Using a classic paper by I. E. Lawton and N. B. Schwartz to consider the array of factors that control luteinizing hormone production

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Bauer-Dantoin AC, Hanke CJ. Using a classic paper by I.E. Lawton and N.B. Schwartz to consider the array of factors that control luteinizing hormone production. Adv Physiol Educ 31: 318–322, 2007; doi:10.1152/advan.00055.2007.—Two significant benefits derived from reading and discussing classic scientific papers in undergraduate biology courses are 1) providing students with the realistic perspective that science is an ongoing process (rather than a set of inarguable facts) and 2) deepening the students’ understanding of physiological processes. A classic paper that is useful in both of these regards is by I. E. Lawton and N. B. Schwartz (A circadian rhythm of luteinizing hormone secretion in ovariectomized rats. Am J Physiol 214: 213–217, 1968). The primary objective of the study is to determine whether tonic (pulsatile) secretion of luteinizing hormone (LH) from the pituitary gland exhibits a circadian rhythm. While this hypothesis seems relatively straightforward, its in vivo investigation necessitates an awareness of the multitude of factors, in addition to the circadian clock, that can influence plasma LH levels (and a consideration of how to control for these factors in the experimental design). Furthermore, discussion of the historical context in which the study was conducted (i.e., before the pulsatile nature of LH secretion had been discovered) provides students with the realistic perspective that science is not a set of facts but rather a systematic series of attempts by scientists to understand reality (a perspective that is difficult to convey using a traditional textbook alone). A review of the historical context in which the study was conducted, and a series of discovery learning questions are included to facilitate classroom discussions and to help deepen students’ understanding of the complex nature of pituitary hormone regulation.

incorporating discussions of the primary scientific literature into the curriculum of undergraduate biology courses is known to benefit student learning in a variety of ways. When students analyze and discuss research articles, they begin to learn actively and think critically as though they are participants in the scientific process (12, 22). They gain confidence in their ability to analyze scientific literature (10) and to interpret tables and graphs (22), and they gain familiarity with the techniques utilized by scientists to test hypotheses (8). Above all, reading primary scientific articles gives students the more accurate perspective that science is an ongoing process rather than a set of inarguable facts (5).

Yet another benefit of providing undergraduate students with the opportunity to explore primary scientific literature is the depth that it provides to their understanding of physiological processes. For example, exploration of a topic such as pituitary hormone synthesis and secretion can be noticeably enriched through introducing students to classic papers that detail scientists’ initial characterization of pituitary regulatory factors. Physiology textbooks typically present a limited view of this topic. While most pay homage to the influence of classic hypothalamic releasing factors and steroid feedback, few delve into the complex interplay between these regulatory mechanisms and others, such as clearance rates or the indirect input of the circadian clock. An analysis of classic papers that characterize these additional regulatory mechanisms is an excellent way to enrich students’ understanding of this topic.

A classic paper that is useful in this regard, and the one that is reviewed in this article, comes from the laboratory of one of the pioneers within the field of reproductive endocrinology, Dr. Neena B. Schwartz. The study (11), entitled “A circadian rhythm of luteinizing hormone secretion in ovariectomized rats,” was published in the American Journal of Physiology in 1968, and is useful for encouraging students to think about the complex interplay of regulatory mechanisms that govern pituitary luteinizing hormone (LH) secretion. The primary objective of the study was quite simple: to determine whether tonic (pulsatile) LH secretion exhibits a circadian rhythm or 24-h periodicity. However, in vivo investigation of this hypothesis necessitates an awareness of the multitude of factors, in addition to the circadian clock, that can influence plasma LH levels. These factors include, but are not limited to, gonadal steroids, adrenal steroids, hormonal clearance rates, and rates of LH synthesis. When considering the design of the study and interpreting the data generated, the authors (and readers) are by necessity drawn into a consideration of the extent to which these factors can be controlled or accounted for in order to draw conclusions about the circadian control of tonic LH secretion.

