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A central nervous system defect in biosynthesis of corticotropin-releasing hormone is associated with susceptibility to streptococcal cell wall-induced arthritis in Lewis rats

(enkephalin/interleukin 1/peptidoglycan/hypothalamic–pituitary–adrenal feedback loop/Fischer rats)

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ABSTRACT **We have recently found that susceptibility to streptococcal cell wall (SCW)-induced arthritis in Lewis (LEW/N) rats is due, in part, to defective inflammatory and stress mediator-induced activation of the hypothalamic–pituitary–adrenal (HPA) axis. Conversely, the relative arthritis resistance of histocompatible Fischer (F344/N) rats is related to their intact responses to the same stimuli. Specifically, LEW/N rats, in contrast to F344/N rats, have markedly impaired plasma corticotropin and corticosterone responses to SCW, recombinant human interleukin 1α, the serotonin agonist quipazine, or synthetic rat/human corticotropin-releasing hormone (CRH). To explore the mechanism of this defect, we examined the functional integrity of the hypothalamic CRH neuron in LEW/N rats compared to F344/N rats. LEW/N rats, in contrast to F344/N rats, showed profoundly deficient paraventricular nucleus CRH mRNA levels and hypothalamic CRH content in response to SCW. Compared to F344/N rats, there was no increase in LEW/N hypothalamic CRH content or CRH release from explanted LEW/N hypothalami in organ culture in response to recombinant interleukin 1α. These data provide strong evidence that the defective LEW/N corticotropin and corticosterone responses to inflammatory and other stress mediators, and the LEW/N susceptibility to experimental arthritis, are due in part to a hypothalamic defect in the synthesis and secretion of CRH. The additional finding of deficient expression in LEW/N rats of the hypothalamic enkephalin gene, which is coordinately regulated with the CRH gene in response to stress, suggests that the primary defect is not in the CRH gene but is instead related to its inappropriate regulation.**

Inbred Lewis (LEW/N) female rats develop severe proliferative and erosive arthritis, which mimics human rheumatoid arthritis, in response to a single intraperitoneal injection of group A streptococcal cell wall peptidoglycan group-specific polysaccharide (SCW). Histocompatible Fischer (F344/N) rats, on the other hand, do not develop arthritis in response to the same SCW stimulus (1–8). We previously found that the susceptibility of LEW/N rats to SCW arthritis is related to their defective hypothalamic–pituitary–adrenal (HPA) axis responses to inflammatory and other stress mediators and that the relative SCW arthritis resistance of F344/N rats is related to their intact HPA responses to the same stimuli (9). Specifically, LEW/N rats, in contrast to F344/N rats, have markedly impaired plasma corticotropin (ACTH) and corticosterone responses to intraperitoneally injected SCW, to recombinant human interleukin 1α (rIL-1α), to the serotonin agonist quipazine, and to synthetic rat/human corticotropin-releasing hormone (CRH). In addition, LEW/N rats, compared to F344/N rats, have smaller adrenal glands and larger thymuses, consistent with chronic lack of stimulation by ACTH and suppression by corticosterone, respectively (9). Furthermore, arthritis and severe inflammation can be induced in otherwise SCW arthritis-resistant F344/N rats, by interruption of the HPA axis at its effector end point, with the glucocorticoid receptor antagonist RU 486 (9). Taken together, these data indicate that LEW/N rats' pituitary and adrenal hyporesponsiveness to inflammatory and other stress mediators is a major factor contributing to their susceptibility to SCW arthritis, and possibly to other experimental inflammatory diseases (10–17). This model is consistent with evidence that inflammatory-mediator activation of glucocorticoid secretion is one mechanism by which the immune response is appropriately regulated and restrained (18–21).

Pituitary ACTH hyporesponsiveness can be primary or secondary to lack of hypothalamic stimulation (22). To determine whether the impaired ACTH and corticosterone responses of LEW/N rats were hypothalamic in origin, we compared the ability of SCW or rIL-1α to affect *in vivo* hypothalamic CRH mRNA expression in the paraventricular nucleus (PVN), *in vivo* hypothalamic CRH content, and *in vitro* hypothalamic CRH secretion in LEW/N and F344/N rats. Results of these studies show that the LEW/N HPA-axis defect involves the hypothalamus. In contrast to F344/N rats, neuronal synthesis and secretion of CRH within the PVN were markedly impaired.

