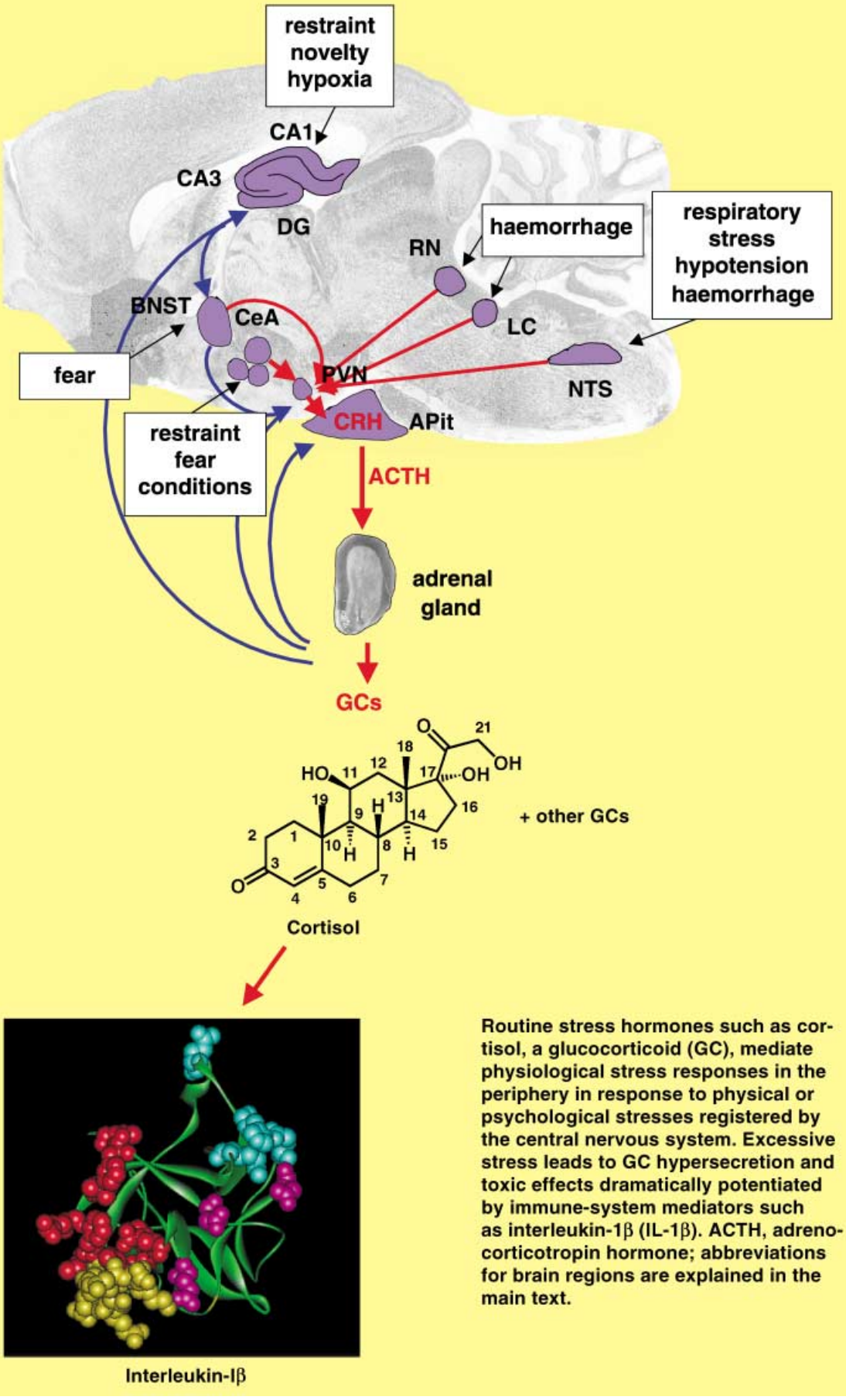

Routine stress hormones such as cortisol, a glucocorticoid (GC), mediate physiological stress responses in the periphery in response to physical or psychological stresses registered by the central nervous system. Excessive stress leads to GC hypersecretion and toxic effects dramatically potentiated by immune-system mediators such as interleukin-1β (IL-1β). ACTH, adrenocorticotropin hormone; abbreviations for brain regions are explained in the main text.

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Chemical Neuroimmunology: Health in a Nutshell

Bidirectional Communication between Immune and Stress (Limbic-Hypothalamic-Pituitary-Adrenal) Systems

Natalya Lozovaya[b] and Andrew D. Miller*[a]

Stress is a ubiquitous and pervasive part of modern life that is frequently blamed for causing a plethora of diseases and other discomforting medical conditions. All higher organisms, including humans, experience stress in the form of a wide variety of stressors that range from environmental pollutants and drugs to traumatic events or self-induced trauma. Stressors registered by the central nervous system (CNS) generate physiological stress responses in the body (periphery) by means of the limbic-hypothalamic-pituitary-adrenal (LHPA) axis. This LHPA axis operates through the use of chemical messengers such as the stress hormones corticotropin-releasing hormone (CRH) and glucocorticoids (GCs). Under conditions of frequent exposure to acute stress and/or chronic, long-term exposure to stress, the LHPA axis becomes dysfunctional and in the process frequently overproduces both CRH and GCs, which results in many mild to severely toxic side effects. Bidirectional communication between the LHPA axis and immune/inflammatory systems can dramatically potentiate these side effects and create environments in the CNS and periphery ripe for the triggering and/or promotion of tissue degeneration and disease. This review aims to present as far as possible a molecular view of the processes involved so as to provide a bridge from the diffuse range of studies on molecular structure and receptor interactions to the burgeoning biological and medical literature that describes the empirical interplay between stress and disease. We hope that our review of this fast-growing field, which we christen chemical neuroimmunology, will give a clear indication of the striking range and depth of current molecular, cellular and medical evidence linking stress hormones to degeneration and disease. In so doing, we hope to provide encouragement for others to become interested in this critical and far-reaching field of research, which is very much at the heart of many important disease processes and very much a critical part of the crucial interface between chemistry and biology.

KEYWORDS:

cytokines · hormones · immunology · neurochemistry · stress

Introduction

Stress is generated in many ways. For instance, environmental pollutants, alcohol or addictive drugs, traumatic events, self-induced traumas created by socioeconomic, psychosocial handicaps, or anxious/defeatist personality conditions, are all stressors that will be processed by the central nervous system (CNS) and relayed to the body (periphery) to create physiological stress response reactions. These stress response reactions to stresses and their accompanying stressors are frequently related anecdotally to disease states and pathological conditions. However, until the recent advent of the field of neuroimmunology there has been little to formally link stress to disease. Nowadays, evidence from neuroimmunology is mounting that shows there are indeed impressive connections between sustained stress, immune/inflammatory systems and disease and that these connections can increasingly be understood at a molecular level.

The primary focus of this review is that facet of neuroimmunology, sometimes known as neuroendocrinimmunology but which we prefer to christen chemical neuroimmunology, that revolves around the bidirectional communication between the immune/inflammatory and limbic-hypothalamic-pituitary-adrenal (LHPA) systems, facilitated by a common set of chemical messengers/mediators. The LHPA axis is the main "information superhighway" linking CNS to periphery and represents the primary means by which the brain receives the emotional, psychological and sensory information generated by stresses and associated stressors and relays them into instructions in the form of chemical messengers that signal physiological stress responses within the body in response to stress stimuli (Figure 1).[1] Excitation of the LHPA axis is driven by several central

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brain circuits that all project directly into the paraventricular nucleus (PVN) of the hypothalamus.[2, 3] Parvocellular neurosecretory neurons of the PVN project towards the median eminence (ME) and release corticotropin-releasing hormone (CRH, **1**; Scheme 1) into the hypophysial portal circulation. Following this hormone release, activation of CRH receptors in corticotropic cells of the anterior pituitary (APit) then stimulate the release of adrenocorticotropin hormone (ACTH, **2**) into wider circulation. The process is complete when ACTH docks with cognate receptors of the adrenal cortex, which causes the release of glucocorticoids (GCs) such as cortisol (**3**) in humans or corticosterone (**4**) in rats. In the classical model of brain function, this system is essentially a one way process from brain to adrenal cortex with the existence of negative feedback loops from GCs to the pituitary gland, hypothalamus and limbic system (which includes the hippocampus, prefrontal cortex and lateral septum) to prevent overexcitation of the LHPA axis and overproduction of GCs (Figure 1).

