MINIREVIEW

Unraveling the Molecular Details Involved in the Intimate Link between the Immune and Neuroendocrine Systems

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During systemic infections, the immune system can signal the brain and act on different neuronal circuits *via* soluble molecules, such as proinflammatory cytokines, that act on the cells forming the blood-brain barrier and the circumventricular organs. These activated cells release prostaglandin of the E_2 type (PGE_2), which is the endogenous ligand that triggers the pathways involved in the control of autonomic functions necessary to restore homeostasis and provide inhibitory feedback to innate immunity. Among these neurophysiological functions, activation of the circuits that control the plasma release of glucocorticoids is probably the most critical to the survival of the host in the presence of pathogens. This review revisits this issue and describes in depth the molecular details (including the emerging role of Toll-like receptors during inflammation) underlying the influence of circulating inflammatory molecules on the cerebral tissue, focusing on their contribution in the synthesis and action PGE_2 in the brain. We also provide an innovative view supporting the concept of "fast and delayed response" involving the same ligands but different groups of cells, signal transduction pathways, and target genes.
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The recent spark of interest in research concerning the molecular links among the nervous, endocrine, and immune systems has caused an explosion of new knowledge concerning the fine mechanisms that orchestrate the integrated response to an immune challenge. This response encompasses not only the cross talk between macrosystems, but the complex and precise interaction of molecular processes where, for example, an inflammatory ligand can bind to and signal in cells of the central nervous system (CNS) (1). The past 5 years of research have clarified some of the intricacies of how these signaling events lead to key neurophysiological functions that are necessary to reestablish homeostasis after the clearance of an immune threat. New players have been discovered, and some controversies still remain about the molecular details involved in the bilateral talk between the immune and the nervous systems. This review discusses some of the recent discoveries that have shed light on the mechanisms that mediate autonomic responses during immune stimuli and revisits some unresolved issues that still remain to be clarified.

Toll-Like Receptors and Proinflammatory Signaling to Pathogens

The immune response that follows systemic infections involves a large and varied cellular and molecular cast (Fig.1). At the forefront of this response are the dendritic cells and macrophages, cells of myeloid origin that process antigens and become the primary antigen-presenting cells

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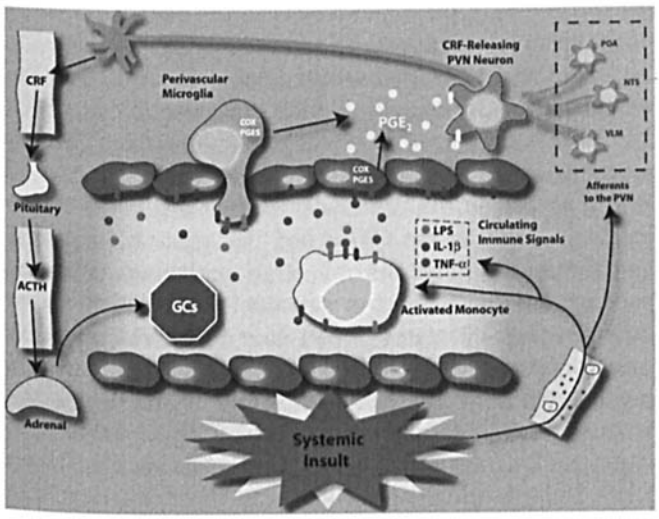

Figure 1. Systemic immune reaction and the hypothalamic–pituitary-adrenal (HPA) axis. When an immune threat (in this case, lipopolysaccharide, [LPS]) is present in the body, activated monocytes and the proinflammatory cytokines (e.g., IL-1β, TNF-α) they release travel through the blood, along with LPS, and interact with the blood-brain barrier (BBB). The endothelial cells and the associated perivascular microglia synthesize prostaglandin E_2 (PGE_2) using cyclooxygeneases (COX) and prostaglandin E synthases (PGES). The PGE_2 is released in the parenchyma and acts on the corticotropin-releasing factor (CRF)-secreting neurons of the paraventricular nucleus of the hypothalamus (PVN). The CRF enters the circulation and reaches the adenohypophysis part of the pituitary gland and causes the release of adrenocorticotropic hormone (ACTH) into the systemic circulation. ACTH then reaches the adrenal gland and triggers the synthesis and release of glucocorticoids (GCs), which may act as negative feedback on the inflammatory response. A similar response takes place at distal sites that project to the PVN (preoptic area [POA], nucleus of the solitary tract [NTS], ventrolateral nucleus of the medulla [VLM]). Activation of these circuits results in an integrated response to immune stimuli, which are directly under the control of the final product of this neuroendocrine axis, GCs.

(APCs) of the body. This first line of defense is called the innate immune response and is characterized by the interaction of specific molecular patterns found in microbes with their cognate receptors expressed on the surface of APCs. These patterns can be found in lipopolysaccharide (LPS), present in the cell wall of gram-negative bacteria, and in many other structural components of pathogens such as gram-positive bacteria, fungi, viruses, and other microorganisms. These molecules are denominated pathogen-associated molecular patterns (PAMPs), and their respective receptors are known as pattern recognition receptors (PRRs) (2, 3). Pattern recognition receptors can be divided into three functional classes: signaling receptors, endocytic receptors, and secreted proteins (4, 5). The release of inflammatory mediators by myeloid cells in response to PAMPs requires sequential signaling steps. The details of these events have been described recently with the characterization of Toll-like receptors (TLRs), which are prototype signaling PRRs.

