ezt vissza ! 185

Lipopolysaccharide is able to bypass corticotrophin-releasing factor in affecting plasma ACTH and corticosterone levels: evidence from rats with lesions of the paraventricular nucleus

I. J. Elenkov, K. Kovács, J. Kiss, L. Bertók* and E. S. Vizi

Institute of Experimental Medicine, Hungarian Academy of Sciences, P.O. Box 67, H-1450 Budapest, Hungary
*'F. Joliot-Curie' National Research Institute for Radiobiology and Radiohygiene, P.O. Box 101, H-1775 Budapest, Hungary

RECEIVED 13 September 1991

ABSTRACT

Stimulation of the immune system or experimental conditions (bacterial lipopolysaccharide (LPS) treatment) provoke a broad spectrum of physiological responses. It was recently shown that one of them is the activation of the hypothalamic-pituitary-adrenal (HPA) axis. The mechanism and the site or sites through which LPS stimulates the HPA axis are not well understood.

To establish whether the effect of bacterial LPS is related *in vivo* to the presence of hypothalamic hypophysiotrophic peptides (corticotrophin-releasing factor-41, arginine vasopressin, etc.), plasma ACTH and corticosterone levels were monitored in intact and sham-operated rats, and in rats with paraventricular nucleus lesions in order to remove the main source of these neuropeptides. Evidence was obtained that 4 h after treatment, LPS was able to activate the hypophysial-adrenal system in the absence of hypophysiotrophic neuropeptides of paraventricular origin.

It is suggested that, *in vivo*, LPS could have a direct effect on the pituitary gland or that it acts through an extrapituitary, non-paraventricular pathway to activate the HPA axis.

Journal of Endocrinology (1992) **133,** 231–236

INTRODUCTION

The response of the organism to disturbances of its homoeostasis caused by microbial invasion, tissue injury, immunological reaction and inflammatory processes is referred to as the acute-phase response (reviewed in Heinrich, Castell & Andus, 1990). The neuroendocrine and immune systems are involved in functionally relevant cross-talk, and serve to restore the equilibrium of the 'milieu interieur' in response to these disturbances. The activation of the hypothalamic-pituitary-adrenal (HPA) axis plays an important role in this process. Bacterial lipopolysaccharide (LPS), also known as endotoxin, a component of the surface of the outer membrane of all Gram-negative bacteria (Bertók, 1983), has been used to study the acute-phase response and the mechanisms through which stimulation of the immune system activates the HPA axis (Egdahl, Melby & Spink, 1959; Makara, Stark & Meszaros, 1971; Moberg, 1971; Yasuda & Greer, 1978; Suzuki, Oh & Nakano, 1986; Rivier, Chizzonite & Vale, 1989*a*).

However, the mechanisms through which LPS stimulates the HPA axis and the exact site or sites of action within the HPA axis are unclear. While some authors using hypothalamic lesions (Yasuda & Greer, 1978) or pharmacological blockade of corticotrophin-releasing factor (CRF) release (Moberg, 1971) suggested that the hypothalamus mediates stimulation of the HPA axis by LPS, others (Makara *et al.* 1971; Stark, Makara, Marton & Palkovits, 1973/1974) observed that LPS could stimulate corticosterone secretion even after removal of the medial hypothalamus. Recently, interleukin-1 (IL-1) (Besedovsky, Del Rey, Sorkin & Dinarello, 1986; Uehara, Gottschall, Dahl & Arimura, 1987; Rivier, Vale & Brown, 1989*b*; Gwosdow, Kumar & Bode, 1990), tumour necrosis factor (TNF) (Sharp, Matta, Peterson *et al.* 1989; Bernardini, Kamilaris, Calogero *et al.* 1990) and IL-6 (Navarra, Tsagarakis, Faria *et al.* 1991), polypeptides produced mainly by activated macrophages and monocytes during stimulation with LPS or other immunological stimulus, have been implicated in the activation of the HPA axis.

