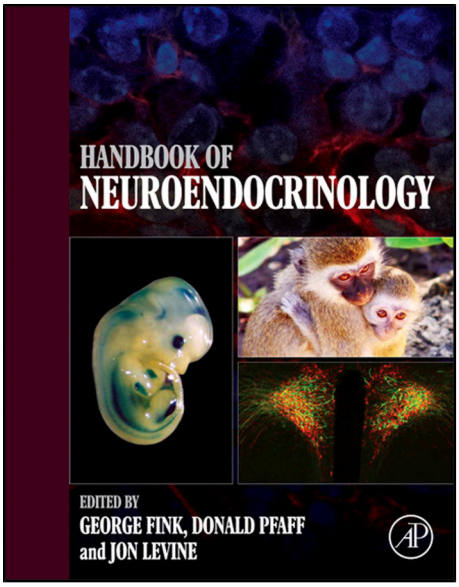

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CHAPTER

3

Neuroendocrine Feedback Control Systems: An Introduction

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OUTLINE

Summary

Feedback control systems are fundamental for the normal physiological functioning and homeostasis of the body. There are two types of feedback, negative and positive, of which the former is the more common. Removal of the main pituitary target glands, the adrenal, gonads and thyroid, the most reproducible and reproduced experiment in classical endocrinology, demonstrates that the secretion of pituitary adrenocorticotropin (ACTH), the gonadotropins (luteinizing hormone, LH, and follicle-stimulating hormone, FSH), and thyrotropin (TSH) is controlled by negative feedback exerted by the adrenal corticosteroids, gonadal steroids and thyroid hormones, respectively.

Positive feedback, whereby the output of a system increases the output of the stimulator (gain in the system), is far less common than negative feedback, possibly because, taken to its logical conclusion, a positive feedback system, uncontrolled, will eventually self-destruct. Positive feedback is exemplified by (a) estrogen stimulation of gonadotropin secretion, which results in ovulation, and (b) the release of oxytocin induced during parturition by pressure of the fetal head on the uterine cervix. Estrogen stimulation of gonadotropin secretion is reinforced by the servomechanism of the "priming effect" of GnRH, whereby a small pulse of GnRH, by further amplifying pituitary responsiveness to itself, ensures the occurrence of a massive ovulatory gonadotropin surge.

Crucial for homeostasis, negative feedback control mechanisms comprise a system in which the output moderates the strength of the controller to a predetermined set-point level. Negative feedback control operates widely throughout the body

at the molecular (for example, end-product inhibition of enzyme activity), cellular and whole system/body levels. The mechanisms by which the set point is determined, functionally and anatomically, vary between systems and species.

Here, attention will focus on the principles of negative feedback control using the hypothalamic–pituitary–adrenal (HPA) system as example. The HPA system, together with the sympathetic–medullary system, plays a pivotal role in the neuroendocrine response to stress. Homeostasis within the hypothalamic HPA is maintained by a precise negative feedback system by which the adrenal glucocorticoids (afferent limb), cortisol or corticosterone, moderate ACTH synthesis and release (efferent limb). Allostasis – that is, change in HPA activity to cope with increased stress load – is thought to be brought about by change in feedback set-point.

INTRODUCTION

Feedback control systems are fundamental for the normal physiological functioning of the body. There are two types of feedback, negative and positive, of which the former is the more common. Removal of the main pituitary target glands, the adrenal, gonads and thyroid, the most reproducible and reproduced experiment in classical endocrinology, demonstrates that the secretion of pituitary adrenocorticotropin (ACTH), the gonadotropins (luteinizing hormone, LH, and follicle-stimulating hormone, FSH), and thyrotropin (TSH) is controlled by negative feedback exerted by the adrenal corticosteroids, gonadal steroids and thyroid hormones, respectively[1–3]; (see also Chapters 1 and 5 in this volume). Interruption of the negative feedback loop caused, for example, by surgical or pharmacological adrenalectomy, gonadectomy or thyroidectomy, or by an enzymatic defect in steroid or thyroid hormone biosynthesis, results in hypersecretion of pituitary ACTH, gonadotropins or TSH (see Chapters 5, 8–11, 19, 21, 30 and 31).

Not discussed here is the leptin feedback system. First characterized in 1994 by Jeffrey Friedman and his colleagues,[4] leptin is a hormone secreted from adipocytes, the body's fat depot. Its discovery galvanized the neuroendocrinology of feeding and metabolism by providing a new perspective for examining how hormones interact with the brain. For a detailed account of leptin and its feedback system, the reader is referred to Chapter 14, and the relatively massive literature in the field (for example, Fehm *et al*.[5]).

Crucial for homeostasis, negative feedback control mechanisms comprise a system in which the output moderates the strength of the controller to a predetermined set-point level[6] (Figs 3.1, 3.2). The set point–stimulator complex contains a "comparator" (error detector), which compares the strength of the feedback signal with a preset level.[7] An increase in the strength of the feedback signal above the preset level reduces the output of the stimulator, whereas a decrease in the strength of the feedback signal below the preset level results in an increase in output of the stimulator and corrects the "error".[7] Negative feedback control operates widely throughout the body at the molecular (for example, end-product inhibition of enzyme activity), cellular and whole system/body levels. The mechanisms by which the set point is determined, functionally and anatomically, vary between systems and

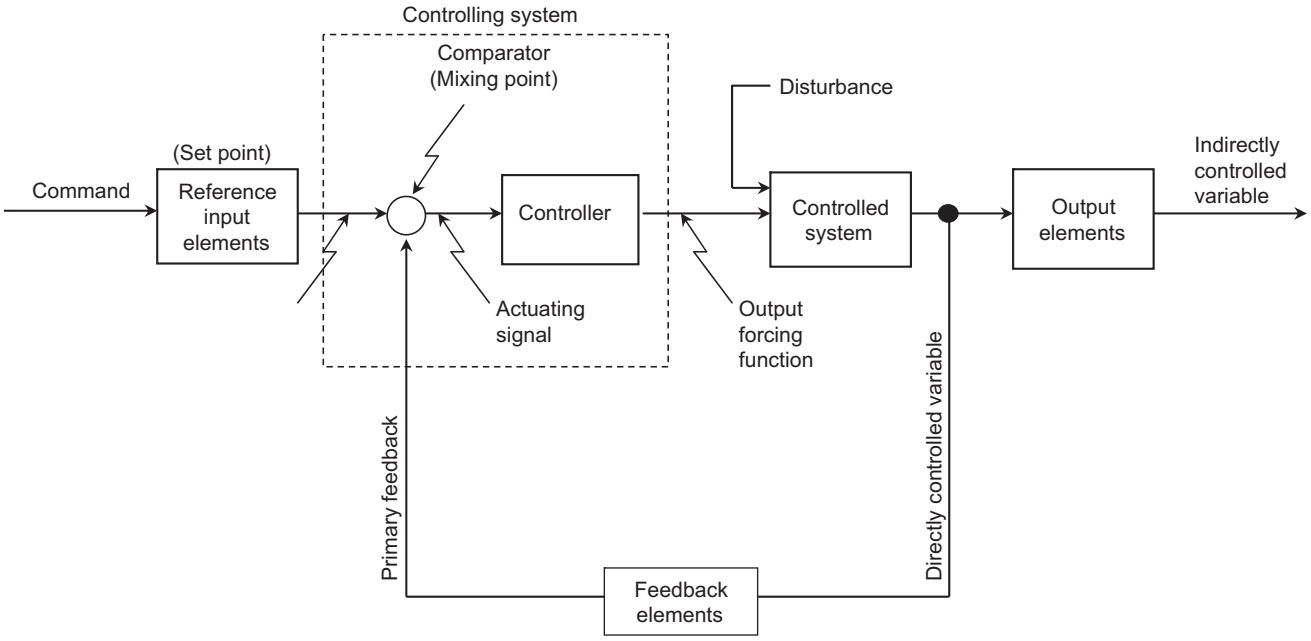

FIGURE 3.1 A generalized feedback control system. *Modified from Milhorn (1966).*[6]