historical background

At the time of this study (11), very little was known about the nature of LH secretion at times other than the preovulatory LH surge. Great strides had been made, however, toward identifying the key factors (ovarian steroids, a hypothalamic releasing factor, and the circadian clock) that control the ovulation-inducing LH surge. Dr. Schwartz’s laboratory had just completed its seminal work in the characterization of the role that ovarian steroids play in regulating cyclic LH release (17, 20). Dr. Schwartz’s laboratory had determined, through carefully timed ovariectomies, that ovarian secretions between the morning of diestrus and the morning of proestrus are critical in order for the preovulatory LH surge to take place on late proestrus in female rats. When the ovaries of female rats are removed during this time frame, positive feedback levels of ovarian steroids are eliminated, and the LH surge is blocked. For this important work describing the critical role of ovarian steroids in the ovulation-inducing LH surge, Dr. Schwartz was invited to give the prestigious Gregory Pincus Memorial lecture at the 1968 Laurentian Hormone conference (18).
By this time, evidence had also emerged that the circadian clock played a key role in the generation of the LH surge. The LH surge was known to occur at the same time of day—a "critical period"—on the afternoon of proestrus in adult female rats, a finding that initially suggested a role for the circadian clock in the timing of the LH surge (3). A later study (25) demonstrated that LH surges induced in prepubertal female rats through treatment with pregnant mare’s serum gonadotropin (PMSG; which stimulates follicle development and gonadal steroid synthesis) also occur during the same critical period. Finally, when hypothalamic activity (and thus the signal from the circadian clock) is blocked in proestrus females through an injection of the barbiturate phenobarbital, the LH surge is delayed for a full 24 h rather than for the few hours of barbiturate sedation (2). These results, when considered along with Dr. Schwartz’s findings regarding the role of ovarian steroids in the generation of the LH surge, established that a daily, surge-inducing signal is emitted from the circadian clock and that this signal only results in the generation of an LH surge when positive feedback levels of ovarian steroids are present (i.e., on the afternoon of proestrus).

Although significant progress had been made in characterizing the LH surge at the time of the study of interest (11), LH secretion at other times of the female cycle was poorly understood. While a hypothalamic releasing factor for LH had been identified (14), the pulsatile nature of its secretion, as well as the pulsatile nature of LH secretion, would not be discovered for quite some time. A major limiting factor in the characterization of tonic (pulsatile) LH secretion was the sensitivity of the bioassay method utilized for measuring LH at the time. The ovarian ascorbic acid depletion assay was not nearly as sensitive as the present-day radioimmunoassay for detecting LH, and thus it required large volumes of plasma for conducting LH measurements. All studies were done using only terminal blood samples. Later development of sensitive radioimmunoassays allowed for frequent, repeated blood samples from the same animal. This eventually allowed the discovery of the pulsatile nature of LH secretion (19).

Even though the precise nature of tonic LH secretion was yet to be discovered at the time that Dr. Schwartz’s laboratory conducted the study of interest [LH secretion at times other than proestrus was thought to be “constant” rather than pulsatile (11)], the role of ovarian steroids in regulating the tonic secretion of both LH and follicle-stimulating hormone (FSH) was fully recognized. Dr. Schwartz’s laboratory (21) as well as others (16) had demonstrated that at times of the cycle other than proestrus, gonadal steroids exert a negative feedback action on the secretion of LH and FSH and that removal of the gonads resulted in an elevation of plasma LH and pituitary LH content. Given that the tonic (albeit elevated) secretion of LH was known to continue in the absence of gonadal steroids (but the cyclic preovulatory LH surge was not), the experimental model chosen for the present study was that of the ovariec- toneized female rat. Use of this model ensured that expression of the circadian signal involved in the generation of the preovulatory LH surge would be prohibited (due to the fact that this event only occurs in the presence of positive feedback levels of gonadal steroids). Thus, any circadian pattern of LH secretion observed in the present study could be attributed to the input of the circadian clock in the regulation of tonic, rather than cyclic, LH secretion.

