Regulation of the Hypothalamic-Pituitary-Adrenal Axis by Cytokines: Actions and Mechanisms of Action

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I. INTRODUCTION

A. Hormones and Cytokines: Definitions

Defining what is meant by the term hormone, and what is meant by the term cytokine, is certainly no easy task. Indeed, the work reviewed in this article has changed many people’s opinion as to what our definitions of these classes of cell-cell signaling molecules should be. However, we are faced at the outset with conveying to the reader what “we” mean when we use the terms cytokines and hormones throughout this review.

A classical endocrinology textbook definition of a hormone is “a biomolecule, which is produced by a specialized cell type, is secreted from a ductless gland directly into the bloodstream, and acts on distant target cells/tissues, to regulate pre-existing cellular activities.” Chemically, hormones are small to large polypeptides, proteins, glycoproteins, derivatives of aromatic amino acids, or steroids. In the case of the pituitary peptide hormone adrenocorticotropic hormone (ACTH), it is produced by corticotropes (specialized cell type) within the anterior pituitary (a ductless gland), is secreted into the general circulation, and acts predominantly on the adrenal cortex (a distant target) to enhance glucocorticoid secretion. This is perhaps a narrow view of a hormone, because many “classical” hormones are also synthesized and act within the brain and are produced and act locally within the periphery. On the other hand, it is perhaps too broad, because under this definition CO₂ produced by exercising muscle and stimulating respiration might have to be considered a hormone also. However, it is a fairly accurate description of what most people understand when thinking of a hormone acting in a “classical endocrine fashion.”

In contrast to hormones that have most commonly been associated with the “endocrine system,” cytokines have been classically associated with the “immune system.” Defining a cytokine is even more difficult than defining a hormone. For the purposes of the work described within this review, we found the definition used in The Cytokine Handbook (862) most useful. Here cytokines are defined as “regulatory proteins secreted by white blood cells and a variety of other cells in the body; the pleiotropic actions of cytokines include numerous effects on cells of the immune system and modulation of inflammatory responses.” This definition is somewhat narrow in that it probably overemphasizes the importance of the immune system as a source and target, but probably reflects most accurately people’s first thoughts when they think of cytokines. It certainly reflects best the definition that would have been applied at the time when the majority of the work described in this article was performed. Under any definition, the term cytokine encompasses the “monokines” (monocyte/macrophage-derived mediators) and “lymphokines” (lymphocyte-derived mediators), which were terms commonly used in the earlier studies described in this review.

Table 1 was compiled of what is presently known of the features of hormones and cytokines. Although this is again not definitive, it is fair to say that if the majority of characteristics of the substance under consideration fit in the cytokine column, then the substance is a cytokine, and if the majority of characteristics fit in the hormone column, then it is a hormone. It should be pointed out that the same chemical substance could be classified differently depending on the “setting” under consideration. For example, prolactin produced by the pituitary and acting on the mammary gland is clearly acting in an “endocrine hormone” fashion. However, prolactin can also be produced by, and act on, lymphocytes, a situation in which it might be better classified as a cytokine. Perhaps the key difference between cytokines and hormones that we indicate in Table 1 is that cytokines are regulators of predominantly local tissue processes, whereas hormones function as regulators predominantly of “systemic” or “whole body” homeostasis.

B. Concept of Bidirectional Communication Between Immune and Neuroendocrine Systems: a Historical Perspective

Regulation of the immune system by the adrenal gland was observed as early as the middle of the 19th century when Thomas Addison (3) documented that a patient with adrenal insufficiency had an excess of circulating lymphocytes. In agreement with this observation, removal of the adrenal gland of the rat was found to produce hypertrophy of the thymus (an organ responsible for the manufacture of mature lymphocytes) (363). Perhaps the best known of early experimental studies were those of Hans Selye (764, 765), who found that enlargement of the adrenal gland and involution of the thymus were communal features of an animal’s response to stress, regardless of the nature of the injurious insult. These early studies clearly suggested a close association between adrenal gland physiology and immune activity.

The isolation of the active principal of the adrenal cortex, cortisone, by Kendall and Reichstein in the late
TABLE 1. Features of cytokines and classical endocrine hormones

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Classical Endocrine Hormone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td>Large polypeptides, proteins, or glycoproteins</td>
</tr>
<tr>
<td>Cell sources</td>
<td>Secreted by white blood cells (and many other cells in numerous types of tissues and organs)</td>
</tr>
<tr>
<td>Concentrations in healthy, stress-free subjects</td>
<td>Very low (virtually absent); increase markedly during tissue disease, injury, or repair</td>
</tr>
<tr>
<td>Location of action relative to secretion</td>
<td>Act predominantly locally, in a paracrine or autocrine manner</td>
</tr>
<tr>
<td>Range of activities</td>
<td>“Pleiotropic,” multiple target cell types and broad spectrum of actions</td>
</tr>
<tr>
<td>“Redundancy”</td>
<td>Display great overlap of biological activities (i.e., “redundancy”)</td>
</tr>
<tr>
<td>Function</td>
<td>Function predominantly as regulators of local tissue processes</td>
</tr>
</tbody>
</table>

TSH, thyroid-stimulating hormone; ACTH, adrenocorticotropic; T₃, 3,3',5-triiodothyronine; T₄, thyroxine.

1940s, and the demonstration of its ability to suppress inflammation (335), gave support to the hypothesis that adrenal glucocorticoid (GC) secretion plays a significant role in regulating immunologic processes. However, even though marked elevations in the plasma blood concentration of GC are observed after all types of stressful stimuli, studies showing that GC produce immunosuppression were assumed (by the majority of workers) to be of pharmacological, rather than of physiological, significance. These findings advertised the widespread use of GC-based therapies for autoimmune and inflammatory disease. It was not until the late 1970s and the pioneering work of Besedovsky, del Rey, Sorkin, and colleagues that a physiological role for GC in preventing overactivity, and preserving the specificity, of immune reactions became established (67, 191). In a significant review article of the early 1980s, Munck et al. (567) reinforced this concept. These authors proposed the now commonly held view that endogenous GC act to prevent “overshoot” of immune/inflammatory responses, thus limiting the host defense response to fighting the aggressor (e.g., invading pathogen) without the deleterious effects to the host of a hyperactive immune system (e.g., autoimmunity). More recent work has indicated that the influence of GC on immunologic processes is more complex than a generalized suppression of immune activity and depends on the type of immune activity and the subset of immunologic cells involved (see Ref. 531 for extensive review). However, it is clear that endogenous GC are key regulators of immune system function.

Further work by Besedovsky et al. (73) suggested that not only do GC have a profound impact on immune activity, but that the converse is also true and immune activity influences GC secretion. This hypothesis grew out of experimental observations that during the development of an immune response to a foreign antigen (sheep red blood cells), rats mount a parallel endocrine response characterized by elevated plasma levels of GC (corticosterone) (73). Furthermore, mice injected with supernatants from concanavalin A-stimulated peripheral blood or spleen cells produce one or more GC-increasing factors (GIF) that increase the blood concentrations of corticosterone (68). These studies therefore suggested the existence of an immune-neuroendocrine regulatory feedback mechanism in which immune cells limited their own activity by secreting molecules that stimulate the secretion of adrenal GC.

The concept of “bidirectional communication” between immune and endocrine systems became firmly established with the seminal works of Edwin Blalock and co-workers in the early 1980s. These workers began to describe molecular basis for such bidirectional communication (reviewed in Refs. 80–82, 952, 953). Their early studies showed a commonality in the pathways of action of immunoregulators (e.g., interferon) and hormones (e.g., norepinephrine) (84). This group went on to discover that a number of classical hormones are not only secreted by classical endocrine glands (e.g., pituitary) but are also made by cells of the immune system (e.g., lymphocytes). For example, they showed that lymphocytes synthesize ACTH, the pituitary hormone which is the major physiological regulator of adrenal GC secretion (792). In addition, they demonstrated that not only do lymphocytes produce hormones such as ACTH, endorphins, thyrotropin, and growth hormone (792, 794, 951) but that these hormones were able to influence immunologic processes (79, 83, 370, 371). Subsequent studies have demonstrated that a number of hormones (e.g., prolactin, insulin-like growth factor) are produced by lymphocytes (372, 556, 557, 621).
The finding that lymphocytes are able to synthesize an ACTH-like molecule, combined with the demonstration that mice whose pituitary gland had been removed (hypophysectomy) still produced a corticosterone response to infection with the Newcastle disease virus (NDV), led Blalock and co-workers (793) to propose the concept of lymphoid-adrenal axis. According to this hypothesis, ACTH produced by virus-stimulated lymphocytes acts on the adrenal to increase corticosterone secretion. However, subsequent studies have failed to replicate the persistence of an NDV-induced corticosterone response in hypophysectomized mice (64, 218, 221, 604), and the hypothesis of a lymphoid-adrenal axis involving lymphoid production of ACTH molecule has fallen out of favor. Furthermore, Besedovsky et al. (71) showed that stimulated lymphocytes secrete GIF that increase plasma ACTH and corticosterone levels in rats and that this corticosterone response was prevented by hypophysectomy. Given that the electrical and neurochemical activities of the hypothalamus are also altered during the course of an immune response (69, 72), Besedovsky et al. (71) proposed that the effects of such GIF on adrenal GC secretion were probably mediated at the hypothalamic component of the hypothalamic-pituitary-adrenal (HPA) axis, rather than on the pituitary or adrenal glands directly.

The chemical identity of putative “GIF” became apparent with the recognition that classical endocrine hormones are not the only class of mediators involved in immune-endocrine communication. Indeed, in the mid 1980s, it became readily apparent that immunoregulatory cytokines also form a key link between immune and neuroendocrine systems (70, 331, 532, 966). Blalock and co-workers (966) showed that the monokines interleukin (IL)-1 and IL-6 (or hepatocyte-stimulating factor) stimulate ACTH secretion from the corticotropic tumor cell line AtT20.1 A year later, Besedovsky et al. (70) demonstrated that systemic administration of monocyte-derived or recombinant IL-1 increases plasma ACTH and GC concentrations in normal mice. Furthermore, Besedovsky et al. (70) demonstrated that neutralization of endogenous IL-1 inhibits the GC response to experimental viral infection (NDV) in rats. This latter experiment clearly indicated that the observations of stimulatory effects of cytokines on neuroendocrine secretion were not merely pharmacological phenomena and suggested that IL-1 plays an important endogenous role in regulating the HPA axis during viral disease. Indeed, these landmark studies by Besedovsky and Blalock indicated that cytokines could be the extrahypothalamic corticotropin-releasing factors (CRF) released by injured tissue which had previously been reported by Brodhis and co-workers during the 1970s (112, 113, 497).

Subsequent work by three independent laboratories resulted in articles being published back to back in a 1987 issue of Science (55, 62, 730). One of these studies (62) demonstrated a direct action of IL-1 on ACTH secretion from primary cell cultures of rat anterior pituitary cells, thus supporting the earlier studies by Blalock and co-workers (966) suggesting a direct action of IL-1 on the pituitary gland to secrete ACTH. Conversely, the other two Science papers (55, 730) found that IL-1 does not stimulate ACTH secretion from anterior pituitary cells in primary culture, despite the fact that IL-1 in vivo elevates plasma ACTH and GC concentrations. These latter two groups (55, 730) showed that IL-1-induced ACTH secretion in vivo is dependent on the secretion and action of the hypothalamic 41-amino acid peptide CRF, which is the major hypothalamic ACTH secretagogue. These findings clearly implicate the hypothalamus as the site at which the HPA axis response to IL-1 is mediated and gave great support to the idea that immunoregulators could influence the activity of the central nervous system (CNS).

Controversy over the likely primary site of IL-1 action (CNS, pituitary gland, or possibly adrenal glands) in stimulating pituitary-adrenal secretion has continued for many years and is considered in detail in sections IV and V. However, a large body of evidence has now accumulated that indicates that IL-1 and other cytokines can signal the brain. In parallel with studies investigating the relationship between the immune system and HPA axis, a large number of studies indicated that fever caused by invading pathogens occurs as a result of the elaboration from immune cells of an “endogenous pyrogen” capable of signaling the CNS (reviewed in Refs. 23, 420). This endogenous pyrogen was putatively identified as IL-1 (reviewed in Ref. 420). Furthermore, administration of IL-1 produces many CNS-mediated changes including changes in behavior (reduced exploration, reproductive activity, food-motivated behavior, and increased sleep), changes in autonomic outflow, metabolic rate, and the activity of a number of neuroendocrine axes (see Refs. 54, 65, 408, 439, 440, 529, 683, 708 for relevant reviews). Collectively, these studies have provided strong evidence for the regulation by IL-1 of CNS responses to peripheral changes in immune activity. Furthermore, the common mediator of the effects on various CNS responses provides a molecular basis for the observations of the stereotypical responses to immune challenges of diverse origins. This “acute phase response” to sickness is characterized by fever, appetite suppression, anorexia, alterations in plasma cation concentrations, synthesis of specific liver proteins (known as acute phase proteins), and changes in neuroendocrine secretion (441). It is now firmly established that acute phase responses are produced by the actions of, and complex interactions between, IL-1 and numerous other cytokines.

Since the landmark studies by the groups of Besedovsky and Blalock, it has become apparent that IL-1 has potent effects on the secretion of the majority of hor-
mones under neuroendocrine control (see Table 2). Furthermore, more recent studies have shown that alterations in neuroendocrine secretion are produced not only by IL-1, but also by many other immunoregulatory cytokines. The HPA axis has remained the most extensively studied neuroendocrine system with respect to the influence of cytokines, and the ability to increase the secretory activity of this axis is a biological property of several interleukins, tumor necrosis factors, chemokines, hematopoietins, interferons, growth factors, and neurotrophic factors.

This article reviews published findings that demonstrate 1) which cytokines influence hormone secretion from the HPA axis, 2) under what physiological/pathophysiological circumstances endogenous cytokines may influence HPA axis secretory activity, 3) at which level (hypothalamus, pituitary, or adrenal) cytokines primarily act, and 4) what anatomic and pharmacological pathways mediate the actions of cytokines on the neuroendocrine hypothalamus. To achieve this aim, we have divided this article into sections corresponding to these overall objectives. We begin by providing brief introductions to relevant aspects of cytokine biology (see sect. 1C) and the functional anatomy of the HPA axis (see sect. 1D).

C. Cytokines

1. Cytokines and cytokine receptor families

Cytokines are large (8–60 kDa), soluble polypeptide mediators that regulate growth, differentiation, and function of many different cell types (see Table 3). The majority of cytokines have been classically associated with the regulation of immune and/or inflammatory processes, and within the immune system, their actions are generally exerted in paracrine or autocrine fashions. However, because of the demonstration that immune, central nervous, and neuroendocrine systems share a common chemical language, much more diverse actions of cytokines in host defense are now recognized. Accordingly, the expression of these polypeptides and their receptors is not restricted to cells of the immune system but is also found in many other tissues (including the brain and endocrine glands). Furthermore, many cytokines exert potent actions on a variety of physiological activities outside of immunoregulation; for example, many cytokines induce fever, sleep, anorexia, malaise, and alterations in neuroendocrine secretions. Finally, the ability of some cytokines to regulate homeostatic processes at tissues distant from their site of production has firmly established cytokines as key regulators of coordinated local and systemic responses to tissue trauma, infection, and disease.

The classification of cytokines into families has proven somewhat arbitrary. With the exception of a few homologous peptides (e.g., IL-1α and -1β; interferon-α and -β; and tumor necrosis factor-α, -β) most cytokines share little sequence similarity. Consequently, classification of cytokines has been based on either functional attributes, target receptors, or cells of origin. Most commonly, cytokines have been classified into families of interleukins, tumor necrosis factors (TNF), interferons (IFN), chemokines, hematopoietins (or neutrophoietins), and colony-stimulating factors (CSF). Because of their similar actions particularly within the CNS and peripheral nervous system, growth factors (GF) and neurotrophins (NT) have also been considered to fall under the umbrella term cytokine. Their overlapping actions lead to a number of cytokines belonging to more than one family (see Table 3). For example, IL-6 is not only an interleukin, but also a member of a family of either hematopoietic or (neuro)poietic factors that utilize an identical receptor subunit (gp130) for cell signaling (416). Furthermore, IL-1, IL-3, IL-5, and IL-6 are also CSF.

One of the striking features of cytokines is their ability to exert many different actions (a property known as “pleiotropy”) and, conversely, that many different cytokines exert the same biological actions (a property known as “redundancy”) (162, 633). Cytokine pleiotropy presumably relates to the widespread distribution of cytokine receptors on numerous cell types and the ability of signal transduction mechanisms activated by cytokines to alter expression of a wide variety of target genes. The functional redundancy of various cytokines has, at least partially, been explained by the identification and molecular cloning of many cytokine receptors. Some, although certainly not all, cytokine receptors consist of a multiunit complex, including a cytokine-specific ligand binding component and a “class”-specific signal transduction unit (416, 733). In addition to the gp130 signaling cytokines, common receptor subunits have also been demonstrated for IL-2, IL-4, and IL-7 (733) and also for IL-3, IL-5, and granulocyte-macrophage CSF which share the signal transduction subunit KH7 (552). However, cytokine redundancy cannot be totally explained by the sharing of
TABLE 3. Cytokine families

<table>
<thead>
<tr>
<th>Family</th>
<th>Members</th>
<th>Major Ascribed Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukins</td>
<td>IL-1 to IL-18</td>
<td>Categorization as an IL does not imply function; IL have numerous and diverse immunoregulatory actions; some IL have clearly proinflammatory effects (e.g., IL-1a, IL-1β, IL-8, IL-9), whereas others have anti-inflammatory effects (e.g., IL-1ra, IL-4, IL-10, IL-13). Many IL also induce systemic aspects of acute phase response (e.g., fever)</td>
</tr>
<tr>
<td>Tumor necrosis factors</td>
<td>TNF-α, TNF-β</td>
<td>Tumor cytotoxicity; broad-ranging immunologic activities; induction of many other cytokines; immunostimulant; proximal mediator of inflammatory response履行</td>
</tr>
</tbody>
</table>
interactions is illustrated by the large number of different cytokines that may be produced by a single threat to cellular/tissue homeostasis. For example, endotoxemia has been reported to cause the increased synthesis and/or secretion of IL-1α, IL-1β, IL-1ra, IL-6, IL-8, IL-10, IL-12, TNF-α, MIP, macrophage migrating inhibitory factor (MIF), IFN-γ, leukemia inhibitory factor (LIF), and granulocyte-macrophage CSF. It is therefore apparent that during the course of a threat to tissue homeostasis, the physiological outcome is determined by the net effect of the interactions between a number of cytokines.

Although many different cytokines have been shown to influence HPA axis secretory activity, by far the majority of studies have focused on the cytokines IL-1, IL-6 and TNF-α. These three cytokines share many biological activities (see Table 4). Section iC2 gives a brief outline of their structure, biosynthesis, and receptors.

2. IL-1, IL-6, and TNF-α

There are at least three distinct glycoproteins that constitute the IL-1 family. The two agonists, IL-1α and IL-1β, share ~25% sequence homology, are distinct gene products, and exhibit the same activities in numerous biological test systems (205). Both are synthesized as 31-kDa precursor molecules. Pro-IL-1β is biologically inactive and requires proteolytic cleavage by the IL-1β converting enzyme (ICE, also known as caspase-1) (401). In addition, an endogenous antagonist at IL-1 receptors (IL-1ra) has been described, which shares significant homology with IL-1α and IL-1β, binds IL-1 receptors, but lacks intrinsic biological activity (225, 318). Interleukin-1ra has been used extensively as a pharmacological tool to explore the role of IL-1-IL-1 receptor interactions in physiological responses (203). More interestingly, however, endogenous IL-1ra is secreted by similar cell types, and in response to similar stimuli, as those which produce the IL-1 agonists, and endogenous IL-1ra plays an important role in regulating the physiological responses to endogenous IL-1 (260, 485). Recently, a fourth member of the IL-1 family has been proposed. Structural alignment of mouse IL-18 (also known as IFN-γ inducing factor, IFIG) demonstrated a 12 and 19% structural homology of this newly cloned cytokine with IL-1β and IL-1α, respectively (46). Interleukin-18 was thus tentatively termed IL-1γ (46). Although there is, as yet, little information about this new cytokine (850), it is known that lipopolysaccharide (LPS)-stimulated production of mature IL-18/IL-1γ requires ICE (249, 287, 307). Furthermore, IL-18/IL-1γ and IL-1 itself share similar signaling pathways (428) and functional activities (351), again indicating a close relationship between this novel cytokine and the IL-1 family.

Two distinct mammalian, membrane-bound IL-1 receptors have been described and designated IL-1R1 and IL-1R2 (784). Both are glycoproteins belonging to the immunoglobulin supergene family and possess a single transmembrane domain. Each receptor binds IL-1α, IL-1β, and IL-1ra, but with differing affinities (784). It has been proposed that the biological actions of IL-1 are mediated exclusively through IL-1R1 (442, 785), with IL-1R2 functioning solely as a decoy receptor that limits the availability of IL-1 for interaction with IL-1R1 (163, 784, 786). In contrast, some studies have demonstrated that a monoclonal antibody (ALVA 42) raised against IL-1R2 inhibits some actions of IL-1 within the brain (493, 548), although the ability of this antibody to bind IL-1R2 has been questioned (282). In addition to the two receptor isoforms, an accessory protein (IL-1RAcP) has been identified that enhances binding of IL-1 to IL-1R1 (304, 482) and plays a critical role in cell signaling through this receptor (348, 433, 994). Several additional members of the IL-1 receptor family have been identified on the basis of sequence homology (53, 94, 278, 488, 550, 627, 989). One of these proteins (IL-1 receptor-related protein, IL-1Rrp) has recently been identified as a functional receptor for IGIF/IL-18/IL-1γ (869).

Tumor necrosis factor also occurs in α- and β-forms, which share ~50% homology. Tumor necrosis factor-β (lymphotoxin-α) is produced predominantly by activated lymphocytes. In contrast, TNF-α (also known as cachectin) is expressed on a wide variety of hemopoietic and nonhemopoietic cells as a 26-kDa membrane-associated molecule. This can be processed to give a secreted 17-kDa soluble form that mediates a range of inflammatory and cellular immune responses. Tumor necrosis factor is one of 10 known members of a family of ligands that activate a family of structurally related receptors. These include receptors for TNF-α and TNF-β, lymphotoxin-β, Fas ligand, nerve growth factor (NGF), and CD40 ligand (47). All the ligands for these receptors consist of three polypeptide chains, and the majority are transmembrane proteins that act mainly through cell-to-cell contact. However, TNF, as indicated, is also secreted.

Actions of TNF-α are exerted through interactions with two distinct receptors: the 55-kDa (TNF-R1) and 75-kDa (TNF-R2) receptors (47). These two receptors are both transmembrane proteins with a single transmembrane span and are expressed at low levels on most cell types. Although the extracellular domains of these two receptors show a similar architecture, the intracellular domains of these two receptors bear no significant homology, suggesting that they utilize separate signaling pathways (467). Indeed, studies of the effects of receptor-specific agonistic antibodies (233, 241, 283, 851, 968) and of TNF-R-deficient mice (234, 642, 707) indicate that these two receptors mediate effects that are largely, but not exclusively, nonoverlapping. Recent molecular studies have shed a considerable light on the activities of the two receptors (reviewed in Ref. 182). Binding of TNF to either receptor activates the proinflammatory transcription fac-
tor NFκB. In the case of TNF-R2, signal transduction occurs via heterodimerization of the receptor with two TNF-R2 associated factors, TRAF1 and TRAF2, and it is TRAF2 that appears to mediate TNF-R2-induced activation of NFκB. In contrast, TNF-R1, upon ligand binding, recruits a protein called TRADD. Like TRAF2, TRADD causes NFκB activation but, unlike TRAF2, also causes apoptosis via an ICE-like protease. This explains why TNF-R1, but not TNF-R2, causes apoptosis. However, the NH2-terminal domain of TRADD interacts directly with TRAF2, and overexpression of a dominant negative TRAF2 blocks not only TNF-R2 but also TNF-R1-induced NFκB activation. Thus activation of the two TNF receptors elicits separate signaling pathways that can interact with one another, thus explaining the distinct and overlapping signals generated by the two TNF receptors.