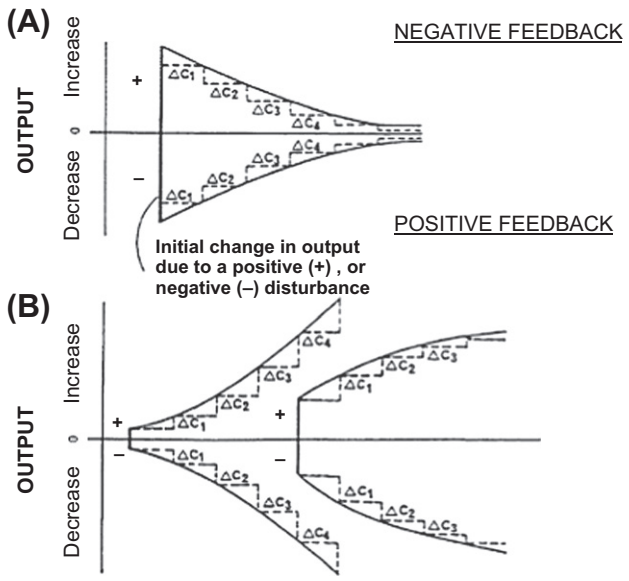

FIGURE 3.2 (**A**) Negative feedback minimizes the disturbance to a regulator, resulting in a system in which the output tends to remain constant. In this case, the ratios of the decrements of the controlled variable (C) (C_2/C_1, C_3/C_2) are less than unity. (**B**) In positive feedback (left-hand curves) an initial disturbance results in a continuous increase in output ("vicious cycle"). The increments of the controlled variable (C_2/C_1, C_3/C_2) are greater than unity. When the response does not result in a vicious cycle, the ratios of C_2/C_1, C_3/C_2, and so on are less than unity (see right-hand curves in B). *Reproduced from Milhorn HTJ.*[6] The Application of Control Theory to Physiological Systems. *Philadelphia: Saunders; 1966:386, with permission.*

species. In higher orders, the level of set point is thought to be regulated predominantly by the central nervous system. In the case of the hypothalamic–pituitary–adrenal axis (HPA) feedback system, for example, GABAergic, glutamatergic and monoamine neural projections from the limbic system and other brain regions to the paraventricular nucleus (PVN) of the hypothalamus may play a key role in set-point regulation[8,9]; (see below).

Positive feedback, whereby the output of a system increases the output of the stimulator (gain in the system) (Fig. 3.2B), is far less common than negative feedback, possibly because, taken to its logical conclusion, a positive feedback system, uncontrolled, will eventually self-destruct. Some systems loosely termed "positive feedback" are in fact "servomechanisms." A servomechanism is a closed-loop control system that increases significantly the power of a small signal. Positive feedback is exemplified by (a) estrogen stimulation of gonadotropin secretion, which results in ovulation[1–3]; (see also Chapter 5), and (b) the release of oxytocin induced during parturition by pressure of the fetal head on the uterine cervix (see Chapter 6). The latter triggers volleys of impulses that, by way of a multisynaptic pathway to the hypothalamus, stimulate oxytocin release. Oxytocin stimulates further contraction of the uterus, which results in a further increase in pressure on the uterine cervix. This "vicious cycle" is only broken when the fetus is expelled. Servomechanisms are illustrated by the way that just before ovulation in the human as well as in other spontaneously ovulating mammals, elevated plasma estrogen concentrations increase the responsiveness of the anterior pituitary gland to gonadotropin-releasing hormone (GnRH) by 20- to 50-fold. This estrogen effect is reinforced by the "priming effect" of GnRH, whereby a small pulse of GnRH, by further amplifying pituitary responsiveness to itself, ensures the occurrence of a massive ovulatory gonadotropin surge in response to a small surge or increased pulse frequency of GnRH.[1–3,10] For a detailed discussion of positive feedback and servomechanisms, see Chapter 5.

The present chapter focuses on the principles of negative feedback control using the hypothalamic–pituitary–adrenal (HPA) system as an example. While our discussion concentrates on negative feedback maintenance of homeostasis by glucocorticoids (cortisol in the human and corticosterone in rodents), the same mechanisms are involved in allostasis, which is brought about by a change in set point presumed to enable the organism to anticipate and deal with the physiological challenge or stress.[11–13] Allostatic regulation emphasizes feedforward regulatory systems, anticipatory and essential for adaptive social behaviors.[14]

THE NEUROENDOCRINE HPA CONTROL SYSTEM

Pattern of ACTH and Glucocorticoid Secretion

In the normal state, with the negative feedback loop intact, the set point in the brain–pituitary module maintains the secretion of ACTH within a relatively narrow bandwidth. Within this bandwidth, the basal secretion of ACTH is pulsatile, and is cleared rapidly from the blood by both metabolic degradation and distribution into several body compartments. Pulsatile, ultradian ACTH secretion is responsible for driving pulsatile glucocorticoid secretion,[15] and this is associated with parallel changes in steroidogenic acute regulatory protein (StAR) and P450scc heteronuclear RNA levels. The pulsatile pattern of StAR and P450scc is paralleled by pulsatile transcription of the melanocortin 2 receptor accessory protein.[15] Plasma ACTH concentrations show a circadian rhythm, with a peak in the morning of diurnal animals and a trough approaching a nadir around midnight. In nocturnal animals, such as rodents, the phase of this rhythm is reversed so that plasma ACTH concentrations reach a peak just before the onset of darkness. The circadian rhythm of ACTH results in a circadian rhythm in the plasma concentrations of

adrenal corticosteroids: cortisol in humans and corticosterone in rodents. A key feature in both diurnal and nocturnal animals is that corticosteroid plasma concentrations reach a peak just before the animal is due to wake from sleep (see cortisol awakening response, below); the levels are lowest just before or during sleep.

The glucocorticoids are bound rapidly by albumin and a specific corticosteroid binding-protein, "transcortin," and metabolized by several organs, especially the liver and kidney (Fig. 3.3). Only unbound (free) glucocorticoids inhibit ACTH release; therefore, the degree of glucocorticoid binding and metabolism, as well as the magnitude of adrenal secretion (Fig. 3.3), determines the strength of the negative feedback signal. The HPA feedback system might also be influenced by a binding protein in plasma that has a high and selective affinity for human but not ovine corticotropin-releasing factor (CRF-41).[16] This CRF-binding protein may explain the brief biological action of hCRF as compared to ovine CRF in man, and why high concentrations of plasma immunoreactive hCRF in women during third-trimester pregnancy do not cause increased ACTH secretion.[16] The hCRF-binding protein was subsequently shown to be a 37-kDa protein.[17–19] Synthesized in the liver, the physiological role of the CRF binding (glyco) protein awaits determination. In the human, CRF binding protein concentrations in plasma increase during the third trimester of pregnancy.

Cortisol Awakening Response

In man, waking is closely associated with a cortisol awakening response (CAR) which typically involves an increase in salivary cortisol concentrations peaking around 30 minutes after waking.[20] The CAR has been increasingly studied in psychoneuroendocrinology in recent years, since the advent of sampling devices has allowed saliva samples to be collected by research participants at home. The CAR is a response to waking, rather than a reflection of any non-specific rise in cortisol levels in the early hours of the day.[21] The magnitude of the CAR has been associated with a number of psychosocial factors, including work stress and other forms of chronic life stress, psychological traits, depression, fatigue and post-traumatic stress disorder, with both increases and decreases being described (see, for example, Dockray *et al.*;[20] Chida and Steptoe[21]). Chronotype has a limited impact on the diurnal cortisol profile of healthy women, and may be somewhat impervious to individual preferences for morning or evening activity.[22] Cortisol, both total output and the awakening response, has consistently been shown to be lower among

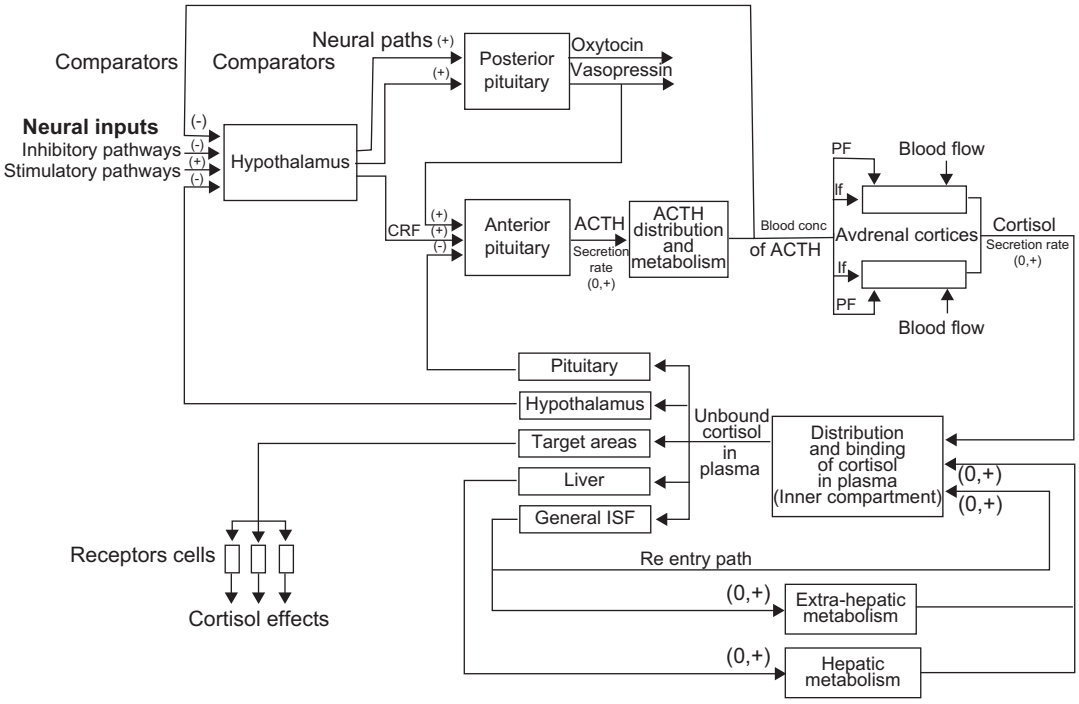

FIGURE 3.3 **Block diagram of the hypothalamic–pituitary–adrenal glucocorticoid control system.** If, input forcing of adrenal by ACTH; PF, parametric forcing of adrenal (hypertrophic effect) caused by ACTH over a longer time period. The parametric effect of changes in adrenal blood flow is also indicated. The designators 0, + and 0, − indicate that signals in pathways are restricted in values (e.g., there are no negative masses or frequencies, and removal processes or inhibitors are negative in effects). *Reproduced from Yates FE, Maran JW. In: Knobil E, Sawyer WH, eds.* Handbook of Physiology. *Washington, DC: American Physiological Society; 1975:367–404, with permission.*

