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CRF-DEPENDENT AND CRF-INDEPENDENT MECHANISMS INVOLVED IN HYPOPHYSIAL-ADRENAL SYSTEM ACTIVATION BY BACTERIAL ENDOTOXIN

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The immune system and the hypothalamic-pituitary-adrenal (HPA) axis play important role in the overall inflammatory response. The mechanism through which lipopolysaccharide (LPS, endotoxin) stimulates the HPA axis is not well understood. In order to clarify the role of hypophysiotropic peptides of paraventricular origin in the effect of LPS on ACTH and corticosterone secretion, the effect of LPS was studied on rats with lesions of hypothalamic paraventricular nucleus (PVN). It was shown that 90 min after 2 mg/kg LPS i.p. the ACTH, but not the corticosterone response was effectively blunted in PVN-lesioned rats, as compared to sham operated animals. However, in PVN-lesioned rats 240 min after treatment with LPS a significantly higher plasma ACTH and corticosterone level was monitored.

It is, therefore, suggested that in response to LPS activation of HPA both CRF(s)-dependent and CRF(s)-independent mechanisms are involved, even a direct effect of the adrenal cortex should be taken into account.

Keywords: hypothalamic-pituitary-adrenal axis, endotoxin, cytokines, median eminence, neuro-immune communication

Evidence has been accumulated that there is an interaction between the neuroendocrine and the immune system, and they are able to regulate each other's function. Recently, application of bacterial lipopolysaccharide, a subcellular component of Gram-negative bacteria [4], has been often used to study how the stimulation of the immune system activates the hypothalamo-pituitary-adrenal axis

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(HPA) [8, 14, 16, 31]. Interleukin-1 (IL-1) [5, 21] and tumor necrosis factor (TNF) [2, 22] cytokines produced mainly by LPS-activated macrophages and monocytes have been shown to be implicated in the activation of HPA. It was shown [19] that stimulation of ACTH secretion in mice treated with LPS, is at least partially mediated by or dependent upon the action of IL-1.

However, the mechanism through which LPS and related cytokines stimulate the HPA and the exact site(s) of their action still remains an enigma. While some authors using hypothalamic lesions [31] or pharmacological blockade of CRF release [16] have suggested that hypothalamus mediates endotoxin stimulation of HPA, others [14, 24] have observed that LPS could stimulate corticosterone secretion even after removal of the medial hypothalamus. There is now increasing consensus that the main site of action of IL-1 [1, 21] and TNF [2] is located in the hypothalamus, through the production of corticotropin releasing factor (CRF). However, it is currently under debate whether IL-1 or other cytokines may act directly on the pituitary level [3] or not [1, 21]. To clarify whether *in vivo* the endogenous CRF is crucial in the action of LPS on ACTH and corticosterone secretion, the effect of LPS was studied in rats with hypothalamic paraventricular nucleus (PVN) lesion. This nucleus is the major source of neuropeptides (CRF-41, vasopressin, etc.) involved in regulation of ACTH synthesis/release from the pituitary gland.

Material and methods

Male Wistar rats (weighting 200–250 g) were housed under controlled conditions (lights on: 07.00-19.00; temperature: 24±1 °C; humidity 65%) and fed laboratory rat chow and water *ad libitum*.

Paraventricular nucleus lesion (PVL): Five days before the experiment the rats were anaesthetized by i.p. injection of 4 mg/100 g body weight pentobarbital. The hypothalamic paraventricular nucleus was lesioned surgically as described [13, 14]. Briefly, animals were placed in a stereotaxic frame. A specially designed microknife was lowered to the base of the skull through a burrhole behind the Bregma suture, rotated 360° and withdrawn. Rostrally this lesion usually damaged the anterior comissural nucleus; posteriorly it extended into the dorsomedial nucleus. Laterally it did not reach beyond the fornix. For sham-operation the skull was opened and the knife was lowered to the base of the skull as in the lesioned animals, but not rotated. The placement of the lesions was checked after the decapitation by registering the trace of the knife on the basal hypothalamus.

One day before the experiment the rats were weighed and placed into individual cages. On the next morning the animals were injected i.p. either with *E. coli* 0101/RG/W LPS (produced in "F. Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene by the method of Westphal, using phenol extraction) 2 mg/kg or with saline. 90 and 240 min later they were decapitated under minimal stress. The trunk blood was collected into chilled tubes containing Na2EDTA and centrifuged. The plasma was stored at −20 °C until assayed.

Radioimmunoassays: Plasma adrenocorticotropin (ACTH) and corticosterone were determin by RIA, as previously described [12].

Statistical analysis: Data were transformed to logarithms before analysis of variance, which was followed by Dunn's test for multiple comparisons.

