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CYTOKINES AS MODULATORS OF THE HYPOTHALAMUS–PITUITARY–ADRENAL AXIS

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Summary—The hypothalamus–pituitary–adrenal (HPA) axis is stimulated during the course of certain immune, inflammatory and neoplastic processes. IL-1 is an important immunologically derived cytokine mediating the stimulation of this axis, although not the only one. We have compared the relative potencies of the cytokines IL-1, IL-6 and tumor necrosis factor (TNF), which share several biological actions, for stimulating ACTH and corticosterone output in freely-moving rats. Although all three cytokines can stimulate the HPA axis, IL-1 was the most potent. This effect of IL-1 was also present during the neonatal period, when the response of the HPA axis to acute stress is reduced in rodents. The results support the existence of an immune–HPA axis circuit. The biological and clinical relevance of this circuit is discussed.

INTRODUCTION

It is now generally accepted that the immune system has been acquired during evolution as a host defense system, to cope with the impact of infective and other external agents and the expression of modified self antigens on general homeostasis. Although pathological processes involving the immune system are often restricted to a given tissue or organ, the whole organism is usually affected, as reflected by the neuro-endocrine and metabolic alterations which are frequently noticed in the host. Such host responses may be mediated by either the causal agent(s) of the disease and the stress of being sick, or by products from activated immunological cells. To discriminate between these two possibilities we stimulated the immune system with innocuous antigens, to elicit an immune response not related to disease states. Using this procedure, changes in endocrine, autonomic and CNS functions were observed, showing that the immune response itself is involved in causing these changes [1]. Thus, products which are able to affect neuro-endocrine mechanisms must be released from or expressed by activated immunological cells during the course of immune responses. Since one of the effects of immune stimulation was elevation of glucocorticoid blood levels [2–4], we searched for products derived from immune cells that could mediate this effect [5, 6]. For this purpose, we originally used conditioned media from immune cells stimulated *in vitro*, which contain several immune-derived substances including lymphokines and monokines. As soon as these cytokines became available in pure form, we started to test their capacity to stimulate the hypothalamus–pituitary–adrenal (HPA) axis. The first pure lympho-monokine natural or recombinant found to stimulate ACTH and glucocorticoid release by mechanisms integrated at hypothalamic levels was IL-1 [7–10]. Later, other cytokines such as tumor necrosis factor (TNF) and IL-6, both involved in the acute phase response during infectious and inflammatory processes, were also reported to exert a similar effect [11, 12]. We have therefore conducted studies to compare their potencies with that of IL-1 and the results are reported in this paper. Furthermore, we provide data showing that IL-1 is already capable of stimulating the HPA axis during the neonatal period.

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EXPERIMENTAL

Animals

Wistar albino male rats (8–10 weeks old, 200–250 g body wt) were purchased from Zentralinstitut für Versuchstierzucht Gmbh, (Hannover, Germany). C57Bl/6J mouse breeding stocks were obtained from the same source and littermates were reared in our animal

facilities at the Kantonsspital (Basel, Switzerland). Rats were caged individually for 7 days before being implanted with a canula and kept isolated throughout the experiment. Animals were fed *ad libitum* and housed in temperature- and light-(12 h cycles)controlled rooms.

Canulation

Rats were chronically canulated according to standard procedures. Briefly, silicon canulas were inserted into one of the jugular veins under anesthesia, the tip of the canula being placed at the entrance of the atrium. The whole procedure was carried out under conditions as sterile as possible. The implanted canulas were checked for their patency at least twice a week and animals were only used for the experiments 5 or more days after operation.

Cytokines

Recombinant human IL-1 (β form, M_w 17.5 kDa) was kindly provided by Dr A. Shaw (Glaxo Institute for Molecular Biology S.A., Geneva). It had sp. act. = 2.5×10^7 U/mg in an LAF assay. Recombinant human IL-6 (M_w 26 kDa), kindly provided by Dr M. Schreier (Sandoz AG, Basel), had sp. act. = 3.3×10^7 U/mg as determined in a B13–29 assay. Recombinant human TNFα (M_w 17 kDa), kindly provided by Dr O. Brokehaus (Hoffman-La Roche AG, Basel), had sp. act. = $5–10 \times 10^8$ U/mg, as determined in a cytotoxic assay using WEHI 164 cells.

