CHAPTER

55

Endocrine Effects on the Brain and Their Relationship to Behavior

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OUTLINE

INTRODUCTION

The brain undergoes changes in its chemistry and structure in response to changes in the environment. Circulating hormones of the adrenals, thyroid and gonads play an important role in this adaptation, because the brain controls the endocrine system through the pituitary gland (Fig. 55-1). This control allows environmental signals to regulate hormonal secretion. Furthermore, circulating hormones act on the brain as well as on other tissues and organs of the body to modify their structure and chemistry via two mechanisms: (i) intracellular receptors that bind to DNA and alter gene expression and (ii) cell-surface receptors that modulate ion channels and second-messenger systems.

Hormonal actions occur during sensitive periods in development, in adult life during natural endocrine cycles and in response to experience as well as during the aging process (see Ch. 28). As a result of their fundamental actions on cellular processes and genomic activity and of the control of their secretion by environmental signals, steroid and thyroid hormone actions on the brain provide unique insights into the plasticity of the brain and behavior (see also Ch. 56).

Awareness of endocrine influences on brain function is as old as endocrinology itself. In 1849, Berthold described striking behavioral changes resulting from castration of roosters, and the reversal of these changes after testes had been transplanted into the castrated animals (see (Becker et al., 2002)). Nearly 100 years later, Beach published *Hormones and Behavior*

(see (Becker et al., 2002)), which has served to instruct generations of investigators and to motivate them to explore in depth the interactions of hormones and brain. Spectacular growth of the field of neuroendocrinology (see also Ch 20) offers the present generation of neurobiologists unparalleled opportunities to explore with great sophistication the influence of neural activity on endocrine secretion, and the effect of hormones, in turn, on neural activity and behavior.

This chapter focuses on the neurochemical and molecular aspects of the influences of hormones on the nervous system and behavior, after first considering the chemical signals, behavioral events and underlying neural activity that regulate hormonal secretion.

BEHAVIORAL CONTROL OF HORMONAL SECRETION

The hypothalamic releasing factors regulate release of the anterior pituitary trophic hormones

As summarized in Figure 55-1, the releasing factors are produced in various neuronal groups within the hypothalamus and are transported to the median eminence for release into the portal circulation to the anterior pituitary. Neurons in the hypothalamus also produce the hormones oxytocin and vasopressin, which are released by the posterior pituitary into the blood. Therefore, it is not surprising that behavior and experience, which influence the hypothalamus, sometimes alter the secretion of these hypothalamic releasing factors and hormones.

Secretion of pituitary hormones is responsive to behavior and effects of experience

Consider, for example, the phenomenon of lactation, in which the sucking stimulus to the nipple triggers the release of oxytocin, which facilitates milk ejection, and of prolactin, which helps the mammary gland to replenish the supply of milk (Ganong, 1977). The phenomenon of stress also illustrates the behavioral and emotional control of anterior pituitary hormone secretion. Conditions associated with tissue injury and surgical trauma, as well as the so-called psychic stresses of fear, novelty and even joy, can activate the release of adrenocorticotropic hormone (ACTH), which in turn stimulates the secretion of adrenal glucocorticoids (Ganong, 1977). The behavioral, emotional stimuli are mediated by neural pathways that can be modified readily by learning.

In the female rabbit, copulation activates spinal reflex pathways that stimulate the secretion of luteinizing hormone (LH), which leads to ovulation (McEwen, 1981). In the male rabbit, copulation also activates the secretion of LH and increases plasma testosterone (McEwen, 1981). Social stimuli also modify gonadotropin secretion. Olfactory cues between female mice can interrupt normal estrous cycles and lead to pseudopregnancies or to periods of prolonged diestrus, termed the Lee–Boot effect; olfactory cues from male mice can shorten the estrous cycle and either cause rapid attainment of estrus, termed the Whitten effect, or terminate pregnancy in a newly impregnated mouse, termed the Bruce effect (McEwen, 1981). In male rhesus monkeys, sudden, decisive defeat by other males leads to prolonged reduction in plasma testosterone, which can be reversed in the defeated male by the introduction of a female companion (McEwen, 1981). In men, the anticipation of sexual intercourse has been reported to increase beard growth, a process under the control of circulating androgen (McEwen, 1981).

Hormones secreted in response to behavioral signals act in turn on the brain and on other tissues

Functional changes caused by hormones secreted in response to behavioral signals include modifications of behavior. With the sex hormones, these changes strengthen and guide the reproductive process. Thus, aggressive encounters between male birds or mammals in defense of territory during the mating period stimulate gonadotropin and testosterone secretion. Increases in these hormones further increase readiness for sexual activity by enhancing supplies of sperm and seminal fluid. This analysis was taken further by Lehrman (see (Becker et al., 2002)), who showed that among doves the behavioral sequence of courtship, mating, nest building and parenting involves complex behavioral interplay between the partners that triggers further hormonal secretion, which leads to the next phase of behavior and hormonal secretion.

Regarding the adrenal steroids, the behavioral activation of hormonal secretion in stress is part of a mechanism for restoring homeostatic balance. For example, an encounter with a predator may require rapid evasive action, in which neural activity and rapidly mobilized hormones such as epinephrine play a role. Adrenal steroid secretion is slower, reaching a peak minutes after the stressful event, and therefore is not expected to play a role in coping with the immediate situation. If the evasive action is successful and the animal survives, it will have to re-establish homeo-stasis; presumably, it also will learn from the experience to minimize the chances of another such encounter. Adrenal steroids facilitate such long-term adaptation; that is, they facilitate the acquisition as well as extinction of a conditioned avoidance response (Korte, 2001). Suppose an animal has learned to avoid a certain place where previously it was punished; adrenal secretions facilitate this learning in order to help 'keep it out of trouble'. Yet, if the animal later discovers that being in that place no longer results in punishment, and if that place also contains a food or water supply, it is in the best interest of the animal to extinguish the avoidance response in order to take advantage of the available food or water. Adrenal steroids have, in fact, been found to facilitate such extinction and, thus, can be said to facilitate a form of behavioral adaptation (Korte, 2001). Adrenal steroids also appear to play a role in both selective attention and consolidation of a variety of learned information related to episodes or events in daily life (Korte, 2001). Another aspect of adaptation in which stress-induced secretion of adrenal steroids participates, concerns the ability of the organism to cope with repeated stressful events through a variety of neurochemical changes (McEwen, et al., 1993).

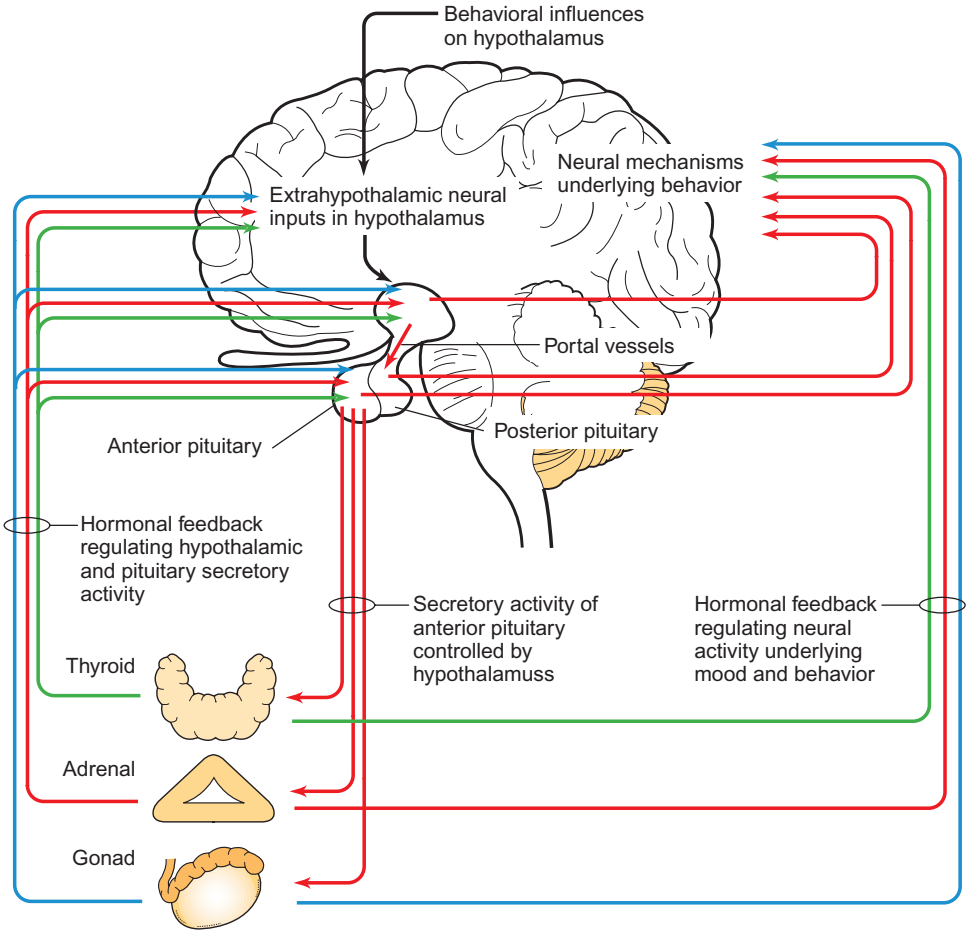


FIGURE 55-1 **Schematic representation of possible and known reciprocal interactions among hypothalamic, pituitary, thyroid, adrenal, and gonadal hormones.**

Besides stress, adrenal steroids are secreted in varying amounts according to the time of day, and in this capacity they perform an important role in coordinating daily activity and sleep patterns with food-seeking and processing of information (McEwen, et al., 1993). In nocturnally active animals, such as the rat, adrenal steroids are secreted at the end of the light period prior to onset of darkness. In humans and monkeys, adrenal steroid secretion precedes waking in the morning to begin daily activity. Thus, in both rats and primates, adrenal steroid secretion precedes the waking period, and appears to contribute, during waking, to optimal synaptic efficacy in the hippocampus for long-term potentiation, a correlate of learning. It is this aspect of adrenal steroid action that contributes to enhanced attention and improved retention of episodic memories (Lupien & McEwen, 1997) (see Ch. 56). Moreover, adrenal steroid elevation prior to waking also increases food-seeking behavior and enhances appetite for carbohydrates (McEwen, et al., 1993).