individuals with higher levels of positive affect. The beneficial effects of positive mood on cardiovascular function, including heart rate and blood pressure, and the immune system have also been described. The influence of positive affect on these psychobiological processes is independent of negative affect, suggesting that positive affect may have characteristic biological correlates that may be partly responsible for the protective effects of positive affect on health outcomes.[22]

Glucocorticoid Negative Feedback Control in the HPA: Interactions with Neural Circuits

Glucocorticoids (cortisol in the human, corticosterone in rodents) secreted by the adrenal cortex exert their inhibitory action both on the brain and on the pituitary gland (Figs 3.3, 3.4). The paraventricular nuclei of the hypothalamus contain the final common pathway neurons that mediate the neural control of pituitary ACTH synthesis and release (see also Chapter 5). Many data point to PVN as the main component of the HPA set point. Thus, for example, our own data showed that glucocorticoid negative feedback in the rat is blocked by lesions of the PVN.[22] The PVN receives stimulatory and inhibitory neural inputs and an important projection from the suprachiasmatic nucleus (SCN), the key circadian oscillator in brain. PVN drive is mediated by the "stress neurohormones," CRF-41 and arginine vasopressin (AVP), which are released into hypophysial portal vessel blood and trigger pituitary ACTH synthesis and release.[24–28] ACTH, released into the systemic circulation, stimulates adrenal corticosteroid synthesis and release.

In addition to glucocorticoids, PVN activity is either moderated or stimulated by higher central nervous components. Thus, lesion studies of the hippocampus suggested that hippocampal efferents from the ventral subiculum and ventral CA1 exert tonic inhibition of the CRFmRNA and AVPmRNA synthesis in the PVN.[29] Furthermore, the posterior bed nucleus of the stria terminalis (BNST) is involved in inhibition of the HPA axis, whereas the anteroventral BNST activates the HPA axis. The BNST contains functional subdomains that play different roles in integrating and processing limbic information in response to stress.[30–32] The posterior medial BNST is likely to be a major component of the brain circuitry involved in both normal and pathological stress adaptation.[30,33,34]

In summary, the relationship between limbic structures and control of ACTH and glucocorticoid release suggests that: (a) in general, the hippocampus and anterior cingulate/prelimbic cortex inhibit stress-induced

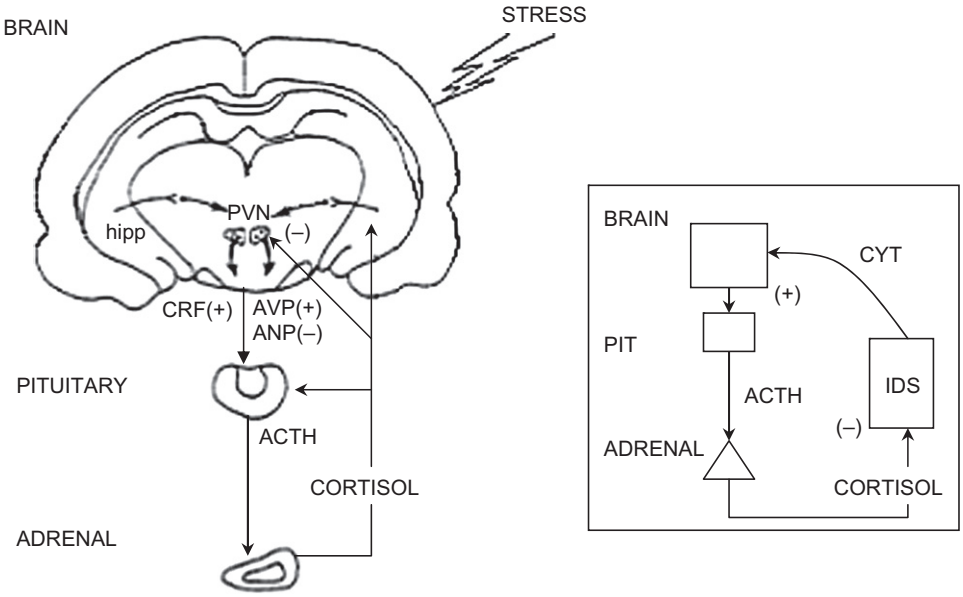

FIGURE 3.4 **Schematic diagram of the elements involved in glucocorticoid negative feedback.** The paraventricular nucleus (PVN) contains the main stress (final common pathway neurons) secreting both CRF and AVP. The negative feedback action of cortisol (corticosterone in rodents) is exerted mainly on the PVN and the pituitary corticotropes. However, long-term effects mediated through the hippocampus (Hipp) and amygdala (not shown) cannot be excluded. There are several possible indirect connections between the hippocampus and PVN. Neural control of ACTH secretion may also be mediated by a corticotropin inhibitory peptide, atrial natriuretic peptide (ANP). The inset shows the important inhibitory action of cortisol on the immune defense system (IDS). The IDS produces cytokines (CYT), which act on the brain to stimulate ACTH secretion. Cytokine secretion is inhibited by the negative feedback effect of glucocorticoids. *Reproduced with permission from Fink G Mechanisms of negative and positive feedback of steroids in the hypothalamic–pituitary system. In: Bittar EE, Bittar N, eds. Principle of medical Biology, Vol. 10A. New York: JAI Press; 1997:29–100.*

HPA activation, whereas the amygdala and perhaps the infralimbic cortex may enhance glucocorticoid secretion; (b) the role of limbic structures is both region- and stimulus-specific; (c) limbic sites have minimal direct projections to the HPA effector neurons of the paraventricular nucleus (PVN); (d) hippocampal, cortical and amygdala efferents apparently relay with neurons in the bed nucleus of the stria terminalis, hypothalamus and brainstem to access CRF neurons; (e) hippocampal, cortical and amygdala projection pathways show extensive overlap in regions such as the bed nucleus of the stria terminalis, hypothalamus and perhaps brainstem, implying that limbic information may be integrated at subcortical relay sites prior to accessing the PVN; and (f) all regions express both glucocorticoid and mineralocorticoid receptors, allowing for glucocorticoid modulation of limbic signaling patterns.[34,35] Our hypophysial portal blood studies showed that: (a) electrical stimulation of the PVN induced massive release of CRF-41; (b) stimulation of the amygdala inhibited CRF-41 release into portal vessel bood; and (c) hippocampal stimulation had no significant effect on CRF-41 release.[36]

INTERACTION BETWEEN NEGATIVE FEEDBACK AND CIRCADIAN RHYTHM IN THE HYPOTHALAMIC–PITUITARY–ADRENAL (HPA) SYSTEM

Already mentioned above, the circadian rhythm of ACTH and glucocorticoid secretion, which has a peak at the end and a nadir at the beginning of the sleep phase, is driven by a neural mechanism mediated mainly by the stress neurohormones. The twofold increase in the ACTH signal between the nadir and the peak of the circadian rhythm in the rat results in a nine-fold increase in corticosterone due to an increase in the responsiveness of the adrenal cortex.[3]

In the unstressed state the HPA system operates in an approximately linear domain, with all the loop variables (Fig. 3.3) showing circadian periodicity.[7] ACTH and cortisol are also released in pulsatile fashion, with circadian and ultradian rhythms governing secretion of these hormones.[37] ACTH and cortisol pulses are released approximately hourly, with ACTH pulses preceding cortisol pulses by approximately 10 minutes. In addition to these approximately hourly pulses, an ultradian rhythm of cortisol of 90–110 minutes has been found, which is linked to the basic rest–activity cycle, particularly the alternating arousal–sleep cycle, which continues throughout the day.[37] Van Cauter *et al*.[38] found a strong bidirectional link between cortisol secretory episodes and arousal.

