Interleukin-1 Stimulates the Secretion of Hypothalamic Corticotropin-Releasing Factor

ROBERT SAPOLSKY,* CATHERINE RIVIER, GAYLE YAMAMOTO, PAUL PLOTSKY, WYLIE VALE

There is now evidence that the immune system, during times of infectious challenge, can stimulate the secretion of glucocorticoids, the adrenal steroids that mediate important aspects of the response to stress. Specifically, secretion of interleukin-1 (IL-1), a monocyte lymphokine secreted after infection, appears at least in part responsible for this effect. Glucocorticoids are secreted in response to a neuroendocrine cascade involving, first, the brain, then the pituitary, and finally the adrenal gland. In this report, human IL-1 is shown to activate the adrenocortical axis at the level of the brain, stimulating the release of the controlling hormone corticotropin-releasing factor (CRF) from the hypothalamus. Infusion of IL-1 induced a significant secretion of CRF into the circulation exiting the hypothalamus, whereas immunoneutralization of CRF blocked the stimulatory effect of IL-1 on glucocorticoid secretion. IL-1 appeared to have no acute direct stimulatory effects on the pituitary or adrenal components of this system. Furthermore, IL-1 did not cause a nonspecific release of other hypothalamic hormones. Thus, the lymphokine acts in a specific manner to activate the adrenocortical axis at the level of the brain; this effect appears to be unrelated to the known pyrogenic effects of IL-1 within the hypothalamus.

THE SECRETION OF GLUCOCORTICOIDS during stress by the adrenal cortex is central to most of the physiological adaptations that constitute the stress response. Glucocorticoid secretion is stimulated by adrenocorticotropic hormone (ACTH); the secretion of this pituitary hormone, in turn, is stimulated by the hypothalamic hormone corticotropin-releasing factor (CRF), as well as by a number of additional hypothalamic substances (such as vasopressin and norepinephrine). Finally, such hypothalamic secretion is activated by neural perception of a stressor (*1*).

One of the hallmarks of glucocorticoid action is its capacity to regulate immune function (*2*). There is a growing belief that the immune system can, in turn, activate the adrenocortical axis and provide a shortcut by which immune recognition of an infectious challenge rapidly activates the stress response. During times of antigenic challenge to the immune system, glucocorticoid secretion is enhanced in parallel with the intensity of the immune response (*3*). A controlling role for the immune system in this correlation was suggested by the observation that lymphokines (chemical mediators of immunologic activation) will provoke glucocorticoid secretion (*4*). There are a multitude of such lymphokines, and Besedovsky *et al.* (*5*) reported that it is interleukin-1 (IL-1), a lymphokine released by stimulated macrophages and monocytes, which provokes the secretion of ACTH and corticosterone (the predominant glucocorticoid of rodents). IL-1 stimulated secretion even in athymic nude mice, demonstrating that the effect did not involve T cell–derived immune constituents. That study, however, did not demonstrate the level within the adrenocortical axis at which IL-1 acts. We now report that IL-1 releases substantial quantities of CRF from the hypothalamus. Furthermore, this appears to be the sole site of IL-1 action within the adrenocortical axis.

Initially, we elaborated on the finding by Besedovsky *et al.* (*5*) that intraperitoneal injection of rat IL-1 (rIL-1) stimulated ACTH and corticosterone secretion in mice. We found that either intravenous (Fig. 1) or intracerebroventricular injection of human IL-1 (hIL-1) (*6*) also provoked dose-related increases in ACTH and corticosterone concentrations in the rat. Both routes of administration caused significant and similar stimulation of the adrenocortical axis within 10 minutes.