Cyclic changes in hormonal secretion, which are under the control of daily and seasonal light–dark rhythms, are important not only for the adrenals but for the gonads as well. Estrous cycles, menstrual cycles and seasonal breeding patterns represent adaptations of individual species to climatic conditions of the environment (Becker et al., 2002). The feedback actions of gonadal and adrenal hormones, which are secreted in response to rhythmic output of hypothalamic and pituitary hormones, prime or activate the nervous system to perform the appropriate behavioral responses. It is important to stress that hormones themselves do not cause behaviors; rather, hormones induce chemical changes in particular sets of neurons, making certain behavioral outcomes more likely as a result of the strengthening or weakening of particular neural pathways.

CLASSIFICATION OF HORMONAL EFFECTS

Hormonal actions on target neurons are classified in terms of cellular mechanisms of action

Hormones act either via cell-surface or intracellular receptors, although this distinction has been somewhat blurred, as will be described below. Peptide hormones and amino-acid derivatives, such as epinephrine, act on cell-surface receptors that do such things as open ion-channels, cause rapid

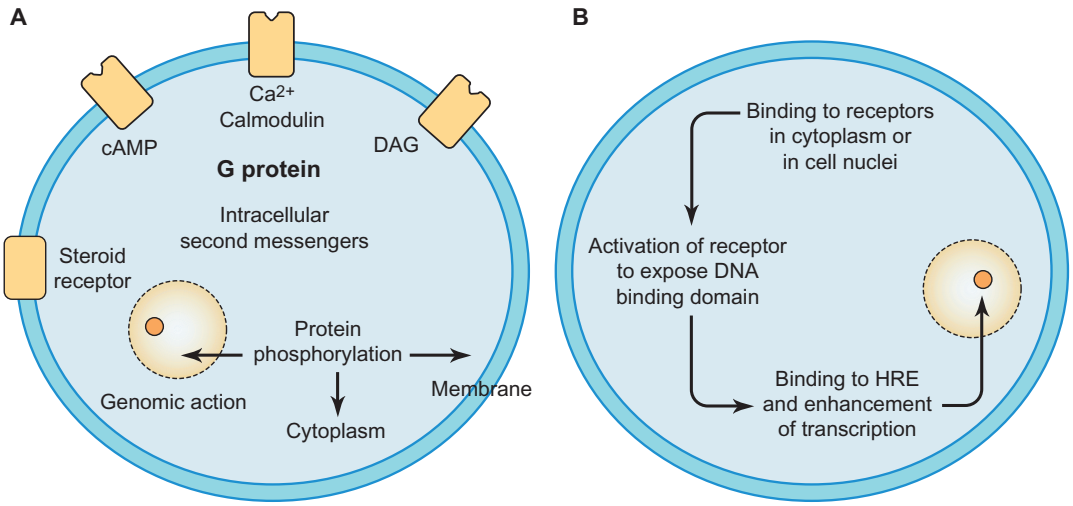


FIGURE 55-2 **There are two modes of hormonal action. (A)** Activation of cell-surface receptors and coupled second-messenger systems, with a variety of intracellular consequences. **(B)** Entry of hormone into the target cell, binding to and activation of an intracellular receptor and binding of the receptor-hormone complex to specific DNA sequences to activate or repress gene expression. *DAG*, diacylglycerol; *HRE*, hormone-response element.

electrical responses and facilitate exocytosis of hormones or neurotransmitters. Alternatively, they activate second-messenger systems at the cell membrane, such as those involving cAMP, Ca^{2+}/calmodulin or phosphoinositides (see Chs. 22-24), which leads to phosphorylation of proteins inside various parts of the target cell (Fig. 55-2A). Steroid hormones and thyroid hormone, on the other hand, act on intracellular receptors in cell nuclei to regulate gene expression and protein synthesis (Fig. 55-2B). Steroid hormones can also affect cell-surface events via receptors at or near the cell surface.

The various modes of hormonal action summarized in Figure 55-2 may be distinguished from each other by time course. The fastest effects, in both latency and duration, are those involving direct opening of ion channels and stimulation of exocytosis. Intermediate effects involve phosphorylation of enzymes, ion channels, receptors or structural proteins, which may last for minutes or even hours. The slowest and most enduring effects are those that alter gene expression and lead to induction or repression of enzyme or receptor proteins, growth responses and even the structural remodeling of tissues.

As summarized in Figure 55-3, steroid/thyroid hormone receptors bind to other proteins as well as to DNA [7–9]. In the simplest type of action (Fig. 55-3A), the steroid/thyroid hormone receptor becomes activated after the hormone binds to it; activation results in conformational changes that include shedding of other proteins, such as heat-shock proteins, and exposing the DNA-binding domain. The receptor then binds to the specific sequence of DNA, called a 'hormone-response element' or enhancer, located on the coding strand of DNA; this enhances transcription by permitting other transcription factors as well as the RNA polymerase to bind to the promoter region (McEwen, 1999). A second scheme (Fig. 55-3B) is for the hormone receptor to bind with high affinity to another protein transcription factor, in this case the c-fos-c-jun complex, removing both protein complexes from binding to DNA (McEwen, 1999). Such a result also blocks the enhancement of transcription by either agent, although it also could reduce inhibitory effects produced by the hormone receptor through the scheme shown in Figure 55-3C. A variant on this theme, not shown in the figure, is 'squelching' in which multiple transcription factors compete with each other for a limited supply of soluble ligands that enhance their activities (McEwen, 1999). A third scheme, shown in Figure 55-3C, is for the steroid receptor to compete with another transcription factor for binding sites in the promoter regions. These other factors may be COUP or the cAMP-dependent transcription factors that bind to the adapter protein-1 (AP-1) response element. The result of this competition is inhibition of transcription since, in this situation the other transcription factor enhances transcription, whereas the hormone receptor does not do so when it binds to the coding DNA strand (McEwen, 1999).

As we have noted, second-messenger systems, through phosphorylation of nuclear proteins, can influence gene expression. There is evidence that even the classical steroid receptors are subject to regulation by phosphorylation and that phosphorylations promoted by a neurotransmitter such as dopamine (Ch. 14) are able to cause nuclear translocation of a steroid receptor in the absence of the steroid (McEwen, 1999).

So far, the best understood examples of genomic regulation of neuronal function stem from the actions of gonadal and adrenal steroids and thyroid hormone, and many of these actions are involved in the plasticity of behavior that results from hormonal secretion, such as changes in aggressive and reproductive behavior and adaptation to repeated stress. In fact, hormonal actions that involve the genome are pervasive throughout the life cycle.

We can distinguish four major types of hormonal actions on the nervous system: (i) developmental actions, such as those involved in sexual differentiation and the effects of

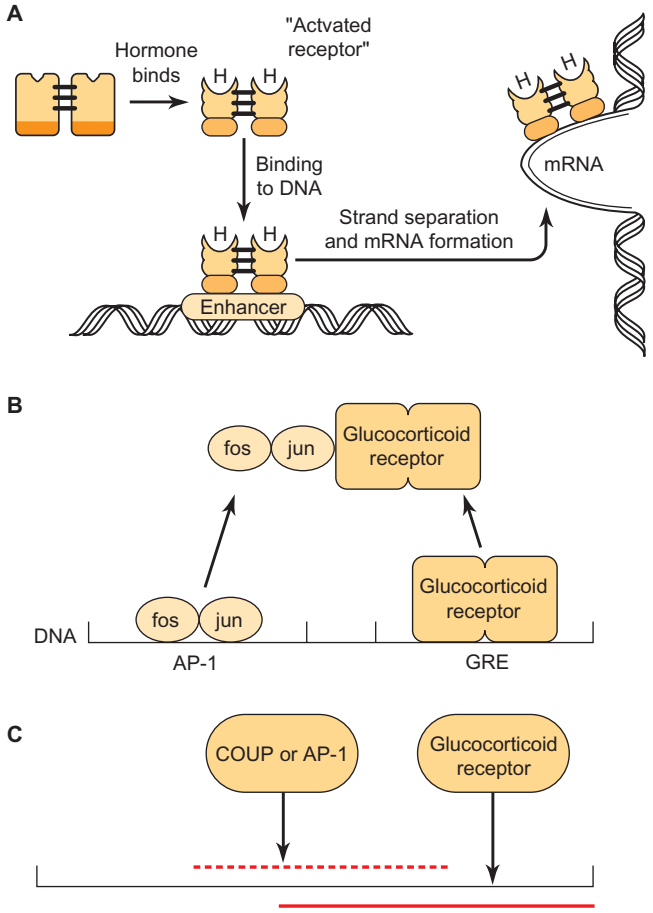


FIGURE 55-3 **Intracellular receptors mediate at least three distinct types of actions on gene expression. (A)** Binding of the hormone-receptor complex to a hormone-response element, a DNA sequence that is placed in a position where receptor binding to it can enhance unwinding of the double helix and attachment of other transcription factors. **(B)** Binding of the steroid receptor to another protein transcription factor, for example, the fos-jun complex through protein–protein interactions, removing both transcription factors from binding to DNA. *GRE*, glucocorticoid response element. **(C)** Binding of the hormone receptor to a hormone-response element located in the middle of a site for binding of another transcription factor-response element, such as the adaptor protein-1 (AP-1) or chicken ovalbumin upstream promoter-transcription factor (COUP sites), resulting in inhibition of transcription normally activated by transcription factors acting on these response elements.

thyroid hormone and retinoic acid; (ii) reversible, and often cyclical, effects on the structure and function of neurons and glial cells that underlie corresponding cyclical changes in behavior, such as in reproduction and the daily rhythms of sleep and waking; (iii) experiential effects involving environmentally induced changes in hormonal secretion that evoke adaptive or maladaptive changes in the brain, as in stress; and (iv) effects that protect neurons or potentiate damage and lead to cell death.

It will be seen below that, in addition to genomic actions, there are nongenomic effects of steroids that modulate neurotransmitter release as well as ion traffic across the cell membrane as well as calcium buffering by mitochondria and direct activation of protein synthesis as well as cytoskeletal modification, and do so frequently in coordination with genomic actions.

