

Protein hormones usually act relatively rapidly, within seconds to minutes. (Although rapid for a hormone, this action is much slower than neural activity.) There can also be prolonged effects. For example, ACTH promotes the proliferation and growth of some adrenal cells, thereby increasing their long-term capacity to produce hormones. A cell may increase or decrease the number of hormone receptors it makes, and these changes are sometimes referred to as *up-regulation* and *down-regulation*, respectively.

STEROID HORMONES Steroid hormones typically act more slowly than protein or amine hormones, requiring hours to take effect. The specificity of action of steroid hormones is determined by the receptors that reside *inside* target cells. These receptors are truly ancient, in an evolutionary sense; estrogen receptors may have arisen in primordial invertebrate species a billion years ago (Bridgham et al., 2006; J. W. Thornton et al., 2003).

Steroid hormones pass in and out of many cells in which they have no effect. If appropriate receptor proteins are inside, however, these receptors bind to the hormone, and the receptor-steroid complex then binds to DNA, so the complexes become concentrated in the nuclei of target cells (**Figure 5.8b**). Thus, we can study where a steroid hormone is active by injecting radioactively tagged molecules of the steroid and observing where they accumulate. For example, tagged estradiol accumulates not only in the reproductive tract (as you might expect), but also in the nuclei of some neurons throughout the hypothalamus. Because neurons producing hormone receptors are found in only a limited number of brain regions, we can begin to learn how hormones affect behavior by finding those brain sites and asking what happens when the hormone arrives there. This strategy for learning about hormones and behavior is discussed in **Box 5.1**.

By altering protein production, steroids have slow but long-lasting effects on the development or adult function of cells. We will discuss such effects of steroids further in Chapter 12. There is a large “superfamily” of steroid receptor genes (Ribeiro et al., 1995), and some steroids act on more than one receptor. For example, a second estrogen **receptor isoform** (steroid receptor subtype) has been described and named estrogen receptor β (Kuiper et al., 1996) to distinguish it from the previously discovered estrogen receptor α . The brain contains both types of estrogen receptors, and they differ in their anatomical distribution within the brain. Scientists are trying to tease apart their separate effects on behavior.

Simple possession of appropriate steroid receptors is not enough to ensure that a given neuron will respond to the presence of that particular steroid hormone. Cells make a wide variety of **steroid receptor cofactors** that may be required, along with the bound steroid receptors, in order for the cell to respond. Furthermore, the nature of the target cell’s response may be determined by the type of cofactor present: two different cells containing the same steroid receptors may respond quite differently to the steroid hormone if they are producing different steroid receptor cofactors (Molenda-Figueira et al., 2006; Tetel, 2000).

Steroids can also affect cells through mechanisms other than the classic nuclear steroid receptor. For example, estradiol, in addition to its slow, long-lasting action on gene expression, can have a rapid, brief effect on some neurons without affecting gene expression. This rapid **nongenomic effect** of steroids involves a separate class of receptors in the neuronal *membrane* (Toran-Allerand, 2005), modulating neural excitability. Similarly, androgenic steroids like testosterone can have effects that are too rapid to involve the transcription of genes and appear instead to involve androgen receptors localized in axons and other sites, distant from the cell nucleus and its DNA (DonCarlos et al., 2006).

Feedback control mechanisms regulate the secretion of hormones

One of the major features of almost all hormonal systems is that they don’t just manufacture a hormone; they also detect and evaluate the effects of the hormone.

receptor isoform A version of a receptor protein (in this context, a hormone receptor) with slight differences in structure that give it different functional properties. Conceptually similar to a receptor subtype.

steroid receptor cofactors Proteins that affect the cell’s response when a steroid hormone binds its receptor.

nongenomic effect An effect of a steroid hormone that is not mediated by direct changes in gene expression.