METHODS

Animals. Eighty- to 100-g, virus antibody-free, age-matched female Fischer (F344/N) and Lewis (LEW/N) rats were obtained from Harlan Sprague Dawley (Indianapolis) and were maintained in microisolator cages in laminar flow units. They were acclimatized to a 12-hr on/12-hr off light cycle and maintained in a relatively stress-free environment for a minimum of 24 hr prior to study.

Drugs and Inflammatory Mediators. SCW was prepared in sterile phosphate-buffered saline (PBS) as described (1) and was injected intraperitoneally at a dose of 2 mg of cell wall

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Abbreviations: SCW, streptococcal cell wall peptidoglycan group-specific polysaccharide; HPA, hypothalamic–pituitary–adrenal; ACTH, corticotropin; CRH, corticotropin-releasing hormone; iCRH, immunoreactive CRH; rIL-1α, recombinant human interleukin 1α; PVN, paraventricular nucleus; PBS, phosphate-buffered saline; ANOVA, analysis of variance.

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rhamnose per 100-g rat. rIL-1α (23) was a kind gift from P. Kilian and P. Lomedico (Hoffmann–LaRoche, Nutley, NJ). It was injected intraperitoneally at a dose of 1 μg per 100-g rat. In *in vitro* studies, it was diluted in tissue culture medium and used in final concentrations ranging from 1 fM to 1 μM. Specific activity was 3×10^8 units/μg. One unit of IL-1 activity was defined in the D10 cell bioassay (23). Endotoxin levels in the final concentrations injected were <0.0013 endotoxin unit/100 μl, as determined by *Limulus* amoebocyte lysate assay (Associates of Cape Cod, Woods Hole, MA).

***In Situ* Hybridization.** F344/N and LEW/N rats were injected intraperitoneally with SCW or rIL-1α. The brains were removed 0, 2, 4, and 7 hr after injection, and 4-mm coronal slices containing the PVN were frozen and stored at −80°C until 12-μm frozen sections were cut and thaw-mounted onto twice gelatin-coated slides. Sections were then processed for *in situ* hybridization with ^{35}S-labeled CRH or enkephalin oligonucleotide probes as described (24–26). The hybridizations were performed at 37°C for 20–24 hr in 600 mM Tris·HCl, pH 7.5/50% (vol/vol) formamide/4 mM EDTA/0.1% sodium pyrophosphate/0.2% SDS/10% dextran sulfate containing heparin at 0.2 mg/ml. The probes had specific activities of 10,000–15,000 Ci/mmol (1 Ci = 37 GBq). Copies of probe hybridized per PVN section were determined by using an image analysis system (24–26).

Radioimmunoassays. Plasma corticosterone was quantitated by a radioimmunoassay kit (Radioassay Systems Laboratories, Immunochem, Carson, CA). Plasma ACTH was determined by radioimmunoassay, as described (27, 28). Inter- and intraassay control variability for corticosterone was 1.2% and 3.4%, respectively; inter- and intraassay control variability for ACTH was 8.0% and 2.8%, respectively. Immunoreactive CRH (iCRH) in tissue culture supernatants was quantitated by a sensitive radioimmunoassay (29). Total hypothalamic iCRH content was quantitated by radioimmunoassay (30).

Brain Extracts. Four hours after intraperitoneal injection of SCW (2 mg of cell wall rhamnose per rat), rIL-1α (1 μg per rat), or PBS (sterile, endotoxin-free, GIBCO), LEW/N and F344/N rats were decapitated, and hypothalami were rapidly removed, quick-frozen on dry ice, and extracted. Total iCRH was quantitated by radioimmunoassay (30).

Hypothalamic Explant Tissue Culture. Hypothalami were rapidly removed from untreated rats, as described (29, 31, 32), and the explants were incubated overnight at 37°C, 5% CO_2, in medium 199 (M199, GIBCO) with 0.1% bovine serum albumin (grade V, Sigma). The hypothalami, incubated in 48-well plates (Costar), were then serially transferred every 20 min through a series of 6 wells containing one of the following additives, in order: control M199 (3 wells, total 60 min), M199 plus rIL-1α (1 fM to 1 μM; 2 wells, total 40 min), or 60 mM KCl (1 well, 20 min). iCRH was assayed directly in the media, as described (29). Only results from hypothalamic explants that showed a ≥90% iCRH increase over baseline in response to depolarization with 60 mM KCl were included in the analyses. We have previously confirmed by trypan blue exclusion and light and electron microscopy that the PVN cells of the majority of the hypothalamic explants incubated for 18 hr or less are alive, albeit edematous and friable (31).