BIOCHEMISTRY OF STEROID AND THYROID HORMONE ACTIONS

Steroid hormones are divided into six classes, based on physiological effects: estrogens, androgens, progestins, glucocorticoids, mineralocorticoids and vitamin D

As shown in Figures 55-2 and 55-3, steroid hormone action on the brain and on other target tissues involves intracellular receptor sites that interact with the genome (Becker et al., 2002). There are also important metabolic transformations of certain steroids, occurring in the nervous system, that either generate more active metabolites or result in the production of less active steroids. Such transformations are particularly important for the actions of androgens, of lesser importance for estrogens and progestins, and of practically no importance for glucocorticoids and mineralocorticoids. For vitamin D, the principal transformation to an active metabolite occurs in the kidney and liver (Garcion et al., 2002). Some metabolites, such as allopregnanolone and allotetrahydrodeoxycorticosterone, produce nongenomic effects on the $GABA_A$ receptor (Reddy, 2010).

Some steroid hormones are converted in the brain to more active products that interact with receptors

The brain, like the seminal vesicles, is able to reduce testosterone to 5α-dihydrotestosterone (DHT); and, like the placenta, the brain aromatizes testosterone to estradiol (Fig. 55-4). Neither conversion occurs equally in all brain regions. The aromatization reaction is discussed below. Regional distribution of 5α-reductase activity toward testosterone in rat brain reveals that the highest activity is found in the midbrain and brainstem, intermediate activity is found in the hypothalamus and thalamus, and the lowest activity is found in the cerebral cortex (Becker et al., 2002). The pituitary has higher 5α-reductase activity than any region of the brain, and its activity is subject to changes as a result of gonadectomy, hormone replacement and postnatal age (Becker et al., 2002). 5α-DHT has been implicated in the hypothalamus and pituitary as a potent regulator of gonadotropin secretion, but it is relatively inactive toward male rat sexual behavior (Becker et al., 2002; McEwen, 1981). Labeled metabolites with Rf values of 5α-DHT have been detected in extracts of hypothalamic and pituitary tissue after [^{3}H]-testosterone administration in both adult and newborn rats. It is interesting that progesterone inhibits 5α-reductase activity toward [^{3}H]testosterone and that [^{3}H]progesterone is converted to [^{3}H]5α-dihydroprogesterone (Fig. 55-4). Progesterone competition for 5α-reductase may explain some of the antiandrogenicity

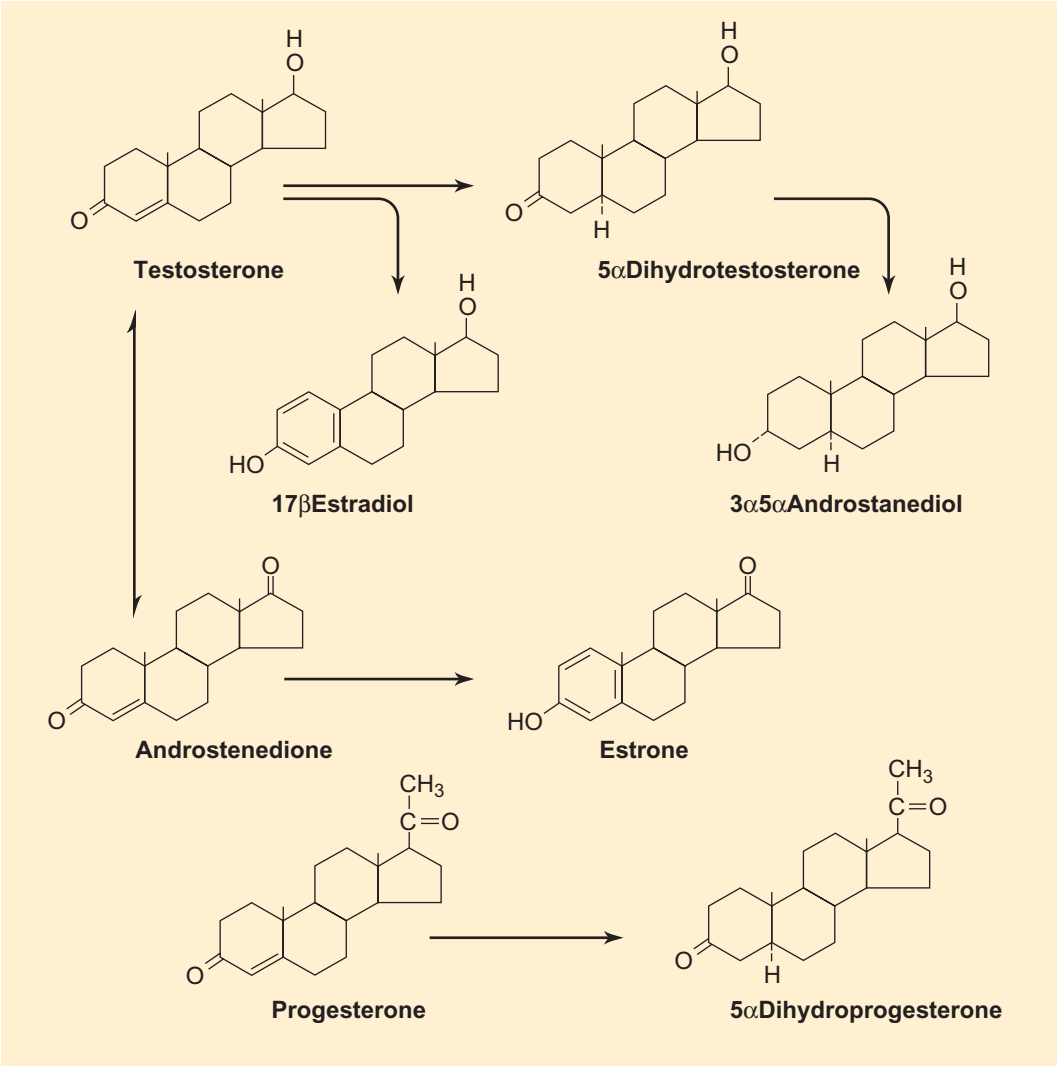


FIGURE 55-4 **Some steroid transformations that are carried out by neural tissue.**

of this steroid (Becker et al., 2002). 5β-DHT is a metabolite of testosterone formed in the avian CNS, as is 5α-DHT. 5β-DHT is inactive toward sexual behavior and is believed to represent an inactivation pathway for testosterone.

The Aromatization of Testosterone

To form estradiol, and of androstenedione to form estrone (Fig. 55-4), has been described in brain tissue *in vitro* and *in vivo* (Becker et al., 2002; Hojo et al., 2004)]. Aromatization is higher in hypothalamus and limbic structures than in cerebral cortex or pituitary gland, and, in noncastrated animals, it is higher in the male than in the female brain. Aromatization has been found in reptile and amphibian brain as well as in mammalian brain (Becker et al., 2002). The capacity to aromatize testosterone and related androgens, therefore, may be a general property of vertebrate brains. The functional role of aromatization has been studied most extensively in the rat. Male sexual behavior is facilitated by estradiol (Becker et al., 2002), and testosterone facilitation of male sexual behavior can be blocked by a steroidal inhibitor of aromatization (Becker et al., 2002; McEwen, 1981). There are indications that a similar situation exists in birds, amphibians and reptiles; that is, testosterone and estradiol can stimulate male and female heterotypical sexual behavior. Curiously, not all mammals are like the rat; for example, male sexual behavior of guinea pig and rhesus monkey is restored by the nonaromatizable androgen DHT (Becker et al., 2002; McEwen, 1981).

Both aromatization and 5α-reductase are regulated by gonadal steroids. In mammals such as the rat, it is principally the neural aromatase activity that is upregulated by androgens acting via neural androgen receptors (Becker et al., 2002). In birds, both neural aromatase and 5α-reductase are induced by testosterone, and this regulation provides a way by which androgens can regulate CNS hormone sensitivity without regulating receptor number (Becker et al., 2002).

Both estrogens and glucocorticoids appear to act on brain cells without first being metabolized because both [^{3}H]estradiol and [^{3}H]corticosterone are recovered unchanged from their cell nuclear binding sites in brain (McEwen, 1981). However, estradiol is subject to conversion to the catechol

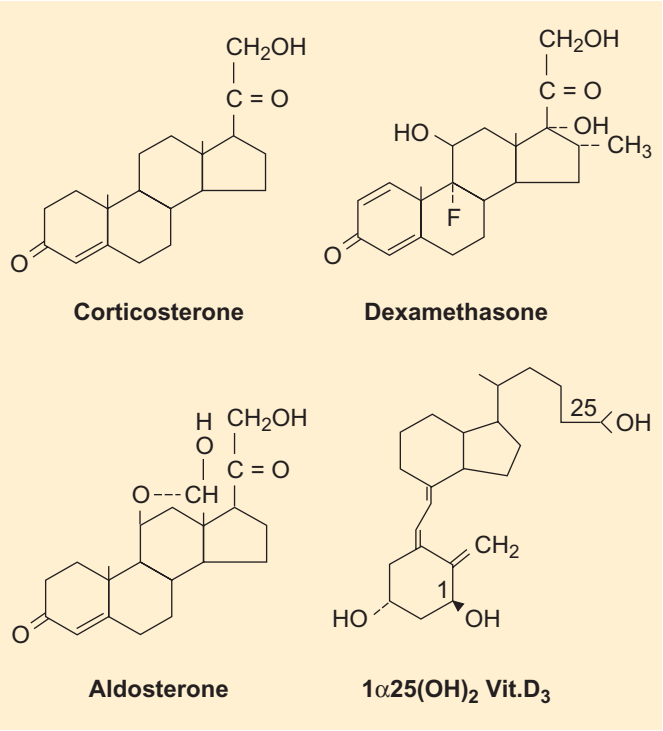


FIGURE 55-5 **Formulas of four steroid hormones.**

estrogen 2-hydroxyestradiol, and this metabolite is both a moderately potent estrogen via intracellular estrogen receptors, as well as an agent capable of interacting with cell-surface receptors such as those for catecholamines, albeit at fairly high concentrations (McEwen, 1999) (see Ch. 14). Glucocorticoids are inactivated by the enzyme 11-hydroxysteroid-dehydrogenase-2 (e.g. cortisol is converted to cortisone) and cortisone is re-activated to cortisol by the enzyme 11-hydroxysteroid-dehydrogenase-1 [(Seckl & Walker, 2001). Organs such as the liver and brain have the ability to carry out this reactivation, which can lead to obesity via the liver and cognitive impairment via the brain (Flier, 2001).