Interleukin-6 is a single 21- to 28-kDa glycoprotein produced by both lymphoid and nonlymphoid cells and regulates immune responses, acute-phase protein synthesis, and hematopoiesis. Human IL-6 is synthesized as a precursor polypeptide of 212 amino acids that is processed by cleavage of a 28-amino acid NH2-terminal signal sequence into a mature form of 184 amino acids.

One IL-6 receptor has thus far been identified. This IL-6 specific receptor (IL-6Rα) is responsible only for binding of its ligand (IL-6). Interleukin-6 belongs to a family of cytokines that includes ciliary neurotropic factor (CNTF), oncostatin M (OM), LIF, IL-11, and cardiotropin-1 (CT-1), which share a common signal-transducing mechanism (reviewed in Refs. 415, 416). All these cytokines are bound by receptors that interact with the common cell-surface protein gp130. Ligand-receptor complexes that share gp130 trigger signaling by the formation of either homodimers of gp130 or heterodimers between gp130 and LIFR. In the case of IL-6, signaling is initiated by the homodimerization of gp130 induced by the interaction with the IL-6/IL-6Rα complex. Either homodimerization of gp130 or heterodimerization of gp130 with LIFR activates JAK kinases, followed by the tyrosine-specific phosphorylation and nuclear translocation of a member of the STAT family (STAT3) of transcription factors. In addition, there is another signaling pathway that involves the activation of the RAS-MAP kinase cascade followed by the activation of transcription factors such as nuclear factor IL-6 (NF-IL-6). Such sharing of receptor complexes and subsequent activation of similar signaling pathways by members of the IL-6 family of cytokines is a clear example of how a number of different cytokines display similar biological activities (i.e., cytokine redundancy).

Receptors for IL-1, IL-6, and TNF-α occur not only in membrane-bound forms, but also as truncated soluble products, which are capable of binding their ligand (257, 332, 703). These receptors are generated either by proteolytic cleavage at the cell surface or are synthesized as alternatively spliced mRNA species. The ability of IL-1 and TNF soluble receptors to bind their ligands limits the availability of either IL-1 or TNF-α for interaction with their membrane-bound receptors and therefore confers antagonistic properties to these truncated receptors. Indeed, coincubation of IL-6 with its soluble receptor has been demonstrated to confer IL-6 sensitivity to previously IL-6-insensitive cells and enhances the effectiveness of IL-6 in vivo (506, 641, 747). Thus the biological activity of a particular cytokine is determined not only by its own concentration and the concentration of cytokines which influence its activity but also by the presence of its soluble receptor.

D. Hypothalamic-Pituitary-Adrenal Axis

1. HPA axis organization

Over the last 10–15 years, there have been over 1,000 published articles concerning the activation of the HPA axis by cytokines. This relative abundance of work is due to the large number of cytokines discovered, the complexity of the organization of the HPA axis (see Fig. 1), and the functional importance of activation of the HPA axis during stressful situations. Basal secretion of GC is necessary for the normal function of most tissues, and even small deviations from normal circulating levels of these steroids produce changes in a wide variety of physiological and biochemical parameters. Interactions between the endocrine system and the CNS result in a diurnal rhythm of GC secretion with a peak occurring at the time of awakening and a nadir during the first few hours of sleep. Blood levels of circulating GC increase in response to virtually any type of stimulus that poses, or is perceived to pose, a threat to bodily homeostasis. Glucocorticoids act on multiple targets to enhance or inhibit various cellular activities, actions that are aimed at providing the altered metabolic, endocrine, nervous, cardiovascular, and immunologic needs that promote survival. Not surprisingly, therefore, the regulation of blood levels of GC is subject to diverse sensory inputs, and this information is integrated at the level of the hypothalamus.

The primary CNS nucleus involved in the regulation of pituitary-adrenal axis is the paraventricular nucleus (PVN) of the hypothalamus. The PVN is the principal CNS source of the 41-amino acid peptide CRF, which is the major physiological regulator of pituitary ACTH secretion (691). The CRF hypophysiotropic neurons from the PVN project to the external zone of the median eminence (ME) and release CRF into a specialized capillary network. The anterior pituitary (or adenohypophysis) is vascularized by hypophysial portal vessels that arise from these median eminence capillary beds. Within the anterior pituitary, CRF interacts with a specific G protein-coupled receptor
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REGULATION OF HPA AXIS BY CYTOKINES

ing rise to long descending projections to brain stem autonomic structures (738). The mPVN together with the supraoptic nucleus (SON) constitute the magnocellular neurons that are the major cell sources of arginine vasopressin (AVP) and oxytocin released into the general circulation from neurons terminating in the posterior pituitary. The more medially situated pPVN is the major source of hypophysiotropic CRF neurons, which release CRF into the hypophysial portal circulation. The cell groups projecting to autonomic structures contain all three peptides (CRF, AVP, and oxytocin). However, although these subdivisions of PVN are anatomically discrete, the PVN does display considerable peptide phenotype plasticity. The CRF hypophysiotropic neurons also produce a number of additional peptides, most notably AVP (740, 864), which interacts synergistically with CRF to stimulate ACTH secretion (693), and whose synthesis in these neurons can be enhanced during increased pituitary-adrenal activity (738). Furthermore, AVP and oxytocin derived from sources other than the pPVN may also contribute to the pool of ACTH secretagogues in hypophysial portal blood (15, 648, 651). Therefore, although it is generally agreed that CRF arising from the pPVN is the major means of stimulating ACTH secretion, this is not an absolute, with AVP (and possibly oxytocin) secretion from either pPVN or magnocellular neurons contributing to an extent that varies with the nature of the physiological threat.

Consistent with the extreme diversity of stressful stimuli that give rise to activation of the HPA axis, the pPVN receives diverse inputs from regions of the brain conveying visceral, somatosensory, auditory, nociceptive, and visual information and also from limbic regions involved in the integration of cognitive and emotional influences (738). These inputs include projections from other nuclei within the hypothalamus (e.g., medial preoptic anterior hypothalamus). Extrahypothalamic inputs include areas both within (e.g., nucleus of the solitary tract (NTS) and other medullary catecholaminergic cell groups) and outside (organum vasculare of the lamina terminalis (OVLT) and subfornical organ (SFO)] the blood-brain barrier (BBB) (738). There are, therefore, multiple levels at which the activity of the HPA axis may be modulated, and indeed, virtually all the regulatory processes described above have been proposed as sites at which cytokines regulate HPA axis activity.

2. Experimental assessment of the HPA axis secretory activity

The measurable end points and experimental methodologies that have been used to examine the secretory activity of the HPA axis in response to cytokines are extremely diverse and shall be considered here briefly. The net result of increased HPA axis secretory activity is the
elevated concentrations of ACTH and GC in blood. Temporal measures of immunoreactive levels of these hormones have been widely adopted as means of studying the influence of cytokines on the HPA axis and are the main method suitable for use in humans. Furthermore, the determination in laboratory animals of the effects of various surgical, pharmacological, or genetic manipulations on the plasma hormone response to a given cytokine provides a valuable means to elucidate the anatomic and neurochemical mechanisms involved in the activation of the HPA axis by a particular cytokine.

To assess the activity of neuronal components of the HPA axis in response to a particular cytokine, a number of methods have been employed, including the use of electrophysiological recordings and the histochemical determination of the expression of cellular immediate early genes (cIEG), such as c-fos. The demonstration of c-fos as an inducible and widely applicable marker of neuronal activity (558) has afforded a means of mapping neuronal activation in response to a variety of stimuli, including the administration of cytokines. In particular, the stress-induced induction of c-fos mRNA and/or Fos protein within the PVN has been thoroughly examined and validated as a means to identify activation of neurosecretory neurons (152, 436). Another cIEG, NGFI-B, has been used for similar purposes (152, 436). Identification of the peptide phenotype of cells within the PVN expressing cIEG provides a powerful means to identify the activation of particular neurosecretory neurons. However, it should be noted that at present we do not know how the induction of such transcription factors relates to transcriptional activity within the PVN. Indeed, of the three peptides CRF, AVP, and oxytocin, only the AVP gene includes an AP-1 response element that binds Fos-Jun dimers, but all three genes contain potential NGFI-B response elements (152).

Functional assessments of the relative neurosecretory rates of peptides from hypophysial PVN neurons have also been performed by measuring 1) peptide concentrations directly in portal blood, 2) peptide concentrations in the perfusates from push-pull cannulas or microdialysis probes within the ME, and 3) peptide content of the ME of either normal animals or animals pretreated with colchicine to block axonal transport. Finally, determination of the expression of steady-state mRNA or primary transcript RNA (hnRNA) levels of either CRF or AVP within the PVN have also been well documented.

To determine the direct effects of cytokines on particular components of the HPA axis, a number of in vitro methodologies have also been used. Only one cell line has been available to study the action of cytokines on the HPA axis, namely, the AtT20 mouse corticotrope tumor line, which has been used as a model for investigating the direct effects of cytokines on anterior pituitary corticotropes. By far the majority of in vitro studies have utilized static or perfused primary preparations of either hypothalami, anterior pituitaries, or adrenals, with the tissue being intact (whole), in segments or slices, or in dispersed monolayer cell culture. These methodologies produce a more isolated environment in which to define direct actions of applied substances.

II. CYTOKINE INFLUENCE ON HYPOTHALAMIC-PITUITARY-ADRENAL AXIS SECRETORY ACTIVITY IN VIVO

A. Animal Studies

Numerous studies have confirmed and extended the original findings that the administration of either IL-1α or IL-1β to rats or mice stimulates ACTH and GC secretion, as well as many other indices of HPA activation (see Table 5). In addition, IL-1 has been demonstrated to increase the secretory activity of the HPA axis of chickens (959), sheep (916), baboons (674), and humans (see sect. nB). Studies addressing interactions between cytokines and neuroendocrine systems in an invertebrate species (snail) have demonstrated the presence of a rudimentary stress system involving CRF-, ACTH-, and bioamine-like molecules in immunocytes (613–616). The snail immunocyte stress system contains, and is responsive to, cytokines, including IL-1, IL-2, and TNF-α (612, 615–617). Activation of the HPA axis (or invertebrate equivalent) by IL-1 has therefore been highly conserved throughout evolution in different species and taxa, indicating the importance of this adaptive response to survival (615).

In mammals, the ACTH response to intravenous IL-1 is usually prompt, commencing within 5–10 min, and of relatively short duration (~1 h). In comparison, the plasma ACTH response to intraperitoneal injection of IL-1β is slower in onset, but usually of longer duration (at least 2 h). Finally, the response to IL-1 administered directly into the brain (intracerebroventricularly) is of intermediate latency and lasts for several hours (usually greater than 3–4 h). The majority of studies have found IL-1β to be more potent than IL-1α in the rat (525, 574, 690, 695). Studies utilizing a panel of monoclonal antibodies have demonstrated that amino acids in the domain 66–85 on the recombinant rat IL-1β molecule are critical for its ACTH-releasing capacity (751). In the rat, IL-1β stimulates ACTH secretion at all stages of postnatal development of both males and females, although the magnitude of the ACTH response depends on age and gender (59, 192, 466, 598, 686).

A single administration of IL-1β not only acutely elevates plasma ACTH and corticosterone concentrations in the rat, but has been demonstrated to produce a long-lasting (at least 3 wk) increase in the coexpression of AVP in hypothalamic CRF neurons and a hyperresponsiveness
of the HPA axis (744). Long-term administration of IL-1β to rats enhances CRF- and ACTH-like immunoreactivities in the hypothalamus and pituitary, respectively, increases adrenal weight (573), and elevates plasma ACTH concentrations for at least 7 days (573, 830, 908).

In addition to IL-1, a number of other cytokines have been demonstrated to influence HPA axis secretory activity in experimental in vivo paradigms (see Table 5). Activation of the HPA axis is not restricted to cytokines produced predominantly by myeloid (e.g., monocyte, macrophage) cells (e.g. IL-1), but also by cytokines produced by lymphoid (e.g., T lymphocytes) cells (e.g., IL-2).

TABLE 5. Acute effects of cytokines on the HPA axis of laboratory animals

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Route</th>
<th>Effect</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>iv</td>
<td>† CRF in portal blood</td>
<td>223, 525, 574, 629, 730</td>
</tr>
<tr>
<td></td>
<td></td>
<td>† Plasma ACTH, corticosterone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ip</td>
<td>† CRF mRNA</td>
<td>639, 640, 824</td>
</tr>
<tr>
<td></td>
<td></td>
<td>† CRF content of ME</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>† POMC mRNA in anterior pituitary</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>† Plasma ACTH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>icv</td>
<td>† Plasma ACTH</td>
<td>574, 695</td>
</tr>
<tr>
<td>IL-1β</td>
<td>iv</td>
<td>† PVN c-fos mRNA or Fos protein</td>
<td>55, 223, 237, 525, 527, 560, 824, 929, 932, 936, 940</td>
</tr>
<tr>
<td></td>
<td></td>
<td>† PVN CRF mRNA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>† CRF secretion from ME</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>† CRF content of ME</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>† Plasma ACTH, corticosterone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ip</td>
<td>† PVN c-fos mRNA or Fos protein</td>
<td>63, 64, 70, 98, 187, 327, 466, 628, 686, 690, 752, 824</td>
</tr>
<tr>
<td></td>
<td></td>
<td>† CRF mRNA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>† CRF secretion from ME</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>† Plasma ACTH, corticosterone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>icv</td>
<td>† PVN c-fos mRNA or Fos protein</td>
<td>40, 187, 457, 681, 684, 692</td>
</tr>
<tr>
<td>IL-2</td>
<td>iv</td>
<td>† ACTH</td>
<td>574</td>
</tr>
<tr>
<td></td>
<td>icv</td>
<td>† Electrical activity of PVN neurons</td>
<td>76, 317</td>
</tr>
<tr>
<td></td>
<td></td>
<td>† Plasma ACTH*, corticosterone*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ip</td>
<td>† POMC in anterior pituitary</td>
<td>326, 623</td>
</tr>
<tr>
<td></td>
<td></td>
<td>† Hypothalamic vasopressin mRNA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>† Hypothalamic oxytocin mRNA</td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td>ip</td>
<td>† POMC in anterior pituitary</td>
<td>326</td>
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<tr>
<td>IL-6</td>
<td>iv</td>
<td>† PVN Fos protein</td>
<td>527, 575, 589, 909</td>
</tr>
<tr>
<td></td>
<td></td>
<td>† Plasma ACTH, corticosterone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ip</td>
<td>† Plasma ACTH, corticosterone</td>
<td>52, 327</td>
</tr>
<tr>
<td></td>
<td>icv</td>
<td>† Plasma ACTH, corticosterone</td>
<td>499, 527, 859, 909</td>
</tr>
<tr>
<td>IL-11</td>
<td>ip</td>
<td>† Plasma corticosterone‡</td>
<td>52</td>
</tr>
<tr>
<td>IL-12</td>
<td>ip</td>
<td>† Plasma corticosterone</td>
<td>608</td>
</tr>
<tr>
<td>LIF</td>
<td>ia</td>
<td>† Plasma ACTH</td>
<td>5</td>
</tr>
<tr>
<td>OM</td>
<td>ip</td>
<td>† Plasma corticosterone‡</td>
<td>52</td>
</tr>
<tr>
<td>CT-1</td>
<td>ip</td>
<td>† Plasma corticosterone‡</td>
<td>52</td>
</tr>
<tr>
<td>CNTF</td>
<td>ip</td>
<td>† Plasma corticosterone‡</td>
<td>52, 246</td>
</tr>
<tr>
<td>TNF-α</td>
<td>iv</td>
<td>† CRF secretion from ME</td>
<td>58, 66, 223, 771, 772, 938</td>
</tr>
<tr>
<td></td>
<td>icv</td>
<td>† Plasma ACTH, corticosterone</td>
<td></td>
</tr>
<tr>
<td>IFN-α</td>
<td>ip, icv</td>
<td>† or † Plasma corticosterone‡</td>
<td>725, 726, 729</td>
</tr>
<tr>
<td>Activin</td>
<td>icv</td>
<td>† CRF in portal blood</td>
<td>650</td>
</tr>
<tr>
<td></td>
<td></td>
<td>† Plasma ACTH</td>
<td></td>
</tr>
<tr>
<td>EGF</td>
<td>iv</td>
<td>† Plasma ACTH, corticosterone</td>
<td>492, 551</td>
</tr>
<tr>
<td></td>
<td>icv</td>
<td>† Plasma ACTH, corticosterone</td>
<td>551</td>
</tr>
<tr>
<td>NGF</td>
<td>iv</td>
<td>† Plasma ACTH, corticosterone</td>
<td>618, 741, 836</td>
</tr>
<tr>
<td>SCF</td>
<td>iv</td>
<td>† Plasma ACTH, corticosterone</td>
<td>435</td>
</tr>
</tbody>
</table>

Routes of administration: iv, intravenous; ia, intra-arterial; ip, intraperitoneal; icv, intracerebroventricular. PVN, paraventricular nucleus; ME, median eminence; POMC, proopiomelanocortin. Other definitions are as in Table 3. * Chronic (7-day) infusion. Significant increases in plasma ACTH concentration observed only during latter period of dark cycle. † At doses tested, each of these cytokines alone had no effect on plasma corticosterone, but each potentiated the corticosterone response to a submaximal dose of IL-1. ‡ Stimulation of corticosterone secretion was noted only at higher doses of IFN-α.
studies have compared the potencies, IL-1β has generally been found to be the cytokine most potent at stimulating ACTH secretion (e.g., Refs. 66, 358, 527, 772). However, this is not necessarily of physiological significance, since when it is the levels of endogenous cytokines that are elevated, the relative concentrations of each cytokine is a major determinant of which cytokine is of greatest influence. For example, during local inflammation, IL-6, which is generally agreed to be a less potent HPA axis secretagogue than IL-1, is elevated to a greater extent and for greater periods of time than is IL-1. Additionally, at least some cytokines act synergistically to enhance ACTH secretion. For example, all hematopoietic cytokines (IL-6, LIF, OM, CNTF, IL-11, and CT-1) enhance ACTH and/or corticosterone secretion produced by IL-1 to an extent greater than can be accounted for by additive effects (52, 639, 1003). Similarly, TNF-α synergistically enhances ACTH release produced by IL-1β (907).

A number of studies have produced contradictory data with respect to the effects of various cytokines on HPA axis secretory activity. Although a stimulatory effect of TNF-α on the rat HPA axis has not been disputed when the cytokine has been administered peripherally (58, 772, 909), contrasting data have been obtained when TNF-α has been administered directly into the brain (intracerebroventricular). For example, although we showed that intracerebroventricular TNF-α induces marked elevations in plasma ACTH concentrations in rats (881), a number of other investigators have found little or no effect (673, 772, 909). Such discrepancies may, at least in part, be explained by the use of cytokines of different species origin. In the example cited, we used murine TNF-α (881), whereas those reporting little or no effect of intracerebroventricular TNF-α on plasma ACTH levels used human TNF-α (673, 772, 909). Similarly, studies in mice (51) have shown that plasma corticosterone concentrations are elevated to a greater extent by intracerebroventricular murine TNF-α than by intracerebroventricular human TNF-α. Species differences have also been noted with IL-6, and the human IL-6Rα recognizes human IL-6 but not mouse IL-6 (173, 905). The use of human or mouse cytokine preparations has been common due to their wide availability, yet the most commonly used species in the investigation of HPA axis activity is the rat. However, the previous limited availability of recombinant rat cytokines has meant that not many studies have investigated the effects of cytokines in a homologous system (rat cytokine in the rat). When rat IL-1α (574) and rat IL-1β (751) have been tested in rats, the intravenous injection of these cytokines produces a marked elevation in plasma ACTH levels. Recombinant rat cytokines have now become more widely available since the establishment of BIOMED 1 program “Cytokines in the brain” of the European Communities (headed by Dr. R. Dantzer, Bordeaux, France). However, at the time of submitting this review, no studies of the effects on the HPA axis of recombinant rat cytokines generated by this initiative had been published.

Although pharmacological differences of cytokines from different species may seem to complicate interpretation of data, they can actually provide useful pharmacological tools. For example, the difference between the effects of intracerebroventricular mouse and human TNF-α on plasma ACTH concentration may reflect the differing pharmacological profiles of the two identified TNF receptors, TNF-R1 and TNF-R2. Murine TNF-R1 has high affinity for either mouse or human TNF-α, whereas only mouse TNF-α is effective at TNF-R2 (334, 467, 505). Should the same pharmacology be true at rat TNF-α receptors, as has been suggested (917), then the increase in plasma ACTH concentrations induced by intracerebroventricular mouse, but not human, TNF-α indicates that TNF-R2 is the major receptor isoform involved in cerebral TNF-α-induced activation of the rat HPA axis.

Although by far the majority of cytokines tested in animal studies have been found to exert stimulatory actions on the HPA axis (see Table 5), two cytokines (IL-4 and IFN-γ) have been suggested to inhibit HPA axis activity in vivo. The anti-inflammatory cytokine IL-4 dose-dependently inhibits POMC mRNA expression in the anterior pituitary, without affecting CRF mRNA in the pPVN, suggesting a direct inhibitory action at the level of the pituitary (326). Interferon-γ has also been suggested to inhibit HPA axis secretory activity, since the administration of low doses either intraperitoneally or intracerebroventricularly reduces plasma corticosterone levels and the electrical activity of neurons within the PVN (725, 726, 729). However, higher doses of IFN-γ given intracerebroventricularly enhance plasma corticosterone levels (726), suggesting that the qualitative effects of IFN-γ on HPA axis activity are dependent on the dose used.

B. Human Studies

In addition to the numerous studies of the effects of cytokines on the HPA axis of laboratory animals, the clinical trials of a number of cytokines as anticancer strategies have afforded the opportunity to detail their effects on the HPA axis of humans. Such clinical studies have permitted investigation of the effects of cytokines on the HPA axis in a homologous system (i.e., human cytokines in human subjects). Either intravenous or subcutaneous administration of IL-1α (179, 785), IL-1β (176, 179), IL-2 (24, 194, 479–481, 812), IL-6 (519, 520, 809), TNF-α (596) IFN-α (41, 289, 337, 565, 566, 702, 758), IFN-β (596), and IFN-γ (342, 596, 810) elevates plasma ACTH and/or cortisol concentrations. As in laboratory animals, the stimulation of ACTH secretion produced by cytokines occurs rapidly: within 1 h of intravenous infusion or within 1–4 h of subcutaneous treatment.
A series of experiments (519, 520) in which cancer patients of good clinical performance were examined showed that IL-6 is a particularly potent activator of the HPA axis and that the human HPA axis is remarkably responsive to this cytokine. On the first treatment day, IL-6 (30 μg/kg sc) induced marked elevations in plasma ACTH and cortisol concentrations, with peaks occurring at 1 and 2 h, respectively. Plasma ACTH concentrations returned to basal levels within 5 h, whereas plasma cortisol levels remained elevated for 24 h. By the seventh day of treatment, the ACTH response to IL-6 was markedly diminished, an effect that was probably due to increased negative feedback produced by persistently elevated plasma cortisol levels. The sustained secretory activity of the adrenal was accompanied by gross enlargement of the adrenal glands as assessed by computed tomographic scans. Subsequent studies demonstrated that as little as 0.3 μg/kg (intravenous) is already a maximal dose of IL-6 (520). The magnitude of the plasma ACTH response to a first injection of IL-6 was greater than that reported with the standard tests of pituitary-adrenal function such as injection of ovine CRF or the insulin tolerance test. The relatively mild toxic effects of IL-6 led the authors to propose that IL-6 may provide a new means of testing pituitary-adrenal function (519) and to conduct additional studies in normal human subjects (876). In normal healthy males, IL-6 (subcutaneous) again produced substantial elevations in plasma ACTH and cortisol, with peaks in ACTH and cortisol levels observed at 60–90 and 90–120 min, respectively, and a minimal effective IL-6 dose of 1–3 μg/kg (876).

Overall, human and animal studies agree that many exogenously administered cytokines have marked stimulatory actions on HPA axis secretory activity and suggest that endogenous production of cytokines during homeostatic threats may well play a causal role in the elaboration of the accompanying HPA axis response.