Such an enhancement could be due to IL-1 acting at the level of the hypothalamus to release CRF (or the related secretagogues), or to IL-1 acting at the level of the pituitary to release ACTH, or IL-1 could be acting at both sites. We next investigated the possibility that IL-1 was acting directly at the pituitary level. Woloski *et al.* (*7*) reported that murine IL-1 (mIL-1) was a very powerful ACTH secretagogue in mouse AtT-20 pituitary tumor cells. We incubated primary cultures of rat anterior pituitary cells as well as cultures of AtT-20 pituitary tumor cells with either hIL-1 or mIL-1. We found that neither hIL-1 (Fig. 2) nor mIL-1 acutely stimulated ACTH secretion in either culture

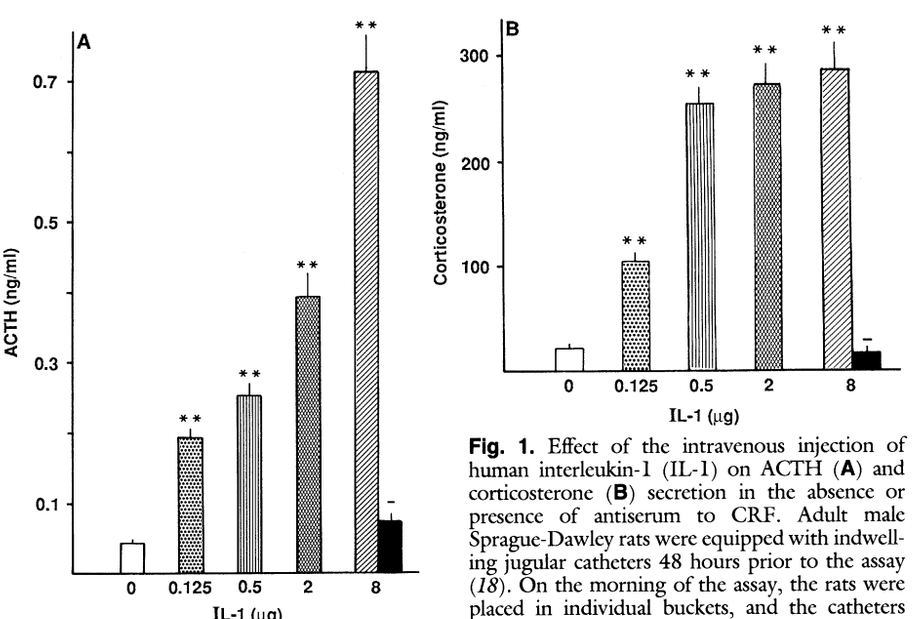

Fig. 1. Effect of the intravenous injection of human interleukin-1 (IL-1) on ACTH (**A**) and corticosterone (**B**) secretion in the absence or presence of antiserum to CRF. Adult male Sprague-Dawley rats were equipped with indwelling jugular catheters 48 hours prior to the assay (*18*). On the morning of the assay, the rats were placed in individual buckets, and the catheters were connected to syringes filled with heparinized saline. After a 3-hour rest period, a first blood sample (0.5 ml) was withdrawn from undisturbed animals. Normal rabbit serum (NRS) or antiserum to CRF was injected intravenously, then immediately followed by IL-1 diluted in saline. A second blood sample was obtained 10 minutes later. From 0 to 2 μg of IL-1 were injected with NRS only; 8 μg of IL-1 were injected with NRS (shaded) or with 0.2 ml of the CRF antiserum (solid). ACTH levels were measured in duplicate samples of individual plasma, by use of materials provided by the NIADDK distribution program. Corticosterone values were measured as previously described (*18*). (−) $P > 0.05$; (**) $P < 0.01$ from control, as calculated by a Duncan test following a one-way analysis of variance. Each point represents the mean ± SEM of five rats.

R. Sapolsky, Department of Biological Sciences, Stanford University, Stanford, CA 94305.
C. Rivier, G. Yamamoto, P. Plotsky, W. Vale, Clayton Foundation Laboratories for Peptide Biology, Salk Institute, La Jolla, CA 92037.

*To whom correspondence should be addressed.