Data Analysis. Results are presented as mean ± SEM throughout. Statistical significance was determined by unpaired two-tailed Student *t* test or one-way analysis of variance (ANOVA) followed by the Duncan multiple range test, as appropriate.

RESULTS

Hypothalamic Expression of CRH and Enkephalin mRNA. The influence of inflammatory mediators on hypothalamic expression of CRH mRNA was quantitated by *in situ* hybridization with a CRH probe in PVN sections from F344/N and LEW/N rats injected intraperitoneally with either SCW

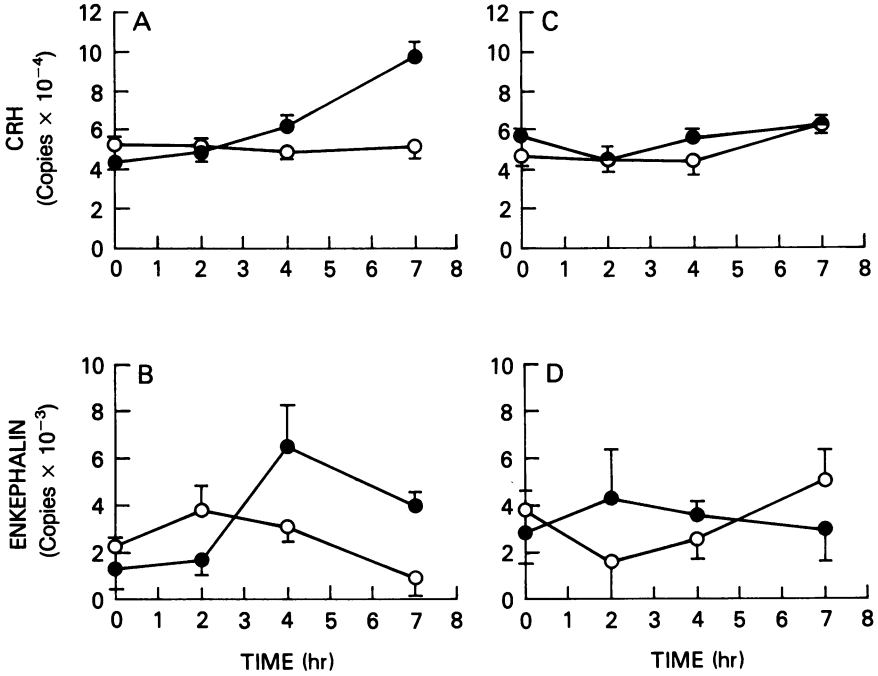

FIG. 1. CRH (*A*) and enkephalin (*B*) transcript levels in the parvocellular neurons of the PVN were increased by SCW administration in F344/N rats (●) but not in LEW/N rats (○). CRH (*C*) and enkephalin (*D*) transcript levels in the PVN were not increased by rIL-1α administration in F344/N (●) or LEW/N (○) rats. Four to 10 rats were used for each experimental condition. The data shown represent the composite data of two separate experiments, sections from which were hybridized at the same time. Experiments in which sections were hybridized separately showed similar results. Bars represent SEM.

or rIL-1α. Between 4 and 7 hr after injection of SCW, CRH mRNA levels clearly increased in the F344/N rats, in comparison with both baseline and LEW/N levels (e.g., two-tailed *t* test, $P < 0.001$ at 7 hr between strains; Fig. 1*A*). When this increase in levels of CRH transcripts was investigated at higher resolution in emulsion-dipped slides (Fig. 2), it was apparent that the individual cells from the F344/N rats were more heavily labeled than those from the LEW/N rats. To determine whether induction of the enkephalin gene by SCW was also defective in LEW/N rats, adjacent sections were examined for enkephalin expression by *in situ* hybridization. The enkephalin mRNA response after SCW injection was significantly greater in the F344/N PVN than in the LEW/N PVN ($P = 0.012$ at 7 hr between strains; Fig. 1*B*). A similar single intraperitoneal injection of rIL-1α (1 μg per rat) did not significantly increase CRH mRNA or enkephalin mRNA in the PVN over baseline in either strain (Fig. 1 *C* and *D*). The same dose of rIL-1α did, however, induce significant ($P < 0.01$) increases in plasma corticosterone and ACTH in F344/N rats compared to LEW/N rats, at 1 hr postinjection (F344/N ACTH = 480 pg/ml, LEW/N ACTH = 70 pg/ml; F344/N corticosterone = 488 ng/ml, LEW/N corticosterone = 78 ng/ml).