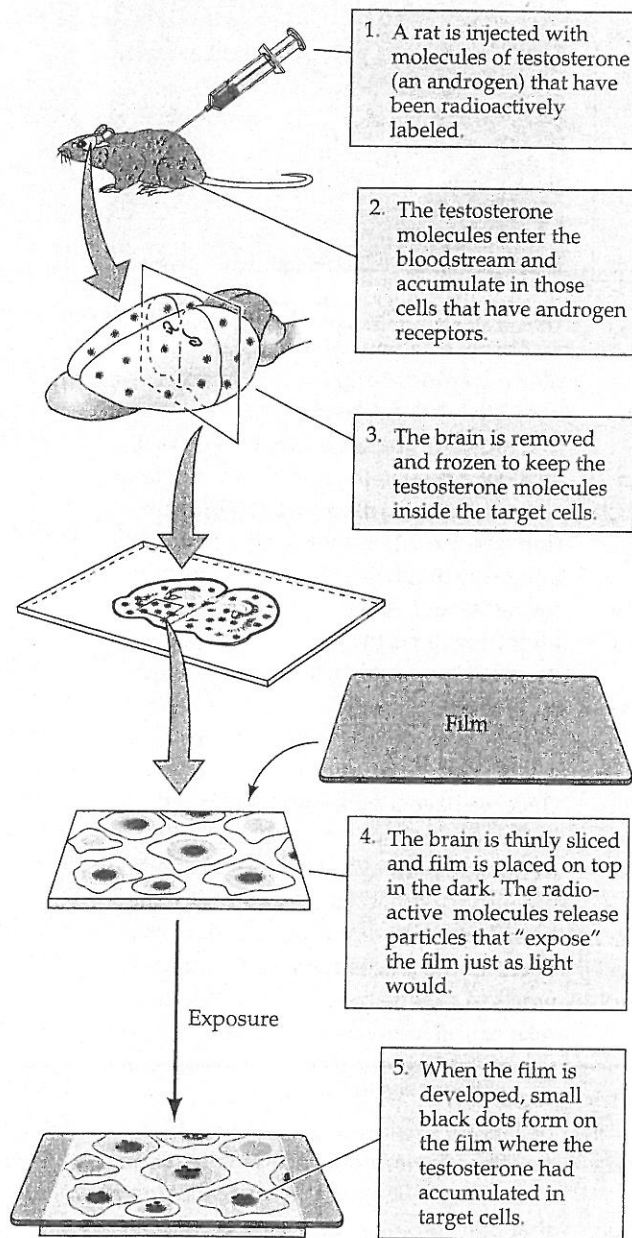
BOX 5.1 Techniques of Modern Behavioral Endocrinology

To establish that a particular hormone affects behavior, investigators usually begin with the type of experiment that Berthold performed in the nineteenth century: observing the behavior of the intact animal, and then removing the endocrine gland and looking for a change in behavior (see Figure 5.2). Berthold was limited to this type of experiment, but modern scientists have many additional options available. Let's imagine that we're investigating a particular effect of hormones on behavior to see how we might proceed.

Which Hormones Affect Which Behaviors?

First we must carefully observe the behavior of several individuals, seeking ways to classify and quantify the different types of behavior and to place them in the context of the behavior of other individuals. For example, most adult male rats will try to mount and copulate with a receptive female placed in their cage. If the testes are removed from the male rat, he will eventually stop copulating with females. We know that one of the hormones produced by the testes is testosterone. Is it the loss of testosterone that causes the loss of male copulatory behavior?

To explore this question, we inject some synthetic testosterone into castrated males and observe whether the copulatory behavior returns. (It does.) Another way to ask whether a steroid hormone is affecting a particular behavior is to examine the behavior of animals that lack the receptors for that steroid. We can delete the gene for a given hormone receptor, making a **knockout organism** (because the gene for the receptor has been "knocked out"), and ask which behaviors are different in the knockouts versus normal animals (see Box 7.3).



(A) Steps in steroid autoradiography

Next we might examine individual male rats and ask whether the ones that copulate a lot have more testosterone circulating in their blood than those that copulate only a little. To investigate this question, we measure individual differences in the amount of copulatory behavior, take a sample of blood from each individual, and measure levels of testosterone with **radioimmunoassay (RIA)**, a technique using an

antibody that binds to a particular hormone. By adding many such antibodies to each blood sample and measuring how many of the antibodies find a hormone molecule to bind, we can estimate the total number of molecules of the hormone per unit volume of blood.