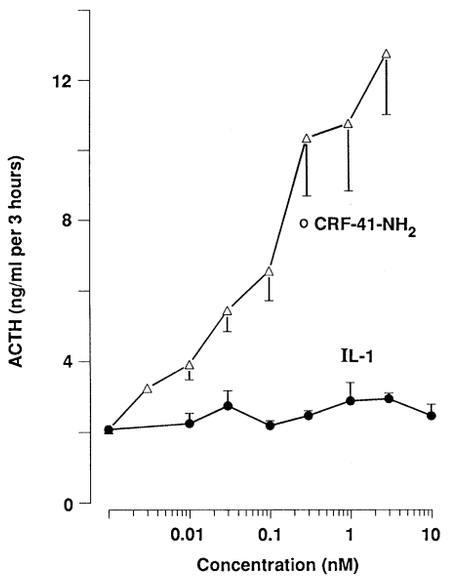

Fig. 2. Effect of CRF and IL-1 on ACTH secretion by rat anterior pituitary (RAP) cells in culture. RAP cells were dissociated and cultured in beta-PJ medium with 2% fetal bovine serum as previously described (*19*). Three days after the dispersion procedure, cells were incubated in beta-PJ medium supplemented with 0.1% bovine serum albumin plus ascorbate (50 μg/ml) and CRF or IL-1 for 3 hours at 37°C. The medium was then collected and assayed for ACTH by radioimmunoassay (*18*). The results represent the means of triplicates ± SEM. The specific activity of the IL-1 was 2.1×10^7 units of thymocyte proliferation activity per milligram of protein. The molar concentrations of CRF and IL-1 were calculated from their protein concentrations (by amino acid analysis) and molecular weights. Similar results were obtained with IL-1 on the AtT20 tumor cell line and with mIL-1 on both RAP cells and the AtT20 cell line.

system. Incubation with CRF, in contrast, stimulated ACTH secretion in a dose-related manner.

This suggested that IL-1 was acting at the hypothalamic level to stimulate the adrenocortical axis. We obtained evidence to support this hypothesis by administering hIL-1 to rats previously injected with an antibody to CRF (*8*). Thus, should IL-1 be releasing hypothalamic CRF, the ability of the latter to provoke ACTH secretion would be blocked; indeed, immunoneutralization of CRF prevented the IL-1–induced rise in ACTH and corticosterone concentrations (Fig. 1).

We then examined more directly whether IL-1 released CRF from the hypothalamus. In a series of anesthetized rats, we surgically exposed the hypophyseal-hypothalamic portal system (the circulatory system by which hypothalamic hormones are transported to the pituitary). Blood was then collected from portal vessels before and after intravenous injection with either hIL-1 or vehicle. In vehicle-treated rats, there was no significant change in portal concentrations of CRF, vasopressin, or oxytocin. In contrast, hIL-1 administration caused a significant increase in the release of CRF (Fig. 3). There was a nonsignificant trend toward increased concentrations of vasopressin, perhaps obscured because of the considerable variability that typically is found in portal concentrations of this hormone (*9*).

The prior work by Besedovsky *et al.* (*5*) gave explicit evidence that the immune system, via release of IL-1, could provoke an adrenocortical stress response. We have replicated this effect and found that such IL-1 action is manifested at the level of the brain, via release of CRF. The stimulatory effects of IL-1 on the axis could be blocked by immunoneutralizing CRF. As more direct evidence, an amount of IL-1 shown to elevate plasma ACTH concentrations also stimulated the release of CRF into the portal circulation. A similar, although nonsignificant, increase by IL-1 of vasopressin concentrations was observed; vasopressin also contributes to the release of ACTH, principally by potentiating the action of CRF at the pituitary (*10*). This pattern did not result from IL-1 merely provoking the indiscriminate release of hypothalamic peptides, since the lymphokine had no effect on oxytocin secretion.

Our results do not support the hypothesis, proposed for other immune mediators (*11*) that the pituitary or adrenal gland are independently stimulated by IL-1. Were IL-1 to possess "ACTH-like" activity, corticosterone secretion would still have been provoked by IL-1, despite immunoneutralization of IL-1–induced CRF; this was not observed (Fig. 1). Were IL-1 able to directly release ACTH from the pituitary, ACTH secretion would have been stimulated by IL-1, despite the immunoneutralization of CRF; again (as shown in Fig. 1), this was not the case. Furthermore, neither hIL-1 nor mIL-1 provoked acute ACTH release from either primary pituitary cultures or pituitary cell lines. This observation conflicts with the finding of Woloski *et al.* (*7*) that mIL-1 releases ACTH from AtT-20 cells. The reason for this discrepancy is not clear, as the IL-1 was from the identical source and was used under identical conditions; our result represents the second failure to demonstrate an IL-1–induced release of ACTH from normal pituitary cells (*12*). In addition, the pituitary does not appear to contain IL-1 receptors (*13*).