Eleven different TLRs have been described to date (6). Peptidoglycan (PGN) and lipoteichoic acid (LTA) from gram-positive bacteria are recognized by TLR2 conjugated to TLR6 or TLR1, double-stranded RNA (dsRNA) viruses are recognized by TLR3, LPS from gram-negative bacteria binds to TLR4, flagellin to TLR5, and CpG bacterial and viral DNA triggers signaling via TLR9 (5, 7, 8). Single-stranded RNAs (ssRNAs) from viruses (e.g., HIV-1 and influenza) are now believed to be the physiological ligands for TLR7 and TLR8 (9, 10), whereas TLR11 is activated by uropathogenic bacteria (11). It is also believed that TLR1, 2, 4, 5, 6, and 11 recognize extracellular PAMPs, whereas TLR3, 7, 8, and 9 are sensors for intracellular PAMPs (e.g., CpG DNA, ssRNA, and dsRNA).

The endotoxin LPS is the most frequently used and most powerful stimulus to trigger the signaling pathways involved in innate immunity. Its recognition involves the binding of LPS to membrane CD14, which transfers LPS to TLR4 in an MD-2–dependent manner (12). The involvement of TLR4 in LPS recognition was substantially accepted because of the identification of a missense mutation in the *lps* locus corresponding to TLR4 in the C3H/HeJ mouse strain, which is largely refractory to LPS (13). Furthermore, TLR4-deficient mice are unresponsive to LPS but not to cell wall components derived from gram-positive bacteria (TLR2) (14). The activation of TLR4 and all other TLRs initiates sequential intracellular signal steps similar to those of IL-1 receptor (IL-1R) because of the conserved domains Toll/IL-1R (TIR). The myeloid differentiation factor 88 (MyD88) is immediately recruited to the TIR domain after that the agonist binds to the receptor, resulting in the activation of serine/threonine kinases from the IL-1R–associated kinase (IRAK) family. This is followed by the recruitment of the adaptor molecule TNF-associated factor 6 (TRAF6) and activation of IκB kinase (IKK) complex, a critical step for the release of nuclear factor NF-κB from its cytoplasmic inhibitor IκB (15). IκB kinase is composed of two catalytic subunits (IKKα and IKKβ) and one regulatory subunit (IKKγ/NEMO). IκB kinase β is the downstream target of signals generated by proinflammatory stimuli that give rise to phosphorylation of IκBα (the predominant and rapidly regulated IκB isoform) at Ser32/36 and proteosomal degradation after polyubiquitination. IκBα itself is a NF-κB target gene, and its *de novo* synthesis is a crucial autoregulatory loop (16).

The recruitment of the MyD88/IRAK/TRAF6 complex can also activate mitogen-activated protein kinase (MAPK) kinases (MKKs) such as the Jun kinase pathway (JNK) that leads to the formation of activator protein-1 (AP-1) complex (17). Nuclear translocation of NF-κB and AP-1 engages the transcription of genes encoding most innate immune proteins, namely cytokines (e.g., IL-1, TNF-α, IL-6), chemokines (e.g., MCP-1, IL-8, RANTES), proteins of the complement system (e.g., C3, C3aR, C5aR, factor B), enzymes (e.g., COX-2, iNOS), adhesion molecules (e.g., ICAM-1,VCAM-1, P/E-selectin), and immune response receptors (e.g., CD14, TLR2, IL-6R). A timely controlled production of these proteins is essential for a proper innate

immune response and the elimination of pathogens. Proinflammatory cytokines (e.g., IL-1β and TNF-α) act as amplifiers of this innate immune response to further increase cell transmigration and phagocytic properties of APCs. A frequently asked question is how such a positive autoregulatory loop is controlled and whether it can lead to detrimental consequences if not properly regulated. There are many factors that play a role in the inhibitory feedback on such a system, but activation of the hypothalamic-pituitary-adrenal (HPA) axis and the release of glucocorticoids (GCs) by the adrenal gland are by far the most powerful endogenous mechanisms to suppress TLR signaling and gene transcription. The details of this neuroendocrine response to innate immunity are described later.