Vitamin D

Prior to acting in the brain, is converted to an active metabolite, 1,25-dihydroxy vitamin D_3, by enzymes in the liver and kidney (Garcion, et al., 2002) (see Fig. 55-5). The nervous system is also capable of cleaving the side chain from cholesterol to generate the same initial series of steroids (Garcion, et al., 2002) that are produced by the adrenals and gonads, namely, pregnenolone, dehydroepiandrosterone and progesterone (Fig. 55-6). In addition, neural tissue converts progesterone via reduction of the delta 4–5 double bond and reduction of the 3 keto group to 3,5α-pregnanolone, which is active on the chloride channel of the $GABA_A$ receptor (Baulieu, 1991) (Fig. 55-7). Glial cells are believed to be the primary sites of both cholesterol side-chain cleavage and generation of pregnanolone from progesterone (Baulieu, 1991). There is also now evidence that the brain is capable of generating testosterone and estradiol from cholesterol via a process that is enhanced by excitatory neurotransmission (Hojo, et al., 2004).

While steroids generated in the brain have been referred to as 'neurosteroids'(Reddy, 2010), another useful term is 'neuroactive steroids' to refer to all steroids that affect brain function via any mechanism and irrespective of site of formation. The term neuroactive steroid also has been used to describe neuroactive steroid drugs.

Genomic receptors for steroid hormones have been clearly identified in the nervous system

The detection of intracellular, DNA-binding steroid receptors became possible with the introduction of tritium-labeled steroid hormones of high specific radioactivity: 20–25 Ci/mmol at each labeled position. The limited number of these sites had escaped detection using steroids labeled with ^{14}C at a much lower specific radioactivity. Tritium labeling also permitted histological localization of steroid receptors because the high spatial resolution of ^{3}H, 1–2 μm in light-microscopic autoradiography, allows cellular and even cell nuclear localization of the radioactivity. More recently, ^{125}I labeled estradiol was used to demonstrate non-nuclear sites of estrogen receptor localization using electron-microscopy combined with autoradiography (Milner et al., 2008)

Cell fractionation procedures were fundamental to the biochemical identification of steroid and thyroid hormone receptors in brain as well as in other tissues. Isolation of highly purified cell nuclei from small amounts of tissue from discrete brain regions generally is accomplished with the aid of a nonionic detergent, such as Triton X-100 (McEwen, 1999). Cytosolic fractions of brain tissue, prepared by centrifugation of homogenates at 105,000 g for 60 min, contain the soluble steroid-hormone-binding proteins, and a variety of methods intended to separate bound from unbound steroid have been used for measuring their binding activity (McEwen, 1981; McEwen, 1999)]. The most commonly employed are gel filtration chromatography and sucrose-density-gradient centrifugation. Dextran-coated charcoal or Sephadex LH20 are frequently used because they effectively absorb unbound steroid and leave intact the complexes between steroid and putative receptor. Other methods, such as gel electrophoresis and precipitation of putative receptor material with protamine sulfate, have more restricted uses.

The objective of such studies is to measure the affinity, capacity and specificity of the hormone–receptor interaction (McEwen, 1981; McEwen, 1999). Measurements of affinity and capacity are accomplished with equilibrium binding analysis. Specificity is based on competition between the labeled and various unlabeled ligands for binding sites.

Because the nervous system is highly heterogeneous from the standpoint of many neurochemical characteristics, including steroid and thyroid hormone receptors, the most useful techniques for mapping these receptors have been histochemical. Steroid autoradiography was the first such method. With purification of receptors and generation of antibodies, immunocytochemistry has been added as a tool. Cloning of steroid and thyroid receptors has opened the way to mapping of receptor mRNAs via *in situ* hybridization histochemistry.

Several criteria determine whether a steroid-hormone-binding site is a putative receptor. First, the

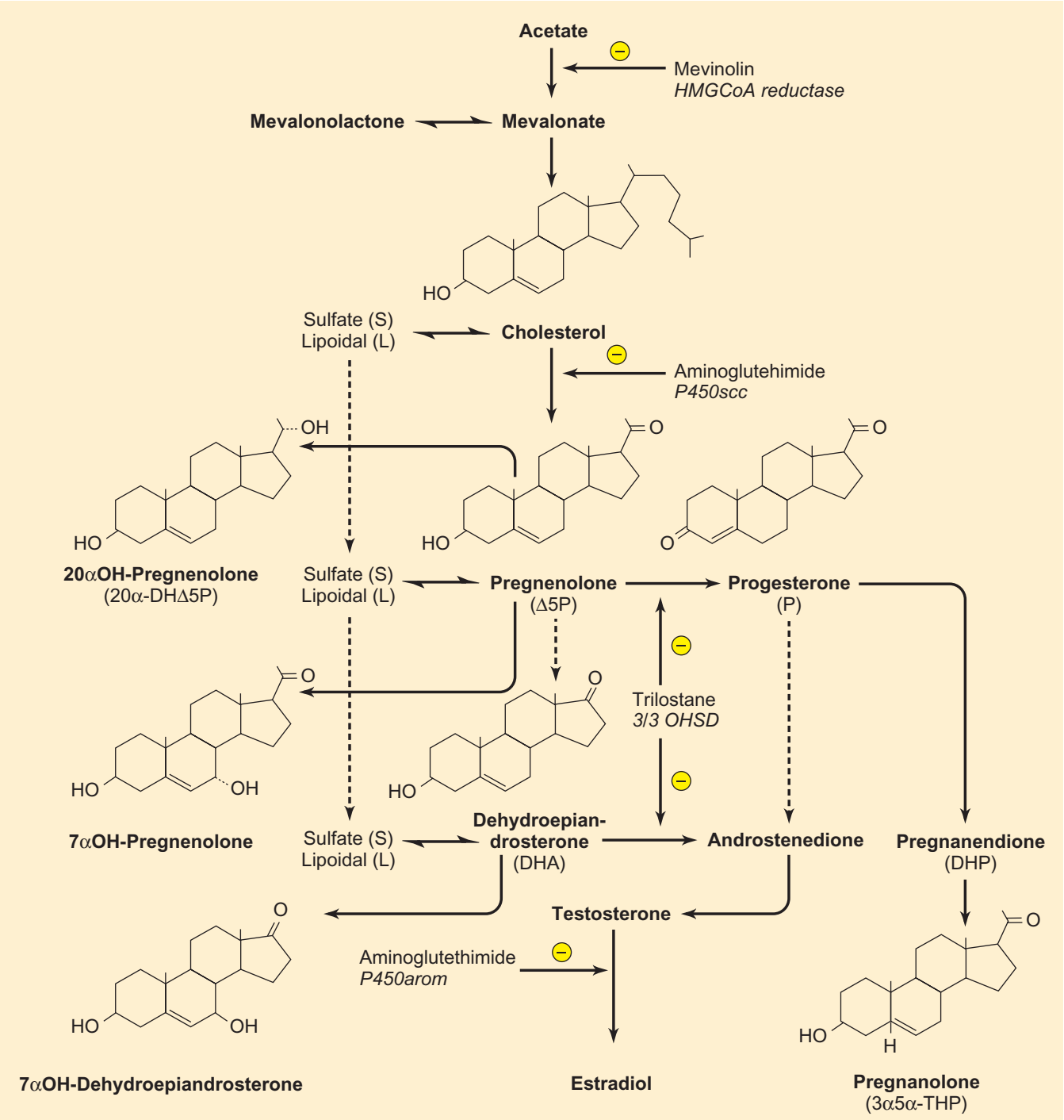


FIGURE 55-6 **Biosynthesis/metabolism of steroids in the CNS.** The conversion of delta5P to dehydroepiandrosterone (DHA) is postulated but not demonstrated. D5P and DHA inhibit and 3α,5α-THP potentiates $GABA_A$ receptor function, as summarized in Figure 55-7. *Solid arrows* indicate demonstrated pathways; *dotted arrows* indicate possible pathways. Metabolic inhibitors of enzymes are indicated by: (Redrawn from Baulieu (1991), with permission.)

steroid- hormone-binding site must be present in hormone-responsive tissues or brain regions, and absent from nonresponsive ones. Second, it should bind steroids that are either active agonists or effective antagonists of the hormone effect, and should not bind steroids that are inactive in either sense.

Understanding of the intracellular localization of steroid receptors has gone through a number of phases, beginning with the view that receptors translocated from cytoplasm to nucleus in the presence of hormone. Indeed, with the exception of thyroid hormone receptors, which are exclusively

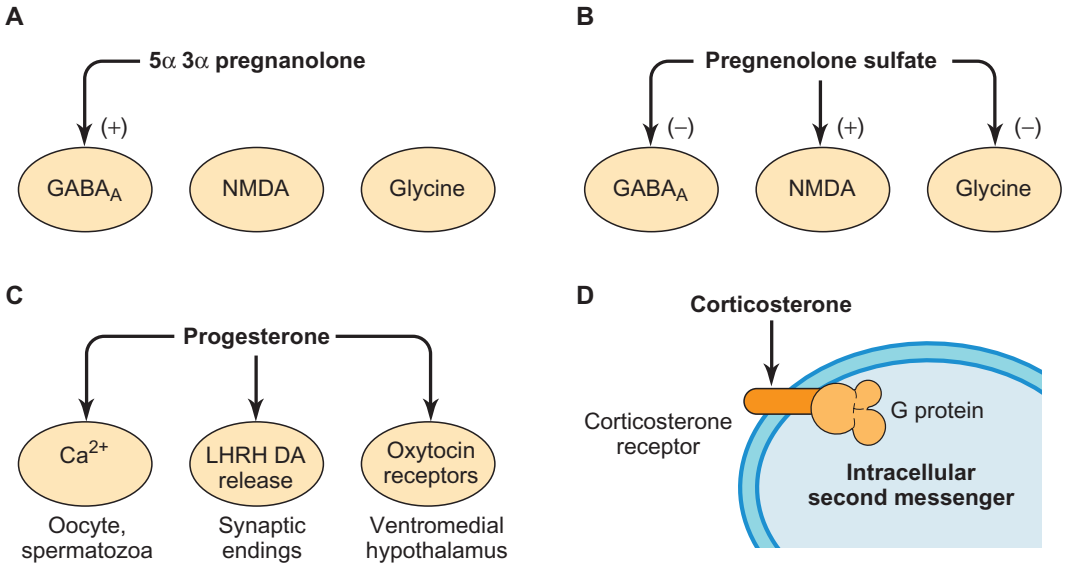


FIGURE 55-7 **Schematic summary of four ways in which steroids affect cell-surface-mediated events and neuronal activity by nongenomic mechanisms. (A)** Activation of $GABA_A$ receptor by 5α3α-pregnanolone. **(B)** Activation of NMDA receptor and inhibition of $GABA_A$ and glycine receptors by pregnenolone sulfate. **(C)** Activation of Ca^{2+} mobilization in oocytes and spermatozoa by progesterone; the same is postulated to happen in certain synaptic endings and may be involved in the rapid, direct effect of progesterone on the oxytocin receptor. **(D)** Corticosterone binds to a cell surface receptor linked to a G protein, and presumably through it to a second-messenger system, in some brain cells. *DA*, dopamine; *LHRH*, luteinizing hormone releasing hormone; *NMDA*, *N*-methyl-D-aspartate.

