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# Locus Coeruleus Stimulation by Corticotropin-Releasing Hormone Suppresses *in vitro* Cellular Immune Responses

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**Previous studies have demonstrated that stressors alter cellular immune system function, and increase the activity of locus coeruleus neurons. Furthermore, stressors increase the release of corticotropin-releasing hormone (CRH) and locus coeruleus neurons are activated by CRH. Thus, the present study examined whether activation of the locus coeruleus by infusion of CRH modulates the function of blood and spleen lymphocytes assessed *in vitro*. CRH (100 ng) was administered into the region of the locus coeruleus in awake rats 1 hr before spleen and peripheral blood lymphocytes were collected for culture with nonspecific mitogens. Unilateral or bilateral microinfusion of CRH into the locus coeruleus produced a decrease in blood and spleen T-lymphocyte mitogenic responses to phytohemagglutinin, ConA, and an antibody to the T-lymphocyte antigen receptor. In contrast, infusion of saline into the locus coeruleus or CRH into the surrounding region of the dorsal pons did not alter spleen or blood lymphocyte responses. Plasma concentrations of adrenocorticotropic hormone, corticosterone, and IL-6 were increased by CRH infusion into the locus coeruleus. These results suggest that CRH-evoked activation of the locus coeruleus stimulates the hypophysial adrenal axis, possibly activates the sympathetic nervous system, and results in immunosuppression. Comparable changes in lymphocyte and hormone responses are produced by an aversive stimulus or a conditioned stressor, suggesting that activation of the locus coeruleus may be a component of stressor-induced immune alterations.**

***[Key words: locus coeruleus, corticotropin-releasing hormone, cellular immunity, rat, adrenocorticotropin hormone, corticosterone, interleukin-6]***

Identifying the CNS substrates that modify immunologic function may lead to a better understanding of how stress influences immune reactivity in infectious, malignant, and autoimmune diseases. Research shows that cellular immune system function is suppressed by psychological and physical stressors and suggests that the modulatory effects of stress on immunologic responses is related to integrated CNS activity leading to activation of hypophysial and sympathetic hormonal systems (Rozman and Brooks, 1985; Solomon, 1987; Rabin et al., 1989; Danzer and Kelly, 1989; Cunnick et al., 1990; Keller et al., 1991).

Increased activity of corticotropin-releasing hormone systems (CRH) concomitant with stress may modulate immunologic responses (Irwin et al., 1987, 1992; Jain et al., 1991; Straubaugh and Irwin, 1992; Irwin, 1993) because autonomic, endocrine, and behavioral responses that are symptomatic of stress can be produced by intraventricular administration of CRH (Britton et al., 1982; Brown et al., 1982; Sutton et al., 1982; Eaves et al., 1985; Koob and Bloom, 1985; Fisher et al., 1989; Dunn and Berridge, 1990). Therefore, like stressors, endogenous CRH systems may modulate CNS transmission to produce regulatory effects on endocrine and immunologic responses.

One site at which CRH likely modulates CNS function during stress is the nucleus locus coeruleus in the dorsal pons (Foote et al., 1980; Butler et al., 1990; Swiergiel et al., 1992). Electrophysiological studies show that the locus coeruleus is activated by CRH (Ehlers et al., 1983; Valentino et al., 1983; Valentino and Foote, 1987, 1988; De Sarro et al., 1992) and exposure to noxious stimuli (Abercrombie and Jacobs, 1987). Furthermore, stressors increase activity of locus coeruleus neurons, as reflected by the expression of the immediate-early gene c-Fos (Pezzone et al., 1993) and the activity of tyrosine hydroxylase, the rate limiting enzyme for norepinephrine biosynthesis (Zigmond et al., 1974; Richard et al., 1988; Weiner et al., 1991; Melia et al., 1992). Therefore, the integrative reactivity of the locus coeruleus and its projections (for review, see Foote et al., 1983; Moore and Card, 1984; Aston-Jones et al., 1991; Valentino et al., 1992) to sensory stimuli is the basis for the present hypothesis, which poses that activation of this nucleus is part of the neural circuit that modifies immunologic function.

Previous studies show that the locus coeruleus is activated by CRH, is innervated by CRH-like immunoreactive fibers (Merchenthaler et al., 1982; Cummings et al., 1983; Swanson et al., 1983; Sakanaka et al., 1987; Valentino et al., 1992) and contains binding sites for CRH (DeSouza, 1987). Therefore, the present study was designed to test whether activation of the locus coeruleus by CRH can modulate *in vitro* cellular immune system function. CRH or vehicle was administered into the locus coeruleus in conscious rats, and spleen and peripheral blood cells were cultured with nonspecific mitogens to evaluate lymphocyte proliferative responses. In addition, plasma samples were as-

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sayed for adrenocorticotrophic hormone (ACTH), corticosterone and interleukin-6 (IL-6) to determine whether CRH-evoked activation of the locus coeruleus modified hypothalamic–pituitary–adrenal function.

