Teaching glucocorticoid negative feedback and adrenocortical regulation using a classic paper by Dr. Dwight Ingle

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Raff, Hershel. Teaching glucocorticoid negative feedback and adrenocortical regulation using a classic paper by Dr. Dwight Ingle. Adv Physiol Educ 29: 141–143, 2005; doi:10.1152/advan.00020.2005.—The American Physiological Society (APS) Legacy Project and its accompanying Essays on APS Classic Papers have allowed the scientific community on-line access to the entire collection of APS publications since their inception in 1898 (http://www.the-aps.org/publications/legacy/ and http://www.the-aps.org/publications/classicals/). The availability of the classic physiological studies provides a unique teaching opportunity. The classic paper of Dr. Dwight Ingle represents just such a study. Dr. Ingle demonstrated that, using only purified extracts of the pituitary (ACTH) and adrenal cortex (corticosterone) and hypophysectomized rats, he could establish several of the basic principles of the control of adrenal function and glucocorticoid negative feedback that are now standard teaching material in endocrinology. An annotated figure from Dr. Ingle’s paper is provided, which, when assigned to undergraduate or graduate students, will allow discovery learning. Furthermore, the brilliance and imagination of the physiologists of the last century are highlighted, which allows an appreciation of the seminal work of our predecessors.

THE CONTROL OF CORTICOSTEROID production from the adrenal gland has been of interest for quite some time. The devastating morbidity from the absence of circulating endogenous corticosteroids (cortisol in humans or corticosterone in rodents) has been known for more than a century. This is now called adrenal insufficiency and is characterized by weakness, fatigue, anorexia, and weight loss (2). It has also been known since early in the 20th century that excess cortisol had serious detrimental consequences leading to Cushing syndrome (1), which is characterized by dramatic weight gain leading to obesity (particularly abdominal), insulin resistance, osteoporosis, hypertension, depression, moon face, and buffalo hump (dorsocervical fat) (2). It is also now appreciated that mild increases in circulating glucocorticoids (i.e., subclinical Cushing syndrome) have negative effects on health (9). A burning question for at least the first half of the 20th century was “What controls the release of glucocorticoids from the adrenal such that they are not too high or too low?”

Dr. Dwight Ingle was an accomplished physiologist who, along with several other prominent scientists, figured this out (7). Mary Dallman (4) has written an elegant essay describing the studies of Dr. Ingle and his collaborators that is part of the American Physiological Society (APS) Legacy Project (http://www.the-aps.org/publications/legacy/) and the Essays on APS Classic Papers (http://www.the-aps.org/publications/classicals/). Dr. Ingle formulated amazingly accurate predictions using purified preparations of adrenal and pituitary extracts and measurement of food intake and adrenal weight (4). As a result, many of the basic principles of the pituitary control of adrenal and corticosteroid negative feedback were established.

This classic paper is particularly useful in teaching the principles of the control of the hypothalamic-pituitary-adrenal (HPA) axis. Figure 1 from Dr. Ingle’s paper is really all you need to teach the fundamentals of the long-term control of adrenal function and the negative feedback control of the HPA axis!

Figure 1 is the original graph from Dr. Ingle’s publication. This page with its figure legend-containing questions can be printed and distributed to your students as a “discovery learning” project.

The y-axis of this graph is combined adrenal weight from rats treated as shown on the x-axis. Why adrenal weight? It measures the integration of the action of ACTH on adrenal function over time. The analogy I use with my students is disuse atrophy. What happens to muscle size (hence, function) when an arm is in a cast? It gets smaller and weaker. The same is true of the adrenal cortex when deprived of activity, i.e., the stimulatory and growth-promoting effects of ACTH.

This provides another great teaching point: ACTH has two actions best described in the time domain. Rapid increases in ACTH result in a rapid stimulation of steroidogenesis such that adrenal glucocorticoid production increases almost immediately. [This is the basis of the synthetic ACTH cosyntropin stimulation test in clinical practice to evaluate potential adrenal insufficiency of any cause (2).] Increases in ACTH over weeks to months results in an increase in adrenal size (adrenal hypertrophy), whereas decreases in ACTH due to, in this case, hypophysectomy, results in a decrease in adrenal size (adrenal atrophy). This is why patients with ACTH-dependent Cushing syndrome have large adrenal glands on imaging with computed tomography (CT scan), whereas patients with secondary adrenal insufficiency have adrenal atrophy and a diminished response to injection of ACTH. These two different actions of ACTH, rapid and long-term, explain why ACTH can be called adrenocorticotropic or adrenocorticotropic hormone, “-tropic” for acting on or stimulating vs. “-trophic” for nutrition (i.e., growth promoting).

Like most good physiological studies, there was at least one control group. The first column in Figure 1 represents adrenal weight in normal rats with ad libitum access to food. The next column represents normal rats with food intake restricted. This was also an essential control group because manipulation of the
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Fig. 1. Original figure from Ingle DJ. (Reprinted with permission from The effects of administering large amounts of cortin on the adrenal cortices of normal and hypophysectomized rats. Am J Physiol 124: 369–371, 1938.)

Hypophysectomy, removal of pituitary gland; cortin, purified adrenal extract containing corticosterone administered via the drinking water; adrenotropic, injection of purified pituitary extract containing ACTH.