nuclear in location, cell fractionation studies have revealed that in the absence of hormone, steroid receptors are extracted in the soluble or cytosolic fraction. However, when steroid is present in the cell, many occupied receptors are retained by purified cell nuclei. Histological procedures, such as immunocytochemistry, have confirmed the largely nuclear localization of occupied receptors, but they also have revealed a nuclear localization of receptors in the absence of hormone in the tissue. This is true for estrogen, progestin and androgen receptors, but the mineralocorticoid and glucocorticoid receptors show a cytoplasmic localization in the absence of hormone. Thus, steroid receptors may exist in nuclei in a loose association that is disrupted during cell fractionation. This is not an uncommon situation for many constituents of cell nuclei [7].

At the same time, there is evidence for membrane- associated steroid receptors that are coupled to second messenger systems . And, at the electron microscopic level, immunocytochemistry with antibodies to intracellular estrogen, progestin and androgen receptors has revealed membrane-associated localization in dendrites, presynaptic terminals, mitochondria, axons and glial cell processes. (see below).

INTRACELLULAR STEROID RECEPTORS: PROPERTIES AND TOPOGRAPHY

Steroid hormone receptors are phosphoproteins that have a DNA-binding domain and a steroid-binding domain

All steroid receptors have a molecular weight of 55,000–120,000. The state of phosphorylation appears to influence functional activity.

Estradiol

The first neuroactive steroid receptor type to be recognized was that for estradiol (McEwen, 1981; McEwen, 1999). *In vivo* uptake of [^{3}H]estradiol, and binding to cell nuclei isolated from hypothalamus, pituitary and other brain regions, revealed steroid specificity closely resembling that of the uterus, where steroid receptors were first discovered (McEwen, 1981; McEwen, 1999). Cytosolic estrogen receptors isolated from pituitary and brain tissue closely resemble those found in uterus and mammary tissue. A hallmark of the estrogen receptor is its existence as an aggregate of subunits that dissociate during steroid-induced transformation to the DNA-binding nuclear form of the receptor. This part of the estrogen-receptor complex was cloned from human breast cancer cells and consists of a 65- to 70-kDa hormone and DNA-binding subunit (McEwen, 1999). The dissociation constant of estradiol binding is approximately 0.2 nM.

Cell nuclear estrogen receptors are found in the adult pituitary, hypothalamus, preoptic area and amygdala. They are principally in neurons, although glial cells also may express these receptors in some brain regions (McEwen & Alves, 1999). The developing rat brain expresses nuclear estrogen receptors in cerebral cortex and hippocampus, but these receptors largely disappear as the brain matures (Goy & McEwen, 1980; McEwen, 1983; McEwen & Alves, 1999). A second form of the estrogen receptor, the β-estrogen receptor, is similar to the α form in affinity and specificity but somewhat different in tissue distribution (Kuiper et al., 1998). Non-nuclear expression of both estrogen receptor types is described below.

Progesterone

Receptors in brain were detected using the synthetic progestin R5020 (promegestrone; 17α, 21-dimethyl-19-norpregna-4,9-diene-3,20-dione), which has a high affinity for

the progestin receptor, with a K_d of 0.4 nM (McEwen, 1999). The progestin receptor, cloned from chick oviduct, consists of a steroid- and DNA-binding subunit of 108 kDa, although one 79-kDa subunit has also been described (McEwen, 1999). Progestin receptors with similar properties are found in pituitary, reproductive tract and most estrogen receptor-containing brain regions; these receptors are inducible by estrogen treatment (McEwen, 1999). There are also progestin receptor sites in brain areas lacking estrogen receptors, such as the cerebral cortex of the rat; these receptors are not induced by estradiol treatment. Nevertheless, such receptors resemble those induced by estradiol (Milner, et al., 2008)]. Another inducer of progestin receptors in brain is testosterone, which works through its conversion to estradiol via aromatization (McEwen, 1999). Progesterone acts rapidly to induce feminine sexual behavior, termed lordosis, in female rats that have been primed with estradiol to induce progestin receptors (McEwen, 1999). The principal site of estradiol and progesterone action is the ventromedial nucleus of the hypothalamus (McEwen et al., 1987). Non-nuclear expression of progestin receptors is described below.

Androgen

Receptors have a steroid-binding subunit estimated to be 120 kDa (McEwen, 1999). The estimated K_d for active androgens is approximately 1–2 nM. Androgen receptors are widely distributed in brain and pituitary tissue, although the highest concentrations are found in hypothalamus, preoptic area and limbic brain tissue. Androgen receptors are deficient in the androgen-insensitivity (Tfm) mutation, and animals with this mutation show defects in sexual behavior, juvenile rough-and-tumble play behavior and certain aspects of neuroendocrine function, thus indicating the actions of testosterone that are mediated by androgen receptors, as opposed to those mediated by aromatization of testosterone to estradiol (see above) and estrogen receptors. Non-nuclear expression of androgen receptors is described below

Glucocorticoid

Adrenal steroid receptors have been subdivided into two categories, one of which is the classical glucocorticoid receptor [(De Kloet et al., 1998; McEwen, 1999). This receptor, cloned from human and rat sources, consists of a steroid- and DNA-binding subunit of 95 kDa (De Kloet, et al., 1998; McEwen, 1999). Such receptors, which have dissociation constants of 4–5 nM for glucocorticoids, are widely distributed across brain regions and are found in neurons and glial cells (De Kloet, et al., 1998; McEwen, 1999) Non-nuclear expression of glucocorticoid receptors is described below

Mineralocorticoid

The other type of glucocorticoid receptor is similar to the mineralocorticoid receptor originally described in the kidney (De Kloet, et al., 1998; McEwen, 1999). In the brain, receptors of this type bind the glucocorticoid corticosterone with high affinity, having a K_d of approximately 1 nM, and they are responsible for the high uptake of tracer levels of [^{3}H] corticosterone by the hippocampus (De Kloet, et al., 1998; McEwen, 1999). These corticosterone receptors, which are found in high concentrations in the hippocampus but are also widely distributed in other brain regions at lower concentrations, may be involved in mediating the effects of diurnally varying concentrations of corticosterone (De Kloet, et al., 1998; McEwen, 1999; McEwen, et al., 1993). Uptake of [^{3}H]aldosterone by brain tissues reveals two types of binding sites: those in the hippocampus, which can be occupied preferentially by corticosterone, and those more diffusely distributed in the brain, which appear to retain [^{3}H] aldosterone preferentially in the presence of the normally higher concentrations of corticosterone (McEwen, 1999). The reasons for this selectivity of an enzyme, 11β-hydroxysteroid dehydrogenase, are that, at least in the kidney-collecting tubules, it converts corticosterone to an inactive metabolite and allows aldosterone access to the mineralocorticoid receptors (Seckl & Walker, 2001).

Vitamin D

Is a steroid hormone, production of which by the body requires the action of light. Therefore, it is often necessary to provide some vitamin D in the diet (Garcion, et al., 2002). Moreover, vitamin D is converted by the kidney and liver to the active metabolite 1,25-dihydroxyvitamin D3 (Fig. 55-5) (Garcion, et al., 2002). Vitamin D3 receptors consist of a hormone- and DNA-binding subunit of 55 kDa (McEwen, 1999; Simerly et al., 1990). Receptor sites for 1,25-dihydroxyvitamin D3 are found in pituitary and brain, especially in the forebrain, hindbrain and spinal cord neurons (Garcion, et al., 2002). In the brain, one site containing vitamin D3 receptors, the bed nucleus of the stria terminalis, responds to exogenous 1,25-dihydroxyvitamin D3 with an induction of choline acetyltransferase, even though the calcium-binding protein that is regulated by vitamin D3 in the intestine is not regulated by this hormone in the brain (Garcion, et al., 2002).

MEMBRANE STEROID RECEPTORS AND SIGNALING PATHWAYS

The known rapid effects of some steroid hormones on neuronal excitability are difficult to explain solely in terms of genomic actions (Kelly & Levin, 2001). Instead, some type of membrane receptor interaction is inferred. Indeed, several types of interaction between neuroactive steroids and neural membranes have been described. Direct binding assays have revealed membrane sites for glucocorticoids and gonadal steroids and one instance of a membrane receptor coupled to a G protein (Fig. 55-7). Additionally, indirect binding assay results have implied interaction of the catechol estrogens with dopamine and with adrenergic receptors (McEwen, 1999). Moreover, studies with specific antibodies at the electron microscope level have revealed estrogen, progestin and androgen receptors with epitopes of the nuclear forms of these receptors in dendrites, presynaptic terminals and glial cell processes (Milner, et al., 2008), and a membrane receptor for progesterone has been cloned from oocytes (Zhu et al., 2003). Besides expression of a form of the nuclear glucocorticoid receptor in the post-synpatic density of amygdala neurons, a G-protein coupled transmembrane glucocorticoid receptor is recognized to drive formation of

endocannabinoids in neurons (ref). Finally, deletion of the nuclear vitamin-D receptor deprives cells of rapid membrane actions of vitamin D, indicating that this receptor may serve a dual function [23].