## Materials and Methods

*Subjects.* Male Wistar rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) were housed individually in stainless steel cages, and were maintained on a 12 hr light/12 hr dark cycle (lights on at 0600). One week after arrival, rats weighing between 271 and 307 gm were anesthetized with an inhalation anesthetic (1.5–3.0% fluothane vaporized with reconstituted breathing air: 21% oxygen balanced with 79% nitrogen) and stereotaxically implanted with bilateral 10 mm, 30 gauge stainless steel intracranial cannulas (Plastics One Inc., Roanoke, VA). Stereotaxic coordinates were, anteroposterior, −0.8 to −1.1 mm from the interaural line; lateral, ±1.1 mm from the midline; and dorsoventral, −4.1 mm from the skull surface at the point of entry. The incisor bar was positioned at −3.4 mm below the interaural line (Paxinos and Watson, 1986). Cannulas were fastened to the skull with dental acrylic cement, secured with two stainless steel screws, and sealed with 10 mm wire stylets. Following a 5 d recovery period, animals were habituated to the microinfusion procedure for 2 d prior to experimentation. Forty-eight hours before testing, the injectors (13 mm, 33 gauge) were inserted through the guide cannulas without extending beyond the length of the guide cannulas; then, 24 hr before testing, the injectors were inserted all the way through the guide cannula aiming at the locus coeruleus.

*Drug administration.* Corticotropin-releasing hormone (CRH, Human, Rat Bachem California, Torrance, CA) was dissolved in 0.9% sterile, pyrogen-free, sodium chloride solution and prepared as 100 ng/0.5 μl. Animals were randomly assigned to receive bilateral microinfusion of either vehicle or CRH solution. While gently restraining the animals, CRH or vehicle solution was administered bilaterally (0.5 μl, 1.5 min) with cannula injectors that were connected to calibrated sterile polyethylene tubing, 10 μl Hamilton syringes (Hamilton, Reno, NV), and a microinfusion pump (Razel Scientific Instruments Inc., Stamford, CN). The injectors were left in place for 90 sec before removal. The dummy stylets were replaced into the guide cannulas and the animals were returned to their home cages, where they remained for 1 hr until the time of sacrifice.

*Blood and spleen collection.* Animals were sacrificed by cervical dislocation and a ventral incision was made to expose the ascending abdominal aorta. For each subject, a blood sample (approximately 3 ml) was collected from the abdominal aorta using a sterile syringe and vacutainer containing sodium heparin (45 USP units; Becton Dickinson, Rutherford, NJ), and then an additional blood sample (2 ml) was collected into a vacutainer containing EDTA (tripotassium salt, 3.6 mg; Becton Dickinson).

For the mitogen-stimulated blood lymphocyte proliferation assays, 0.5 ml of heparinized blood was immediately added to 4.5 ml of RPMI 1640 tissue culture medium (GIBCO) supplemented with 10 mM HEPES, 2 mM L-glutamate, and 50 μg/ml gentamicin sulfate (all from GIBCO). The remaining blood was kept on ice, and then centrifuged (2000 rpm, −4°C, 15 min). Plasma samples were placed into siliconized, sterile, microcentrifuge tubes and stored frozen at −70°C for ACTH, corticosterone, and IL-6 assays.

Spleens were removed and placed in sterile polypropylene tubes containing 7 ml of supplemented culture medium.

*Mitogen-stimulated lymphocyte proliferation assays.* Sterile laboratory equipment was used for the *in vitro* cell proliferation assays. Mitogen-stimulated spleen and blood lymphocyte responses were determined using assays of spleen and blood cell cultures prepared with the T-cell mitogens, phytohemagglutinin (PHA) type HA-16 (Wellcome), and concanavalin-A (ConA; Difco, Detroit, MI), which are plant lectins. Spleen lymphocyte cell cultures were also prepared with a B-cell mitogen, lipopolysaccharide (LPS; Difco), which is component of the cell walls of gram-negative bacteria. In addition, cultures were prepared to measure the spleen T-lymphocyte proliferative responses to a mouse monoclonal antibody to rat α/β T-cell antigen receptor (Ab TCR; total protein, 27 mg/ml; Pharmingen, San Diego, CA). PHA, ConA, LPS, and the Ab TCR were each prepared in supplemented culture medium. Lymphocyte proliferation was stimulated with concentrations of mitogens that produce suboptimal and optimal proliferation in our laboratory (Cunnick et al., 1990; Lysle et al., 1990a).

