Luteinizing Hormone: Action on the Graafian Follicle in vitro

Abstract. *Graafian follicles, dissected intact from the rabbit ovary and incubated for 40 minutes with luteinizing hormone, developed into corpora lutea when autotransplanted under the kidney capsule. Follicles incubated with follicle-stimulating hormone or without hormone degenerated and failed to luteinize. Direct exposure of the mammalian follicle to luteinizing hormone initiates luteinization and formation of the corpus luteum.*

Luteinizing hormone (LH), a gonadotropin secreted by the anterior pituitary gland, causes the Graafian follicle of the mammalian ovary to ovulate, releasing the egg (or ovum) which can then be fertilized. Another important action of this hormone is to initiate formation of the corpus luteum, the structure that develops from granulosa cells within the follicle and that has as a principal function the secretion of progesterone (*1*). Although it has generally been assumed that LH must elicit luteinization through some direct, but unknown, effect on the follicle, this hypothesis has not received experimental verification. Alternatively, LH might initiate luteinization of granulosa cells indirectly, through an action upon nonfollicular tissue (for example, interstitial tissue) which then secretes steroids or other substances in sufficient concentrations to effect changes in the follicle that result in luteinization.

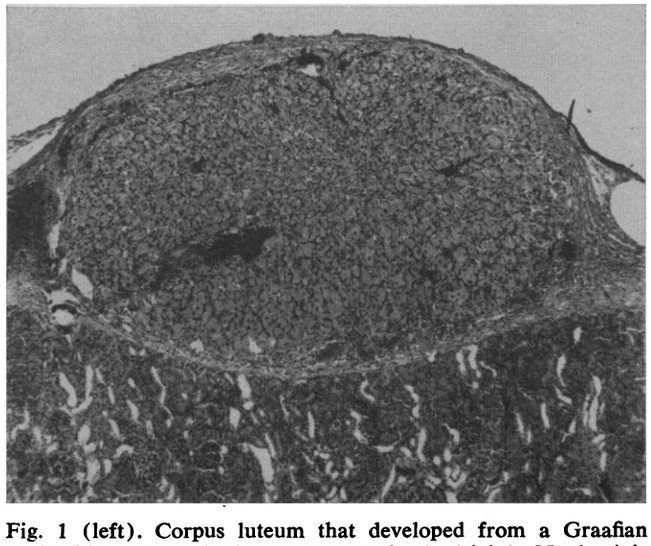
My experiments demonstrate unequivocally the ability of LH to act directly on the Graafian follicle to initiate the physiological events culminating in luteinization and formation of the corpus luteum.

One can autotransplant mature follicles removed from ovaries stimulated by endogenous LH; such follicles will subsequently develop into corpora lutea that secrete progesterone (*2*). This procedure makes it possible to investigate the luteinizing activity of hormones in vitro, since follicles exposed to various hormones and conditions in vitro can be transplanted under the kidney capsule of the donor and observed at a later date to determine whether luteinization has occurred.

I used mature Dutch Belted rabbits in estrus. Large Graafian follicles protruding from the ovarian surface were removed and placed in 0.9 percent saline, and the adhering interstitial tissue was then dissected away exposing the thecal tissue. Care was taken to avoid rupture of the follicles; those ruptured during dissection were discarded. From each of six rabbits, six follicles were dissected and placed into two flasks (three per flask), one containing hormone (NIH-LH-S11; 50, 25, 10, or 1 μg) in 2.5 ml of Krebs-Ringer bicarbonate buffer with glucose (1 mg/ml), the other containing only buffer solution and glucose. The flasks were flushed with a mixture of 95 percent O_2 and 5 percent CO_2, stoppered, and incubated in a Dubnoff metabolic shaker for 40 minutes at 37°C. Follicles were removed from respective media and briefly rinsed in 0.9 percent saline. I then aspirated the follicles into a polyethylene tube filled with saline and "injected" the follicles under the kidney capsules, placing hormone-exposed and control follicles in opposite kidneys. Seven days later the animals were laparotomized, and in five animals I found vascular corpora lutea in the kidneys in which LH-exposed follicles had been placed. Follicles incubated with 1 μg of LH had not luteinized. Control follicles in opposite kidneys had degenerated without luteinizing and were barely visible on the kidney surface.