Because of their lipophilic nature, GCs readily cross the membrane system of the cell and bind to mineralocorticoid (MR or type I) or GC (GR or type II) receptors localized in the cytoplasm. Basal levels of GCs activate only MRs that have higher affinity than GCs, whereas inflammatory and stress-related levels activate both receptors. GR and MR in their inactive form are complexed to heat-shock proteins (hsps). The binding of GCs to their intracellular receptors causes the release of the complex, which is formed from a combination of hsp90 and p23, p60, hsp40, or hsp70. This protein complex maintains the GR or MR in the inactive state and also helps the high-affinity binding of the ligands. Following this step, the complex (steroid/receptor) moves to the nucleus and binds to specific DNA sequences called glucocorticoid response elements (GREs). Activated GRs are able to interfere at numerous levels of the proinflammatory signaling cascades, and many models have been proposed to explain the profound anti-inflammatory action of GCs: (i) repression by a direct action of activated GRs modulating transcription *via* GRE sites; (ii) induction of GRE-responsive genes that inhibit transcription factor signaling (e.g., IκBα); (iii) protein-protein interactions, either by inhibition of transcription factor DNA binding or by association with the factor bound to its DNA site; (iv) competition of GRs with NF-κB and AP-1 for nuclear coactivators such as CREB-binding protein and p300 (CBP/p300); and (v) GR effects at the post-transcriptional level, such as mRNA destabilization of proinflammatory genes. There is no consensus about the mechanisms that play leading roles in suppressing proinflammatory genes, which underlines the complexity of this system (18).

How PAMPs and Circulating Soluble Immune Molecules Signal to the CNS

Circulating pathogens and blood-derived cytokines are unlikely to diffuse across barriers of the CNS in concentrations high enough to activate distant cells, so they have to activate cells that can be reachable from the bloodstream. As a result, three possible sites are the likely direct targets of the systemic immune system and their aggressors: (i) the circumventricular organs (CVOs) and other leaky structures; (ii) cells forming the blood-brain barrier (BBB); and (iii) a direct nerve connection.

***Via* the CVOs and Other Leaky Structures.** The sensorial CVOs contain a rich vascular plexus with specialized arrangements of the blood vessels. The tight junctions normally present between the endothelial cells are shifted in part to the ventricular surface and partly to the boundary between the CVOs and the adjacent structures, explaining the diffusion of large molecules into the perivascular region. Such a structural organization makes them privileged target sites not only for proinflammatory cytokines but also for infectious agents that may have a rapid access to these regions, consisting of four organs: the vascular organ of the lamina terminalis (OVLT), subfornical organ (SFO), median eminence (ME), and area postrema (AP). The subcommissural organ is another CVO located in the posterior wall of the third ventricle that actually forms the dorsal roof of the entrance to the aqueduct of Sylvius. In contrast to the other CVOs, there are no neuronal cell bodies in the subcommissural organ. The functions of this organ remain largely unknown, and it is not found in the human brain. The choroid plexus (chp) and leptomeninges are also recognized as being highly vascularized regions and are very sensitive to infectious agents in exhibiting a rapid transcriptional activation of different inflammatory molecules (19–27). These structures are also devoid of neurons, and they are not considered as being CVOs, but they produce inflammatory ligands that could diffuse throughout the CNS *via* the cerebrospinal fluid (CSF). In the OVLT, the external zone and the pia mater enveloping the microvessel loop extend to the core of the organ and spread out to the ependymal lining cells. Circulating inflammatory molecules may reach specific compartments through the fenestrated capillaries originating from the anterior communicating artery. On the other hand, blood-derived molecules may target specific population(s) of cells of the external lamina *via* the capillaries of the primary plexus that irrigates the basement membrane of the lower palisade layer of the ME. Both the SFO and AP display extensive networks of capillary loops, and the entire organs are exposed to hema milieu. These anatomical features and the fact that neurons innervating the regions that integrate the autonomic outputs are found in these CVOs support the concept that they may be key structures in mediating the cerebral responses to circulating immunogenic agents.

The constitutive expression of immune receptors (e.g., CD14, TLR2, TLR4, IL-1R1, TNFR1) together with a rapid induction of functional indices of cellular activity in response to systemic boluses of cytokines and LPS provide supporting evidence that CVOs act as a route of entry for numerous inflammatory agents that circulate in the bloodstream. The particular distribution of IL-6 mRNA in the CVOs may also offer some clues in understanding the role of this cytokine within the CNS (28). The increase of IL-6 synthesis in the OVLT might be a central mechanism participating in the thermogenic effects of the bacterial

endotoxin; the OVLT/MPOA is a recognized region involved in the appropriate control of thermoregulation (29, 30); IL-6 is a component of the fever response to LPS (31), and the firing rate of thermosensitive neurons of the preoptic area can be influenced by IL-6 (32). Neurons of the OVLT and SFO have direct projections to the paraventricular nucleus (PVN), and those of the AP connect with the nucleus of the NTS, which in turn sends catecholaminergic projections (A2/C2) to the hypothalamic PVN. The PVN is the key integrative nucleus for the autonomic functions and also contains the corticotropin-releasing factor (CRF) neurons that are responsible for the control of the ACTH release from the adenohypophysis. Catecholaminergic neurons that project from the NTS (and other areas receiving input from the CVOs) to the CRF-releasing neurons of the PVN have also been found to be responsive to IL-1β (33). Thus, the CVOs could be possible sites of action through which pathogens and cytokines may exert their actions on the HPA axis. In this regard, the AP and the adjacent NTS have been shown to play a pivotal role in transducing a circulating IL-1 signal into HPA axis activation by a pathway linking the AP and NTS to CRF neurons of the PVN (34). Moreover, it has been suggested that the neuroendocrine effects of cytokines may occur by increasing prostaglandin synthesis at the level of the ME (35). This issue is further developed in the section on cells of the BBB and cyclooxygenase (COX) pathways. Finally, the response wave of proinflammatory cytokine-expressing cells from the CVOs to deeper parenchymal regions may also be involved in these events, although this innate immune reaction takes place essentially within the population of microglial cells, and such a response may not participate in neuronal activity, at least as it relates to the autonomic circuits. We have reviewed these mechanisms elsewhere (26, 27, 36).