It turns out that individual differences in the sexual behavior of normal male rats (and normal male humans) do *not* correlate with differences in testosterone levels in the blood. In both rats and humans, a drastic loss of testosterone, as after castration, results in a gradual decline in sexual behavior. All normal males, however, appear to make more than enough testosterone to maintain sexual behavior, so something else must modulate this behavior. In other words, the hormone acts in a permissive manner: it permits the display of the behavior, but something else determines how much of the behavior each individual exhibits.

Where Are the Target Cells?

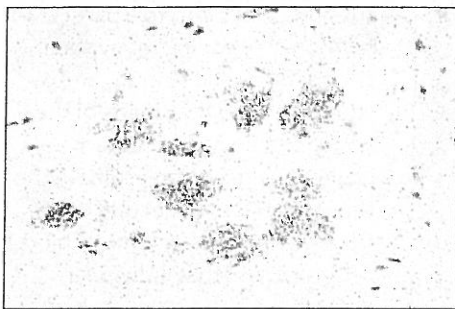
What does testosterone do to permit sexual behavior? One step toward answering this question is to ask another question: Which parts of the brain are normally affected by this hormone? We have several methods at our disposal for investigating this question.

First we might inject a castrated animal with radioactively labeled testosterone and wait for the hormone to accumulate in the brain regions that have receptors for the hormone. Then we can sacrifice the animal, remove

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knockout organism An individual in which a particular gene has been disabled by an experimenter.

radioimmunoassay (RIA) A technique that uses antibodies to measure the concentration of a substance, such as a hormone, in blood.

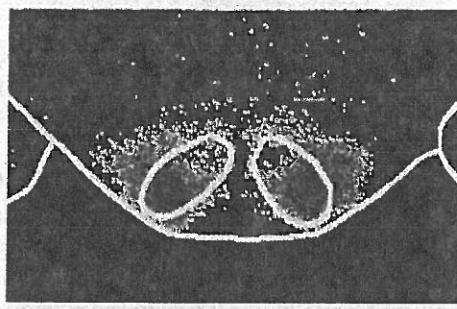
BOX 5.1 (continued)

(B) An autoradiogram showing that spinal motoneurons (purple cell profiles) accumulate radioactive testosterone (small dots)

the brain, freeze it, cut thin sections from it, and place the thin sections on photographic film. Radioactive emissions from the tissue expose the film, revealing which brain regions had accumulated the most labeled testosterone. This method is known as **autoradiography** because the tissue “takes its own picture” with radioactivity (Figure A).

When the labeled hormone is a steroid like testosterone, the radioactivity accumulates in the nuclei of neurons and leaves small black specks on the film (Figure B). When the radiolabeled hormone is a protein hormone such as oxytocin, the radioactivity accumulates in the membranes of cells and appears in particular layers of the brain. Computers can generate color maps that highlight regions with high densities of receptors (Figure C).

Another method for detecting hormone receptors is **immunocytochemistry**. In this method (described in more detail in Box 2.1), we use antibodies that recognize the hormone receptor (Figure D). This method allows us to map the distribution of hormone receptors in the brain. We put the antibodies on slices of brain tissue, wait for them to bind to the receptors, wash off the unbound antibodies, and use chemical methods to visualize the antibodies by creating a tiny dark spot at each



(C) An autoradiogram showing the concentration of oxytocin receptors in the ventromedial hypothalamus (oval outlines)

one. When the antibodies recognize a steroid receptor, chemical reactions cause a dark coloration in the nuclei of target brain cells. We can also use **in situ hybridization** (see Box 2.1) to look for the neurons that make the mRNA for the steroid receptor. Because these cells make the transcript for the receptor, they are likely to possess the receptor protein itself.

What Happens at the Target Cells?

Once we have used autoradiography, immunocytochemistry, or *in situ* hybridization (or, better yet, all three) to identify brain regions that have receptors for the hormone, those regions become candidates for the places at which the hormone works to change behavior. Now we can take castrated males and implant tiny pellets of testosterone into one of those brain regions. We use RIA to ensure that the pellets are small enough that they have no effect on hormone levels in the blood. Then we ask whether the small implant in that brain region restores the behavior. If not, then in other animals we can implant pellets in a different region or try placing implants in a combination of brain sites.