III. PHYSIOLOGICAL/PATHOPHYSIOLOGICAL CIRCUMSTANCES IN WHICH ENDGENOUS CYTOKINES PLAY A ROLE IN REGULATION OF HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

There are a number of examples of injury, infection, and/or disease that are associated with increased cytokine production and concomitant elevations in HPA axis activity: head trauma (427, 611, 976), cerebral ischemia (stroke) (253, 367, 571), a number of autoimmune diseases (175, 239, 322–325, 347, 455, 539, 654, 959), psychiatric and dementia disorders (165, 302, 510, 896), acquired immune deficiency syndrome (AIDS) (636, 661, 763, 827, 954), and antigenic challenges (69, 72, 73). However, direct and clear evidence for the involvement of particular cytokines in the HPA axis response to such insults has been limited to only a few cases (viral or bacterial infection, local tissue damage and inflammation, and acute physical/psychological stress). Furthermore, although numerous cytokines have been shown to influence the secretory activity of the HPA axis, the direct demonstration of cytokine involvement in physiological or pathophysiological HPA axis responses has been restricted largely to the cytokines IL-1, IL-6, and TNF-α. The following sections outline the types of threats to homeostasis that have been demonstrated to elicit activation of the HPA axis via mechanisms that depend critically on the endogenous elaboration of cytokines.

A. Viral Disease

1. Newcastle disease virus

The first direct evidence indicating that IL-1 participates in the HPA axis response to an immune challenge came from studies investigating the neuroendocrine responses to inoculation with NDV (70). Newcastle disease virus is a neurotropic paramyxovirus which, when administered to rodents, produces symptoms of viral disease without the potential hazard of replication. Within 1–2 h of injection, NDV produces marked elevations in ACTH and corticosterone concentrations in the blood of mice (70, 219–221, 604, 793) or rats (685). The majority of studies demonstrate that the increase in corticosterone is abolished by hypophysectomy (218, 220, 221, 604), indicating the importance of the pituitary in the adrenal response. Furthermore, the ACTH response is completely prevented when rats are passively immunized against CRF (685), indicating that hypothalamic CRF regulates the pituitary ACTH response to NDV. The stimulation of the HPA axis by NDV appears to be produced not by the virus itself, but by mediators released by immune cells exposed to the virus. This is evidenced by the fact that the supernatants of cocultures of NDV with either human peripheral blood leukocytes (HPBL) or mouse spleen cells also elevate plasma corticosterone concentrations. Indeed, intraperitoneal injection of supernatant from NDV plus HPBL produces a fourfold increase in plasma corticosterone concentrations in rats (70). This plasma corticosterone response is prevented by preincubation of the NDV plus HPBL supernatant with a rabbit neutralizing anti-human IL-1 antibody (70). More recent studies have demonstrated that intraperitoneal administration of IL-1ra to mice virtually abolishes the elevations in plasma ACTH and corticosterone concentrations 2 h after NDV (221), confirming the obligatory role of IL-1 in the generation of the HPA axis response to this viral challenge.

2. Polyinosinic polycytidilic acid

Polyinosinic polycytidilic acid (Poly I:C) is a synthetic, double-stranded polynucleotide commonly
used to mimic viral exposure. Injection of Poly I:C, like NDV, produces a rapid (within 1–2 h) activation of the HPA axis (544, 716). Although there have been only a few investigations of the HPA axis response to Poly I:C, it is apparent that CRF is an important mediator of this response, because Poly I:C-induced increases in rabbit plasma cortisol concentrations are abolished by pretreatment with a monoclonal anti-CRF antibody (544). Furthermore, the substantial plasma corticosterone response to Poly I:C observed in normal mice is completely absent in mice deficient in IL-6 (716), indicating that Poly I:C-induced activation of the HPA axis is dependent on the elaboration of the cytokine IL-6.

3. Murine cytomegalovirus

Cytomegaloviruses (CMV) are herpes viruses that are a major cause of mortality and morbidity in human transplant recipients, are a serious problem in patients with AIDS, and are the most frequent viral cause of congenital abnormalities. Administration of murine CMV (MCMV) productively infects mice. Recent studies by Ruzeck et al. (716) showed that MCMV induces increased levels of IL-12, IFN-γ, TNF-α, IL-1α, and IL-6, but not IL-1β, in the general circulation at 24–48 h of infection. During this period, there are marked increases in the plasma concentrations of corticosterone and smaller, but statistically significant, increases in plasma ACTH (716). The corticosterone response to MCMV in either normal mice treated with neutralizing anti-IFN-γ antibodies, or in IFN-γ-deficient mice, is comparable to that in control mice infected with MCMV (716). However, IL-1 and IL-6 appear to play important roles in the activation of the HPA axis. The corticosterone response to MCMV is markedly blunted in either normal mice treated with IL-1ra or in IL-6-deficient mice, without either of these “treatments” having a significant impact on viral load. The elevated IL-6 levels produced by MCMV infection are dramatically reduced when mice are treated with IL-1ra, whereas IL-1α levels are normally elevated in IL-6-deficient MCMV mice (716). Consequently, the authors concluded that IL-6 is the pivotal cytokine in the activation of the HPA axis in response to MCMV and that IL-1α plays a secondary role by contributing to IL-6 production (716).

B. Endotoxin Treatment

Endotoxins are LPS constituents of the outermost part of a Gram-negative bacterial cell membrane that are released upon bacterial lysis. Administration of purified preparations of LPS mimics many of the acute phase responses to Gram-negative infection without actively infecting the host (123). Consequently, administration of bacterial endotoxins to laboratory animals has been the most commonly used model to study the mechanisms underlying the neuroendocrine responses to bacterial infection and sepsis (214, 863, 883).

Although mice and rats are relatively insensitive to LPS in comparison with many other species (including humans), the intravenous administration of LPS to laboratory rodents produces marked elevations in ACTH and corticosterone secretion within 30–60 min. It should be emphasized that, quantitatively, physiological responses to LPS can differ depending on its source and preparation (345). We find that doses of ~5 µg/kg LPS (Escherichia coli serotype O26:B6; lot 20H4025, Sigma Chemical) produce a peak elevation in plasma ACTH concentration of 500–1,000 pg/ml (compared with <20 pg/ml in controls) at 90–120 min after intravenous injection in intact, male rats (889). Similarly, LPS stimulates ACTH and corticosterone secretion in mice, and HPA activation can be observed in both rats and mice after either intravenous or intraperitoneal administration. However, the precise mechanisms through which these two routes of LPS administration influence the HPA axis may be substantially different (144, 925).

In addition to elevated blood levels of ACTH and corticosterone, other indexes of HPA activation have been reported after peripheral administration of LPS. Intravenous or intraperitoneal LPS produces increased pPVN expression of c-fos mRNA or Fos protein and of CRF hnRNA or mRNA (230, 387, 456, 680, 718, 924, 925). Rats in which the PVN has been electrolytically lesioned can mount a detectable plasma ACTH response to an extremely large dose of LPS (2 mg/kg ip), but its magnitude is markedly diminished (227), indicating that the PVN plays a pivotal role in LPS-stimulated increases in plasma ACTH. Indeed, it is clear that CRF is an important mediator of LPS-induced ACTH secretion. This is evidenced by the increased secretion of CRF from the ME after systemic treatment with LPS (292) and by the marked attenuation, or abolition, of LPS-induced ACTH secretion produced by doses of LPS that are either very large (2.5 mg/kg ip) or more moderate (2.5 µg/kg ip or 50 µg/kg iv), respectively (25, 750).

These actions of LPS on the HPA axis in vivo are not due to a direct pharmacological interaction of LPS with HPA axis tissues. Lipopolysaccharide has either no effect or an inhibitory action on either CRF secretion from hypothalamic explants (545, 581, 652) or ACTH secretion from rat anterior pituitary cell cultures (117, 886). It also seems unlikely that LPS enhances the pituitary ACTH response to CRF, since LPS reduces the expression of CRF receptors in the pituitary both in vivo and in vitro (25), and ACTH secretion by rat anterior pituitary cell cultures stimulated with CRF is unaffected by cotreatment with LPS (886). Furthermore, mice (C3H/HeJ strain) that are deficient in their production of IL-1 in response to LPS (373, 759, 856) exhibit markedly reduced elevations in the plasma concentrations of ACTH and corticosterone after intraperitoneal LPS (216), sug-
gesting that IL-1 is an important mediator of the effects of LPS. Lipopolysaccharide is a potent inducer of the synthesis and secretion of a number of cytokines (see sect. 1C). In particular, the cytokines IL-1, IL-6, and TNF-α are likely candidate mediators of the effects of LPS on neuroendocrine secretion (863).

After administration of LPS directly into the bloodstream, the plasma concentrations of each of these three cytokines are elevated in a regulated temporal manner with TNF-α first, then IL-1β, and finally IL-6 (174, 189, 290). Not only are the secretions of these three cytokines temporally related, but there is also evidence that they are also causally related. Administration of antibodies to TNF-α blunts the secretion of IL-1 and IL-6 in response to LPS (263), whereas immunoneutralization of IL-1 (487) or frequent administration of large doses of IL-1ra (494) abrogates LPS-induced IL-6 secretion. After local injection of LPS (e.g., intraperitoneally or into an experimentally constructed, subcutaneous air pouch), the local concentrations of all three cytokines are also elevated. However, their levels in blood appear to be dependent on the degree of “overspill” into the general circulation, with only IL-6 levels being consistently increased in systemic blood (541, 542, 844, 998). Local administration of IL-1ra at the site of LPS injection inhibits the rise in plasma IL-6 levels (541), again suggesting a causal relationship between the production of IL-1 and IL-6 after LPS. In light of these data, it is surprising that recent experiments performed in mice have shown that the plasma IL-6 response to LPS appears normal in mutant mice lacking either IL-1β (10, 1001) or IL-1R1 (463). Whether this indicates important roles for IL-1α (or IL-1γ) and the IL-1R2 (or novel IL-1 receptors) clearly warrants investigation.

Comparisons of the time courses of cytokine production and HPA axis activation produced by systemic LPS have produced somewhat conflicting data. The concentrations of TNF-α, IL-1β, and IL-6 in plasma have been reported to lag behind the rise in plasma ACTH after intraarterial LPS regardless of LPS dose (290). However, after low doses of LPS administered intravenously, elevations in plasma TNF-α coincide with the secretion of ACTH, whereas at higher doses, elevations in plasma TNF-α occur after the initial rise in plasma ACTH (223). We find that after LPS (5 μg/kg iv), the TNF-α profile in blood precedes that of ACTH, with a time of onset and peak that occurs 15 min before those of plasma ACTH (889).

The importance of cytokines in the ACTH response to LPS was first directly indicated by the pronounced inhibition of this response when mice were pretreated with IL-1 receptor antibodies (690). Further studies showed that destruction of macrophages produced a marked reduction in circulating IL-1 levels in response to a high dose of LPS (2.5 mg/kg iv) and a 40% inhibition of the ACTH response to a small dose of LPS (2.5 μg/kg iv) (195). Either anti-IL-1 receptor antibodies given to mice (640) or IL-1ra injected into rats (223, 752) reduce the ACTH response to LPS administered intravenously or intraperitoneally. A CNS site of IL-1 action has been suggested by experiments showing that intracerebroventricular infusion of IL-1ra inhibits the increase in CRF mRNA in the PVN of rats 8 h after intraperitoneal LPS (387).

Although the above findings seem to strongly implicate the cytokine IL-1 as a mediator of the activation of the HPA axis by LPS, not all studies support this hypothesis. For example, mice injected with IL-1ra ip, at a dose that inhibited the corticosterone response to either IL-1α or IL-1β, failed to inhibit the corticosterone response to LPS (213). Furthermore, the development of genetically manipulated mice, with deficiencies in various components of the IL-1 system, has raised interesting questions regarding the absolute requirement of IL-1 in acute phase responses to LPS. For example, fever response to LPS is inhibited by either anti-IL-1β antibodies (418, 461, 487) or by IL-1ra (494, 791), clearly implicating IL-1 in the pathogenesis of fever due to LPS. However, mice deficient in IL-1β show only a slightly decreased (10) or even an increased (438) fever in response to intraperitoneal LPS. Similarly, although IL-1β has been implicated in the induction of IL-6 and the cachexia after LPS, neither of these responses is inhibited in IL-1β-deficient mice (10, 247, 250, 438, 1001). Studies in IL-1R1-deficient mice demonstrated that IL-1R1 is essential for all the IL-1 mediated signaling events examined (fever, induction of IL-6, induction of E-selectin) (442), but these animals display normal fever and cachexia induced by intraperitoneal LPS (442, 463). Not surprisingly, therefore, investigations of the HPA axis response to LPS in these mutant mice have also failed to confirm a role of IL-1 in the elaboration of this HPA axis response (10, 247, 250).

Investigations of the effect of immunoneutralizing IL-6 and TNF-α have also suggested physiological roles for these cytokines in the HPA axis secretory response to LPS. Although inhibition of either IL-1, IL-6, or TNF-α abrogates the ACTH response to larger doses of LPS in mice, Perlstein et al. (640) found that only anti-IL-6 antibodies were completely effective at reducing plasma ACTH concentrations produced by lower doses in mice. In contrast, IL-6-deficient mice show a normal plasma corticosterone response to a high dose (1 mg/kg ip) of LPS (254). In rats, anti-TNF-α antisera inhibits the plasma ACTH response to both low and high doses of intravenous LPS (223, 889), whereas TNF-R1-deficient mice display a normal corticosterone response to intracerebroventricular LPS (2.5 μg).

From the above discussion, it is apparent that the precise role played by IL-1, IL-6, and TNF-α in the elaboration of the HPA axis response to LPS is not fully understood. That each of these three cytokines affects the synthesis and secretion of the other, that each is capable of enhancing the others’ effect in a synergistic manner, and
the possible redundancy of each of these cytokines undoubtedly contribute to the lack of clarity of the various studies described. Overall, however, the evidence presented seems to indicate that, depending on experimental paradigm, one or more of IL-1, IL-6, and/or TNF-α may contribute to the HPA axis secretory response to LPS.

C. Local Inflammation

A number of studies have investigated the mechanisms by which acute local inflammation produces activation of the HPA axis (169, 879, 881, 884, 965). One much-studied model consists of the subcutaneous or intramuscular injection of the irritant turpentine into the mouse or rat. Injection of turpentine produces a localized inflammation that is characterized by a centrally necrotic, well-defined abscess and increased vascular permeability (476, 620, 829, 978). This paradigm has been used to investigate a multitude of acute phase responses to local inflammation including fever, hypermetabolism, cytokine synthesis and secretion, protein metabolism, anorexia, neuroendocrine alterations, and hepatic acute phase protein synthesis (27, 167–169, 247, 286, 432, 603, 965, 978, 1001).

The intramuscular injection of turpentine produces a biphasic activation of the HPA axis in the rat (see Fig. 2). Increased ACTH secretion shortly after turpentine administration appears to be due to activation of nociceptive sensory afferents (879) and is not related to cytokine synthesis or secretion (247, 254). By 3 h after turpentine, plasma ACTH concentrations return temporarily to around those of control animals (Fig. 2). This is followed by a second rise in plasma ACTH and corticosterone concentrations that parallels the development of the local inflammation, lasts for ~24 h, and is the HPA axis response to the actual local inflammation per se (884). Turpentine-induced inflammation produces a long-lasting (at least 12 h) increase in the expression of Fos in the PVN (696), and the second rise in plasma ACTH concentration is completely reversed by the administration of anti-CRF antiserum (884), indicating the importance of hypothalamic CRF secretion to the plasma ACTH response.

The generation and role of cytokines during acute phase responses produced by turpentine injection has been well studied. Turpentine-induced local inflammation elicits a marked elevation in the plasma levels of IL-6, but not of IL-1 or TNF-α (167, 248, 495, 881). However, concentrations of IL-1 are elevated at the site of local inflammation induced by turpentine (248), and inhibition of either TNF-α (167) or IL-1 (286, 495, 879) action markedly reduces the levels of IL-6 in blood, suggesting that both these cytokines stimulate the secretion of IL-6 at the local inflammatory site. Although IL-1β or IL-1R1-deficient mice display normal IL-6 responses to LPS, the plasma IL-6 response to turpentine is completely absent in either of these mutants (463, 1001). Interestingly, however, ICE-deficient mice fail to generate mature IL-1β in response to LPS (469) but exhibit normal production of mature IL-1β and display normal plasma IL-6 responses, when injected with turpentine (248). These latter studies clearly indicate that distinct molecular mechanisms of IL-1β and IL-6 elaboration are operative during systemic (LPS) and local (turpentine) inflammation.

Interleukin-6 has been demonstrated to be an obligatory mediator of a number of acute phase responses to local inflammation induced by turpentine, including fever, cachexia, and increased hepatic protein synthesis (87, 247, 254, 432, 437, 603). Because IL-6 is the only identified cytokine in the systemic circulation in significant quantities after turpentine, it seems likely that it is the major circulating signal to the brain. Nevertheless, there is only

![Fig. 2. Plasma concentrations of ACTH, corticosterone, and interleukin-6 (IL-6) in rats after (intramuscular) injection of 50 μl/100 g body wt of either saline (○) or turpentine (■). Thick, broken bar represents dark cycle (lights out). [From Turnbull and Rivier (884); © The Endocrine Society.]](http://physrev.physiology.org/Downloadedfrom)
limited evidence indicating that IL-6 mediates the HPA axis response to turpentine. Plasma IL-6 concentrations correlate well with the second rise in plasma ACTH and corticosterone levels (879, 884) (see Fig. 2), which given the known stimulatory effects of IL-6 on HPA axis secretory activity (327, 499, 519, 520, 527, 574, 575, 809) suggests that IL-6 is a likely circulating mediator of this neuroendocrine response to turpentine (879, 884). However, one report shows that immunoneutralization of systemic IL-6 does not influence the rise in plasma corticosterone in mice 12 h after turpentine (603). It should be noted that in this study (603), administration of IL-6 antibodies resulted in enhanced rather than diminished biological activity of IL-6 in plasma, a finding that has been commonly reported (reviewed in Ref. 790), and which casts doubt on the significance of the lack of effect on plasma corticosterone levels in response to turpentine. Studies in both IL-1β- and IL-6-deficient mice have shown that neither IL-1β nor IL-6 is an obligatory mediator of the elevation in plasma corticosterone concentrations shortly (1.5–2 h) after turpentine (247, 254). However, the time point chosen for investigation by these authors corresponds to the initial, pain-related increase in HPA axis secretory activity (see Fig. 2), and no observation was made at later time points corresponding to the HPA axis response to the actual local inflammatory reaction (6–24 h). We have recently obtained evidence that indicates that IL-6 is an important mediator of the HPA axis response to local inflammation (A. V. Turnbull, S. J. Hopkins, S. Prehar, and C. L. Rivier, unpublished data). At 9–12 h after turpentine, when plasma IL-6 levels are maximally elevated in control animals (see Fig. 2), IL-6-deficient mice display a very markedly reduced (two-thirds inhibited) plasma corticosterone response (Fig. 3). These data together with those demonstrating reduced fever (437), acute phase protein synthesis (432), and cachexia (432, 437) in IL-6-deficient mice collectively indicate that IL-6 is an important circulating mediator during local inflammation, acting as an “SOS” signal and inducing a variety of acute phase responses.

There is also evidence for the involvement of other cytokines in this response. Although intramuscular turpentine in rats appears not to elevate mRNA for either IL-1β, IL-6, or TNF-α within the CNS or pituitary, intracerebroventricular administration of either a neutralizing anti-TNF-α antiserum or a dimeric soluble TNF-α receptor construct markedly inhibits ACTH secretion 6–9 h after turpentine in the rat (881). This indicates an important, although as yet unexplained, role of cerebral TNF-α in this response. The extent to which other CNS-derived cytokines may contribute to the enhanced secretory activity of the HPA axis is presently unknown.

D. Physical and Psychological Stress

The elaboration of cytokines appears not to be restricted to injurious, inflammatory, and infectious insults, since recent studies have indicated that IL-1, IL-6, and brain-derived neurotrophic factor (BDNF) synthesis and/or secretion are altered during acute physical or psychological stresses (462, 536, 546, 777, 778, 797, 1002). For example, plasma IL-6 levels are elevated in rats by exposure to a novel environment (462, 561), conditioned aversive stimuli (1002), electroshock (883, 1002), or restraint (1002). Furthermore, IL-6 mRNA is elevated in the midbrain and IL-6R mRNA is diminished in the midbrain and hypothalamus 4–24 h after restraint stress in the rat (779), whereas immobilization causes increases plasma IL-6 concentrations and elevated hepatic and splenic IL-6 mRNA expression in mice (417). In humans, plasma IL-6 levels increase rapidly after treadmill exercise with peak increases apparent at 15 and 45 min (622). The increases in plasma IL-6 after physical and/or psychological stresses in rats are much more rapid (within 15 min) than those observed after either local (turpentine, 2–3 h) or systemic (LPS, 45–60 min) inflammations and appear to be mediated by an action of catecholamines (196, 800, 852, 910). However, increased blood levels of IL-6 do not appear to directly contribute to the HPA axis response to acute stress, since the rise in plasma IL-6 levels is only modest, and although plasma IL-6 levels increase rapidly, they still lag behind plasma ACTH responses (883, 1002). Furthermore, Ruzek et al. (716) reported that IL-6-deficient mice display an increase in plasma corticosterone levels in response to restraint stress that is comparable to that found in wild-type mice.

Acute restraint or immobilization has also been reported to increase hypothalamic IL-1β mRNA (546, 828), IL-1 protein (778), and IL-1RA mRNA (828) within 30 min of commencement of stress. In addition, chronic physical/pyschological stress elevates both plasma IL-1β concentrations in rats (536) and humans (814) and hypothalamic...
IL-1β mRNA in mice (837). On the basis of studies showing that intrahypothalamic administration of IL-1ra produces a significant reduction of the plasma ACTH response to immobilization in rats, a role for IL-1 in the regulation of the HPA axis response to a stress unrelated to infection or inflammation has now been proposed (777, 778). Indeed, other studies have shown that intracerebroventricular administration of IL-1ra before inescapable shock blocks the subsequent interference with escape learning and enhancement of fear conditioning normally produced by such a stressor (511), suggesting that IL-1 may also mediate some behavioral effects of noninflammatory/infectious stresses.

The findings that cytokines may be elaborated quickly (within minutes) in response to a stimulus that does not appear to result in tissue damage or infection is novel and suggests a role of cytokines in homeostasis that has previously been unrecognized. It should be pointed out that many of the physiological responses to inflammatory/infectious stressors and psychological/physical stressors are common (e.g., activation of the HPA axis, suppression of reproduction, certain changes in behavior, fever, and reduced appetite). Indeed, psychological/physical stress produces many aspects of the acute phase response to sickness. Furthermore, although humans experience psychological/physical stress commonly without the presence of inflammatory/infectious stress, psychological/physical stress commonly precedes inflammatory/infectious stress in animals in the wild (e.g., predator-prey experiences, shortage of food/water supply). That the rapid elaboration of cytokines may contribute to the signal-generating physiological (e.g., neuroendocrine) responses to psychological/physical stressors (778) suggests that, mechanistically, responses to such stressors may not differ markedly from responses to inflammatory/infectious stressors.

### E. Basal Hypothalamic-Pituitary-Adrenal Activity

Perhaps even more surprising than the demonstrated role of cytokines in the regulation of the HPA axis in response to noninflammatory or noninfectious stress is the recent finding that the cytokine LIF may contribute to the regulation of HPA axis secretion under basal, nonstress conditions. Although inhibition or genetic deletion of IL-1, IL-6, or TNF-α does not significantly affect basal plasma ACTH or corticosterone concentrations, recent studies by Melmed and colleagues (6, 7, 667, 926) have demonstrated that a member of the gp130 signaling family of cytokines, LIF, plays an important role in the regulation of basal ACTH secretion. Either normal or 36-h fasted mutant LIF-deficient mice have lower basal plasma concentrations of ACTH than wild-type controls, an effect that can be reversed by administration of exogenous LIF (6). This suggests that either LIF is important in the development and maturation of the HPA axis or that it represents a tonic ACTH secretagogue in the adult animal.