***Via* the BBB and Synthesis of Soluble Mediators.** Cells forming the BBB are in a privileged position to transfer the information from the circulation to the brain parenchyma, and there are exciting new developments regarding the molecular events taking place in the endothelium of the cerebral arterioles, small capillaries, and venules during systemic immune challenges. Cytokines, when secreted by cells of myeloid origin in the circulation, trigger a cascade of events leading to the proinflammatory signal transduction pathways in vascular-associated cells of the CNS. The brain blood vessels exhibit both constitutive and induced expression of receptors for different proinflammatory ligands that can stimulate mitogen-activated protein (MAP) and NF-κB kinases. Depending on the challenges and the cytokines involved, the transduction signal(s) solicited in cells of the BBB may elicit neuronal activity in a very specific manner by activating the transcription and production of soluble factors, such as prostaglandins (PGs). To be sure, during inflammation and CNS trauma, the BBB can be altered and compromised (37), which can further enhance the inflammatory response in the brain.

Biosynthesis of PGs. Prostaglandins are derivatives of arachidonic acid (AA), which, in response to various challenges, including proinflammatory cytokines and LPS, is translocated into the cell cytosol by several phospholipases A_2 (PLA_2). The most well-documented PLA_2 is the cytosolic PLA_2 ($cPLA_2$) that is present and modestly upregulated in the hypothalamus following LPS (38, 39). However, one of the secretory forms of the enzyme, $sPLA_2$-IIA, is also strongly upregulated by LPS, specifically in the brain (133-fold increase in the hypothalamus) compared to the increase in the peripheral organs that process LPS (up to a 9-fold increase in the liver) following its administration in rats (38, 39). The AA released by PLA_2 is converted by cyclooxygenases (COXs) in a two-step process. First, cyclooxygenase activity adds molecular oxygen to the unsaturated fatty acid AA, generating prostaglandin G_2 (PGG_2). PGG_2 is then converted to PGH_2 by the peroxidase activity of the enzyme. Once generated, PGH_2 is rapidly converted to prostaglandins (PGD_2, PGE_2, $PGF_{2\alpha}$), prostacylin (PGI_2), and thromboxane A_2 (TxA_2) by tissue-specific synthases. Prostaglandin E synthase (PGES) has recently been identified, and it catalyzes conversion of COX-derived PGH_2 to PGE_2 (40). Three distinct types of PGES have been characterized in mammals. Cytosolic PGES (cPGES), known as p23, is constitutively and ubiquitously expressed and predominantly converts COX-1-derived PGH_2 to PGE_2 (41). Microsomal PGES-1 (mPGES-1) is an inducible perinuclear enzyme that seems to be linked with COX-2. COX-2 and mPGES-1 are transcriptionally regulated by NF-κB, and both enzymes are essential for the delayed PGE_2 synthesis during inflammation (42). In this regard, mPGES-1 has been shown to play a crucial role in immune-induced pyresis and may be a direct target for the treatment of fever and other PGE_2-dependent acute-phase reactions elicited by the brain (43, 44). Finally, glutathione-nonspecific mPGES-2 is a unique PGES that can be coupled with both COXs and may elicit the production of the PGE_2 involved in both tissue homeostasis and disease (45). The diversity of the tissue-specific synthases and receptors gives rise to a wide range of potential biologic functions for the prostanoids. Prostaglandin G, PGH, PGI, and TxA are chemically unstable and are degraded into inactive products under physiological conditions, with a half-life of 30 seconds to a few minutes. Other PGs, although chemically stable, are metabolized quickly. It is therefore believed that prostanoids work locally, acting only in the vicinity of the site of production to serve as potent autocrine and paracrine mediators in a wide variety of physiological processes.

Circulating PAMPs and cytokines therefore have the ability to bind to their cognate receptors expressed on the surface of endothelial and/or monocytic cells lining the BBB, which would then lead to proinflammatory signaling and transcription of the enzymes responsible of the PGE_2 formation in the cerebral tissue. It is also possible that alternative pathways involving COX-1, cPGES, and mPGES-2 lead to an early rise in PGE_2 levels that would

explain the rapid increase in the activity of neurons involved in the control of autonomic functions (see below). It is interesting to note that systemic inflammatory insults induce COX-2 and mPGES in a rather nonspecific manner across the cerebral blood vessels and small capillaries, whereas the neuronal activity is limited to selective nuclei, including the endocrine hypothalamus. It is thus possible that expression of specific PGE_2 receptors within parenchymal cells adjacent to the site of production determines the action of the PG in the brain. Along with the alternative pathways, these receptors may also mediate the rapid and delayed responses to immune insults (see below).