Progesterone produces direct membrane effects via G-protein coupled and other membrane associated recepors [16]. These include actions that promote maturation of spermatozoa as well as oocytes and facilitation of the release of neurotransmitters such as dopamine and LH-releasing hormone (LHRH) (Fig. 55-7). Membrane actions of progesterone also activate oxytocin receptors in the hypothalamus in a way that enables oxytocin to turn on sexual behavior in the estrogen-primed female rat [3].

Estradiol activates a variety of signaling pathways via membrane-associated receptors in many cell types (Kelly & Levin, 2001) In neurons, rapid actions of estradiol via activation of phosphorylation of Akt and Lim kinase stimulate, respectively, translation of a key protein, PSD-95, involved in synapse formation as well as actin polymerization (Akama & McEwen, 2003).

Besides progestin action via G-protein coupled receptors (Atsak et al., 2011; Di, Maxson et al., 2009; Hill et al., 2010), there are now indications for the interaction of progesterone metabolites with the Cl^- channel of the $GABA_A$ receptor (Fig. 55-7) (Reddy, 2010). The A-ring-reduced steroids, especially those with the 5α,3α configuration, are particularly active on the $GABA_A$ receptor (Baulieu, 1991). By facilitating chloride-channel opening, these steroids produce anesthetic, anxiolytic and sedative–hypnotic effects (see Ch. 18).

Another neurosteroid (Fig. 55-6), pregnenolone sulfate (PS), produces effects that in many ways antagonize those of the steroids that open the $GABA_A$ receptor Cl^- channel (Baulieu, 1991; Reddy, 2010). PS in micromolar concentrations inhibits the $GABA_A$ receptor and the inhibitory glycine receptor, and facilitates activity of the excitatory *N*-methyl-D-aspartate (NMDA) receptor (Fig. 55-7). It is unclear, however, whether these effects are physiologically relevant, since the PS concentration needed to produce them is rather high. However, PS is produced locally in the brain and may reach sufficiently high concentrations in some compartments within the nervous system.

None of these findings undermines the importance of the intracellular genomic actions of steroids. Rather, they increase the richness of the cellular actions of steroid hormones and raise the possibility that there may be connections between genomic and nongenomic actions of steroids. For example, genomic action may induce receptors that mediate nongenomic effects. Moreover, the activation of oxytocin receptors by progesterone is dependent on the ability of estrogen priming to induce the formation of new oxytocin receptors via a genomic mechanism; these receptors are then transported along dendrites to sites where the progesterone action occurs at the membrane level (McEwen, 1981).

BIOCHEMISTRY OF THYROID HORMONE ACTIONS ON BRAIN

Like steroid hormones, thyroid hormones interact with receptors to alter genomic activity and affect the synthesis of specific proteins during development. As with testosterone and progesterone, metabolic transformation of thyroxine (T_4) is critical to its action. Moreover, as with steroid hormones, thyroid hormones alter brain functions in adult life in ways that both resemble and differ from their action during development (Bauer et al., 2003; Bernal, 2002; Forrest et al., 2002; Konig & Moura Neto, 2002).

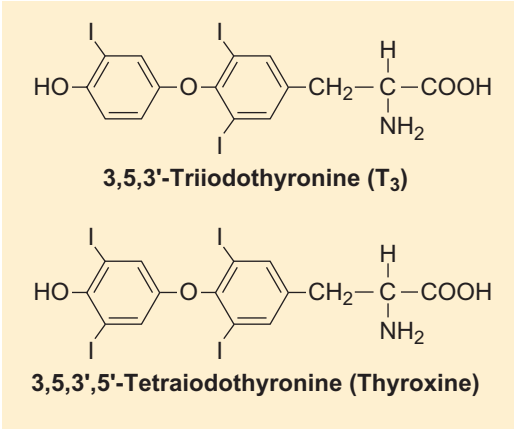


FIGURE 55-8 **Structures for thyroxine (T4) and triiodothyronine (T_3).**

The initial step after cellular uptake of T_4 is metabolic transformation to 3,5,3′-tri-iodothyronine (T_3) (Fig. 55-8), which interacts with cytosolic and nuclear receptors, as well as with synaptosomal membrane binding sites of unknown function (Bernal, 2002). Cytosolic receptors are proteins of 70 kDa that do not appear to undergo translocation to cell nuclei, nor do they appear to be nuclear proteins that have leaked out of cell nuclei during cell rupture; nuclear receptors are proteins of 50–70 kDa that have both DNA- and hormone-binding domains (Bernal, 2002; Forrest, et al., 2002; Konig & Moura Neto, 2002).

Nuclear T_3 receptors are present in higher levels during neural development than they are in adult life. In human fetal brain, nuclear T_3 receptors increase in concentration from 10 weeks of gestation to the sixteenth week, when neuroblast multiplication is high (Bernal, 2002; Forrest, et al., 2002). Glial cells, as well as neurons, contain nuclear T_3 receptors (Bernal, 2002; Forrest, et al., 2002). Functionally, many neurons develop prior to the appearance of significant T_3 receptor levels and, therefore, appear to be independent of large-scale thyroid influence (Bauer, et al., 2003; Bernal, 2002; Forrest, et al., 2002; Konig & Moura Neto, 2002). Other neurons, such as those in the cortex and cerebellum, show a more profound dependence on thyroid function (Bernal, 2002). Although thyroid hormone affects the number of replicating cells in the external granular layer of the developing cerebellum, it is not possible to conclude that T_3 directly affects the mechanism of cell replication (Bernal, 2002). Rather, the most pronounced effect of hypothyroidism is a hypoplastic neuropil, with shortened dendrites and fewer spines. It has been shown that a major effect of T_3 involves development of the neuronal cytoskeleton (Bauer, et al., 2003; Bernal, 2002; Forrest, et al., 2002; Konig & Moura Neto, 2002). Proteins, such as microtubule-associated protein (MAP2) and tau (see Ch. 6), which are polymorphic and affect

microtubular assembly, are differentially affected by T_3 absence or excess (Bauer, et al., 2003; Bernal, 2002)]. Energy metabolism is another aspect of brain function affected by thyroid hormones (Lopez et al., 2010; Straub et al., 2010).

Developmentally, thyroid hormones interact with sex hormones such that hypothyroidism prolongs the critical period for testosterone-induced defeminization (see below); in contrast, the hyperthyroid state prematurely terminates the sensitivity to testosterone. Undoubtedly, an important link in these and other effects is synapse formation. Hypothyroidism increases synaptic density, at least transiently. Interesting parallels with synapse formation are reported for learning behavior in rats; neonatal hypothyroidism impairs learning ability, whereas hyperthyroidism accelerates learning initially, followed by a decline later in life (McEwen, 1981).

The adult brain is endowed with nuclear as well as cytosolic and membrane T_3 receptors that have been visualized by autoradiography and studied biochemically (Bakker et al., 2002; De Vries et al., 2002; Matsumoto et al., 1995; McCullough et al., 2003). Both neurons and neuropil are labeled by [^{125}I]T_3, and the labeling is selective across brain regions. Functionally, one of the most prominent features of neural action of thyroid hormone in adulthood is subsensitivity to norepinephrine as a result of a hypothyroid state (Bauer, et al., 2003). These changes may be reflections of loss of dendritic spines in at least some neurons of the adult brain. Clinically, thyroid hormone deficiency increases the probability of depressive illness, whereas thyroid excess increases the probability of mania (Ch. 60) in susceptible individuals (Bauer, et al., 2003).

DIVERSITY OF STEROID-HORMONE ACTIONS ON THE BRAIN

Steroid-hormone effects on the brain link the environment surrounding the organism with the genome of target brain cells through the process of variable genomic activity (McEwen, 1981; McEwen, 1999; McEwen, 1999). By this we mean that an organism experiences light, dark, heat, cold, fear and sexual arousal. These experiences influence hormonal secretion, and these hormones in turn act on the genome of receptor-containing brain cells to alter their functional state. The genome of brain cells, like that of other cells of the body, is continually active from embryonic life until death and continually responsive to intra- and extracellular signals. This activity can be seen from the high rates of RNA metabolism in neurons. The differential influence of steroid hormones on variable genomic activity is evident from studies showing rapid and brain region-specific induction of ribosomal and mRNA, as well as changes in cell nuclear diameter and chromatin and structure (Becker et al., 2002; McEwen, et al., 1987). However, variable genomic activity changes qualitatively with the state of differentiation of the target cells: embryonic neurons show growth responses that result in permanent changes in circuitry, whereas adult neurons show impermanent responses. Under other circumstances, the same hormonal signals can promote damage and even neuronal loss; under still other conditions, adult neurons can be stimulated by treatment with hormones to grow and repair the damage.

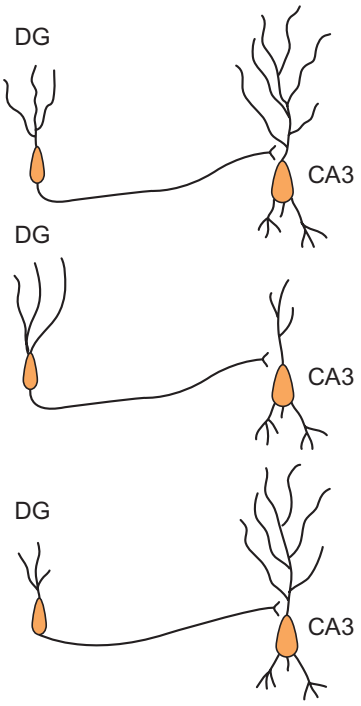
During development, steroid-hormone receptors become evident in target neurons of the brain

These receptors appear within several days of final cell division (Goy & McEwen, 1980; McEwen, 1983). Whether some receptors are also present in dividing neuronal precursors is not clear. After they have appeared, these receptors mediate a variety of developmental actions. For example, glucocorticoids direct differentiation of adrenergic/cholinergic neurons of the autonomic nervous system to develop in the adrenergic direction. Glucocorticoids also increase the number of epinephrine-containing, small, intensely fluorescent cells, often referred to as SIF, in autonomic ganglia and are required for the normal postnatal ontogeny of serotonin neurons in the forebrain (McEwen, 1999). These effects may not all be direct but may involve hormonal modulation of growth factors produced by other cells surrounding the developing neurons.