For the spleen lymphocyte assays, the Ab TCR was diluted in supplemented culture medium (1:200) and was added to the culture plates (96-well, flat bottom, microtiter plates; Costar no. 3696) 24 hr prior to the experiment to allow the antibody to bind to the plates. The microtiter plate wells containing the Ab TCR were washed three times with Hanks' Balanced Salt Solution (GIBCO, Grand Island, NY), and then 100 μl of supplemented culture medium was added to these microtiter plate wells. PHA (5 and 10 μg/ml), ConA (5 and 10 μg/ml), and LPS (10 and 20 μg/ml) were each added in triplicate to the other wells of the microtiter plates.

Spleens were dissociated into a single cell suspension by gently grinding the spleens between the frosted edges of microscope slides with supplemented culture medium that contained 10% heat-inactivated fetal calf serum (GIBCO) (designated as complete medium). The dispersed spleen cells were transferred to polypropylene tubes and allowed to stand for approximately 10 min in 10 ml of complete medium, and the supernatant suspension was then transferred to a new tube. The concentration of nucleated cells in the spleen samples was determined in a Coulter Counter (model ZBI) and adjusted to $5 \times 10^6$ cells/ml using complete culture medium. Aliquots of the single cell suspension (100 μl) for each spleen sample were added to the culture plates. Background level of radioactivity was determined by adding 100 μl of supplemented culture medium instead of mitogens to the microtiter plate wells. All cell cultures were placed into a temperature (37°C) humidity (67%) controlled, $CO_2$ (5%) incubator.

The incorporation of thymidine into newly formed DNA during lymphocyte proliferation, the dependent measure of the *in vitro* cellular immune response, was assayed by adding $^3$H-thymidine (1 μCi/well; specific activity, 6.7 Ci/mmol; DuPont–New England Nuclear) to the spleen cell cultures during the last 5 hr of a 48 hr incubation period. The cell cultures were then harvested onto filtermat paper using a Skatron semiautomatic cell harvester and the incorporation of $^3$H-thymidine was determined by a liquid scintillation counter (Packard Tri-Carb 1500 beta-counter).

Blood lymphocyte cell cultures were processed using a similar assay method with the following differences: aliquots (100 μl) of the blood samples (diluted 1:10 in supplemented medium as described in the blood collection section) were added to microtiter plates containing PHA (2.5 and 5 μg/ml) and ConA (5 and 10 μg/ml), and the $^3$H-thymidine was added to the cultures during the last 18 hr of a 96 hr incubation period. The number of leukocytes per milliliter of whole blood for each sample was determined using a Unopette and hemocytometer.

*Hormone assays.* Plasma samples from blood collected in EDTA-treated and heparin-treated vacutainer tubes were assayed for ACTH and corticosterone, respectively. Plasma samples were assayed in duplicate for ACTH and corticosterone using radioimmunoassay kits (ICN Biomedicals, Inc).

*Plasma assay of IL-6.* Plasma samples were assayed for IL-6 activity using a B9 plasmacytoma cell bioassay. Duplicates of the plasma samples (from blood collected in heparin-treated tubes) were diluted serially (beginning with a 1:4 dilution) with supplemented medium containing 5% heat-inactivated fetal calf serum in 96-well, flat bottom, microtiter plates. B9 cells were washed twice by suspending the cells in 40 ml of Hanks' Salt Solution and centrifugation at 2100 rpm for 10 min. Then, the B9 cells were suspended in complete medium containing $5 \times 10^{-5}$ M 2-mercaptoethanol, and centrifuged again. Finally, the cell concentration was adjusted to $1 \times 10^5$ cells/ml using complete medium containing $5 \times 10^{-5}$ M 2-mercaptoethanol, and added (100 μl per well) to the cultures plates. During the last 4 hr of a 72 hr incubation period, the cultures were incubated with $^3$H-thymidine (1 μCi/well), and harvested. Proliferation values expressed as cpm were compared against a standard curve for recombinant human IL-6 (80 pg/ml) (BRMP, Frederick, MD). Plasma concentrations of IL-6 were calculated as the dilution necessary to achieve 50% of the maximal proliferation in the experimental sample divided by the dilution necessary to achieve 50% of the maximal proliferation in the standard sample, multiplied by 80 pg/ml. The sensitivity of this assay is approximately 1 pg/ml and the specificity of this assay was confirmed using an IL-6 neutralizing antibody (R&D Systems) (Zhou et al., 1993).