Journal of Endocrinology (1992) 133, 231–236 *Printed in Great Britain*
0022–0795/92/0133–0231 $02.00/0

A major source of neuropeptides (CRF-41, arginine vasopressin, etc.) affecting synthesis and/or release of adrenocorticotrophin (ACTH) from the pituitary gland is the hypothalamic paraventricular nucleus (PVN). In the present study, the effect of LPS was studied in rats with lesions of PVN. Lesions were made to clarify whether *in vivo* LPS could affect secretion of ACTH and corticosterone in animals in which the endogenous supply of hypophysiotrophic peptides of paraventricular origin were removed. Evidence was obtained that some of the pathways through which LPS activates the hypophysial-adrenal system may bypass the PVN.

MATERIALS AND METHODS

Animals

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

Paraventricular nucleus lesion

Five days before the experiment the rats were anaesthetized by i.p. injection of 4 mg pentobarbital/100 g body weight. The PVN was lesioned surgically as described by Makara, Stark, Karteszi *et al.* (1981). Briefly, animals were placed in a stereotaxic frame and 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 commissural 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 decapitation by observing the trace of the knife on the basal hypothalamus. Intact rats were neither anaesthetized nor operated upon.

One day before the experiment the rats were weighed and placed in individual cages. Next morning the animals were injected i.p. with either 2 mg/kg *E. coli* 0101/RG/W LPS (produced in 'F. Joliot-Curie' National Research Institute for Radiobiology and Radiohygiene by the method of Westphal, Lüderitz & Bister (1952) using phenol extraction) or 0·9% (w/v) NaCl. Later (30, 60, 90 and 240 min) they were decapitated under minimal stress (five to six animals for each group and four time-points were used). Trunk blood was collected in chilled tubes containing Na_2EDTA and centrifuged. Plasma was stored at −20 °C until assayed.

Radioimmunoassays

Plasma ACTH was determined by direct radioimmunoassay (RIA), as previously described (Kovács & Makara, 1988) using an antiserum raised against ACTH(1–32) conjugated with bovine serum albumin (BSA) in rabbits. The tracer used was ACTH(1–39) (NIAMDD) labelled with ^{125}I by the chloramine T method. The sensitivity of the assay was 0·2 fmol/tube and the intra- and interassay variations were 4 and 13% respectively.

Plasma corticosterone was determined in the plasma without extraction. The antiserum was raised in a rabbit against corticosterone–carboxymethyloxime–BSA. ^{125}I-Labelled corticosterone–carboxymethyloxime–tyrosine–methylester was used as tracer. The interference of plasma transcortin was eliminated by inactivated transcortin at low pH. The sensitivity of the assay was 0·1 pmol/tube and the intra- and interassay variations were 6·4 and 23·8% respectively.

Statistical analysis

Data were transformed to logarithms before analysis of variance, which was followed by Dunn's test for multiple comparisons. Geometric means are presented in the Figures.

RESULTS

Changes in plasma ACTH concentration (Fig. 1*a*)

Plasma ACTH concentrations in intact, sham-operated and PVN-lesioned rats after saline injection were in the range of 8–22 pmol/l throughout the experimental period, corresponding to basal ACTH values routinely obtained using our direct RIA.

In both intact and sham-operated rats, 2 mg/kg i.p. *E. coli* LPS provoked a rise in plasma ACTH levels at 30 min after the treatment. At 60 min, in both intact and sham-operated animals ACTH levels were significantly higher than the corresponding control levels. In both groups the peak ACTH levels in response to LPS were reached at 90 min and remained increased even 4 h after the treatment.

In PVN-lesioned rats, 60 and 90 min after the treatment with LPS the ACTH response was blunted, but not completely suppressed. Four hours after the treatment, however, plasma ACTH rose to a level which was not different from the hormone levels obtained in intact and sham-operated animals at the same time-point.

