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/classics/). 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.

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4. Compare the results of the different experimental groups and make some
observations. For example, “If normal rats have food restriction, then
their adrenal weight...”
a. Hypophysectomy/untreated (no injections).

b. Normal/cortin in the drinking water.
c. Hypophysectomy/adrenotropic injection with cortin in drinking water.
d. Hypophysectomy/adrenotropic injection without cortin.

3. Describe the results of the following experimental groups. State them in
the “If...then” form. For example, “If normal rats have food restriction, then
their adrenal weight...”
a. Hypophysectomy/untreated (no injections).

b. Normal/cortin in the drinking water.
c. Hypophysectomy/adrenotropic injection with cortin in drinking water.
d. Hypophysectomy/adrenotropic 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?

HPA axis is known to alter food intake. In this case, it controlled for the
decrease in food intake observed in rats after
hypophysectomy (removal of the pituitary gland). This allows
one to point out to students the effects of circulating glucocor-
ticoids on food intake (deficiency leads to anorexia, whereas
excess leads to hyperphagia).

The next column, labeled “Hypophysectomy untreated”
shows the combined weight of adrenals from rats after removal
of the entire pituitary gland. These rats were otherwise un-
treated. We now know that this leads to a decrease in adrenal
size (i.e., adrenal atrophy) due to a deficiency in the trophic
action of ACTH. At the time, the identity of this peptide was
not known; Dr. Ingle called it “adrenotropic hormone.” (He
could have called it “adrenotrophic.”) Why did the animals
survive in the absence of ACTH? Another teaching point: we
now know that the synthesis of mineralocorticoid (aldosterone)
release from the adrenal gland is not primarily controlled by
ACTH (5). Therefore, the renin-angiotensin system can main-
tain electrolyte balance in the absence of ACTH and cortisol.

Now it gets really interesting! Administration of a prepara-
tion 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 hypophysec-
tomy. 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 (adreno-
tropic 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 teach-
ing 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 administra-
tion of what we would now consider relatively impure prepa-
rations 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
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-
dependent 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

1. Adrenal weight represents an integration of the “trophic”
   (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.)

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

1. Adrenal weight represents an integration of the “trophic”
   (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