*Assessment of injection site.* At the end of the experiment, brains were rapidly removed and stored frozen. Brains were sectioned coronally at 40 μm intervals on a cryostat, and then mounted onto glass slides and stained with cresyl violet. Infusion sites were verified under a light microscope. Cannula placements for infusion were judged to be within

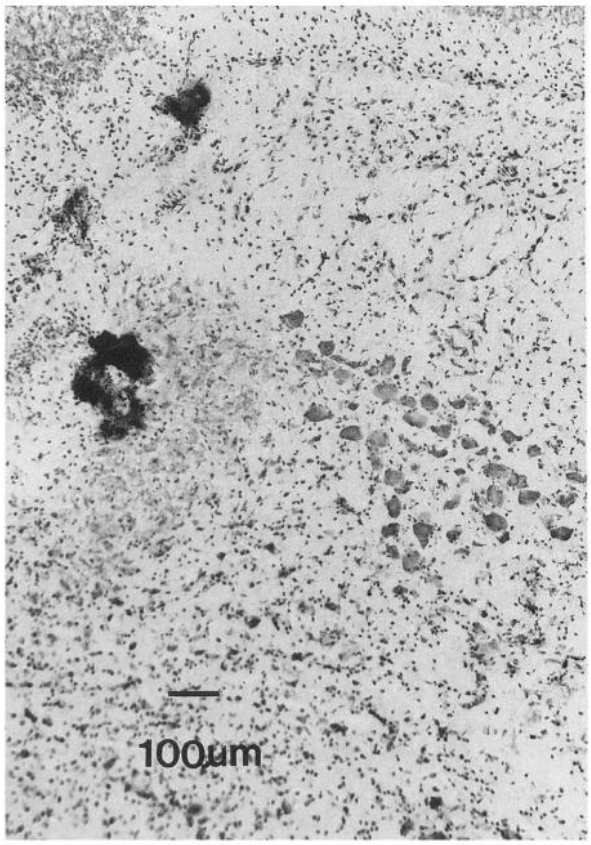


*Figure 1.* Histological localization of cannula placement within the locus coeruleus for animals receiving infusions of vehicle or CRH into the locus coeruleus. Note the cannula track extending into the locus coeruleus. This placement is typical of those included as infusions into the locus coeruleus. Scale bar, 100 μm.

the locus coeruleus when the cannula tracks were clearly within approximately 0.15 mm (medial-lateral) of the locus coeruleus neurons and located −10.3 to −9.16 mm anteroposterior from bregma (Paxinos and Watson, 1986).

*Experimental design.* This study was conducted as a series of four cohorts, each using an identical experimental design, a representative number of subjects in each treatment group and a total of 12–17 animals. In each cohort, a separate group of unhandled rats (unoperated control group) was included to control for the effects of handling and surgery prior to experimentation. Samples for this group were processed identically to sample preparation for the other treatment groups. The study included 54 rats, with 34 of these rats receiving either vehicle or CRH infusions aimed at the locus coeruleus.

*Statistical analysis.* Data for the spleen cell proliferation were expressed as counts per minute (cpm) and data for the blood cell proliferation were normalized to cpm per $10^5$ leukocytes. Data for blood lymphocyte responses in two subjects that received vehicle into the locus coeruleus were uninterpretable due to technical error and were not included in the statistical analyses.

For each experimental sample, the background level of radioactivity (average of the triplicate cpm of unstimulated cell response) was subtracted from the mitogen-stimulated response (average of the triplicate cpm). Blood and spleen lymphocyte responses to each mitogen were analyzed separately using two-way analysis of variance (ANOVA) with a between-subjects factor for treatment group (control, vehicle into the locus coeruleus, and CRH into the locus coeruleus) and a within-subjects factor for mitogen concentration. Individual group means were compared using Newman-Keuls a posteriori test.

Plasma concentrations of ACTH, corticosterone, and IL-6 were transformed to logarithms and these data were analyzed by one-way ANOVA, followed by Newman-Keuls test for pairwise comparisons.

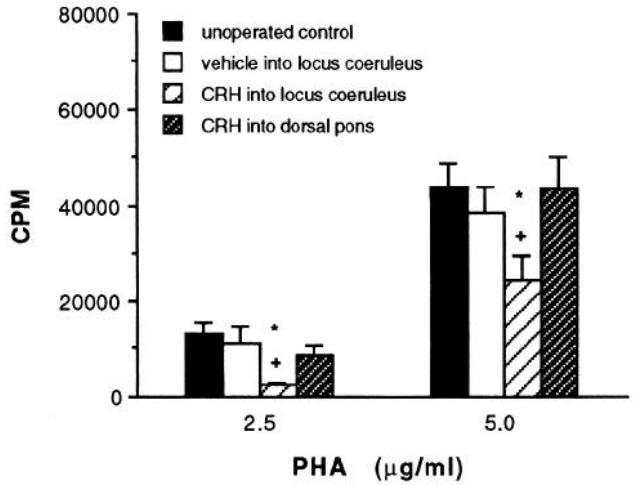


*Figure 2.* Blood lymphocyte mitogenic responses to PHA. The data are expressed as mean (±SEM) specific stimulated proliferative response. *Asterisks* indicate significant differences in responses as compared with responses in the vehicle into the locus coeruleus group (Newman-Keuls test, $p < 0.05$), and *pluses* indicate significant differences in responses relative to the control group ($p < 0.05$).