PGE_2 Sites of Action. Classic prostanoid receptors comprise a family of eight encoding transmembrane G-protein–coupled receptors. These receptors are classified on the basis of selective affinities for naturally occurring prostanoids. There are distinct receptors for TxA_2, PGI_2, $PGF_{2\alpha}$, PGD_2 (namely, TP, IP, FP, and DP, respectively) and four different receptors for PGE_2 (EP_{1-4}). Multiple alternatively spliced isoforms exist for the PGE_2 EP_3 receptor ($EP_{3\alpha}$, $EP_{3\beta}$, $EP_{3\gamma}$). They share common extracellular and membrane-spanning regions but differ in intracellular and carboxy-terminal domains. Each receptor is associated with a unique G-protein and consequently a unique second messenger system, namely, elevation of intracellular Ca^{2+} (EP_1) and stimulation (EP_2, EP_4) or inhibition (EP_3) of adenylate cyclase. Despite the presence of some conserved sequences, overall homology among the prostanoid receptors is quite limited, ranging from 20%–30%. On the other hand, the homology of a given type or subtype of receptor among various species is considerably higher (46, 47). Each of the eight types and subtypes of receptors shows selective ligand binding specificity that distinguishes it from the others. In addition to transmembrane receptors, the peroxisome proliferator-activated receptor-γ (PPAR-γ) is a member of the nuclear receptor family of transcription factors that can be activated by binding to PGD derivatives, such as 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2).

In 1988, the distribution of [^{3}H]PGE_2-binding sites, presumably PGE_2 receptors, was first demonstrated in the monkey diencephalon (48), which was followed by more detailed analysis of [^{3}H]PGE_2-binding sites in rat brain (49, 50). PGE_2-binding sites were located in a number of discrete brain regions, including thalamic and hypothalamic nuclei, hippocampus, central gray, superior colliculus, parabrachial nucleus (PB), locus coeruleus (LC), raphe nuclei, spinal trigeminal nuclei, and nucleus of the solitary tract (NTS). *In situ* hybridization was thereafter used to determine the exact distribution of each PGE_2 receptor subtype. The hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei exhibited EP_1-expressing cells (51), but a wide distribution was found for the gene encoding the EP_3 subtype (52). We and others have reported a very distinct pattern of EP_2- and EP_4-expressing neurons throughout rat brain (53–55); EP_2 receptor mRNA was detected in the bed nucleus of the stria terminalis (BnST), lateral septum (LS), SFO, ventromedial hypothalamic nucleus (VMH), central nucleus of the amygdala (CeA), LC, and AP, whereas EP_4 receptor transcript was located mainly in regions involved in the control of neuroendocrine and autonomic activities. Moderate doses of LPS or IL-1β activate EP_4 neurons, and this activation is prevented when animals are pretreated with COX inhibitors (54).

All four PGE_2 EP receptor mRNAs are expressed in the anteromedial preoptic region that plays a crucial role in the febrile response (53–55). Among these, only EP_4 receptor mRNA is strongly expressed throughout the PGE_2-sensitive regions, including the OVLT, ventromedial preoptic nucleus (VmPO), and median preoptic nucleus (MnPO). These EP_4-expressing neurons are also activated by systemic inflammation, whereas EP_2- (53, 54) and EP_3-positive neurons (55) do not respond. EP_1 receptor mRNA is present in PGE_2-sensitive regions, but its expression level is weak. This led us to believe that EP_4 may be the key binding and functional receptor for PGE_2 in the brain to activate the circuits involved in the autonomic control.

In spite of this, pharmacological and genetic mutation experiments suggest otherwise. Oka and colleagues (56) used drugs with agonist and antagonist properties for each EP receptor in rats. Intracerebroventricular (icv) injection of 17-phenyl-ω-trinor-PGE_2 (an EP_1 and $EP_{3\alpha}$ receptor agonist), but not butaprost, M&B28767, or 11-deoxy-PGE_1 (EP_2, $EP_{3\alpha}$, and EP_4 receptor agonists, respectively), induced fever, and SC19220 (an EP_1 receptor antagonist) prevented the febrile response to PGE_2. In a later study, they found that icv injection of the EP_1 agonist ONO-DI-004 and the EP_3 agonist ONO-AE-248 both produced hyperthermia, whereas the EP_4 agonist ONO-AE1-329 caused a decrease in core body temperature (57). Another pharmacological study indicated that EP_2 or EP_3 receptor might be the receptor necessary to produce fever (58). In mice bearing genetic deletions of the EP_{1-4} receptors, only EP_3-knockout animals failed to show the early phase of fever (up to 1 hr) after LPS iv or PGE_2 icv (59). A later study demonstrated that EP_1-knockout mice had a blunted fever response to ip LPS, and EP_3-knockout mice became hypothermic in response to LPS (60). These studies suggest distinct roles for the receptors in mediating the central thermoregulatory response to PGE_2, but the role of the receptors in mediating the central neuroendocrine response have not been discussed. In this regard, a group has recently shown that both EP_1 and EP_3 are required for adrenocorticotropic hormone release in response to LPS and that these two receptors were colocalized with CRF-positive neurons in the PVN (61). This study was the first to show clear distribution of EP_1 in the PVN, NTS, and the CeA (where it was found to play a crucial role for the activation of the CeA in response to LPS). These data remain somewhat surprising but may be attributable to early and late/delayed responses of this endocrine axis to immune stimuli and the period in which the analysis was performed (see below). It is also