It turns out that such implants can restore male sexual behavior in rats only if they are placed in the medial preoptic area



(D) Immunocytochemistry revealing cells with nuclei that contain androgen receptors (dark circles), to which testosterone can bind. The somata of these neurons have been labeled with the tracers fluorogold (white) and fluororuby (red).

(mPOA) of the hypothalamus. Thus, we have found so far that testosterone does something to the mPOA to permit individual males to display sexual behavior. Now we can examine the mPOA in detail to learn what changes in the anatomy, physiology, or protein production of this region are caused by testosterone. We have more or less caught up to modern-day scientists who work on this very question. Some of the preliminary answers suggested by their research will be discussed in Chapter 12. (Figure C courtesy of Bruce McEwen; D courtesy of Cynthia Jordan.)

autoradiography A histological technique that shows the distribution of radioactive chemicals in tissues.

immunocytochemistry (ICC) A method for detecting a particular protein in tissues in which an antibody recognizes and binds to the protein and then chemical methods are then used to leave a visible reaction product around each antibody.

in situ hybridization A method for detecting particular RNA transcripts in tissue sections by providing a nucleotide probe that is complementary to, and will therefore hybridize with, the transcript of interest.

negative feedback The property by which some of the output of a system feeds back to reduce the effect of input signals.

Thus, secretion is usually monitored and regulated so that the rate is appropriate to ongoing activities and needs of the body. The basic control used is a **negative feedback** system: output of the hormone *feeds back* to inhibit the drive for more of that same hormone. This negative feedback action of a hormonal system is like that of a thermostat, and just as the thermostat can be set to different temperatures

at different times, the set points of a person's endocrine feedback systems can be changed to meet varying circumstances. We'll discuss negative feedback regulation of other processes in Chapter 13 (see Figure 13.1).

In the simplest kind of hormone regulation system, diagrammed in **Figure 5.9a**, an endocrine cell releases a hormone that acts on target cells, but the same hormone also feeds back to inhibit the gland that released it. This is an autocrine response.

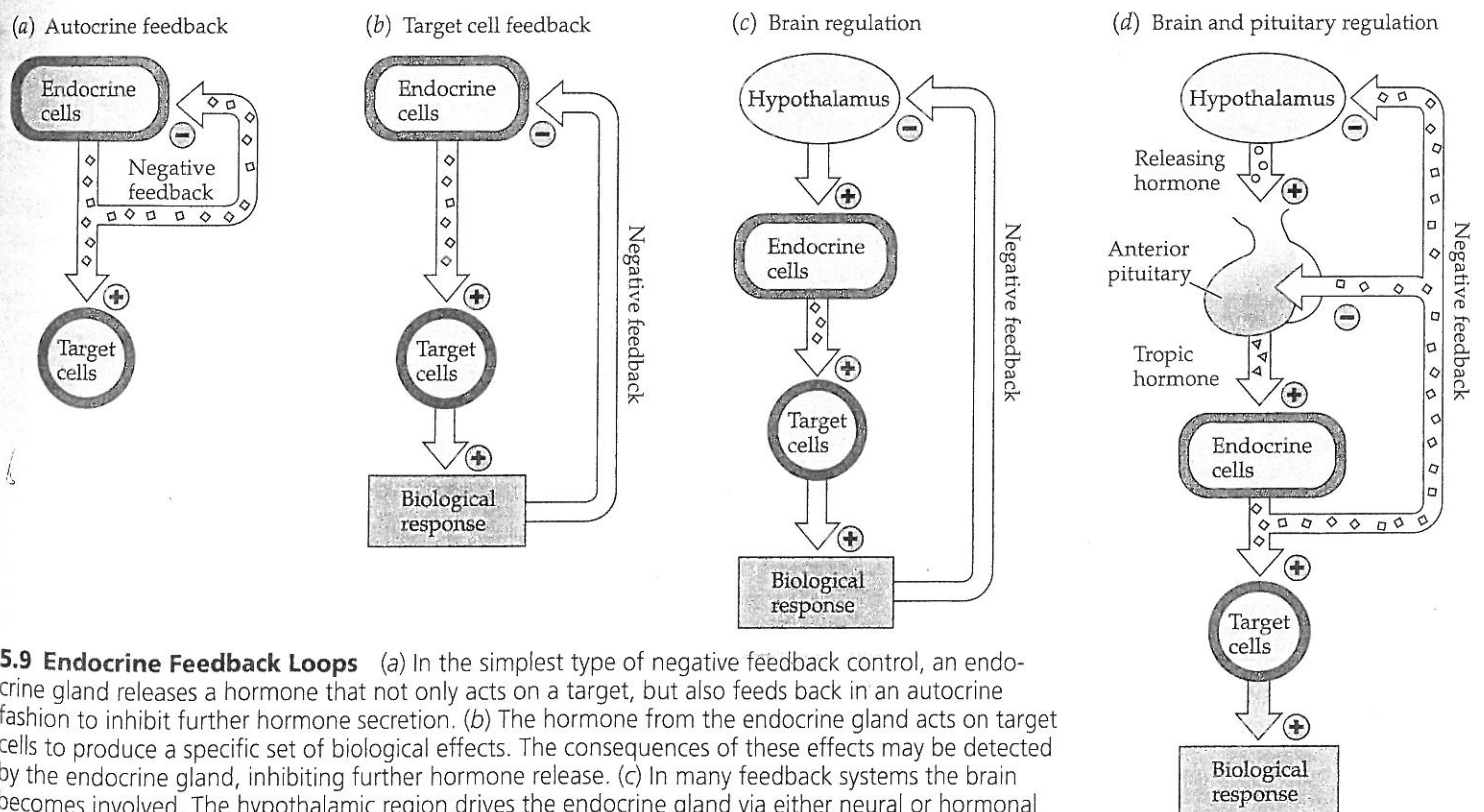
In other cases, the endocrine cell reacts not to its own hormone, but to the biological response that the hormone elicits from the target cells (**Figure 5.9b**). If the initial effect is too small, additional hormone is released; if the effect is sufficient, no further hormone is released. For example, the hormone insulin is released to control the level of glucose circulating in our blood. After a meal, glucose from the food enters the bloodstream, causing insulin to be released from the pancreas. The insulin causes glucose to enter muscle and fat cells. As the level of glucose in the blood falls, the pancreas secretes less insulin, so a balance tends to be maintained (Chapter 13).

