Teaching aldosterone regulation and basic scientific principles using a classic paper by Dr. James O. Davis and colleagues

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Hanke, Craig J., and Angela C. Bauer-Dantoin. Teaching aldosterone regulation and basic scientific principles using a classic paper by Dr. James O. Davis and colleagues. Adv Physiol Educ 30: 141–144, 2006; doi:10.1152/advan.00073.2006.—Classroom discussion of scientific articles can be an effective means of teaching scientific principles and methodology to both undergraduate and graduate science students. The availability of classic papers from the American Physiological Society Legacy Project has made it possible to access articles dating back to the early portions of the 20th century. In this article, we discuss a classic paper from the laboratory of Dr. James O. Davis on the regulation of aldosterone synthesis from the adrenal zona glomerulosa cell. Dr. Davis has conducted much of the seminal research investigating the renin-angiotensin system and the regulation of aldosterone release by angiotensin II. In addition to a characterization of the effects of ACTH on aldosterone regulation, this study is useful for discussing the basic principles of negative feedback pathways of the hypothalamic-pituitary axis. This study also provides examples of early bioassay techniques for the detection of angiotensin II and of the importance of quantitative measurements when investigating physiological responses. Three figures and one table are reproduced from the original article along with a series of discussion questions designed to facilitate discovery learning.

angiotensin; adrenal; adrenocorticotropin; discovery learning

As the mass of biological information continues to grow, the challenge of teaching effectively requires that we continuously evaluate our teaching strategies. Why teach with classic scientific papers? Can we afford the time? As a method of information transmission, discussing a paper is certainly less efficient than lecturing. The data are no longer groundbreaking, and the results of many of the studies can be found clearly presented (with high quality art and/or web animations) in textbooks and cyberspace. The scientists who have performed the experiments are not celebrities (at least as far as most of our students are concerned). Yet, there is something vital in this process. There is the opportunity for our students to connect with the process of science itself, to see the unraveling of a mystery and to examine the methods and clues used to solve a scientific puzzle. Our students are proficient at memorizing and applying the facts, theories, and laws of science, but they lack familiarity with how these principles are tested and verified. How do we explain the problem solving, troubleshooting, and ingenuity that are integral to the scientific process? Students need an opportunity to think as scientists, evaluating data and choosing for themselves the appropriate interpretations. They need an opportunity to evaluate a scientific study and recognize, first hand, scientific advancements being made. This is the advantage of teaching using a classic scientific paper; the history and creativity of the scientific process are really the lessons.

Dr. James O. Davis is internationally recognized for his contributions to our understanding of the renin-angiotensin system and the fundamental role of ANG II in the regulation of aldosterone. Dr. Davis’ career has spanned more than 50 years of research into the hypertensive effects of ANG II (still called “hypertensin II” in some of his early work). His accomplishments include the description of the antihypertensive effects of ANG II receptor blockade and angiotensin-converting enzyme inhibition, strategies now commonly used in the treatment of hypertension. In writing this teaching guide to the classic paper by Dr. Davis and his collaborators, Peter F. Binnion, Torrey C. Brown, and Michael J. Olichney, we hope to provide a background and series of questions useful to both undergraduate and graduate students studying endocrine pathways. The article “Mechanisms regulating aldosterone secretion during sodium depletion” (2) has made important contributions to the field of adrenal steroid regulation in its description of multiple pathways controlling aldosterone synthesis. In this sense, it is useful to the graduate endocrinology student. For the undergraduate student, it is useful to illustrate basic scientific principles, such as the principle of negative feedback regulation, the quantitative nature of physiological studies, and the difference between correlation and causation. For all readers, it is a classic example of the often unexpected complexity of endocrine control systems.

Aldosterone Regulation

The focus of the study, the adrenal cortex, is a complex endocrine tissue subdivided into three layers of steroid-producing cells. The outermost layer of the cortex is composed of a thin layer of zona glomerulosa (ZG) cells, which synthesize the mineralocorticoid hormone aldosterone. Immediately below the ZG cell layer are the zona fasciculata (ZF) cells, which synthesize the glucocorticoid hormones cortisol and corticosterone. The innermost layer of the adrenal cortex is composed of zona reticularis cells, which synthesize small amounts of glucocorticoids and adrenal androgens.