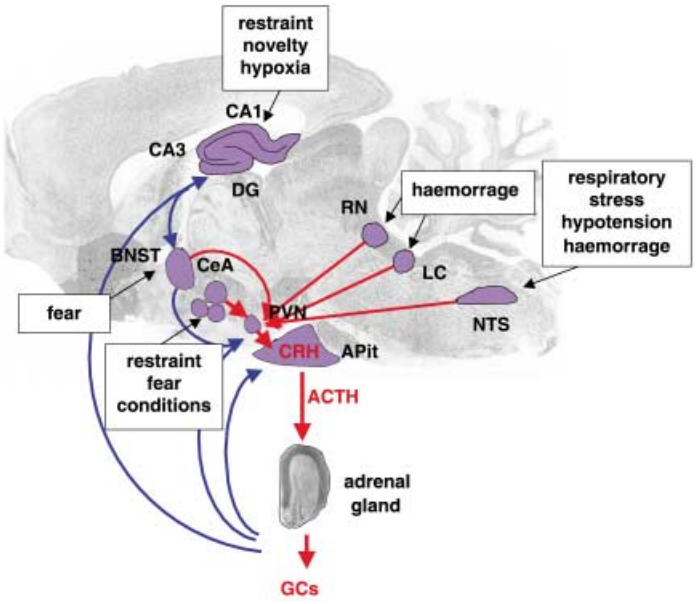

Figure 1. *Diagrammatic summary of the limbic-hypothalamic-pituitary-adrenal (LHPA) axis showing how stressors interact with various brain centres to initiate the production of corticotropin-releasing hormone (CRH), which in turn stimulates the secretion of adrenocorticotropin hormone (ACTH) into the blood stream and results in anabolism of glucocorticoid (GC) hormones in the adrenal gland. Both positive (red arrows) and negative (blue arrows) feedback control loops are shown. Abbreviations used for brain regions are as follows: CA1 and CA3, hippocampal neurons; DG, dentate gyrus; RN, raphe nucleus; LC, locus coeruleus; NTS, nucleus tractus spinalis; CeA, central nucleus of the amygdala; BNST, bed nucleus of stria terminalis; PVN, paraventricular nucleus; APit, anterior pituitary.*

The range and type of central brain circuits that project directly into the PVN and communicate with the LHPA axis are various and reflect the central role of the LHPA axis in stress management. These circuits include the brainstem catecholaminergic pathways, amygdaloid projections from the central, medial and cortical nucleus (CeA),[4] projections from the bed nucleus of the stria terminalis (BNST),[5, 6] catechol-aminergic/noradrenalinergic circuits emanating from the locus coeruleus (LC) and serotoninergic circuits from the raphe nucleus (RN).[7] These are all stress-activated circuits that operate directly in response to a defined stressor. For instance, haemmorhage, respiratory distress and hypotension will activate catecholaminergic input from the brainstem whilst restraint stress and fear conditions will activate amygdaloid input.[8, 9] Hence, the PVN appears to be the crucial locus for collection of stress stimuli in the form of electrical impulses and their onward transformation into chemical messages.

Given the central importance of the LHPA axis in the communication of external stress stimuli to the periphery, LHPA dysfunction presents potentially very serious problems. In short,

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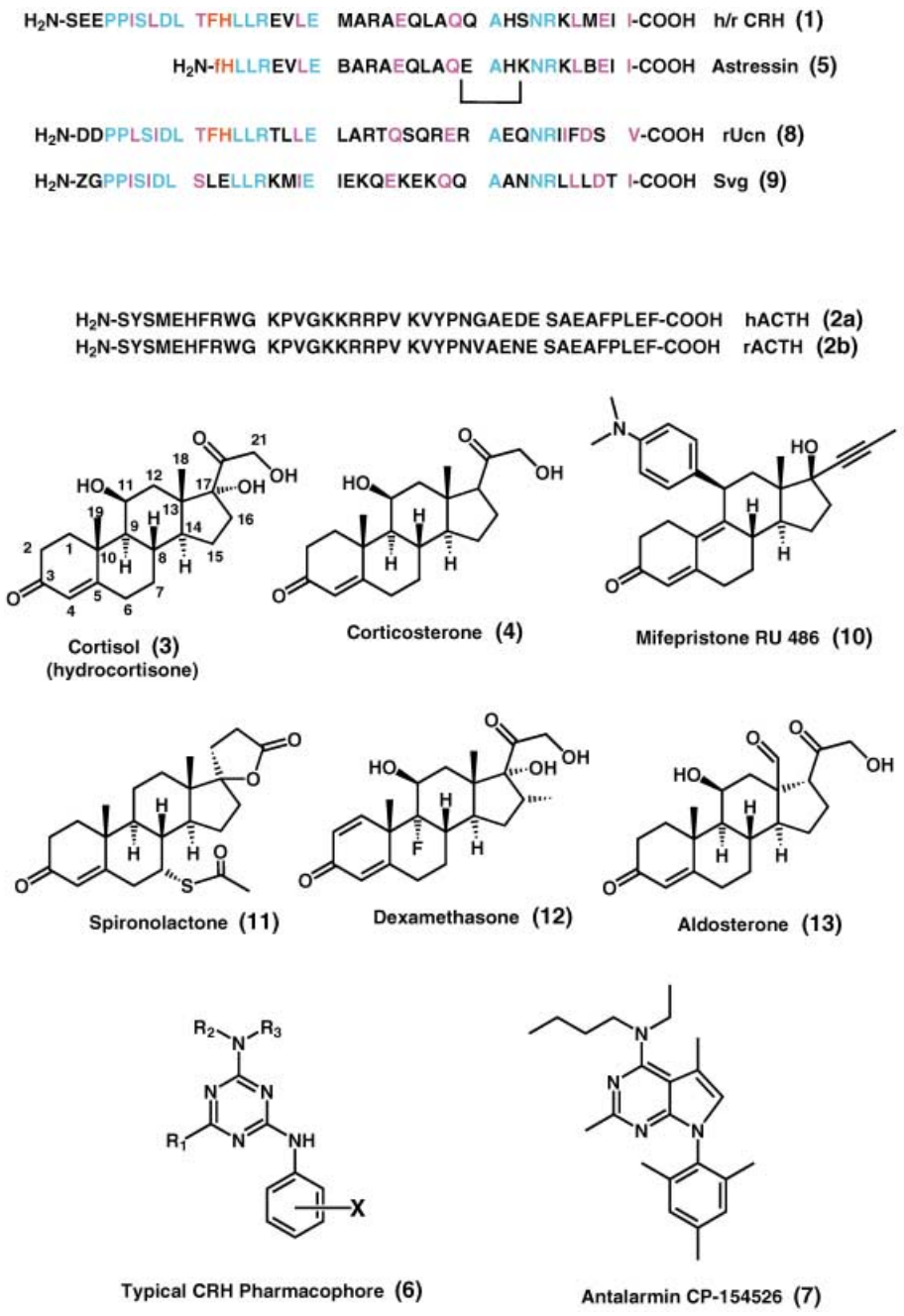

Scheme 1. *Structures of corticotropin-releasing hormone (CRH), glucocorticoids (GCs) and typical CRH antagonists. Red, key functional CRH, astressin and urocortin (Ucn) amino acid residues; light blue, amino acid residues that are identical in* ***1***, ***5***, ***8*** *and* ***9****; purple, similar amino acid residues. Other abbreviations used are as follows: h, human; r, rat; f,* D*-phenylalanine; Z,* L*-pyroglutamic acid; ACTH, adrenocorticotropin hormone; Svg, sauvagine; X, variable substituent.*

whilst the LHPA axis exists for the communication of stress stimuli and the management of routine levels of stress, LHPA-axis dysfunction will result from excessive stress stimulation, which thereby creates conditions able to trigger, promote and/or potentiate a bewildering variety of disease states and pathologies.[10, 11] These disease states and pathologies range from sleep disorders, melancholic depression, anxiety and mental illnesses through to hyperlipidemia, hypertension, type II diabetes, infections and autoimmune disorders. Prolonged LHPA-axis dysfunction may also be the trigger for the neurological effects seen in several significant human pathologies such as obesity, Alzheimer's disease (AD), AIDS, dementia, anxiety disorders, anorexia nervosa and chronic depression. Even severe autoimmune/inflammatory disorders like rheumatoid arthritis (RA) and allergic conditions such as asthma and eczema may be triggered by immune system distortions linked to LHPA axis dysfunction.

The molecular links from LHPA-axis dysfunction to these disease states and pathologies are not yet fully characterised but the key stress hormones, which comprise CRH and the GCs, are now understood to have toxic effects when produced in excess. Moreover, these toxic effects appear to be enhanced in range and scope by the bidirectional interplay between the LHPA axis and immune/inflammatory system mediated by proinflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor α (TNFα). In this review, the interrelated toxic effects of CRH, GCs and cytokines will be discussed in the light of basic chemical and biochemical behaviour in an attempt to advance an understanding of the relationship between stress, LHPA axis dysfunction, immune/inflammatory system and disease at the molecular level. To do this, the review will begin with a discussion about the structures and receptor interactions of the key stress hormones and cytokines, continue with a discussion about their potential toxic effects and conclude with a detailed summary demonstrating how LHPA axis dysfunction can now be linked causally to many major disease states and pathological conditions.

Chemical Messengers of the LHPA Axis

CRH, agonists, antagonists and receptors

CRH is a 41 amino acid single-chain polypeptide (Scheme 1). The secondary structure of human CRH (hCRH) has been characterised by ^{1}H NMR spectroscopy in trifluoroethanol/water (TFE/H_2O; 66:34 v/v) and shown to consist of an α helix from residues 6 to 36 (76% helical).[12] The α-helical region from residues 6 to 20 is

amphiphilic but the remaining C-terminal region is hydrophilic in character. This C-terminal helical region appears to be retained in pure water whilst the amphiphilic helical region is not.[12, 13] Intriguingly, structure – activity studies have revealed that the amino acid residue side chains of residues 5 to 19 are important for receptor binding and activation, whilst the C-terminal region is only present for structural conservation.[14] Since an α-helical CRH (9 – 41) has been shown to be an antagonist of CRH,[12] the ^{1}H-NMR-derived structure described has been proposed to be the conformation of receptor-bound CRH. Therefore, CRH receptor proteins (see below) may have the role of amphiphilic organisers that encourage the formation of the amphiphilic helical region of CRH and lead to receptor activation. Since α-helical CRH (9 – 41) has also been shown to displace CRH from CRH-binding proteins,[15] these binding proteins may well function in a similar way to sequester CRH and control the level of free CRH in neurological tissue.