Results

Changes in plasma adrenocorticotropin concentration (Fig. 1)

In control animals injected with saline in both sham-operated and paraventricular lesioned groups the plasma ACTH concentrations were in the range of 8–22 fmol/ml. This value of ACTH corresponds well to values routinely obtained by our direct radioimmunoassay. Plasma ACTH levels 90 and 240 min after *E. coli* LPS administration were significantly higher in sham-operated animals ($p<0.01$) than in control animals (Fig. 1a).

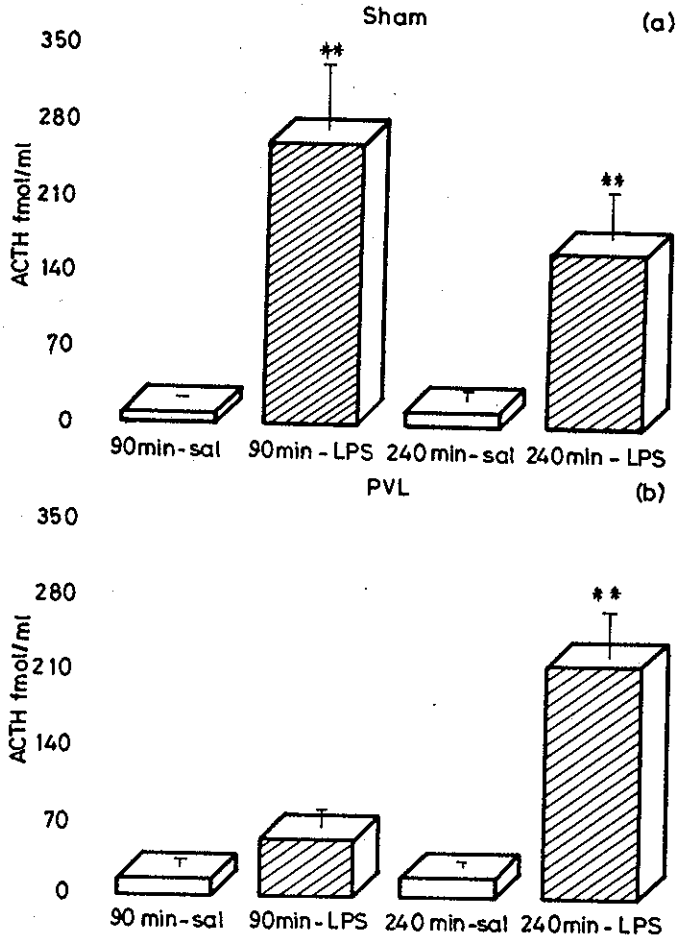

Fig. 1. Effect of 2 mg/kg i.p. bacterial lipopolysaccharide on plasma ACTH level in sham operated rats (Fig. 1a) and in rats with lesions of paraventricular nucleus (PVL) (Fig. 1b). Animals injected with saline (sal) were used as controls. Means ± S.E.M. from six animals in each group. **$p<0.01$ compared with the corresponding saline-treated group

In paraventricular lesioned rats the elevation of plasma ACTH, 90 min after LPS administration, was not significant compared to the saline-treated controls. Four hours later, however, plasma ACTH raised to the level which was not different from the hormone levels measured in sham-operated group at the same time (Fig. 1b).

