ENDOTOXIN, PROSTANOIDS AND CORTICOTROPHIN-RELEASING HORMONE:
AN INTEGRATED VIEW

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INTRODUCTION

While immuno-inflammatory challenges cause marked changes in many aspects of neuroendocrine function, activation of the hypothalamo-pituitary-adrenal (HPA) axis has its major effect on stress responsiveness. In particular, secretion of corticotrophin-releasing hormone (CRH) is primarily responsible for modulating circulating corticosteroids, the dominant endocrine inhibitors of the immune response. Thus, the immune system has access to a pathway whereby it can attenuate its own responses. It has been suggested that the purpose of such a system is to prevent immuno-inflammatory processes from ‘over-shooting’, in a manner analogous to other classical negative feedback pathways. In addition, immunological and inflammatory challenges involve the localized production of vasoactive cytokines which are important mediators of the local inflammatory response. When such responses become generalized, as in endotoxemia, the overriding concern must be the preservation of vascular integrity and blood pressure in order to preserve cerebral function. While chronic immune challenges may elicit significant changes in the growth and reproductive axes, the predominant
survival need is for up-regulation of glucocorticoid function.

This review deals with the mechanism(s) through which endotoxin activates the HPA axis. In this context, some peculiar aspects will be reviewed: i) the pattern of activation of the HPA axis by bacterial endotoxin, and ii) the role of prostanoids as mediators of the effects of cytokines.

ENDOTOXIN

Endotoxin, also referred to as lipopolysaccharide (LPS), is released from the bacterial cell wall during mitosis and after cell death; systemic infections may be associated with plasma levels of endotoxin as high as 100 µg/ml (1). The ability of host organisms to recognize and react to endotoxin released during bacterial infections represents a pivotal mechanism of the so-called innate immune response to bacterial challenge; on these basis, the use of LPS as such became established as a popular model to reproduce and investigate, in vivo and in vitro experimental settings, the immune-inflammatory responses during infectious diseases.

Regardless of the bacterial species and strain they derive from, endotoxins possess a common structure, consisting of a lipid region (lipid A), which is part of the lipid bilayer of the bacterial cell membrane, and a polysaccharide region. The latter is formed by an external oligosaccharide domain (the O-antigen) and a core region that binds the lipid portion of the molecule. While species- and strain-specificity are related to the O-antigen domain, lipid A is responsible for the pathological actions of LPS. In fact, the latter are counteracted by agents quenching lipid A, such as specific antisera or the antibiotic polymixin B (2).

It is now well established that the biological activities of endotoxin are mediated by specific receptors. This issue was thoroughly investigated over the last decade, and several putative receptors, including the glycoproteins CD14 and CD18, have been proposed (2,3). At present, compelling evidence indicates that primary endotoxin receptors are members of the family of Toll-like receptors (TLRs), in particular TLR4. In fact, mice with mutations of the gene encoding TLR4 are unresponsive to LPS or gram-negative bacteria (4,5). Furthermore, other receptors may play a role: binding of LPS, either alone or as a complex with serum LPS-binding proteins (LBP), to CD14 is currently thought to be an important step in the cascade of events of LPS signaling. In fact, CD14-deficient mice show reduced responses to endotoxin challenge and resistance to endotoxic shock (6). Thus, a hypothetical model of the LPS signal transduction complex should involve the assemblage of LPS, LBP, CD14 and TLR4 into the membranes of target cells; indeed, there is evidence that one such mechanism may take place, probably mediated by the general adaptor protein MyD88 (5,7). It should be pointed out that TLRs play a pivotal role in systemic responses to LPS challenges; however, a large body of evidence from in vitro studies on isolated CNS cells, microglia in particular, clearly shows that cellular responses can be elicited by the activation of CD14 only, as these cells are functionally activated by endotoxin even in the apparent absence of TLR expression.

Post-receptor mechanisms are also complex. Several pathways have been described that can be summarized into events such as the activation of NFkB and mitogen-activated protein (MAP) kinase, as well as the induction of pro-inflammatory cytokines. Furthermore, direct and/or cytokine-mediated activation of phospholipase A2 (PLA2) and cyclo-oxygenase-2 (COX-2) and the subsequent production of prostanoids, as well as the increased production of nitric oxide (NO) (8,9), also take place, altogether accounting for the pathological pleiotropic actions of endotoxin.
Endotoxin activation of the hypothalamo-pituitary-adrenal axis