CRH exhibits its activity through G-protein-coupled receptors. CRH Type 1 receptors (CRHR1) are found mainly in the pituitary and brain; examples have been cloned from human, mouse, rat, chicken and frog cells.[16–20] Two splice variants of CRH Type 2 receptors, known as Type 2α (CRHR2α) and Type 2β (CRHR2β), have been found expressed in the periphery in tissue from brain, heart, lung and skeletal muscle.[19, 21–25] In rodents, CRHR2α and CRHR2β are found exclusively in the CNS and periphery, respectively. Recently, it has been proposed that urocortin (Ucn, **8**; Scheme 1), a natural CRH analogue, may be the natural endogenous ligand of has been isolated from the skin of the frog *Phyllomedusa sauvagei* CRHR2.[26] Also, a 40 amino acid peptide, sauvagine (Svg, **9**; Scheme 1), has been isolated from the skin of the frog *Phyllomedusa sauvagei* that is highly homologous to CRH and displays a number of CRH effects also mediated by CRHR2 receptor types.[13, 27]

There has been substantial interest in the design and synthesis of CRH antagonists as well as agonists. Since the discovery of potent peptide antagonists [such as α-helical CRH (9 – 41) and astressin (**5**; Scheme 1)] based upon the N-terminal truncated amino acid sequence of human/rat CRH (h/rCRH), several CRHR1-selective nonpeptide antagonists have been developed.[28, 29] These antagonists have been found to attenuate CRH-mediated conditions such as seizure, interleukin-1β (IL-1β) induced fever, or exhibit anxiolytic activity in vivo.[30, 31] Such CRHR1-selective nonpeptide antagonists usually contain a pharmacophore with a substituted phenyl ring attached to a five- or six-membered nitrogen heterocycle, as in **6**.[32] One notable example of this class of molecule is the CRHR1-selective antagonist antalarmin (CP-154526; **7**).[29, 33] Conceivably, this common feature mimics an important and favourable arrangement of the side chains of the adjacent amino acid residues phenylalanine (F) and histidine (H) that are present in both CRH (**1**) and its peptide antagonists such as astressin (**5**; Scheme 1) and may well form key contacts in the receptor – ligand complexes. Certainly, the available structural evidence suggests that these residues may well be in an exposed, conformationally mobile region of CRH proximal to C-terminal α-helical structures formed upon receptor binding, as described above (also see, ref. [34]). Other CRHR2-selective peptide antagonists have also been designed.[27]

GCs, agonists, antagonists and receptors

The main glucocorticoids (GCs) involved in the LHPA axis are cortisol (**3**) in humans and corticosterone (**4**) in rats. GCs interact primarily with either mineralocorticoid receptors (Type I; MR) or glucocorticoid receptors (Type II; GR),[35–38] which have been known to participate in corticosteroid negative feedback control for a number of years.[39] Corticosteroids, including GCs, have been the subject of research for decades, research which has given rise to a variety of selective receptor agonists and antagonists. For instance, RU486 (also known as RU38486, mifepristone or Mifegyne; **10**) is a GR-selective antagonist,[40, 41] as is RU40555.[42, 43] By contrast, RU28318 and spironolactone (**11**) are MR-selective antagonists.[41–44] In studies of the LHPA axis, the GR-selective agonist known as dexamethasone (DEX; **12**) is critical. This molecule appears to be able to mimic many of the neurological effects of **3** or **4** through GR binding (as described below).[45]

Both corticosteroid receptor types mentioned above belong to the nuclear receptor (NR) family[46] of ligand-induced transcription factors. These molecules are structurally and functionally related and have structures that may be divided into three main domains, which include a C-terminal ligand binding domain (LBD) and a central DNA binding domain.[47] Steroid receptors not bound to their ligand are complexed with molecular chaperone proteins such as Hsp90 and Hsp70 in readiness for GC binding.[48] Following GC binding to the LBD, DNA binding is enabled and DNA transcription triggered.[49–51] Human MR and GR are closely related to one another, with structural and functional similarities that include highly conserved LBD and DNA-binding domains (57 and 94% sequence identity, respectively).[35, 37] MR binds mineralocorticoids such as aldosterone (**13**; Scheme 1), as well as glucocorticoids with high affinity, whereas GR binds only glucocorticoids with high affinity.[35, 36, 52, 53]

The LBD of GR comprises about 250 amino acid residues, which may fold into a hydrophobic pocket to bind glucocorticoids. An homology model of the LBD has been reported in the form of a three-layered antiparallel α-helical "sandwich" structure that undergoes a substantial conformational change involving the folding back of Helix H12 to give a more compact structure upon ligand binding.[54, 55] A similar homology model of the MR LBD has also been described.[56] Such mutagenesis studies that have been performed guided by these structures suggest that general receptor selectivity is determined by crucial binding regions in both GR and MR LBDs that contact the steroid A-ring α,β-unsaturated ketone group (site I), 11-β functionalities in the C ring, and C-17/C-18 functionalities around the D ring (site II; see Scheme 1).[54, 56] However, current mutagenesis evidence also suggests that the key difference between MR and GR selectivity arises from an amino acid residue, Valine 571, in the GR LBD.[54] This residue appears to function as a means to reduce interactions with mineralocorticoids even though there is no direct contact between this residue and the ligand, according to the GR LBD homology model.

Proinflammatory cytokines and receptors

Pivotal cytokines such as TNFα, IL-1β, Interleukin-1α (IL-1α) and IL-6 appear to provide crucial communication links between the immune system and the LHPA system. In other words, TNFα, IL-1β, IL-1α and IL-6 are amongst a select number of cytokines that are central to the facet of neuroimmunology described here.

IL-1β and IL-1α are isoforms of the potent cytokine Interleukin 1 (IL-1). These isoforms have been demonstrated to perform central mediatory roles in immunity, hematopoiesis and inflammation, with consequences for rheumatoid arthritis and septic shock as well as potential consequences for neurodegenerative diseases such as Alzheimer's disease.[57–61] Both IL-1β and IL-1α are expressed as approximately 31-kDa precursors that are processed to mature biologically active proteins of 153 and 159 amino acid residues in length, respectively (both approximately 17.5 kDa).[62, 63] In spite of possessing only about 25% sequence homology, these two molecules have almost identical tertiary structures (Figure 2),[64–66] and act through the same cell surface receptors (IL-1RI, IL-1RII and accessory chain) to elicit similar biological responses, with few exceptions.[67–71] IL-1RI has a molecular mass of approximately 80 kDa and is found at the surface of virtually all cells, including T-cells and endothelial cells. IL-1RII has a molecular mass of 68 kDa and locates to the surface of immune cells such as neutrophils and macrophages. Both receptors are glycosylated and belong to the immunoglobulin superfamily.[57, 58, 72] The determination of the X-ray crystal structure of the complex formed by IL-1β and the soluble IL-1R receptor fragment (sIL-1R)[73] has confirmed that interactions between receptor and cytokine involve contacts that may be grouped into two main regions, Sites A and B. These sites coincide well with the locations of amino acid residues previously identified by site-directed mutagenesis experiments as having a role in mediating receptor binding (Figure 2).[72, 74–76] Mutagenesis studies have also identified three other amino acid residues unimportant for binding but apparently essential for receptor activation and signal transduction.[72] Indeed, one of these residues in particular, aspartate 145 (D145) was found to be especially important for receptor activation. The lysine (K) mutant D145K derived from wild-type IL-1β was shown convincingly to behave as a receptor antagonist able to bind IL-1 receptors in a manner competitive with wild-type IL-1β, but the

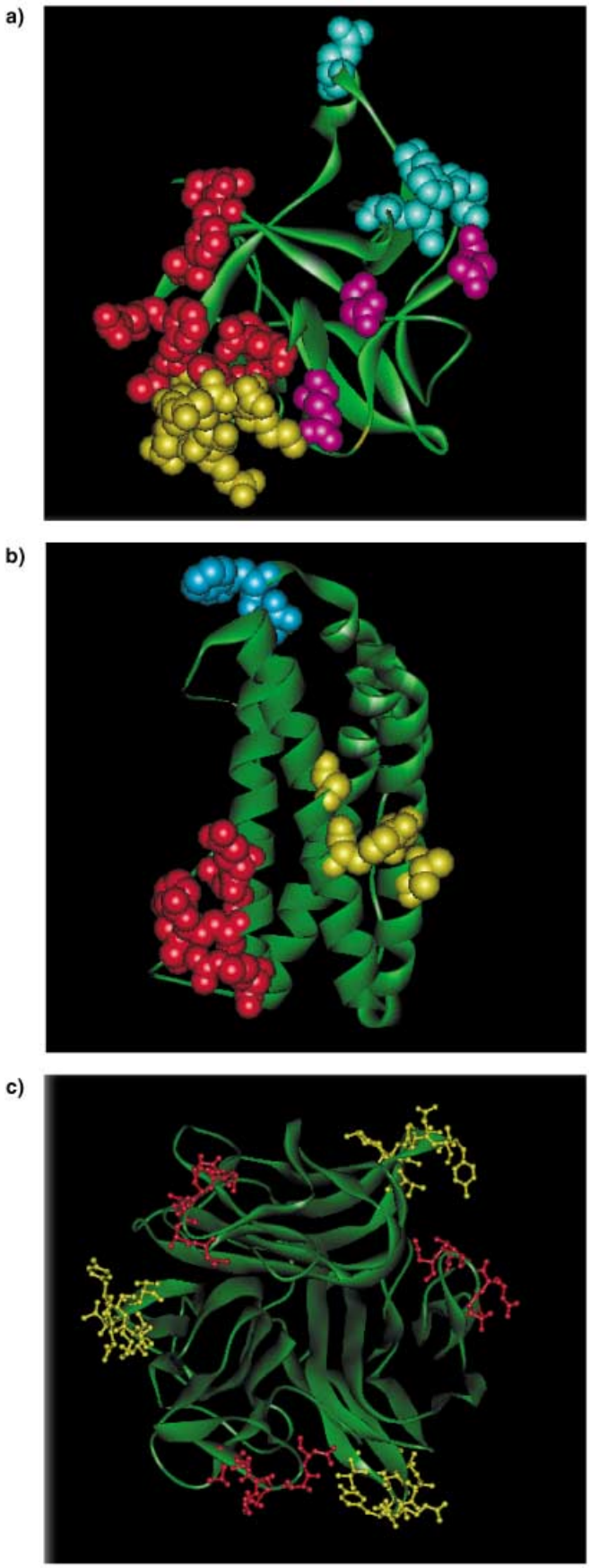

Figure 2. *Cytokine structures with amino acid residues important for receptor binding and/or receptor activation/signal transduction illustrated. a) Top view of IL-1β (Protein Data Bank (pdb) code 1I1B),[64] showing Site A (blue) and Site B (red) receptor binding residues identified by site-directed mutagenesis experiments[72, 74] and confirmed by X-ray crystal structure analysis.[73] The Boraschi-loop (trigger loop; yellow) functions both for binding and early gene activation. Residues essential for receptor activation and not binding (purple) are also shown;[72] b) Side view of IL-6 (pdb code 1ALU) showing Site 1 (red), Site 2 (yellow) and Site 3 (blue) amino acid residues identified by site-directed mutagenesis[85–91] and confirmed by X-ray crystal structure analysis.[84] Site 1 mediates binding to IL-6Rα and Sites 2 with 3 mediate binding to two different molecules of gp130; c) top view of the TNFα homotrimer (pdb code 1TNF)[100] highlighting amino acid residues of primary loop 84 – 91 (yellow) shown by site-directed mutagenesis experiments to be important for TNFR-p75 and p55 interactions.[102] A second loop (29 – 34; red) is also shown and is thought to be important for selective interactions with TNFR-p75.[103]*

mutant was barely able to elicit any biological responses in assays.[75]