Changes in plasma corticosterone concentration (Fig. 1*b*)

The basal corticosterone concentration in the plasma of saline-treated animals was in the range of 32–200 nmol/l. In intact rats, LPS treatment caused a

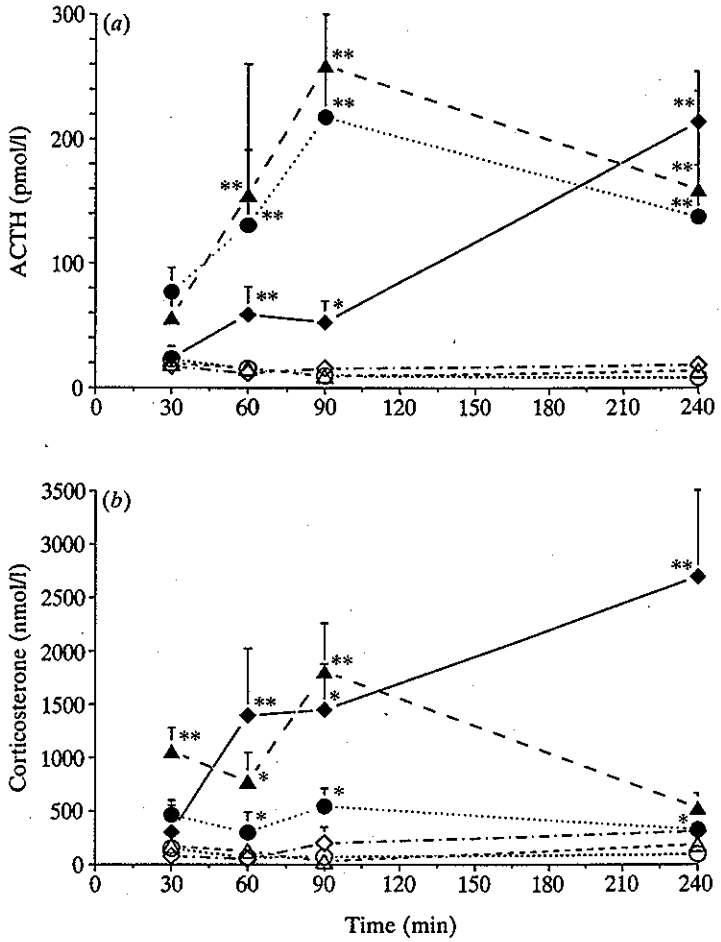

FIGURE 1. Time-course of the effect of 2 mg lipopolysaccharide (LPS)/kg injected intraperitoneally on (*a*) plasma ACTH levels and (*b*) plasma corticosterone levels in intact (●), sham-operated (▲) and rats with lesions of the paraventricular nucleus (PVN) (◆). Intact (○), sham-operated (△) and PVN-lesioned (◇) animals injected with saline were used as controls. Means and S.E.M. of the results from five to six animals are shown for each time-point. Where standard errors are not shown, they were smaller than the symbols. $*P<0{\cdot}05$, $**P<0{\cdot}01$ compared with the corresponding saline-treated group.

three to five times increase in plasma levels of corticosterone above the control levels of saline-treated animals as early as 30 min after i.p. injection of LPS. The peak of the response was at 90 min (544 nmol/l), but 4 h after treatment plasma corticosterone was still three times higher than in the saline-injected controls.

In sham-operated rats, the overall corticosterone response to LPS was higher than that of the intact animals. LPS in these rats induced a 7 to 56 times increase in corticosterone levels above the baseline. The maximal increase was also at 90 min (1810 nmol/l).

The induction of plasma corticosterone by LPS was the highest in PVN-lesioned rats. The highest concentration was measured 4 h after LPS injection (2695 nmol/l). In contrast to plasma ACTH levels measured 1 h after the treatment, plasma corticosterone was increased to a level comparable with that of the sham-operated control (777 vs 1395 nmol/l respectively).

DISCUSSION

The HPA axis plays an important role in the bidirectional communication between the neuroendocrine and immune systems. There is growing evidence that activation of the HPA axis is involved in the acute-phase response caused by infection or immunological reaction or (under experimental conditions)

after LPS treatment (Egdahl *et al.* 1959; Makara *et al.* 1971; Moberg, 1971; Yasuda & Greer, 1978; Suzuki *et al.* 1986; Rivier *et al.* 1989*a*). However, the mechanism and the crucial site or sites of the effect of LPS and related cytokines on the HPA are not well understood.