Based on our current understanding, the regulation of ZG cell aldosterone synthesis is complex and includes a variety of stimulatory and inhibitory factors. The primary stimulator of aldosterone synthesis is the renin-angiotensin system, which, as demonstrated by Binnion et al. (2), is activated in response to changes in blood sodium concentrations. Although not addressed in the study by Binnion et al., the renin-angiotensin system is also activated in response to changes in blood pressure. Decreases in the sodium concentration (as induced by a low-sodium diet and/or treatment with the diuretic mercury-
drin in Ref. 2) and blood pressure are detected by the macula densa and juxtaglomerular cells of the nephron, respectively, and result in the secretion of the enzyme renin by juxtaglomerular cells. In the bloodstream, renin cleaves the plasma protein angiotensinogen, converting it to ANG I. As ANG I circulates through the lungs, it is cleaved by angiotensin-converting enzyme to yield ANG II, which then acts directly on ZG cells to stimulate the synthesis of aldosterone.

Other peptides known to play less significant roles in the stimulation of aldosterone synthesis include the pituitary hormones ACTH (as described by Binnion et al.) and vasopressin (14). Nonpeptide stimulators of aldosterone secretion include potassium ions, which act to directly depolarize the plasma membrane of ZG cells, and eicosanoids, such as prostaglandin E2 (1). Finally, there exist inhibitors of aldosterone synthesis, including dopamine, atrial natriuretic peptide, and nitric oxide (7,8). It has been suggested that complex regulatory pathways exist for aldosterone synthesis and secretion because the mineralocorticoid has multiple functions within the body (direct regulation of both sodium and potassium ion concentrations as well as indirect regulation of fluid retention and blood pressure).

Aldosterone synthesis occurs through a multistep enzymatic cascade beginning with the conversion of cholesterol to pregnenolone. Pregnenolone is then enzymatically converted to progesterone, deoxycorticosterone, corticosterone, and, finally, aldosterone. Thus, the steroid hormones produced by a given cell are determined by the specific set of steroidogenic enzymes expressed by the cell. In the adrenal gland, only the ZG cell expresses the isoform of the aldosterone synthase enzyme necessary for the final conversion of corticosterone to aldosterone. The ZF cell expresses 11β-hydroxylase rather than aldosterone synthase and synthesizes corticosterone and cortisol, rather than aldosterone, using the early portions of the same enzymatic pathway (9).

Aldosterone synthesis is regulated by ACTH and ANG II at multiple points along the enzymatic cascade. The conversion of cholesterol to pregnenolone requires the transport of cholesterol from the outer to inner mitochondrial membranes, a process dependent on the production of steroidogenic acute-regulating (StAR) protein. Modern evidence indicates that ACTH and ANG II stimulate aldosterone synthesis by increasing the production of ZG cell StAR protein (13). ANG II also participates in the later portions of the steroidogenic pathway in ZG cells by stimulating the expression of aldosterone synthase (12).

Elevations in circulating aldosterone concentrations result in sodium reabsorption in the kidney and colon and increased fluid retention by the body. The majority of aldosterone-stimulated sodium reabsorption takes place in the kidney, where aldosterone activates receptors that are located in high concentrations in the distal tubule and cortical collecting ducts of the nephron. The principal cells of the cortical collecting duct respond to aldosterone by increasing sodium reabsorption and potassium excretion and through increased activity of epithelial sodium channels as well as increased expression and activation of sodium-potassium ATPase and potassium ATPase transporters (15).

