une injection unique d'oestradiol chez la Ratte. C.R. Soc. Biol. 151:290-292.

Arimura, A., Debeljuk, L. and A.V. Schally. 1972. LH release by LH-releasing hormone in golden hamsters at various stages of estrous cycle. Proc. Soc. Exp. Biol. Med. 140:609-612.

Arimura, A. and A.V. Schally. 1971. Augmentation of pituitary responsiveness to LH-releasing hormone (LH-RH) by estrogen. Proc. Soc. Exp. Biol. Med. 136:290-293.

Barraclough, C.A. 1966. Modification in the CNS regulation of reproduction after exposure of prepubertal rats to steroid hormones. Recent Progr. Hormone Res. 22:503-539.

Beattie, C.W., Campbell, C.S., Nequin, L.G., Soyka, L.F. and N.B. Schwartz. 1973. Barbiturate blockade of tonic LH secretion in the male and female rat. Endocrinology 92:1634-1638.

Blackwell, R.E. and M.S. Amoss, Jr. 1971. A sex difference in the rate of rise of plasma LH in rats following gonadectomy. Proc. Soc. Exp. Biol. Med. 136:11-14.

Bogdanove, E.M. 1963. Direct gonad-pituitary feedback: An analysis of effects of intracranial estrogenic depots on gonadotrophin secretion. Endocrinology 73:696-712.

Brown-Grant, K. 1967. The effects of a single injection of progesterone on the oestrous cycle, thyroid gland activity and uterus-plasma concentration ratio for radio-iodide in the rat. J. Physiol. 190:101-121.

Brown-Grant, K., Exley, D. and F. Naftolin. 1970. Peripheral plasma oestradiol and luteinizing hormone concentrations during the oestrous cycle of the rat. J. Endocr. 48:295-296.

Brown-Grant, K. and F. Naftolin. 1972. Facilitation of luteinizing hormone secretion in the female rat by progesterone. J. Endocr. 53:37-46.

Caligaris, L., Astrada, J.J. and S. Taleisnik. 1971. Release of luteinizing hormone induced by estrogen injection into ovariectomized rats. Endocrinology 88:810-815.

Chowers, I. and S.M. McCann. 1965. Content of luteinizing hormone-releasing factor and luteinizing hormone during the estrous cycle and after changes in gonadal steroid titers. Endocrinology 76: 700-708.

Cooper, K.J., Fawcett, C.P. and S.M. McCann. 1973. Variations in pituitary responsiveness to luteinizing hormone releasing factor during the rat oestrous cycle. J. Endocr. 57:187-188.

Daane, T.A. and A.F. Parlow. 1971. Periovulatory patterns of rat serum follicle stimulating hormone and luteinizing hormone during the normal estrous cycle: Effects of pentobarbital. Endocrinology 88:653-663.

Davidson, J.M. 1969. Feedback control of gonadotropin secretion, p. 343-388. In Ganong and Martini (eds.) Frontiers in Neuroendocrinology, 1969. Oxford University Press, New York.

Debeljuk, L., Arimura, A. and A.V. Schally. 1972. Effect of estradiol and progesterone on the LH release induced by LH-releasing hormone (LH-RH) in intact diestrous rats and anestrous ewes. Proc. Soc. Exp. Biol. Med. 139:774-777.

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

Doecke, F. and G. Doerner. 1965. The mechanism of the induction of ovulation by estrogens. J. Endocr. 33:491-499.

Dziuk, P.J. and B. Cook. 1966. Passage of steroids through silicone rubber. Endocrinology 78:208-211.

England, B.G., Niswender, G.D. and A.R. Midgley, Jr. 1974. Radioimmunoassay of estradiol-17β without chromatography. Endocrinology (In press).

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

Everett. J.W. 1964. Central neural control of reproductive functions of the adenohypophysis. Physiol. Rev. 44:373-431.

Everett, J.W. and C.H. Sawyer. 1950. A 24-hour periodicity in the "LH-release apparatus" of female rats, disclosed by barbiturate sedation. Endocrinology 47:198-218.

Ferin, M., Tempone, A., Zimmerung, P.E. and R.L. VandeWiele. 1969.

Effect of antibodies to 17β-estradiol and progesterone on the estrous cycle of the rat. Endocrinology 85:1070-1078.

Gay, V.L. and A.R. Midgley, Jr. 1969. Response of the adult rat to orchidectomy and ovariectomy as determined by LH radioimmunoassay. Endocrinology 84:1359-1364.

Gay, V.L., Midgley, A.R., Jr. and G.D. Niswender. 1970. Patterns of gonadotropin secretion associated with ovulation. Fed. Proc. 29: 1880-1887.

Gay, V.L., Rebar, R.W. and A.R. Midgley, Jr. 1969. Constant monitoring of plasma luteinizing hormone by radioimmunoassay in individual rats following injection of hypothalamic extract. Proc. Soc. Exp. Biol. Med. 130:1344-1347.