Total Hypothalamic iCRH. Hypothalamic iCRH content was measured 4 hr after intraperitoneal injection of SCW, rIL-1α, or PBS (Fig. 3). In F344/N rats, hypothalamic content of iCRH increased significantly ($P < 0.0001$) over both noninjected and PBS-injected controls in response to intraperitoneal SCW or rIL-1α. iCRH content of F344/N hypothalami from both rIL-1α- and SCW-treated rats was significantly higher than iCRH content of hypothalami from PBS-treated or untreated F344/N rats and of hypothalami from treated or untreated LEW/N rats (ANOVA followed by Duncan test). There was no significant difference between

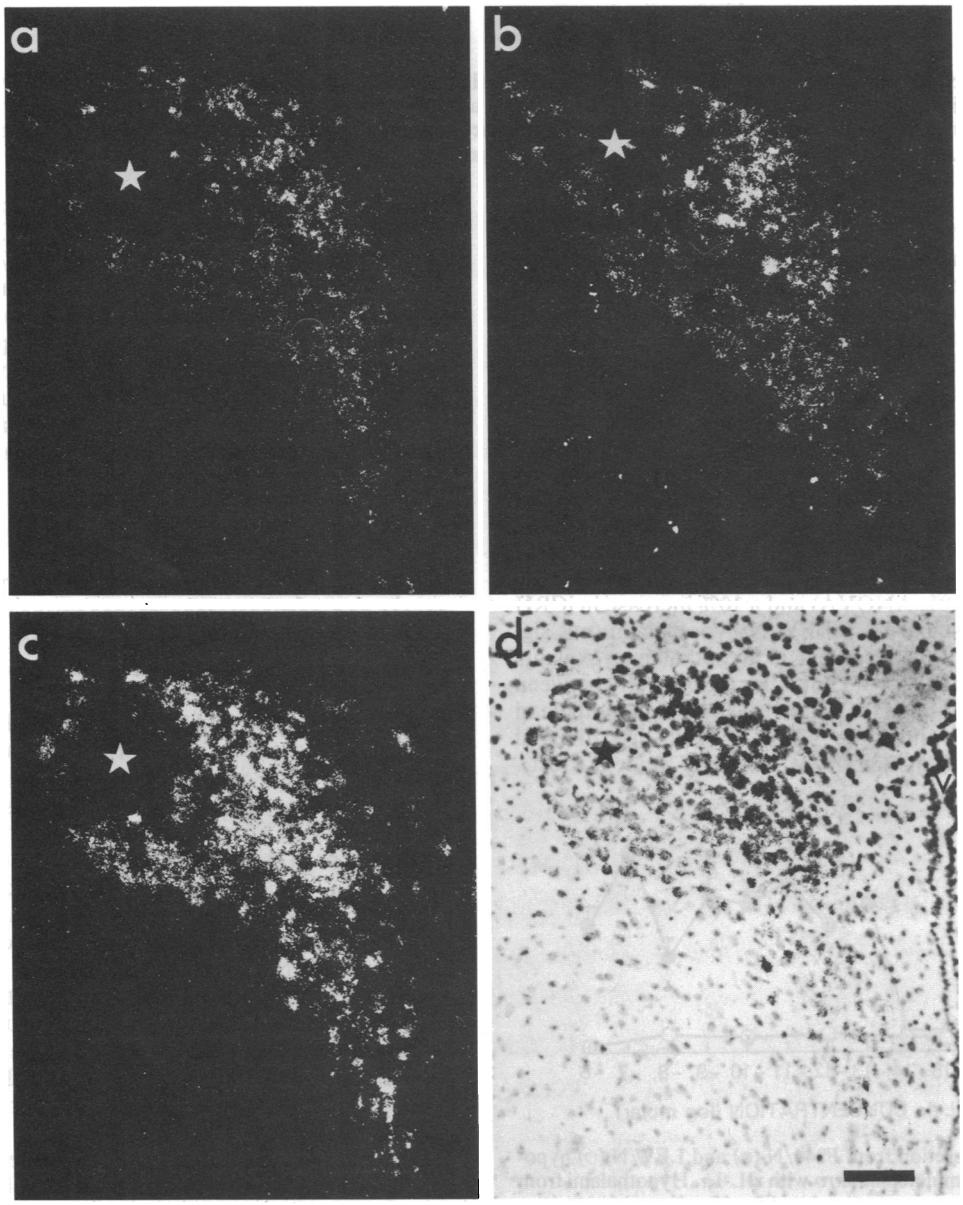

FIG. 2. Photomicrographs of PVN sections labeled *in situ* with CRH oligonucleotide probe. Darkfield micrographs show PVN sections from F344/N rats at time 0 (*a*), LEW/N rats 8 hr after SCW injection (*b*), and F344/N rats 8 hr after SCW injection (*c*). The brightfield micrograph (*d*) shows toluidine staining of the same section shown in *c*. Levels of CRH transcripts are comparable in the PVNs from F344/N rats initially (*a*) and from LEW/N rats at 8 hr after SCW injections (*b*). However, there is an increase in hybridization signal over individual PVN neurons, especially parvocellular ones (to the right of the star), from F344/N rats at 8 hr after SCW injection (*c* and *d*). In the darkfield photomicrographs (*a–c*), individual grains appear white. The star is in the posterior magnocellular region of the PVN. V (at right in *d*), the third ventricle. Exposure was for 6 weeks. (Bar = 100 μm.)

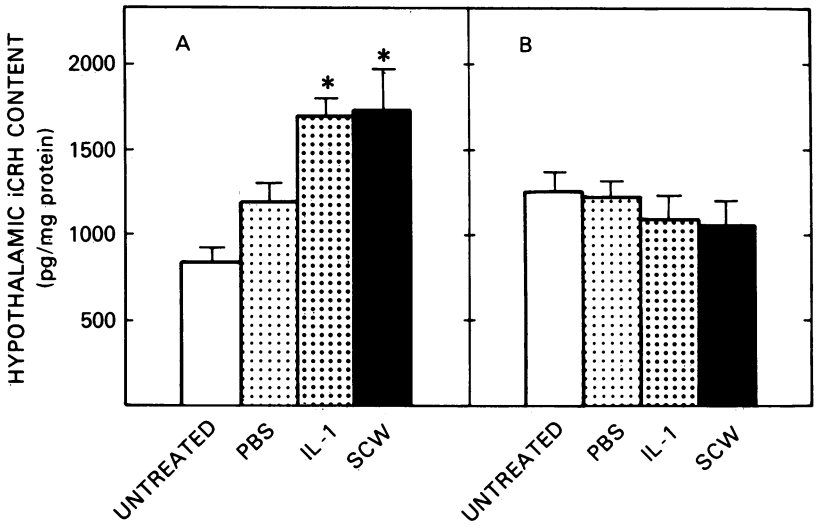