The demonstrated neural effects of IL-1 are not surprising, given its roles as a somnogen, inducing slow-wave sleep (*14*), and as an endogenous pyrogen, inducing the fever typical of infectious states (*15*). IL-1 does not appear to release CRF via its pyrogenic actions. Pyrogenicity is mediated by prostaglandin synthesis and can be blocked by indomethacin administration (*15*), whereas indomethacin does not block adrenocortical activation by IL-1 [rats implanted with a 1-mg cholesterol pellet (IRA, Toledo, Ohio) subcutaneously, 1233 ± 265 pg of ACTH per milliliter 10 minutes after

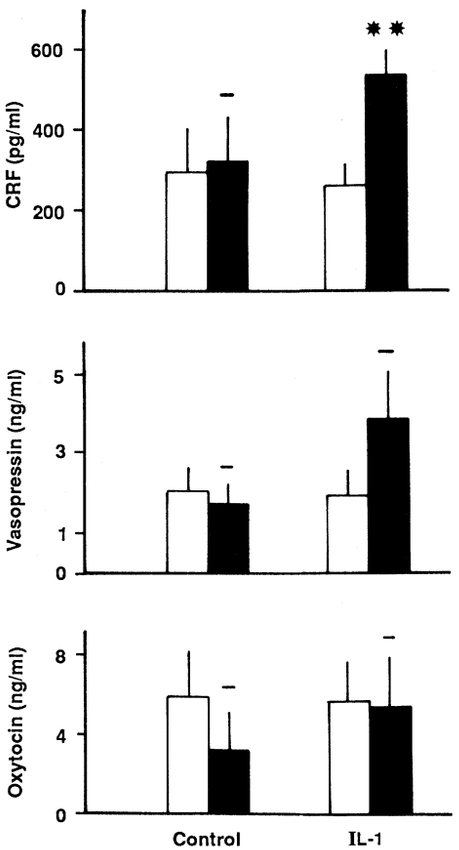

Fig. 3. Increase of concentrations of CRF in the portal blood by intravenous infusion of IL-1. Barrier-derived germ-free male Sprague-Dawley rats (300 g) were anesthetized with urethane (1.1 g per kilogram of body weight, intraperitoneally), placed in a stereotaxic device and, after tracheal intubation, had the ventral surface of the hypothalamus and pituitary exposed to allow cannulation of the hypothalamic-hypophyseal portal vessels (*20*). After the dura mater was cut, rats were allowed 45 minutes to rest before the infundibular stalk was cut at the junction of the anterior pituitary and placement of the cephalid stump of the stalk in a polyethylene cannula. Rats were then given intravenous infusions of 0.4 ml of heparin, and portal blood was collected (8 μl/min). An initial collection was made ("pre-infusion," open bars) for 30 minutes. IL-1–treated rats were then given an injection of 3 μg of hIL-1 in 1 ml of saline as a single bolus through the femoral vein. Control rats were injected with saline alone. A second ("post-infusion," closed bars) sample of 30 minutes was collected. Plasma samples were extracted on Bond Elut C_{18} cartridges before radioimmunoassay for CRF, vasopressin, and oxytocin, each in a single assay (*21*); $n = 7$ for each group. $^{**}P < 0.02$, two-tailed paired t test comparing pre- and post-infusion concentrations. Results of other comparisons were not significant.

infusion of 10 μg of IL-1; rats implanted with 1 mg of indomethacin over a 28-hour period 1225 ± 205; not significant]. As an additional mechanistic note, IL-1 need not act within the hypothalamus or even the brain to release CRF. Potentially, the peptide could be activating afferent pathways to the hypothalamus which normally mediate signals of peripheral stressors.