A more complex endocrine system includes the brain, usually the hypothalamus, as part of the circuit that controls an endocrine gland (**Figure 5.9c**). When we are alarmed, for example, the hypothalamus directs the adrenal medulla to secrete the hormone epinephrine (also called adrenaline), which affects many target cells. The brain detects these effects and exerts negative feedback on the hypothalamus to reduce further hormone output.

An even greater degree of complexity is encountered when the anterior pituitary becomes involved (**Figure 5.9d**). As we'll see in the next section, several anterior pituitary hormones regulate hormone secretion by other endocrine glands; all of these pituitary hormones are called **tropic hormones**. (*Tropic*, pronounced with a long *o* as in *toe*, means "directed toward.") The hypothalamus uses another set of hormones, called **releasing hormones**, to control the pituitary release of

tropic hormones A class of anterior pituitary hormones that affect the secretion of other endocrine glands.

releasing hormones A class of hormones, produced in the hypothalamus, that traverse the hypothalamic-pituitary portal system to control the pituitary's release of tropic hormones.



5.9 Endocrine Feedback Loops (a) In the simplest type of negative feedback control, an endocrine gland releases a hormone that not only acts on a target, but also feeds back in an autocrine fashion to inhibit further hormone secretion. (b) The hormone from the endocrine gland acts on target cells to produce a specific set of biological effects. The consequences of these effects may be detected by the endocrine gland, inhibiting further hormone release. (c) In many feedback systems the brain becomes involved. The hypothalamic region drives the endocrine gland via either neural or hormonal signals. The target organ signals the brain to inhibit this drive. (d) Highly complex feedback mechanisms involve the hypothalamus and the anterior pituitary, as well as the endocrine gland. Feedback is regulated by a variety of hormones via multiple routes.