The circadian rhythm of ACTH secretion is driven by the suprachismatic nucleus (SCN), the master circadian light entrainable oscillator (LEO).[39–41] In addition to the diurnal rhythm of corticosterone, the SCN, located in the hypothalamus, is involved in other key rhythms in the body, such as the sleep–wake cycle, motor activity, thermoregulation, pineal arylalkylamine *N*-acetyl transferase activity (rate-limiting enzyme for melatonin synthesis in the pineal gland), and the regular occurrence of ovulation (see Chapter 5). If any one of these functions is abnormal, then there is a high probability that the others will also be abnormal. The corticosterone rhythm is especially sensitive in that even partial lesions of the SCN which have no effect on any of the other circadian functions disrupt the adrenal rhythm.

A prominent exception to the concept that the SCN is dominant in the control of circadian rhythms in physiology and behavior is the fact that lesions of the SCN do not abolish the ability of rats to anticipate one meal per day. This anticipation is associated with an increase in motor activity, core body temperature and serum corticosterone, and would appear to be driven by another Zeitgeber termed the "food-entrainable oscillator" (FEO), the location of which has still to be determined.[42]

An approach to elucidating a possible link between the LEO and the FEO has recently been reported by Hayasaka *et al*.[42] and is based on G-protein signaling. Regulators of G-protein signaling (RGS) are a multifunctional protein family, which functions in part as GTPase-activating proteins (GAPs) of G-protein alpha-subunits to terminate G-protein signaling. Previous studies have demonstrated that the Rgs16 transcripts exhibit robust circadian rhythms both in the SCN and in the liver. To investigate the role of Rgs16 in the circadian clock *in vivo*, Hayasaka *et al*.[42] generated two independent transgenic mouse lines using lentiviral vectors expressing short hairpin RNA (shRNA) targeting the Rgs16 mRNA. The knockdown mice demonstrated significantly shorter free-running periods of locomotor activity rhythms and reduced total activity compared with wild-type siblings. Furthermore, when feeding was restricted during the daytime, FEO-driven elevated food-anticipatory activity (FAA) observed prior to the scheduled feeding time was significantly attenuated in the knockdown mice. Whereas the restricted feeding in wild-type animals phase advanced the rhythmic expression of the Per2 clock gene in liver and thalamus, this phase shift was not observed in the knockdown mice. The report by Hayasaka *et al*.[42] is the first *in vivo* demonstration that a common regulator of G-protein signaling is involved in the two separate, but interactive, circadian timing systems, LEO and FEO.

The SCN receives afferent projections from (a) the retina (direct as well as indirect after relay in the ventral lateral geniculate nucleus), (b) the 5-hydroxytryptamine

(5-HT) raphe neurons, and (c) the hippocampus by way of the medial corticohypothalamic tract.[43–50] Each of these inputs to the SCN is likely to affect the periodicity and amplitude of the SCN pacemaker. The retinal input affects the time of the light–dark cycle, while the raphe nuclei hippocampal input may be related to the sleep–wake cycle and other behaviors. The raphe input also plays a major role in determining the amplitude of diurnal ACTH oscillations.

Although the central action of glucocorticoids in moderating HPA activity is mainly on the PVN (see later), the noradrenergic locus coeruleus, the serotonergic dorsal raphe and the dopaminergic ventral tegmental area all express corticosteroid receptors and have been the focus of antidepressant research. All three of these nuclei express glucocorticoid receptor (GR), and the locus coeruleus and dorsal raphe have also been shown to express mineralocorticoid receptor (MR).[51] Monaminergic projections from these brainstem nuclei can also influence the activity of the HPA axis.[32,33] Locus coeruleus noradrenergic afferents to the hypothalamus stimulate HPA activity, while serotonergic projections from the raphe nuclei can both facilitate and inhibit HPA activity, depending on the limbic targets of these projections.[32] Dopaminergic projections from the ventral tegmental area affect HPA sensitivity to glucocorticoid feedback inhibition indirectly via the prefrontal cortex.[51]

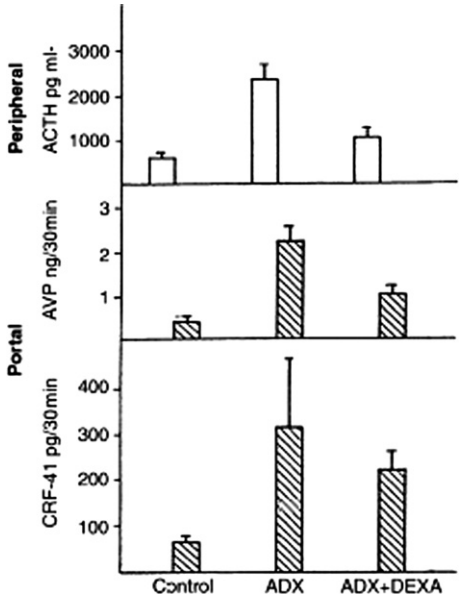

FIGURE 3.5 **The delayed negative feedback action of glucocorticoid in adult female Wistar rats anesthetized with sodium pentobarbitone.** ACTH was measured in plasma taken immediately before sectioning of the pituitary stalk for the collection of hypophysial portal vessel blood. Adrenalectomy (ADX) resulted in a fourfold increase in ACTH concentration and a similar increase in the output of AVP and CRF-41 release relative to that in intact, untreated animals (control). The administration of dexamethasone (DEXA) significantly reduced the levels of ACTH and AVP, but not CRF-41. *Modified from, Fink* et al. *(1988).*[28]

CORTICOSTEROID FEEDBACK ON THE HYPOTHALAMUS AND PITUITARY GLAND: PHASE DIFFERENCES

The seminal modeling studies of Eugene Yates and associates[7] suggested that corticosteroid negative feedback on ACTH release occurs in three phases: a fast and immediate rate-sensitive phase of about 30 minutes, followed by a level-sensitive phase that occurs 2–3 hours after the start of corticosteroid administration, followed by a long-term chronic phase. We investigated the effect of corticosteroids on the release of stress neurohormones into hypophysial portal blood in the intermediate and chronic phases.[28] Our findings were that adrenalectomy induced a three- to fourfold increase in the release of both CRF and AVP into hypophysial portal blood. Administration of the synthetic glucocorticoid dexamethasone 2.5 hours before portal blood collection significantly reduced the release of AVP but not CRF-41 (Fig. 3.5), and also blocked the ACTH response to CRF-41 (Fig. 3.6). These data suggest that "intermediate-delayed" (2- to 3-hour) glucocorticoid feedback is mediated by the blockade of pituitary responsiveness to CRF-41 and a reduction in AVP output into hypophysial portal blood. That is, in this

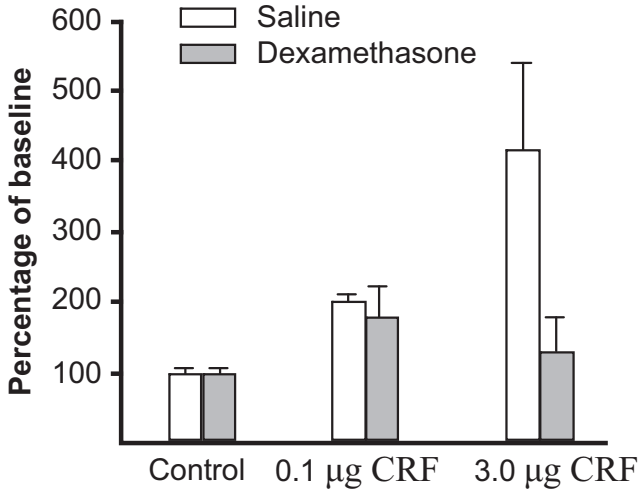

FIGURE 3.6 Mean (± SEM, $n = 5$) percentage increase over the basal concentration of ACTH after the injection of saline, 0.1 μg CRF, or 3 μg CRF. Female Wistar rats that had been adrenalectomized 3 weeks earlier were treated with either saline or dexamethasone 3 h before injection of CRF-41. Note that dexamethasone blocked the ACTH response to 3.0 μg of CRF. *Reproduced from Fink G, Robinson ICAF, Tannahill LA. Effects of adrenalectomy and glucocorticoids on the peptides, CRF-41, AVP and oxytocin in rat hypophysial portal blood.* J Physiol. *1988;401:329–345, with permission of the authors and Cambridge University Press.*

situation AVP is the regulatory or signaling neurohormone, whereas CRF-41 is the "permissive" neurohormone. In contrast, our studies on the long-term effects of corticosterone administered by a subcutaneous pellet suggested that long-term negative glucocorticoid feedback is due to a decreased release of CRF-41, as well as AVP, into portal blood.[52] That is, both stress neurohormones are sensitive to the long-term effects of corticosteroids.