Follicle-stimulating hormone (FSH) promotes the growth of follicles, and highly purified preparations have relatively insignificant LH activity (*1*, *3*). To confirm the lack of "luteinizing" activity and to investigate in part the specificity of the in vitro response to LH observed in these experiments, I tested the ability of FSH (NIH-FSH-S6) to cause luteinization. Follicles in one flask were incubated with FSH (50 or 25 μg) and those in a second flask with equal quantities of LH. When the three animals used in this study were laparotomized, corpora lutea were flourishing in each animal, but in only one kidney—the one to which LH-exposed follicles had been transplanted. Follicles incubated with FSH were atretic. Figures 1 and 2 show appearances of transplanted tissues incubated 7 days previously with 25 μg of LH and 25 μg of FSH, respectively.

These experiments establish, beyond doubt, that luteinization of the Graafian follicle can be initiated in vitro in response to a direct action of LH. Under these conditions it is apparent that



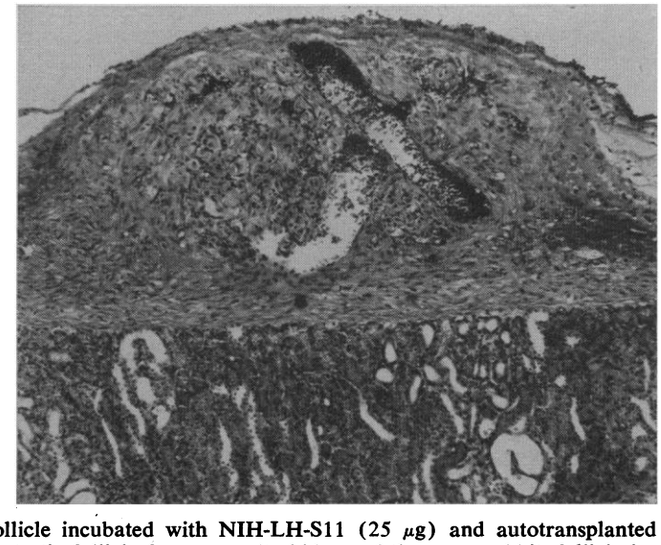


Fig. 1 (left). Corpus luteum that developed from a Graafian follicle incubated with NIH-LH-S11 (25 μg) and autotransplanted under kidney capsule (× 42). Fig. 2. (right). Nonluteinized atretic follicle from opposite kidney of the same rabbit; follicle incubated with NIH-FSH-S6 (25 μg) (× 69).

nonfollicular ovarian tissues or secretions thereof are not essential for LH to have an initial effect on the follicle. The similarity between the effect of LH in vitro and in vivo is illustrated by the normal histologic appearance of corpora lutea developing under the kidney capsule.

The site and physiologic mechanism of action of LH on the Graafian follicle are obscure. The hormone may act on the peripheral (thecal) tissues of the follicle or it may enter the follicle to stimulate the granulosa cells directly. An action of LH on the follicle wall might account, at least in part, for the propensity of LH-stimulated follicles to vascularize when autotransplanted. The importance of capillary penetration in the early stages of luteinization is unknown, but it is apparent from results of this study that absence of a blood supply does not preclude the initial action of LH on the follicle.

The rabbit is an especially useful species in which to investigate luteinization, since the follicles remain in readiness to ovulate and luteinize. However, the procedure described here can be adapted to investigations of luteinization and corpus luteum formation in other species, including those that ovulate spontaneously.