IL-1 activity is normally controlled within biological systems by mechanisms that involve a third member of the IL-1 family, namely the interleukin-1 receptor antagonist (IL-1ra),[63, 77, 78] which binds competitively to IL-1 cell surface receptors without eliciting a comparable biological response to that induced by IL-1.[79] IL-1ra possesses a very similar tertiary structure to IL-1β and IL-1α, minus a critical β-bulge structure present in both IL-1β and IL-1α structures but completely absent from that of IL-1ra.[80, 81] This β-bulge structure, also known as the trigger or Boraschi loop (Figure 2), has been implicated in receptor binding but also as an "early trigger" of gene expression following IL-1 binding to receptors.[73, 76, 82]

Cytokine IL-6 is a frequent counterpart to IL-1 and is known to exercise a comparable variety of roles in immunity, inflammation and hematopoiesis.[83] This cytokine consists of 185 amino acid residues and possesses a vastly different tertiary structure to that of the IL-1 family. Determination of the X-ray crystal structure of IL-6[84] has provided the first real clues about receptor interactions involving this cytokine in the face of a complex and rather contradictory set of site-directed mutagenesis data (Figure 2).[85–91] The mechanism of receptor activation and signal transduction appears to have little connection to that of the IL-1 family. The IL-6 receptor complex is now known to consist of two polypeptides known as the α chain (IL-6Rα; 80 kDa) and the β chain, the latter of which is a transmembrane glycoprotein (gp130) that binds IL-6 only weakly but binds the IL-6/IL-6Rα complex with high affinity leading to signal transduction and appropriate biological responses.[92] An ordered and sequential mechanism appears to exist in which IL-6 binds first to IL-6Rα, mediated by amino acid residues in Site 1 (Figure 2), and then to gp130 on the cell surface through the amino acid residues of Site 2 (Figure 2). A heterotrimeric complex is then formed. Heterotrimers may well dimerise further to form a hexameric complex as a result of a third "cross-linking" binding event in which the IL-6 molecule of one heterotrimer interacts with the gp130 of the other heterotrimer.[93] This third binding event appears to involve amino acid residues in Site 3 (Figure 2).[84]

Cytokine TNFα represents yet another substantial twist in the theme of cytokine structure and receptor interactions. TNFα is widely appreciated to be the principle mediator of systemic responses to sepsis and injury[94] and is produced by inflammatory cells in response to diverse infectious stimuli and tissue injury so as to generate a cascade of mediators that direct immunological functions.[95] In large excess, TNFα may induce the equivalent of toxic shock,[96] and slightly lower levels have been implicated as contributing towards anorexia and cachexia conditions.[97] TNFα effects are mediated through two main TNFα receptors, namely the 55-kDa TNFR-p55 and the 75-kDa TNFR-p75.[98, 99] The X-ray crystal structure of TNFα reveals a homotrimeric structure comprised of three identical 17-kDa polypeptide subunits (Figure 2).[100] This homotrimeric structure is now considered likely to be the biologically active form[101] and certainly appears to be essential for TNFα binding and activation of either of the two main receptors.[102] Whilst at least four regions of TNFα appear to be critical for receptor binding and signal transduction, two exposed surface loops corresponding with residues 84 – 91 and 29 – 34 appear to be of particular significance (Figure 2).[102] The former region harbours tyrosine 87, an amino acid residue that may be mutated to result in complete retention of the homotrimeric quaternary structure but almost complete loss of receptor binding and activation. The latter loop region appears to have special significance for mediation of binding to the TNFR-p75.[103] Activation of this particular receptor type is responsible for the systemic toxicity that characterises the biological effects of excess TNFα.

Cytokine/receptor interactions, in common with many such interactions between proteins and their cognate receptors, are clearly complex and involve multiple discontinuous contact points. For this reason, direct inhibitors have been difficult to obtain. However, there have been some notable successes, particularly with IL-1. Recent attempts to inhibit IL-1 effects have included the use of recombinant IL-1ra, soluble receptor antagonists, pyridinylimidazole inhibitors and even complementary peptide "mini receptor" inhibitors.[80, 104–108] This very same complementary peptide "mini receptor" inhibitor approach has even been found to yield some useful results with TNFα[107a] and some other cytokines involved in neuroimmunological cascades.[107b] Such inhibitors used in conjunction with inhibitors of CRH could become very useful tools in the future to control the excesses of the LHPA axis with arguably beneficial consequences for mental and physical health, as we shall reveal in the next sections of this review.

LHPA-Axis Control; The Negative Feedback Loop

Control of the LHPA axis is essential given the problems associated with dysfunction alluded to in the Introduction. Therefore, this section is devoted to understanding the negative feedback loop and how this may degrade, which leads to LHPA dysfunction and hence to disease states and pathologies. GC-mediated negative feedback has been known for some time to emanate primarily from the hippocampus.[38, 109] Substantial numbers of GR and MR complexes reside in the hippocampus. Activation of these receptors by GC binding provides the negative feedback inhibition of the LHPA axis necessary to return the system to basal activity after exposure to stress stimuli.[110]

MRs have approximately 10-fold higher affinity than GRs for GCs so that at normal basal GC levels the majority of MRs are occupied (over 70 – 90%) by GCs, even at resting levels during the circadian trough.[38, 111–113] Since GRs have lower affinity for GCs, these only become substantially occupied during periods of high circulating GC levels, such as during stress or at a circadian (diurnal) peak.[38, 112, 114] The general opinion appears to be that MRs are needed to maintain basal LHPA-axis activity during the circadian (diurnal) trough and provide sensitivity to the stress response,[39, 42, 112, 115] whilst GRs are needed to suppress excitability (that is, constrain the LHPA axis) during the circadian peak and acute stress hence facilitating recovery from stress post exposure to stimuli.[42, 116–118] The constraining role of GRs has been supported by data obtained during experiments with transgenic mice expressing an impaired GR function.[119] How-

ever, there is evidence to suggest that MR activation plays an important role in facilitating GR-dependent regulation of the LHPA axis by corticosterone (**4**).[42] The results of other recent studies also point to a more central role for MRs in constraint and control of the LHPA axis during stress.[44, 120, 121]

There do appear to be several alternative means to modulate MR and GR activity, which include the use of the ability of noradrenergic (NA) pathways to exercise direct control over hippocampal MR levels and the affinity of hippocampal GRs for GCs.[122] Cholinergic pathways are also known to regulate LHPA-axis activation.[123] By contrast, GC binding to a steroid-family receptor complex known as nur77 in rats (NAK-1 in humans) is known to antagonise LHPA-axis negative feedback.[124]

Stress and negative feedback

If the normal response of the LHPA axis reflects the net influence of stimulatory drive and GC negative feedback, then the simplest model of LHPA-axis dysfunction is loss of negative feedback leading to an unopposed stimulatory drive. Evidence acquired from studies using dexamethasone (**12**) suggests that loss of negative feedback control is an important consequence of intensive, acute stress or chronic stress conditions. Dexamethasone has been used extensively in the context of the dexamethasone suppression test (DST) to probe the LHPA axis for defects in GC negative feedback.[125] Dexamethasone (**12**) administration normally provokes a substantial fall in the levels of ACTH and GCs in plasma through GR-mediated agonist effects.[45] A loss of sensitivity towards dexamethasone administration is regarded as indicative of reduced GC negative feedback control of the LHPA axis. In rats, intensive, acute stress has been found to cause prolonged loss of sensitivity towards dexamethasone (**12**) in DSTs that correlated closely with long-lasting decreases in GR and then MR binding of GCs in hippocampal tissue.[126]

The GR-agonist properties of dexamethasone (**12**) also make repeated and excessive administration of this compound a useful mimic of excessive GC secretion generated by intensive, acute bouts of stress stimulation. When rats were exposed to excessive repeat administrations of **12**, they exhibited a host of symptoms such as significantly decreased levels of GR mRNA levels in the hippocampus, increased levels of CRH mRNA levels in the PVN and prolonged ACTH secretion. Such symptoms are all consistent with a scenario in which excessive levels of dexamethasone (**12**) erode GC negative feedback control of the LHPA axis by a mechanism that involves reductions in MR and GR mRNA levels and possibly reductions in receptor–ligand avidities as well, leading to LHPA-axis hyperactivity and stress hormone hypersecretion.[127] Results such as these have also been seen in the adult offspring of dexamethasone-treated pregnant animals. In rats, fetoplacental exposure to maternally administered dexamethasone was found to result in permanent attenuation of GR and MR mRNA expression in hippocampal tissue, accompanied by increased basal levels of corticosterone.[128] Furthermore, other studies involving chronic stress have revealed a similar combination of adrenal hypertrophy and baseline hypersecretion of both GCs and CRH, concurrent with significant downregulation of MR and GR mRNA levels.[129, 130]

The clear implication of all these experiments is that intensive, acute bouts of stress stimulation or chronic stress stimulation could directly cause LHPA-axis dysfunction by impairing GC negative feedback control at the level of the corticosteroid receptors.[126] Furthermore, excessive stress stimulation in pregnancy can lead to permanent LHPA-axis dysfunction in offspring by a similar mechanism. Such suggestions are completely consistent with the GC cascade hypothesis,[131] according to which stress stimuli are proposed to directly induce down-regulation of MRs and GRs in the hippocampus and impair GC feedback control on stress-induced LHPA-axis activation, leading to an increase in the basal level of GCs, ACTH and CRH that may develop into long-term hypersecretion depending upon the severity of stress stimulation.