FIG. 3. Total hypothalamic iCRH measured 4 hr after intraperitoneal injection of various agents. F344/N (*A*) or LEW/N (*B*) rats were either untreated or were injected intraperitoneally with PBS, rIL-1α, or SCW, and hypothalamic iCRH was quantitated by radioimmunoassay. At least 10 rats were used for each experimental condition. Statistical significance was determined by one-way ANOVA followed by Duncan's multiple range test. *, $P < 0.0001$, ANOVA; $P < 0.05$, Duncan's multiple range test.

untreated and PBS-treated F344/N rats, nor between any treatment group of LEW/N rats. Baseline levels of iCRH of LEW/N hypothalami were not significantly different from baseline iCRH content of F344/N hypothalami (ANOVA followed by Duncan test).

Hypothalamic Explant Stimulation. To determine whether IL-1 stimulation of CRH secretion is also defective in LEW/N rats, we compared the ability of rIL-1α to induce iCRH release *in vitro* from LEW/N versus F344/N hypothalamic explants. Hypothalamic explants, obtained from untreated age-matched F344/N or LEW/N rats, were cultured in the presence of various concentrations of rIL-1α, and release of iCRH into the culture medium was quantitated by radioimmunoassay (Fig. 4). rIL-1α (1 fM to 1 μM) induced a 150% increase in iCRH secretion over baseline from F344/N hypothalami ($P < 0.001$, ANOVA) and a 10% increase in iCRH secretion over baseline from LEW/N hypothalami (not significant, ANOVA). The percent of hypothalamic explants responding to 60 mM KCl did not differ significantly between strains: 63 ± 3% (mean ± SEM) of F344/N hypothalami responded to KCl and 53 ± 4% of LEW/N hypothalami responded to KCl (not significant, unpaired Student *t* test).