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References and Notes

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Neuronal Controls of a Behavioral Response Mediated by the Abdominal Ganglion of Aplysia

Abstract. *Tactile stimulation of the siphon and mantle shelf in* Aplysia *causes a characteristic withdrawal response of the external organs of the mantle cavity. A similar response also occurs spontaneously. Both responses are mediated by the abdominal ganglion and therefore provide an opportunity for correlating cellular functioning and behavior in a relatively simple and well-studied neuronal system. The withdrawal responses are controlled by five identified motor cells which receive two types of synaptic inputs. One set of excitatory connections, activated by tactile stimulation of the siphon and mantle shelf, mediates the defensive withdrawal reflex. A second set of connections is activated by a spontaneous burst of activity in a group of closely coupled interneurons which are excitatory to some of the motor cells and inhibitory to the others. This second set of connections mediates the spontaneous withdrawal response. These two inputs can therefore switch the same population of motor cells from a simple reflex to a more complex, internally organized response.*

The cellular neurophysiological analysis of behavior and learning requires that neurons mediating a particular behavioral sequence be identified and their interconnections be specified. It is difficult to meet this requirement in the vertebrate central nervous system, but it is becoming increasingly more feasible in certain invertebrates. The nervous systems of higher invertebrates have the advantage of containing relatively few cells, many of which are directly accessible for investigation. For example, in arthropods, one can record from individual identified sensory, interneuronal, and motor elements and describe their functional properties (*1*). One of the striking findings to emerge from this work is that single central neurons (command elements) can initiate complex behavioral sequences (*2*). This fortunate circumstance, which also occurs in mollusks (*3, 4*), reduces the task of a behavioral analysis. However, the advantages offered by arthropods are somewhat counterbalanced by the difficulty in recording synaptic potentials from their central neurons. As a result, it has generally not been possible to analyze the mechanisms involved in the synaptic transformations of neural information within the arthropod central nervous system. This limitation can be overcome in certain mollusks, particularly in marine gastropods, whose nervous system contains many large cells which are easy to impale with microelectrodes and from which it is possible to continuously record synaptic potentials for hours (*5, 6*) and even days (*7*). The molluscan preparation in which synaptic physiology has been studied in particular detail is the abdominal ganglion of *Aplysia*. This ganglion contains about 1800 cells of which perhaps 1000 have been categorized as members of different functional clusters and 30 have been identified as unique individuals that can be recognized reliably from preparation to preparation (*6*). A number of the central connections between different identified cells have been mapped and several interneurons common to the identified cells have been either identified or inferred (*8*).

Despite a substantial background of neurophysiological information on *Aplysia* (for reviews, see *6, 9*), relatively few studies have examined the behavior of this animal (*10, 11*) and little is known about the control of behavior by the nervous system. Our report here is part of a larger study designed to investigate the role of the abdominal ganglion in the behavior of *Aplysia* (*4, 6, 11*) and to delineate neural systems that control behavioral reflexes which might prove modifiable.

A striking and reliable response in the intact *Aplysia* is a defensive withdrawal reflex of the external organs of the mantle cavity. When the siphon or the mantle shelf is touched, the siphon, the mantle shelf, and the gill contract and withdraw into the mantle cavity (Fig. 1, A1 and A2). This response has a short latency but occasionally a second, long latency response occurs. The excitatory receptive field of this reflex is centered on the siphon and on the margin of the mantle shelf (Fig. 1, A3). A similar but not quite identical behavioral response also occurs spontaneously, sometimes recurring at intervals of 40 to 300 seconds. Both the evoked and the spontaneous withdrawal responses persist after the connectives leading to the rest of the central nervous system are cut, and both are abolished by removal of the abdominal ganglion, an indication that these responses are controlled by cells in this ganglion.

To study the cellular basis of the evoked and spontaneous withdrawal responses we used a semi-intact preparation (Fig. 1B). The animal was prepared by slitting it open and removing the digestive system. The parapodia were cut back and pinned, and the