It is important to consider the physiological relevance of these observations. The IL-1–induced doubling of CRF concentrations is similar to that occurring after a stressor such as hypotension (*9*). It is difficult to translate the bolus injection of IL-1 at 10 μg per kilogram of body weight (Fig. 3) into the amount of IL-1 secreted during an infectious challenge, as monocytes are likely to secrete the lymphokine continuously, and glia also contain IL-1 (*16*). However, the demonstration that infection is associated with corticosterone secretion in parallel with the extent of immune activation (*3*) and that IL-1 can produce this effect in the absence of other immune constituents (*5*) suggests that the present observations may be physiologically applicable. If so, this supports the emerging view that the immune system can regulate neural and endocrine events traditionally viewed as far outside its sphere of influence. Furthermore, the specific type of regulation demonstrated here suggests a novel route by which the immune system can rapidly activate the adrenocortical axis when challenged by infection. The principal effect of glucocorticoids on the immune system is an inhibitory one (*2, 17*) and a number of theories have been proposed as to the logic of stress-induced immunosuppression by glucocorticoids (*2*). Regardless of the reason, the present and other observations suggest that the immune system has a novel and active role in promoting the adrenocortical stress response during times of infectious challenge.

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Corticotropin-Releasing Factor–Producing Neurons in the Rat Activated by Interleukin-1

FRANK BERKENBOSCH, JOEP VAN OERS, ADRIANA DEL REY, FRED TILDERS, HUGO BESEDOVSKY

Intraperitoneal administration of human recombinant interleukin-1 (IL-1) to rats can increase blood levels of corticosterone and adrenocorticotropic hormone (ACTH). The route by which IL-1 affects pituitary-adrenal activity is unknown. That the IL-1–induced pituitary-adrenal activation involves an increased secretion of corticotropin-releasing factor (CRF) is indicated by three lines of evidence. First, immunoneutralization of CRF markedly attenuated the IL-1–induced increase of ACTH blood levels. Second, after blockade of fast axonal transport in hypothalamic neurons by colchicine, IL-1 administration decreased the CRF immunostaining in the median eminence, indicating an enhanced release of CRF in response to IL-1. Third, IL-1 did not stimulate ACTH release from primary cultures of anterior pituitary cells. These data further support the notion of the existence of an immunoregulatory feedback circuit between the immune system and the brain.

THERE IS INCREASING SUPPORT FOR the view that a bidirectional communication exists between neuroendocrine systems and the immune system (*1*). For instance, glucocorticoid-associated immunoregulatory mechanisms are implicated in a constant surveillance of the activity of immune cells (*2*). In addition, opioid peptides derived from different opioid precursors, and also sex steroids, prolactin, and catecholamines affect immune competence (*3*). Conversely, immune cell–derived products such as lymphokines and monokines have been proposed to influence brain function. Interleukin-1 (IL-1), a protein produced predominantly by activated macrophages and monocytes, has an important role in the regulation of immune defense (*4*) as well as several nonimmunological effects (*5*). In a recent study, subpyrogenic doses of IL-1 were found to activate the pituitary-adrenal system of mice and rats independently of a secondary release of products from mature T cells (*6*). Studies involving immunoneutralization of IL-1 support the notion that IL-1 may be a key factor mediating the increased pituitary-adrenal activation in animals undergoing immunological responses (*6, 7*). In this report, we show that the IL-1–induced pituitary-adrenocortical response in rats is mediated by the secretion of corticotropin-releasing factor (CRF) from hypothalamic neurons.

Immunoneutralization studies with antisera to CRF or studies with CRF antagonists clearly demonstrate that CRF plays a key role in the pituitary-adrenal activation in response to stress (*8, 9*). To determine whether CRF may also play a role in the IL-1–mediated pituitary-adrenal activation, we treated intact male Wistar rats with antiserum to rat CRF during the course of the IL-1–induced adrenocorticotropic hormone (ACTH) response. Administration of the antiserum markedly neutralized the IL-1–induced ACTH response (53.7 ± 3.5 versus 543.0 ± 164.0 pg/ml; mean ± SEM;

F. Berkenbosch, J. van Oers, F. Tilders, Department of Pharmacology, Medical Faculty, Free University, Van der Boechorststraat 7, 1081 BT, Amsterdam, the Netherlands.
A. del Rey and H. Besedovsky, Schweizerisches Forschungsinstitut, Medizinische Abteilung, 7270 Davos-Platz, Switzerland.