Experimental procedures

The first blood sample (time 0) was withdrawn at about 10 a.m. and immediately after, 200 μl of control medium with or without different doses of each cytokine were injected i.v. into rats through the implanted canulas. Subsequent blood samples, obtained at the times indicated in the figures, were collected in EDTA-coated tubes, immediately centrifuged and aliquoted from hormone determinations. 3-Day-old mice which had received either 2 ng/g body wt of IL-1 or control medium injected i.p., were killed by decapitation 2 h later and trunk blood was collected into EDTA-coated tubes. A third group of mice was separated from the mother, kept at 11°C for 30 min (cold stress) and killed as indicated above.

Hormone determinations

ACTH levels in plasma were determined by radioimmunoassay (RIA) using the kit and prescriptions supplied by Radioassay System Labs (Carson, CA). Corticosterone plasma levels were also determined by RIA as described previously [6], however ethanol precipitation was replaced by heating the samples, previously diluted with the buffer, at 98°C for 15 min.

RESULTS

IL-1 stimulates the HPA axis

An i.v. injection of IL-1 induced dose-dependent increases in corticosterone and ACTH blood levels. As shown in Fig. 1, this is a quick effect since it is noticed within 15 min following i.v. administration.

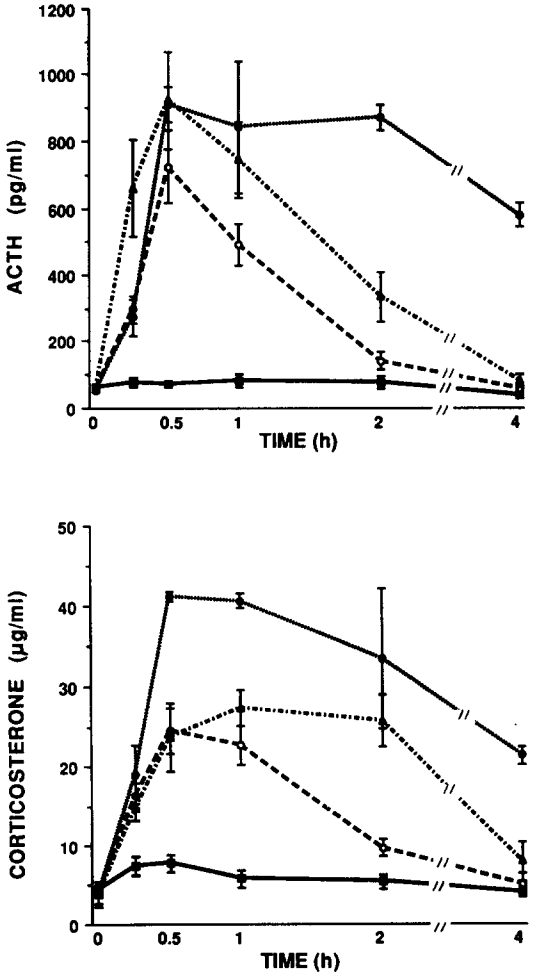

Fig. 1. IL-1 stimulates the HPA axis in freely-moving rats. Different doses of IL-1 were injected i.v. into rats. Blood samples were obtained at the times indicated and ACTH and corticosterone blood levels determined by RIA. Each point in the curves represents the mean ± SEM from 5–10 determinations. —■— Control; – –○– – IL-1 (0.25 μg/rat); -·-▲-·- IL-1 (1 μg/rat); ··●·· IL-1 (5 μg/rat)

Comparison of studies on the effects of IL-1, IL-6 and TNF on the HPA axis

The effect of various doses of IL-1, IL-6 and TNF on corticosterone blood levels 1 h after the administration of the cytokines is shown in Fig. 2. The molecular weights of the three cytokines are within the same range. Thus, the data clearly show that, on a molecular weight basis, IL-1 is the most potent of the three cytokines in inducing increased corticosterone levels in blood. Only the highest doses of IL-6 and TNF increased corticosterone levels significantly 1 h after administration.

The time courses of the increases in ACTH and corticosterone levels following administration of different doses of IL-6 and TNF are shown in Figs 3 and 4, respectively. The effect induced by the lowest dose of IL-1 is also shown for comparison. IL-6 and TNF were both capable of stimulating ACTH and corticosterone output. However, these effects were delayed compared with that of IL-1, and even about 20-fold higher doses of IL-6 and TNF were not capable of eliciting an endocrine response similar in magnitude to that of IL-1.