Given that ACTH regulates portions of the aldosterone, cortisol, and corticosterone synthesis pathways, secretion of aldosterone can at times change in parallel with the secretion of cortisol and corticosterone. Unlike its supportive role in regulating aldosterone secretion, ACTH is the primary regulator of cortisol and corticosterone secretion from the adrenal glands, stimulating both cholesterol transport and enzymes involved in the final conversion steps (12). Within the context of the hypothalamic-pituitary axis, ACTH secretion is stimulated by corticotropin-releasing hormone (CRH) release from the hypothalamus. At the level of ZF and ZR cells, ACTH stimulates glucocorticoid synthesis and secretion through activation of its transmembrane receptors. The hypothalamic-pituitary axis of this pathway demonstrates both long- and short-loop feedback inhibition, with cortisol secretion inhibiting the release of both CRH and ACTH, and ACTH inhibiting the release of CRH from hypothalamic neurons.

**Historical Background**

In an effort to better understand any historical scientific study, it is helpful to be aware of the state of the field at the time that the study was conducted. By the late 1930s, nearly 30 years before the publication of “Mechanisms regulating aldosterone secretion during sodium depletion,” most of the glucocorticoids produced by the adrenal cortex had been isolated and characterized. However, it wasn’t until 1948, approximately 10 years later, that Deane et al. (4) confirmed that a mineralocorticoid (then termed “electrocortin”) was also secreted from the adrenal cortex and that its secretion was triggered by a low-sodium diet or elevated potassium. By 1953, the British researchers Simpson and Tait (11) had developed a highly sensitive bioassay that allowed for the isolation and crystallization of aldosterone from bovine adrenal glands. The availability of the bioassay then allowed for significant progress to be made in the characterization of aldosterone control systems, and, in 1961 (3 years before the publication of the study of Binnion et al.), Mulrow and Ganong (10) confirmed that ANG II was a major stimulus for aldosterone secretion. A subsequent study by Ganong and Mulrow (6) suggested that ACTH, already known to be the major stimulus for cortisol and corticosterone synthesis and secretion, could also stimulate the secretion of aldosterone.

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for glucocorticoid secretion, might also contribute to the production of aldosterone. The purpose of the study of interest by Binnion et al. (2) was to further elucidate the role of the hypothalamic-pituitary axis in the regulation of aldosterone.

Teaching Points

We chose three figures and one table from the study (2) for a detailed review. In Fig. 1, the authors examined the effect of sodium depletion on aldosterone and corticosterone synthesis. The results indicated that sodium depletion was correlated with increased aldosterone synthesis. Next, Binnion et al. determined the effect of sodium depletion on renin activity (measured as vasoconstriction due to ANG II) using three different methods (Table 1). A discussion of the data shown in Table 1 provides an opportunity to consider the importance of internal validation within a scientific study. The students should consider why ANG II measurements were even needed for the study, and a discussion of the differences between correlation and causation (so often confused in popular science) might follow. The students might also consider why the authors used three different techniques to measure changes in renin activity.

The principle of a real “bioassay” technique, using the vasoconstriction of live tissue to determine the presence of a compound like ANG II, is not something with which many of our students are familiar. Finally, the students may be asked to consider why sodium is excreted if aldosterone is being synthesized. The mechanisms of the kidney tubule and the effects of a diuretic/natriuretic such as mercuhydrin could then be discussed.

Having determined that sodium depletion results in increased renin activity, and being well aware of the effect of ANG II on aldosterone synthesis, Binnion et al. next examined the regulation of corticosterone by ANG II. Although ACTH was known to be the major stimulus for corticosterone secretion at that time, a previous study (3) had indicated that ANG II increased corticosterone synthesis as well as aldosterone synthesis. Thus, the authors examined the regulation of corticosterone (and aldosterone) secretion in the absence of ACTH, i.e., in hypophysectomized dogs. Figure 2 presents corticosterone and aldosterone synthesis during sodium depletion in these animals. The students will quickly notice that corticosterone secretion increased in response to sodium depletion,

<table>
<thead>
<tr>
<th>Total Negative Sodium Balance for Sodium-Depleted Dogs, mEq</th>
<th>Method of Warzynski et al.</th>
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<tbody>
<tr>
<td>Bumpus Method Normal dogs</td>
<td>Helmer Method Normal dogs</td>
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<tr>
<td>Normal dogs</td>
<td>Sodium depleted</td>
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<tr>
<td>Mean</td>
<td>112</td>
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<tr>
<td>SEM</td>
<td>±1.2</td>
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<td>N</td>
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Values for both normal and sodium-depleted dogs, in all three methods, are expressed as ng angiotensin-like activity formed per ml plasma. *Sodium depletion was effected with a low-Na diet plus 2 ml of mercuhydrin im daily.

