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

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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;

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$P < 0.01$) (Fig. 1). A statistically significant difference was found in the concentration of ACTH in the blood of control rats and IL-1–injected rats treated with antiserum (19.3 ± 0.2 versus 53.7 ± 3.4 pg/ml; $P < 0.01$). This small residual ACTH response to IL-1 may be due to an inability of the antiserum to fully neutralize all of the available secreted CRF or, alternatively, may be due to a contribution of other unknown factors structurally distinct from CRF. Nevertheless, the results demonstrate that an endogenous CRF-like molecule plays a crucial role in the activation of the pituitary-adrenal axis in response to IL-1 administration.

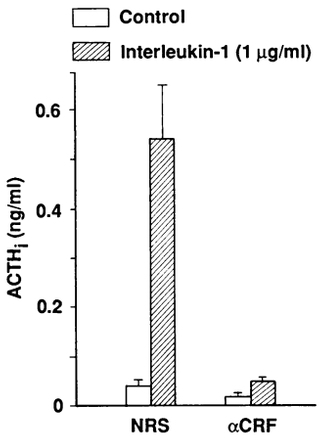

Fig. 1. Effect of normal rabbit serum (NRS) or antiserum to CRF on IL-1–mediated secretion of immunoreactive ACTH ($ACTH_i$) in rats. Male Wistar rats (160 to 180 g) were housed and adapted to experimental conditions as described (*20*). On the day of the experiment (between 9 and 10 a.m.) rats received intraperitoneal injections of 1 ml of control medium (0.15% NaCl and 0.01% human serum albumin dissolved in water) or control medium containing 1 μg of human recombinant IL-1 (*p*I 7, the beta form, kindly provided by C. A. Dinarello, Boston, Massachusetts). Recombinant IL-1 was expressed in *Escherichia coli* as described (*21*) and contained amino acids 112 to 269 (molecular size 17,500) of the percursor sequence. Antiserum to CRF was obtained by immunizing New Zealand rabbits with a conjugate of rat CRF coupled to thyroglobulin as described (*22*). Crude immunoglobulin fractions (sodium sulfate–precipitated) of NRS or of antiserum to CRF (binding capacity, 7.5 n*M*/ml), were dissolved in saline and injected intravenously in a dose equivalent to 650 μl of undiluted serum 80 minutes after administration of control medium or IL-1. Fifty minutes later, the animals were decapitated for collection of blood and median eminence tissue. ACTH was determined by radioimmunoassay after plasma extraction with Vycor as described elsewhere (*20*), with the only difference being the use of a new antiserum. This ACTH antiserum [code, ACTH(2–3)] was raised in New Zealand rabbits to a conjugate of hACTH(11–17) coupled to bovine thyroglobulin with glutaraldehyde and was used in a final dilution of 1:66,000; cross-reactivities with melanocyte-stimulating hormone, corticotrophin-like intermediate lobe peptide, human β-endorphin at concentrations up to 5 μg/ml were less than 0.01%. Assay sensitivity was 2 pg per tube. NRS or antiserum to CRF did not interfere in the plasma extraction or ACTH radioimmunoassay. For determination of the CRF concentrations, median eminence tissue was extracted in a medium consisting of 1 mg of ascorbic acid per milliliter of 0.01*N* HCl, and CRF concentrations in the extracts were determined by radioimmunoassay as described (*23*). No differences were found in CRF concentrations 2 hours after IL-1 administration (see text). Each bar represents the mean and SEM of six to eight animals. Data were evaluated by modified *t* statistics with Bonferroni corrections for multiple comparisons (*24*).