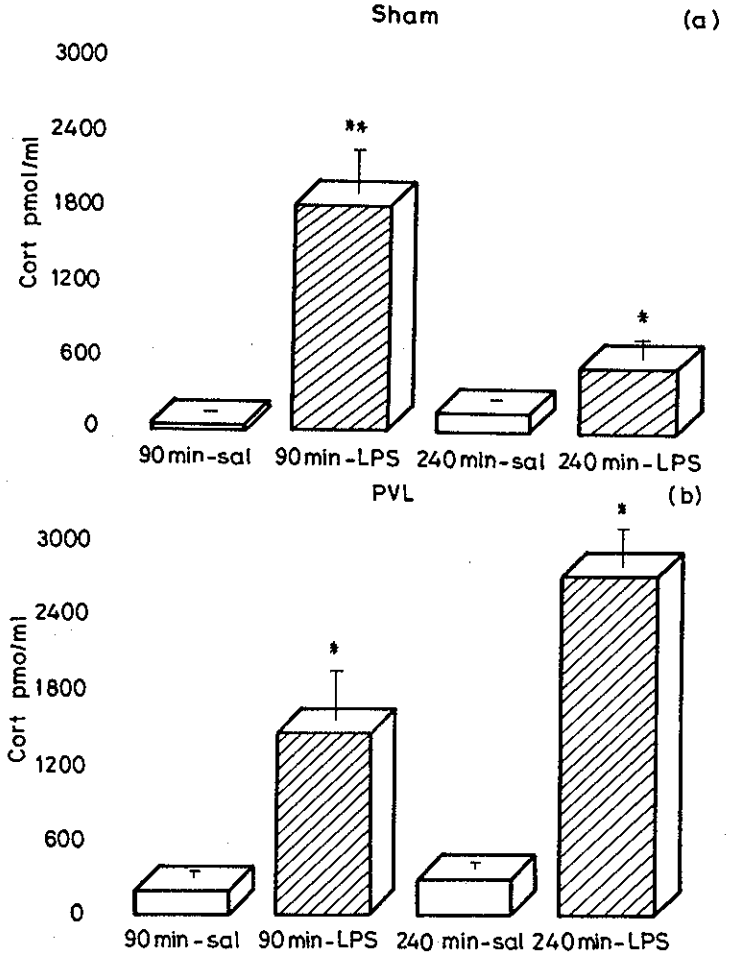

Fig. 2. Effect of 2 mg/kg i.p. bacterial lipopolysaccharide on plasma corticosterone level in sham-operated rats (Fig. 2a) and in rats with lesions of paraventricular nucleus (PVL) (Fig. 2b). Animals injected with saline (sal) were used as controls. Means ± S.E.M. from six animals in each group. *$p<0.05$ and ** $p<0.01$ compared with the corresponding saline-treated group

Changes in plasma corticosterone concentration (Fig. 2)

The basal corticosterone concentration in the plasma of saline-treated animals was in the range of 32–200 pmol/ml. In sham-operated rats LPS induced 7–56-fold elevation of corticosterone levels above the baseline. The maximal elevation was at 90 min (1810 pmol/ml) (Fig. 2a).

In paraventricular lesioned rats, 90 and 240 min after i.p. injection of LPS, significantly higher corticosterone levels were monitored, as compared to the controls. In contrast to the not significant elevation of plasma ACTH 90 min after the treatment, plasma corticosterone was induced to reach the a level comparable to that of the sham-operated control (Fig. 2b).

Discussion

In the present study evidence was obtained that *E. coli* LPS directly or indirectly is able to activate hypophysial-adrenal system even in the absence of hypophysiotropic neuropeptides of paraventricular origin. In PVN-lesioned rats, 90 min after LPS treatment, the elevation of plasma ACTH was blocked. This finding suggest that the action of LPS at this time requires CRF-41, AVP or other releasing factors of the paraventricular nucleus. However, after four hours a significant increase of ACTH level was observed indicating that the effect of LPS is not mediated through hypophysiotropic factors of paraventricular origin. Therefore, two different mechanisms might be involved in the effect of LPS: an early, CRF(s)-dependent, and a late, CRF(s)-independent mechanism. Our data are supported by the observation of Moberg [16]. He has shown that lesion of median eminence (ME), a hypothalamic structure where neurosecretory projections from the PVN terminate, totally abolished the effect of LPS. Therefore, it is conceivable to suggest, that *in vivo* the effect of LPS and/or related cytokines is located at the level of ME.

LPS and/or related cytokines released in the periphery may be able to reach ME, since this structure is devoid of blood-brain barrier. It has recently been shown that IL-1 [1, 21] and TNF [2] cytokines, which are released during LPS stimulation of the immune system, activate HPA through a CRF-dependent mechanism. In support if this hypothesis Sharp et al. [22] showed that intraparenchymal injections of IL-1β, adjacent to the hypothalamic ME, resulted in an increase of ACTH level. This finding also indicates that CRF-containing nerve terminals in the ME are the targets. Another possibility is that CRF/ACTH release [reviewed in 18] is modulated by atecholamines. Recently substantial stimulation-evoked and frequency-dependent release of NA from isolated ME was demonstrated [25]. The release was not subject to so-called alpha-2 adrenoreceptor mediated negative feedback modulation and it has been suggested that NA in this region exerts sustained tonic modulation through

alpha-2 adrenoreceptors exclusively located on the axon terminals of the hormone-containing neurons [25]. Using isolated ME, we have shown, that TNF was able to inhibit the stimulation-evoked release of NA from isolated ME [Elenkov, Kovách, Duda, Stark and Vizi, unpublished results]. These data suggest that TNF might influence the noradrenergic inputs in the ME and consequently modulate the release of CRF. Since central noradrenergic inhibitory influences on CRF release were proposed by Ganong [9], desinhibition of this inhibitory pathway by TNF might result in an increase of CRF release and subsequently ACTH production. This suggestion is supported by the findings of Matta et al. [15] who demonstrated that chronic neurotoxic ablation of central catecholaminergic neurones and acute depletion of central catecholamine pools were both associated with a substantial reduction in the ACTH secretory response to IL-1β instilled into ME. This means that this response depends on the availability of catecholamines to be released. It is also interesting that the ACTH response to intraventricular injection of IL-1 was effectively blocked by prazosin [30], an alpha-1 and alpha-2B adrenoreceptor antagonist.