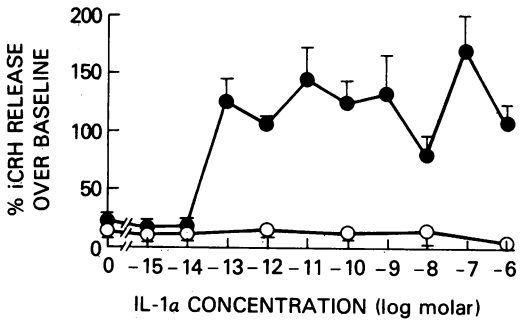

FIG. 4. iCRH secretion from F344/N (●) and LEW/N (○) hypothalamic explants stimulated *in vitro* with rIL-1α. Hypothalami from LEW/N or F344/N rats were stimulated for 20-min periods *in vitro* with control medium or with rIL-1α (1 fM to 1 μM). iCRH in the culture medium was quantitated by radioimmunoassay. Hypothalami from F344/N rats showed an increase of 150% over baseline iCRH secretion, compared to a 10% increase over baseline by LEW/N hypothalami ($P < 0.001$). Statistical significance was determined by Duncan's multiple range test. At least seven rats were used for each experimental condition.

F344/N hypothalami secreted statistically significantly higher amounts of iCRH in response to KCl than did LEW/N hypothalami (F344/N, 395 ± 16%; LEW/N = 299 ± 11%; $P < 0.001$, unpaired Student *t* test). Because F344/N hypothalami were more responsive to KCl than LEW/N hypothalami, we also included both KCl-responsive and KCl-nonresponsive hypothalami in calculations of the effect of rIL-1α on iCRH secretion from hypothalami of both strains. In these calculations, F344/N hypothalami also released significantly more iCRH than LEW/N hypothalami in response to rIL-1α ($P = 0.008$, ANOVA).

DISCUSSION

We previously showed that the LEW/N rats' defective ACTH and corticosterone responsiveness to inflammatory and other stress mediators is one critical factor in their susceptibility to SCW-induced arthritis (9). Our current findings suggest that the deficient LEW/N ACTH and corticosterone responses, and their associated susceptibility to arthritis, are related to a lack of hypothalamic synthesis and secretion of CRH in response to inflammatory and other stress mediators. This lack of a CRH biosynthetic response to SCW in LEW/N rats could be secondary to a defect in the CRH gene or in steps leading to its activation. Lightman and Young (33, 34) have described coordinate regulation of the CRH and enkephalin genes in the PVN after application of two different stresses. The coordinate defect in LEW/N enkephalin mRNA synthesis in response to SCW provides evidence that the CRH biosynthetic defect of LEW/N rats is not specific to the CRH gene but may result from a defect in its regulation. The results presented here suggest that a common pathway that activates the CRH and enkephalin genes in the PVN in F344/N and other normal rats (33, 34) is defective in LEW/N rats.

Bacterial endotoxin (lipopolysaccharide, similar to SCW) and IL-1 have been shown to augment levels of CRH, ACTH, and corticosterone through stimulation of the HPA axis at multiple levels (35–44). Since SCW induces IL-1 release (45), IL-1 is one probable mediator of SCW-induced HPA-axis stimulation. The discrepancy, reported here, between the ability of rIL-1α to augment plasma ACTH and corticosterone and its inability to augment CRH mRNA levels in the PVN in F344/N rats suggests that rIL-1α, at the doses used,

may stimulate secretion but not transcription of CRH. Alternatively, any increase in transcript levels induced by rIL-1α may be below the level of sensitivity of the *in situ* hybridization assay. The increase of CRH mRNA levels induced by SCW, in contrast to the lack of CRH mRNA response to rIL-1α, could be related to two mechanisms: (*i*) the more sustained nature of the SCW stimulus compared to rIL-1α or (*ii*) the multiple factors released by SCW, which could stimulate the CRH neuron via multiple pathways.