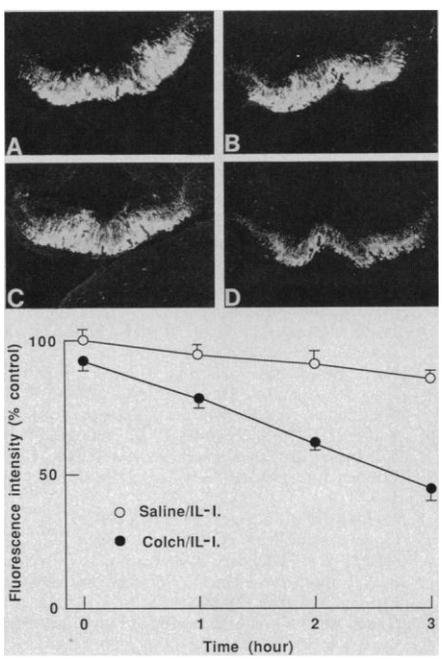

Fig. 2. Photomicrographical and quantitative representation of the effect of IL-1 on CRF immunofluorescence (CRF-QICC) in the median eminence after blockade of axonal transport by intracisternal administration of colchicine. Male Wistar rats were housed and adapted to experimental conditions as indicated in the legend to Fig. 1. On the day of the experiment, groups of rats ($n = 6$) received intracisternal injections of 10 μl of saline or saline containing 5 μg of the axonal transport blocker colchicine under mild ether anesthesia. Control medium or control medium (1 ml) containing 1 μg of IL-1 was injected intraperitoneally 2, 3, and 4 hours after intracisternal injections. Control groups injected with saline or colchicine did not receive IL-1 or control medium and are designated as saline controls or colchicine controls. Five hours after saline or colchicine administration, rats were killed for collection of blood and hypothalamus tissue. Hypothalamic processing for CRF immunocytochemistry was performed as described (*23*).The photomicrographs (top) represent CRF immunofluorescence in the median eminence of (**A**) saline controls, (**B**) colchicine controls, (**C**) saline-treated rats 3 hours after IL-1 administration, and (**D**) colchicine-treated rats 3 hours after IL-1 administration. Immunofluorescence intensities (bottom) were determined with an automated microfluorimeter (MPV II, Leitz) connected to a microcomputer (Rockwell A1M-65) as described earlier (*23, 25*). Data are expressed as percentage of values for saline controls. Each point represents the mean and SEM of five or six animals. Data were evaluated by modified *t* statistics with Bonferroni correction for multiple comparison.

The next experiments were conducted to differentiate between the possibilities that the IL-1–induced ACTH response involves an increased secretion of CRF or that CRF acts as a permissive factor allowing IL-1 to stimulate ACTH secretion at the level of the pituitary gland. We first measured CRF concentrations by radioimmunoassay in median eminence extracts obtained from rats injected with IL-1 or control medium. No differences were found in CRF concentrations 2 hours after administration of control medium or IL-1 (9.8 ± 1.7 versus 12.7 ± 0.6 ng per median eminence). Since the lack of effect may be due to replenishment of released CRF-like material by fast axonal transport of newly synthesized CRF, we determined CRF concentrations in the median eminence at various time intervals after IL-1 administration in rats treated earlier with the axonal transport blocker colchicine (*10*). Intracisternal administration of colchicine (5 μg) did not affect the time course and amplitude of the IL-1–mediated ACTH response. Changes of the CRF immunofluorescence intensity (CFR-QICC) in the median eminence, as determined by quantitative immunocytochemistry, served as index for changes in the CRF content (*11*) (Fig. 2). IL-1 did not markedly affect CRF-QICC in the median eminence of rats treated with an intracisternal injection of saline; only a small reduction was observed 3 hours after IL-1 administration (224.5 ± 4.5 versus 264.4 ± 10.7 instrumental units; $P < 0.05$). In contrast to its marginal effects in saline-treated rats, IL-1 decreased the CRF-QICC at an approximate rate of 15% per hour in the median eminence of rats treated with an intracisternal injection of colchicine. If it is assumed that the time-related changes of CRF-QICC are not due to increased internal degradation of CRF but reflect changes in CRF secretion, calculated CRF concentrations in the hypophyseal portal plasma will reach values of 0.5 n*M* (*12*). This concentration compares favorably with CRF concentrations measured in portal blood during stress (*13*) and is within the range to evoke ACTH release from anterior pituitary cells (*14*). In summary, these data support the view that IL-1 markedly increases the secretory activity of hypothalamic CRF-producing neurons.

IL-1 did not affect ACTH secretion from primary cultures of anterior pituitary cells (Fig. 3). Furthermore, in the presence of