Age and negative feedback

Age-related effects appear to be very similar to stress-related effects on the LHPA axis. Aging is associated with erosion of negative feedback control of the LHPA axis heralding crucial changes in long term functioning and the advent of neurodegeneration. Hippocampal neurodegeneration, loss of corticosteroid receptors and concomitant LHPA-axis dysfunction and lability following stress are all associated with the aging process.[132, 133] Studies with aged rats demonstrate that MR and GR binding of GCs by the aged hippocampus is up to 50% lower than that achieved by the young hippocampus, comparable with a 30–40% reduction in MR and GR steady-state mRNA levels.[133] Other studies suggest that MR binding capacities are principally affected by aging.[132] Such reductions explain the slower, corrupted response of the negative feedback loop in aged as opposed to young animals, which leads to ACTH and GC hypersecretion in aged animals.[132, 134, 135] Similar effects have also been observed in aged dogs as well as in aged rats and lead to similar conclusions.[136] Other age-related effects involving neurotransmitter interactions may also impinge negatively upon LHPA-axis control.[137] The very similarities between age- and stress-related effects on LHPA-axis function have led to the suggestion that stress stimulation and GCs may at least facilitate if not be a primary cause of the aging process in rats and also in humans, although the evidence is less categoric in the latter case.[131] In any event, it should now be clear that stress and aging cause parallel effects and are inextricably linked at the level of the LHPA axis.

Recently, Wang et al. provided a devastating review of the morphological and pathological changes associated with age-related LHPA dysfunction.[131] Reduction in sensitivity to the DST with aging clearly suggests that loss of negative feedback control is crucial to the development of the pathology. Furthermore, raised levels of CRH, ACTH and GCs in aged humans or animals are clearly accompanied by hypertrophy in the adrenal gland, neuronal loss in the hypothalamus and loss of GC receptors in the hippocampus. In addition, oxidative enzyme activities are also elevated by aging. Mechanisms for the toxic and pathological consequences of excessive secretion of stress

hormones, including potential mechanisms for neurological damage, are described in detail later on in this review. Interestingly, there has been a report that prolonged oestrogen therapy of aged rats increases GR levels in the hippocampus and restores the efficacy of the DST, which suggests that a practical reversal of aging effects has taken place.[138]

LHPA-Axis Control; The Role of Cytokines

Research in the field of neuroimmunology during the last few years has shown convincingly how proinflammatory cytokines such as IL-1, IL-6 and TNFα mediate bidirectional communication between the immune/inflammatory and LHPA systems (Figure 3). The range and depth of these communication links is far from being completely understood but there is now a growing appreciation, as is described below, that LHPA-axis dysfunction caused by intensive, acute bouts of stress or chronic stress stimulation can have significant consequences for immune system function owing to the existence of direct communication links between the LHPA and immune/inflammatory systems. Moreover, these direct communication links are very much bidirectional and cytokines can also directly influence the functioning of the LHPA axis even to the point of contributing to LHPA-axis dysfunction.[139]

Central to bidirectional communication between immune/inflammatory and LHPA systems is a negative feedback loop wherein cytokines such as IL-1, IL-6 and TNFα released from sites of inflammation and infection in the periphery cross the blood–brain barrier (BBB) and stimulate the LHPA axis in order to release GCs into the circulation, where these GCs may exert systemic antiinflammatory effects.[140–142] IL-1 and possibly IL-6 appear to influence MR affinity for GCs in the hippocampus and shift the MR/GR balance so as to promote stress hormone secretion by impairing GC negative feedback control at the level of the corticosteroid receptors.[116, 143, 144] The similarity between this apparent mechanism of cytokine-mediated LHPA-axis stimulation and the consequences of excessive stress stimulation for the LHPA axis (as described above) suggest key synergies may exist between the effects of excessive stress stimuli and the immune/inflammatory system that could provide the means to provoke and exacerbate LHPA-axis dysfunction.

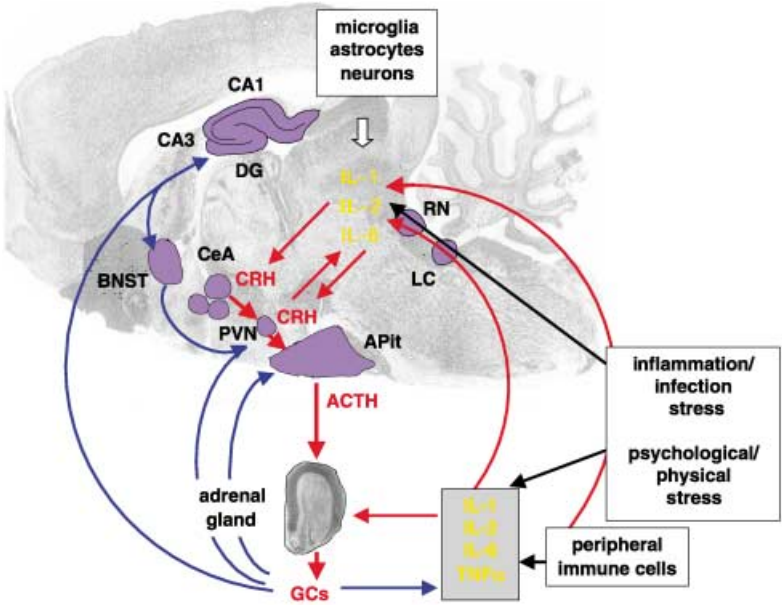

Figure 3. *How key pro-inflammatory cytokines (IL-1, IL-2, IL-6 and TNFα) interact with and stimulate elements of the LHPA axis. Both positive (red arrow) and negative (blue arrow) feedback control loops are shown.*

Peripheral origins of cytokines

The potential for these synergies becomes all the more significant given the fact that physical and psychological stress increases peripheral production of cytokines directly. Such data are crucial in that they suggest a direct link from stress stimuli to control of the LHPA axis through the peripheral immune system. There have been clear demonstrations that plasma IL-6 levels increase in rats exposed either to a novel environment,[145] physical restraint[146] or conditioned aversive stimuli.[146] Chronic restraint stress has also been found to elevate levels of rat plasma IL-1β.[147] Similarly, Spivak et al. have noted an elevation in IL-1 levels as a result of combat-related post-traumatic stress disorder.[148] These cytokines produced in the periphery can affect neuronal activity and the LHPA axis by accessing the CNS within the circumventricular organs (CVO) and specific regions that are relatively devoid of BBB restrictions owing to high local density and permeability of capillaries. Specific regions include the PVN, the organum vasculosum laminae terminalis (OVLT), the area postrema (AP), the median eminence (ME), the CeA and the BNST.[149–151] In addition, there are now known to be specific saturable BBB transport systems for IL-1α,[152] IL-1β,[153] IL-1ra,[154] TNFα[155] and IL-6,[156] but not apparently for IL-2. This latter deficit may be made up for by the fact that peripheral immune cells such as macrophages, T-cells and neutrophils can enter the CNS as a result of a breakdown in the BBB following insult or infection that may itself be cytokine mediated.[11, 157–159] These cells then generate all the main proinflammatory cytokines such as IL-1, IL-6 and TNFα in situ along with IL-2. Furthermore, lymphocytes can travel into the CNS through the BBB even in the absence of any obvious pathology, although to a lesser extent.[160, 161]

CNS origins of cytokines

Data has also been accumulating to suggest that a link may also exist between stress stimuli and control of the LHPA axis by local CNS immune system activity. All types of glial cells and neurons are now understood to produce or have the capacity to produce cytokines.[57, 161–167] IL-1, IL-6 and TNFα immunoreactivities have been found in a variety of regions in rats, including the hypothalamus, hippocampus, brainstem and cerebral cortex, under "normal" condi-

tions.[143, 162–164, 168, 169] This basal level of cytokine expression is dramatically increased in the CNS as a result of conditions such as brain trauma, infection, inflammation, cerebral ishaemia and Alzheimer's disease.[11, 143, 159, 161, 167]

Stress stimuli are also major inducers of cytokine production, especially in CNS regions closely associated with the LHPA axis. For instance, hypothalamic expression of IL-1 has been found to increase after rats have been exposed to immobilisation and restraint stress, whilst IL-6 expression is increased in the midbrain.[170, 171] Furthermore, receptors for a variety of cytokines, which include IL-1, IL-2, IL-6 and TNFα, have been found in the brain, with the highest levels in the hippocampus and hypothalamus.[161, 172] Given this fact, stress stimuli appear to be more than capable of inducing effective, local proinflammatory cytokine production in the CNS that could cooperate with and enhance the capacity of cytokines produced in the periphery to stimulate the LHPA axis.

Mechanism of interactions with the LHPA axis

Proinflammatory cytokines activate the LHPA axis during the acute-phase response to infection, injury and stress conditions, which determines the pattern of hormone secretion (Figure 3). In addition to the effects of IL-1 and IL-6 on glucocorticoid negative feedback in the hippocampus (described above), other studies demonstrate that cytokines, especially IL-1 and IL-6 but also IL-2, IL-8, TNFα and IFNα, potently stimulate the secretion of CRH from the hypothalamus.[141, 149, 150, 173–180] The mechanism(s) by which these cytokine effects are mediated probably involve a number of different pathways both local and long range.