### IV. CYTOKINE ACTIONS ON THE CENTRAL NERVOUS SYSTEM, PITUITARY, AND ADRENAL

The effects of both administration of cytokines to normal, healthy subjects and the consequences of inhibiting cytokine action during infectious, inflammatory, or stressful threats imply that cytokines may play a physiological role in the regulation of the secretory activity of the HPA axis. However, cytokines also produce a number of systemic acute phase responses that themselves could elicit HPA activation, raising the question of whether the effects of cytokines on HPA axis secretory activity are direct or secondary to stress produced by other acute phase responses. For example, IL-1α causes fever, sickness behavior, increases in heart rate, increased blood flow to certain vascular beds, activation of the sympathetic nervous system, and changes in intermediary metabolism. These physiological changes are themselves challenges to the maintenance of homeostasis, and each could, if pronounced, activate secretion by the HPA axis. The effects of some cytokines on plasma ACTH concentrations have nevertheless been dissociated from a number of these other acute phase responses. For example, in the original account by Besedovsky et al. (70), IL-1 induced activation of the HPA axis of mice kept at an ambient temperature that did not result in these animals mounting a febrile response. Indeed, IL-1β analogs with markedly reduced pyrogenicity still stimulate ACTH secretion (572). In addition, enhanced ACTH secretion is observed after peripheral administration of IL-1β at doses of IL-1β that have no, or only modest, effects on the secretion of other hormones, such as luteinizing hormone, catecholamines, and prolactin, whose secretion is markedly altered by many other types of stressors (399, 695, 886). Similarly, doses of IL-6 that markedly elevate plasma ACTH and cortisol concentrations in humans have only moderate effects on other acute phase responses (519, 520). Finally, studies described in section IV.E clearly indicate that regulation of ACTH secretion by LIF can be demonstrated in healthy animals. These findings suggest cytokines target the HPA axis in a specific manner.

The original Science papers of 1987 suggested that the influence of IL-1 on pituitary ACTH secretion was attributable to actions either at the level of hypothalamic CRF release (55, 730) or directly on the pituitary itself (62) to stimulate ACTH secretion. Furthermore, although original hypotheses regarding a lymphoid-adrenal axis whereby ACTH produced by lymphocytes represented the
link between activation of the immune system and adrenal hormone secretion (793) has fallen out of favor, other studies (e.g., Ref. 698) suggest that cytokines may act on the adrenal directly.

The following sections describe the evidence that indicates the potential site(s) of cytokine action on HPA axis secretory activity. In presenting arguments for each level of the HPA axis (CNS, pituitary, and adrenal), we consider three lines of evidence. First, we describe the localization of cytokine receptors within the HPA axis. The a priori condition for a particular cytokine to influence HPA axis secretory activity by an action at a particular level is the expression of functional receptors for that cytokine by the tissue under consideration (e.g., hypothalamus) or at least in functionally related tissues (e.g., other regions of the CNS which send projections to the hypothalamus). Second, we describe the expression of cytokines by tissues of the HPA axis. It was originally hypothesized that the influence on neuroendocrine function of endogenous cytokines resulted from the exposure of the CNS and pituitary to cytokines produced by circulating or tissue-resident cells such as macrophages, monocytes, fibroblasts, and B and T lymphocytes. However, studies over the last decade have demonstrated that many IL-1, chemokines, TNF, IFN, CSF, and growth factors are produced by numerous cell types, including those found within neuroendocrine systems. Therefore, it appears that cytokines may play a paracrine or autocrine role in the regulation on neuroendocrine function, as they do in the immune system. In general, levels of cytokines in resting, healthy, unstressed animals or humans are low, but the expression of a number of cytokines can increase dramatically during injury, infection, disease, or physical/psychological stress. The following sections therefore outline what is known about the synthesis of cytokines within the CNS, pituitary, and adrenal and how this expression may be regulated. Finally, we discuss the numerous in vivo and in vitro studies that have investigated the effects of cytokines on each component of the HPA axis.

A. Evidence That Cytokines Activate the Hypothalamic-Pituitary-Adrenal Axis Primarily at the Level of the Central Nervous System

1. Cytokine receptors within the CNS

Receptors to many cytokines have been localized within the CNS or described in primary cell cultures or cell lines derived from brain tissue. These include receptors for IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, interferons, TNF, growth factor, CSF, growth factors, and neurotrophins (for reviews, see Refs. 65, 344, 762) (Table 6). Of most relevance to this review article is the distribution throughout the rodent CNS of the receptors for the cytokines IL-1, IL-6, and TNF-α, and these are discussed in more detail here.

A) IL-1 receptors in the CNS. Early studies investigating the localization of IL-1 receptors in the brain indicated a fairly widespread distribution. High levels of specific binding of 125I-labeled IL-1β were found in the choroid plexus, dentate gyrus, hippocampus, cerebellum, and olfactory bulb, with low levels in the hypothalamus and ME in rat brain slices (251). Specific binding of 125I-labeled IL-1β to membrane preparations of rat hypothalamus and cortex were also reported (398). Subsequent studies have confirmed the existence of IL-1 receptors within the rodent CNS, and IL-1R1 mRNA or IL-1 binding have been demonstrated on neurons (see below), astrocytes (31, 714, 868), cerebrovascular endothelia (904), neuroblastoma cells (625), and glioblastoma cells (303), but not on microglia (31, 868). However, the localization within the rat brain appears to differ somewhat from that which was originally reported, in particular with respect to the presence of IL-1 receptors within the hypothalamus. Furthermore, there are marked differences in the distribution of IL-1 receptors in rat and mouse brains.

Overall, the mouse brain exhibits very low densities of IL-1 receptors as assessed by binding of 125I-labeled IL-1α, IL-1β, or IL-1ra. However, very high levels of labeling are found consistently in the hippocampus (dentate gyrus, but not CA1 to CA4 pyramidal regions), choroid plexus, and meninges (30, 841, 848). Within the dentate gyrus, IL-1 binding appears to be predominantly to neurons (30, 848). Most notably, none of the more recent studies reported substantial IL-1 binding in the hypothalamus (30, 848). In situ hybridization histochemical analyses of mouse brains have indicated that IL-1R1 mRNA is expressed predominantly in the granule cell layer of the dentate gyrus, the entire midline raphe system, the choroid plexus, and endothelial cells, but not in the hypothalamus (177, 178). In contrast, IL-1R2 mRNA has been undetectable in normal mouse brain using in situ hybridization histochemical procedures (202). As in the mouse, the rat choroid plexus, but not hypothalamus, shows significant IL-1 binding (516, 845, 848). However, in marked contrast to the robust IL-1 binding in the mouse hippocampus, the rat hippocampus displays no binding of either IL-1α, IL-1β, or IL-1ra (using either heterologous or homologous ligands) (516, 845, 848). Similarly, in situ hybridization signal for IL-1R1 mRNA over the rat hippocampus has been described as either weak (972, 982) or background (238), with no study demonstrating significant signal in the dentate gyrus. The IL-1R1 mRNA in rat brain is confined largely to nonneuronal cells, with the ependymal cells lining the ventricular system, the choroid plexus, the leptomeninges, and in particular endothelial/perivascular cells being the major sites of IL-1 receptor expression (178, 238, 969, 970, 972, 982). A few neuronal groups in the rat brain do, however, display low...
TABLE 6. Cytokines and their receptors in the CNS

<table>
<thead>
<tr>
<th>Cytokine</th>
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<th>Induced by Systemic LPS</th>
<th>Receptor</th>
<th>Reference No.</th>
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<td>+</td>
<td>38, 125, 184, 185, 475, 797, 798</td>
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Definitions are as in Table 3. Reference numbers with regard to IL-8 include the homologous cytokines cinc/gro/NAP-1.

to moderate levels of IL-1R1 mRNA: the basolateral nucleus of the amygdala, the arcuate nucleus of the hypothalamus, the trigeminal and hypoglossal motor nuclei, and the area postrema (238). As in the mouse, IL-1R2 mRNA is undetectable in the rat brain by in situ hybridization histochemistry (592), although the expression of IL-1R2 mRNA is induced by systemic treatment with kainic acid (592).

The low levels of IL-1R1 mRNA observed in the hippocampal formation of the rat are in apparent contrast to the localization in the rat of IL-1RAcP. The IL-1RAcP mRNA is undetectable in both mouse (304) and rat brain (482). In situ hybridization studies of rat brains demonstrated that in contrast to IL-1R1 mRNA, IL-1RAcP mRNA is highly expressed in the granule cell layer of dentate gyrus, a region in which IL-1R1 mRNA is expressed in mice but not in rats (482). Furthermore, IL-1R1 and IL-1RAcP mRNA are differentially regulated by peripheral LPS treatment (271, 353). It therefore seems possible that the IL-1RAcP in the brain may also serve as an accessory protein to novel IL-1 receptor(s) or has functions unrelated to IL-1 receptor signaling.

In addition to the radioligand binding and in situ hybridization studies described above, RNase protection assay (RPA) (238, 280, 352, 353) and RT-PCR (270, 625) procedures have been used fairly extensively to investigate the distribution and regulation of IL-1R1 and IL-1R2 mRNA in both rat and mouse brain. Parnet et al. (625) found transcripts for both IL-1R1 and IL-1R2 in whole mouse brain but showed that the murine neuroblastoma cell line C1300 expresses only IL-1R1 mRNA. Indeed, Gabellec et al. (270) also demonstrated transcripts for both IL-1 receptors in the mouse brain but found that IL-1R1 mRNA was the predominant species in brain, whereas IL-1R2 mRNA was the most abundant in the spleen. Although previous work showed that systemic LPS reduces IL-1 binding in rat and mouse brain (29, 321, 517, 840, 842, 843), RT-PCR and RPA analyses have shown that either
LPS or IL-1β increases the expression of IL-1R1 and IL-1R2 mRNA (270, 271, 281, 353, 354, 357, 646, 668). These differences between binding studies and mRNA analyses presumably relate to either increased receptor occupancy after LPS treatment (see sect. 4A2b) or decreased translation of receptor mRNA into receptor protein.

B) IL-6 RECEPTORS IN THE CNS. Specific IL-6 binding sites have been demonstrated in both astrocytoma and glioblastoma cell lines (835), as well as in extracts of bovine hypothalamus (170). Messenger RNA encoding the α-subunit of the IL-6 receptor (IL-6Ra) has also been detected in neurons, microglia, and astrocytes from either normal brain tissue, primary cell cultures, or tumor cell lines (735, 834). The IL-6Ra mRNA is expressed in several brain regions in the untreated rat (273, 274, 746, 749, 900, 988). The IL-6Ra mRNA is most abundant in the pyramidal cells of the CA1 and CA4 regions of the hippocampus and in the granule cell layer of the dentate gyrus (900) and has also been detected in the hypothalamus, cerebellum, hippocampus, striatum, neocortex, and pons/medulla (273, 274, 746, 749, 988). Specific hybridization signals are observed in glial cells of the lateral olfactory tract, in ependymal cells of the olfactory and anterior lateral ventricle, and in neurons of the piriform cortex, medial habenular nucleus, neocortex, hippocampus, and hypothalamus (746). Within the hypothalamus, IL-6Ra mRNA is present in the ventromedial and dorsomedial regions including the periventricular hypothalamus and in the medial preoptic nucleus. However, the anterior hypothalamus and PVN display no specific hybridization signal for IL-6Ra mRNA (746).

The expression of IL-6Ra mRNA in the rat brain appears to be developmentally regulated, with marked increases in its expression, particularly in the striatum, hypothalamus, hippocampus, and neocortex, between 2 and 20 days of age (273, 274). Furthermore, LPS administration markedly elevates IL-6Ra mRNA levels in several rat brain regions (area postrema, bed nucleus of the stria terminalis, amygdala, cerebral cortex, caudal, hypothalamus, ME, piriform cortex, septohippocampal nucleus, PVN, SFO, and OVL7) (900). Furthermore LPS (intraperitoneal) or IL-1β (intravenous) stimulates the expression of IL-6Ra mRNA over blood vessels throughout the brain (900).

In addition to IL-6Ra, the signal-transducing component of the IL-6 receptor, gp130, has also been localized within the rat CNS (900, 927). Gp130 is found in glial, neuronal, oligodendrocyte, and ependymal cell types. The distribution of immunoreactive gp130 in the rat brain overlaps that which has been observed for IL-6Ra but is more widespread, consistent with its role in signal transduction for other members of the IL-6 family (927). Indeed, gp130 mRNA has been detected throughout the normal rat brain, with positive hybridization signals detectable in almost all brain areas (900). These brain areas include the hypothalamus and, of particular interest, the PVN (900). Gp130 mRNA is also expressed by cerebrovascular elements of LPS-treated rats (900).

C) TNF RECEPTORS IN THE CNS. Currently, there is little known of the distribution or physiological role of either TNF receptor within the CNS. Studies utilizing radioligand binding of 125I-tnfα to sections of whole mouse brain have demonstrated only weak specific binding throughout the entire surface of brain tissue (967), and binding assays of tissue homogenates have shown specific binding in the mouse brain stem, cortex, thalamus, basal ganglia, and cerebellum (414). In vitro studies have shown that both TNF-R1 and TNF-R2 mRNA are present in mouse cerebrovascular endothelium (49). In microglia, astrocytes, and oligodendrocytes, both TNF-R1 and TNF-R2 can be expressed in humans (788, 823a, 834, 963), whereas at least one receptor subtype is present in rats and mice (17, 209, 855). Both undifferentiated and differentiated clonal murine neuroblastoma cells (N1E cells) express TNF-R1, but not TNF-R2, mRNA (787). The TNF-R1 immunoreactivity has also been demonstrated in neurons in the substantia nigra of humans and both TNF-R1 and TNF-R2 immunoreactivities shown in human hippocampal and striatal neurons (92). However, there is presently no evidence that either TNF-α receptor subtype is localized to hypothalamic regions directly involved in the regulation of HPA axis secretory activity.

2. Cytokine expression in the CNS

The production and actions of cytokines within the CNS have been reviewed extensively in a number of recent excellent review articles (65, 344, 710, 711, 745, 762), and only a brief overview pertinent to the discussion of cytokine influence on the HPA axis is given here.

A) BASAL EXPRESSION. Many cytokines are synthesized within the brain (see Table 6), although in most cases their expression is in healthy, stress-free subjects is low. Nonetheless, a number of studies have reported the distributions of IL-1, IL-6, and TNF-α immunoreactive or biologically active protein or mRNA in the brains from normal untreated subjects. In the human brain, IL-1β immunoreactivity is found within neuronal elements of the hypothalamus, including periventricular regions, the pPVN, and the ME (101). This distribution is consistent with a role of IL-1β as a neuroregulator of acute phase responses, and in particular, of the HPA axis (101). Although studies in rats also detected neuronal immunoreactive IL-1β in similar hypothalamic regions, more prominent staining was found in extrahypothalamic sites, particularly the hippocampus (454). Interleukin-1β immunoreactivity has also been reported in the rat hypothalamus (672). Interleukin-1 biological activity in the rat brain has been either undetectable (264, 655) or detected in brain stem, cerebral cortex, diencephalon, and hippocampal homogenates (32,
656, 778). In the normal rat or mouse hypothalamus, IL-1β is detectable using sensitive immunoassays (314). However, the majority of in situ hybridization studies have found that the brain parenchyma lacks a readily distinguishable IL-1β mRNA signal (124, 338, 546, 981), whereas constitutive expression of IL-1β in the cerebrovasculature of control animals is readily observable (969, 970). Similarly, the majority of Northern blot hybridization or RT-PCR studies have found extremely low or undetectable levels of IL-1α or IL-1β mRNA in the brains of control rats or mice (197, 277, 338, 453, 507, 644), although sufficient sensitivity to demonstrate small diurnal variations in rat brain IL-1β mRNA expression was achieved in one study (838).

Messenger RNA of the gene encoding ICE, the enzyme responsible for cleavage of pro-IL-1β to mature, active IL-1β, has been demonstrated in murine microglia (990), whole brain homogenates (401), homogenates of hypothalamus and hippocampus (452, 865, 866), and blood vessels (arterioles and venules) throughout the brain parenchyma (970) of control rats. Interleukin-1 receptor antagonist mRNA is also present within the rat brain (269, 281, 353–357, 472, 485, 970, 971), with positive in situ hybridization signal present in the hypothalamus (particularly the PVN), hippocampus, cerebellum, choroid plexus, and blood vessels throughout the brain (472, 970).

Tumor necrosis factor-α immunoreactivity is found in the hypothalamus, caudal raphe nuclei, and along the ventral surface of the brain in the pons and medulla in the normal mouse brain (105). On the basis of morphological observations, the majority of staining observed appears neuronal, with two principal fiber pathways being noted: a periventricular pathway which coursed along the ventricular system and a pathway associated with the medial forebrain bundle (105). Within the hypothalamus, the PVN represents one of the terminal fields of fibers originating from the most intensely stained cell groups within the bed nucleus of the stria terminalis. On the basis of in situ hybridization studies, there is only a weak signal over regions that coincided with immunoreactive TNF-α (105). Finally, IL-6 mRNA has been either undetectable (900) or shown to be colocalized with IL-6Ra mRNA within several regions of the normal rat brain, including hypothalamus, cerebellum, hippocampus, striatum, neocortex, and pons/medulla (746).

B) INDUCED EXPRESSION. The expression of a number of cytokines within the CNS increases dramatically upon cellular damage. Accordingly, local concentrations of IL-1β, IL-6, and TNF-α, in particular, are elevated during CNS bacterial or viral infections, brain trauma, cerebral ischemia, and convulsions (344, 745). In addition, their expression is increased during a number of chronic CNS disorders such as multiple sclerosis, Down’s syndrome, and Alzheimer’s disease (344, 745). In general, when induction of cytokine synthesis within the brain has been demonstrated, microglia appear to be the major “brain-resident” cell type that synthesizes ILs, chemokines, TNF, and IFN, although vascular cells, astrocytes, and neurons also contribute to cytokine production.

Of particular significance to the field of immune-nervous system interactions has been the hypothesis that the synthesis of cytokines in brain may be induced by stimuli other than those resulting in direct cellular challenge to the CNS, and consequently that cytokines may act as neuroregulators within the brain in a manner akin to classical neuropeptides. Indeed, in response to the peripheral administration of LPS, the CNS expression of a number of cytokines is elevated (see Table 6). In response to large doses of LPS (0.4–4 mg/kg ip or iv), the mRNA encoding IL-1α, IL-1β, IL-1ra, IL-6, and TNF-α are elevated in homogenates of several regions of the mouse brain as assessed by RT-PCR and Northern blot hybridization methodologies (28, 269, 277, 453, 570). These elevations have been noted within 1 h, and peak at ~6 h. However, an important question regarding the induction of cytokines in the brain by LPS is whether the stimulus causing increased synthesis is of peripheral origin, because large doses of LPS could, for example, penetrate the BBB in sufficient quantities to stimulate cytokine synthesis directly. Indeed, LPS is a potent stimulus of IL-1, IL-6, and TNF-α synthesis after its intracerebroventricular administration (197, 338, 655, 881) and induces cytokine synthesis by glial cells in cell culture (770). In addition, large doses of LPS may disrupt the BBB (91, 199, 483, 496, 781, 878), thus permitting the entrance from the periphery of cells (e.g., macrophages) that may contribute to the cytokine signal. The majority of RT-PCR studies have been performed using high doses of LPS, and the fact that these have not fully addressed issues regarding contamination with blood cells and possible disruption of the BBB makes conclusions difficult. However, recent studies by Pitossi and co-workers (643, 644) used semi-quantitative RT-PCR to measure cytokine production after the injection of LPS to mice, at a dose (20 µg/kg ip) that is reported not to influence BBB integrity. Importantly, these authors also quantified possible contaminant by peripheral blood cells. This work showed marked induction of IL-1β, IL-6, TNF-α, and IFN-α mRNA in several brain regions (cortex, cerebellum, thalamus/striatum, hippocampus, brain stem, and hypothalamus), with peak increases observed 2–4 h after LPS administration (644). Because the authors concluded that these increased signals could not be accounted for by contamination with peripheral blood cells, it is clear that these cytokines are induced in brain by doses of LPS that do not disrupt the BBB.

A number of in situ hybridization and immunocyto-chemical studies have investigated the distribution of IL-1β mRNA or IL-1β protein after systemic (iv or ip) LPS in rats and rabbits and have yielded very similar results. “Barrier-related” regions have been the most
consistently observed sites of IL-1\(\beta\) expression after systemic LPS (124, 576–578, 971). These include the circumventricular organs [CVO; including OVLT, SFO, ME, area postrema (AP), and pineal gland], meninges, and choroid plexus. The cell types expressing IL-1\(\beta\) in these regions include macrophages, microglia, and perivascular cells. Marked induction of IL-1\(\beta\) in microglia throughout the entire brain has also been observed, but this trend to occur only at larger doses of LPS (>2.5 mg/kg vs. <1 mg/kg for barrier related) (124, 902, 971). Furthermore, the induction of IL-1\(\beta\) expression in barrier-related regions seems to occur faster (by 1–2 h) than that in brain parenchyma (peak ~6–8 h). Immuno- and bio-assays have also been used to document increases in brain cytokine concentrations. Lipopolysaccharide administered intraperitoneally increases the immunoreactive concentration of IL-1\(\beta\) within the rat hypothalamus (314, 340). The minimum doses of LPS required to measure significant increases in hypothalamic IL-1\(\beta\) have been reported to be between 0.15 and 1 mg/kg (intraperitoneal), and elevations have been detected as early as 1 h after LPS, with a peak increase apparent at 4–10 h (314, 340). Similarly bioactive IL-1 has been detected in the brains of rats (655) and mice (264) after LPS treatment, and again the doses used were large and the time required before a significant increase was measurable was long (5–6 h).

The topographical, temporal, and cellular induction of TNF-\(\alpha\) mRNA by LPS treatment in mice appears to be similar, but not identical, to that described for IL-1\(\beta\) (102). At early time points (1.5 h), hybridization signal is most prevalent over perivascular and neuronal elements in the CVO (OVLT, AP, and ME) and in the meninges. Increased hybridization signal within the brain parenchyma is not obvious 6 h after LPS, with marked induction of TNF-\(\alpha\) mRNA in the hypothalamus and NTS being apparent only at 9–18 h after LPS (102). Increased immunoreactive TNF-\(\alpha\) concentrations in rat CSF have been detected as early as 0.5 h after a huge dose of LPS (30 mg/kg iv) (483), and significant increases in the concentrations of bioactive TNF-\(\alpha\) and IL-6 in push-pull perfusates from the anterior hypothalamus have also been demonstrated within 1–3 h of LPS (20–50 \(\mu\)g/kg ip) injection in rats or guinea pigs (365, 418, 419, 705). Similar to IL-1 and TNF-\(\alpha\), IL-6 mRNA is induced in CVO (particularly the SFO, OVLT, ME, and AP) and the choroid plexus 3–6 h after LPS (intraperitoneal) (900).

The effect of discrete localized inflammation within the periphery on cytokine expression in the brain has been less well studied. We have found no increase in either IL-1\(\beta\) or TNF-\(\alpha\) mRNA using in situ hybridization or IL-1\(\beta\), IL-6, or TNF-\(\alpha\) mRNA by competitive RT-PCR at 5–8 h after intramuscular turpentine (881). Furthermore, we have been unable to detect any increase in brain homogenate content of either IL-6 or TNF-\(\alpha\) during turpentine-induced inflammation (Ref. 881 and unpublished observations).

Recent studies have suggested that cytokine synthesis in brain may also be induced by stressors unrelated to infection or inflammation. Hypothalamic expression of IL-1\(\beta\) mRNA (546, 828), IL-1ra mRNA (828), and IL-1 bioactivity (778) is increased within 30 min of immobilization stress in the rat, and IL-6 mRNA is elevated in the midbrain 4–24 h after restraint stress (779). Similar paradigms produce increases in IFN-\(\gamma\) mRNA expression in mouse brain homogenates (849). Acute (2 h) or repeated immobilization stress in rats increases BDNF mRNA expression in the pPVN and the lateral hypothalamus (797) and decreases BDNF mRNA in the hippocampus, whereas repeated stress increases NT-3, but not NT-4, mRNA in the hippocampus (798).