For statistical analyses, data for vehicle infusions that were not localized to the locus coeruleus were excluded. Data for the CRH infusions into the surrounding regions of the locus coeruleus dorsal pons formed a separate group to control for the specificity of CRH's actions on locus coeruleus activity, and responses in this group were compared with responses in the CRH into the locus coeruleus group using Student's *t* test.

## Results

Data for the LC infusion groups were based on histological determination of infusion sites that were within approximately 150 μm of the LC neurons (Fig. 1). Histological examination of infusion sites showed that 10 of the 16 vehicle infusions were localized to the locus coeruleus. Of the 18 rats receiving CRH infusions aimed at the locus coeruleus, histological analysis showed that five of CRH infusions were administered bilaterally, seven CRH infusions were administered unilaterally into the locus coeruleus, and six CRH infusions were administered into the region of the dorsal pons surrounding the locus coeruleus. Mitogenic responses of spleen and blood lymphocytes after unilateral or bilateral infusion of CRH into the locus coeruleus were equivalent (Student's *t* test; all comparisons, $p > 0.05$); therefore, these data were pooled for statistical analyses. The numbers of subjects for each of the statistical tests were $n = 20$ for the unoperated control group, $n = 12$ for CRH infusion into the locus coeruleus, and $n = 6$ for the CRH infusion into the dorsal pons group. Data from 10 subjects that received vehicle infusion into the locus coeruleus were used for all the statistical tests except for analyses of blood lymphocyte responses, where $n = 8$ subjects.

Infusion of CRH into the locus coeruleus decreased peripheral blood lymphocyte mitogenic responses to PHA (Fig. 2). ANOVA revealed that there was a significant main effect of treatment on blood lymphocyte responses to PHA [$F(2,37) = 4.55$; $p < 0.05$], a main effect of PHA concentration [$F(1,37) = 84.59$; $p < 0.0001$], and no significant treatment × PHA concentration interaction [$F(2,37) = 0.73$; $p = 0.49$]. The differences in blood lymphocyte responses to PHA after CRH infusion into the locus

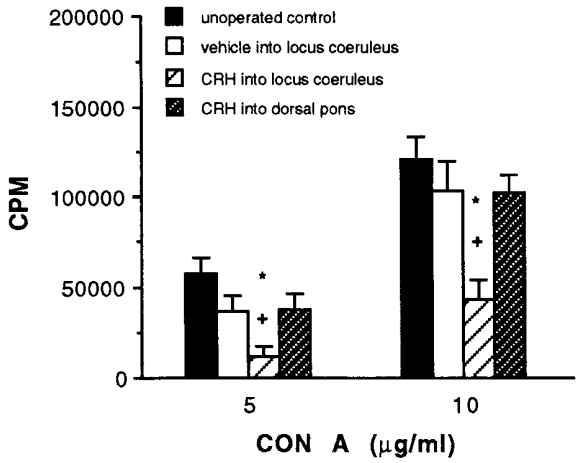


*Figure 3.* Blood lymphocyte mitogenic responses to ConA. The data are expressed as mean (±SEM) specific stimulated proliferative response. *Asterisks* indicate significant differences in responses as compared with responses in the vehicle into the locus coeruleus group (Newman-Keuls te t, $p < 0.05$), and *pluses* indicate significant differences in responses relative to the control group ($p < 0.05$).

coeruleus as compared with CRH infusion into the dorsal pons were statistically significant [$t(16) = -3.4$ and $-2.2$, $p < 0.05$, for 2.5 and 5 μg/ml PHA, respectively].

Infusion of CRH into the locus coeruleus also decreased peripheral blood lymphocyte mitogenic responses to ConA (Fig. 3). ANOVA revealed a significant main effect of treatment on blood lymphocyte responses to ConA [$F(2,37) = 9.35$; $p < 0.001$] and ConA concentration [$F(1,37) = 48.62$; $p < 0.001$], and no significant treatment × ConA concentration interaction [$F(2,37) = 2.12$; $p = 0.13$]. The differences in blood lymphocyte responses to CON A after CRH infusion into the locus coeruleus as compared with CRH infusion into the dorsal pons were statistically significant [$t(16) = -2.76$ and $-3.4$, $p < 0.05$, for 5.0 and 10 μg/ml ConA, respectively].