Cytokine-mediated excitation of the LHPA axis may be driven in part by selected central-neuronal, stress-activated afferent inputs terminating in the hypothalamus (see Figure 1). These afferent inputs appear to involve neuronal input from catecholaminergic/noradrenalinergic cell groups, located in the nucleus trachtus spinalis (NTS) in particular, and possibly from serotoninergic cell groups located in the dorsal raphe nucleus (these include the B7, B8 and B9 groups). Disruption of the catecholaminergic/noradrenalinergic projection into the PVN of the hypothalamus has been found to inhibit production of both ACTH and corticosterone (**4**) in response to central or peripheral administration of IL-1β.[181, 182] Furthermore, there is evidence to show that catecholamines, serotonin[183, 184] and even histamine[185, 186] may all have roles in LHPA-axis activation in the hypothalamus. In contrast, there is some suggestion that catecholaminergic/noradrenalinergic projections may not necessarily be important to mediate cytokine effects.[187] Therefore, the picture is not completely clear.

Cytokine activation of the LHPA axis appears also to involve eicosanoid-mediated pathways. Cytokines are known to induce expression of prostaglandin endoperoxide synthase (PHS), which is a key enzyme system that converts arachidonic acid into eicosanoids such as the thromboxanes and prostaglandins and which exhibits cyclooxygenase (COX) activity.[188] Crucially, administration of the COX inhibitor indomethacin appears to prevent CRH release induced either by IL-1α, IL-1β or IL-6.[173, 179, 189, 190] Therefore, it appears likely that arachidonic acid metabolism is an important mediator of LHPA-axis activation in response to cytokine stimulation.[161] Prostaglandins are probably the most significant COX product in this regard. In the hypothalamus, prostaglandins such as PGE2 stimulate LHPA activity locally in response to IL-1α, IL-1β, IL-6 or TNFα receptor stimulation of endothelial and perivascular microglial cells. Prostaglandins may also exercise long-range effects given the fact that peripheral administration of certain cytokines such as IL-1β leads to an increase in PGE2 concentrations throughout the brain in regions such as the PVN of the hypothalamus, hippocampus, OVLT and also the medial preoptic area (MPOA).[191, 192] Intriguingly, there appears to be a link between prostaglandins and catecholaminergic/noradrenalinergic projections to the PVN. Recently it has been proposed that prostaglandins released from perivascular cells in the medulla as a result of systemic IL-1 stimulation activate the catecholaminergic/noradrenalinergic projection to the PVN through prostanoid receptors.[193] Such a proposal neatly couples together the mediatory roles of prostaglandins and catecholaminergic/noradrenalinergic projections to the PVN and suggests a mechanistic pathway from initial long-range cytokine effects to LHPA-axis stimulation.

Nitric oxide (NO), another diffusible agent of the CNS, has been suggested to act as an alternative mediator of cytokine effects on the LHPA axis. Certainly, local nitric oxide synthase (NOS) expression in the PVN of the hypothalamus is known to be upregulated by either intracerebroventricular injection of IL-1β or systemic administration of lipopolysaccharide (LPS) leading to increases in CRH secretion.[194] Furthermore, CRH-releasing neurons in the hypothalamus will express NOS and there is evidence of local NO activity in the hypothalamus as well.[139] However, in vitro studies using NO donors and inhibitors to study effects on CRH secretion have given far more ambiguous results. Both stimulatory and inhibitory effects of NOS inhibitors have been demonstrated in hypothalamic explants.[139] Therefore, the role of NO in mediating stimulatory cytokine effects upon the LHPA axis remains essentially unproven, in contrast to the roles of prostaglandins and catecholaminergic/noradrenalinergic projections to the PVN of the hypothalamus.

Alternative interactions with the LHPA axis

Lower down the LHPA axis, IL-1 appears to be able to alter the release state of anterior pituitary hormones (Figure 3). At the peripheral end of the LHPA axis, cytokines may also stimulate intramedulary CRH secretion in the adrenal glands. Cytokines such as IL-1 and IL-6 have been found in the adrenocortex and adrenomedulla, whilst cells in the adrenal glands are equipped with specific receptors for cytokines including IL-2 and IL-6.[195–198] Furthermore, in vitro and in vivo studies with adrenal cell and slice preparations or with isolated adrenal glands have clearly demonstrated that IL-1, IL-2 and IL-6 can directly cause an increase in GC secretion mediated by prostaglandin and/or catecholamine/noradrenalinergic-related pathways.[199–204] The similarity between CNS and peripheral stimulation of stress hormone secretion in response to cytokine production is certainly striking and is a key hallmark of neuroimmunology.

Model of LHPA-Axis-Mediated Neurodegeneration

Thus far, we have established the connections between stress stimuli and LHPA-axis dysfunction, as well as the capacity of stress and other stimuli to induce cytokine production, which leads to further stimulatory pressure upon the LHPA axis and potentially contributes to further dysfunction. The initial result is poorly controlled secretion of stress hormones, which leads to hypersecretion. Previously, we noted that key stress hormones such as CRH and GCs may have toxic effects when produced in excess. Those toxic effects are discussed in detail in this section of the review. Mechanistically, GCs could promote hippocampal atrophy by enhancing the accumulation of excitatory amino acids (EAAs) found in extracellular spaces.[10, 205–207] One of the most devastating EAAs in this respect is glutamate. Two main mechanisms have been proposed to account for GC-mediated increases in extracellular glutamate. The first mechanism is based upon direct GC-mediated anoxic depolarisation associated with excess Ca^{2+} ion influx into cells.[208] The second process revolves around GC-mediated inhibition of glutamate uptake into glial cells.

Inhibition of glutamate uptake is well known as a major source of glutamate accumulation in the hippocampus under stress conditions,[209] and GCs have now been established to inhibit the uptake of glutamate into hippocampal neurons and glial cells such as astrocytes by inhibiting glucose uptake and utilisation by these cells.[210–212] The implications of high extracellular glutamate levels are serious (Figure 4). Recent experiments with the hippocampal slice model have shown that increased glutamate release conditions can lead to a dramatic increase in the *N*-methyl-D-aspartate (NMDA)-receptor-mediated component of glutaminergic exitatory synaptic transmission.[213–215] According to the glutamate volume transmission hypothesis, there is a large pool of extrasynaptic NMDA receptors, inactive under normal conditions, but propelled into activity by excessive glutamate release owing to the higher affinity that NMDA receptors have for glutamate in comparison with less pathogenic α-amino-3-hydroxy-5-methyl-4-isoxazole propanoic acid (AMPA) receptors. Activation of such extrasynaptic NMDA receptors leads to substantial increases in NMDA-receptor-mediated Ca^{2+} ion influx into hippocampal neurons culminating in neuronal damage.

There is evidence that GCs may induce upregulation of the NMDA receptor system as well as inhibit glutamate uptake. For instance, administration of excess corticosterone to rats has been found to cause an increase in the levels of mRNA from several NMDA receptor subunit subtypes, namely NR2A and NR2B but not NR1 subtypes, in all regions of the hippocampus.[216] Furthermore, there is evidence that GC-induced changes in γ-aminobutyric acid (GABA) receptor subunit composition may also impair the regulatory GABAergic system that might otherwise modulate glutamate excitation and minimise the possibilities for neuronal damage.[217] Once neuronal damage exists, this damage may further induce cytokine-mediated inflammatory effects causing even more substantial neurodegenerative problems.[218] This possibility that GC-mediated neuronal damage could provoke subsequent cytokine activity establishes a key catastrophe scenario. If GC secretion induces neuronal damage that stimulates cytokine production, then further cytokine-mediated stimulation of the LHPA axis (as described above) could establish a positive feedback loop in which further GC secretion is induced, which leads to yet more neuronal damage and increased cytokine production. Such interplay between the effects of stress stimuli and immune/inflammatory system responses could be critical in establishing an environment in the CNS appropriate for sustained neurological damage with severe pathological consequences, as is discussed below.

This very catastrophe scenario could be stoked by alternative mechanisms of GC-mediated toxicity. For instance, the basal levels of antioxidant enzymes such as Cu^{+}/Zn^{2+} superoxide dismutase (Cu/Zn SOD) and glutathione peroxidase (GSPx) have been found to be significantly lower in all brain regions of GC-treated rats, which leads to an increase in peroxide levels and increased possibilities for oxidative damage.[219] GCs also prevent catalase induction and suppress GSPx in the presence of excitotoxins such as EAAs in the hippocampus. Therefore, GCs appear to actively increase the vulnerability of neurons to insult from trauma, metabolism and other stressors. Such a situation must surely enhance the opportunities for GC-mediated neuronal

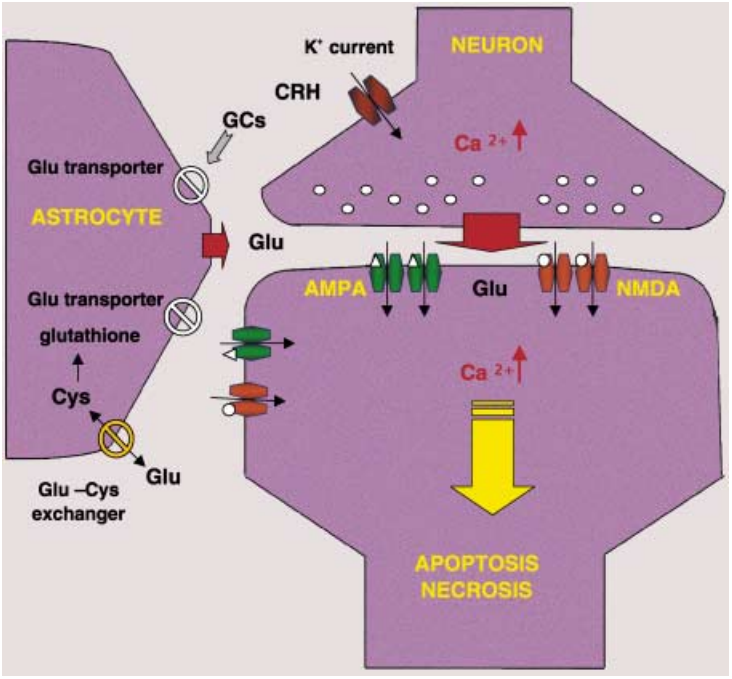

Figure 4. *The process of glutamate toxicity. Hyperactivation of high-affinity NMDA glutamate receptors, as opposed to lower affinity AMPA glutamate receptors, is perceived to be the primary consequence of GC inhibition of glutamate uptake by glial cells. Hyperactivation leads to substantial increases in NMDA-receptor-mediated Ca^{2+} ion influx into hippocampal neurons, culminating in neuronal damage. Abbreviations used are as follows: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; NMDA, N-methyl-D-aspartate.*

damage and hence the key catastrophe scenario described above.

