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Persistent Changes in Corticotropin-Releasing Factor Neuronal Systems Induced by Maternal Deprivation

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ABSTRACT

There is considerable evidence that CRF-containing neurons integrate the endocrine, autonomic, immune, and behavioral responses to stress. In this study we examined long term effects of early stress on developing hypothalamic and extrahypothalamic CRF neural systems in the rat brain and subsequent responses to stress in the adult. Specifically, we sought to determine whether adult male rats previously isolated for 6 h daily during postnatal days 2–20 react in a biochemically distinct manner to a mild foot shock stress compared to controls. Four treatment groups were examined: nondeprived (NDEP)/no shock, NDEP/shock, deprived (DEP)/no shock, and DEP/shock. Compared to the NDEP group, DEP rats exhibited an increase in both basal and stress-induced ACTH concentrations. Moreover, DEP rats exhibited a 125% increase in immunoreactive CRF concentrations in the median eminence and a reduction in the density of CRF receptor binding in the anterior pituitary compared to those in all NDEP rats. Alterations in extrahypothalamic CRF systems were also apparent in DEP *vs.* NDEP animals, with an observed 59% increase in the number of CRF receptor-binding sites in the raphe nucleus and an 86% increase in immunoreactive CRF concentrations in the parabrachial nucleus. These results indicate that maternal deprivation before weaning in male rats produces effects on CRF neural systems in both the central nervous system and pituitary that are apparent several months later and are probably associated with persistent alterations in behavioral responses in adult rats. (*Endocrinology* **137:** 1212–1218, 1996)

THERE IS INCREASING evidence of a preeminent role for psychosocial stressors and life events in the development of affective disorders. This stress diathesis model for the major mood disorders is at least partly based on the findings that stress often precedes the onset of affective episodes in genetically vulnerable individuals (1–3). Not only does it appear that there is a significant correlation between environmental stress and the subsequent onset of an episode of an affective disorder, but there is a marked similarity between the cardinal features of depression in humans and the neuroendocrine and behavioral responses to stress in laboratory animals (4). A putative link between these studies is the neuropeptide CRF. Although long known as the primary mediator of the hypothalamic-pituitary-adrenal (HPA) axis by virtue of its role as a hypothalamic-hypophysiotropic hormone, it was not isolated and sequenced until 1981 (5).

Research continues to accumulate which suggests that patients with major depression exhibit hyperactivity of the HPA axis probably driven by hypersecretion of CRF. Clinical studies have repeatedly shown that drug-free depressed patients exhibit elevated concentrations of serum cortisol, cortisol nonsuppression after administration of the synthetic glucocorticoid dexamethasone (6, 7), increased concentrations of cerebrospinal fluid CRF (8), decreased density of CRF-binding sites in the frontal cortex (9), a blunted ACTH response to administration of CRF (10, 11), and enlarged pituitary and adrenal glands (12). These HPA axis alterations, all plausibly a result of increased hypothalamic CRF secretion, appear to represent a state, rather than a trait marker, of depression, because hypercortisolemia and elevated cerebrospinal fluid CRF concentrations normalize after treatment with electroconvulsive therapy or after clinical recovery (13, 14).

In addition to the role CRF may play in the HPA axis alterations in depression, CRF has a putative role in the behavioral symptoms of this disorder. Specifically, in laboratory animals, centrally administered CRF produces many of the signs and symptoms of depression, including decreased appetite, disrupted sleep, psychomotor alterations, and decreased sexual behavior (15, 16).

Given this burgeoning evidence for a major role for CRF in the pathogenesis of affective disorders, coupled with the observed psychiatric impact of early trauma (17), we hypothesized that early stress, such as that induced by maternal deprivation in neonatal rats, may modify hypothalamic and extrahypothalamic CRF neurons in such a way as to produce augmented and possibly detrimental neurochemical, endocrine, and behavioral stress responses in adults. We, therefore, sought to determine whether adult male rats maternally deprived as neonates react in a neurochemically distinct manner to a mild foot shock stressor.

Materials and Methods

Animals

Animal studies were approved by the Emory University institutional care and use committee under NIH guidelines. Thirteen timed pregnant

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Sprague-Dawley dams with similar parity were obtained from Charles River Laboratories (Raleigh, NC) on day 14 of gestation. The dams gave birth approximately 1 week later within 36 h of each other. Litters were housed in clear plastic cages in a temperature- and humidity-controlled room on a 12-h light, 12-h dark cycle (lights on from 0700–1900 h). Dams received rat chow and water *ad libitum*, and cages were changed only once weekly to avoid excessive handling.