3. Cytokine actions at the level of the CNS

Consistent with the CNS as a primary target of IL-1 action in eliciting pituitary ACTH secretion, administration of either IL-1\(\alpha\) or IL-1\(\beta\) directly into the cerebroventricles (intracerebroventricular) of rats markedly elevates plasma ACTH concentrations. Elevation in plasma ACTH concentrations produced by intracerebroventricular IL-1 generally occurs at considerably lower (5- to 20-fold less) doses than those required by intravenous IL-1 (399, 684, 695, 909). Similarly, IL-2, IL-6, TNF-\(\alpha\), and epidermal growth factor (EGF) have been shown to elevate plasma ACTH and/or corticosterone concentrations when administered via the intracerebroventricular route (see Table 5). Interleukin-1 infused directly into several brain sites, including the PVN (40, 939), ME (525–527, 530, 939), and hippocampus (477), also increases pituitary ACTH secretion.

The effectiveness of cytokines when administered directly into the brain, and in particular the fact that lower doses are usually required to stimulate HPA axis secretory activity than when administered peripherally, have been interpreted as evidence that the activation of the HPA axis by peripherally administered cytokines occurs via an action within the CNS. This assumption based on such dose-response studies alone is at least questionable, given the differences in dilution of the cytokine when administered via these two routes. Indeed, at least in the cases of IL-1\(\beta\) and TNF-\(\alpha\), activation of the HPA axis is associated with patterns of gene expression within the PVN and/or pharmacological profiles that differ according to whether the cytokine was administered peripherally or centrally (457, 684, 688, 881). This indicates that the mechanisms by which these routes of cytokine administration stimulate HPA axis secretory activity are distinct. However, a number of lines of evidence do suggest that the stimulation of pituitary ACTH and adrenal GC secretion by either centrally or peripherally administered cytokine
is due to an action at or above the level of the hypothalamus. Indeed, surgical lesioning studies indicate the importance of an intact hypothalamus (619) to the elaboration of a plasma ACTH response to IL-1β in the rat. Electrolytic obliteration of the rat PVN also markedly reduces the rise in plasma ACTH concentrations produced by a number of cytokines, with inhibition being complete in the cases of intracerebroventricular IL-1β (681) or intravenous IL-6 (434). ~70% after intravenous TNF-α (434), and ~50% when IL-1β is injected intravenously (434).

Studies assessing the expression of cIEG and neuropeptide mRNA within the rat pPVN after administration of IL-1β have also suggested a CNS site of action of IL-1 administered via either peripheral or central routes. Interleukin-1β administered intravenously (237, 588, 914) or intraperitoneally (98, 128, 187, 831) induces c-fos mRNA or Fos protein expression in the rat pPVN. The Fos signal in the pPVN colocalizes with CRF immunoreactivity or mRNA after either peripheral route of IL-1β treatment (128, 237, 914), indicating cellular activation of CRF-containing neurons. Peripheral administration of large doses of IL-1β also increases CRF mRNA in the PVN (98, 237, 327). Similarly, increases in c-fos mRNA or Fos protein are elicited in the pPVN in response to intracerebroventricular IL-1β (153, 187, 588, 682, 684) and are accompanied by increased expression of not only CRF (457, 682) but also AVP mRNA in the pPVN (457).

In addition to IL-1β, IL-1α also stimulates Fos expression in the pPVN after its intracerebroventricular administration (153, 381). Interleukin-1α administered intraperitoneally has been reported not to influence the expression of pPVN CRF mRNA (327). However, the fact that the doses of IL-1α used in this latter study were not sufficient to produce significant increases in plasma ACTH concentrations casts doubt on the significance of these negative findings (327). In contrast to the impact of a single injection of IL-1β on pPVN cIEG expression, a single injection of IL-6 intravenously (899), intracerebroventricularly (899), or intraperitoneally (128) has no impact on pPVN cIEG expression. It should be noted, however, that the half-life of IL-6 (like other cytokines) in circulation after intravenous injection is very short. Indeed, Castell et al. (146) described a biphasic disappearance of human IL-6 from rat plasma, with an initial half-life of just 3 min. However, during inflammation, plasma IL-6 concentrations are elevated for hours or days. Therefore, the findings that constant infusion of IL-6 (589) does induce Fos expression in the pPVN (in a cyclooxygenase-dependent fashion) are clearly of relevance and indicate that circulating IL-6 can influence pPVN activity.

The different effects of a single injection of IL-1β or IL-6 on pPVN neuronal activity, as assessed by Fos and CRF mRNA expression, would seem to indicate that activation of the pPVN may be a factor responsible for elevated HPA axis secretory activity produced by acute exposure to IL-1β, but not IL-6. However, this contrasts with the findings of PVN lesioning studies that indicate an obligatory role of the PVN in the elaboration of the plasma ACTH response to a single injection of IL-6 (434). The relationship between changes in mRNA levels of neuropeptides and cIEG in the PVN and hypothalamic release of peptides and subsequent pituitary ACTH secretion thus remains unclear. Whereas ACTH secretion is observed within 5–10 min of peripheral administration of IL-1, the earliest time point reported for induction of c-fos mRNA in the PVN after peripheral IL-1β has been 30 min (187), and the earliest time reported for CRF mRNA is 1 h (98). Furthermore, the minimum dose of IL-1β required to induce Fos expression in CRF-containing neurons in the PVN is an order of magnitude greater than the minimum dose required to elicit ACTH secretion (237). Finally, marked increases in plasma ACTH concentrations (from <20 pg/ml basal to >700 pg/ml) can be elicited by doses of peripheral IL-1β that do not produce measurable changes in either CRF or AVP mRNA in the pPVN (457). This disparity in minimum doses could be due to differences in the sensitivity of the detection of elevations in plasma ACTH versus the detection of induction of Fos or CRF mRNA. Alternatively, it may indicate that ACTH secretion can occur at doses of IL-1β that do not elicit changes in gene expression in the PVN. Indeed, the transcriptional/translational changes that have been observed within the pPVN may well occur as a consequence of, rather than as a cause of, increased secretory activity of PVN neurons. Consequently, the absence of detectable changes in cIEG or neuropeptide mRNA expression in the pPVN does not necessarily indicate a lack of PVN contribution to observed increases in HPA axis secretory activity.

Strong evidence that IL-1 stimulates the secretory activity of the HPA axis primarily by an action on the CNS comes from studies that have demonstrated that IL-1 rapidly stimulates the secretion of CRF from the ME into hypothypophysial portal blood vessels. Corticotropin-releasing factor is depleted from the ME of colchicine-treated rats within 1 h of intraperitoneal IL-1β (55), and CRF concentrations in portal blood are elevated within 30 min of intravenous IL-1β (730). In the perfusates from push-pull cannulas placed within the ME, CRF concentrations are elevated within 5 min of either intracerebroventricular or intra-PVN IL-1β (40) and precede the rise in plasma ACTH after intravenous IL-1β (936, 940). Similarly, intravenous TNF-α produces an immediate rise in CRF secretion (938). Histological examination of hypothysiotropic nerve terminals suggests that AVP may (958) or may not (54) be cosecreted with CRF in response to peripheral IL-1, whereas electrophysiological data indicate that increased activity in the PVN is selective for neurons containing CRF only (728). However, in one study, the mean portal blood concentrations of AVP were almost twofold elevated by 30 min after intravenous IL-1β, although this increase did not achieve statistical significance (730). Fur-
Cytokines increase CRF secretion from the hypothalamus in vitro

<table>
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Definitions are as in Table 3. The majority of studies have quoted cytokine concentrations in terms of molarity. Only a few studies quote their concentrations in the more appropriate biological or international standard units, but these have not been included here, because without their widespread usage, they do not afford easy comparison between different studies.

thermore, either intravenous, intra-PVN, or intra-ME IL-1β causes prompt increases in both CRF and AVP concentrations in ME push-pull perfusates (939). In contrast, the intravenous injection of IL-1β does not influence hypothalamic portal blood oxytocin concentrations (730).

Despite the fact that no IL-1 receptors have been demonstrated in the PVN, a number of studies demonstrate that IL-1 stimulates the secretion of CRF from the hypothalamus in vitro (see Table 7). Incubation with subnanomolar doses of IL-1α or IL-1β has consistently been reported to rapidly (within minutes) increase the release of CRF from rat hypothalamic explants, superperfused hypothalamic tissue, as well as dispersed hypothalamic cell cultures (see Table 7). In addition, IL-1β increases the CRF content of hypothalamic explants, indicating an increase in CRF peptide production (315). Most (121, 491, 545, 991, 992, 999), although not all, investigators (813) have noted concomitant increases in AVP secretion. However, it should be noted that AVP release from the hypothalamus may be destined, in vivo, for secretion from the posterior pituitary, rather than direct interaction with corticotropes. Similarly, IL-2, IL-6, IL-8, TNF-α, IFN-γ, and EGF potently induce rapid increases in CRF (see Table 7) and/or AVP secretion (339, 658, 741) from rat hypothalami in vitro. A few studies have also demonstrated synergistic effects of various cytokine combinations on hypothalamic neuropeptide secretion. Buckingham et al. (121) demonstrated that the release of CRF and AVP by conditioned media from LPS-stimulated rat peritoneal macrophages (which contains multiple cytokines) is far greater than that observed with either IL-1α, IL-1β, IL-6, IL-8, or TNF-α alone. Indeed, a subthreshold dose of TNF-α markedly potentiates the IL-1β-induced release of AVP from hypothalamic explants (121), while the increase in CRF secretion due to IL-1β plus IL-2 is greater than the addition of their individual effects (131). Such synergistic effects mirror the cytokine interactions that take place within the immune system (see sect. I C) and underscore the importance of applying to experimental paradigms the in vivo reality of multiple cytokine production.

Although the above discussion clearly indicates that a number of cytokines influence hypothalamic neurosecretory activity, probably the best evidence that the CNS is the primary target of cytokine action on the HPA axis in vivo is derived from experiments where CRF (or AVP) has been immunoneutralized. Immunoneutralization of CRF in the rat inhibits the rise in plasma ACTH or corticosterone concentrations produced by intravenous IL-1α or -1β, IL-6, and TNF-α (55, 58, 457, 575, 730, 894, 909). The reduction in intravenous IL-1β-induced ACTH secretion produced by anti-CRF antisera/antibodies has been found to range from an 84% reduction to a complete blockade (55, 457, 730, 894). Interleukin-1β administered either intraperitoneally or intracerebroventricularly elicits ACTH secretion that can be inhibited by 85 and 73%, respectively (457). When a complete blockade of intravenous IL-1β-induced ACTH secretion has been achieved, the rise in plasma corticosterone concentrations has also been totally abolished (730). However, some authors have reported only marginal effects of CRF antibodies on the plasma corticosterone response to IL-1β (intravenous) (909), but the absence of measurement of plasma ACTH levels in this study precludes determination of whether these findings can be interpreted as indicating direct actions of IL-1 on the pituitary and/or adrenal glands.

Corticotropin-releasing factor antisera/antibodies/receptor antagonists also inhibit the rise in plasma ACTH concentrations produced by a number of other cytokines. Consistent with the effects of PVN lesioning, anti-CRF pretreatment totally abolishes elevations in both plasma
ACTH and corticosterone concentrations produced by IL-6 (575, 909), whereas inhibition of intravenous TNF-α-induced ACTH secretion by anti-CRF is nearly complete (58), with only a small corticosterone response remaining (58, 909). Administration of the CRF receptor antagonist α-helical CRF-(9–41) inhibits the rise in plasma ACTH due to EGF (551), but not NGF (741). However, the low potency of this receptor antagonist at the pituitary CRF receptor (888, 891) makes interpretation of incomplete inhibition of ACTH secretion difficult.

The effect of immunoneutralization of AVP on cytokine-induced ACTH secretion has been less well studied. We have consistently observed a 15–20% decrease in the plasma ACTH response to peripherally administered IL-1β when rats are pretreated with anti-AVP, although these effects are not always statistically significant (457, 687; unpublished observations). However, we also find that immunoneutralization of AVP inhibits the ACTH response to CRF itself by ∼20–30% (892). Thus the role of AVP in peripheral IL-1β-induced ACTH secretion is probably only permissive and is required only to permit the full expression of the ACTH secretagogue capacity of CRF. However, when IL-1β is administered intracerebroventricularly, the effects of AVP neutralization are more pronounced (40% inhibition), a finding consistent with the stimulatory effects of intracerebroventricular IL-1β on pPVN AVP mRNA and which suggests an activation role of AVP in the ACTH response to intracerebroventricular IL-1β (457).

Collectively, the rapid effects of IL-1 and other cytokines on hypothalamic CRF secretion in vivo and in vitro, together with the reduction of plasma ACTH responses to cytokines produced by inhibiting the actions of CRF, provide an extremely strong case for the CNS as a primary site of cytokine action in the stimulation of HPA axis secretory activity. Nevertheless, a large number of in vitro studies have also indicated the possibility of direct effects of cytokines on pituitary ACTH secretion and adrenal GC secretion.

B. Evidence for Direct Effects of Cytokines on Pituitary Adrenocorticotropic Hormone Secretion

1. Cytokine receptors within the pituitary

A number of cytokine receptors have been localized in the pituitary (see Table 8). For example, 125I-labeled human IL-1α (29, 30, 177, 843, 846, 847), IL-1β (30) or IL-1ra (841), or rat IL-1β (516) bind specifically to the anterior lobe of the mouse pituitary gland, with little or no IL-1 binding apparent in the mouse posterior pituitary (29, 30, 516). Pituitary IL-1 binding in the mouse is decreased by systemic treatment with LPS (840, 843) and increased by immobilization stress (29), ether-laparotomy stress (846, 847), and long-term treatment (7 day) with GC (29). Interestingly, the increase in IL-1 binding observed in the pituitary after ether-laparotomy stress can be prevented by treating mice with a CRF receptor antagonist (847), suggesting that activation of the HPA axis secretion per se may upregulate pituitary IL-1 receptors. Although there have been many studies investigating IL-1 binding in mouse pituitary, there is only one report demonstrating IL-1 binding in the rat (516). In contrast to the mouse, both anterior and posterior lobes of the rat pituitary bind 125I-labeled rat IL-1β (516).

In addition to studies demonstrating IL-1 binding by the anterior pituitary, RT-PCR experiments have demonstrated the presence of both IL-1R1 and IL-1R2 mRNA in the whole mouse pituitary (626). In situ hybridization histochemistry experiments agree with IL-1 binding studies and show that IL-1R1 mRNA is present in the mouse anterior, but not posterior, pituitary (178). Interleukin-1 binding and IL-1R1 and IL-1R2 mRNA have also been demonstrated in the mouse corticotropic tumor cell line AtT20 (110, 111, 423, 872, 947). Interestingly, and in accordance with the stress-induced increases in IL-1 binding in the anterior pituitary of the mouse (847), CRF increases IL-1α binding in AtT20 cells (947), while IL-1β and TNF-α increases the expression of both IL-1R1 and IL-1R2 mRNA (110). However, the expression of IL-1R1 and IL-1R2 by AtT20 cells does not necessarily indicate that normal corticotropes contain cell-surface IL-1 receptors. Indeed, when IL-1R1 and IL-1R2 immunoreactivities were localized to particular endocrine cell types in the normal mouse anterior pituitary, no evidence of colocalization of IL-1 receptor with ACTH was apparent (267). Immunolabeling using a panel of IL-1 receptor antibodies demonstrated that IL-1R1 and IL-1R2 were abundantly expressed in the mouse anterior pituitary, were always coexpressed, and were predominantly localized to a single cell type, the somatotrope (growth hormone-producing cells) (267). However, the possibility that IL-1 receptor expression by corticotropes is below the limits of the immunohistochemical techniques employed, or that the level of IL-1 receptor expression may be induced (as demonstrated in AtT20 cells), cannot be excluded.

Much less work has focused on the presence of IL-6 and TNF-α receptors within the pituitary. Rodent anterior pituitaries exhibit binding of 125I-labeled IL-6 (601), and IL-6Ra mRNA is expressed in normal rat (601, 915) and fetal and adult human pituitaries (774, 915). Interleukin-6Ra is also expressed in human ACTH and growth-hormone secreting tumors (675, 915). In addition, the IL-6 signaling subunit gp130 is present in human fetal pituitary cells (774). High concentrations of binding sites for rmTNF-α have also been demonstrated in the mouse and rat anterior pituitaries (967), AtT20 cells (422), and the folliculostelate cell line TtT/GF (422).
TABLE 8. Cytokine receptors in pituitary

<table>
<thead>
<tr>
<th>Cytokine Receptor</th>
<th>Localization</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1</td>
<td>AtT20 cells</td>
<td>29, 30, 110, 111, 178, 267, 423, 516, 517, 626, 840, 843, 846, 847, 872, 947</td>
</tr>
<tr>
<td></td>
<td>Normal mouse anterior pituitary</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal rat anterior and posterior pituitary</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>AtT20 cells</td>
<td>22, 706</td>
</tr>
<tr>
<td></td>
<td>Human corticotropic adenoma</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>Normal rat anterior pituitary</td>
<td>601, 675, 774, 775, 915</td>
</tr>
<tr>
<td></td>
<td>Human fetal pituitary (IL-6Rα and gp130)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal human pituitary</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Human pituitary tumors</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>Normal rat and mouse anterior lobe</td>
<td>422, 967</td>
</tr>
<tr>
<td></td>
<td>AtT20 cells</td>
<td></td>
</tr>
<tr>
<td>LIF</td>
<td>Folliculostellate cells</td>
<td>7, 774</td>
</tr>
<tr>
<td>OM</td>
<td>Human pituitary fetal cells</td>
<td>774</td>
</tr>
<tr>
<td></td>
<td>AtT20 cells</td>
<td>136, 155, 521</td>
</tr>
<tr>
<td></td>
<td>Normal rat pituitary</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GH3 rat pituitary tumor cells</td>
<td></td>
</tr>
<tr>
<td>EGF</td>
<td>Rat anterior lobe</td>
<td>151, 244, 562</td>
</tr>
<tr>
<td></td>
<td>Sheep anterior lobe</td>
<td></td>
</tr>
<tr>
<td>FGF</td>
<td>Normal anterior and posterior lobes</td>
<td>298</td>
</tr>
<tr>
<td>PDGF (α and β)</td>
<td>Human pituitary adenomas</td>
<td>464</td>
</tr>
<tr>
<td></td>
<td>Normal human anterior lobe</td>
<td></td>
</tr>
<tr>
<td>TGF-α</td>
<td>Rat anterior lobe</td>
<td>244</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Rat anterior pituitary, lactotropes</td>
<td>155, 188</td>
</tr>
<tr>
<td></td>
<td>GH3 rat pituitary tumor cells</td>
<td></td>
</tr>
<tr>
<td>Neurotrophin receptors</td>
<td>Rat anterior pituitary</td>
<td>431, 631</td>
</tr>
</tbody>
</table>

References are as in Table 3.

2. Cytokine expression in the pituitary

The pituitary has been shown to produce a diverse range of cytokines (see Table 9 and an excellent review by Ray and Melmed, Ref. 666). Some of these cytokines (e.g., IL-2, IL-10, LIF, and MIF) have been localized to corticotropes or demonstrated in the corticosterone cell line AtT20 (7, 22, 61, 119, 350). In vivo mRNA encoding the cytokines IL-1α, IL-1β, IL-6, LIF, IFN-γ, and TNF-α mRNA in the pituitary are all elevated by 45 min to 6 h after treatment with LPS (277, 424, 453, 570, 644, 748, 865, 926). Anterior pituitary IL-6 mRNA is also increased by chronic, local inflammation (732, 818). The constitutive expression of IL-1ra in the pituitary (269, 277, 471, 971) is of particular interest given its ability to antagonize the effects of IL-1 agonists, suggesting that the responsiveness of the pituitary to IL-1 may be modulated at a local level. Interleukin-1ra mRNA has been reported to be either unaffected (277) or induced (269, 471, 971) by systemic treatment with LPS.

One cytokine in particular, MIF, has been proposed to serve as a pituitary hormone (60, 118–120, 126). Bucala and co-workers (60) isolated a 12.5-kDa protein from the conditioned media of LPS-stimulated mouse pituitary cells, which was subsequently sequenced, its cDNA cloned, and identified as the mouse homolog of human MIF (60, 61, 118, 119). The mouse pituitary contains large amounts of preformed, intracellular pools of MIF (possibly located within corticotropes) which are released into the systemic circulation after treatment with LPS. Macrophage migrating inhibitory factor appears in the blood of normal mice within 2 h of LPS treatment, and its serum concentration continues to rise for ~20 h. No MIF is detectable in the serum of hypophysectomized mice 20 h after treatment, suggesting a pituitary origin of MIF found in serum. Macrophage migrating inhibitory factor appears to play a critical role in host defense to endotoxemia, since LPS-induced lethality in mice is potentiated by coadministration of MIF, whereas an anti-MIF antibody confers protection from lethal doses of LPS (60). Corticotropin-releasing factor increases the secretion of MIF from AtT20 cells, at doses lower than those required to stimulate ACTH secretion, suggesting that MIF secretion may increase in parallel with activation of the HPA axis (591). Although itself proposed as a pituitary hormone, the effects of MIF on the secretion of other pituitary hormones (e.g., ACTH) have not been characterized.

The regulation of IL-6 secretion from anterior pituitary cell cultures has been investigated extensively. Anterior pituitary cells constitutively produce IL-6 (807), and the secretion of this cytokine can be induced within 6 h of treatment with LPS (801, 806, 886), IL-1α, IL-1β (801,
In addition to secretion from anterior pituitary cells, IL-6 is released by neurointermediate lobe cells in culture, with IL-1β and LPS again being potent secretagogues (801).

The cell source within the normal anterior pituitary does not appear to be a classical endocrine cell type. Rather, folliculostellate (FS) cells are the major sources of IL-6 (11, 912, 913). Folliculostellate cells are of monocytic lineage and are thought to be involved in paracrine regulation of hormone secretion from the pituitary (26). Recently, an FS-like cell line has been isolated (TTT/GF), and in accordance with previous studies on whole anterior pituitaries, TTT/GF cells constitutively secrete IL-6, and IL-6 secretion is enhanced by TNF-α, VIP, PACAP (422, 522) or IL-1β (L. Bilezikjian and A. V. Turnbull, unpublished observations).

### 3. Direct effects of cytokines on pituitary ACTH secretion in vitro

With the exception of activin, which inhibits POMC mRNA expression and ACTH secretion in the corticotropic tumor cell line AtT20 (74), and reduces ACTH secretion from primary cultures of rat anterior pituitary cells (75), all other cytokines studied have been reported to either enhance or have no effect on either ACTH secretion or POMC mRNA expression in otherwise untreated pituitary cells (see Table 10). The effects of cytokines on the mouse anterior pituitary tumor cell line AtT20 are reasonably undisputed. Similarly, IL-1β, IFN-γ, and GM-CSF stimulate ACTH secretion from cultured human pituitary adenoma cells from patients with Cushing's disease (512). The duration of exposure of AtT20 cells and human pituitary adenoma cells to cytokines required to elicit statistically significant increases in ACTH secretion has, in general, been long. Although one report indicates increases in ACTH produced by either IL-1 or IL-6 within 2 h (966), the majority have used incubation times ranging from 6 to 72 h (7, 115, 243, 268, 667, 816). The fact that many cytokines have a stimulatory effect on pituitary tumor cells indicates the ability of signal transduction pathways activated by cytokine-cytokine receptor interaction to influence POMC expression and/or ACTH secretion. However, it is unclear to what extent corticotrope tumors accurately represent normal anterior pituitary corticortropes. For example, although AtT20 cells possess both IL-1RI and IL-1RII mRNA (110, 198, 872, 947), immunostaining of normal mouse pituitary does not show significant IL-1 receptor expression by corticotropes (257). Most important to the present discussion, however, is that although most cytokines tested increase ACTH secretion from AtT20 cells, their effects on normal rat anterior pituitaries have been extensively debated (see Table 10).