Our findings agree broadly with those of Plotsky and associates, which showed that pharmacological "adrenalectomy" with metyrapone and aminoglutethimide (which block glucocorticoid biosynthesis) resulted, after 3 days, in a significant increase in the release of both CRF and AVP into hypophysial portal blood.[53] The intravenous infusion of corticosterone inhibited nitroprusside-evoked CRF release into portal blood, but had no effect on AVP release.[54]

Data in the rat are complemented by those obtained in the sheep. Thus, the concentrations of CRF-41 and AVP in hypophysial portal blood collected from conscious sheep were (a) similar to those in the rat, (b) increased by volume depletion, fear-associated audiovisual stimuli and by insulin-induced hypoglycemia, and (c) inhibited by dexamethasone.[55,56]

GLUCOCORTICOID FEEDBACK EFFECTS ON STRESS NEUROHORMONE BIOSYNTHESIS

Glucocorticoids have potent inhibitory effects on CRF-41 and ACTH biosynthesis and release.[57–66] Thus, adrenalectomy is followed by a significant increase in CRF-41 mRNA levels in the parvocellular PVN, and this increase can be reduced by either corticosterone or dexamethasone. As assessed by CRF-intron (CRFin) *in situ* hybridization, the stimulation of CRF gene transcription can be detected as early as 15–30 minutes after the injection of the glucocorticoid synthesis inhibitor metyrapone. This increase in CRF-41 gene transcription in the PVN was associated with a coincident increase in c-fos mRNA in the PVN. An increase in the levels of CRF-41 mRNA in the PVN after metyrapone injection was delayed by about 60 minutes, possibly a function of the high resting levels of CRF-41 mRNA and the time taken to assemble mRNA from the CRF-41 primary transcript.

The ACTH precursor pro-opiomelanocortin (POMC) has in its promoter one of the best characterized negative glucocorticoid response elements (nGRE).[58] POMC plays an important role in the regulation of the HPA axis (see above). The POMC promoter is stimulated by CRF and repressed by glucocorticoids. ACTH, which is the major POMC gene product in corticotropes, is generated by processing of the 266-amino acid POMC precursor.[58] The nGRE in the POMC promoter plays a key role in glucocorticoid negative feedback action in the HPA.

The effects of corticosterone[67] and dexamethasone are dose dependent, and can be demonstrated by systemic administration of the steroid, as well as by implantation of steroid pellets into the brain. This effect of glucocorticoids is cell-specific, in that the glucocorticoid-induced decrease in CRF-41 mRNA was localized to the dorsomedial parvocellular neurons of the PVN, the major source of CRF-41 fiber projections to the median eminence. In contrast, glucocorticoid increased the levels of CRF-41 mRNA in parvocellular neurons that project to the brainstem and the spinal cord. The implantation of dexamethasone micropellets in cerebral cortex, dorsal hippocampus, ventral subiculum, lateral septum or amygdala had no effect on CRF-41 mRNA levels in the PVN. The molecular mechanism by which glucocorticoids regulate CRF-41 gene expression remains unclear. Recent data show that the the repressor isoform of the cAMP response element modulator (CREM) is involved in CRF-41 gene regulation, but that stress-induced glucocorticoids do not limit CRF-41 gene transcription.[68] The latter may also be affected by GABAergic, glutamatergic and monoaminergic projections to the PVN from the forebrain and hindbrain limbic system (see also "Glucocorticoid Negative Feedback at the Pituitary Level," below).

The concentration of AVP mRNA in the dorsomedial parvocellular neurons of the PVN parallel those of CRF-41 mRNA – i.e., levels increased after adrenalectomy and decreased after treatment with glucocorticoids.[69] In unstressed intact rats, PVN CRF hnRNA, but not AVP hnRNA, showed a clear circadian rhythm that was correlated with plasma corticosterone concentrations. However, AVP hnRNA levels in the PVN did show a circadian rhythm in adrenalectomized rats that was moderated by corticosterone.[66] Corticosterone treatment also produced a modest reduction in the levels of enkephalin mRNA in all four parts of the PVN, but glucocorticoid manipulation had no significant effect on the PVN concentrations of the mRNAs for angiotensin, cholecystokinin, preprotachykinin and tyrosine hydroxylase.

Data on the release and synthesis of stress neurohormones suggest that even though corticosteroid receptors are present in high concentration in parts of the brain remote from the hypothalamus, including the hippocampus, amygdala and the monoaminergic nuclei of the hindbrain, central inhibition of CRF-41 and AVP synthesis and release may be due in large part to corticosteroid action mainly at the level of the PVN (but see "Possible Role of 11β-Hydroxysteroid Dehydrogenase," below).

ROLE OF HIPPOCAMPUS AND AMYGDALA IN GLUCOCORTICOID NEGATIVE FEEDBACK

It has long been assumed that the hippocampus and the amygdala, major components of the forebrain limbic system, play a key role in the stress response. The forebrain limbic system together with the thalamus and neocortex and brainstem structures forms an analyzer–integrator system involved in neuroendocrine control. The hypothalamus can be considered an intermediate relay station in the reciprocal circuits between the limbic forebrain and brainstem structures, receiving inputs from both. The forebrain limbic system is thought to be involved in stressors that require analysis by higher brain structures ("processive" stressors), whereas direct brainstem projections to the PVN subserve stressors that pose an immediate physiological threat ("systemic" stressors).[70–74]

Projections to the PVN from the hippocampus are mainly multisynaptic, involving (a) the hippocampal fimbria–fornix system including the lateral septum, bed nucleus of the stria terminalis (BNST) and anterior hypothalamus, which all project to the parvocellular PVN, and (b) the medial corticohypothalamic tract from the anteroventral subiculum to the ventromedial, arcuate and suprachiasmatic nuclei of the hypothalamus. The PVN also receive direct projections from the amygdala, as well as projections that relay in the BNST (Fig. 3.7).

Many but not all studies in which HPA activity was assessed by the assay of corticosterone, and, less frequently, ACTH, suggest that the hippocampus inhibits HPA activity. Hippocampal inhibition of the

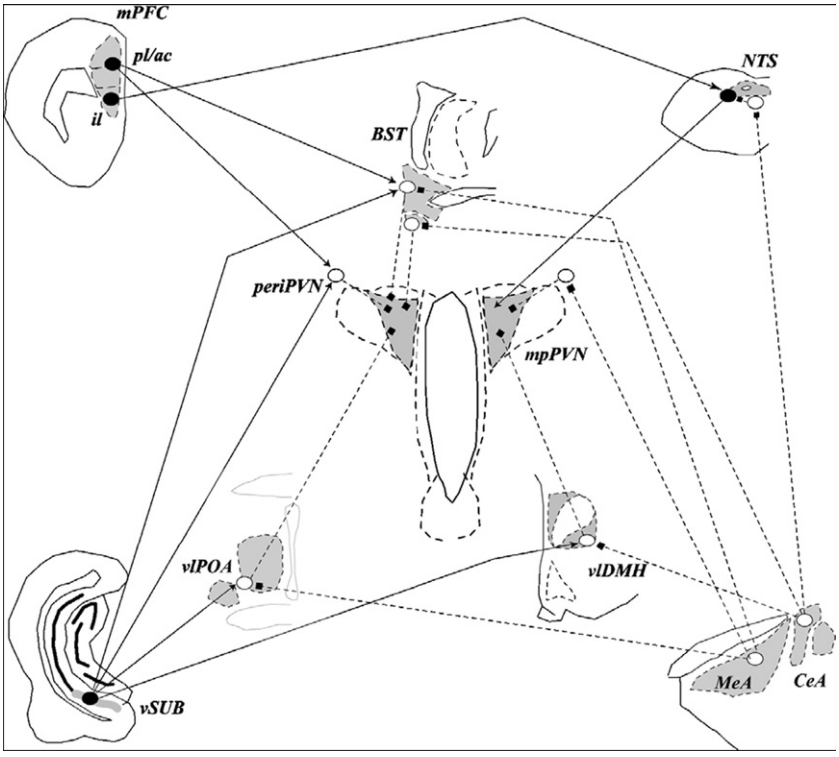

FIGURE 3.7 **Diagrammatic representations of limbic stress-integrative pathways from the prefrontal cortex, amygdala and hippocampus.** The medial prefrontal cortex (mPFC) subsumes neurons of the prelimbic (pl), anterior cingulate (ac) and infralimbic cortices (il), which appear to have different actions on the HPA axis stress response. The pl/ac send excitatory projections (designated as dark circles, filled line with arrows) to regions such as the peri-PVN zone and bed nucleus of the striaterminalis (BST), both of which send direct GABAergic projections to the medial parvocellular PVN (delineated as open circles, dotted lines ending in squares). This two-neuron chain is likely to be inhibitory in nature. In contrast, the infralimbic cortex projects to regions such as the nucleus of the solitary tract (NTS), which sends excitatory projections to the PVN, implying a means of PVN excitation from this cortical region. The ventral subiculum (vSUB) sends excitatory projections to numerous subcortical regions, including the posterior BST, peri-PVN region, ventrolateral region of the medial preoptic area (vlPOA) and ventrolateral region of the dorsomedial hypothalamic nucleus (vlDMH), all of which send GABAergic projections to the PVN and are likely to communicate transynaptic inhibition. The medial amygdaloid nucleus (MeA) sends inhibitory projections to GABAergic PVN-projecting populations, such as the BST, vlPOA and peri-PVN, eliciting a transynaptic disinhibition. A similar arrangement likely exists for the central amygdaloid nucleus (CeA), which sends GABAergic outflow to the ventrolateral BST and to a lesser extent, the vlDMH. The CeA also projects to GABAergic neurons in the NTS, which may disinhibit ascending projections to the PVN. *Reproduced from Herman JP, Ostrander MM, Mueller NK, Figueiredo H. Limbic system mechanisms of stress regulation: hypothalamo-pituitary–adrenocortical axis.* Prog Neuropsychopharmacol Biol Psychiatry *2005;29(8):1201–1213, with permission.*