In a similar vein to that described for GCs, CRH is also associated with neurotoxicity when in excess although the reverse appears to be true when levels are more modest. CRH has been demonstrated to have neuroprotective effects under some circumstances. For instance, primary neuronal cultures expressing CRHR1 were apparently protected from oxidative cell death by administration of CRH.[220] This protective effect of CRH was apparently blocked by selective and nonselective CRHR1 antagonists and by AMP-dependent protein kinase inhibitors. Other in vitro studies have demonstrated that CRH modulates glutamatergic synaptic transmission in hippocampal circuits. Accordingly, CRH may influence normal neuronal functions (memory and learning) by this mechanism, but should CRH levels become significantly elevated because of LHPA-axis activation, then CRH may instead promote abnormal EAA excitation and neurotoxicity,[221] effects comparable with the GC-mediated effects described above (Figure 4).

CRH may also have properties comparable with EAAs as excitatory neurotransmitters with potentially toxic characteristics when released in excess.[222] CRH-like immunoreactivities and high-affinity CRHRs are widely distributed within the CNS in extrahypothalamic locations (Figure 5).[223–227] Therefore, stress-related responses may be mediated by CRH at extrahypothalamic sites significantly beyond the boundaries of the classical

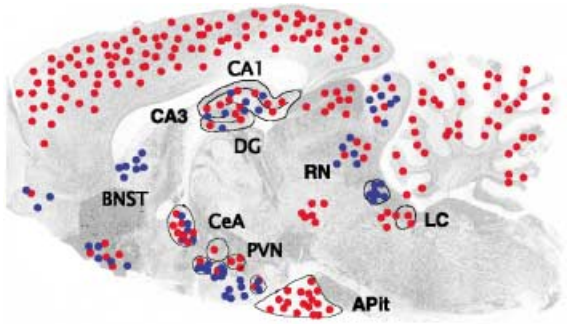

Figure 5. *Distribution of CRH receptors in various brain regions. CRHR1 are represented by red dots, CRHR2 by blue dots.*

LHPA axis.[228–230] Excess CRH has been shown to induce neuronal loss in several limbic structures, including the CA3 region of the hippocampus. After CRH-induced epilepticus in infant rats, electron microscopic analysis revealed the presence of CA3 pyramidal cells in various states of degeneration with intact cell membranes but dense nuclei and cytoplasm. The shrunken dendrites of these cells had spines and these were found to be associated with large mossy fibre afferents of an immature appearance.[231] Dense CRH-immunoreactive fibres have also been seen in close proximity to degenerating neurons. Frequently, areas undergoing neurodegeneration have been shown to exhibit substantial amounts of CRH that is not present under normal conditions.[222] In addition to all these observations, induction of CRH mRNA in endangered brain regions following necrotic insults has also been observed and this process appears to have toxic consequences. Accordingly, CRH antagonists have been found to have considerable neuroprotective effects against ischaemic or excitotoxic damage to neurons. For instance, antagonist astressin (**5**) has been shown to have considerable neuroprotective potential against kainic-acid-induced excitotoxic seizures. Intracerebroventricular infusion of **5** before and after seizures decreased damage in some hippocampal cell fields by as much as 84% and was effective even if administered only 10 minutes after excitotoxin exposure.[232]

LHPA-Axis Dysfunction and Disease

The toxic properties of stress hormones exacerbated by responses of the immune/inflammatory system are now becoming clear. Therefore, we should be in a position to begin to consider the impact of stress hormone hypersecretion, when operating in concert with the immune/inflammatory system, on major disease states and their pathologies. The previous section suggests a key hypothetical principle, namely that LHPA-axis dysfunction created by the combined effects of excessive stress stimuli with concurrent and subsequent immune/inflammatory system responses to these and other stimuli, creates an environment suitable for the initiation and propagation of disease states and their pathologies. This principle is explored in this final section.

Mild but persistent LHPA-axis hyperactivity exhibited in rats with hypothalamic lesions has been shown to have a number of clear pathological and disease traits, which include obesity, hyperinsulinemia, hyperglycemia, hypertension and tachycardia.[233] More pronounced LHPA dysfunction associated with persistently high levels of cortisol in humans (a condition known as Cushing's Syndrome) is now known to diminish the size of the hippocampus, a brain region essential to memory function, which leads to disruption of memory and verbal recall as well as having other affects on mood and behaviour.[234, 235] In animals, pronounced LHPA-axis dysfunction is known to produce chronic hippocampal inflammation (astroglial) and result in cell death, thereby reducing brain volume.[234] Evidence such as this points firmly to LHPA-axis dysfunction aligned with immune/inflammatory system responses as a major risk factor if not the trigger for an impressive variety of disease and dependency states as diverse as, say, arthritis,[236] bulimia,[237] bipolar affective disorder,[238] post traumatic stress disorder (PTSD) [239] and drug abuse.[240]

Depression and anxiety

A link from excessive stress stimuli through to LHPA-axis dysfunction, immune/inflammatory system responses and depression is yet to be properly established. Nevertheless, there is little doubt that LHPA-axis dysfunction is closely associated with depression. Abnormalities in the LHPA axis are the most consistently demonstrated biological markers of depressive illness, which suggests that LHPA-axis overdrive is a key aetiological feature of depression.[241] The classic test for depression is the DST (see above). A decreased responsiveness

to dexamethasone (**12**) within the context of the DST owing to a weakening of the negative feedback loop has been repeatedly found in nearly 50% of individuals with depression.[125, 242] Furthermore, atrophies of the hippocampus, frontal cortex, cerebellum and striatum are commonly found in major depression, findings that closely parallel the pathophysiological effects of Cushing's Syndrome, which typifies the consequences of LHPA-axis dysfunction and hypersecretion of cortisol (**3**). Another characteristic of major depression is immunosuppression of cellular immunity, in particular a blunting of natural killer (NK) cell activities compared with those in healthy individuals.[243] Such an effect is typical of cortisol (**3**) hypersecretion, as described below in the section on immunity and infection, but may be linked with CRH hypersecretion as well.[244]

Activation of the LHPA axis and the presence of brain atrophies are also consistent with the results of studies suggesting that both IL-1β and IL-6 levels are elevated in depressed patients.[245, 246] We have previously described how stress stimuli may induce cytokine production as well as the capacity of such cytokines to contribute towards hyperactive LHPA functioning of the type associated with depression. Conceivably, increases in IL-1β and IL-6 levels in response to stress stimuli could be a trigger for major depression; however, increases in cytokine levels could equally well be the result of inflammatory responses to neurological damage, as mentioned previously.[247] Similarly elevated cytokine production has also been observed in psychotic disorders, which include schizophrenia and manic depression.[247–249] Once again, whilst increases in cytokine production could be a trigger for psychotic illness through LHPA-axis dysfunction, they could equally well be the result of inflammatory responses to neurological damage. At this stage, there is insufficient evidence to separate cause from effect.

By contrast, stronger evidence is available to suggest that LHPA-axis dysfunction may act as a direct trigger for major depression.[250] For instance, direct administration of CRH to the CNS has been found to produce a number of behavioral and physiological effects reminiscent of both an organism's response to stress and of the symptoms of patients with major depression. These include diminished food consumption, decreased sexual behavior, disturbed sleep, alterations in locomotor activity and sympathetic nervous system activation.[251] Hypersecretion of CRH also takes place from hypothalamic and extrahypothalamic neurons in patients with major depression, such that the concentration of CRH in the CSF is significantly elevated compared with normal levels.[251–253] Furthermore, postmortem brain tissue from depressed patients shows a marked increase in the number of hypothalamic PVN neurons that express CRH. Abnormalities in CRH-mediated limbic neurotransmission may also contribute towards depression.[254]

A number of depressive states appear to be associated with LHPA-axis hypo- rather than hyperactivity. For instance, sufferers of post traumatic stress disorder (PTSD) and childhood and/or adolescent sexual abuse (CSA) are known to show an enhanced response to the dexamethasone suppression test, consistent with very low basal secretion of cortisol (**3**) and a hypersensitive negative feedback loop, perhaps owing to increased GR sensitivity.[255–259] Such a pattern of LHPA-axis dysfunction may well be characteristic of psychiatric disorders that occur following a range of sustained heavily traumatic experiences. Patients are typically hyper-reactive and hyper-responsive probably as a consequence of LHPA-axis hypersensitivity. Clearly, this is completely different from the situations described above for major depressive disorders.