Total hypothalamic CRH content reflects the balance between CRH synthesis and secretion. The lack of change in total hypothalamic iCRH content observed in LEW/N rats in response to *in vivo* administration of SCW or rIL-1α is consistent with the LEW/N rats' defective CRH mRNA response to these mediators. The SCW-induced increase in hypothalamic iCRH in F344/N rats is consistent with their ability to increase CRH mRNA levels in response to SCW. The capacity of rIL-1α to increase hypothalamic iCRH content in F344/N rats, but not CRH mRNA levels in the PVN of these rats, suggests that rIL-1α may increase the rate or efficiency of CRH mRNA translation and/or posttranslational processing without causing detectable increases in CRH transcript levels in these rats.

The data presented here suggest that the LEW/N hypothalamic defect is at the level of regulation of CRH gene expression and could be related to a defect in any of the known regulatory pathways of the CRH gene, including trans-synaptic signals (i.e., neurotransmitter pathways), transduction-pathway components, or glucocorticoid receptor-related mechanisms. The present findings also suggest a model for mammalian autoimmune disease, in which a central nervous system defect results in an illness characterized by inadequate immune/inflammatory counterregulation. Such a mechanism may be relevant to human illnesses such as rheumatoid arthritis and other autoimmune/inflammatory diseases.