Finally, little was known regarding the prepubertal regulation of LH secretion when the classic study was conducted. Thus, in addition to examining whether a circadian pattern of LH secretion exists in ovariec toneized adult female rats, the present study also investigated the nature of LH secretion in immature (prepubertal) female rats. This investigation in prepubertal rats was broader than that conducted in adults in that it included not only 1) a characterization of a possible circadian pattern of LH secretion before puberty but also 2) an examination of whether gonadal steroid feedback is operational prior to puberty as well as 3) an assessment of whether changes in pituitary responses to removal of negative feedback (i.e., gonadectomy) prior to puberty can be inhibited by pretreatment with gonadotropins [either PMSG or human chorionic gonadotropin (hCG)].

**Teaching Points**

Reading and understanding the paper of interest (11) requires prior knowledge of the hypothalamic-pituitary-gonadal axis as well as its regulation by gonadal steroid feedback (both negative and positive) and the circadian clock (see question 1 in Questions for Discovery Learning for encouraging students to review these fundamental aspects of reproductive physiology). Given the background knowledge required, discussion of this paper would be most appropriate in undergraduate biology courses in which a significant amount of time is spent reviewing these aspects of reproduction (courses such as human physiology, mammalian reproduction, and/or endocrinology). Textbooks that provide a useful overview of these topics include Vander’s Human Physiology: the Mechanisms of Body Function (26), Essential Reproduction (9), and Endocrinology (7). Since the typical undergraduate physiology student will find the paper of interest to be a challenging read, we do not recommend requiring students to read any additional classic papers that provide a historical context for the discussions.

A worthwhile topic to discuss prior to delving into the data from the study is a comparison of present-day methods for measuring LH [radioimmunoassays (RIAs) or ELISAs] versus the method used in the study of interest (question 2). At the time that the study was conducted, a RIA for the measurement of LH had not yet been developed, and thus researchers like Dr. Schwartz had to rely on the ovarian ascorbic acid bioassay for assessing LH levels in their experimental samples. This labor-intensive bioassay was conducted in immature female rats that had been made “pseudopregnant” by treatment with PMSG (which exhibits FSH-like activity) and hCG (which exhibits LH-like activity (15)). This hormonal regimen induces follicle growth, ovulation, and the formation of corpora lutea in prepubertal animals (thereby inducing a pseudopregnant state). Further treatment of pseudopregnant rats with samples containing LH (by an injection of either standards or unknowns into the tail vein of pseudopregnant rats) depletes the corpora lutea of ascorbic acid, and the extent of the depletion is correlated with the concentration of LH found in the sample. To this day, the physiological basis for LH-induced ovarian ascorbic acid depletion is not completely understood, but it is likely related to ascorbic acid’s roles in LH-induced progesterone and collagen synthesis in the corpus luteum (reviewed in Ref. 13).

Regardless of the explanation for LH-induced depletion of ovarian ascorbic acid, the bioassay was found to be a reliable method for assessing LH concentrations in experimental sam-
should consider why the authors chose to use the ovariectomized animal as their experimental model for determining if there exists a circadian rhythm in pituitary LH content (question 3). Students might also be predicted to predict how mean LH levels will change after ovariectomy and to compare their predictions with the findings presented in the study (question 4).

Next, students can be asked to examine the data shown in Fig. 1B, which illustrates pituitary LH content across a 24-h time period in mature rats ovariectomized at 71 days of age and in immature rats that had been ovariectomized prepubertally (at 32 days of age); these data were generated in experiment 2. Was a circadian pattern of pituitary LH content detected, either in prepubertal or postpubertal animals? Next, students can be asked to study the data generated in experiment 1 (Fig. 1A) and consider whether prior activation of the reproductive axis in prepubertal rats, by pretreatment with PMSG or hCG, had any influence on pituitary LH content, either with respect to absolute levels or a circadian change in LH content (question 5). Why did the authors choose to include these experimental groups (PMSG- or hCG-treated immature females) in the study? Finally, students might wish to consider what the variability in pituitary LH content from time point to time point reflects in Fig. 1, A and B (hint: remember the nature of “tonic” LH secretion; question 6). When group means were generated for the data (Fig. 1C), did the variability persist? Why or why not?