Early reports showed that bacterial infections are associated with increased adrenocortical secretion (10). This phenomenon was found to be caused by endotoxin through a mechanism involving increased prostaglandin production within the hypothalamus (11,12). These findings raised the question of whether endotoxin is capable of crossing the blood-brain barrier (BBB), thereby activating the HPA axis via a direct effect on hypothalamic CRH. In experimental models involving the administration of LPS to otherwise normal animals, endotoxin is unable to cross the intact BBB (reviewed in 13). However, circulating LPS causes morphological and functional changes at the interface between the systemic circulation and brain parenchyma, i.e., at the level of endothelial cells of brain capillaries and the surrounding glia; these changes include the induction and synthesis of prostaglandin E2 (PGE2) and interleukin-1 (IL-1) (14,15). Induction of the cytokine after peripheral injection of LPS is also observed in other brain regions (16), in particular the hypothalamus (17). In brain areas devoid of BBB, such as the circumventricular organs (CVOs), LPS is able to induce CD14 biosynthesis, for example, in the parenchyma surrounding the CVOs (18). If stimulation persists, this process tends to spread throughout the brain, involving primarily CD14 expression in microglial cells. However, expression of TLR4 is not induced, except at the level of the CVOs and a few other brain areas (18). Altogether, the above evidence indicates that endotoxin can influence brain function, including the central control of HPA axis, via indirect mechanisms that do not imply crossing of the BBB and widespread diffusion within the parenchyma.

In keeping with this notion, one other mechanism accounting for endotoxin stimulation of hypothalamic CRH release is via the increase in systemic production of pro-inflammatory cytokines (13). In this regard, probably the most important cytokine mediator of the LPS effect is IL-1. Increases in systemic levels of IL-1 during endotoxemia are primarily accounted for by activated macrophages, but other cell types may contribute to systemic IL-1 production; in fact, macrophage depletion does not totally prevent the HPA activation induced by high doses of LPS (19). Endotoxin stimulation of the HPA axis might also be mediated by other inflammatory cytokines, such as IL-6 (whose circulating levels during experimental pathology increase to a greater extent than those of IL-1) (20), tumor necrosis factor and interferons. However, only in the case of IL-1 and IL-6 is there unequivocal evidence of a direct stimulatory action on CRH release (21,22) (see below). Thus, the question of whether LPS crosses the BBB to directly activate CRH release is now subsumed within the more important and intriguing problem of whether circulating cytokines cross the BBB. Because of their large molecular size (over 15 kDa), IL-1 and the other pro-inflammatory cytokines do not enter the CNS via simple diffusion. Systemic IL-1 is currently thought to exert its action on the CNS via three non-mutually exclusive mechanisms: i) via peripheral afferent nerves, in particular the vagus: this pathway is involved in the signaling of IL-1 produced in the splanchnic region, as shown by experiments with intraperitoneal injection of the cytokine; ii) via the brain vasculature, through the induction of secondary mediators such as prostaglandins or NO in perivascular glia; and iii) crossing the BBB via specific carrier-mediated transport, or entering the CNS at sites lacking a BBB (see 23 for a review).

Another line of evidence supporting the lack of direct effects of endotoxin on CRH release derives from in vitro studies on hypothalamic explants. In this experimental model, the effects of endotoxin on CRH neurons can be measured in the absence of systemic influ-
ences. Under these conditions, our group and others have demonstrated that LPS does not stimulate CRH release (22, 24-26), and may even produce inhibitory effects, depending on the dose administered and the length of incubation (25,26). The absence of stimulatory effects observed in these studies might be explained by the lack of TLR4 receptors within the hypothalamus. However, other studies on hypothalamic explants showed that LPS is able to elicit acute increases in the production and release of bioactive IL-1 and IL-6, as well as PGE2 (26-28), these findings being consistent with the fact that LPS can trigger a signal transduction process in the CNS in spite of the absence of TLR4 expression (see above). Therefore, the lack of stimulation observed in the previously cited studies may have been caused by the presence of other endotoxin-induced factors that counteract the effects of stimulatory mediators by inhibiting CRH release.

The most likely candidate for an endotoxin-induced inhibitor of CRH is NO. A large body of evidence has accumulated in the last 10 years concerning the interaction between NO and CRH in the control of the HPA axis function; this topic has recently been reviewed in detail (29). Briefly, it emerges that NO may have a dual capacity (inhibition or stimulation) in regulating the HPA axis, depending on the nature of the stressor. In fact, inhibitors of NO synthase further enhance the activation of the HPA axis following systemic endotoxemia or local inflammation in the rat and the mouse (29), whereas NO appears to attenuate the response to physico-emotional stressors. It is difficult at the present time to reconcile the above discrepancies: a finely-tuned balance between levels of NO produced, hypothalamic sites of production [periventricular nuclei (PVN) versus median eminence], NO synthase isoforms involved and time-course of such production, could account for the fact that the same molecule plays opposite roles under broadly similar conditions.