Effect of neonatal administration of IL-1 on the HPA axis

Administration of 2 ng/g body wt of IL-1 to 3-day-old mice resulted in a 15-fold increase in corticosterone blood levels (Fig. 5). In contrast, 30 min of cold stress did not induce an elevation of corticosterone blood levels.

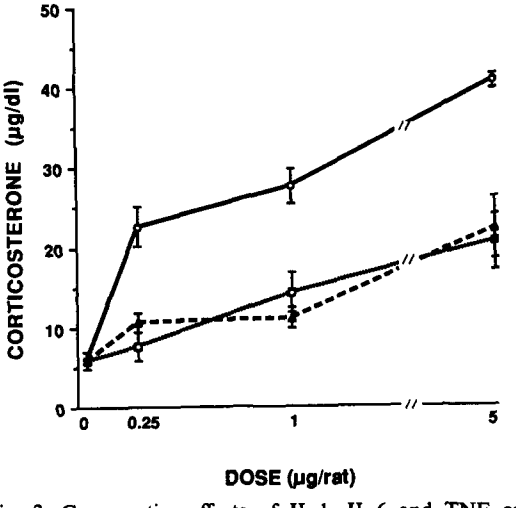

Fig. 2. Comparative effects of IL-1, IL-6 and TNF on corticosterone blood levels. Different doses of IL-1, IL-6 and TNF were injected i.v. into rats and blood samples were obtained 1 h after injection. Each point in the curves represents the mean ± SEM from 5–10 determinations, —○— IL-1; ··□·· IL-6; --▲-- TNF.

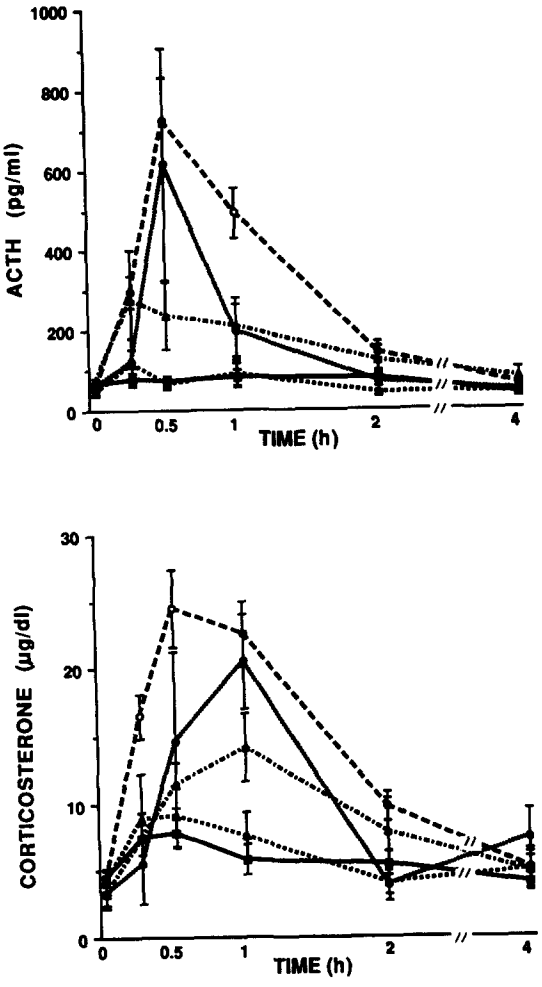

Fig. 3. Comparative effects of IL-1 and different doses of IL-6 on ACTH and corticosterone blood levels. Different doses of IL-6 (--△-- 0.25 µg/rat; ···▲··· 1 µg/rat; ··●·· 5 µg/rat), 0.25 µg/rat of IL-1 (--○--) or control medium (—■—) were injected i.v. into rats. Animals were bled at different times and ACTH and corticosterone plasma levels were determined by RIA. Each point in the curves represents the mean ± SEM from 5–10 determinations.

DISCUSSION

As shown in this paper, IL-1, IL-6 and TNF can stimulate the HPA axis. However, IL-1 induces a more intense and persistent effect than IL-6 and TNF. Furthermore, as can be seen in Figs 3 and 4, the effects of IL-6 and TNF are delayed compared with that of IL-1. It has been shown that TNF is able to induce IL-1 release. Therefore, at least for TNF, it can not be excluded that the effect on the HPA axis attributed to this cytokine, is actually exerted by the secondary induction of IL-1.