important to note that EP_4-deficient mice do not survive, and they have to be intercrossed in a different background. It is, therefore, quite difficult to compare them with the other gene-deficient mice, and conditional EP_4-knockout mice will be essential to clearly define the role of this receptor in the brain of adult animals. The relative lack of specificity of EP_4 antagonists and agonists in the species studied may also explain the pharmacological data. Finally, it is possible that the circuits unraveled by functional indices of neuronal activity participate in more subtle physiological responses, which were masked by the previous experimental designs.

The Fast and Delayed Response to Centrally Produced PGE_2

In order to activate PGE_2 production in the brain, inflammatory ligands (e.g., IL-1β, TNF-α, LPS) need to bind to their receptors on cells of the brain endothelium or perivascular microglia (62). This triggers a quick production of PGE_2 within the CNS parenchyma, and this response is essential for CRF production by the PVN (63). We suggest that the production of PGE_2 that is released in the brain occurs *via* two pathways (Figs. 2 and 3): (i) a fast transient production in endothelial cells and CVOs dependent on activation (possibly through MAPK p38 phosphorylation) of available constitutive PGE_2 synthesis enzymes ($cPLA_2$, COX-1, cPGES, mPGES-2), independent of transcription and NF-κB activation, and (ii) a slow, sustained PGE_2 synthesis in the endothelial cells and perivascular microglia (that are better equipped to respond to cytokines and pathogens) involving the robust induction of the principal enzymes involved in PGE_2 synthesis (especially $sPLA_2$-IIA, COX-2, and mPGES-1) that are NF-κB–mediated gene products. The slow transcription-mediated response would be massive, but could offer a way through which GCs would then exert their negative feedback on NF-κB activity to shut down the CNS production of PGE_2. The fast response could be involved in priming the later delayed response and preventing drastic changes to homeostatic processes that the delayed response would have problems overcoming. A study looking at the effects of specific COX-1 and COX-2 inhibitors supports such a role for the COX-1–dependent early response because the ablation of COX-1 activity following LPS administration led to a fast hypothermic responsive, which could not be counterbalanced by the later COX-2–dependent fever response (64). This study emphasizes the importance of the coordination between the fast and delayed responses.

The populations of cells containing the enzymes responsible for PGE_2 synthesis during systemic inflammation are still debated. Numerous papers have provided clear evidence that both COX-2 and mPGES-1 transcripts and proteins are expressed essentially within the endothelium of the cerebral capillaries (44, 62, 65–73). On the other hand, few studies have shown that perivascular myeloid cells are responsible for delivering PGE_2 in response to systemic

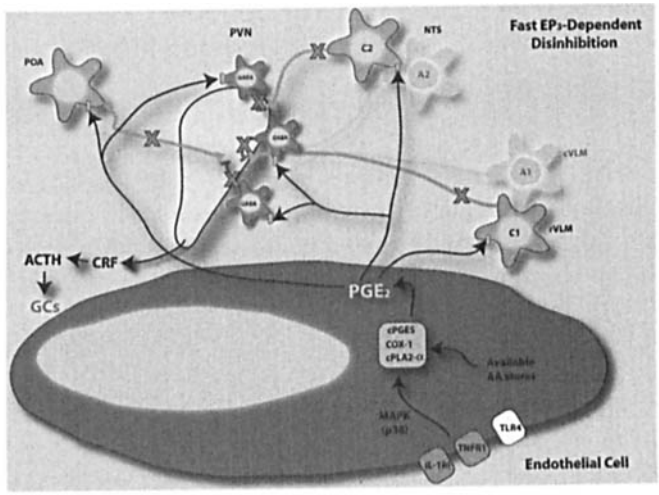

Figure 2. Hypothetical model for the fast EP_3-dependent PGE_2 response in the brain. Circulating inflammatory factors (IL-1, TNF, LPS) bind to their receptors expressed on the surface of endothelial cells of the brain microvasculature. This leads to a rapid use of available constitutive enzymes (cyclooxygenase [COX]-1, cytosolic phospholipase A_2 [$cPLA_2$], cytosolic prostaglandin E synthase [cPGES]) and arachidonic acid (AA) stores to transiently produce and release PGE_2 (possibly through MAPK p38 mechanisms). This PGE_2 acts on its EP_3 receptor on GABAergic afferents to the PVN, which rapidly turns off the inhibition of the corticotropin-releasing factor (CRF)-releasing neurons. A similar scenario may happen at the medial preoptic area (POA), C1 catecholaminergic neurons of the rostral ventrolateral medulla (VLM) and C2 catecholaminergic neurons of the solitary tracts nucleus (NTS), where the binding of PGE_2 to the EP_3 receptor inhibits excitation of these GABAeric projections of PVN neurons.