**PROSTANOIDS**

Prostaglandins and the hypothalamo-pituitary-adrenal axis

Prostaglandins do not exert hormone-like actions, since they are rapidly metabolized in the pulmonary and systemic circulations. Thus, their effects within the HPA axis result from the sum of local actions at hypothalamic, pituitary and adrenal levels. Early studies, based on systemic administration of various prostaglandins (PGs) in rats with median eminence lesions showed that PGs can stimulate the HPA axis via an action within the CNS. Furthermore, the administration of indomethacin (INDO, an inhibitor of PG synthesis) in various hypothalamic areas, especially in the anterior hypothalamus, inhibited ACTH secretion, thus supporting a role for PGs in regulating HPA axis function. In earlier reports, the stimulatory role of PGs was reported not to be specific, since PGs of the E, F, A and B series were all able to elicit increased ACTH secretion when injected into the medial basal hypothalamus (reviewed in 30). However, in later studies only PGE2 was shown to stimulate plasma ACTH release, whereas other PGs tested (PGD2 and PGE1) had no effect. Pre-treatment with CRH antiserum counteracted the increase in plasma ACTH observed after intra-hypothalamic injection of PGE2 (31). While most in vivo studies show that PGE2 is specifically involved in the central activation of the HPA axis, evidence obtained in vitro indicates that PGF2a is also able to stimulate CRH release from the isolated rat hypothalamus (32).

At the pituitary level, however, PGE2 in -hibits CRH-stimulated, but not basal, release of ACTH (33). Prostaglandin E2 synthesis in the pituitary gland is stimulated by CRH or vasopressin, and is inhibited by lesions of the PVN (34). Thus, PGE2 may act as a locally-produced negative feedback signal within the anterior pituitary gland.

Other COX and lipoxygenase (LO) deriv-
atives seem to play no role in the control of ACTH release from the pituitary gland in vitro. However, the activation of PLA2 in isolated pituitary cells causes increased ACTH secretion from the pituitary gland, mediated by arachidonic acid (AA) metabolites of the cytochrome P450 epoxygenase pathway (35). Injection of INDO directly into the pituitary gland increases ACTH secretion (36); this effect can be ascribed to reduction of the local PGE2 inhibitory tone, or to the shunting of AA toward other metabolic pathways, such as oxidation via the cytochrome P450.

Prostaglandins and cytokines

Renewed interest in the involvement of PGs in the control of the HPA axis was kindled by the observation that the former have been shown to mediate many biological effects of IL-1 (37). The sites of HPA axis activation by IL-1 and other pleiotropic cytokines have been investigated in detail; although there is evidence that IL-1 can stimulate the HPA axis by direct effects at the pituitary and adrenal level, there is a general consensus that the acute response of the HPA axis to cytokines during immune-inflammatory processes occurs via a direct stimulatory action on CRH release from the hypothalamus. Evidence supporting this concept arises in part from studies conducted on isolated rat hypothalami demonstrating that IL-1 is able to modulate hypophysiotropic peptide release (reviewed in 38).

While PGs have also been shown to influence the HPA axis at hypothalamic, pituitary or adrenal levels, there is no evidence that they modulate the effects of cytokines on the adrenal gland. They also appear not to be involved in the acute IL-1-induced release of ACTH from isolated pituitary cells, although they may be involved in longer term regulation (39). Thus, most of the studies on the interplay between PGs and cytokines in the control of the HPA axis have focused on the actions of these substances on the hypothalamus, and on the control of CRH release in particular.

A key role for PGE2 in the control of IL-1-induced ACTH release has been demonstrated in several in vivo studies in the rat. Katsuura et al. (40) observed that INDO blunts the increase in circulating ACTH induced by i.v. or i.c.v. IL-1β. Micro-injection of PGE2 into the pre-optic area of the hypothalamus produces a marked increase in plasma ACTH, and both INDO and a PGE2 receptor antagonist, injected into the anterior hypothalamus, counteracted the increase in plasma ACTH induced by i.v. IL-1β, while systemic pre-treatment with INDO also antagonized the rise in plasma ACTH induced by IL-1α. Evidence has also been provided that both IL-6 and tumor necrosis factor stimulate the HPA axis in vivo via a pattern involving the central activation of PG biosynthesis.