Although the effects of a number of cytokines on in vitro anterior pituitary preparations have been investigated (see Table 10), by far the best studied has been the

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### Table 9. Cytokines in the pituitary

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Localization</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>Whole rat pituitary</td>
<td>277</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Rat anterior pituitary</td>
<td>277, 424, 453, 644</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>Human pituitary adenomas</td>
<td>471, 734, 971</td>
</tr>
<tr>
<td>IL-2</td>
<td>AT-T20</td>
<td>22</td>
</tr>
<tr>
<td>IL-6</td>
<td>Human corticotropic adenoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rat, mouse, pig, or sheep anterior or whole pituitaries</td>
<td>1, 11, 277, 453, 570, 644, 818</td>
</tr>
<tr>
<td></td>
<td>Normal rat anterior pituitary cell cultures</td>
<td>145, 508, 579, 802</td>
</tr>
<tr>
<td></td>
<td>Human pituitary tumors</td>
<td>806, 853, 986</td>
</tr>
<tr>
<td></td>
<td>Human pituitary adenoma</td>
<td>375–378, 675, 874, 895</td>
</tr>
<tr>
<td></td>
<td>Folliculostellate cells</td>
<td>801, 912, 913</td>
</tr>
<tr>
<td>IL-8/cinc/gro</td>
<td>Rat anterior pituitary</td>
<td>426</td>
</tr>
<tr>
<td>IL-10</td>
<td>Mouse pituitary</td>
<td>350, 662</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Whole mouse or rat pituitary</td>
<td>277, 453, 644, 756</td>
</tr>
<tr>
<td>LIF</td>
<td>Normal mouse pituitary</td>
<td>7, 258, 756, 774, 926</td>
</tr>
<tr>
<td>MIF</td>
<td>Normal sheep pituitary</td>
<td>60, 118, 119</td>
</tr>
<tr>
<td>INF-γ</td>
<td>Corticotrophs and thyrotrophs</td>
<td>644</td>
</tr>
<tr>
<td>Activin</td>
<td>Gonadotropes</td>
<td>75</td>
</tr>
<tr>
<td>BDNF</td>
<td>Rat anterior and intermediate lobes</td>
<td>431, 797</td>
</tr>
<tr>
<td>EGF</td>
<td>Rat anterior pituitary</td>
<td>244, 245, 562</td>
</tr>
<tr>
<td>bFGF</td>
<td>Rat folliculostellate cells</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Rat anterior pituitary</td>
<td>208</td>
</tr>
<tr>
<td></td>
<td>Whole human pituitary</td>
<td>518</td>
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<tr>
<td>NGF</td>
<td>Rat anterior pituitary</td>
<td>164, 549, 631, 632</td>
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<td>PDGF (A and B)</td>
<td>Human pituitary adenomas</td>
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<td>TGF-α</td>
<td>Rat anterior pituitary</td>
<td>244</td>
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<tr>
<td></td>
<td>Bovine anterior pituitary</td>
<td>564</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Rat anterior pituitary</td>
<td>731</td>
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</tbody>
</table>

Definitions are as in Table 3.
TABLE 10. Direct effects of cytokines on ACTH secretion from the pituitary

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>AtT-20</th>
<th>Anterior Pituitary Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>115, 268</td>
<td>402, 491, 629, 730, 825*</td>
</tr>
<tr>
<td>IL-2</td>
<td>115, 796</td>
<td>268</td>
</tr>
<tr>
<td>IL-6</td>
<td>268, 966</td>
<td>499, 774, 491, 498, 583</td>
</tr>
<tr>
<td>IL-8</td>
<td>350</td>
<td>737*</td>
</tr>
<tr>
<td>IL-10</td>
<td>7, 95, 667, 816</td>
<td>774</td>
</tr>
<tr>
<td>LIF</td>
<td>7, 95, 667, 816</td>
<td>774</td>
</tr>
<tr>
<td>OM</td>
<td>7, 95, 667, 816</td>
<td>774</td>
</tr>
<tr>
<td>TNF-α</td>
<td>422</td>
<td>540, 402, 583</td>
</tr>
<tr>
<td>Activin</td>
<td>74</td>
<td>897</td>
</tr>
<tr>
<td>EGF</td>
<td>156</td>
<td>492, 551</td>
</tr>
<tr>
<td>IFN-α</td>
<td>74</td>
<td>289, 583</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>385</td>
<td>583</td>
</tr>
<tr>
<td>NGF</td>
<td>741</td>
<td>915*</td>
</tr>
<tr>
<td>ACTH</td>
<td>402, 825</td>
<td>730, 825, 873</td>
</tr>
</tbody>
</table>

Reference numbers for corresponding studies are given. Unless otherwise stated, effect of cytokines is to increase either ACTH secretion or proopiomelanocortin (POMC) mRNA expression. a Neither IL-1α nor IL-1β affected ACTH secretion or POMC mRNA expression after 3 h incubation, but either IL-1α or IL-1β increased ACTH secretion after 3 h, with only IL-1β producing a statistically significant increase in POMC mRNA. b Significant increases observed only at doses of 100 nM and greater. c Stimulation described as "weak" or "slight." d References with regard to IL-8 include the homologous cytokines cinc/gro/NEF-1. e TNF-α had no effect on basal ACTH secretion but inhibited CRF- or hypothalamic extract-induced ACTH secretion. f Unlike all other cytokines listed, activin decreases POMC mRNA expression and ACTH secretion in AtT20 cells and rat anterior pituitary cells. g IFN-γ did not affect basal but inhibited CRF-stimulated ACTH secretion. Definitions are as in Table 3.

influence of IL-1. However, this literature is confusing. Indeed, of the three original articles discussing the potential effects of IL-1 on ACTH secretion in the rat, one described a direct effect on pituitary cells in culture (62), whereas the other two found no effect (55, 730). Subsequent studies have not clarified this apparent discrepancy (see Table 10). Although some have shown stimulatory effects of IL-1β but not IL-1α (893), others have reported that either will enhance ACTH secretion directly from the anterior pituitary in vitro (402, 825). A greater proportion of positive findings have been obtained when using pituitary tissue obtained from female rats (62, 402, 873, 893). However, when directly compared, the male and female pituitary responded to IL-1β in an identical manner (893), and there have been reports of a stimulatory influence of IL-1 in male pituitaries (116, 132, 329) and no effect in females (55). The use of different anterior pituitary prepa-
C. Evidence for Direct Actions of Cytokines on Adrenal Glucocorticoid Secretion

1. Cytokine receptors within the adrenal

To our knowledge, there are only very few published studies of the presence of cytokine receptors within the adrenal gland. The adrenal gland displays no positive in situ hybridization signal for IL-1R1 mRNA (178), whereas IL-6α mRNA has been detected mainly in the zona glomerulosa and fasciculata in human adrenals (630) and also in the adrenal medulla (272).

2. Cytokine expression in the adrenal gland

In the adrenal gland, large constitutive pools of IL-1α and IL-1β have been identified (42, 753, 754). Interleukin-1α, IL-1β, and IL-1ra immunoreactivities have been demonstrated in adrenal chromaffin cells (42, 753, 754). In addition, IL-1α, IL-1β, ICE, and IL-1ra mRNA are present in the adrenal cortex and are markedly elevated in both adrenal medulla and cortex 90 min after intravenous or intraperitoneal LPS (594, 754, 866). Interestingly, the IL-1-related cytokine IL-18/IL-1γ is also synthesized within the rat adrenal cortex, mainly in the zona reticularis and fasciculata, and is strongly induced by acute cold stress (166). Interleukin-6 mRNA is also present throughout the human adrenal cortex (299, 630), and rat adrenal gland extracts contain IL-6 mRNA (272, 570, 748), which is markedly induced 2 h after intraperitoneal LPS (570). Although TNF-α mRNA has been described throughout the cortex of human adult adrenals, particularly in steroid-producing cells (300), TNF-α immunoreactive protein has been detected in only 12 of 22 fetal, and in 0 of 7 adult, human adrenals (361). A number of growth factors are also present in the adrenal: basic fibroblast growth factor mRNA or protein is present in whole human adrenals or in rat or bovine cortex or medulla (44, 301, 306, 341, 537, 757, 955), TGF-β1 mRNA and protein in bovine adrenal cortex and mouse whole adrenal (255, 341, 410, 861, 961), and NT-3 protein has been reported in whole rat adrenal (396).

Studies of primary cultures of dispersed adult rat adrenal glands have indicated the likely cellular sources and major secretagogues of the cytokines IL-6 and TNF-α within the adrenal gland. Although the zona fasciculata/reticularis produce small amounts of TNF-α and IL-6, the primary source of IL-6 in the rat adrenal is the zona glomerulosa (382–385). The secretion of TNF-α and IL-6 from adrenal glomerulosa cells is stimulated in a dose-dependent manner by LPS, IL-1α, and IL-1β (383–385). Furthermore, IL-6 secretion from these cells is enhanced by protein kinase C activators, the calcium ionophore ionomycin, prostaglandin E2, forskolin, and angiotensin II (382, 385). Unlike most cell types that produce IL-6, dexamethasone does not influence either basal or IL-1β-stimulated IL-6 secretion in zona glomerulosa cells (383). Intriguingly, ACTH increases the release of IL-6 from zona glomerulosa cells but not zona fasciculata/reticularis, despite similar ACTH-stimulated cAMP levels in each cell type (383). Such ACTH-induced IL-6 release from the adrenal suggests that activation of the HPA axis per se may cause IL-6 secretion from the adrenal (383). Like IL-6, TNF-α secretion is stimulated by protein kinase C activators and ionomycin (384). However, in stark contrast to IL-6, basal and stimulated TNF-α release is dose dependently inhibited by ACTH and dibutyryl cAMP (384). Similarly, differential effects on IL-6 and TNF-α secretion from zona glomerulosa cells are observed with serotonin and adenosine (676, 677).

3. Direct effects of cytokines on adrenal glucocorticoid secretion

Despite a lack of evidence for the presence of IL-1, IL-6, or TNF-α receptors within the adrenal gland, direct actions of these cytokines on GC secretion have been demonstrated. In vivo, peripheral administration of IL-1β has been reported to either stimulate (14) or exert no effect (310) on corticosterone secretion in hypophysectomized rats and to induce secretion of GC in anesthetized rats with isolated adrenal glands (698). The doses required to observe direct effects of IL-1β on adrenal corticosterone secretion in vivo have, however, been extremely high (35 μg/rat, Ref. 698), which casts doubt on the physiological relevance of this effect.

In vitro studies have indicated that IL-1β does not influence either basal or ACTH-stimulated GC production from human fetal adrenal tissue either in cell or organ culture (328). However, either IL-1α or -1β increases GC secretion from rat quartered adrenals (311), rat adrenal slices (14), and rat, bovine, and human dispersed adrenal cells (311, 528, 597, 867, 957, 964). Similarly, IL-2, IL-3, and IL-6 induce GC secretion from various adrenal cell preparations (180, 630, 867, 946), and IL-6 potentiates corticosterone secretion induced by low concentrations of ACTH (722). Tumor necrosis factor-α stimulates cortisol secretion from adult adrenocortical cells (180) but inhibits ACTH-induced cortisol secretion from human fetal adrenal tissue (361) and cultured cells (362). Interferon-γ stimulates corticosterone secretion from primary dispersed rat adrenal cells (289) and normal human adrenal slices (143), whereas NGF does not influence either basal or ACTH-stimulated corticosterone production from dispersed rat adrenal cell cultures (741).

These in vitro studies provide strong evidence that some cytokines may influence GC secretion directly. However, it should be noted that of the studies cited,
the majority required incubations in excess of 12 h to observe significant effects of cytokines on GC secretion. Indeed, in a comprehensive series of studies by van der Meer et al. (906), either IL-1α, IL-1β, IL-2, IL-6, or TNF-α had no significant effect on GC secretion from isolated rat adrenal cells inside 60 min, but a small stimulation was apparent ~4 h after completion of a 6-h incubation with IL-1β. Thus, as with the effects of cytokines directly on the pituitary, it seems unlikely that a direct action of a cytokine on the adrenal can account for the rapid in vivo effects of administered cytokines on plasma GC concentrations.

V. MECHANISMS OF HYPOTHALAMIC-PITUITARY-ADRENAL AXIS ACTIVATION BY INTERLEUKIN-1

The above discussion details the ability of a number of cytokines from various families to significantly influence the activity of the HPA axis. Furthermore, the distributions of various cytokines and their receptors throughout the brain, pituitary, and to a lesser extent the adrenal provide an anatomic basis for the hypothesis that cytokines influence the function of these organs. However, in general, the evidence that cytokine receptors, and IL-1 receptors in particular, are expressed by secretory cells (hypophysiotropic CRF neurons, normal corticotropes, GC-producing adrenocortical cells) is not strong. This has suggested the involvement of structural and/or pharmacological intermediates in activation of the HPA axis by cytokines. Studies aimed at elucidating the mechanisms by which particular cytokines may activate the HPA axis during a particular threat to homeostasis have focused on the cytokine IL-1 and to a much lesser extent IL-6 and TNF-α. The following sections discuss the proposed anatomic and pharmacological pathways by which IL-1 generated in response to a particular threat to homeostasis may influence the HPA axis and are summarized in Figure 4. The studies described in section IV indicate that the CNS is probably the primary site of IL-1 action in eliciting increased HPA axis secretory activity. Because the question of how IL-1 signals the brain is relevant to all CNS-mediated acute phase responses, such as fever, hypermetabolism, cachexia, suppression of reproduction, and sickness behavior, knowledge derived from studies of such responses is mentioned wherever pertinent. Furthermore, what information is available for other cytokines, in particular IL-6 and TNF-α, is also discussed.

A. Direct Actions on Pituitary and Adrenal

The majority of in vivo data indicate that stimulation of pituitary ACTH and adrenal GC secretion in response to the presence of IL-1 is produced by enhanced secretion of hypothalamic ACTH secretagogues, in particular CRF. This is evidenced by the marked reductions in plasma ACTH and corticosterone concentrations when CRF is immunoneutralized. However, incomplete inhibition of either ACTH or corticosterone secretion after CRF immunoneutralization has been observed (e.g., Ref. 909). Furthermore, marked elevations in plasma corticosterone concentrations have been noted even when ACTH secretion produced by IL-1 has been markedly reduced (e.g., Ref. 390). However, decisive evidence that CRF has been completely immunoneutralized or that the ACTH secretion remaining is unable to produce marked elevations in plasma corticosterone concentrations is often lacking. Although plasma ACTH concentrations can be elevated to levels of 1,000 pg/ml by intravenous IL-1β, much lower levels (100–200 pg/ml) of ACTH are sufficient to stimulate corticosterone secretion maximally. Indeed, the adrenals of both rats and dogs have been shown to respond to very small elevations (≤10 pg/ml) in plasma ACTH with significant elevations in plasma GC (388, 405, 975).

The data discussed in section IV indicate that a number of cytokines, and IL-1 in particular, may be capable of having direct actions on the pituitary to enhance the secretion of ACTH and on the adrenal cortex to increase GC secretion. Receptors for IL-1 are clearly present in the anterior pituitary, although it is unlikely that these are expressed on normal corticotropes. The pituitary and adrenals clearly would be exposed to IL-1 if its concentrations in blood were elevated (e.g., severe endotoxemia). In addition, IL-1 can be synthesized locally within these tissues. However, as discussed above, it seems clear that prolonged exposure of the pituitary or adrenals to IL-1 is necessary to elicit the release of ACTH or GC, respectively. Therefore, direct actions of IL-1 on either the pituitary or adrenal do not appear to account for a significant component of the hormone secretion observed in response to acute exposure to IL-1 in vivo. In contrast, circumstances involving prolonged increases in cytokines, for example, during chronic inflammation (732, 818), may well involve direct actions of IL-1 or other cytokines on the pituitary ACTH and/or adrenal GC secretion. Furthermore, enhanced pituitary or adrenal synthesis of IL-1 may regulate these glands’ growth and development (21, 669, 819, 1004). Similarly, a number of other interleukins (e.g., IL-2, IL-6) and growth factors (e.g., EGF) have been shown to influence the growth of the pituitary or adrenal glands (20, 21, 157, 637).

A large body of evidence indicates that the level of the HPA axis primarily affected by IL-1 is the hypothalamus. This is true whether IL-1 has been administered directly into the brain or into the periphery. This raises the question of how a blood-borne, large, hydrophilic peptide such as IL-1 accesses the CNS to influence hypo-
Fig. 4 Proposed models by which interleukin-1 influences secretory activity of hypothalamic-pituitary-adrenal axis. AP, area postrema; BBB, blood-brain barrier; CVO, circumventricular organs; ME, median eminence; OVLT, organum vasculosum of lateral terminalis; PVN, paraventricular nucleus; CNS, central nervous system; NTS, nucleus tractus solitarius.

1. Direct actions on pituitary and adrenal.
2. Transport across the BBB.
   a. Diffusion through disrupted BBB.
   b. Specific transport mechanisms.
3. Role of Prostaglandins
4. Induction of intermediates at BBB
5. Action at CVOs.
6. Activation of medullary catecholaminergic cell groups.
7. Stimulation of vagus nerve.
8. Synthesis within CNS

B. Penetration of Cytokines Into Brain

The transport of solutes out of vascular compartments and into perivascular tissue (or vice versa) occurs via either paracellular or transcellular mechanisms. Within the cerebrovasculature, the paracellular route is particularly impermeable due to the presence of the BBB. The BBB consists primarily of nonfenestrated endothelial cells that are connected by tight junctions and thus form a continuous cell layer that has the permeability properties of a continuous plasma membrane (664). The paracellular ultrafiltration of solutes into and out of tissues that occurs in peripheral vascular beds does therefore not occur in most cerebrovascular beds, at least while the BBB remains intact. Furthermore, the large molecular size (8–65 kDa) and hydrophilic nature of cytokines preclude

Thalamic secretions, and the models which have been proposed to address this question are discussed below. These include the possibilities that IL-1 penetrates the BBB to enter brain parenchyma (see sect. vB) or that IL-1 stimulates the production of intermediary signals (see sect. vC), by actions at the BBB interface (see sect. vD), at regions of the brain relatively devoid of a BBB (see sect. vE), at brain stem medullary cell groups (see sect. vF), and/or peripheral afferent nerves (see sect. vG). The hypothesis that IL-1 generated within the brain itself produces activation of the HPA axis is also considered (see sect. vH). Finally, the possibility that increased secretion of IL-1 locally in damaged or diseased tissue can activate the HPA axis indirectly by inducing the synthesis and secretion of a circulating factor is also discussed (see sect. vI).
their movement transcellularly by simple diffusion to any appreciable extent. Indeed, early studies concluded that the BBB was impermeable to IL-1 (85, 161, 204). However, transport of cytokines via the paracellular route can occur when BBB integrity is compromised (see sect. vB1), and saturable transcellular transport mechanisms for a number of cytokines have now been described (see sect. vB2).

1. Cytokines and blood-brain barrier integrity

Loss of BBB integrity may occur during inflammatory insults to the brain such as those accompanying CNS disease (e.g., multiple sclerosis, meningitis, brain tumors, AIDS dementia), brain trauma, cerebrovascular lesions, or seizures (368). Furthermore, administration of large doses of LPS can increase BBB permeability (91, 199, 483, 496, 781, 878). Such disruption of the BBB enables not only the passage of large peptides such as cytokines, but also augments the rate of entry of cells, such as macrophages, monocytes, lymphocytes, and neutrophils, which are capable of cytokine synthesis and secretion, but whose passage into the normal, healthy brain is very limited.

The association between peripheral inflammatory events and the CNS production of cytokines has led to a number of studies investigating the possible influence of cytokines on BBB permeability. In monolayer cultures of cerebral endothelial cells, either LPS or IL-1β, IL-6 or TNF-α produces a decline in transendothelial electrical resistance data supportive of an elevation in BBB permeability (199, 200). In vivo studies have demonstrated that intracerebroventricular TNF-α increases BBB permeability in the rat (413) and pig (535), and enhanced brain TNF-α production has been linked to the increased BBB permeability associated with a number of CNS inflammatory conditions (768, 769, 780). However, the ability of systemic administration of cytokines (IL-1α, IL-1β, IL-2, IL-6, TNF-α) to influence BBB integrity has been debated (33, 229, 413, 719). Furthermore, experiments demonstrating increased BBB after peripheral administration of LPS have either used exceedingly high doses (483) or have observed increased BBB permeability only over a protracted time course (496). Enhanced BBB permeability as a result of either CNS or severe peripheral infection may therefore permit the entry of cytokines themselves or cytokine-producing cells into the CNS, and cytokines so derived may contribute to CNS-mediated acute phase responses. However, it is clear that the initial neuroendocrine effects of peripherally administered cytokines or LPS can be observed more quickly, and at lower doses, than can be accounted for by damage to the BBB.

2. Carrier-mediated transport of cytokines across the blood-brain barrier

Work by Banks et al. (34) has shown that transcellular, saturable transport mechanisms afford a means of cytokine entry into the brain, even when BBB integrity is not compromised. These include saturable transport mechanisms for IL-1α (35), IL-1β (37), IL-1ra (309), IL-6 (36), and TNF-α (308) but not IL-2 (923). Such transport mechanisms have been described by injecting 125I-labeled cytokines intravenously in mice and measuring radioactivity in either whole brain, whole brain perfused free of blood contamination, CSF, or brain parenchymal homogenates depleted of brain capillaries. It should be noted that at the doses of cytokines used, no perturbation of BBB integrity was apparent as indicated by the low and unaltered rates of 125I-labeled albumin entry into the CNS. Not all radioactive material found in brain after intravenous cytokine chromatographically elutes as authentic cytokine. In particular, only 16% of radioactivity in brain parenchyma after intravenous 125I-labeled IL-6 was intact IL-6 (36), which raises the question of how much of the 125I-labeled IL-6 entering the brain is actually biologically active. Nevertheless, multiple time regression analyses and competition with unlabeled cytokines has demonstrated that such transport of radioactivity into brain is saturable. The members of the IL-1 family, IL-1α, IL-1β, and IL-1ra share the same transporter(s) (309, 647), but events and the influence of unlabeled IL-1α or TNF-α (36), whereas competition studies with IL-1α, IL-1β, IL-6, and MIP-1α have demonstrated selectivity of the TNF-α transporter (308).

These studies by Banks et al. (34) have demonstrated that peak values between 0.05 and 0.3% of the total dose of either 125I-labeled IL-1α, IL-1β, IL-1ra, IL-6, or TNF-α is found in each gram of whole brain tissue by 20–60 min after their intravenous injection. Many have questioned whether this amount of cytokine entry into brain is physiologically significant (678, 711, 943). Indeed, it seems unlikely that such a small proportion of cytokine entering the brain via a saturable transport mechanism can exert rapid effects, such as those observed after intravenous injection of recombinant cytokine. Furthermore, it is improbable that transport of IL-1 across the BBB significantly accounts for the effects of peripherally administered IL-1 on the HPA axis, since the pattern of neuropeptide gene and cIEG expression and the temporal profile of ACTH secretion differs markedly between peripheral and central administration (see sects. II.A and IV.A3). It nevertheless seems plausible that such transport mechanisms across the BBB may play a significant role when peripheral blood levels of endogenous cytokines are elevated for longer periods of time. This would seem particularly pertinent when considering chronic inflammation where plasma IL-6 levels can remain elevated for prolonged periods (hours to weeks).

C. Role of Readily Diffusible Intermediates

The apparent lack of IL-1 receptors within neuronal elements in the pPVN and the limited entry of IL-1 into...
the CNS have led to the hypothesis that IL-1 stimulates the HPA axis via enhancing the production of intermediates that directly interact with hypothalamic neurosecretory processes. These intermediates include classical neurotransmitters, such as catecholamines (see sect. Vf), serotonin (285, 477, 478), histamine (293, 421, 638), and more readily diffusible agents such as the lipid autacoids, eicosanoids, and the gaseous mediator nitric oxide (NO) (see sect. Vc2).

1. Eicosanoids

Eicosanoids are formed by the metabolism of arachidonic acid. The rate-limiting step in the production of eicosanoids is the conversion of arachidonic acid to PGH2 by the enzyme cyclooxygenase (COX; also known as PGH synthase/cyclooxygenase and PG endoperoxide synthase). Two forms of COX have been characterized: a form (COX-1) and a more recently described second form (COX-2) that is induced by various factors including mitogens, hormones, serum, and, of most relevance to this discussion, cytokines (374, 406, 609, 980, 985). Conversion of the resulting PGH2 by specific synthases results in the generation of three eicosanoid families: the prostaglandins (PG), the thromboxanes (Tx), and prostacyclins. The role of PG in the activation of the HPA axis by IL-1 has been particularly well studied largely because of the recognition that PG are critical mediators of IL-1 actions within most peripheral tissues studied (343).