Gonadal hormones, however, are involved in sexual differentiation of the reproductive tract and brain (Becker et al., 2002; Goy & McEwen, 1980; McEwen, 1983). Mammals, among which animals have X and Y chromosomes, undergo sexual differentiation under the impetus of testosterone secreted by the testes during a period of perinatal life; in humans, this period is in midgestation, while in rats it is from the end of gestation into neonatal life. Key features of sexual behavior in birds are determined in the reverse manner, in keeping with the fact that the female has the chromosomal heterogeneity: females produce either estradiol or testosterone, either of which feminizes the brain, which otherwise would develop a masculine pattern in the absence of gonadal steroids (Becker et al., 2002; McEwen, 1983).

As for the mechanisms of sexual differentiation, we must consider the metabolism of the hormone receptor types involved and the primary-receptor-mediated events. Testosterone, as noted above, is a prohormone that is converted into either 5α-DHT or estradiol within the brain; these products exert effects on brain sexual differentiation via androgen and estrogen receptors, respectively (Goy & McEwen, 1980; McEwen, 1983). Masculinization of sexual and aggressive behavior involves either 5α-DHT alone or a combination of 5α-DHT and estradiol acting on different cells. Besides masculinization, there is in some mammals a process of defeminization, in which feminine responses that would develop in the absence of testosterone are suppressed by its presence during the critical period. Conversion to estradiol appears to be involved in this process (Goy & McEwen, 1980; McEwen, 1983). Progesterone plays no major role in brain sexual differentiation, but it does have the ability to antagonize actions at both androgen and estrogen receptors and, thus, can moderate the degree of masculinization and defeminization.

As to the primary developmental actions of testosterone, growth and differentiation appear to be involved. Testosterone or estradiol stimulates outgrowth of neurites from developing hypothalamic neurons that contain estrogen receptors (Goy & McEwen, 1980; McEwen, 1983). This is believed to be one of the principal aspects of testosterone action that increases the number and the size of neurons within specific hypothalamic nuclei in males, compared to females 5α-DHT may have a similar effect on



CA3 pyramidal neurons receive the mossy fiber input from the granule neurons of the dentate gyrus. This connection is part of the 3-cell circuit of the hippocampus that is believed to be involved in learning and memory.

Chronic corticosterone treatment or repeated restraint stress promotes atrophy of apical dendrites of CA3 pyramidal neurons.

Adrenalectomy causes neurons of the dentate gyrus to die and to be replaced by new neurons, whereas pyramidal neurons are unaffected. Low levels of adrenal steroids prevent this and stabilize the dentate granule neuron population; they do this via Type I adrenal steroid receptors.

FIGURE 55-9 **Summary of adrenal steroid effects on neurons of the hippocampus in male rats, illustrating their ability to protect and stabilize the population of the dentate granule neurons and to potentiate damage caused by excitatory amino-acid release upon pyramidal neurons.** *DG*, dentate gyrus. From (McEwen, 1991), with permission.

androgen-sensitive neurons. Differentiation of target neurons also occurs; in adult brain tissue, hormones like estradiol can evoke responses that differ between adult male and female rats (Becker et al., 2002; Goy & McEwen, 1980; McEwen, 1983).

Because of new research with genetic manipulations, there are other aspects of sexual differentiation that must now be incorporated into our thinking. First is that female sexual differentiation depends in part on aromatization of androgens, since knockout of the aromatase gene deprives the female of a number of normal sociosexual behavior patterns in adult life (Bakker, et al., 2002). Second, is that genetic sex plays a role in brain and body sexual differentiation; more specifically, genes on the Y chromosome are believed to contribute to sex differences in the midbrain dopaminergic system, among other brain regions (De Vries, et al., 2002).

The response of neural tissue to damage involves some degree of structural plasticity, as in development

Collateral growth and reinnervation of vacant synaptic sites is facilitated in some cases by steroid hormones (Matsumoto, et al., 1995). In the hypothalamus, estrogen treatment after knife cuts that destroy certain inputs promotes increases in the number of synapses. In the hippocampus, glucocorticoid treatment promotes homotypical sprouting of serotonin fibers to replace damaged serotonin input. It has also been noted that androgens enhance the regrowth of the severed hypoglossal nerve. One interpretation of these steroid effects is that injury reactivates programs of steroid-responsive genomic activity that normally operate during the phase of synaptogenesis in early development (Matsumoto, et al., 1995).

Estrogens are also neuroprotective against ischemic damage (McEwen & Alves, 1999), and aromatization of androgens to estrogens plays a role even in females, where knockout of the aromatase gene increases the vulnerability of female mice to stroke damage by a process that is prevented by estradiol administration (McCullough, et al., 2003).

Another aspect of neuroprotection by steroids is stabilization of neurons against death and replacement. In the dentate gyrus of both the neonate and the adult rat, neurons are born and die; rates of both birth and death are increased by adrenalectomy, and these increases are prevented by low doses of adrenal steroids acting via mineralocorticoid receptors, which also exert trophic effects to promote growth and branching of dendrites of existing granule neurons (Cameron & Gould, 1996) (Fig. 55-10). Regulation of the turnover rate of dentate gyrus neurons may provide the hippocampal formation of the adult with the potential to increase and decrease its volume and functional capacity, as occurs in relation to seasonal or other long-term changes in the environment. This process also occurs in the developing dentate gyrus (Cameron & Gould, 1996).

Activation and adaptation behaviors may be mediated by hormones

Hormonal secretion by the adrenals and gonads is controlled by endogenous oscillators, ie. Clock genes (see

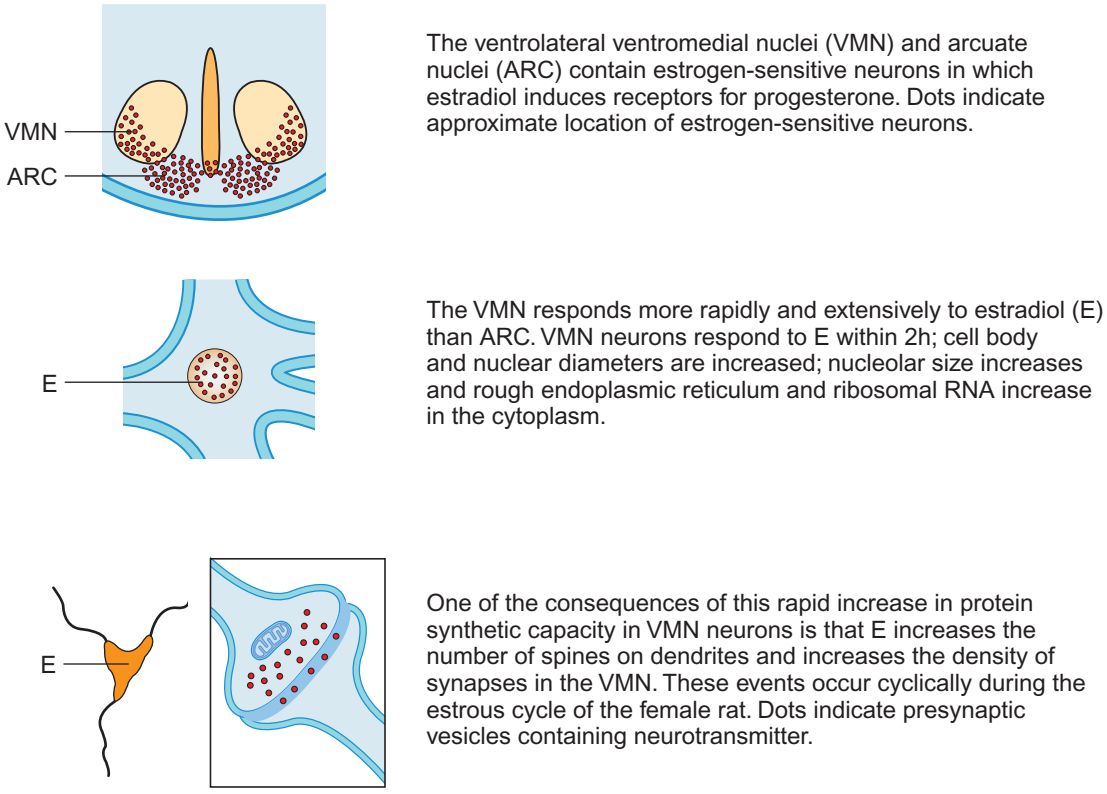


FIGURE 55-10 **Summary of estrogen effects on the ventromedial nuclei related to its activation of female sexual behavior in the rat.** *E*, estrogen. From (McEwen, 1991) with permission.

chapter 57), that can be entrained by environmental cues such as light and dark. The actions of cyclically secreted hormones on behavior and brain function are referred to as activational effects. In addition to the cyclic mode, there is another mode of secretion initiated by such experiences as stress, fear and aggressive and sexual encounters. In this case, actions of adrenal steroid hormones secreted in response to experience lead to adaptive brain responses, which help the animal cope with stressful situations (De Kloet, et al., 1998; McEwen, 1999; McEwen, et al., 1993). The activational and adaptational effects are largely reversible and involve a variety of neurochemical changes, most of which appear to be initiated at the genomic level. For example, estradiol is secreted cyclically during the estrous cycle in the female rat and triggers the surge of LH, which induces a surge of progesterone from the ovary. Progesterone, in turn, stimulates proceptivity and enhances sexual responsiveness to the male rat (Becker et al., 2002).

Estradiol action to promote feminine sexual behavior in the rat involves a cascade of induced protein synthesis in specific hypothalamic neurons accompanied by morphological changes indicative of increased genomic activity (Becker et al., 2002; McEwen, et al., 1987; Pfaff, 1980). Among the induced proteins are receptors for progesterone (see above), crucial for activating sexual behavior; receptors for acetylcholine and oxytocin that are active in enabling the hypothalamic neurons to respond to afferent input; proteins that are axonally transported from the hypothalamus to the midbrain, where they may be involved in neurotransmission; and structural proteins that contribute to formation of new synapses that come and go during the estrous cycle (Becker et al., 2002; McEwen, et al., 1987; Pfaff, 1980) (Fig. 55-10).

Estradiol also induces synapses in the hippocampus and this contributes to enhanced capacity for learning and memory that is dependent on the hippocampus. Estradiol exerts many other nonreproductive actions on the brain, such as fine motor coordination, seizure susceptibility, mood, protection from ischemic damage. Many of these actions occur in brain regions that show little, if any, nuclear estrogen receptors, and the nongenomic estrogen receptor described above are involved (McEwen & Alves, 1999).