In the present study, evidence was provided that the LPS-induced activation of the HPA axis may be partially independent of the ACTH-releasing factors produced by the hypothalamic PVN.

The time of the paraventricular lesion (5 days before the experiment) was chosen because at that time rats have recovered from surgical trauma but their capacity to release either ACTH or corticosterone in response to different stressors is minimal (Dohanics, Kapocs, Janaky *et al.* 1986). Immunohistochemical investigations showed that in these rats there is a dramatic decrease of CRF-41- and arginine vasopressin (AVP)-positive nerve terminals in the zona externa of the median eminence (Antoni, Kovács, Dohanics *et al.* 1988). The CRF-41 concentration in this region fell to about 5–10% of that in sham-operated rats (Makara, Stark, Kapocs & Antoni, 1986). In intact and sham-operated rats, the activation of pituitary hormone secretion was seen as early as 60 min after LPS treatment and reached its maximum at 90 min. In contrast, in PVN-lesioned rats, the increase in plasma ACTH was substantially blunted in the early phase (30–90 min) and it reached its maximum after 4 h. This could be explained by a delayed activation of the pituitary ACTH secretion in PVN-lesioned rats. However, no similar phenomenon has been observed when studying the stress response to ether in these rats (Makara *et al.* 1981). The fact that the early ACTH peak is missing in PVN-lesioned rats suggests that the action of LPS at this time requires CRF-41, AVP or other releasing factors from the PVN. The delayed activation (after 4 h) of ACTH secretion in PVN-lesioned rats could also indicate that the effect of LPS (or mediators or factors that are involved) at this time is not mediated through hypophysiotrophic factors of paraventricular origin. Therefore our results suggest that *in vivo* the activation of the HPA axis by LPS might involve two phases, an early, CRF(s)-dependent, and a late, CRF(s)-independent phase. Another question is whether CRF originating outside the PVN is also involved in the ACTH response to LPS in PVN-lesioned animals, since CRF immunoreactivity has also been described in other brain regions (Joseph, Pilcher & Knigge, 1985; Hornby & Piekut, 1989) and in the periphery (Hashimoto, Murakami, Hattori *et al.* 1984). In fact different populations of CRF neurones might be activated at different time-points after treatment with LPS since it was shown (Hornby & Piekut, 1989) that CRF neuronal groups are not homogenous in that each population receives a characteristic neural input. An increase in pituitary sensitivity to CRF-41 and AVP should be also taken into account (Bruhn, Plotsky & Vale, 1984). All these questions need further investigation, however. In this respect, the use of CRF antiserum might be helpful.

Since it was reported by Blalock & Smith (1981) that lymphocytes are able to synthesize an ACTH-like molecule, we did not exclude this possibility in our system, although it remains unclear whether this ACTH could be released from the lymphocytes.

Nevertheless, our data suggest that LPS and/or related cytokines, *in vivo*, might have a direct effect on the pituitary gland to release ACTH. However, LPS alone (Yasuda & Greer, 1978) was unable to induce ACTH secretion by cultured pituitary cells. Recently it was shown (Rivier *et al.* 1989*a*) that stimulation of ACTH secretion in mice treated with LPS is at least partially mediated by, or dependent upon, the action of IL-1. While there is increasing consensus that the main site of action of IL-1 is located in the hypothalamus, through the production of CRF (Berkenbosch, van Oers, Del Rey *et al.* 1987; Sapolsky, Rivier, Yamamoto *et al.* 1987; Berkenbosch, de Goeij, Del Rey & Besedovsky, 1989; Blatteis, 1990; Hermus & Sweep, 1990), it is currently under debate as to whether IL-1 or other cytokines may act directly at the pituitary level (Bernton, Beach, Holaday *et al.* 1987) or not (Berkenbosch *et al.* 1987; Sapolsky *et al.* 1987).