TABLE 5.2 Examples of Major Classes of Hormones

Gland	Hormones	Principal effects
POSTERIOR PITUITARY (storage organ for certain hormones produced by hypothalamus)	Oxytocin	Stimulates contraction of uterine muscles; stimulates release of milk by mammary glands
	Vasopressin (AVP), or antidiuretic hormone (ADH)	Stimulates increased water reabsorption by kidneys; stimulates constriction of blood vessels
ANTERIOR PITUITARY	Growth hormone (GH)	Stimulates growth
	Thyroid-stimulating hormone (TSH)	Stimulates the thyroid
	Adrenocorticotrophic hormone (ACTH)	Stimulates the adrenal cortex
	Follicle-stimulating hormone (FSH)	Stimulates growth of ovarian follicles and of seminiferous tubules of the testes
	Luteinizing hormone (LH)	Stimulates conversion of follicles into corpora lutea; stimulates secretion of sex hormones by gonads
	Prolactin	Stimulates milk secretion by mammary glands
HYPOTHALAMUS	Releasing hormones	Regulate hormone secretion by anterior pituitary
	Oxytocin; vasopressin	See under "Posterior pituitary" above
PINEAL	Melatonin	Regulates seasonal changes; regulates puberty
ADRENAL CORTEX	Glucocorticoids (corticosterone, cortisol, hydrocortisone, etc.)	Inhibit incorporation of amino acids into protein in muscle; stimulate formation and storage of glycogen; help maintain normal blood sugar level
	Mineralocorticoids (aldosterone, deoxycorticosterone, etc.)	Regulate metabolism of sodium and potassium
	Sex hormones (especially androstenedione)	Regulate facial and body hair
ADRENAL MEDULLA	Catecholamines (epinephrine, norepinephrine)	Prepare body for action
GONADS		
Testes	Androgens (testosterone, dihydrotestosterone, etc.)	Stimulate development and maintenance of male primary and secondary sexual characteristics and behavior
Ovaries	Estrogens (estradiol, estrone, etc.)	Stimulate development and maintenance of female secondary sexual characteristics and behavior
	Progestins (progesterone)	Stimulate female secondary sexual characteristics and behavior; maintain pregnancy
THYROID	Thyroxine (tetraiodothyronine); triiodothyronine	Stimulate oxidative metabolism
	Calcitonin	Prevents excessive rise in blood calcium
PANCREAS	Insulin	Stimulates glycogen formation and storage
	Glucagon	Stimulates conversion of glycogen into glucose
STOMACH	Secretin	Stimulates secretion of pancreatic juice
	Cholecystokinin (CCK)	Stimulates release of bile by gallbladder
	Gastrin	Stimulates secretion of gastric juice
	Ghrelin	Provides appetite signal to the hypothalamus appetite controller
HEART	Atrial natriuretic peptide	Promotes salt loss in urine

tropic hormones. Thus, the brain's releasing hormones affect the pituitary's tropic hormones, which affect the release of hormones from endocrine glands. Negative feedback in this case goes from the hormone of the endocrine gland to both hypothalamus and the anterior pituitary (**Figure 5.10**).

5.10 An Example of Complex Endocrine Regulation The brain funnels information to the hypothalamus, which then controls the anterior pituitary, which in turn stimulates the thyroid gland. Note that three hormones and at least four cell groups are interacting in this instance.

Each Endocrine Gland Secretes Specific Hormones

We will restrict our account in this chapter to some of the main endocrine glands because a thorough treatment would fill an entire book (e.g., Hadley and Levine, 2006). **Table 5.2** gives a fuller but far from complete listing of hormones and their functions. We will discuss hormones from the pancreas and stomach in Chapter 13 when we consider hunger. Keep in mind that most hormones have more functions than are mentioned here and that several hormones may act together to produce effects in the same target cells.