HPA appears to be due mainly to corticosteroid negative feedback inhibition, although there is also evidence that the hippocampus may exert an inhibitory tone on the HPA independently of corticosteroid feedback. However, the hippocampus is neither the only nor necessarily the major site of corticosteroid negative feedback, as removal of its input to the hypothalamus reduces, but does not abolish, the efficacy of corticosteroid inhibition. Thus, for example: (a) although electrical stimulation of the PVN resulted in a two- to threefold increase in CRF-41 release into hypophysial portal blood, stimulation of the hippocampus had no effect on the release of CRF-41, AVP, or oxytocin;[36] (b) transection of the fornix, the major hippocampal–hypothalamic connection, had no significant effect on either basal or stress (nitroprusside-induced hypotension) evoked CRF-41 release into hypophysial portal blood; and (c) implantation of dexamethasone pellets in hippocampus had no effect on CRF-41 synthesis in the PVN. Nevertheless, fornix section did elevate AVP concentrations in portal blood and blocked corticosterone reduction of elevated CRF, but not AVP, levels in portal blood during hypotensive stress.[75]

Further evidence that the hippocampus normally moderates the synthesis of stress neurohormones is suggested by the finding that lateral fimbria–fornix lesions increased CRF-41 mRNA and AVP mRNA in medial parvocellular PVN and plasma ACTH concentrations.

Feldman and Weidenfeld[76] have shown that in freely moving male rats bearing cholesterol implants in the hippocampus, photic and acoustic stimuli depleted the CRF-41 content of the median eminence with a concomitant increase in plasma ACTH and corticosterone levels. This was inhibited by the systemic administration of dexamethasone, an effect inhibited by hippocampal implants of glucorticoid and, to a lesser extent, mineralocorticoid receptor antagonists. Thus, in freely moving conscious animals the hippocampus may play a role in corticosteroid feedback moderation of ACTH release in response to photic and acoustic stimuli.

Herman and associates have underscored the role of the BNST as a nucleus that integrates or processes hippocampal and amgydaloid modulation of the HPA. This hypothesis is based, first, on the anatomical connections of the BNST, in that the nucleus receives rich projections from the amygdala and hippocampus and projects to the parvocellular PVN. Second, lesion of the anterior BNST resulted in a 30% decrease in CRF-41 mRNA levels in the PVN, whereas lesion of the posterior BNST resulted in a 13% increase in the level of CRF mRNA in the PVN.[29,64] On the basis of these data, Herman and colleagues inferred that the anterior BNST integrates excitatory inputs mainly from the amygdala, whereas the posterior BNST integrates inhibitory inputs mainly from the hippocampus.[29,77] Lesion studies of the amygdala showed that glucocorticoid feedback was unaffected by lesions of the amygdala.[78]

In summary, the hippocampus plays an important role in moderating the HPA both by mediating corticosteroid negative feedback and by exerting an endogenous inhibitory tone on the HPA. Corticosteroid negative feedback is also exerted directly on the PVN and the pituitary gland. The hippocampus is a heterogeneous structure; thus, for example, Dunn and Orr found that electrical stimulation of the CA1 region increased corticosterone levels, whereas stimulation of the CA3, dentate and subiculum decreased plasma corticosterone concentrations.[79] This heterogeneity will need to be considered in the design of further studies on the role of the hippocampus in negative feedback control of the HPA.

GLUCOCORTICOID NEGATIVE FEEDBACK AT THE PITUITARY LEVEL

As well as exerting a central effect, corticosteroids inhibit ACTH synthesis and release by a profound action at the level of the anterior pituitary gland. This is illustrated by Fig. 3.6, in which dexamethasone completely blocked the ACTH response to a bolus injection of CRF-41.[28] As already mentioned in "Glucocorticoid Feedback Effects on Stress Neurohormone Biosynthesis," above, the ACTH precursor proopiomelanocortin (POMC) has in its promoter one of the best-characterized negative glucocorticoid response elements (nGRE).[58] POMC plays an important role in the regulation of the HPA axis (see above).

Studies on dispersed pituitary cells with inhibitors of mRNA and protein synthesis have shown that both the rapid and the delayed glucocorticoid inhibition of ACTH release depend upon mRNA and protein synthesis. Glucocorticoids exert potent inhibitory effects on the expression of POMC in the anterior lobe of the pituitary gland. In male Sprague-Dawley rats, transcription assays showed that dexamethasone inhibited POMC gene transcription by 10-fold within 30 minutes of a single injection of the glucocorticoid. Inhibition of POMC transcription was paralleled by a dramatic fall in plasma ACTH concentrations. The same study showed that CRF-41 stimulated POMC transcription by nearly twofold within 15 minutes, which coincided with a massive increase in ACTH release. The action of dexamethasone is cell-specific in that the steroid had no effect on the transcription rate of POMC in primary cultures of neurointermediate lobe cells.[80,81]

Studies in transgenic mice showed that no more than 769 base pairs of the rat POMC promoter are required for cell-specific expression and glucocorticoid inhibition of the POMC gene in the anterior pituitary gland.[63] A

good deal is known about the glucocorticoid response elements in the POMC promoter and the transcription factors involved in POMC expression, but the precise molecular mechanism of glucocorticoid inhibition of POMC transcription remains to be elucidated.

POSSIBLE ROLE OF 11β-HYDROXYSTEROID DEHYDROGENASE

Two isozymes of 11β-hydroxysteroid dehydrogenase play a pivotal role in glucocorticoid synthesis and metabolism. 11beta-HSD type 1 (11β-HSD1) is an isozyme that predominantly catalyzes the reduction of inert cortisone to active cortisol in intact cells and organs. 11β-HSD type 2 (11β-HSD2) is an isozyme that catalyzes the rapid dehydrogenation of active cortisol to inert cortisone (11-dehydrocorticosterone). These two isozymes can play a key role in glucocorticoid feedback. For details, the reader is referred to the reviews by Seckl and colleagues.[82,83]

FUNCTIONAL IMPORTANCE OF GLUCOCORTICOID NEGATIVE FEEDBACK

Glucocorticoid feedback inhibition of ACTH release protects the organism against the deleterious effects of hypercortisolemia (excessive concentrations of cortisol in blood). Whether due to endocrine disorders such as Cushing's syndrome, or other causes such as trauma or chronic stress, hypercortisolemia is associated with at least three major deleterious effects. First, it suppresses the immune-inflammatory defense system, and so incapacitates the animal's ability to respond to infection by pathogenic microorganisms, or to chemical or physical insult. Second, persistent hypercortisolemia has major adverse effects on intermediary metabolism, resulting eventually in all the features of Cushing's syndrome: that is, android obesity, diabetes mellitus, hyperlipidemia, hypertension and osteoporosis. Third, hypercortisolemia and/or stress are thought to be associated with reduction in hippocampal volume ("atrophy") in several neuropsychiatric disorders, such as depression and post-traumatic stress disorder, as well as in Cushing's syndrome. In all three disorders, the hippocampal atrophy is associated with explicit memory deficits. For details regarding these stress-induced cognitive deficits and the mechanism of action of stress or hypercortisolemia on the hippocampus, the reader is referred to papers by Sapolsky.[84–86] Notwithstanding the technical excellence of the experimental work that has demonstrated the neurotoxicity of sustained high levels of glucocorticoids, especially in the hippocampus, translation of these findings to the human in terms of reduced hippocampal size as assessed by brain imaging might require further study.[87]

CLINICAL MANIFESTATIONS OF DISORDERED GLUCOCORTICOID FEEDBACK REGULATION OF THE HPA SYSTEM

No attempt is made here to give a detailed account of the clinical effects of disruption of feedback control within the HPA system (see Chapter 29). Rather, we consider two clinical examples that underscore the principles of negative feedback and illustrate the consequences of disruption of normal feedback control in the HPA system. The first example is of enzyme defect in the adrenal cortex that results in the absence or deficiency of the afferent glucocorticoid limb of the HPA negative feedback system, whereas the second is probably due to an alteration in the central set point of the negative feedback control system.