It was demonstrated that ACTH response induced by IL-1 is mediated by hypothalamic PgE through CRF secretion [29]. In fact it was shown [17] that PgE levels in the ME were remarkably higher than in the medial basal hypothalamus and the anterior pituitary. Since PgE reduces the release of NA [10], and LPS and TNF enhances PgE_2 production in the hypothalamus of rats [6] it is conceivable to suggest the following (see Fig. 3): LPS or other immunological stimulus provokes the release of TNF and other interleukins mainly from monocytes and macrophages. TNF and probably also other cytokines being increased in the serum, act at the level of ME and release PgE. The increased production of PgE in turn inhibits the release of NA in the ME, thereby resulting in an increase of CRF release and subsequently ACTH and corticosterone production. This fine tuning of CRF release, based on non-synaptic communication [26, 27] and presynaptic transmitter modulation [27, 28], might result in a considerable increase in ACTH and corticosterone levels. The elevated serum level of hormones could prevent the excessive production of cytokines. This feedback modulation between IL-1 and glucocorticoids was proposed by Besedovsky et al. [5].

Concerning the CRF-independent mechanism in the late phase of the response, indicated by excessive ACTH response after four hours in PVL animals, apparently can be suggested that there is an other pathway involved in this mechanism. Our data suggest, however, that LPS or related cytokines, *in vivo*, during this time, might have a direct effect on the pituitary gland to release ACTH. However, LPS alone [31] was unable to induce ACTH secretion from culture pituitary cells and other data available concerning direct effect at the pituitary level of different cytokines related to LPS are still contradictory [1, 3, 21]. Another possibility is, that LPS acts through an extrapituitary, non-paraventricular pathway. To explain

the phenomenon of "non-CRF" stimulation of ACTH production by LPS needs further evidence. It was shown recently that opioids are released in two secretory pools during endotoxin treatment [7]. In addition, intrinsic pituitary IL-1 and IL-6 was found to be induced by bacterial LPS [11, 23]. Therefore endogenous opioids and intrinsic interleukins might be involved in this process, however, this and other possibilities for the CRF-independent mechanism of activation of hypophysial-adrenal system needs further investigation.

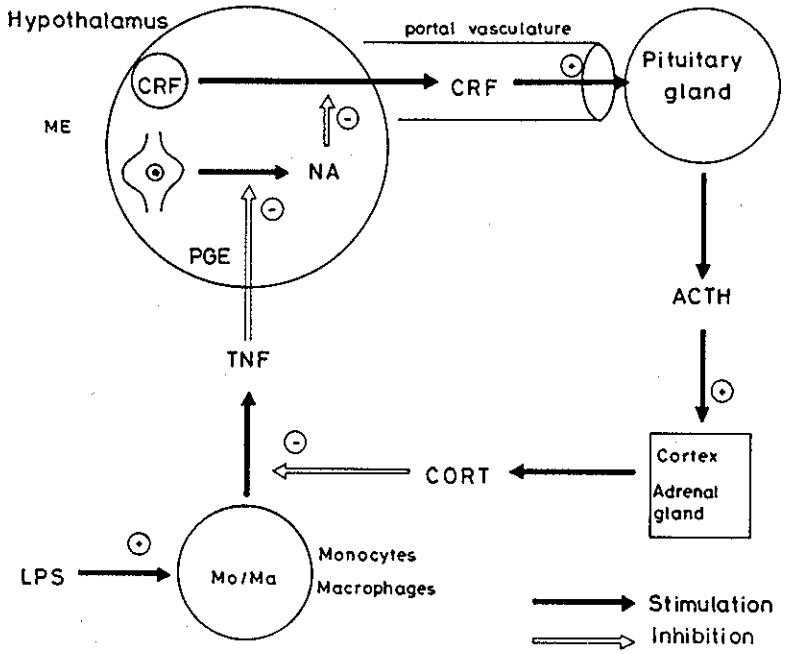

Fig. 3. Proposed model for CRF-dependent mechanism of activation of hypophysial-adrenal system by bacterial lipopolysaccharide and related cytokines. For more details, see the text

An increased plasma corticosterone level was observed after endotoxin treatment [8, 16]. The fact, that in our experiments there was an apparent corticosterone response in PVN-lesioned rats after 90 min, and at the same time the ACTH response was substantially blunted, suggests, but does not prove, that LPS and/or related cytokines have a direct effect on the adrenal steroid secretion. This possibility has been proposed for the effect of TNF [2].

In summary our results suggest that both CRF-dependent and CRF-independent mechanisms are involved in pituitary-adrenal axis activation by bacterial endotoxin, mechanisms in which ME might play an important interface role in the bidirectional communication between the neuroendocrine and immune systems.

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