Links from excessive stress stimuli to anxiety states through LHPA-axis dysfunction appear to be more straightforward than for depression. For instance LHPA-axis hypersecretion resulting from prenatal stress has been found to cause attention deficits, hyperanxiety and disturbed social behaviour in human infants and experimental animals.[260] In addition, the development and expression of anxiety now appears to be mediated by either MRs[261] and/or GRs[262] in the hippocampus. Studies with the nonpeptide CRH antagonist antalarmin (**7**; CP-154526) have also suggested a role of the LHPA axis in the development of anxiety states. In these studies, antalarmin (**7**) was found to cause a significant reversal of escape deficit in the rat "learned helplessness procedure", a result consistent with the role of CRH-receptor events in mediating anxiety.[263] Other studies with antalarmin have also revealed that CRH in addition mediates the induction and expression of fear responses.[229]

Obesity, metabolic syndrome and growth

Abnormalities in the functioning of the LHPA axis are consistently being demonstrated in obesity and growth problems. Moreover, excessive stress stimuli leading to LHPA-axis dysfunction appear to be fundamental triggers for these conditions. Dietary fat is now known to be a background form of chronic stress that provokes LHPA-axis hyperactivity.[264] Once initiated, LHPA-axis hyperactivity (maintained by a collapse in GC-mediated negative regulation) appears to be intimately involved in the development of abdominal obesity, and is accompanied by diminished secretion of sex steroids and growth hormones thereby affecting growth rates.[265–267] The appearance of hyperandrogenicity in obese women is probably of adrenal origin and another consequence of LHPA-axis hyperactivity. Elevated cortisol, and low sex steroid and growth hormone secretions are likely to be jointly responsible for the direction of fat storage to visceral depots and contribute towards the high circulating levels of free fatty acids linked to insulin resistance (Type I diabetes), non-insulin-dependent diabetes (Type II diabetes) and cardiovascular problems.[266, 267] Normalisation of the LHPA axis appears to lead to clear improvements in the condition of obesity,[266] which underlines the causal link from stress stimuli to obesity through LHPA-axis dysfunction.

Particularly intriguing risk factors for abdominal obesity and metabolic syndrome are stressors such as self-induced traumas created by socioeconomic, psychosocial handicaps or anxious/defeatist personality conditions.[265, 267, 268] The importance of these stressors has been shown by studies with monkeys subjected to mild psychosocial stress. These monkeys appeared to show psychological, anthropometric, endocrine and metabolic abnormalities similar to those found in humans with abdominal obesity, which includes signs of diabetes and

cardiovascular disease.[265] The link from stress stimuli to cardiovascular disease has also been established in studies with the spontaneously hypertensive rat.[269] Prenatal treatment of rats with dexamethasone (**12**) has been found to generate hypertensive offspring with low birth weights, further characterised by elevated basal levels of corticosterone and a disrupted negative feedback loop, associated with permanently attenuated MR and GR mRNA levels in the hippocampus.[128] Such experiments suggest that excessive GC secretion generated by intensive, acute bouts of stress stimulation can be sufficient to permanently effect the long-term cardiovascular condition of offspring as well as adults.

Immunity and infection

Excessive stress stimulation is well known to bring about GC-mediated immunosuppression.[270] However, these immunosuppressive effects are complex. Recent data indicates that GCs secreted in response to acute, sub-acute and chronic stress might actually suppress $CD8^+$ cellular immunological responses but boost $CD4^+$ humoral-antibody immune responses.[271, 272] Furthermore, GCs appear to affect the balance of the $CD4^+$ T-cell subsets Th1/Th2 in favour of Th2 cells.[273] Th1 cells produce cytokines associated with cell-mediated immune responses against intracellular pathogens and induce organ-specific autoimmune diseases. Th2 cells produce cytokines that are associated with atopic and allergic conditions.[274] Such selective inhibitions may account for the way in which LHPA hypersecretion induced by stress stimuli can decrease resistance to viral and bacterial infections,[275, 276] whilst increasing certain allergic or autoimmune/inflammatory responses (see below).[273]

Although GCs are the dominant agents of long-term immunomodulation, they are not the only agents. Links are now emerging between the peptide messengers of the LHPA axis, some neurotransmitters and immune cells.[271, 272] Such neuroendocrine modulators are now understood to be able to attain access to cells of the immune system either through the peripheral circulation or through direct innervation of lymphoid organs. Indeed, postganglionic sympathetic as well as peripheral sensory afferent nerve fibres innervate primary and secondary lymphoid organs. Once in the periphery, a wide range of interactions is possible, with consequences yet to be fully characterised. Both lymphocytes and monocytes express receptors for CRH, ACTH, cortisol (**3**), noradrenaline and adrenaline.[141, 273] CRH has been found to inhibit IFNα release from Th1 cells and INFγ from monocytes, which is consistent with suppression of Th1 responses[271, 277] and similarly consistent with the immunosuppressive effect of GCs that shift the $CD4^+$ Th1/Th2 response balance in favour of Th2 cell responses, as described above. In addition, CRH has been found to stimulate the activation and degranulation of mast cells by CRHR1, which leads to the release of histamine followed by a marked increase in vasodilatation and vascular permeability. Accordingly, excessive stress, including psychological stress that leads to LHPA-axis dysfunction, could have the potential to exacerbate or even trigger allergic or autoimmune phenomena such as eczema, uticaria, atopic dermatitis, psoriasis, and asthma.[141, 271, 272, 278]

There remains the question of the reverse impact of infection and immune reactions upon the LHPA axis. Ever since the discovery that gastric ulcers can be caused by *Helicobacter pylori* infection of the gut, evidence has been accumulating to suggest that other pathogens may play a role, even a causal one, in chronic diseases like Alzheimer's disease (AD). The particulars of AD are addressed in the next section, however evidence has been advanced to suggest that *Chlamydia pneumoniae* infection of brain tissue may be associated with AD; another infectious agent implicated is human herpes virus 6 (HHV-6).[279] Such infections would naturally result in CNS production of cytokines that could also contribute to LHPA-axis stimulation alongside stressor-induced LHPA-axis stimulation. In the light of much of the foregoing discussion, the combined effect of CNS infection-induced cytokine production and concurrent stress stimuli could be yet another means to promote LHPA-axis overdrive sufficient to establish an environment in the CNS appropriate for the creation of sustained neurological damage of the type seen in AD. This theme is taken up further below.

Inflammation

GCs act as potent inhibitors of inflammatory responses by acting in part to suppress the activity of activator protein 1 (AP-1) and the transcription factor NF-κB.[280] Therefore, GCs can be described as antiinflammatory as well as immunosuppressive. GC-mediated antiinflammatory effects are an important part of complex, regulatory negative feedback loops that regulate cytokine production and place a natural brake on inflammatory processes.[139, 281, 282] In the process, GCs suppress levels of pro-inflammatory cytokines (such as IL-1, IL-6 and TNFα), whilst levels of antiinflammatory cytokines (such as IL-4 and IL-10) are upregulated along with their cognate receptors.[270, 283, 284] However, as described previously, overproduction of GCs in response to excessive stress stimuli has the capacity to cause excitotoxic neurodegeneration that in turn may provoke cytokine release and further LHPA-axis stimulation in response. A positive feedback loop would then be established that would create a sustained inflammatory environment in the CNS that could contribute towards the pathogenesis of a number of well-known neurological conditions such as AD (see below), Parkinson disease, ischaemia and psychiatric disorders such as depression and schizophrenia.[172, 247–249, 285–288] Cognitive, mood and memory dysfunction have been clearly shown to result from pro-inflammatory cytokine overproduction in the brain as well.[249, 289, 290] The situation with GCs could easily be compounded by CRH, since CRH induces the production of pro-inflammatory cytokines such as IL-1 in the CNS as well.[166] Furthermore, CRH can stimulate the proliferation of peripheral B- and T-lymphocytes as well as IL-2 receptor expression,[291, 292] not to mention the release of IL-1 and IL-2 from mononuclear cells,[293] leading to a variety of additional pro-inflammatory responses in the periphery. Strikingly, rheumatoid arthritis, osteoarthritis, thyroiditis and ulcerative colitis patients have CRH present in their inflamed tissues, which is consistent with a role for stress stimuli and stress hormone activities in the

development of these autoimmune/inflammatory conditions as well.[141, 294, 295]

Cytokine stimulation is well known to lead to overproduction of amyloid (Aβ), a direct mediator of neurodegeneration and pathogenesis in AD.[59–61, 296–303] In addition, abnormalities of CRH-mediated limbic neurotransmission may contribute to AD pathology.[15, 304] Therefore, we might easily envisage a scenario in which LHPA-axis dysfunction could "light the blue touch paper" of AD pathophysiology. Once overproduction of GCs and CRH is established as a result of the combined effects of excessive stress stimuli and concurrent cytokine stimulation acting on the LHPA axis, substantial GC/CRH-induced neurological damage and further CRH-mediated pro-inflammatory effects would be possible culminating in a sustained state of cytokine release in the CNS sufficient to provoke Aβ overproduction and further LHPA-axis overdrive. Consistent with this scenario, at least 50% of AD patients show decreased responsiveness to dexamethasone (**12**) within the context of the DST, which suggests a weakening of the negative feedback loop and LHPA-axis dysfunction.[242, 305] Furthermore, mice overexpressing a mutant protein precursor of Aβ exhibit classic AD symptoms in hippocampal and cortical brain regions and are reported to have a dysfunctional LHPA axis with high levels of CRH in brain regions prone to degeneration in AD.[220] Given this observation, might not the most appropriate way to combat AD be a duel approach combining an antiinflammatory strategy with efforts to modulate LHPA-axis dysfunction?

Conclusion

There should now be no doubt that stress acting through the LHPA axis and its bidirectional communication pathways with the immune/inflammatory system can be pathogenic. Feedback mechanisms usually ensure that the LHPA axis and immune/inflammatory system are carefully regulated to avoid catastrophe, but all too often these defences appear to be breached, which leads to LHPA-axis dysfunction and toxic side effects resulting from the synergism of stress hormone and cytokine actions. Given the weight of evidence in favour of a central role for stress in degeneration and disease, it is likely that an ever more detailed appreciation of the mechanisms involved could lead to a more holistic appreciation of the origins of disease. Naturally, this understanding could also lead to the development of strategic therapeutic treatments with applicability to a wide range of chronic and degenerative diseases. In the meantime, such diseases will continue to blight ever more lives as global populations become ever more aged, but perhaps we should all appreciate that prevention may be better than cure; a reduction of stress in the world would surely be the best treatment of all and certainly the most cost effective!

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