Table 1 illustrates plasma LH levels across a 24-h time period in ovariectomized females that received no hormone treatment prior to ovariectomy (experiment 2) or were injected either with PMSG or hCG prior to ovariectomy (experiment 1). Since the authors observed no difference in plasma LH levels between pre- and postpubertal ovariectomized females in experiment 2, data from the two groups were combined. Ask students to examine mean plasma LH levels observed across a 24-h time period in experiment 2. Did they exhibit a circadian pattern? If so, at what time of day were plasma LH levels elevated (question 7)? Was the same pattern of plasma LH levels detected across a 24-h time period in PMSG- or hCG-treated animals (experiment 1)?

Table 1. Plasma LH concentration over a 24-hr period in chronically ovariectomized rats

<table>
<thead>
<tr>
<th>LH, μg/2 ml Plasma</th>
<th>Experiment 1</th>
<th>Experiment 2 Combined Data†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autopsy Time*</td>
<td>PMSG-Treated‡</td>
<td>HCG-Treated‡</td>
</tr>
<tr>
<td>10 AM 1</td>
<td>.045 ± .010 (4)</td>
<td>.385 ± .178 (3)</td>
</tr>
<tr>
<td>2 PM</td>
<td>.035 ± .007 (2)</td>
<td>.142 ± .047 (4)</td>
</tr>
<tr>
<td>6 PM</td>
<td>.045 ± .006 (3)</td>
<td>.130 ± .048 (3)</td>
</tr>
<tr>
<td>10 PM</td>
<td>.079 ± .013 (4)</td>
<td>.123 ± .080 (3)</td>
</tr>
<tr>
<td>2 AM</td>
<td>.082 ± .069 (2)</td>
<td>.088 ± .030 (2)</td>
</tr>
<tr>
<td>6 AM</td>
<td>.317 ± .153 (4)</td>
<td>.287 ± .084 (2)</td>
</tr>
<tr>
<td>10 AM 2</td>
<td>.157 ± .127 (3)</td>
<td>.089 ± .012 (3)</td>
</tr>
</tbody>
</table>

Values are given as mean ± se of the mean. Figures in parentheses indicate no. of samples assayed. *5 months after ovariectomy. †50 IU PMSG at 24 days of age, and ovariec- tomized at 32 days of age. §25 IU HCG at 24 days of age, and ovariectomized at 32 days of age. $Untreated; one group ovariectomized at 32 days of age, the other at 71 days of age.

Shown is the original Table 1 from the classic paper of Lawton and Schwartz (11). LH, luteinizing hormone; PMSG, pregnant mare’s serum gonadotropin; HCG, human chorionic gonadotropin.

Dr. Schwartz made the following comment regarding the laborious nature of the bioassay (19):

I can still remember the grueling days of using the ovarian ascorbic acid bioassay to measure the amount of LH in the pituitaries of rats. After a full day’s autopsying of 50 prepubertal rats that had been primed with PMSG, injecting homogenized pituitary extracts into their tail veins and then removing the ovaries for ascorbic acid determinations, my technicians and I were exhausted. All we had to show for the hard day’s labor were six estimates of pituitary LH content and six 95% confidence limits! Unless you have done such laborious bioassays, you cannot know what it means to set up a 1,000 tube [RIA] for serum LH and have the LH in ng/ml of each sample within a day or so.