Figure 5 shows that the HPA axis of 3-day-old mice can be stimulated by IL-1. Assuming that this effect is mediated in the same way as in adult animals through increased CRF

release [8–10], these experiments show that the HPA axis is already mature enough to mount an effective response at this stage of development. However, the response to acute stress, e.g. cold stress is not, or only marginally present during the neonatal period [13–15]. This suggests that the neural connections between the structures that integrate sensorial inputs leading to stress, and those that control the HPA axis are not yet completely mature.

The data reported here, together with the fact that innocuous antigens [2, 3] and infective agents [16, 17] cause increased corticosterone blood levels, reinforce the view that immune-derived cytokines integrate the immune–HPA axis circuit. Increased levels of ACTH and glucocorticoids are known to affect the function

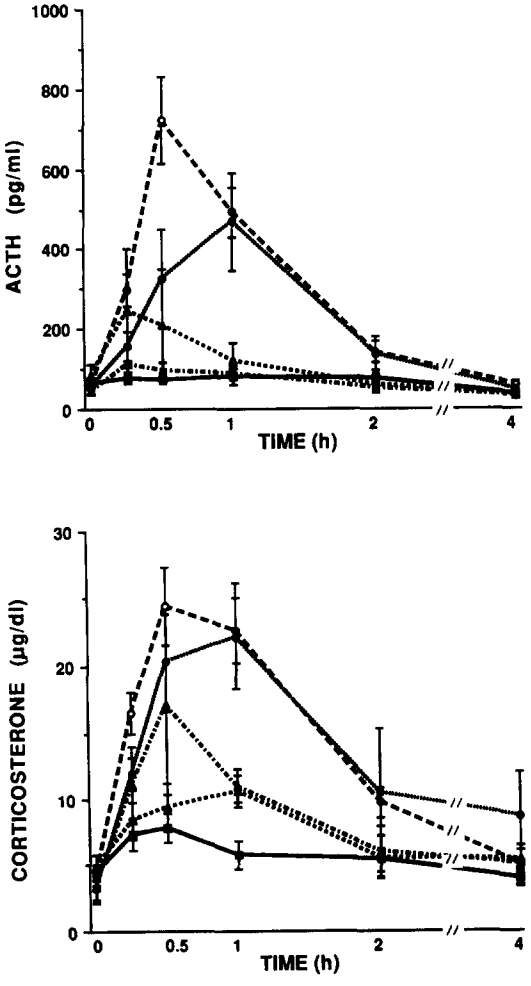

Fig. 4. Comparative effects of IL-1 and different doses of TNF on ACTH and corticosterone blood levels. Different doses of TNF (- -△- - 0.25 μg/rat; - · -▲- · - 1 μg/rat; · ·●· · 5 μg/rat), 0.25 μg/rat of IL-1 (– –○– –) or control medium (—■—) were injected i.v. into rats. Animals were bled at different times and ACTH and corticosterone plasma levels were determined by RIA. Each point in the curves represent, the mean ± SEM from 5–10 determinations.

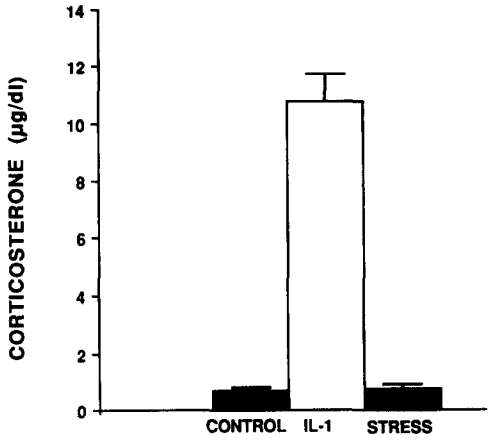

Fig. 5. Effect of IL-1 or cold stress on corticosterone blood levels of 3-day-old mice. Animals received either 2 ng/g body wt of IL-1 or control medium injected i.p., or were subjected to cold stress (see Experimental). Corticosterone plasma levels were determined 2 h after injection or 30 min after stress. Bars indicate the mean ± SEM from 10 (control), 7 (IL-1) and 5 (stress) determinations.

of different immunological cells. However, one of the most conspicuous effects of glucocorticoids on the immune system is the control of the production and action of lympho-monokines, including those known to affect the HPA axis [18–21]. This reveals the existence of a feedback mechanism that involves glucocorticoid hormones on the one hand and immune-derived cytokines on the other.