Splenic lymphocyte mitogenic responses to PHA were decreased by infusion of CRH into the locus coeruleus (Fig. 4). ANOVA revealed that there was a main effect of treatment on spleen lymphocyte responses to PHA [$F(2,39) = 4.50$; $p < 0.05$], a main effect of PHA concentration [$F(1,39) = 24.24$; $p < 0.001$], and no significant treatment × PHA concentration interaction [$F(2,39) = 1.38$; $p = 0.26$]. The proliferative responses to PHA after CRH infusion into the locus coeruleus were not statistically different from the proliferative responses to PHA after CRH infusion into the dorsal pons [for 5.0 and 10 μg/ml PHA, respectively: $t(16) = -1.59$, $p = 0.13$; and $t = -1.7$, $p = 0.11$].

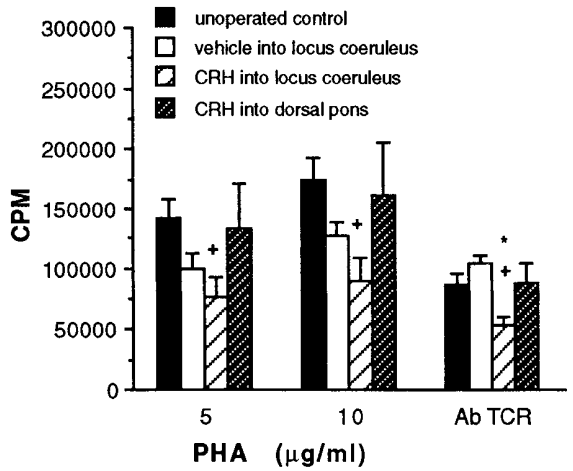


*Figure 4.* Spleen lymphocyte proliferative responses to PHA and the Ab TCR. The data are expressed as mean (±SEM) specific stimulated proliferative response. *Asterisks* indicate significant differences in responses as compared with responses in the vehicle into the locus coeruleus group (Newman-Keuls test, $p < 0.05$), and *pluses* indicate significant differences in responses relative to the control group ($p < 0.05$).

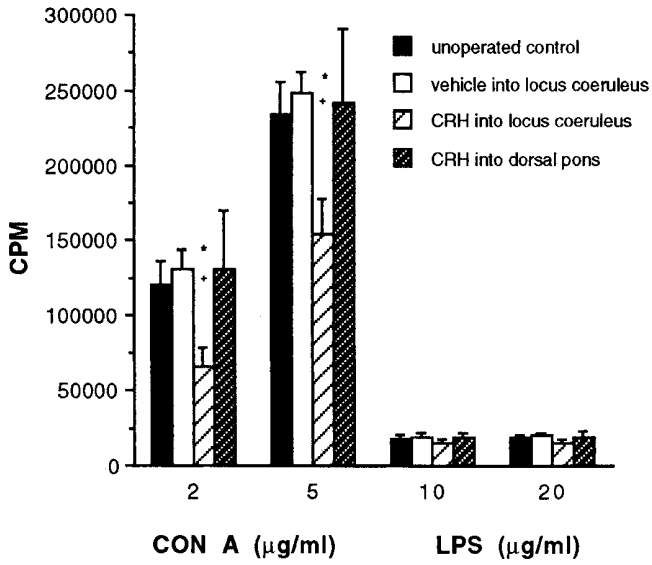


*Figure 5.* Splenic lymphocyte mitogenic response to ConA and LPS. The data are expressed as mean (±SEM) specific stimulated proliferative response. *Asterisks* indicate significant differences in responses as compared with responses in the vehicle into the locus coeruleus group (Newman-Keuls test, $p < 0.05$,) and *pluses* indicate significant differences in responses relative to the control group ($p < 0.05$).

The splenic lymphocyte proliferative response to the Ab TCR was decreased by CRH administration into the locus coeruleus [$F(2,39) = 6.81$; $p < 0.005$; see Fig. 4]. Splenic lymphocyte responses to the Ab TCR after CRH infusion into the locus coeruleus were significantly different from responses after CRH infusion into the dorsal pons ($t = 2.25$; $p < 0.05$).

The splenic lymphocyte proliferative response to ConA was decreased by CRH administration into the locus coeruleus. For lymphocyte responses to ConA, there was a main effect of treatment [$F(2,39) = 5.31$; $p < 0.01$], a main effect of ConA concentration [$F(1,39) = 198.93$; $p < 0.001$], and no significant treatment × ConA concentration interaction [$F(2,39) = 1.29$; $p = 0.28$]. The differences in splenic lymphocyte responses to ConA after CRH infusion into the locus coeruleus as compared with CRH infusion into the dorsal pons were marginally significant (for 2 and 5 μg/ml ConA, respectively: $t = -2.0$, $p = 0.06$; and $t = -1.9$, $p = 0.08$).

In contrast, CRH administration into the locus coeruleus did not alter LPS-stimulated spleen lymphocyte responses (Fig. 5). ANOVA on splenic lymphocyte responses to LPS showed no significant effects of treatment [$F(2,39) = 1.0$; $p = 0.36$] or LPS concentration [$F(1,39) = 1.3$; $p = 0.26$], and no treatment × LPS concentration [$F(2,39) = 0.18$; $p = 0.83$].