Goding, J.R., Catt, K.J., Brown, J.M., Kaltenbach, C.C., Cumming, I.A. and B.J. Mole. 1969. Radioimmunoassay for ovine luteinizing hormone. Secretion of luteinizing hormone during estrus and following estrogen administration in the sheep. Endocrinology 85:133-142.

Gorski, R.A. 1971. Gonadal hormones and the perinatal development of neuroendocrine function, p. 237-290. In Martini and Ganong (eds.) Frontiers in Neuroendocrinology, 1971. Oxford University Press, New York.

Halasz, B. 1969. The endocrine effects of isolation of the hypothalamus from the rest of the brain, p. 307-342. In Ganong and Martini (eds.) Frontiers in Neuroendocrinology, 1969. Oxford University Press, New York.

Hori, T., Ide, M. and T. Miyake. 1968. Ovarian estrogen secretion during the estrous cycle and under the influence of exogenous gonadotropins in rats. Endocr. Jap. 15:215-222.

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

Kalra, S.P., Kalra, P.S. and R.M. Abrams. 1973b. Peripheral blood LH, estrogen (E_2), and progestins (P) during the rat estrous cycle: Correlation with pituitary and hypothalamic function. Fed. Proc. 32:282. (Abstract)

Karsch, F.J., Dierschke, D.J., Weick, R.F., Yamaji, T., Hotchkiss, J. and E. Knobil. 1973a. Positive and negative feedback control by estrogen of luteinizing hormone secretion in the rhesus monkey. Endocrinology 92:799-804.

Karsch, F.J., Weick, R.F., Butler, W.R., Dierschke, D.J., Krey, L.C., Weiss, G., Hotchkiss, J., Yamaji, T. and E. Knobil. 1973b. Induced LH surges in the rhesus monkey: Strength-duration charac-

teristics of the estrogen stimulus. Endocrinology 92:1740-1747.

Kincl, F.A. and H.W. Rudel. 1971. Sustained release hormonal preparations. Acta Endo Suppl. 151.

Knobil, E., Dierschke, D.J., Yamaji, T., Karsch, F.J., Hotchkiss, J. and R.F. Weick. 1972. Role of estrogen in the positive and negative feedback control of LH secretion during the menstrual cycle of the rhesus monkey, p. 72-86. In Saxena, Beling and Gandy (eds.) Gonadotropins. John Wiley and Sons, Inc. New York.

Kobayashi, F., Hara, K. and T. Miyake. 1969. Effects of steroids on the release of luteinizing hormone in the rat. Endocr. Jap. 16: 251-260.

Krey, L.C., Tyrey, L. and J.W. Everett. 1973a. The estrogen-induced advance in the cyclic LH surge in the rat: Dependency on ovarian progesterone secretion. Endocrinology 93:385-390.

Krey, L.C., Butler, W.R., Weiss, G., Weick, R.F., Dierschke, D.J. and E. Knobil. 1973b. Influences of endogenous and exogenous gonadal steroids on the actions of synthetic LRF in the rhesus monkey, p. 10-18. In Gual and Rosemberg (eds.) Hypothalamic Hypophysiotropic Hormones. Excerpta Medica International Congress Series 263.

Labhsetwar, A.P. 1970. Role of estrogens in ovulation: A study using the estrogen-antagonist, ICI 46,474. Endocrinology 87:542-551.

Legan, S.J., Coon, G.A. and A.R. Midgley, Jr. 1973a. Evidence for a 24-hour periodicity of LH and FSH release in estrogen-treated ovariectomized rats. Fed. Proc. 32:240. (Abstract)

Legan, S.J., Gay, V.L. and A.R. Midgley, Jr. 1973b. LH release following steroid administration in ovariectomized rats. Endocrinology 93:781-785.

Martin, J.E., Tyrey, L., Everett, J.W. and R.E. Fellows. 1973. Variation in pituitary response to synthetic luteotropin releasing factor (LRF) in the cycling rat. Fed. Proc. 32:240. (Abstract)

McCann, S.M., Dhariwal, A.P.S. and J.C. Porter. 1968. Regulation of the adenohypophysis. Ann. Rev. Physiol. 30:589-640.

Miyake, T. 1969. Causal relationship between ovarian steroid secretion and pituitary luteinizing hormone release in the rat estrous cycle. Endocr. Jap. Suppl 1:83-92.

Monroe, S.E., Jaffe, R.B. and A.R. Midgley, Jr. 1972. Regulation of human gonadotropins. XII. Increase in serum gonadotropins in response to estradiol. J. Clin. Endocr. 34:342-347.

Naftolin, F., Brown-Grant, K. and C.S. Corker. 1972. Plasma and pitu-

itary luteinizing hormone and peripheral plasma oestradiol concentrations in the normal oestrous cycle of the rat and after experimental manipulation of the cycle. J. Endocr. 53:17-30.

Neill, J.D. 1972. Sexual differences in the hypothalamic regulation of prolactin secretion. Endocrinology 90:1154-1159.