Shown is original Table 1 from Binnion et al. (2). [Reprinted with permission from the American Physiological Society.]
indicating that ANG II regulates corticosterone. The key to understanding the physiological significance of this effect, however, is a careful examination of the scale of the y-axis. Although corticosterone secretion was increased in response to sodium depletion in hypophysectomized dogs, this change is minute relative to the concentrations of corticosterone in intact dogs. The baseline synthesis of corticosterone decreased from ~0.7 to 0.07 µg/min in intact versus hypophysectomized dogs. The removal of the pituitary resulted in a loss of ACTH-stimulated corticosterone (synthesized by the ZF cell) and the uncovering of a much smaller amount of corticosterone inadvertently released during the production of aldosterone by the ZG cell. Careful consideration of the scale changes the interpretation of Fig. 2. What appears to be a notable increase in corticosterone synthesis in response to ANG II may be physiologically insignificant.

Finally, in Fig. 3, Binnion et al. directly examined the effect of hypophysectomy on aldosterone synthesis. The amount of aldosterone synthesis stimulated by sodium depletion was reduced by hypophysectomy. The students should consider the implication of hypophysectomy decreasing aldosterone synthesis. Because hypophysectomy does not alter renin-angiotensin system activity, the decrease in aldosterone synthesis is likely due to a loss of ACTH. In fact, we now know (thanks to the efforts of Binnion et al.) that chronic ACTH stimulation of the ZG cell is critical to maintaining adequate levels of aldosterone synthesis. Recent data have indicated that ACTH stimulates the production of cholesterol transport proteins required for the maximal delivery of cholesterol to the steroidogenic enzymes of the ZG cell.

Forty years later, the complexity of aldosterone regulatory pathways continues to be unraveled. We now know that in addition to the classic regulators of aldosterone synthesis, there exists a network of paracrine regulators of aldosterone synthesis within the adrenal gland itself. New evidence has suggested that the adrenal gland contains all of the components of the renin-angiotensin system and produces ANG II itself.

Although additional aldosterone regulatory pathways continue to be explored, the contributions of Binnion et al. continue to be central to our understanding of the regulation of ZG function. Thus, recognition and careful examination of their discoveries will not only provide students with a solid foundation in their understanding of this complex endocrine tissue but will also foster an understanding and appreciation for the process that leads to such important scientific discoveries.

Questions for Discovery Learning

**Question 1.** Diagram the renin-angiotensin-aldosterone pathway. How is this pathway an example of feedback inhibition? Based on the results of Binnion et al., what other hormone appears to play a role in the control of aldosterone synthesis?

**Question 2.** What is the effect of sodium depletion on aldosterone secretion as shown in Fig. 1 of the study? Which of the control systems is affected by sodium depletion?

**Question 3.** Consider the results shown in Table 1. How did the authors measure ANG II production? Why were the different methods used? How would we carry out these experiments differently today?

**Question 4.** What is the effect of aldosterone on the cells of the nephron tubule? Why was sodium excreted if aldosterone increased?

**Question 5.** What is a hypophysectomy and what would be the effects of this procedure on the two aldosterone control pathways discussed in this study? What other side effects would you expect in a hypophysectomized animal?

**Question 6.** Compare the aldosterone and corticosterone responses in Figs. 1 and 3. Does hypophysectomy alter secretion of either steroid? What cell type is the primary source of corticosterone and what happens to this cell after hypophysectomy?

**Question 7.** The primary source of corticosterone in the intact dog is the ZF cell. However, the renin-angiotensin system stimulates a small amount of corticosterone release from the ZG cell. Keeping in mind that ZG cells are a source of both steroid hormones, can you explain the effects of hypophysectomy on changes in aldosterone and corticosterone synthesis? Why does sodium depletion appear to stimulate corticosterone production only after hypophysectomy?

REFERENCES