In the study of interest (11), the ovarian ascorbic acid depletion assay was used to assess whether plasma LH levels and pituitary LH content exhibit a circadian pattern in female rats. Figure 1 shows results from experiments assessing pituitary LH content across a 24-h period. All of the LH measurements illustrated in Fig. 1 were obtained from rats that had been ovariectomized 5 mo prior to LH measurements. Students
Another facet of the data worth mentioning is the fact that, in general, plasma LH levels were significantly lower in PMSG- and hCG-treated ovariectomized rats than in ovariectomized rats that received no hormone treatment (Table 1). The authors attributed this difference to a “failure of the LH secretory mechanism to respond fully following withdrawal of the steroidal negative feedback” (11). They hypothesized that pretreatment with gonadotropins (especially PMSG, which has FSH-like activity and would be most effective in stimulating follicle development) caused an elevation in ovarian steroid secretion, which then exerted lasting effects on the central pathways involved in the generation of tonic LH secretion. Despite the effects of gonadotropin pretreatment on pituitary responses to steroid withdrawal, it is important to note that a circadian rhythm of plasma LH levels still persisted in these animals.

After a thorough discussion of the data shown in Fig. 1 and Table 1, ask students to consider what the circadian pattern in plasma LH levels and lack thereof in pituitary LH content might reflect regarding a circadian pattern of LH biosynthesis (question 8). Might a circadian pattern of LH biosynthesis exist that is not revealed by the experimental design and methods utilized in the study? What modern-day techniques could be used to directly address this question? Also worth discussing when comparing data from experiments analyzing plasma LH versus pituitary LH content is the utility of statistics for determining when variability among groups likely represents a true physiological phenomenon versus random variation.

The authors mention the possibility that the 24-h pattern of plasma LH levels observed in the study might not reflect an influence of the circadian clock on tonic LH secretion but rather might indicate the existence of a circadian change in LH clearance rates or perhaps the influence of adrenal steroids (which exhibit a circadian pattern of secretion) on the pituitary. Challenge students to work in small groups to design experiments that would allow them to test these alternative hypotheses (question 9).

Before ending the discussion of the Lawton and Schwartz paper (11), ask students to restate the original hypothesis of the study (question 10). Then, reflect on the fact that in addition to generating data in support of this original hypothesis, the study also provides data that support or refute several other hypotheses (e.g., hypotheses regarding patterns of LH secretion prior to puberty or the impact of gonadotropin pretreatment on pituitary responses to steroid withdrawal). Ask students to list all of the hypotheses that they feel the experimental design addressed in the Lawton and Schwartz study.

Finally, several approaches can be taken to assess the impact on student learning of classroom discussions of primary scientific papers such as that of Lawton and Schwartz (25). Our approaches to assessing the impact of these discussions have included scoring students based on 1) the frequency and accuracy with which they answer questions posed by the instructor and/or other students during classroom discussions; 2) their performance on homework assignments that include providing written answers to the questions for discovery learning or providing written summaries of findings illustrated in Fig. 1 and Table 1 of the original paper; 3) their performance on exam questions that ask them to summarize the findings illustrated in Table 1 or Fig. 1 from the original paper; and/or 4) their performance on exam questions that ask them to employ higher-order thinking skills by using the knowledge they gained from studying the original paper (about a physiological principle or laboratory technique) to design a logical followup study or to critique a hypothesis or experimental design provided by the instructor. Finally, in addition to assessing the impact of studying classic scientific papers on student learning of physiology, it is also worthwhile to assess the impact of the exercise on the affective components of learning (in other words, did students like the exercise?). We have typically conducted this type of assessment by soliciting student opinions on classic paper discussions in their end of semester course evaluations. Analysis of anecdotal evidence gained from these assessments indicates that while students find classic paper discussions to be one of the more challenging aspects of the course, they enjoy the “active learning” aspects of the discussions and feel they are valuable for enhancing the depth of their understanding of basic principles of physiology and the historical context in which these principles were discovered.