Congenital Adrenal Hyperplasia: Failure of Glucocorticoid Negative Feedback

There are several types of inherited enzymatic defects in cortisol synthesis known to result in congenital adrenal hyperplasia (CAH), also known as the adrenogenital syndrome. By far the most common form is due to a deficiency of $P450c_{21}$ (21-hydroxylase; see Fig. 3.8),[88] which leads to a deficiency in cortisol biosynthesis. Excessive androgen secretion results from a failure of glucocorticoid negative feedback and consequent, uncontrolled, high ACTH secretion. Excessive androgen levels may lead to virilization of females *in utero*. About two-thirds of patients also have mineralocorticoid deficiency, resulting in salt wasting. If not obvious during the neonatal period, androgen excess may appear in early infancy, resulting in sexual precocity in boys, and clitoral enlargement and pubic hair growth in girls. Excess androgen accelerates linear growth and epiphyseal closure, leading ultimately to diminished adult height. In adult women with untreated CAH, reproductive function is impaired due to (a) the disturbance of normal menstrual cycles as a consequence of the high plasma progesterone and androgen concentrations, and (b) labial fusion, which prevents successful coitus. The former can be corrected by glucocorticoid replacement therapy whereas the latter can be treated surgically.

The $P450c_{21}$ deficiency is transmitted as a single gene autosomal recessive trait linked to the major histocompatibility complex locus on the short arm of chromosome 6. An allelic variable of classical 21-hydroxylase

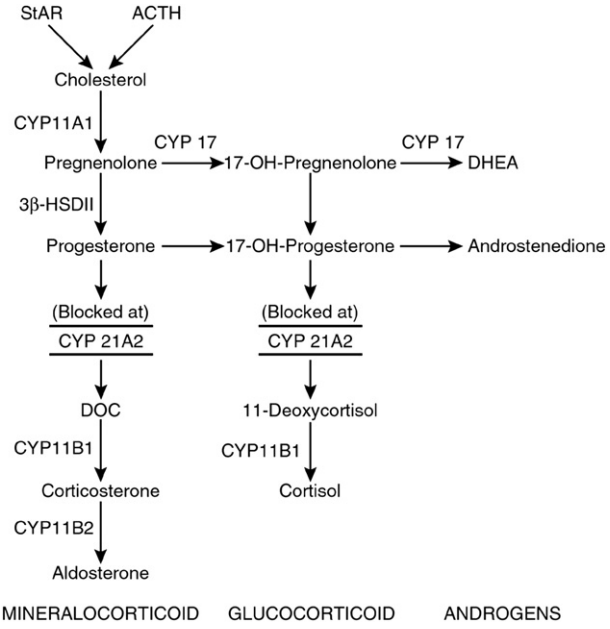

FIGURE 3.8 Congenital adrenal hyperplasia related to 21-hydroxylase deficiency. The normal synthesis of cortisol is impaired, and adrenocorticotropic hormone (ACTH) levels increase because of loss of normal negative feedback inhibition resulting in an increase in adrenal steroid precursors proximal to the block. The results are cortisol deficiency, mineralocorticoid excess related to excessive deoxycorticosterone (DOC) secretion, and excessive secretion of adrenal androgens. DHEA, dehydroepiandrosterone; StAR, steroidogenic acute regulatory protein. *Reproduced from Stewart PM. The adrenal cortex. In: Reed Larsen P, Kronenberg HM, Melmed S, Polonsky KS, eds.* Williams Textbook of Endocrinology, *10th edn. Philadelphia: Saunders; 2003:367–404, with permission.*

deficiency results in a late-onset type, which frequently presents with clinical features similar to those of polycystic ovarian disease.

Deficiency of P450c_{11} (11β-hydroxylase) is a much less common cause of CAH. As in the case of P450c_{21} deficiency, it is transmitted as an autosomal recessive disorder, but is not linked to the HLA locus. Also as in the case of P450c_{21} deficiency, a deficiency in P450c_{11} results in impaired glucocorticoid feedback and a consequent hypersecretion of ACTH and adrenal androgens. The condition is treated with glucocorticoid replacement therapy.

Much rarer forms of CAH are produced by deficiencies of 17α-hydroxylase and 3β-hydroxysteroid dehydrogenase, which result in defective adrenal androgen, as well as glucocorticoid secretion.

Hypercortisolemia in Major Depression: Possibly Due to an Altered Set Point in Glucocorticoid Negative Feedback

Major depressive disorder is characterized by a significant increase in plasma cortisol concentrations (hypercortisolemia), which is most prominent at the nadir of the circadian rhythm, toward midnight. It was first thought that hypercortisolemia and resistance to the suppression of endogenous cortisol secretion by dexamethasone were specific features of major depression, which led to the hope that the dexamethasone suppression test could be used as a specific biological marker of depression. However, extensive studies have shown that hypercortisolemia and resistance to dexamethasone suppression are also associated with other types of psychoses, such as schizoaffective disorder and organic dementia, including Alzheimer's disease.[89–91]

Hypercortisolemia in major depression is associated with a three-fold increase in the mean plasma concentration of β-endorphin.[92] In fact, resistance of β-endorphin to dexamethasone suppression appears to be a more robust marker of major depression than cortisol. Thus, in a study by Young and associates of 73 patients with major depressive disorder, 39 (53%) showed β-endorphin "non-suppression" to dexamethasone while only 8 (11%) showed cortisol "non-suppression".[93] These findings suggest that hypercortisolemia in major depression is due to a resistance of the brain–pituitary–ACTH module to glucocorticoid negative feedback: that is, an elevation of the set point for glucocorticoid feedback. The precise mechanism remains to be determined, but decreased responsiveness of the limbic system, PVN and/or pituitary gland to glucocorticoid negative feedback is a likely explanation. Reduced responsiveness of the PVN could be caused by transynaptic changes triggered by changes in function of the limbic system and frontal cortex. Because the serotonergic raphe neurons determine the amplitude of the circadian excursions of plasma ACTH and corticosterone, it is also conceivable that hypercortisolemia reflects dysregulation of serotonergic function, which seems to occur in major depression.

HPA FEEDBACK CONTROL: SUMMARIZED

The HPA system, together with the sympathetic–medullary system, plays a pivotal role in the neuroendocrine response to stress. Homeostasis within the HPA is maintained by a precise negative feedback system by which the adrenal glucocorticoids (the afferent limb) – cortisol in humans or corticosterone in rodents – moderate ACTH synthesis and release (efferent limb). Allostasis – that is, change in HPA activity to cope with increased stress load – is brought about by change in feedback set point. The major sites of negative feedback are the PVN, where glucocorticoids inhibit CRF and AVP synthesis and release, and the pituitary gland, where they block the ACTH response to CRF and inhibit

POMC/ACTH synthesis. The limbic system of the brain, especially the hippocampus and amygdala, plays an important role in glucocorticoid negative feedback.

Disruption of the HPA negative feedback system has serious deleterious effects, a point illustrated by the congenital adrenogenital syndrome and hypercortisolemia associated with serious mental illnesses. The adrenogenital syndrome, due to defective or absent cortisol secretion (loss of the afferent limb of the feedback system) consequent on a congenital enzyme defect in the adrenal cortex, results in massive uncontrolled pituitary ACTH release. The latter induces excessive androgen production, which in turn causes precocious puberty in males and masculinization of females. Hypercortisolemia, a prominent feature of major depressive disorder and other psychoses and organic dementias, is probably due to elevation of the set point of the glucocorticoid negative feedback in the HPA system. Elucidation of the precise cause of this change in feedback set point may provide insight into the central disorder in depression. Hypercortisolemia may exert adverse effects by (a) inhibiting immune-inflammatory defence mechanisms, (b) disrupting intermediary metabolism, (c) inducing effects akin to Cushing's syndrome that lead to obesity, type 2 diabetes and osteoporosis, and (d) compromising the viability of hippocampal structure, neurogenesis and function that might lead to cognitive impairment.

RELEVANCE FOR THE HYPOTHALAMIC–PITUITARY–THYROID AND –GONADAL AXES: SET POINTS

The principles set out in the HPA feedback system apply equally to the other two major hypothalamic pituitary axes. That is, for the major part all three systems are predominantly under negative feedback control. Negative feedback is mediated through the target hormones. As is the case for the glucocorticoids, a substantial proportion of the gonadal steroids and the thyroid hormones, tri-iodothyronin (T3) and thyroxin (T4), are bound to plasma proteins: it is only the small fraction of free target hormone that exerts a negative feedback action.

The key variant among the three hypothalamic–pituitary systems is the hypothalamic–pituitary–gonadal axis, in which positive feedback plays an essential role in triggering the ovulatory gonadotropin surge. Thus, in rodents, the arcuate nucleus and the anterior pituitary gland[94] tend to be the main site for estradiol negative feedback, while the preoptic area tends to be the main site of estradiol action in triggering positive feedback and the ovulatory GnRH/gonadotropin surge. For details, the reader is referred to Chapters 5, 9 and 19.