inflammatory stimuli (74–76). The action of this prostaglandin may therefore be dependent on its cellular source, the endogenous pool of PGE_2 in endothelial cells, and the receptor subtypes expressed on their cellular targets. It has been shown that EP_4 is present on the postsynaptic sites of hypothalamic stimulatory neurons, whereas γ-aminobutyric acid (GABA) neurons contain presynaptic EP_3 receptors in their terminals (77). Activation of both EP_3 and EP_4 receptors is actually involved for the excitatory regulation of supraoptic neurons by PGE_2 (77). Taking these data into consideration, we propose that both receptors contribute to the activation of PVN neurons during systemic inflammation, but at different times. Stimulation of the $cPLA_2$–COX-1–cPGES pathway would lead to a rapid secretion of PGE_2 from endothelial cells and then cause an immediate activation of the CRF neurons through inhibition of GABA afferents that contain EP_3 receptors. The hypothalamic PVN receives robust innervation from GABA neurons that play a critical role in the control of the HPA axis during stress (78–83). Inhibition of GABAergic neurons by EP_3 may take place locally at the level of the nerve terminals within the PVN or through distal inhibition of GABA projections originating from different areas, such as the preoptic area (POA), parabrachial nucleus (PB), rostral ventrolateral medulla (rVLM), and/or NTS.

PGE_2 synthesis under the control of the $sPLA_2$-IIA–COX-2–mPGES-1 pathway would take the relay and trigger

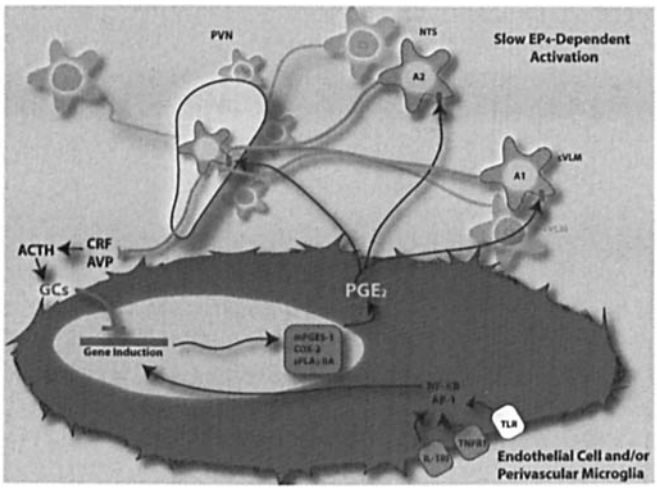

Figure 3. Delayed EP_4-dependent PGE_2 response in the brain during systemic immune insults. The delayed PGE_2 response requires transcription of the inducible enzymes (secretory phospholipase A_2 ($sPLA_2$-IIA), cyclooxygenase (COX)-2, microsomal prostaglandin E synthase (mPGES-1) through NF-κB– and AP-1–mediated transcriptional mechanisms. These enzymes permit the massive production of PGE_2, which acts on the EP_4 receptors in the paraventricular nucleus (PVN) and at the adrenergic A1 region of the caudal ventrolateral medulla (cVLM) and A2 region of the nucleus of the solitary tract (NTS) to enhance corticotropin-releasing factor (CRF) and vasopressin (AVP) release into the infundibular system. This leads to corticotroph adrenocorticotropic hormone (ACTH) release and subsequently to biosynthesis and secretion of glucocorticoids (GCs) from the adrenal gland. The latter hormone exerts negative feedback on the transcription of the enzymes responsible for the production of PGE_2 and prevents overproduction of inflammatory molecules during innate immunity.

different populations of neurons. Enzymes of this pathway are not constitutively present and have to be induced by the proinflammatory signal transduction pathways. It is also possible that both endothelial and microglial cells contribute to the PGE_2 production in the delayed phase. Once secreted, PGE_2 would maintain CRF activity *via* EP_4 stimulation either directly or through activation of the A1 and A2 catecholaminergic circuits from the cVLM and NTS, respectively. These mechanisms fit nicely with the hypothesis of fast and delayed PGE_2 responses, where the fast phase could be associated with the disinhibition of CRF-releasing neurons of the PVN, without the need for time- and energy-hungry transcription. This response could also prime the PVN to become more receptive to the massive release of PGE_2 by endothelial and perivascular microglial cells with their newly transcribed PGE_2-synthesizing enzymes. In support of this hypothesis is the specific increase in EP_4 mRNA levels in CRF neurons and catecholaminergic afferents to PVN following a systemic immune challenge (53, 54). The sustained release of GCs provides direct inhibitory feedback on these events at the level of transcriptional machinery (see above), which is the most powerful endogenous immunosuppressive mechanism. This innovative dual regulation of PGE_2 production and action in the brain could have important implications for an appropriate control of fever, HPA axis, and other autonomic functions.