Another possibility is that LPS activates the HPA in PVN-lesioned animals by an extrapituitary, non-paraventricular pathway. One such pathway is through noradrenergic modulation of ACTH release (reviewed in Plotsky, Cunningham & Widmaier, 1989; Al-Damluji, 1988). Taking our results into account together with those of Moberg (1971), that lesions of the median eminence (ME) totally abolished the effect of LPS, we suggest that *in vivo* the effect of LPS is at the level of the ME. The recent data of Sharp *et al.* (1989) support this hypothesis. Using intraparenchymal injections of IL-1β adjacent to the hypothalamic ME, they observed increased ACTH levels and suggested that CRF-containing nerve terminals in the ME are the targets. It was shown that, in the system we used, PVN lesion caused a dramatic decrease in CRF-41 content of the ME (Dohanics *et al.* 1986); however, noradrenergic nerve terminals were not damaged, since they originate from the lateral tegmental system and locus coeruleus. The other possible target in the ME might therefore be the noradrenergic nerve terminals. It has been shown (Vizi, Harsing, Zimanyi & Gaal, 1985) that the release of noradrenaline (NA) from these nerve terminals in the isolated ME is not subject to so-called 'negative feedback' modulation, and it was suggested that NA released from such nerve terminals might act not only upon adjacent

elements, to exert tonic control on hormone-containing nerve terminals, but also more diffusely, influencing vast assemblies of neurones (Vizi & Lábos, 1991). It was demonstrated by Matta, Singh, Newton & Sharp (1990) that chronic neurotoxic ablation of central catecholaminergic neurones and acute depletion of central catecholamine pools are both associated with a substantial reduction in the ACTH secretory response to IL-1β instilled into the ME, suggesting that this response depends on the local release of catecholamines. In fact we have recently shown that TNF was able to inhibit the stimulation-evoked release of NA from the isolated ME (I. J. Elenkov, K. Kovács, E. Duda, E. Stark & E. S. Vizi, unpublished results), suggesting that LPS-related cytokines might be able to affect NA release in the ME directly.

According to the findings described in the literature plasma corticosterone was also elevated after LPS treatment. The effect of LPS on corticosterone levels was seen as early as 30 min after the injection, preceding the peak of plasma ACTH, suggesting that LPS, *in vivo*, has a direct effect on adrenal steroid secretion. This possibility has also been suggested for the effect of TNF (Bernardini *et al.* 1990). In our experiments, the sham-operated animals exhibited the same ACTH responses to LPS as intact animals but showed enhanced corticosterone responses. We have no explanation for this difference in the responses at presence. The time difference between the induction of corticosterone and ACTH is more conspicuous in our PVN-lesioned rats, where plasma corticosterone levels were elevated between 30 and 90 min after injection, in spite of the blunted ACTH secretion. This phenomenon fits the general concept that mediators of the immune response produce counter-regulatory elevations in the secretion of adrenal steroids, which are known to inhibit either the immune or the hypothalamo-pituitary responses. It is noteworthy that the peak of the corticosterone feedback signal precedes ACTH induction. However, the possibility that the adrenal sensitivity to ACTH in PVN-lesioned rats is enhanced should also be taken into account.

In summary, we have shown that LPS could activate the hypophysial-adrenal system in the absence of hypophysiotrophic neuropeptides of paraventricular origin.

ACKNOWLEDGEMENTS

We thank Miss E. Hegedüs for excellent technical assistance.

REFERENCES

Al-Damluji, S. (1988). Adrenergic mechanisms in the control of corticotrophin secretion. *Journal of Endocrinology* **119**, 5–14.

Antoni, F. A., Kovács, K. J., Dohanics, J., Makara, G. B., Holmes, M. C. & Mazurek, M. F. (1988). Hypophysiotropic function of vasopressin and oxytocin. *Brain Research Bulletin* **20**, 729–736.

Berkenbosch, F. B., de Goeij, D. E. C., Del Rey, A. & Besedovsky, H. O. (1989). Neuroendocrine, sympathetic and metabolic responses induced by interleukin-1. *Neuroendocrinology* **50**, 570–576.