Thyroid negative feedback control is more complex than originally thought. It seems that most of thyroid negative feedback is actioned by an effect on TRH at the level of the paraventricular nucleus.[95] Furthermore, as reported by Ghamari-Langroudi *et al*.[96] TRH gene expression is influenced by leptin. Fasting-induced suppression of thyroid hormone levels is an adaptive response to reduce energy expenditure in both humans and mice. This suppression is mediated by the hypothalamic–pituitary–thyroid axis through a reduction in TRH levels expressed in neurons of the paraventricular nucleus of the hypothalamus. *TRH* gene expression is positively regulated by leptin. Whereas decreased leptin levels during fasting lead to a reduction in *TRH* gene expression, the mechanisms underlying this process are still unclear. Indeed, evidence exists that TRH neurons in the PVN are targeted by leptin indirectly via the arcuate nucleus, whereas correlative evidence for a direct action exists as well. The activity of the hypothalamic–pituitary–thyroid axis is regulated by both direct and indirect leptin regulation, with both leptin and α-MSH inducing significant neuronal activity mediated through a postsynaptic mechanism in TRH-expressing neurons of the PVN.[96]

For reasons outlined in Box 3.1, the nature of set points can be complex and defy precise definition. However, broadly, for the hypothalamic–pituitary adrenal system, the set point is comprised of the paraventricular nucleus and the anterior pituitary gland. In the case of the hypothalamic–pituitary–thyroid system, the set point seems to be comprised mainly of the paraventricular nucleus. The arcuate nucleus plus the anterior pituitary gland seem to comprise the set point for negative feedback in the hypothalamic–pituitary–gonadal system, and the preoptic area plus the anterior pituitary gland comprise the set point for estrogen positive feedback.

Glossary

allostasis Maintaining stability (or homeostasis) through change – term introduced by Sterling and Eyer in 1988 (see ref[11]) to describe cardiovascular adjustments to resting and active states.

homeostasis The maintenance of equilibrium, or constant conditions, in a biological system by means of automatic mechanisms (generally feedback systems) that counteract influences tending toward disequilibrium (term introduced by Walter Cannon in 1932[97]).

hypercortisolemia Excessively high concentrations of adrenal corticosteroids in plasma.

hypophysial portal vessels A small system of venules that connects a primary plexus of capillaries in the median eminence of the hypothalamus (base of the brain) with a secondary plexus of sinusoids in the pituitary gland. Blood flowing in these vessels, which run down the pituitary stalk, transports hypothalamic–pituitary regulatory neurohormones released at nerve

BOX 3.1

HOW DO WE KNOW THE VALIDITY OF THE CONCEPT OF THE "SET POINT"?

Unlike the classical example of the room thermostat, which is an all-in-one command and control center, most biological feedback set points and comparators are more complex.

By Analogy with Body Temperature Control

Consider, for example, body temperature control.[1] The concept of feedback of the thermal state of the body, conveniently represented by a high-weighted core temperature (T(c)) and low-weighted peripheral temperatures (T(s)) is equivalent to the control concept of "auxiliary feedback control," using a main (regulated) variable (T(c)), supported by an auxiliary variable (T(s)). This concept implies neither regulation of T(s) nor feedforward control. Steady-states result in the closed control-loop, when the open-loop properties of the (heat transfer) process are compatible with those of the thermoregulatory processors. No set-point and no comparison of signals (e.g., actual set value) are necessary. Metabolic heat production and sweat production, though receiving the same information about the thermal state of the body, are independent effectors with different thresholds and gains. Coordination between one of these effectors and the vasomotor effector is achieved by the fact that changes in the (heat transfer) process evoked by vasomotor control are taken into account by the metabolic/sweat processor.

Control theory deals primarily with the dynamic properties of control-loops (feedback systems). In contrast to such a feedback control, the simpler direct actuation, modification or triggering of a variable (without any feedback of such an action) is referred to as feedforward control. A synonymous term of feedback control is regulation. The term "regulation" is generally recommended in thermal physiology. Deep body temperatures are not as constant as possibly desired, and to a certain extent are dependent on the amount of environmental and internal disturbances, in spite of regulation or feedback control. This fact was interpreted as a consequence of additional aspects of biological complexity and plasticity. Only recently, it was proposed that the term "homeostasis" would be more adequate than "regulation," in view of the amount of integration and flexibility in biological systems. The sequence of controversies started with the erroneous assumption that every "regulator" needs as input a difference of signals, classically the difference between the actual and the reference value of the regulated variable, and that the aim and the consequence of regulation is a difference of zero, thus defining a "set point". Although it was hypothesized that temperature-insensitive neurons could play the role of the reference generators, there was ample evidence that the steady-states reached in temperature regulation were not due to stable reference generators. To solve this apparent discrepancy, a series of questionable theories, explaining the necessary difference of signals and the observed deviations of body temperature in spite of regulation, were developed, among them an "adjustable set point", a set point defined by the balance of warm and cold sensor signals or at least of signals representing information from sensors with different temperature coefficients, a concept of heat flow regulation, and of "comparators" instead of sensors. The term "set point" still is very popular in thermal physiology, although it was shown early that it is not necessary to explain the functional processes of temperature regulation, and indeed may be erroneous.

Romanovsky[2] argues that such a term is ambiguous and misleading in analyzing the system of temperature regulation. Romanovsky rejects the notion that deep body temperature (Tb) is regulated by a unified system with a single controller:

> It is proposed that Tb is regulated by independent thermoeffector loops, each having its own afferent and efferent branches. The activity of each thermoeffector is triggered by a unique combination of shell and core Tbs. Temperature-dependent phase transitions in thermosensory neurons cause sequential activation of all neurons of the corresponding thermoeffector loop and eventually a thermoeffector response. No computation of an integrated Tb or its comparison with an obvious or hidden set point of a unified system is necessary. Coordination between thermoeffectors is achieved through their common controlled variable, Tb.

Most of those controversies, though not in every detail, can be clarified now. Some physiological experimenters still tend to propose concepts which cannot be backed by the logics and physics of system and control theory, and, on the other hand, there are interested engineers still developing ideas and "models" without taking the physiological reality properly into account. None of the definitions of set point ("steady-state of body temperature," "central reference signal," "thermoneutral" or "null zone," or "thermoeffector threshold") have a biological basis.

Relevance for Neuroendocrine Control Systems

There are similarities in principle in the above examples and feedback control systems within the neuroendocrine systems. Specifically, the "set point" refers to a concept concerning a cluster of neuroanatomical

BOX 3.1 *(cont'd)*

structures whose function is modulated by a variety of neural and endocrine inputs. Within each "set point" complex there does seem to be a rough "benchmark" for determining the output of the main pituitary hormone within a known band width. Furthermore, internal and external environmental factors can alter the "set point." Nevertheless, the precise understanding of neuroendocrine "set points" still offers a major research challenge. In the case of the HPA and hypothalamic–pituitary–thyroid axis, for example, does each paraventricular neuron act independently of all other paraventricular neurons? How do neural or neuroendocrine inputs alter the sensitivity and responsiveness of individual paraventricular neurons, and do the latter respond coherently? In terms of the hypothalamic–pituitary–gonadal system, a large question remains as to how the alleged set points for negative (arcuate neurons in rodents) and positive (preoptic neurons) feedback sense and respond completely oppositely to relatively small differences in estrogen concentration?

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terminals in the median eminence of the hypothalamus to the anterior pituitary gland, where they stimulate or inhibit the release of anterior pituitary hormones.

limbic system An extensive brain region that includes the cingulate and parahippocampal cortex, the hippocampus, amygdala, hypothalamus, septal nuclei and other structures. The precise interaction of the several components of the system remains poorly understood, but since the proposal by Papez, the limbic system has been implicated in emotion. The connections between the limbic system and the neocortex, hypothalamus, and the olfactory bulb via the olfactory tract suggest that it serves as an important analyzer–integrator of signals, which it conveys from the neocortex to the hypothalamus. The input of olfactory information to the limbic system has led to its alternative name, the rhinencephalon (olfactory brain), and possibly reflects the strong emotive effects of smell in many animals. The structure and relative size of the limbic system have remained remarkably constant through evolution; in humans it is overshadowed by the massive development of the neocortex.

neurohormones Chemical neurotransmitters released from nerve terminals into the hypophysial portal vessels or the systemic circulation at neurohemal junctions and conveyed to their target cells by the blood stream. This contrasts with "classical neurotransmitters" that reach receptors on target cells by crossing synaptic clefts or neuromuscular junctions.

suprachiasmatic nuclei Two small hypothalamic nuclei located immediately above (dorsal to) the optic chiasm that are responsible for regulating most circadian rhythms of the body.

zeitgeber Generic term for the generator of bodily rhythms (from the German meaning time keeper or pacemaker).

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