The Vagus Nerve

Vagal afferents originating in the periphery were proposed by numerous studies to monitor circulating immune molecules because neurons within the NTS are primary recipients of sensory information from the vagus nerve, and this region of the CNS is quite sensitive to circulating proinflammatory components. However, a recent study investigated whether vagal connections with the brainstem were necessary for LPS-induced activation of dorsal vagal complex (DVC) neurons and found that systemic exposure to LPS elicited a significant activation of c-*fos* in neurons in the NTS and area postrema regardless of the integrity of the vagal nerve (84). Another group reported that low doses of LPS stimulated expression of CRF mRNA in rats subjected to axotomy of the gastric or celiac branches of the vagus nerve but did not change the intensity of autoradiographic labeling in animals with transected hepatic branches (85). High doses of the endotoxin, on the other hand, enhanced expression of the neuropeptide in vagotomized rats of all groups (85). The role of the vagus nerve of the parasympathetic system in mediating the effects of LPS and inflammatory cytokines would then arise only when the endotoxin is injected ip and at a very low dose. Indeed, vagotomy can prevent neuronal activation in response to fairly low doses of LPS and IL-1 injected ip, but not following moderate to high doses and not when the endotoxin and proinflammatory cytokines are injected directly into the circulation (for a review see Ref. 86). The exact contribution of the vagus nerve has also been challenged by numerous studies that failed to prevent the cerebral responses even in presence of fairly low doses of cytokines injected ip. Other groups have found that behavioral responses to immune stimuli can be attenuated by selective transection of the gastric, celiac, and hepatic branches of the vagus nerve without significantly altering other parameters, such as increased activity in the HPA axis or CRF and c-*fos* transcription in the PVN as well as in other regions of the brain. It can be concluded that the participation of vagal sensory mechanisms in mediating the neuronal response to immune challenge is, therefore, quite limited to local peritoneal inflammation and may not be considered a general feature by which circulating proinflammatory molecules trigger the cascade of events taking place in the CNS during systemic innate immunogenic stimuli.

Concluding Remarks

The recent developments on interactions between immune regulators and the brain have given a clearer shape to the neuroimmune and neuroendocrine pathways involved in establishing a proper control of the immune response. Although the story is far from complete, the presence of the

TLRs and cytokine receptors as well as molecules for the recognition of immune cells on the brain vessel endothelia and at the CVOs clearly establishes these areas as the gateways for immune signals into the brain. The emergence of the polyvalent TLR family has also shed light on a possible mechanism by which the brain can customize its response to various immune insults and offers a tangible explanation for a cytokine-independent blood-to-brain signaling cascade. The elucidation of the intricacies of PGE_2 synthesis and action in the brain not only has helped clarify the importance of this soluble mediator in affecting both neuronal and immune cell function, but has led to the development of many nonsteroidal anti-inflammatory drugs (NSAIDs) that target its biosynthetic enzymes (87–89). The further understanding of the essential role of GCs in modulating the whole body and maintaining brain integrity during the central immune response reminds us of the importance of the strong regulatory feedback needed to maintain the balance between the "good" and "bad" immune response in the brain (90). It is interesting to note that activation of the HPA axis is directly under the control of the immune ligands, which are in return in close check by the final product of this reaction (e.g., GCs). A very small defect in such interplay was recently found to have devastating consequences for the organism following a simple challenge with LPS (90).

Although the mediators, both molecular and cellular, described in this review are of great (if not essential) importance in most inflammation-related processes in the brain, there exist many other factors in the brain that could play influential roles in mediating the immune response in the brain. For example, polyamines, which usually play a role in growth and differentiation in the brain as well in neurodegeneration and NMDA channel gating, could be major players in the control of inflammation in the CNS. Inhibition of polyamine biosynthesis was found to rescue mice from the neurotoxic LPS/RU486 cocktail and attenuated inflammation in the brain following LPS administration (91). Emerging interest has also been put in heat-shock proteins (especially hsp70 and hsp90), which can play critical roles in GR assembly (92) and inflammation (93). Furthermore, hsp60 and hsp70 have been proposed as endogenous ligands for TLRs (94). Thus, possible interactions between emerging new contributors in the field and the classical neuroimmune factors (cytokines, PGs, GCs, etc.) need to be investigated. Another point of debate is the emergence of the hypothesis of two types of microglia in the brain: one that is resident and evolved from neuroectodermal origin and others that would be monocytes that have invaded the brain from the blood (95, 96). These invading cells express markers that would make them potent APCs and thus stimulate the adaptive response in the brain and have detrimental effects in the CNS (97). It is possible that these cells express both COX-2 and mPGES-1 and contribute to the delayed PGE_2 secretion. In future studies, it will be important to determine the role of each type of cells (endothelial versus perivascular microglial cells derived from bone marrow stem cells) in the endocrine control of innate immunity. We could then be able to develop tools that would select the exact source of PGE_2 response to trigger the HPA axis and GCs. Furthermore, the prevention of factors that could cause the recruitment and maintenance of the harmful glial population in the brain (e.g., possibly early life clinical or subclinical infections or trauma) could prevent inflammation-related neuropathologies later in life. Taking all of these factors into consideration, we are left to conclude that the communication between the brain and the immune response not only rests on automatic responses between specific receptors and static neuroendocrine pathways but adapts to and requires many cellular, molecular, and neural factors and interactions in order to have a controlled and specialized response to each threat it encounters.

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