Several factors that modulate glucocorticoid output and/or the effect of increased glucocorticoid levels should also be included in the immune–HPA axis circuitry. One such factor can suppress ACTH-induced steroidogenesis *in vitro* and is present in supernatants of LPS-treated macrophages [22]. Another, present in supernatants of mitogen-stimulated spleen cells and distinguishable from IL-1, IL-2, IL-3 and interferon, seems to selectively block the suppressive action of glucocorticoids on helper cells [23]. Similarly, IL-1 protects specific T helper cells but not suppressor and cytotoxic T cells [24] from inhibition by glucocorticoids.

Antigen presentation and the release of certain lympho-monokines are sensitive to glucocorticoids. However, since these early events of the immune response precede the increase in the levels of endogeneous glucocorticoids, they will not be affected. As the immune response proceeds, the stimulation of the HPA axis will result in inhibition of the production of certain lympho-monokines [18–21]. As mentioned above, specific helper T cells might be protected by IL-1 and other cytokines from the

inhibitory effects of glucocorticoids. In this way, the consequences of increased glucocorticoid levels for already activated cells with high affinity for the antigen may be different from those for accessory cells or resting or low affinity lymphocytes. Since the latter are more sensitive to glucocorticoids than activated lymphocytes, we have postulated that the function of this circuit could be to prevent the excessive expansion of cells which are recruited under the polyclonal influence of lymphokines and monokines.

The variety of factors which contribute to the immune-mediated stimulation of the HPA axis provide evidence to the redundancy of these mechanisms. Redundant mechanisms are usually associated with regulatory effects of high biological relevance. In the following, we shall comment briefly on some examples of the biological and clinical relevance of cytokine-induced stimulation of the HPA axis:

1. Glucocorticoids exert protective effects during sepsis. LPS administration, frequently used as a model of sepsis, results in increased glucocorticoid levels and it has been shown that this effect is mediated through IL-1 [25]. Impediment of such hormonal changes following LPS inoculation results in increased mortality [26].
2. Endogeneous glucocorticoids contribute to the control of excessive inflammatory responses. We have already demonstrated that during inflammatory processes, the cytokine-induced increase in the endogeneous production of glucocorticoids plays a role in moderating inflammation [4]. Furthermore, Lew/N female rats, which are susceptible to experimentally induced arthritis, have a defective HPA axis that cannot be stimulated by cytokines like IL-1 [27]. A clinical counterpart of these studies shows that patients with rheumatoid arthritis have a disturbed HPA axis [28].
3. As mentioned above, the immune–HPA axis circuit may contribute to the control of excessive cumulative expansion of immune cell mass and activity. Disturbances in this circuit may contribute to the expression of lymphoproliferative and autoimmune diseases in genetically predisposed individuals, especially since these pathological events are the product of multiple factors which progressively disrupt immune cell homeostasis. In support of this view is the finding that obese strain (OS) chickens with spontaneous autoimmune thyroiditis, in contrast to normal chickens, do not respond to immunization with sheep erythrocytes with any detectable elevation in corticosterone blood levels [29]. Furthermore, although OS chickens are capable of producing a factor which increases glucocorticoid blood levels upon injection into normal animals, they themselves are not able to respond to this factor with a similar hormonal change. Another example of the relevance of the immune–HPA axis circuit in the prevention or moderation of autoimmune diseases derives from studies on experimental allergic encephalomyelitis (EAE), an animal model of multiple sclerosis. Before and during the overt clinical expression of the experimentally induced disease, increased glucocorticoid levels, which are probably induced by immune cytokines, are observed. Such increased levels help to moderate the clinical course of the disease, since most adrenalectomized rats with EAE die during the first attack [30, 31].
4. The immune-mediated glucocorticoid output may not necessarily be beneficial for the host. We have demonstrated, for example, that IL-1 mediates the stimulation of the HPA axis after virus inoculation [32]. This increase may contribute to the immunosuppression observed during viral diseases, which often results in the appearance of opportunistic superinfections.

There is evidence that the immune–HPA axis circuit is not the only one involving immune components and neuro-endocrine mechanisms and that the immune system interacts at several levels with mechanisms integrated by the CNS. The operation of a complex network of immune–neuro–endocrine interactions contributes to immunoregulation and to the homeostatic adjustments necessary during diseases that involve the immune system.

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