Prostaglandins exert effects on HPA axis secretory activity at multiple levels. In the rat, elevated plasma ACTH concentrations are observed within 10 min of intravenous injection of PGE2, PGE2a, or PGF2a, but not PGD2 (560, 580, 928, 935). Similarly, increases in plasma ACTH concentration are produced by administration of PGE2 directly into the several brain sites, including the preoptic anterior hypothalamus (POAH) (397, 931), the OVLT (397), and the ME (530). Intracerebroventricular administration of PGE2 elicits c-fos mRNA in CRF-containing neurons within the pPVN as well as increased pPVN CRF hnrNA (445). Indeed, PG receptors of the EP-1 subtype (45), but not EP-3 subtype (236, 826), have been demonstrated within the PVN. However, the majority of interest in potential sites of PG action within the CNS has focused on the POAH. Prostaglandin E2 injected into the POAH induces Fos expression in the pPVN (742), and ACTH secretion induced by PGE2 delivered into the POAH can be prevented by prior treatment with an anti-CRF antisemur (931), indicating the importance of activation of hypothalamic CRF secretion. However, in vitro studies investigating the release of CRF from hypothalamic explants have disagreed, with PGE2 being reported to either stimulate or have no effect on CRF secretion (57, 135, 652). Despite the fact that intravenous PGE2 elevates plasma ACTH concentrations from the pituitary, the direct effect of PGE2 on pituitary ACTH secretion in vitro appears to be inhibitory (921). Collectively, these data suggest that the stimulatory actions of PG on ACTH secretion in vivo are exerted at the level of the hypothalamus or elsewhere within the CNS.

The majority of studies investigating the effects of IL-1 or LPS on PG formation have focused on PGE2, which is elevated in the systemic blood of either rats or rabbits injected with LPS (712, 935). Prostaglandin E2 can readily cross the BBB (183, 224), raising the possibility that PGE2 may enter the brain and stimulate neurosecretory activity in the pPVN. However, although the onset of the increase in blood PGE2 levels was reported to occur simultaneously with the onset of fever after either LPS or IL-1 in the rabbit (712), it is not significantly elevated in the rat until after the peak of the ACTH response to IL-1/β has been reached (935). Furthermore, administration of doses of PGE2 that result in blood levels 100- to 400-fold greater than those observed in blood after IL-1/β, has a much smaller effect on plasma ACTH concentrations than induced by IL-1/β itself (935). Collectively, these data indicate that increased entry of PGE2 into the brain from the systemic circulation is unlikely to be a major intermediary step by which peripheral IL-1 stimulates HPA axis secretory activity.

In addition to increased circulating levels of PGE2, the brain itself represents a potential source of PGE2 in response to peripheral injection of IL-1 or LPS. Both isoforms of COX are present in the brain of normal rats or mice and are expressed predominantly in neurons (100, 104). The patterns of immunostaining for COX-1 and COX-2 are distinct and are most prominent in regions of the brain subserving the processing and integration of visceral and special sensory inputs, and in the elaboration of autonomic, endocrine, and behavioral responses (100, 104, 400, 877). In the rat, the PGE2 concentrations in the OVLT, the POAH, PVN, hippocampus, and CSF of the lateral ventricle are all increased within 20 min of intravenous injection of IL-1/β (429, 940). All these neuronal structures contain both COX-1 and COX-2 immunoreactivities (100, 104). Ex vivo measurements indicate that mouse hippocampal slices produce more PGE2 after either intraperitoneal or intracerebroventricular treatment with either IL-1α or IL-1β (950), whereas in vitro studies have shown that IL-1/β and IL-6 increase the release of PGE2, but not PGF2α, TxB2, or 6-keto-PGF1α from rat hypothalamic explants (582). Similarly, peripheral administration of LPS in vivo increases hypothalamic PGE2 production (789, 799), whereas IL-6 or acute local inflammation increase CSF levels of PGE2 (169, 207). The cellular source of PGE2 in the CNS in response to LPS, turpentine-induced local inflammation, or IL-1 is most likely the cerebrovasculature and/or associated perivascular, structures in which COX-2 mRNA is markedly induced by these treatments (140--
Inhibitors of COX activity, such as indomethacin or ibuprofen, have been the most commonly used method of investigating the role of PG in HPA axis responses to IL-1. As such, these studies indicate the importance of arachidonic acid metabolites but do not specifically identify PG as the principal players. Peripheral administration of indomethacin abrogates the rise in plasma ACTH concentrations produced by either IL-1 or TNF-α (560, 569, 688, 684, 771, 880, 930). The inhibition observed is not always complete and, in particular, appears to be short-lived, even when indomethacin or ibuprofen is administered several times (C. Rivier, unpublished data). Similar abrogation of IL-1-induced ACTH secretion is produced by indomethacin when IL-1 is injected intracerebroventricularly (399, 688, 930) or locally into the ME (530), but results have been inconsistent when IL-1β has been injected intraperitoneally (215, 685). In response to peripheral LPS or local inflammation, administration of COX inhibitors reduces ACTH secretion, an effect that cannot be accounted for by effects of COX inhibition on the inflammation per se (880, 884). Peripheral administration of indomethacin also inhibits the expression of Fos in the pPVN of rats injected intravenously, intraperitoneally, or intracerebroventricularly with either IL-1β or LPS (443, 588, 718, 925) or of rats infused continuously with IL-6 (intravenous) (589).

Because indomethacin or ibuprofen can elevate basal GC secretion, it has been suggested that the inhibitory effects of COX inhibitors on cytokine- or inflammation-induced ACTH secretion may be due to enhanced GC feedback. However, a number of lines of evidence indicate that this is probably not the case. For example, elevations of plasma ACTH concentrations produced by either IL-1β (694) or TNF-α (771) in adrenalectomized rats are also abrogated by indomethacin inhibitors, whereas ACTH secretion due to a variety of other stimuli, including intravenous CRF (771, 880), electrofootshock (880), immobilization (399), or insulin-induced hypoglycemia (771), is unaffected by COX inhibition. In vitro studies have also demonstrated that CRF release from hypothalamic explants produced by either IL-1α, IL-1β, or IL-6 is prevented by indomethacin (56, 135, 500, 583). Finally, in addition to the abrogating effects of COX inhibitors on IL-1-induced ACTH secretion, prior intracerebroventricular passive immunoneutralization with antibodies to either PGE1, PGE2, or PGF2α also produces a significant reduction in IL-1-induced elevations in plasma ACTH (937). Collectively, these data argue for a direct role of PG in the mediation of IL-1 induced activation of the HPA axis.

Although the generation of PG in response to IL-1, and a number of other cytokines (e.g., TNF-α, IL-6), is generally accepted to be a critical step in their stimulatory actions on HPA axis secretory activity, the anatomic sites and cell types responsible for PG synthesis are less clear. Two possible sites, the cerebrovasculature and CVO, for which there is substantial evidence that PG play a role in the transduction of a peripheral IL-1 signal into a CNS response are discussed in sections vD and vE.

2. Nitric oxide

Nitric oxide is now considered a putative neuromodulator within the mammalian CNS (107, 186, 555). The localization of NO synthase (NOS), the enzyme responsible for NO formation, within the cerebrovasculature and the neuroendocrine hypothalamus (885), the readily diffusible nature of NO, and the proposed role of NO in the mediation of the effects of cytokines (e.g., Refs. 284, 509, 948) provided good evidence for the hypothesis that NO may play a role in the regulation of the HPA axis by cytokines, in a manner akin, or as an adjunct, to PG. However, it appears that during activation of the HPA axis by IL-1 or other inflammatory stimuli, the primary effect of NO is in restraining the HPA axis response (172, 458, 679, 684, 885, 887, 993). The following paragraphs describe the distribution and regulation of NOS within the HPA axis and also the experiments investigating the effects of manipulation of NOS on the HPA axis response to IL-1.

Nitric oxide synthase is the enzyme that catalyzes the conversion of L-arginine to L-citrulline and the gaseous mediator NO. Several isoforms of NOS have been identified (265). Nitric oxide synthase I is expressed in the central and peripheral nervous systems and is otherwise known as brain or neuronal NOS. Nitric oxide synthase II is found in many cell types, such as hepatocytes, macrophages, smooth muscle cells, and glia. Finally, NOS III is synonymous with endothelial NOS. These three isoforms are distinct gene products, differ in terms of their pattern of expression (NOS I and III are constitutively expressed, whereas NOS II is present only after induction by cytokines or endotoxin), are either calcium/calmodulin dependent (NOS I and III) or independent (NOS II), and exhibit different kinetic properties. That NO acts as a neuromodulator was first indicated by the demonstration that inhibitors of NOS block the stimulation of cGMP synthesis in brain slices by glutamate acting at NMDA receptors (276). However, NO clearly does not behave like a conventional neurotransmitter, since it is neither stored in nerve terminals nor does it influence its target cell via interaction with a cell-surface receptor. Rather, it diffuses from nerve terminals and forms covalent linkages with several potential postsynaptic intracellular targets (e.g., guanylyl cyclase).

Nitric oxide synthase-like activity, NOS immunoreactivity, and NOS mRNA are present within the PVN and SON of the hypothalamus (19, 107, 127, 129, 147, 456, 917-
In the pPVN, NOS is found within a subpopulation of CRF-expressing neurons (782, 870). Circumstances known to alter the activity of the pPVN, e.g., immobilization stress (129), intracerebroventricular CRF injection (459), intracerebroventricular IL-1β (459), or systemic LPS treatment (456), upregulate NOS expression in this nucleus, suggesting a role for NO in the regulation of HPA axis. In addition, like the components of the IL-1 system, NOS mRNA is expressed in cerebrovascular elements (969, 974). With regard to localization within the pituitary gland, NOS is found in both the posterior (106, 107) and anterior (148) pituitary. In the anterior pituitary, NOS mRNA and protein are expressed at only low levels in normal animals but are markedly induced by systemic LPS (974). Nitric oxide synthase is also present in the hypophysial portal vasculature (149), which delivers hypothalamic releasing factors to the anterior pituitary.

Possible roles of NO in biological processes have been determined using NO donors (L-arginine, nitroprusside), inhibitors of NOS activity (arginine derivatives), and NO scavengers (hemoglobin). Inhibitors of NOS activity are generally low molecular mass (150–500 Da), and the BBB does not appear to hinder their diffusion. These inhibitors have therefore been used after either systemic or central injection, the latter being chosen when effects on systemic parameters, in particular, blood pressure, were to be avoided.

Intravenous pretreatment of rats with the NOS substrate L-arginine blunts the rise in plasma ACTH due to intravenous IL-1β (692). Conversely, the NOS inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) (intravenous) exaggerates and prolongs the ACTH and corticosterone responses to IL-1β, an action which is specific to the L-isomer of NAME (the isomer active at NOS), can be reversed by competition with L-arginine, and is unrelated to its hypertensive effects (687, 692, 885, 887). Thus endogenous NO appears to inhibit the ACTH response to systemic IL-1β. Potentiation of ACTH secretion by L-NAME is also observed during responses to turpentine-induced local inflammation and systemic LPS treatment, but interestingly, not to IL-1β administered directly into the brain (intracerebroventricular) (692, 884).

The mechanisms by which NO influences the HPA response to IL-1β, inflammation, and endotoxemia are far from clear, but a number of findings have shed some light on likely candidates. Because CRF is an obligatory mediator of the ACTH response to IL-1, a number of in vitro studies have investigated whether NO influences hypothalamic CRF/AVP secretion. However, in hypothalamic explants, NO inhibitors have been reported to either blunt (116, 724) or exacerbate (172, 993) the increase in CRF/AVP secretion produced by IL-1. In vivo, L-NAME does not markedly influence ACTH secretion produced by treatment with CRF but potentiates the ACTH response to the two less potent ACTH secretagogues, AVP and oxytocin (692). These latter findings raise the possibility that increased sensitivity of corticotropes to AVP and/or oxytocin after L-NAME accounts for its potentiation of IL-1β-induced ACTH secretion. However, administration of neutralizing antisera to AVP does not influence the exacerbation of IL-1-induced ACTH secretion produced by L-NAME (687). Recent studies (887) with partially selective NOS inhibitors identify endothelial (type III) NOS as the most likely source of NO that inhibits the HPA axis response to IL-1β, raising the possibility of functional interactions between NO and IL-1 signaling pathways in cerebrovascular elements (see sect. IV). On the other hand, administration of combined adrenergic antagonists, propranolol and prazosin, partially reverses the effects of L-NAME on IL-1-, but not AVP-, induced ACTH secretion (687), suggesting that the inhibitory influence of NO on HPA activity may be due to a suppressive effect on catecholaminergic pathways (see sect. vF).

It is interesting to note that the suppressive effect of NO on the HPA axis response to systemic IL-1 has proinflammatory consequences because of the diminution of endogenous GC levels. This proinflammatory neuroendocrine action of NO is in agreement with its local actions as an inflammatory mediator at the site of tissue damage or infection (449). Therefore, NO appears to represent a new class of mediator that belongs to the large families of cytokines, neurotransmitters, and hormones that constitute the common chemical language of the immune and neuroendocrine systems.

D. Induction of Intermediates at Blood-Brain Barrier Interface

Although there has been much debate as to the precise location of IL-1 receptors within neuronal elements of the brain (see sect. IV A A), all studies agree that the cerebrovasculature and perivascular elements are the most prominent loci of IL-1R1 mRNA expression in rat or mouse brain (178, 238, 969, 970, 972, 982). Intense signal for IL-1R1 mRNA expression has been described over endothelial cells of the postcapillary venules throughout the entire brain (178, 238, 972, 982), and a number of lines of evidence support a role for such endothelial IL-1 receptors in the transmission of IL-1 signal from blood to brain. Immunoreactive IL-1β is found on the luminal side of endothelial cells lining cerebral venules in rats treated peripherally with LPS (901), whereas increased PGE2 immunostaining has been detected in the cerebral microvasculature after peripheral injection of either IL-1β (904) or LPS (903). Indeed, perivascular elements (probably perivascular microglia) are the major site of COX-2 mRNA induction after systemic treatment with LPS, turpentine, or IL-1 (103, 140–142, 231, 444). Perivascular cells were identified as the primary cell group within the CNS that
express both IL-1R1 and IL-1β-induced cIEG expression 1 h after intravenous injection of IL-1β (238). Finally, IL-1R1 expression by perivascular cells, but not neuronal elements, exhibits the expected ligand-induced downregulation after intravenous IL-1β (238).

The microvasculature has long been recognized as a target and source of cytokines, with a number of studies focusing specifically on the cerebrovasculature. Such studies have shown that cerebral endothelial cells express cytokine ligands (e.g., IL-1α, IL-1β, IL-6), cytokine-related enzymes (ICE), or receptors (e.g., TNF, IL-1) and are sites of cytokine action (49, 51, 171, 190, 242, 380, 713, 970). Cytokine induction of readily diffusible intermediates, such as NO (412, 969) or PG (77, 78, 201, 904), represents a possible means by which cytokines within the bloodstream can influence cerebral function.

A number of in vitro paradigms have been developed to investigate PG formation in the cerebrovasculature. Addition of LPS to isolated feline cerebral microvessels (consisting mainly of capillaries) has been shown to increase their secretion of PGE2 but not 6-keto-PGF1α (77, 78). Studies by Van Dam et al. (904) have demonstrated that cultured rat cerebral endothelial cells (RCEC) bind rat IL-1β specifically and express IL-1R1, but not IL-1R2, mRNA. Prostaglandin E2 is the main arachidonic acid metabolite found in rat cerebral endothelial cells (201), and IL-1β increases their secretion of PGE2 and 6-keto-PGF1α, but not TxB2, within 3 h of incubation. As with IL-1β, IL-6 stimulates PGE2 and 6-keto-PGF1α secretion within a period of 3 h (201).

Induction of PGE2 secretion through the serosal surface of endothelial cells by luminally confined IL-1 (or possibly other cytokines) represents an extremely plausible means by which IL-1 in the circulation could influence the neuroendocrine hypothalamus. Prostaglandin formation in endothelial/perivascular cells expressing IL-1 receptors within the PVN (178, 982) may provide a relatively local and direct mechanism of HPA axis activation in response to blood-borne IL-1. However, other lines of evidence also indicate that CVO and/or medullary catecholaminergic cell groups are important anatomic structures in mediating the effects of IL-1 on the HPA axis, and there is considerable evidence that PG formation (possibly in perivascular elements) occurs in these regions in response to IL-1.

E. Actions at Circumventricular Organs

In addition to cytokine entry into the CNS by way of carrier-mediated transport or diffusion through a disrupted BBB, a number of regions of CNS relatively devoid of a BBB permit cytokine interaction with neuronal elements. These CVO include structures lining the anteroventral border of the third ventricle (AV3V) (namely, the OVLT and SFO), the ME, and the AP, posterior lobe of the pituitary, subcommissural organ, and the pineal gland. Their capillaries do not form tight junctions and are thus far more readily penetrable via the paracellular route (305). Circumventricular organs not only contain capillaries with far greater permeability than the rest of the CNS, but the capillary density in these regions is extraordinarily high (369). Penetration of cytokines into brain parenchyma within the CVO does not imply that they may diffuse to interact with deeper brain structures, however, because tight junctions between modified ependymal cells in these regions form a diffusion barrier between CVO and the rest of the brain (389). But these “leaky” sites do provide a means with which cytokines such as IL-1 can influence neuronal activity in these regions of the CNS.

The CVO that have been proposed as potential sites of IL-1 action are the structures lining the AV3V region (in particular the OVLT), the ME, and the area postrema, and these are considered further in sections E1–3.

1. Structures lining the anteroventral border of the third ventricle

Substantial evidence has accumulated that the OVLT may serve as an interface between the blood and the brain during the process of an inflammatory response (568, 821). In particular, the role of the OVLT during fever induced by various cytokines or inflammatory stimuli has been well documented, and the OVLT has been proposed as a “pathway” for circulating IL-1 to influence neuronal activity (86, 568, 820, 822).

Efferent projections from both the OVLT and the SFO to the PVN provide good anatomic evidence indicating an influence of these structures on the activity of CRF neurosecretory neurons (369, 474, 783). A number of anatomic studies have therefore investigated whether the AV3V region may be a site responsive to the systemic administration of IL-1β. Induction of c-fos mRNA in the OVLT and SFO has been noted within 30–60 min of intraperitoneal injection of IL-1β (98, 187), and Fos protein expression is apparent at 3 h after intravenous IL-1β (237). Similarly, c-fos mRNA has been detected in the OVLT and SFO after intravenous injection of IL-6 (899) and after peripheral injection of LPS (232, 679, 718). The SFO is one of the select regions of the rat brain that displays changes in protein synthesis within 1 h after subcutaneous injection of IL-1β (962), whereas AV3V neurons identified electrophysiologically as projecting to the PVN are responsive to IL-1β within 15 min of its intra-arterial injection (610). Experiments injecting colloidal gold-labeled IL-1β (intravenous) into rabbits demonstrated that within 10 min of its intravenous injection, IL-1β is bound to the luminal surface of the endothelium within the OVLT (330). This is followed by internalization of IL-1β and transport to the basal membrane, although IL-1β was not observed...
in the neighboring POAH (330). In addition to being responsive to IL-1β, the AV3V regions have been demonstrated to be a site of production of IL-1β in response to systemic treatment with LPS (see sect. IV A2b).

Consistent with a role for the AV3V in mediating the effects of systemic IL-1β action on the HPA axis, placement of either radiofrequency or kainic acid-induced lesions in the OVLT markedly reduces ACTH secretion in response to intravenous IL-1β, but not immobilization stress (397). However, there is little or no evidence for the existence of IL-1 receptors within neuronal elements within the AV3V. With the consideration of the density of vasculature in this region and the demonstration of IL-1 receptors on endothelial and perivascular cells, these may well represent the cellular targets of IL-1β within the AV3V. Consistent with the idea of PG formation being the principal action of IL-1β in the AV3V, enhanced PGE2 formation in this region has been noted after systemic treatment with IL-1β or LPS (429, 903, 940). Furthermore, microinjection of indomethacin into the OVLT markedly attenuates the rise in plasma ACTH concentrations due to IL-1β (397). That adjacent neuroanatomic structures such as the POAH represent the target of PG action is supported by a number of observations. The POAH (but not the OVLT itself) contains a high density of PGE2 binding sites (523, 524, 933, 934), mRNA for the PG receptor EP-3 (236, 826), and the plasma ACTH response to intravenous IL-1β is inhibited by either electrolytic or kainic acid-induced lesions of the POAH or by microinjection of a PG antagonist into this brain region (397).

Although there is substantial evidence that the AV3V, and in particular the OVLT, may serve as a neuroanatomic site of action of circulating IL-1β, none of the experiments investigating the effects of either surgical or pharmacological manipulation of the OVLT showed complete inhibition of the plasma ACTH response to IL-1β (397). Furthermore, the dose of IL-1β used in these studies was extremely high (10 μg/kg iv). In more recent dose-response studies, the minimum dose of IL-1β required to induce Fos protein in the OVLT (3.58 μg/kg) was found to be ~10-fold higher than that required to stimulate Fos expression in the pPVN (237), suggesting that the involvement of the OVLT in the HPA axis response to systemic IL-1β may be restricted to high circulating levels of IL-1β.

2. Median eminence

The ME contains the terminal projections from CRF secretory neurons arising from the pPVN. Being an area of the hypothalamus that is richly supplied by vasculature relatively devoid of a BBB, the ME is a potential site by which circulating factors can enhance CRF secretion by interacting with pPVN nerve terminals, and stimulating CRF release without directly stimulating CRF cell bodies in the pPVN. Evidence supporting the concept that the ME is a primary site of action of circulating IL-1 in the stimulation of CRF secretion derives from a number of experimental findings. For example, microinjection of IL-1α, IL-1β, or IL-6 into the ME rapidly stimulates ACTH secretion (525–527, 530, 772). The doses of IL-1 or IL-6 required to elicit increases in plasma ACTH concentration after microinjection into the ME are significantly, although not markedly (2- to 5-fold), less than those required to elicit ACTH after intravenous administration of cytokine. As demonstrated for intravenous or intracerebroventricular injection of IL-1, the plasma ACTH response to intra-ME IL-1β is markedly reduced by indomethacin (530), suggesting the importance of PG formation as an intermediate step. However, in vitro studies attempting to determine whether the enhanced CRF release observed after incubation of hypothalamic explants (which include the ME) can be accounted for by actions on the ME have yielded conflicting data (583, 813, 863).

Although intravenous IL-1β induces c-fos mRNA and Fos protein in the PVN at 60–180 min, the observation that intracerebroventricular and intravenous injection of doses of IL-1β elicit differential c-fos mRNA expression in the PVN at 30 min suggested that the ME may be the primary target after intravenous IL-1β (684). This conclusion was based on the fact that both routes of IL-1β administration rapidly stimulate ACTH secretion, yet c-fos mRNA in the pPVN 30 min after IL-1β was observed only after intracerebroventricular IL-1β. This suggests that pPVN cell bodies represent an early target of IL-1β after its intracerebroventricular, but not intravenous, administration. Because both routes of IL-1β administration elicit rapid (within minutes) increases in CRF secretion, it seemed likely that IL-1β administered intravenously acts at the level of the ME (684). However, subsequent neuroanatomic data supporting this hypothesis are not strong. In situ histochemical studies have described the expression of IL-1R1 mRNA in the rat ME to be either weak (982) or absent (238, 972), whereas in the mouse, only labeling on “scattered cells” is apparent (178). Radioligand binding studies have similarly shown either low (251) or no (516) signal within the rat ME and no specific signal in the mouse ME (30, 848). Indeed, although LPS induces c-fos mRNA in several layers of the ME (679), c-fos mRNA or Fos protein induction has not been observed in the ME in response to systemic IL-1β (98, 187, 237, 588).

3. Area postrema

Recent studies implicated the AP in the mediation of HPA activation in response to IL-1β administered intravenously (238). Whereas other CVO do not contain IL-1 receptors to any appreciable extent (at least in neuronal elements), the AP was shown to contain positive hybridization signal for IL-1R1 mRNA, although the cell type
expressing IL-1R1 could not be identified. Most importantly, the AP is the only CVO that reliably displays intravenous IL-1β-induced c-IEG expression (237, 238). However, ablation of the AP does not influence IL-1β-induced CRF mRNA or Fos expression within the PVN (235), suggesting that it is not a critical relay site in the activation of the hypothalamic PVN in response to IL-1β.