Berkenbosch, F. B., van Oers, J., Del Rey, A., Tilders, F. & Besedovsky, H. O. (1987). Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1. *Science* **238**, 524–526.

Bernardini, R., Kamilaris, T. C., Calogero, A. E., Johnson, E. O., Gomez, M. T., Gold, P. W. & Chrousos, G. P. (1990). Interactions between tumor necrosis factor-α, hypothalamic corticotropin-releasing hormone, and adrenocorticotropin secretion in the rat. *Endocrinology* **126**, 2876–2881.

Bernton, E. W., Beach, J. E., Holaday, J. W., Smallridge, R. C. & Fein, H. G. (1987). Release of multiple hormones by a direct action of interleukin-1 on pituitary cells. *Science* **238**, 519–521.

Bertók, L. (1983). Bacterial endotoxins and nonspecific resistance. In *Traumatic Injury: Infection and other Immunologic Sequelae*, pp. 119–143, Ed. J. L. Ninnemann. Baltimore, MD: University Park Press.

Besedovsky, H. O., Del Rey, A., Sorkin, E. & Dinarello, C. A. (1986). Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. *Science* **233**, 652–654.

Blalock, J. E. & Smith, E. M. (1981). Human lymphocyte production of corticotropin and endorphin-like substances: association with leukocyte interferon. *Proceedings of the National Academy of Sciences of the U.S.A.* **78**, 7530–7534.

Blatteis, C. M. (1990). Neuromodulative actions of cytokines. *The Yale Journal of Biology and Medicine* **63**, 133–146.

Bruhn, T. O., Plotsky, P. M. & Vale, W. W. (1984). Effect of paraventricular lesions on corticotrophin-releasing factor (CRF)-like immunoreactivity in the stalk-median eminence: studies on the adrenocorticotropin response to ether stress and exogenous CRF. *Endocrinology* **114**, 57–62.

Dohanics, J., Kapocs, G., Janaky, T., Kiss, J. Z., Rappay, G., Laszlo, F. A. & Makara, G. B. (1986). Mechanism of restoration of ACTH release in rats with long-term lesions of the paraventricular nuclei. *Journal of Endocrinology* **111**, 75–82.

Egdahl, R., Melby, J. C. & Spink, W. W. (1959). Adrenal cortical and body temperature responses to repeated endotoxin administration. *Proceedings of the Society for Experimental Biology and Medicine* **101**, 369–372.

Gwosdow, A. R., Kumar, M. S. A. & Bode, H. H. (1990). Interleukin-1 stimulation of the hypothalamic-pituitary-adrenal axis. *American Journal of Physiology* **258**, E65–E70.

Hashimoto, K., Murakami, K., Hattori, T., Niimi, M., Fujimo, K. & Ota, Z. (1984). Corticotropin-releasing factor (CRF)-like immunoreactivity in the adrenal medulla. *Peptides* **5**, 707–712.

Heinrich, P. C., Castell, J. V. & Andus, T. (1990). Interleukin-6 and the acute phase response. *Biochemical Journal* **265**, 621–636.

Hermus, A. R. M. M. & Sweep, C. G. J. (1990). Cytokines and the hypothalamic-pituitary-adrenal axis. *Journal of Steroid Biochemistry and Molecular Biology* **37**, 867–871.

Hornby, P. J. & Piekut, D. T. (1989). Opiocortin and catecholamine input to CRF-immunoreactive neurons in rat forebrain. *Peptides* **10**, 1139–1146.

Joseph, S. A., Pilcher, W. H. & Knigge, K. M. (1985). Anatomy of the corticotropin-releasing factor and opiomelanocortin systems of the brain. *Federation Proceedings* **44**, 100–107.

Kovács, K. J. & Makara, G. B. (1988). Corticosterone and dexamethasone act at different brain sites to inhibit adrenalectomy-induced adrenocorticotropin hypersection. *Brain Research* **474**, 205–210.