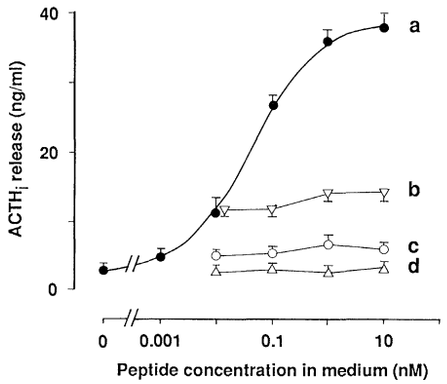

Fig. 3. Effect of IL-1, CRF, or both on secretion of immunoreactive ACTH ($ACTH_i$) from primary cultures of anterior pituitary cells. Anterior lobes obtained from female Wistar rats (180 to 200 g) were dissociated enzymatically with dispase (grade I, 2.4 U/ml; Boehringer Mannheim) for 2 hours at 37°C. Dissociation was facilitated by mechanically dispersing the cells for 1 minute every 30 minutes with a plastic pipette. After centrifugation (100*g* for 5 minutes at room temperature) and washing, cells were plated in a density of 2×10^5 viable cells per well (multiwell plates, Costar) in 0.5 ml of Dulbecco's minimal essential medium (MEM) [2 m*M* L-glutamine, 1% nonessential amino acids, 10% fetal calf serum (Flow)] and were cultured for 4 days (37°C, 6.5% CO_2 in air). Subsequently, the cells were washed in Dulbecco's modified Eagle's medium without fetal calf serum for 20 to 30 minutes. The incubation was initiated by introducing 450 μl of fresh culture medium containing various concentrations of CRF, IL-1, or both (IL-1 was taken from the same stock as used for the in vivo studies). Four hours later, the incubation was terminated by quickly transferring the medium to test tubes. The samples were stored at −20°C until used for ACTH determinations. Data represent the mean and SEM of four wells and are evaluated by modified *t* statistics with a Bonferroni correction for multiple comparisons. The curves represent (a) CRF, (b) IL-1 + CRF (0.01 n*M*), (c) IL-1 + CRF (0.001 n*M*), and (d) IL-1.

suboptimal CRF concentrations in the incubation medium, no synergistic effects of IL-1 could be seen. This observation excludes additional effects of IL-1 at the level of the pituitary gland. Recently, IL-1 was shown to stimulate ACTH secretion from the mouse AtT-20 pituitary tumor cell line (*15*). Although this cell line has provided valuable information on intracellular mechanisms (*16*), its value for the study of physiological mechanisms is limited, because functional receptors are expressed that are normally not present on anterior pituitary corticotrophs (*17*).

After intraperitoneal injection, IL-1 may activate hypothalamic CRF neurons indirectly via visceral afferents in the gut. However, in the in vivo experiments presented, IL-1 did not affect blood levels of prolactin, melanocyte-stimulating hormone, or growth hormone. Furthermore, IL-1 did not induce a concomitant decrease of vasopressin of the external zone of the median eminence in colchicine-treated rats, as is found after various other conditions that induce a persistent ACTH response (*18*). This indicates that the effect of IL-1 is highly selective and clearly differs from stimuli generating a more general stress response. In view of the presence of IL-1–binding sites in the hypothalamus (*19*), we speculate that IL-1 may induce CRF secretion by a direct action in the hypothalamus, possibly on CRF neurons.

The finding that IL-1 administration in subpyrogenic doses activates hypothalamic CRF neurons, leading to a complete pituitary-adrenal activation, is consistent with the existence of a feedback circuit involving products of immunocompetent cells such as IL-1 and the hypothalamo-pituitary-adrenal system.

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10. Colchicine is administered intracisternally in a nontoxic dose (5 μg) that has been reported to arrest transport of vasopressin and oxytocin in hypothalamic neurons of the rat [D. C. Parish, E. M. Rodriquez, S. D. Birkett, B. T. Pickering, *Cell Tissue Res.* **220**, 809 (1981)]. In intact rats, intracisternal administration of colchicine does not affect the CRF content in the median eminence as measured by radioimmunoassay or quantitative immunocytochemistry for up to 6 hours. Conversely, in adrenalectomized rats, a linear decline of the median eminence CRF stores at an average rate of 10% per hour can be found after colchicine administration, which can be prevented with physiological concentrations of corticosterone (F. Berkenbosch and F. J. H. Tilders, *Brain Res.*, in press). These observations are in accordance with other data suggesting an increased turnover of CRF in the median eminence after removal of circulating glucocorticoids by adrenalectomy [(*8*); N. Jangami, S. Matsukura, S. Numa, H. Imura, *Endocrinology* **117**, 1314 (1985); J. Schipper, T. R. Werkman, F. J. H. Tilders, *Brain Res.* **293**, 111 (1984); P. C. Wynn *et al.*, *Peptides* **5**, 1077 (1984)]. In intact rats, an increased secretory activity of CRF producing neurons in response to a variety of stressors can be unmasked after blockade of axonal transport in central neurons [F. Berkenbosch and F. J. H. Tilders, *Neurosci. Lett. Suppl.* **26**, S616 (1986); F. J. H. Tilders and F. Berkenbosch, *Acta Endocrinologica Suppl.* **276**, 63 (1986)].
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