Discovery of the luteinizing hormone of the anterior pituitary gland

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This essay looks at the historical significance of an APS classic paper that is freely available online:


It is now widely known that normal function of the ovaries and testes is regulated by two pituitary gonadotropic hormones, the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH), which provide the one-two punch that drives gamete and gonadal hormone production. In females FSH stimulates follicular growth and maturation while LH triggers ovulation and luteinization. However, in the years leading up to publication of “The gonad stimulating and the luteinizing hormones of the anterior lobe of the hypophysis [sic]” (4), the question of how the pituitary might control ovarian function was not at all well understood. Only five years earlier P. E. Smith (5, 6) provided definitive proof that the pituitary plays a crucial role, when he showed that the ovarian atrophy that follows removal of the hypophysis was reversed within just a few days by pituitary implants. In this classic paper, Fevold, Hisaw, and Leonard isolated from acetone-dried hog anterior lobes two protein hormones with distinct actions on the rat ovary. One, FSH, stimulated ovarian follicular development and caused precocious sexual maturity in immature rats. The other, LH, had no effect when given alone but caused follicles that were stimulated by FSH to luteinize. Given together, the two accounted for all of the ovarian effects of whole pituitary extracts.

This research was led by Frederick Hisaw (Fig. 1), then Professor of Zoology at the University of Wisconsin and later at Harvard. Not only is Hisaw known for his own monumental contributions to reproductive biology, but many of the 20th century giants of endocrine research trace their intellectual lineage to him. Fevold was the chemist. Leonard was the graduate student, and this work was part of his thesis project.

The idea that two hormones might be required for normal ovarian function originated earlier with Zondek and Ascheim who showed that extracts of the urine of postmenopausal women (7), which is rich in FSH, produced a predominantly follicle-stimulating effect, whereas extracts of urine of pregnant women had strong luteinising activity (1). The existence of a separate luteinizing hormone ran counter to the ideas of H. M. Evans, who was already established as a major investigator in endocrinology. Evans contended that luteinization resulted from interaction of a single ovarian stimulating hormone with the growth hormone. This view was based on his earlier findings that the extraordinary growth of rats produced by daily treatment with whole pituitary extracts was accompanied by excessive growth and luteinization of the ovaries (3). The sometimes bitter rivalry between the Hisaw and Evans laboratories continued for the next two decades, fueled by related controversies relating to the gonadotropins, even after the Evans group later isolated an even purer preparation of LH (2).

Although the hormones prepared by Fevold, Hisaw, and Leonard cannot be considered as anything but crude by today’s standards, their isolation nevertheless was a major technical, as well as theoretical, achievement. FSH and LH are both glycoproteins comprised of two noncovalently linked glycoprotein subunits that are products of separate but closely related genes. In fact, they both contain the same α-subunit coupled to distinct β-subunits that confer biological specificity. Their chemical and physical properties are thus quite similar. In addition, their abundance in the dried anterior lobe powder is less than one part per thousand. Chromatographic and electrophoretic methods that would later become the standard techniques of protein hormone purification were not yet invented, and removal of solvents depended on evaporation in a drying oven rather than lyophilization.

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Fig. 1. Frederick Hisaw. Courtesy of the Endocrine Society.
Though protein chemistry was in a primitive state, it was well known that different proteins have relatively unique solubility properties in water and other solvents. The challenge to the chemist was to find the appropriate mixtures of solvents in which particular proteins would selectively be dissolved or precipitated as the pH and ionic strength were manipulated. Neither activity could be extracted from dried anterior lobe powder with any pure neutral solvent, and significant deviation from neutrality was not well tolerated. Follicle-stimulating activity was quickly lost in alkaline solutions, while luteinizing activity could not withstand even mildly acidic conditions. However, both activities could be extracted quantitatively in 50% aqueous pyridine “without injury to either.”

The dried pyridine extract was repeatedly extracted with distilled water to separate the two activities, which in retrospect proved to be remarkably simple. “The gonad stimulator is taken up in the water while most of the luteinizing hormone remains in the water insoluble fraction.” Purification of FSH included alcohol precipitation from the aqueous solution followed by resolubilization and acidification to remove an acid-insoluble precipitate. The luteinizing hormone in the water-insoluble fraction of the pyridine extract was dissolved in dilute alkali. After centrifugation to remove remaining insoluble material, the LH was precipitated with alcohol, dried, and redissolved in slightly alkalinized water.

Isolation of active hormone from the mixture of unknown proteins present in the dried anterior pituitary powder is necessarily guided by bioassay. The physiological activities of the various fractions had to be tested at every step along the way. Judging from the rat numbers listed in the data tables and assuming that 5 or 6 rats were used in each experiment, at least 100 bioassays had to be performed. “The chief criteria used were the ability of the extracts to produce follicular and lutein development in the ovaries and opening of the vaginal orifice” in 20- to 25-day-old immature rats. A dose equivalent to 20 mg of the whole dried pituitary extract injected daily for five consecutive days produced vaginal opening in all animals tested and a greater than 10-fold increase in ovarian weight. Histological examination revealed massive luteinization and the presence of numerous follicles. When the equivalent dose of the follicle-stimulating extract was tested in the same protocol, the ovaries increased two- to threefold in weight due to follicular development, but contained no corpora lutea. An equivalent doses of LH produced no change in ovarian weight or vaginal opening. Combining the two fractions in the same protocol gave mixed results, but when FSH was given for 2 days followed by 2 days of LH, the ovaries became massively luteinized.

REFERENCES