Adrenal steroids secreted in the diurnal cycle are responsible for reversibly activating exploratory activity, food-seeking behavior, carbohydrate appetite and synaptic efficacy (Korte, 2001; Lupien & McEwen, 1997; McEwen, et al., 1993). These appear to do so by acting on the hippocampus, where there are many mineralocorticoid and glucocorticoid receptors (see above). Adaptational effects of adrenal steroids that result from stress appear to operate via the classical glucocorticoid receptor found not only in hippocampus but also in other brain regions (De Kloet, et al., 1998; McEwen, 1999). Changes in synaptic vesicle proteins, high-affinity GABA transport, neurotransmitter-stimulated cAMP formation and central serotonin and noradrenergic sensitivity accompany repeated glucocorticoid elevations (De Kloet, et al., 1998; McEwen, 1999; McEwen & Alves, 1999). One view of these changes induced by

GENDER DIFFERENCES IN RESPONSE TO CHRONIC STRESS

Scott T. Brady, Hanwu Liu

The 1950 Nobel Prize for Medicine was awarded to Hench, Kendall and Reichstein for their work leading to the discovery of glucocorticoids, as well as elucidation of their structure and biological effects. A key event leading to the discovery of glucocorticoids and their anti-inflammatory action was the observation that female patients with rheumatoid arthritis often had a dramatic remission when they became pregnant (Hench, 1977). Once the endogenous factor producing this remission, cortisol or hydrocortisone, was identified, studies showed significantly increased plasma glucocorticoids during pregnancy. Upon reflection, the adaptive value of increased glucocorticoids during pregnancy is apparent. Many changes in normal physiology occur in conjunction with the hormonal changes during pregnancy to support development of a healthy fetus. A number of these changes are consistent with known effects of elevated glucocorticoid, including changes in the immune system, cardiovascular function, fluid balance and energy metabolism.

However, chronically elevated glucocorticoids can have profound effects on the normal nervous system function in both positive and negative ways (McEwen, 1998). Some of these effects are reversible over a period of weeks (Luine et al., 1994; Magarinos & McEwen, 1995), but more prolonged exposure may lead eventually to permanent changes (R. Sapolsky et al., 1985). The health related effects of chronic stress are of considerable interest, because a variety of stress-related disorders result in chronic elevation of glucocorticoids, including mild cognitive impairment in aging patients, posttraumatic stress disorder, Cushing's syndrome, and depression (R. M. Sapolsky, 1996). In addition, stress from life events and socioeconomic status or even longterm clinical treatment for chronic inflammatory conditions may have pathological consequences. How can these deleterious effects of chronically elevated glucocorticoids be reconciled with the exposure of women to elevated glucocorticoids for nine months during pregnancy?

One of the best characterized of these pathological changes in the nervous system is atrophy of neurons in the CA3 region of the hippocampus in male rats and mice (Liu et al., 2006; Watanabe et al., 1992b). The hippocampus plays a role in control of the hypothalamus-pituitary-adrenal gland response to stress and is an important target for glucocorticoids in the nervous system. Hippocampal neurons are important for adaptive behavior as well as certain forms of memory and learning. Structural differences between males and females have been described in the hippocampus. Males have a greater total number of granular neurons in the dentate gyrus and more mossy fiber synapses in the hilus than females, whereas females exhibit a greater number of mossy fiber synapses in the CA3 region (Madeira & Paula-Barbosa, 1993; Madeira et al., 1991). Functional differences between male and female hippocampal function have also been reported, including long-term potentiation and performance of hippocampal-dependent tasks (Roof et al., 1993). However, the physiological and molecular basis for these differences were not well delineated.

Regardless, the chronic elevation of glucocorticoids during pregnancy raised the question of whether males and females also differed in neuronal responses to chronic elevation of corticosterone. Comparisons of neuronal morphologies, neurotransmitter receptor composition and synaptic protein levels in male and female rodent hippocampus exposed to normal and chronically elevated corticosterone indicate that females are remarkably resistant to the effects of chronically elevated glucocorticoids (Galea et al., 1997; Liu, et al., 2006). The ability of the antiepileptic drug phenytoin (Watanabe, et al., 1992a) to block the effects of chronically elevated glucocorticoids on neuronal activity in hippocampal neurons in males, and certain other observations, implicate excitatory amino acid neurotransmitter receptors in the actions of glucocorticoids (Magarinos & McEwen, 1995). An analysis of glutamate receptor isotypes expressed in male and female mouse hippocampus in response to normal and chronically elevated glucocorticoids have revealed a striking gender-dependent differential shift in the expression of glutamate receptors. Females exhibited increased relative expression of NR2A and GluR2 receptor isotypes compared to males (Liu, et al., 2006). Strikingly, NMDA receptors with NR2A subunits require higher levels of glutamate for activation and exhibit increased sensitivity to inhibition by Zn^{2+} and glycine (Cull-Candy et al., 2001). Similarly, AMPA receptors containing GluR2 subunits are essentially impereant to Ca^{2+} (Swanson, et al, 1997) Kamboj, & Cull-Candy, 1997 (See also Chapter 17). Such changes may reduce the likelihood of excitotoxic damage that is thought to be associated with chronically elevated glucocorticoids (R. Sapolsky, 1990). Understanding the molecular basis for neural damage due to chronic stress may provide new therapeutic strategies to limit such damage. In addition, the recognition of gender specific differences in response to environmental stressors may have important implications for the clinic.

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glucocorticoids is that they counter-regulate some of the immediate and persistent neural effects of stress and are, therefore, part of the mechanism of adaptation (De Kloet, et al., 1998; Korte, 2001; Lupien & McEwen, 1997; McEwen, 1998; McEwen, 1999; McEwen, et al., 1993). This and other aspects of adaptation protect the organism; without adrenal secretions, the body probably would not survive many events in daily life.

The price of adaptation often includes some wear and tear and damage, called allostatic load (McEwen, 1998). Allostasis, meaning to achieve stability through change, is aword to describe the active process of adaptation. The wear and tear is the result of the inefficiency or overactivity of allostatic systems, including those like the adrenal cortex and autonomic nervous system, which respond to challenge and promote adaptation. This takes four forms: (i) repeated activation by many stressful events; (ii) failing to shut off after the challenge is over; (iii) failure to habituate to repetition of the same mild stressor; and (iv) inability to be activated adequately, allowing other systems that are normally counter-regulated to become overactive, for example, inflammatory cytokines. In each of these situations, there is a cumulative as well as an immediate effect on many organs, including the heart and brain, as well as on the immune system.

Enhancement of neuronal atrophy and cell loss during aging by severe and prolonged psychosocial stress are examples of allostatic load

Repeated psychosocial stress in primates and rodents causes pathological changes in various body organs, including neuronal damage in the hippocampus (Sapolsky, 1992). Shorter exposure of rats to restraint stress, psychosocial stress or corticosterone causes atrophy of neurons in the hippocampus, particularly in the CA3 region, which receives heavy input from the dentate gyrus mossy fiber system (Fig. 55-9). The mossy fiber input is also responsible for kainic-acid- and seizure-induced damage of the CA3 region and strongly suggests the importance of excitatory amino acids (Sapolsky, 1992).

Indeed, the stress-induced atrophy is blocked by NMDA receptor blockers and by an anticonvulsant drug, phenytoin. The presence of elevated glucocorticoids at the time of hypoxic damage or kainic-acid lesions (see Chapter 35) to the brain potentiates the necrosis produced by these treatments, especially in the hippocampus. Adrenalectomy reduces such damage and retards loss of neurons with age. Thus, adrenal steroids operate in conjunction with neural excitability to produce damage, and it has been suggested that they do so by compromising the ability of the brain to obtain nutrients to support ATP generation (Sapolsky, 1992).

Yet, the effect of repeated stress on the hippocampus is reversible if the stress is terminated after 3 weeks in rats, and it is best referred to as 'adaptive plasticity', since other brain areas, such as the amygdala, show a growth response to repeated stress (McEwen, 2003). The concept of allostatic load implies that there is a paradox in the actions of adrenal steroids: they exert protection in the short run and have the potential to cause damage in the long run if the allostatic, that is, the adaptation-promoting, responses are not managed efficiently. 'Good stress' is therefore the efficient management of an allostatic response, whereas 'bad stress', or being 'stressed-out', involves the persistence or otherwise inefficient operation of these normally adaptive responses.

Stressful early life events, involving abuse or neglect, can have a life-long influence on the stress response, and lead to elevated levels of allostatic load for the lifespan. Overactivity of the stress hormone axis has been linked to prenatal stress or poor maternal care in rodent models, and this overactivity

contributes to increased rates of brain and body aging (Caldji et al., 2000).

There are gradients of health status across income and education (referred to as 'socioecomic status' or 'SES') that are not explained by access to health care or other simple explanations (Adler et al., 1999). Therefore, it may be of great relevance in the future to understand the role of such factors as sense of control, helplessness, persistent fear and anxiety, diet, exercise, and the impact of the living and social (e.g. family and work) environments in regulating the allostatic systems; these factors could cause allostatic systems to operate inefficiently and lead to an acceleration of genetic predispositions towards disease.

SUMMARY

In mammalian species, the brain is a major 2-way conduit of environmental influences on all the organ systems through control of the hypothalamic-pituitary-endocrine axis. Specific releasing factors produced in specialized hypothalamic neurons are secreted into the portal circulation of the anterior pituitary gland where these hypothalamic factors regulate secretion of growth hormone and specific pituitary hormones that circulate in the blood stream to regulate, in turn, the secretion of hormones from the thyroid, adrenal, and gonadal tissues. What makes the brain a 2-way conduit is that these pituitary and peripheral hormones have feed-back regulatory effects at the pituitary, hypothalamic and extra-hypothalamic brain regions. Thus, environmental-nervous system interactions that are expressed or experienced through emotion, cognition and behavior are themselves integrated into hormonal control of the biochemistry and function, not only of the nervous system, but, actually of the whole organism. The integration of hormonal and nervous system responses to environment are manifested from modifications of genome expression, through cellular development, plasticity and responses to pathologic factors, both internal and external and broadly in the cognitive, emotional, gender-related and behavioral spheres of brain function. This chapter deals with the progress of neuroscience toward identifying the molecular processes of hormonal action at the transcriptional, biochemical and morphological levels in mammalian brain function.

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