Plasma concentrations of ACTH, corticosterone and IL-6 were increased after infusion of CRH into the locus coeruleus as

**Table 1. Plasma concentrations of ACTH, corticosterone, and IL-6 after CRH infusion into the locus coeruleus**

| Treatment group | ACTH (pg/ml) | Corticosterone (μg/dl) | IL-6 (pg/ml) |
|---|---|---|---|
| Unoperated control | 25.20 (2.51) | 6.52 (1.90) | 2.61 (1.40) |
| Vehicle into the locus coeruleus | 58.66 (20.63) | 12.03 (5.61) | 48.30 (16.48)* |
| CRH into the locus coeruleus | 196.11 (49.26)* | 45.04 (6.03)* | 172.88 (59.77)* |
| CRH into dorsal pons | 129.48 (29.77) | 27.27 (6.30) | 68.23 (14.58) |

Data are presented as mean (±SEM).

* Significant differences in plasma hormone concentrations as compared with the unoperated control group or the group that received vehicle infusion in the locus coeruleus (Newman-Keuls test, $p < 0.01$).

compared with vehicle infusion into the locus coeruleus or the treatment control groups (Table 1). Plasma concentrations of ACTH, corticosterone and IL-6 were transformed to logarithms due to the unequal error variance across treatment groups (see Table 1). ANOVA on logarithmic transformed data showed a main effect of treatment for ACTH [$F(2,39) = 25.75$; $p < 0.0001$], corticosterone [$F(2,39) = 22.65$; $p < 0.0001$], and IL-6 [$F(2,39) = 40.07$; $p < 0.0001$]. The average concentrations of ACTH, corticosterone, and IL-6 were greater after CRH infusion into the locus coeruleus than after CRH infusion into the dorsal pons; however, these differences did not reach statistical significance [$t(16) = 0.7, 1.7, 1.7$, $p > 0.1$, for ACTH, corticosterone, and IL-6, respectively].

## Discussion

The results of the present study show that infusion of CRH into the locus coeruleus decreased spleen and peripheral blood lymphocyte mitogenic responses. This response is specific to T-lymphocytes because CRH-evoked activation of the locus coeruleus decreased lymphocyte responses to mitogens that selectively stimulate T-lymphocyte proliferation (PHA, ConA, and an antibody to the T-cell receptor). Furthermore, CRH infusion into the locus coeruleus did not alter splenic lymphocyte proliferative responses to LPS, a mitogen that selectively stimulates differentiation of B-lymphocytes. Comparable alteration of lymphocyte function are produced by stress (Lysle et al., 1988, 1990a; Maier et al., 1988; Cunnick et al., 1990). However, more research is needed to understand why T-cell responses are consistently altered by stressors and B-cell responses are not (Komori et al., 1987; Lysle et al., 1990a,b). Thus, the data are consistent with the notion that CRH infused into the locus coeruleus mimics the immunosuppressive effects induced by stress and are consistent with the hypothesis that CRH into the locus coeruleus is involved in stressor-induced immune alterations.

The hypothesis that CRH in the locus coeruleus mediates the effects of stress on immune system function is supported by additional evidence. Locus coeruleus neurons are activated by CRH and stress (Korf et al., 1973; Ehlers et al., 1983; Valentino et al., 1983; Abercrombie and Jacobs, 1987; Valentino and Foote, 1987, 1988; Lachuer et al., 1991; Weiner et al., 1991; De Sarro et al., 1992; Curtis et al., 1993; Pezzone et al., 1993). Also, responses that are symptomatic of stress are produced by administration of CRH into the brain (Britton et al., 1982; Brown et al., 1982; Sutton et al., 1982; Eaves et al., 1985; Koob and Bloom, 1985; Fisher et al., 1989). The locus coeruleus receives CRH-containing afferents (Merchenthaler et al., 1982; Cummings et al., 1983; Swanson et al., 1983; Sakanaka et al., 1987; Valentino et al., 1992), and these afferents are influenced by stress, as indicated by an increase in the concentration of CRH in the locus coeruleus in response to stress (Chapell et al., 1986). Furthermore, the effects of certain noxious stimuli on locus coeruleus neuronal activity can be blocked by antagonists of the CRH receptor (Valentino and Wehby, 1988; Valentino et al., 1991).