While there is compelling in vivo evidence that PGE2 mediates the effects of IL-1 on the HPA, in vitro experiments have provided conflicting results. In studies conducted on isolated hypothalamic explants, many groups have shown that IL-1α and IL-1β are able to stimulate CRH release in short-term experiments, a finding in keeping with the cytokine ability to induce a rapid rise in plasma ACTH in vivo (reviewed in 41). There is also general agreement that IL-6 is able to stimulate CRH release, while there are discordant findings regarding CRH release in relation to IL-2, IL-8 and TNF treatments. Both IL-1- and IL-6-induced CRH release could be inhibited by dexamethasone, by specific inhibitors of COX, such as INDO and naproxen, and by an inhibitor of the epoxygenase pathway, clotrimazole, but not by a selective inhibitor of the lipoxygenase pathway, BW A4C. This suggests that IL-1 and IL-6 activate in sequence the PLA2, COX and possibly the epoxygenase pathways to release CRH. The next question is: can IL-1 and IL-6 stimulate the production of PGs from the isolated hypothalamus? Indeed,
both IL-1β and IL-6 selectively increase the production and release of PGE2, but they have no effect on PGF2α, thromboxane A2 and prostacyclin (42).

The fact that the increase in CRH secretion from hypothalamic explants treated with IL-1β or IL-6 is associated with a parallel selective increase in PGE2 release suggested a role for the latter in mediating the stimulatory effects of the cytokine. However, the addition of PGE2 into the incubation medium of hypothalamic explants failed to reproduce increases in CRH secretion as great as those induced by IL-1β or IL-6 (26). Prostaglandin F2α, which was also shown to stimulate CRH release from the isolated hypothalamus (see above), does not seem to be involved in the release of CRH induced by IL-1, since its hypothalamic synthesis and secretion were not increased by IL-1 either in vitro (42) or in vivo (43).

Modeling

The evidence reviewed here suggests that there are two sites within the hypothalamus where PGs, more specifically PGE2, may play a role in signaling between cytokines and the HPA axis: i) the organum vasculosum laminae terminalis (OVLT, a vascular structure devoid of a BBB that is located within the anterior wall of the third ventricle) in the anterior hypothalamus, and ii) the PVN CRH neurons themselves (perikarya or median eminence terminals), where CNS-borne IL-1 and IL-6 act to release CRH. While most of the evidence for the involvement of the OVLT in the signaling system between cytokines and the HPA axis has been obtained in vivo, the isolated hypothalamus is also a good model for studying the molecular events taking place at the levels of neurons producing CRH.

It is worth noting that in all studies where PGE2 elicited HPA axis activation, injections were placed in the anterior hypothalamus. This led Katsuura et al. (31) to hypothesize that PGE2 is the intra-hypothalamic mediator of IL-1β-induced ACTH secretion, and that the OVLT, where the highest density of PGE2 binding sites is found (44), is the specific site at which circulating IL-1 increases PGE2. Subsequent studies confirmed that PGE2 was markedly increased in the OVLT and, to a lesser extent, in other hypothalamic regions by i.v. injection of IL-1β. The mechanism by which a local increase in PGE2 in the anterior hypothalamus stimulates neurons within the PVN to release CRH is still unknown. Prostaglandin E2 might diffuse from the anterior hypothalamus to the PVN or a PGE2-activated pathway might communicate between the anterior hypothalamus and the PVN. Within this model, elevations in anterior hypothalamic PGE2 induced by circulating IL-1, or other ‘pyrogenic’ cytokines such as IL-6 (45), could represent a fundamental mediator in various phenomena which take place during the acute phase response: these include activation of the HPA axis as well as febrile and anorectic responses, all of which also require the mediation of CRH.

As far as the second site of interaction (CRH neurons) is concerned, the finding that dexamethasone inhibits IL-1- and IL-6-induced release of both CRH and PGE2 raises the possibility that the cytokines might stimulate CRH release through the activation of PLA2. This hypothesis was confirmed in an elegant study by Loxley et al. (22) which showed that dexamethasone suppresses IL-1- and IL-6-stimulated CRH release by inducing lipocortin 1 in the hypothalamus. Interleukin-1 acutely stimulates PLA2 through the activation of the sphingomyelin pathway (46), although PLA2 activation does not appear to be the only IL-1-operated post-receptor event. In fact, there is evidence to suggest that IL-1 can trigger several transduction mechanisms simultaneously in the same cell. This seems to be the case as regards the effect of IL-1 on CRH release as well, since both protein kinase A and protein kinase C pathways have also been shown to play a role.
in CRH release from hypothalamic neurons in vitro (47). It is worth noting that the inhibition of each of these pathways by specific antagonists is able to inhibit IL-1-induced CRH release completely (22,47), since all the pathways have to be activated simultaneously for the complete secretory response of CRH to IL-1 to occur, and the blocking of only one of them inhibits the whole signaling mechanism. Activation of PLA2, which possibly involves surrounding glial cells, in turn activates COX, and the resultant increase in PGE2 might subsequently reinforce cytokine-induced CRH release through activation of its own receptor. Within this model, PGE2 should not be thought of as a classical intracellular second messenger of IL-1 and IL-6, but rather as a positive modulator of the cytokines’ signal transduction complex on CRH release.

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