Since the publication of the classic paper by Lawton and Schwartz (25), several other laboratories have confirmed that a circadian pattern of tonic LH secretion exists in rats (24) as well as in humans (1, 4, 6, 23). In human females, the circadian pattern of LH secretion manifests itself during puberty as an increase in LH pulse amplitude at night (1), whereas in adult human females, it manifests itself during the follicular phase of the menstrual cycle as a slowing of LH pulse frequency during sleep (4, 23). This circadian pattern of LH secretion, at least in rats, may reflect circadian changes in the production of the hypothalamic releasing factor gonadotropin-releasing hormone (GnRH), given that measurements of GnRH mRNA in adult male rats have indicated a circadian pattern of expression, with GnRH mRNA levels rising between 1000 and 1400 hours and remaining elevated until 2200 hours (6). The physiological significance of a circadian pattern of pituitary LH secretion for an individual’s reproductive status remains to be determined.

In conclusion, classroom discussion of this classic study by Lawton and Schwartz (11) provides students with a wonderful opportunity to consider the complex nature of the regulatory mechanisms governing pituitary LH secretion. Moreover, as students consider the historical context and technical limitations experienced by the researchers at the time the study was conducted, they gain an appreciation not only for the process of science but also for the persistence, dedication, and innovative spirit of the scientists whose discoveries created the foundation for our understanding of basic physiological processes. Thus, incorporating discussion of classic scientific articles into the curriculum of undergraduate biology courses is well worth the time and effort and provides a vital learning experience that cannot be replicated by simply considering the “set of facts” presented in traditional textbooks.

Questions for Discovery Learning

**Question 1.** Draw a diagram of the hypothalamic-pituitary-gonadal axis and include the major hormones produced at each level of the axis (GnRH, LH, FSH, and estrogen). How and when does gonadal steroid feedback operate within the axis? How and when is positive feedback operational? At what level of the axis is input from the circadian clock received?
Question 2. These experiments used a bioassay to determine LH concentrations in the pituitary and plasma of experimental animals. What specifically was measured in the bioassay, and why was it a reflection of LH levels in the experimental samples? Why did they have to measure this molecule instead of measuring LH directly? What other factors in the experimental blood sample could interfere with this assay?

Question 3. What impact do gonadal steroids have on expression of the circadian signal that allows for the preovulatory LH surge? Describe the rationale for studying the impact of the circadian clock on tonic LH secretion? Do gonadal steroids play a similar role in regulating circadian signals involved in the tonic secretion of LH?

Question 4. Describe the changes in pituitary LH content and plasma LH levels that occurred in immature rats after gonadectomy (comparisons between intact females and females gonadectomized prior to puberty is not included in Fig. 1 but rather is described in the text in RESULTS). Do gonadal steroids play a similar role in regulating circadian signals involved in the tonic secretion of LH?

Question 5. Were the responses of immature rats to gonadectomy (pituitary LH content and plasma LH levels) different when they were pretreated with gonadotropins (PMSG or hCG)? If so, explain what these differences might reflect.

Question 6. Given what is now known about the nature of LH secretion at times other than during the LH surge, what likely accounts for the fluctuation in individual pituitary LH content observed across time (Fig. 1; A and B)? When group means are generated for these time points, do these fluctuations come to represent a circadian rhythm in pituitary LH content (Fig. 1C)?

Question 7. Describe the circadian pattern of plasma LH levels observed in experiments 1 and 2 (Table 1). At what time of day were LH levels elevated?

Question 8. Pituitary LH content remained constant over a 24-h period in both immature and mature ovariectomized rats, whereas plasma LH levels exhibited a circadian rhythm. What might this difference reflect regarding circadian changes in LH biosynthesis?

Question 9. The authors of the paper mention in the discussion of their results that a 24-h rhythm of plasma LH levels was observed in the study. Further experiments were necessary to determine whether this pattern of secretion truly represented an input of the circadian clock or whether it reflected a 24-h rhythm of other factor(s) influencing plasma LH levels (e.g., LH clearance rates or influence of adrenal steroids). Design two experiments to determine whether the circadian pattern of plasma LH levels observed in the Lawton and Schwartz study reflects 1) a 24-h rhythm of LH clearance rates or 2) a circadian pattern in the influence of adrenal steroids on pituitary LH secretion.

Question 10. Generate hypothesis statement(s) for this study.

REFERENCES