The present results also indicate the infusion of CRH into the locus coeruleus produces another response similar to that evoked by stressful stimuli: activation of the hypothalamic–pituitary–adrenal axis. Activation of this system following administration of CRH into the locus coeruleus is reflected by increases in plasma concentrations of ACTH, corticosterone, and IL-6, and confirms a previous report that CRH infused into the locus coeruleus increases plasma corticosterone levels (Butler et al., 1990). The increase in the circulating concentrations of each of these hormones is similar to that observed following exposure of rats to moderate stressors, such as low-intensity footshock (Zhou et al., 1993). Activation of the hypothalamic–pituitary–adrenal axis by infusion of CRH into the locus coeruleus is consistent with previous reports suggesting that activation of locus coeruleus noradrenergic neurons increases the release of ACTH and corticosterone (Plotsky et al., 1989; Carlson and Gann, 1991; Mezey and Palkovits, 1991; Thrivikraman et al., 1993).

Activation of the hypothalamic–pituitary–adrenal axis by infusion of CRH into the locus coeruleus may contribute to the suppression of blood lymphocyte mitogenic responses. Previous work in our laboratory shows that hormone secretion from the adrenal is an essential mediator of stress-induced suppression of mitogenic responses of peripheral blood lymphocytes (Cunnick et al., 1990). Furthermore, an adrenal-dependent release of IL-6 comprises part of the hormonal responses to stress (Zhou et al., 1993), suggesting that the increase in plasma concentrations of IL-6 by infusion of CRH into the locus coeruleus may reflect an increase in adrenal secretion of IL-6 (Judd et al., 1990). Therefore, stress and locus coeruleus activation share an ability to increase hormone secretion from the pituitary and adrenal glands.

Adrenal cortical responses may contribute to the decreased mitogenic responses of blood lymphocytes. However, further study is necessary to determine whether the decreases in blood lymphocyte responses by CRH-evoked activation of the locus coeruleus are dependent upon corticosterone, because some studies show that blood lymphocyte responses are independent of corticosterone (Keller et al., 1983; Jain et al., 1991; Pezzone et al., 1992). In addition, the present findings showed increased plasma concentrations of corticosterone (also ACTH) in some

animals after vehicle infusion into the locus coeruleus and CRH infusion into the dorsal pons that were not associated with decreased mitogenic responses of blood lymphocytes (see Table 1, Figs. 2, 3). The increases in hormone concentrations in the vehicle-treated and the CRH dorsal pons groups probably reflect activation of the hypophysial–adrenal axis that resulted from handling during infusion. Furthermore, the increase in plasma concentrations of corticosterone and ACTH after infusion of CRH into the dorsal pons suggest that some CRH infusions that were just outside the region of the locus coeruleus had effected the function of locus coeruleus neurons. Further study is necessary to determine the mechanisms by decreased blood lymphocyte responses occur as a function of the extent of locus coeruleus activation.

The decreased mitogenic response of splenic T-lymphocytes elicited by CRH infusion into the locus coeruleus most likely results from activation of the sympathetic nervous system. Previous studies have shown that stress-induced suppression of spleen lymphocyte mitogenic responses can be blocked by transection of the splenic nerve (Wan et al., 1993) or pharmacological inhibition of the sympathetic input to the spleen with $\beta$-adrenergic receptor antagonists (Cunnick et al., 1990). However, data on the effects of activating the locus coeruleus neurons on sympathetic neural activity are controversial. Whereas several studies have demonstrated sympathetic activation in response to electrical stimulation of the region of the locus coeruleus (Philippu et al., 1974; Ward and Gunn, 1976), the noradrenergic neurons of the locus coeruleus do not appear to be responsible for this effect (Sved and Felsten, 1987). In addition, stimulation of the locus coeruleus in anesthetized rats by local injection of excitatory drugs elicits a decrease in blood pressure (Sved and Felsten, 1987), and sympathetic neural activity (Miyawaki et al., 1991). Thus, future studies will be required to clarify the role of the sympathetic nervous system in mediating the suppression of lymphocyte responses that is produced by increased locus coeruleus activity.

The present findings may lead to clinical questions concerning the competency or reactivity of the immune system in patients who have functional changes in locus coeruleus activity. Relevant populations include patients experiencing chronic stress (Chapell et al., 1986; Richard et al., 1988; Melia and Duman, 1991), or neuropsychiatric disorders that may be associated with a hypersection of CRH (Holsboer et al., 1984; Nemeroff et al., 1984; Gold et al., 1986), and CNS diseases that are associated with a depletion of locus coeruleus neurons (Tavalato and Argenteiro, 1980; Singh et al., 1987; Chan-Palay, 1991; German et al., 1992).

In summary, the present results suggest that CRH and the locus coeruleus may function as an integral part of a regulatory system that is capable of modulating immunologic responses. Increases in activity within this neural substrate may contribute to the modulatory effects of stressors or CNS CRH systems on cellular immune responses. Further research is necessary to characterize how alterations in brain function during immunologic challenges effect the dynamic equilibrating interactions of the central nervous, endocrine, sympathetic nervous, and immune systems.

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