Makara, G. B., Stark, E., Kapocs, G. & Antoni, F. A. (1986). Long-term effects of hypothalamic paraventricular lesions on CRF content and stimulated ACTH secretion. *American Journal of Physiology* **250,** E319–E324.

Makara, G. B., Stark, E., Karteszi, M., Palkovits, M. & Rappay, Gy. (1981). Effects of paraventricular lesions on stimulated ACTH release and CRF in stalk-median eminence of the rat. *American Journal of Physiology* **240,** E441–E446.

Makara, G. B., Stark, E. & Meszaros, T. (1971). Corticotropin release induced by *E. coli* endotoxin after removal of the medial hypothalamus. *Endocrinology* **88,** 412–414.

Matta, S. G., Singh, J., Newton, R. & Sharp, B. M. (1990). The adrenocorticotrophin response to interleukin-1β instilled into rat median eminence depends on the local release of catecholamines. *Endocrinology* **127,** 2175–2182.

Moberg, G. P. (1971). Site of action of endotoxins on hypothalamic-pituitary-adrenal axis. *American Journal of Physiology* **220,** 397–400.

Navarra, P., Tsagarakis, S., Faria, M. S., Rees, L. H., Besser, G. M. & Grossman, A. B. (1991). Interleukin-1 and -6 stimulate the release of corticotropin-releasing hormone-41 from rat hypothalamus *in vitro* via the eicosanoid cyclooxygenase pathway. *Endocrinology* **128,** 37–44.

Plotsky, P. M., Cunningham, E. T., Jr & Widmaier, E. P. (1989). Catecholaminergic modulation of corticotropin-releasing factor and adrenocorticotropin secretion. *Endocrine Reviews* **10,** 437–458.

Rivier, C., Chizzonite, R. & Vale, W. (1989*a*). In the mouse, the activation of the hypothalamic-pituitary-adrenal axis by lipopolysaccharide (endotoxin) is mediated through interleukin-1. *Endocrinology* **125,** 2800–2805.

Rivier, C., Vale, W. & Brown, M. (1989*b*). In the rat, interleukin-1α stimulates adrenocorticotropin and catecholamine release. *Endocrinology* **125,** 3096–3102.

Sapolsky, R., Rivier, C., Yamamoto, G., Plotsky, P. & Vale, W. (1987). Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. *Science* **238,** 522–524.

Sharp, B. M., Matta, S. G., Peterson, P. K., Newton, R., Chao, C. & McAllen, K. (1989). Tumor necrosis factor-α is a potent ACTH secretagogue: comparison to interleukin-1β. *Endocrinology* **124,** 3131–3133.

Stark, E., Makara, G. B., Marton, J. & Palkovits, M. (1973/1974). ACTH release in rats after removal of the medial hypothalamus. *Neuroendocrinology* **13,** 224–233.

Suzuki, S., Oh, C. & Nakano, K. (1986). Pituitary-dependent and -independent secretion of CS caused by bacterial endotoxin in rats. *American Journal of Physiology* **250,** E470–E474.

Uehara, A., Gottschall, P. E., Dahl, R. R. & Arimura, A. (1987). Interleukin-1 stimulates ACTH release by an indirect action which requires endogenous corticotropin releasing factor. *Endocrinology* **121,** 1580–1582.

Vizi, E. S., Harsing, L. G., Jr, Zimanyi, I. & Gaal, G. (1985). Release and turnover of noradrenaline in isolated median eminence: lack of negative feedback modulation. *Neuroscience* **16,** 907–916.

Vizi, E. S. & Lábos, E. (1991). Nonsynaptic interactions at presynaptic level. *Progress in Neurobiology* **37,** 145–163.

Westphal, O., Lüderitz, O. & Bister, F. (1952). Uber die Extraction von Bacterien mit Phenol-wasser. *Zeitschrift für Naturforschung* **7b,** 148–151.

Yasuda, N. & Greer, M. A. (1978). Evidence that the hypothalamus mediates endotoxin stimulation of adrenocorticotropic hormone secretion. *Endocrinoogy* **102,** 947–953.