The pituitary gland releases many important hormones

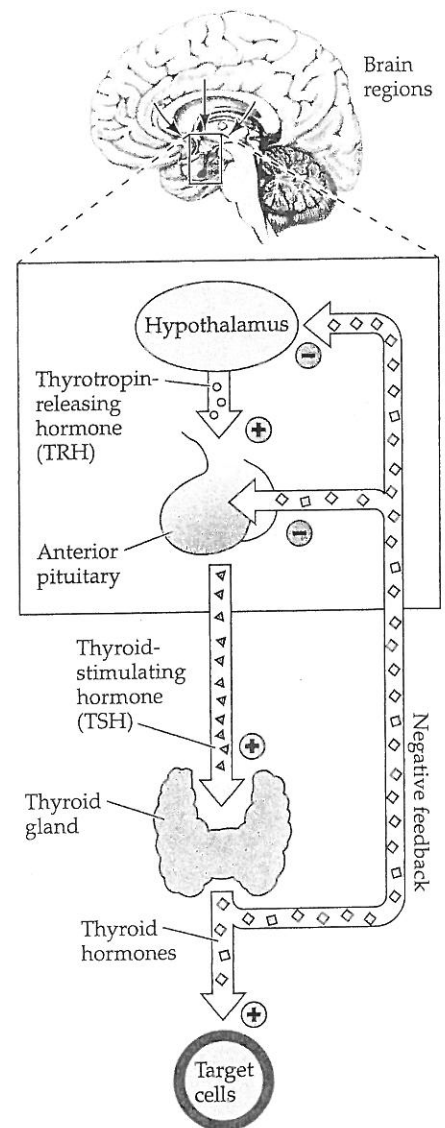
Resting in a socket in the base of the skull is the **pituitary gland** (or **hypophysis**; see Figure 5.1), occupying a volume of about 1 cm³ and weighing about 1 g. The hypothalamus sits just above it. The term *pituitary* comes from the Latin *pituita*, "mucus," reflecting the outmoded belief that waste products dripped down from the brain into the pituitary, which secreted them out through the nose. (The ancients may have thought you could literally sneeze your brains out!) The pituitary used to be referred to as the *master gland*, a reference to its regulatory role with regard to several other endocrine glands. But this gland is itself enslaved by the hypothalamus above it, as we'll see.

The pituitary gland consists of two main parts: the **anterior pituitary** (or **adenohypophysis**) and the **posterior pituitary** (or **neurohypophysis**). The anterior and posterior pituitary develop from different embryonic tissues and are completely separate in function. The pituitary is connected to the hypothalamus by a thin piece of tissue called the **pituitary stalk** or **infundibulum** (see Figure 5.11). The stalk contains many axons and is richly supplied with blood vessels. The axons extend only to the posterior pituitary, which we will consider next. The blood vessels, as we will see later, carry information exclusively to the anterior pituitary.

THE POSTERIOR PITUITARY The posterior pituitary gland secretes two principal hormones: **oxytocin** and **arginine vasopressin (AVP)**, often called just **vasopressin**. Neurons in various hypothalamic nuclei, especially the supraoptic nuclei and the paraventricular nuclei, synthesize these two hormones and transport them along their axons to the axon terminals (**Figure 5.11**). Action potentials in these hypothalamic neurosecretory cells travel down the axons in the pituitary stalk and reach the axon terminals in the posterior pituitary, causing release of the hormone from the terminals into the rich vascular bed of the neurohypophysis. The axon terminals abut capillaries (small blood vessels), allowing the hormone to enter circulation immediately.

Some of the signals that activate the nerve cells of the supraoptic and paraventricular nuclei are related to thirst and water regulation, which we will discuss in Chapter 13. Secretion of vasopressin increases blood pressure by causing blood vessels to contract. Vasopressin also inhibits the formation of urine, so it is sometimes called *antidiuretic hormone*, or **ADH** (a *diuretic* is a food or drug that promotes urination). This action of vasopressin helps conserve water. In fact, the major physiological role of vasopressin is its potent antidiuretic activity; it exerts this effect with less than one-thousandth of the dose needed to alter blood pressure.

Oxytocin is involved in many aspects of reproductive and parental behavior. One of its functions is to stimulate contractions of the uterus in childbirth (the word *oxytocin* is derived from the Greek *oxys*, "rapid," and *tokos*, "childbirth"). In



pituitary gland or hypophysis A small, complex endocrine gland located in a socket at the base of the skull.

anterior pituitary or adenohypophysis The front division of the pituitary gland; secretes tropic hormones.

posterior pituitary or neurohypophysis The rear division of the pituitary gland.

pituitary stalk or infundibulum A thin piece of tissue that connects the pituitary gland to the hypothalamus.

oxytocin A hormone, released from the posterior pituitary, that triggers milk letdown in the nursing female.

arginine vasopressin (AVP) or vasopressin Also called *antidiuretic hormone (ADH)*. A peptide hormone from the posterior pituitary that promotes water conservation.