Nillius, S.J. and L. Wide. 1972. Variation in LH and FSH response to LH-releasing hormone during the menstrual cycle. J. Obstet. Gynaec. Brit. Comm. 79:865-873.

Niswender, G.D., Midgley, A.R., Jr., Monroe, S.E. and L.E. Reichert, Jr. 1968. Radioimmunoassay for rat luteinizing hormone with antiovine LH serum and ovine LH-^{131}I. Proc. Soc. Exp. Biol. Med. 128:807-811.

Piacsek, B., Schneider, T.C. and V.L. Gay. 1971. Sequential study of luteinizing hormone (LH) and "progestin" secretion on the afternoon of proestrus in the rat. Endocrinology 89:39-45.

Piacsek, B.E. and J. Meites. 1966. Effects of castration and gonadal hormones on hypothalamic content of luteinizing hormone releasing factor (LRF). Endocrinology 79:432-439.

Powers, J.B. and I. Zucker. 1969. Sexual receptivity in pregnant and pseudopregnant rats. Endocrinology 84:820-827.

Ramirez, V.D. and C.H. Sawyer. 1965. Fluctuations in hypothalamic LH-RF (luteinizing hormone-releasing factor) during the rat estrous cycle. Endocrinology 76:282-289.

Reeves, J.J., Arimura, A. and A.V. Schally. 1971a. Pituitary responsiveness to purified luteinizing hormone-releasing hormone (LH-RH) at various stages of the estrous cycle in sheep. J. Anim. Sci. 32:123-126.

Reeves, J.J., Arimura, A. and A.V. Schally. 1971b. Changes in pituitary responsiveness to luteinizing hormone-releasing hormone (LH-RH) in anestrous ewes pretreated with estradiol benzoate. Biol. Reprod. 4:88-92.

Rippel, R.H., Johnson, E.S. and W.F. White. 1973. Ovulation and serum luteinizing hormone in the cycling rat following administration of gonadotropin releasing hormone. Proc. Soc. Exp. Biol. Med. 143: 55-58.

Roser, S. et Cl. Aron. 1973. Variations du taux de l'oestradiol-17β produit par l'ovaire au cours de cycles de 5 jours chez la Ratte. C. R. Soc. Biol. 167:341-343.

Scaramuzzi, R.J., Tillson, S.A., Thorneycroft, I.H. and B.V. Caldwell. 1971. Action of exogenous progesterone and estrogen on behavioral estrus and luteinizing hormone levels in the ovariectomized ewe.

Endocrinology 88:1184-1189.

Schneider, H.P.G. and S.M. McCann. 1970. Estradiol and the neuroendocrine control of LH release in vitro. Endocrinology 87:330-338.

Schwartz, N.B. 1964. Acute effects of ovariectomy on pituitary LH, uterine weight and vaginal cornification. Amer. J. Physiol. 207: 1251-1259.

Schwartz, N.B. 1969. A model for the regulation of ovulation in the rat. Recent Progr. Hormone Res. 25:1-55.

Shaikh, A.A. 1971. Estrone and estradiol levels in the ovarian venous blood from rats during the estrous cycle and pregnancy. Biol. Reprod. 5:297-307.

Spies, H.G., Frantz, R.C. and G.D. Niswender. 1972. Patterns of luteinizing hormone in serum following administration of stalk-median eminence extracts to rhesus monkeys. Proc. Soc. Exp. Biol. Med. 140:161-166.

Swelheim, T. 1965. The influence of a single high dose of oestradiol benzoate on the ICSH-content in the serum of gonadectomized male and female rats. Acta Endocr. 49:231-238.

Uchida, K., Kadowaki, M., Miyake, T. and K. Wakabayashi. 1972. Effects of exogenous progesterone on the ovarian progestin secretion and plasma LH and prolactin levels in cyclic rats. Endocr. Jap. 19:323-333.

Weick, R.F. and J.M. Davidson. 1970. Localization of the stimulatory feedback effect of estrogen on ovulation in the rat. Endocrinology 87:693-700.

Yamamoto, M., Diebel, N.B. and E.M. Bogdanove. 1970. Analysis of initial and delayed effects of orchidectomy and ovariectomy on pituitary and serum LH levels in adult and immature rats. Endocrinology 86:1102-1111.

Yen, S.S.C. and C.C. Tsai. 1971. The biphasic pattern in the feedback action of ethinyl estradiol on the release of pituitary FSH and LH. J. Clin. Endocr. 33:882-887.

Yen, S.S.C. and C.C. Tsai. 1972. Acute gonadotropin release induced by exogenous estradiol during the mid-follicular phase of the menstrual cycle. J. Clin. Endocr. 34:298-305.

Yen, S.S.C., VandenBerg, G., Rebar, R. and Y. Ehara. 1972. Variation of pituitary responsiveness to synthetic LRF during different phases of the menstrual cycle. J. Clin. Endocr. 35:931-934.