Makara, G. B., Stark, E., Kapocs, G. & Antoni, F. A. (1986). Long-term effects of hypothalamic paraventricular lesions on CRF content and stimulated ACTH secretion. *American Journal of Physiology* **250,** E319–E324.

Makara, G. B., Stark, E., Karteszi, M., Palkovits, M. & Rappay, Gy. (1981). Effects of paraventricular lesions on stimulated ACTH release and CRF in stalk-median eminence of the rat. *American Journal of Physiology* **240,** E441–E446.

Makara, G. B., Stark, E. & Meszaros, T. (1971). Corticotropin release induced by *E. coli* endotoxin after removal of the medial hypothalamus. *Endocrinology* **88,** 412–414.

Matta, S. G., Singh, J., Newton, R. & Sharp, B. M. (1990). The adrenocorticotrophin response to interleukin-1β instilled into rat median eminence depends on the local release of catecholamines. *Endocrinology* **127,** 2175–2182.

Moberg, G. P. (1971). Site of action of endotoxins on hypothalamic-pituitary-adrenal axis. *American Journal of Physiology* **220,** 397–400.

Navarra, P., Tsagarakis, S., Faria, M. S., Rees, L. H., Besser, G. M. & Grossman, A. B. (1991). Interleukin-1 and -6 stimulate the release of corticotropin-releasing hormone-41 from rat hypothalamus *in vitro* via the eicosanoid cyclooxygenase pathway. *Endocrinology* **128,** 37–44.

Plotsky, P. M., Cunningham, E. T., Jr & Widmaier, E. P. (1989). Catecholaminergic modulation of corticotropin-releasing factor and adrenocorticotropin secretion. *Endocrine Reviews* **10,** 437–458.

Rivier, C., Chizzonite, R. & Vale, W. (1989*a*). In the mouse, the activation of the hypothalamic-pituitary-adrenal axis by lipopolysaccharide (endotoxin) is mediated through interleukin-1. *Endocrinology* **125,** 2800–2805.

Rivier, C., Vale, W. & Brown, M. (1989*b*). In the rat, interleukin-1α stimulates adrenocorticotropin and catecholamine release. *Endocrinology* **125,** 3096–3102.

Sapolsky, R., Rivier, C., Yamamoto, G., Plotsky, P. & Vale, W. (1987). Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. *Science* **238,** 522–524.

Sharp, B. M., Matta, S. G., Peterson, P. K., Newton, R., Chao, C. & McAllen, K. (1989). Tumor necrosis factor-α is a potent ACTH secretagogue: comparison to interleukin-1β. *Endocrinology* **124,** 3131–3133.

Stark, E., Makara, G. B., Marton, J. & Palkovits, M. (1973/1974). ACTH release in rats after removal of the medial hypothalamus. *Neuroendocrinology* **13,** 224–233.

Suzuki, S., Oh, C. & Nakano, K. (1986). Pituitary-dependent and -independent secretion of CS caused by bacterial endotoxin in rats. *American Journal of Physiology* **250,** E470–E474.

Uehara, A., Gottschall, P. E., Dahl, R. R. & Arimura, A. (1987). Interleukin-1 stimulates ACTH release by an indirect action which requires endogenous corticotropin releasing factor. *Endocrinology* **121,** 1580–1582.

Vizi, E. S., Harsing, L. G., Jr, Zimanyi, I. & Gaal, G. (1985). Release and turnover of noradrenaline in isolated median eminence: lack of negative feedback modulation. *Neuroscience* **16,** 907–916.

Vizi, E. S. & Lábos, E. (1991). Nonsynaptic interactions at presynaptic level. *Progress in Neurobiology* **37,** 145–163.

Westphal, O., Lüderitz, O. & Bister, F. (1952). Über die Extraction von Bacterien mit Phenol-wasser. *Zeitschrift für Naturforschung* **7b,** 148–151.

Yasuda, N. & Greer, M. A. (1978). Evidence that the hypothalamus mediates endotoxin stimulation of adrenocorticotropic hormone secretion. *Endocrinoogy* **102,** 947–953.