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1. Wilder, R. L., Allen, J. B. & Hansen, C. (1987) *J. Clin. Invest.* **79,** 1160–1171.
2. Wilder, R. L., Calandra, G. B., Garvin, A. J., Wright, K. D. & Hansen, C. T. (1982) *Arthritis Rheum.* **25,** 1064–1072.
3. Allen, J. B. & Wilder, R. L. (1985) *Arthritis Rheum.* **28,** 1318–1319.
4. Anderle, S. K., Allen, J. B., Wilder, R. L., Eisenberg, R. A., Cromartie, W. J. & Schwab, J. H. (1985) *Infect. Immun.* **49,** 836–837.
5. Allen, J. B., Calandra, G. B. & Wilder, R. L. (1983) *Infect. Immun.* **42,** 796–801.
6. Wilder, R. L., Allen, J. B., Wahl, L. M., Calandra, G. B. & Wahl, S. M. (1983) *Arthritis Rheum.* **26,** 1442–1072.
7. Ridge, S. C., Zabriskie, J. B., Osawa, H., Diamantstein, T., Oronsky, A. & Kerwar, S. S. (1986) *J. Exp. Med.* **164,** 327–332.
8. van den Broek, M. F., van Bruggen, M. C. J., van den Berg, W. & van den Putte, L. B. A. (1988) *Arthritis Rheum.* **31,** S91 (abstr.).
9. Sternberg, E. M., Hill, J. M., Chrousos, G. P., Kamilaris, T., Listwak, S. J., Gold, P. W. & Wilder, R. L. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 2374–2378.
10. Kohashi, O., Kohashi, Y., Takahashi, T., Ozawa, A. & Shigematsu, N. (1986) *Arthritis Rheum.* **29,** 547–553.
11. Griffiths, M. M. & DeWitt, C. W. (1984) *J. Immunol.* **132,** 2830–2836.
12. Hill, J. L. & Yu, D. T. (1987) *Infect. Immun.* **55,** 721–726.
13. Beraud, E., Reshef, T., Vanderbark, A. A., Offner, H., Friz, R., Chou, C. H. J., Bernard, D. & Cohen, I. R. (1986) *J. Immunol.* **136,** 511–515.
14. Caspi, R. R., Roberge, F. G., McCallister, M., El-Sared, M., Kuwabara, T., Gery, I., Hanna, E. & Nussenblatt, R. B. (1986) *J. Immunol.* **136,** 928–933.
15. Davis, J. K., Thorp, R. B., Maddox, P. A., Brown, M. B. & Cassell, G. H. (1982) *Infect. Immun.* **36,** 720–729.
16. Allen, J. B. & Wilder, R. L. (1987) *Infect. Immun.* **55,** 674–679.
17. Lehman, T. J. A., Allen, J. B., Plotz, P. H. & Wilder, R. L. (1984) *Arthritis Rheum.* **27,** 939–942.
18. Besedovsky, H., del Ray, O. A. & Sorkin, E. (1983) in *Immunoregulation*, eds. Fabris, N., Garaci, E., Hadden, J. & Mitcheson, N. A. (Plenum, New York), pp. 315–339.
19. Munck, A., Guyre, P. M. & Holbrook, N. J. (1984) *Endocr. Rev.* **5,** 25–44.
20. Blalock, E. J. & Smith, E. M. (1985) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **44,** 108–111.
21. Sternberg, E. M. (1988) in *The Year in Immunology*, eds. Cruse, J. M. & Lewis, R. E. (Karger, Basel), Vol. 4, pp. 1–13.
22. Chrousos, G. P., Schulte, H. M., Oldfield, E. H., Gold, P. W., Cutler, G. B., Jr., & Loriaux, D. L. (1984) *N. Engl. J. Med.* **310,** 622–626.
23. Kilian, P. L., Kuffka, K. L., Stern, A. S., Woehle, D., Benjamin, W. R., Dechiara, T. M., Gubler, U., Farrar, J. J., Mizel, S. B. & Lomedico, P. T. (1986) *J. Immunol.* **136,** 4509–4514.
24. Young, W. S., III, Mezey, E. & Siegel, R. E. (1986) *Neurosci. Lett.* **70,** 198–203.
25. Young, W. S., III, Bonner, T. I. & Brann, M. R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 9827–9831.
26. Lightman, S. L. & Young, W. S., III (1987) *J. Physiol. (London)* **394,** 23–39.
27. Gross, H. A., Ruder, J. J., Brown, K. S. & Lipsett, M. B. (1972) *Steroids* **20,** 681–695.
28. Chrousos, G. P., Schulte, H. M., Oldfield, E. H., Gold, P. W., Cutler, G. B., Jr., & Loriaux, D. L. (1984) *N. Engl. J. Med.* **310,** 622–626.
29. Calogero, A. E., Gallucci, W. T., Chrousos, G. P. & Gold, P. W. (1988) *J. Clin. Invest.* **82,** 839–846.
30. Vale, W., Vaughan, J., Yamamoto, C., Bruhn, T., Douglas, C., Dalton, D., Rivier, C. & Rivier, J. (1983) *Methods Enzymol.* **103,** 565–577.
31. Calogero, A. E., Bernardini, R., Margioris, A. N., Bagdy, G., Gallucci, W. T., Munson, P. J., Tamarkin, L., Tomai, T. P., Brady, L., Gold, P. W. & Chrousos, G. P. (1989) *Peptides*, in press.
32. Calogero, A. E., Gallucci, W. T., Gold, P. W. & Chrousos, G. P. (1988) *J. Clin. Invest.* **82,** 767–774.
33. Lightman, S. L. & Young, W. S., III (1987) *Nature (London)* **328,** 643–645.
34. Lightman, S. L. & Young, W. S., III (1988) *J. Physiol. (London)* **403,** 511–523.
35. Fontana, A., Weber, E. & Dayer, J.-M. (1984) *J. Immunol.* **133,** 1696–1698.
36. Woloski, B. M. R. N. J., Smith, E. M., Meyer, W. J., III, Fuller, G. M. & Blalock, J. E. (1985) *Science* **230,** 1035–1037.
37. Bernton, E. W., Beach, J. E., Holaday, J. W., Smallridge, R. C. & Fein, H. G. (1987) *Science* **238,** 519–521.
38. Sapolsky, R., Rivier, C., Yamamoto, G., Plotsky, P. & Vale, W. (1987) *Science* **238,** 522–524.
39. Berkenbosch, F., Oers, J. V., Del Rey, A., Tilders, F. & Besedovsky, H. (1987) *Science* **238,** 524–526.
40. Besedovsky, H., Del Rey, A., Sorkin, E. & Dinarello, C. A. (1986) *Science* **233,** 652–654.
41. Uehara, A., Gottschall, P. E., Dahl, R. R. & Arimura, A. (1987) *Biochem. Biophys. Res. Commun.* **146,** 1286–1290.
42. Uehara, A., Gottschall, P. E., Dahl, R. R. & Arimura, A. (1987) *Endocrinology* **121,** 1580–1582.
43. Calogero, A. E., Luger, T., Gallucci, W. T., Gold, P. W. & Chrousos, G. P. (1987) *Endocrinology* **120,** Suppl. 271 (abstr. 1001).
44. Nakamura, H., Motoyoshi, S. & Kadokawa, T. (1988) *Eur. J. Pharmacol.* **151,** 67–73.
45. Wilder, R. L. (1987) *Rheum. Dis. Clin. North Am.* **13,** 293–306.