Questions for Discovery Learning

1. Draw the control of the hypothalamic-pituitary-adrenal (HPA) axis.
2. Describe the control groups for this study. Why are they necessary?
3. Describe the results of the following experimental groups. State them in “If...then” form. For example, “If normal rats have food restriction, then their adrenal weight...”
   a. Hypophysectomy/unreated (no injections).
   b. Normal/cortin in the drinking water.
   c. Hypophysectomy/adrenotropin injection with cortin in drinking water.
   d. Hypophysectomy/adrenotropin injection without cortin.
4. Compare the results of the different experimental groups and make some conclusions.
5. Suggest some clinical implications of these findings.
   a. What would plasma ACTH concentration be (high or low) in patients taking high doses of glucocorticoids for arthritis or asthma? What about their adrenal size?
   b. What would plasma ACTH concentration be (high or low) in patients who have had both adrenal glands removed?
   c. Will adrenal glands be an increased size in a patient with a tumor making too much ACTH? What about in a patient whose pituitary gland has been destroyed?

Now it gets really interesting! Administration of a preparation of adrenal extract (we now know contained corticosterone, which Dr. Ingle called “cortin”) in the drinking water of the rats led to a decrease in adrenal weight similar to hypophysectomy. Dr. Ingle correctly hypothesized that cortin was shutting off the pituitary release of adrenotropic hormone (i.e., negative feedback). However, he did not know whether cortin could directly shut off its own release. He then demonstrated that an injection of a purified preparation of pituitary extract (adrenotropic hormone) restored adrenal weight whether cortin was given or not, thereby proving that cortin decreases adrenal weight via inhibition of ACTH. Other than the obvious teaching point of negative feedback and ACTH stimulation of adrenal growth, one can also teach that cortin (corticosterone in this case) is absorbed in the gastrointestinal tract because it is a steroid hormone. If it were a peptide, it would be destroyed in the lumen of the gastrointestinal tract.

These are amazingly simple but elegant experiments! With only an accurate balance (to weigh adrenals) and administration of what we would now consider relatively impure preparations of corticosterone and ACTH, Dr. Ingle figured out that 1) cortin (i.e., corticosterone) inhibits adrenotropic hormone (i.e., ACTH) via negative feedback and 2) adrenals atrophy after loss of pituitary function due to a deficiency of ACTH (secondary adrenal insufficiency). This figure foreshadowed the use of oral exogenous cortisol (hydrocortisone) to treat adrenal insufficiency (2). Of course, Dr. Ingle did not know much about the hypothalamic control of the pituitary gland and many of the subtleties of the control of the HPA axis (6, 8).

Regardless, every single conclusion he drew from the results was accurate without a single hormone measurement.

Implications of these findings are profound. The restoration of normal adrenal weight and function during weaning from high-dose glucocorticoid therapy requires great patience and often takes months or longer (3). Furthermore, primary adrenal insufficiency (loss of adrenal function due to destruction of the adrenal gland itself) is diagnosed by the measurement of an elevated ACTH level (release of the pituitary corticotrope from glucocorticoid negative feedback) (2). ACTH-dependent Cushing syndrome results in adrenal hypertrophy, and ACTH-independent Cushing syndrome results in a suppression of ACTH (negative feedback) (2, 8).

In summary, Dr. Ingle’s three-page paper with one table and figure has an enormous number of teaching points. Figure 2 is a duplicate of Dr. Ingle’s figure with the following annotated teaching points.

Teaching Points

The following teaching points are illustrated as boxes 1–5 on Fig. 2:

1. Adrenal weight represents an integration of the “trrophic” (growth promoting) action of ACTH on the adrenal gland. In the absence of ACTH (e.g., during high-dose glucocorticoid therapy or primary loss of pituitary function), the adrenal glands atrophy and can take a long time to recover when glucocorticoid therapy is discontinued. (You might also ask students what would happen to testicular weight and function in athletes taking high doses of anabolic steroids! This will generalize to the control of all anterior pituitary hormones.)
2. Control groups are mandatory for all physiological studies. The food restriction group is necessary to compare with hypophysectomized rats because hypophysectomy (glucocorticoid deficiency) decreases food intake. One can extend this teaching point to the fact that glucocorticoid excess causes hyperphagia and is responsible for some of the phenotypic weight gain observed in exogenous or endogenous Cushing syndrome.

3. Hypophysectomy decreased adrenal weight due to loss of the “trophic” action of ACTH on adrenal size. It is vital to make the distinction between the acute effects of ACTH on steroid production and the chronic effects of ACTH on adrenal size.

4. Administration of cortin (corticosterone) decreased adrenal weight. This loss of adrenal mass (and function) is a common occurrence in patients treated with glucocorticoid therapy for a variety of reasons.

5. Injection of adrenotropic hormone (ACTH) restored adrenal weight in hypophysectomized rats whether cortin was given or not. This closes the feedback loop between ACTH stimulation of corticosterone from the adrenal and corticosterone inhibition of ACTH.

This very short paper by Dr. Dwight Ingle with or without the separate handout of Fig. 1 and its associated legend (which contains questions for discovery learning) can be distributed to your undergraduate or graduate students taking endocrinology or physiology. Your students will not only learn the essence of the control of adrenal function but will also gain an appreciation for the elegance of experimentation before the technological explosion of the last 50 years. Imagine trying to publish a paper on the HPA axis in the 21st century without any measurements of hormones!

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REFERENCES