F. Potential Role of Catecholamines and Evidence of Activation of Medullary Cell Groups

In addition to PG, there is a second class of intermediates for which there exists substantial evidence in the mediation of IL-1-induced activation of the HPA axis, namely, monoamines. Monoamine utilization and/or content in the hypothalamus and other regions of the CNS is altered by immunologic challenges, such as those produced by inoculation with sheep red blood cells (69, 767, 997), NDV (220, 221), Poly I:C (221), and influenza virus (219) and by peripheral administration of LPS (193, 212, 222, 450, 478). Intraperitoneal administration of either IL-1α or IL-1β to mice also elevates cerebral metabolism of norepinephrine (NE) and serotonin, with effects being most marked in the hypothalamus (211, 222). Similarly, intravenous or intraperitoneal IL-1β increases NE turnover in the hypothalamus (including PVN) as well as other regions of the rat brain (386, 553, 790). Intrahypothalamic or intracerebroventricular infusion of IL-1β also modulates hypothalamic monoamine levels (554, 773, 776, 860). As for other cytokines, intraperitoneal injection of IL-2 increases hypothalamic NE turnover in mice (996), and peripheral administration of IL-6 produces either small (222) or no increases (213, 858, 996) in hypothalamic NE and serotonin turnover. Intraperitoneal TNF-α was found to have no effect on hypothalamic NE turnover in mice (213) or rats (858) but was reported to inhibit NE release from the isolated ME in vitro (226).

There is extensive evidence that noradrenergic innervation of the hypothalamus influences the secretion of CRF from the hypothalamus (reviewed in Refs. 9, 649, 727, 739, 833). The PVN and SON represent two of the most prominent terminal fields of catecholaminergic neurons. These projections derive from medullary cell groups, in particular the NTS. Noradrenergic and adrenergic neurons from the rostral and caudal regions of the NTS project preferentially to the pPVN, as does the major projection field of the NTS, the C1 adrenergic cell group (739). Lesion of pathways from medullary catecholaminergic cell groups to the PVN has been reported to disrupt HPA axis responses to diverse stimuli (181, 832).

The use of adrenergic receptor antagonists to explore the role of catecholamines in IL-1-induced activation of the HPA axis has produced inconsistent data (134, 695, 949). On the other hand, neurotoxic lesions of ascending medullary catecholaminergic fibers (using 6-hydroxydopamine) consistently produce a marked depletion of hypothalamic NE concentrations and inhibit the plasma ACTH or corticosterone response to intracerebroventricular or intraperitoneal IL-1β (160, 949). A similar inhibitory effect has been noted when 6-hydroxydopamine is infused into the lateral ventricle (526, 949) or directly into the PVN (160). Discrete lesions of ascending catecholaminergic inputs to the hypothalamus (affecting either dorsal, ventral or intermediate axons) showed that the plasma ACTH responses to either intra-arterial IL-1β or LPS, or intra-PVN IL-1β, is dictated by brain stem catecholaminergic input to the hypothalamus and that the effect of lesion was dependent on the route of cytokine administration and precise location of the lesion (39, 291).

Recent studies have provided even more substantial evidence for the critical role of medullary catecholaminergic innervation of the hypothalamus in the activation of the HPA axis in response to systemic IL-1. Administration of IL-1β, either intravenously or intraperitoneally, results in marked expression of c-fos mRNA or Fos protein in the NTS of the rat (98, 187, 237). Similarly, all studies investigating the CNS distribution of either Fos protein of c-fos mRNA in response to systemic LPS have noted induction of this early-immediate gene expression in (likely catecholaminergic) medullary cell groups within 1–3 h of LPS injection (230, 232, 678, 679, 718, 924). Ericsson et al. (237) described Fos protein expression throughout the rostrocaudal extent of the medial and commissural nuclei of the NTS, as well as in the rostral ventrolateral medulla and in the dorsal motor nucleus of the vagus within 3 h of intravenous IL-1β. These authors demonstrated that Fos expression in the NTS is very sensitive to IL-1β and is induced at doses lower than those required to elicit a specific signal within the pPVN. Retrograde tracing studies indicated that the NTS and rostral ventrolateral medulla are the sources of projections to the PVN that most consistently express IL-1β-induced Fos protein (237). The majority of these projections are catecholaminergic (237). Furthermore, unilateral surgical disruption of ascending catecholaminergic projections from the medulla to the PVN prevents intravenous IL-1β-induced expression of Fos protein and CRF mRNA in the ipsilateral, but not contralateral, pPVN (237, 468). Finally, although such a surgical disruption of medullary-PVN fibers does not prove a catecholaminergic involvement, a recent study demonstrated that 6-hydroxydopamine lesions also substantially reduce intraperitoneal IL-1β-induced Fos expression in the PVN of the mouse (831).

The activation of medullary catecholaminergic cell groups seems unlikely to be because of a direct activation by IL-1, since these cell groups do not express IL-1RI mRNA (238). Given the demonstrated critical role of PG in the HPA axis response to IL-1 (see sects. vC1 and vD), it is not surprising that there has been considerable inter-
est in the possibility that PG are an important intermediary between IL-1 action and activation of medullary catecholaminergic neurons. Accordingly, the induction of medullary Fos protein (235) and the changes in hypothalamic monoamine turnover (534, 857, 858, 860) produced by IL-1 have been shown to be prevented by COX inhibitors. Indeed, the PG receptor subtype EP-3 is present in inflammatory response, since LPS-induced expression of cytokines in tissues other than the brain is unaffected by SDVX (90, 451). This suggests that it is the transduction of a cytokine signal to the brain that is impaired in SDVX animals. Indeed, it has now been shown that the fever (607, 942), increased sleep (319), NE turnover (261), hyperalgesia (945), behavioral effects (88, 89, 294), and induction of IL-1 mRNA in the CNS (320) produced by intraperitoneal IL-1 are all diminished or absent in SDVX animals. Similarly, SDVX inhibits hyperalgesia and conditioned taste aversion induced by intraperitoneal TNF-α (294, 941). Although SDVX inhibits CNS-mediated effects of intraperitoneal TNF-α (e.g., hyperalgesia, activation of the HPA axis), “peripheral organ” effects (e.g., reduction of glucocorticoid binding globulin) are unaffected by this surgical procedure (262), suggesting that the role of the vagus is specific to CNS-mediated acute phase responses.

The vagus also appears to play a role in the activation of the HPA axis in response to peripheral LPS or cytokine. In rats, SDVX attenuates the rise in plasma ACTH and corticosterone concentrations produced by intraperitoneal IL-1β (261, 390). The number of CRF containing neurons in the pPVN that stain positive for Fos and the rise in plasma ACTH concentration produced by LPS are likewise attenuated in SDVX animals (279), indicating that vagotomy interferes with the activating signal to the neuroendocrine hypothalamus. Similarly, SDVX blocks the rise in plasma corticosterone caused by intraperitoneal TNF-α (262).

A number of studies have addressed the question of how SDVX interferes with the generation of CNS-mediated acute phase responses to either LPS or IL-1. The SDVX procedure is a major surgical intervention resulting in SDVX animals being less “healthy” than sham-operated or control rats. It has been suggested that the inability of SDVX rats to respond normally to inflammatory stimuli may relate to their overall health status rather than to a specific effect of SDVX on “immune-to-brain” communication. However, even in SDVX rats receiving special perioperative care to prevent malnutrition, the febrile response to intraperitoneal LPS is still impaired (699). In addition, SDVX animals respond with elevations in body temperature (543, 700), hyperalgesia (941), and activation of the HPA axis (390, 943) in a manner similar to sham-operated animals when the stimulus is not an inflammatory one (e.g., insulin- or electrofootshock-induced elevations in plasma ACTH concentrations).

Although the importance of vagal afferents in the mediation of CNS-mediated acute phase responses to abdo-
inal inflammation is not undisputed (e.g., Ref. 755), the weight of evidence supporting this hypothesis is now substantial, and a number of studies have begun to unravel how cytokines might interact with the vagus. Administration of IL-1 into the hepatic portal vein produces increased electrical activity of the hepatic branch of the vagus (586, 587), suggesting the possible importance of this vagal branch. However, in contrast to SDVX, selective sectioning of the hepatic vagus does not inhibit the rise in either plasma corticosterone or hypothalamic NE turnover in response to intraperitoneal IL-1 (261), or the suppression of food intake in response to LPS (447). Similarly, the hypothesis that C-fiber afferents are the principal vagal fiber type important in the vagal signal to the brain has been disproven (109). What does seem clear at the present time is that while abdominal vagal afferents themselves do not appear to contain functional IL-1 receptors, abdominal paraganglia, which are in close proximity to and synapse with vagal fibers, bind biotinylated IL-1ra specifically (296). Furthermore, macrophages are found close to vagal fibers and paraganglia after treatment with LPS (205), suggesting a close association between IL-1-producing cell types, IL-1 receptors, and abdominal vagal afferents.

The vagus appears to be important in signaling the brain specifically during intra-abdominal/peritoneal inflammation. This is evidenced by the lack of effect of SDVX on CNS-mediated acute phase responses, such as activation of the HPA axis (235, 399), when either IL-1 or LPS is administered via routes other than into the abdomen/peritoneum (88, 89, 235, 297, 399, 645). However, global depletion of C-fiber afferents (by repeated systemic treatment with capsaicin during adulthood) inhibits the plasma ACTH response to intravenous IL-1/β (932). Furthermore, the initial (within 45 min) rise in plasma ACTH concentrations produced by intramuscular turpentine is reduced in rats treated neonatally with capsaicin (879). Therefore, it seems possible that sensory fibers, other than vagal abdominal/peritoneal afferents (e.g., from skin or muscle), are capable of signaling the brain of the occurrence of high local concentrations of cytokines in the periphery.

H. Cytokine Synthesis Within Brain

The previous discussions focussed on answering the question of how IL-1 generated within the periphery may signal the brain to elicit HPA activation. Recent studies have demonstrated that IL-1, as well as other cytokines, are also generated within the brain (see sect. ivA2), thus raising the question of whether such brain-derived IL-1 may influence HPA axis secretory activity. The present data regarding the distribution of IL-1 receptors within the brain would seem to argue against a role for cerebral IL-1 in regulating the activity of the neuroendocrine hypothalamus, since IL-1 receptors are located predominantly in barrier-related regions (e.g., perivascular elements) suited to transducing an IL-1 signal from blood to the brain, but not to mediating effects of IL-1 generated within brain. The only hypothalamic nucleus that expresses IL-1 receptors on neuronal elements is the arcuate nucleus, which is known to influence HPA axis secretory activity (238), but whose role in mediating the effects of IL-1 has not been determined. Despite a lack of evidence for an action of cerebral IL-1 on HPA axis secretory activity based on the distribution of IL-1 receptors within the brain parenchyma, IL-1 administered directly into the brain induces Fos expression in the pPVN, elevates PVN CRF mRNA, enhances CRF secretion from the ME, and increases plasma concentrations of ACTH and corticosterone (see sect. ivA3). Furthermore, the doses of IL-1 needed to stimulate HPA axis secretory activity are lower than those required when the cytokine is administered peripherally, clearly indicating a CNS site of action of cerebrally administered IL-1. Finally, incubation of the hypothalamus with IL-1 in vitro elicits CRF secretion (see sect. ivA3). Collectively, these data argue strongly that when CNS production of IL-1 is increased it is likely to stimulate HPA axis secretory activity.

Pathologies comprising direct cellular insults (infection, trauma, ischemia, and disease) to the CNS clearly induce the synthesis of IL-1 and other cytokines within the brain. However, only very few studies have investigated cytokine regulation of the HPA axis during such pathologies, although the work performed has implicated brain-derived IL-1 in the activation of the HPA axis in response to CNS viral disease. For example, intracerebral infusion or transgenic expression of gp120, the envelope protein of the human immunodeficiency virus, causes an increase in brain IL-1 biological activity and mRNA and elevates plasma ACTH and corticosterone concentrations (112, 355, 657, 661, 827). Coinfusion of α-melanocyte-stimulating hormone (which inhibits IL-1 actions, but not synthesis) completely prevents the elevation in plasma corticosterone concentrations, suggesting that IL-1 generated in brain as a consequence of gp120 infusion produces activation of the HPA axis (827). Similarly, induction of IL-1β within the brain has been proposed as the mechanism responsible for increased adrenocortical activity in rats inoculated with the neurotropic herpes simplex virus-1 (50).

Many studies have begun to address a question of much broader significance to the concept to neuroimmune regulation, i.e., whether events unrelated to direct cellular insults to the CNS can induce the synthesis within the brain of cytokines, which subsequently regulate CNS activity, and play a physiological role in orchestrating adaptive responses. In particular, work has focused on the possibility that an immune/inflammatory response...
within the periphery is paralleled by, or even initiates, cytokine production within the CNS. As detailed in section IV.A2B, the peripheral administration of LPS has been shown to induce cytokine and, in particular, IL-1 synthesis within the brain. However, by far the majority of studies which investigated cellular localization of cytokines demonstrated that IL-1 mRNA and protein are only induced within brain parenchyma at doses (hundreds of micrograms to milligrams) well above those required to observe physiological responses such as activation of the HPA axis (<1 µg/kg). Furthermore, cytokine induction within brain parenchyma is observed only several hours after LPS administration (compare increases in ACTH secretion at 30–45 min). Indeed, the location of IL-1 mRNA and protein that is induced fastest and at lowest doses appears to be in barrier-related regions such as the CVO and perivascular sites, suggesting a site of action on the blood side of the BBB. Therefore, cytokine synthesis within brain parenchyma would appear not to be an absolute requirement for at least those responses that occur rapidly after LPS administration.

Nevertheless, studies have demonstrated that inhibition of IL-1 action within the brain by intracerebroadministration of anti-IL-1β antibodies can inhibit acute phase responses to peripheral LPS (418, 709). However, such experiments failed to demonstrate that the IL-1β inhibited is generated within the brain and does not enter from the periphery (see section V.B) and also have not necessarily indicated that the site of action of the injected antibodies is within the brain. We have recently found that pretreatment of rats intracerebroventricularly with an anti-TNF-α antiserum delays the onset of the rise in plasma ACTH concentrations produced by intravenous injection of 5 µg/kg LPS, suggesting an action of TNF-α within the brain (889). However, intravenous administration of the same volume of antiserum produced qualitatively and quantitatively the same effect as that observed with intracerebroventricular anti-TNF-α (889). Subsequent studies indicated that antisera rapidly dissipate to the periphery after intracerebroventricular injection and can produce immunoneutralizing concentrations within the periphery within only a very short time period (~1–4 h) (890). Experiments using injections of antisera/antibodies or receptor antagonists to inhibit the action of cytokines within the brain clearly need to demonstrate that effects observed cannot be accounted for by actions of the inhibitors in the periphery, particularly given the high concentrations of cytokines that can occur in blood and peripheral tissues during inflammation/infection.

One tightly controlled study has indicated that IL-1 may well act within the brain to stimulate HPA axis activation in response to peripheral administration of LPS (387). Kakucska et al. (387) demonstrated that continuous intracerebroventricular infusion of human IL-1ra completely prevents the rise in pPVN CRF mRNA observed 8 h after LPS (2.5 mg ip). These authors simultaneously measured the quantities of human IL-1ra that escaped into the general circulation and in separate experiments demonstrated that these levels in plasma had no effect on the increase in CRF mRNA in response to LPS. Therefore, it would appear that IL-1 acts within the brain to stimulate the increase in CRF mRNA in response to peripheral LPS, although concomitant plasma ACTH levels were not measured. However, whether or not the IL-1 responsible for elevating CRF mRNA was of CNS rather than peripheral origin can still not be assumed.

We also found evidence indicating that TNF-α may act within the brain to activate the HPA axis in response to local inflammation. The intracerebroventricular, but not intravenous, administration of either 5 µl anti-TNF-α antiserum or 1–50 µg of a soluble TNF receptor construct inhibits the second rise in plasma ACTH concentrations produced by intramuscular turpentine (881). Because no increases in plasma TNF-α are apparent during discrete, localized inflammation, we assumed that TNF-α not only acted within the brain but that the CNS was the major source of TNF-α. However, we found no evidence of elevated TNF-α, IL-1, or IL-6 synthesis within the rat brain, as assessed by in situ hybridization histochemistry or semiquantitative PCR. Whether increased release of pre-stored TNF-α (105) or synergistic actions of TNF-α with PG (whose levels in the brain are elevated) (256) accounts for these observations is not known.

Surprisingly, more convincing evidence that IL-1 generated within the CNS plays a role in stimulating HPA axis secretory activity comes from studies investigating the neuroendocrine responses to immobilization stress (546, 777, 778). Minami et al. (546) reported that IL-1β mRNA expression was induced in the rat hypothalamus within 30 min of the commencement of immobilization stress. Interleukin-1β mRNA peaked at 60 min and was still elevated at 120 min. These results are particularly surprising given that these authors detected IL-1β mRNA using Northern blot hybridization, whereas the more sensitive technique of RT-PCR has been required to reliably demonstrate increases CNS expression of IL-1β mRNA in response to systemic LPS. Nonetheless, this group subsequently reported increases in hypothalamic IL-1 biological activity within 30–60 min of immobilization stress and furthermore demonstrated that microinjection of IL-1ra (2 µg) into the hypothalamus inhibits the stress-induced rise in plasma ACTH concentrations by as much as 50% (778). On the other hand, we have found no significant effects of either IL-1ra (100 µg icv) or anti-TNF-α antiserum on the rise in plasma ACTH concentrations produced by electrofootshock in rats (Ref. 881 and unpublished data), whereas others (716) have found that IL-6 deficiency does not influence the HPA axis response to immobilization in mice. These data would seem to indicate that the participation of cytokines in psychological/physical
stress-induced HPA activation is probably limited to specific cytokines and specific stressors.

Overall, although there is substantial evidence that IL-1 can be produced in the brain during CNS insults, severe endotoxemia, and possibly also acute physical/psychological stress, there is only limited data implicating CNS-derived IL-1 in the regulation of the associated HPA axis secretory responses. Direct testing of the physiological role of CNS-derived cytokines in the regulation of the HPA axis awaits the development of appropriate gene-targeting strategies that selectively mutate cytokine synthesis in a region- or cell type-specific manner (875).

I. Local Interleukin-1 Induction of Circulating Interleukin-6

Finally, it seems pertinent to consider the possibility that IL-1 induced by peripheral infection/inflammation can activate the HPA axis by a mechanism that does not involve a “direct” interaction of IL-1 with any of the above mechanisms. There is substantial evidence that the effect of IL-1 on the HPA axis during peripheral infection/inflammation is mediated via the induction of high circulating levels of IL-6 and that it is IL-6 that actually interacts with components of the HPA axis. Indeed, IL-1 potently stimulates the synthesis and secretion of IL-6 (e.g., Refs. 470, 585, 766, 871). Studies of the HPA axis response to viral infection (MCMV; see sect. III A3) clearly indicate that systemic administration of IL-1ra inhibits the rise in plasma corticosterone levels (716). Because IL-1β was undetectable in the general circulation, plasma IL-1α concentrations were not elevated during MCMV, and the marked rise in plasma IL-6 levels was reduced by >75% by treatment with IL-1ra, the authors concluded that IL-6 is a circulating mediator of IL-1 actions (716). Indeed, they showed that IL-6-deficient mice also display a markedly reduced corticosterone response to MCMV (716). Similarly, turpentine-induced local inflammation induces elevated levels of IL-1β locally but not systemically, IL-1 is responsible for inducing high circulating levels of IL-6 in plasma, and IL-6-deficient mice display a markedly reduced HPA axis response (see sect. III C). Such studies strongly suggest that circulating IL-6 may mediate the effects on the HPA axis of locally increased levels of IL-1. Indeed, IL-6 has been suggested to mediate a number of other IL-1 actions, including inhibition of proteoglycan synthesis in cartilage, induction of thymocyte proliferation, and the production of fever (97, 150, 333, 585, 602). Therefore, there is substantial evidence that during pathophysiological circumstances IL-6 may well be a major mediator of IL-1 action.

It seems unlikely that intravenous administration of exogenous IL-1 results in an IL-6-dependent activation of the HPA axis, since elevations of plasma ACTH concentrations can be observed within 5–10 min of IL-1 administration. However, there is evidence that IL-1 administered intraperitoneally stimulates ACTH secretion in the mouse in an IL-6-dependent fashion (584, 640). It is unfortunate, therefore, that given the much higher incidence of high levels of IL-6 rather than IL-1 during pathophysiological states, there is a relative paucity of information regarding the mechanisms of activation of the HPA axis in response to exogenous IL-6 than there is in response to exogenous IL-1 (see sects. IV A–IV F). At least part of the reason why this is the case is because of the relative impotency of IL-6 compared with IL-1 when these cytokines have been administered to animals (66, 527, 909, 1003). Whether this reflects a true difference in the potencies of the two cytokines to interact with components of the HPA axis, or reflects the fact that IL-1 also induces prolonged high circulating levels of IL-6, is not presently known. Nevertheless, it is clearly important to determine whether the mechanisms described above for IL-1 are also relevant for, or are in fact mediated by, IL-6. Such future studies would clearly benefit from the use of infusions of IL-6 to mimic the levels of IL-6 actually observed during pathological conditions.

VI. CONCLUSIONS

The landmark discoveries of Besedovsky’s and Bla-lock’s groups culminated with their demonstrations that IL-1 is an important endogenous regulator of the HPA axis. Since that time, a tremendous growth in the study of cytokine biology has provided several major advancements in the understanding of immune-neuroendocrine interactions. First, we now know that, in addition to IL-1, numerous other cytokines are capable of influencing HPA secretory axis activity, with most having a stimulatory action. Cytokine receptors have been cloned, characterized, and localized to many neuroendocrine (among other) tissues. Furthermore, it is now recognized that the HPA axis exposure to cytokines is not restricted to those carried within the vascular supply, since the CNS, pituitary, and adrenal are all capable of synthesizing a variety of cytokines, whose levels are increased during endotoxemia. Furthermore, neural afferents, such as those carried within the vagus nerve, may be a target of cytokine action that conveys information from inflammatory sites to the brain. Finally, recent studies suggesting that cytokine regulation of the HPA axis may occur not only during infection, inflammation, and trauma, but also during periods of psychological and/or physical stress unrelated to the presence of tissue disease or damage, have given a new perspective on the nature of immune-neuroendocrine interactions.

Clearly, the pituitary and adrenal glands represent potential targets of cytokine action on the HPA axis when these organs are exposed to prolonged elevated cytokine
levels. However, the majority of evidence indicates that either direct or indirect stimulation of hypothalamic CRF secretion is the primary means by which cytokines (at least IL-1, IL-6, and TNF-α) activate the HPA axis. The neuroanatomic and neurophysiological pathways by which IL-1 has been proposed to influence the neuroendocrine hypothalamus are numerous and diverse. Accurate models of the mechanisms by which IL-1 activates the HPA axis have to take into account the consistently reported inhibitory effects of either inhibitors of PG synthesis or disruption of catecholaminergic input into the hypothalamus.

In the situation where IL-1 levels are elevated endogenously (rather than by exogenous administration), the mechanism by which IL-1 activates the HPA axis is determined largely by the anatomic location of the tissues and bodily fluids that possess markedly increased IL-1 concentrations. In this respect, distinct proposed mechanisms seem most appropriate to the situations where IL-1 is increased in blood, peripheral tissue, or the brain. In reality, however, the extent to which elevations in cytokine concentrations can be compartmentalized on an anatomic basis is unclear. For example, it might be assumed that during a local inflammatory insult when IL-1 levels are elevated only at the local site of tissue damage, a neural afferent mechanism seems the most likely candidate pathway for activation of the HPA axis. However, because IL-1 induces the secretion of IL-6, which subsequently gains access to the systemic circulation, it seems likely that IL-6 is, at least partly, responsible for activation of the HPA axis. An additional complication is that none of the proposed mechanisms of activation of the HPA axis by cytokines takes into account the fact that elaboration of a single cytokine in response to a homeostatic threat is an unlikely event. Furthermore, there are no systematic studies of the mechanisms by which multiple cytokines (e.g., IL-1 plus IL-6) may induce HPA axis activation. Perhaps the most striking feature of the numerous studies investigating the mechanisms of IL-1 induced activation of the HPA axis is the tremendous diversity of plausible immune-neuroendocrine interactions. This diversity ensures that the occurrence of challenges to cellular, tissue, or system homeostasis is reliably conveyed to the neuroendocrine hypothalamus.

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