Maternal deprivation

The day of birth was designated day 0; on day 1, all pups were randomized to each of the mothers, with four pups of each sex placed with each mother. Three investigators collaborated in the determination each pup's sex, and each pup received similar handling during this procedure. The rats were assigned to experimental groups by litter to avoid differential maternal treatment toward deprived pups. Beginning on day 2, neonates belonging to the maternally deprived group (DEP; litters 1–6; n = 48) were placed within a temperature- and humidity-controlled incubator (W-1 ICS Warmer, Thermocare, Incline Village, NV) into individual cardboard compartments containing 1.5 cm cage shavings for a set period of time, after which they were returned to their mothers. During days 2–5, pups were isolated for 4 h each, increasing to 6 h on days 6–20. Due to limited space within the incubator, we isolated pups in two shifts, one beginning at 0900 h and the second at 1500 h, alternating shifts to randomize circadian and external variables. To reduce handling to a minimum, pups were housed within 1.5 m of the incubator and transferred to and from their cages quickly and gently. Aside from this, deprived pups received no other handling except that required to change the bedding in their cages once weekly. Nonmaternally deprived rat pups (NDEP; litters 8–13; n = 48) remained with their mothers and siblings during this period and received no special handling other than that necessary to change the bedding in their cages once weekly. To compensate for the mother's body heat, we gauged the temperature of the incubator with respect to the age of the neonates in accordance with previously published procedures for maternal deprivation (18). The ambient temperature was as follows: 35–36 C, days 2–5; 34–35 C, days 6–8; 33–34 C, days 9–11; 31–32 C, days 12–15; and 29–30 C, days 16–20. On day 21, we culled all female pups, whereas male pups were weaned from their mothers and housed as pairs with surrogate siblings for 11 weeks. At this time, we realized that six male DEP rats had been initially misidentified as female on day 1. Thus, we continued the study with a total of 30 male DEP rats and 24 male NDEP rats.

Foot shock stress and tissue collection

On day 108, we weighed all 54 rats to determine whether differential growth had occurred. To avoid the compounded stress of handling before death, all subjects were handled for approximately 5 sec during each of the 3 days preceding decapitation. At this time, each surrogate pair was divided into 2 experimental groups, stressed (STRESS) and control (CON), resulting in 4 treatment groups: NDEP + STRESS (n = 12), NDEP + CON (n = 12); DEP + STRESS (n = 15), and DEP + CON (n = 15). All rats were killed between 0930–1030 h on days 114 and 115. The animals in the two stressed groups received 0.5-sec foot shocks of 400 μA in an enclosed chamber on a 25-sec variable interval schedule for 15 min just before decapitation, whereas those in the nonstressed group were killed immediately after removal from their cage. Brains and pituitaries were removed within 1 min of decapitation and frozen immediately on dry ice. Trunk blood was collected into plastic centrifuge tubes or EDTA-treated glass centrifuge tubes for corticosterone and ACTH measurements, respectively.

Corticosterone and ACTH RIA analysis

Trunk blood for measurement of corticosterone was centrifuged in a high speed centrifuge at 17,000 × *g* at 4 C for 10 min. Five hundred microliters of serum from each sample were placed in a 1.5-ml centrifuge tube and frozen until assay. Plasma for measurement of ACTH was centrifuged for 10 min at 1,600 × *g* at 4 C; 1 ml plasma from each sample was combined with 200 μl 1 M HCl in a 1.5-ml centrifuge tube and frozen until assay. Measurement of corticosterone was performed on 10 μl unextracted serum, run in duplicate. The corticosterone assay (Radioassay Systems Laboratories, Carson, CA) has a sensitivity of 6.25 ng/tube, with 9% and 7% inter- and intraassay coefficients of variation, respectively. The sensitivity of the ACTH assay was less than 1 pmol/liter (19), and the inter- and intraassay coefficients of variation were 14% and 11%, respectively.

CRF receptor binding

Single point CRF receptor binding assays were performed on individual tissue samples from the anterior pituitary, prefrontal cortex, cerebellum, hippocampus, amygdala, and raphe nucleus at a near-saturating concentration of [^{125}I]ovine CRF (1 nmol/liter final concentration; 0.1 nmol/liter [^{125}I]ovine CRF plus 0.9 nmol/liter ovine CRF). Specific CRF receptor binding was calculated by subtracting the mean counts per min in triplicate pellets incubated with [^{125}I]ovine CRF in the presence of 1 μmol/liter unlabeled rat CRF. The K_d of [^{125}I]ovine CRF binding in our laboratory ranges from 0.25–0.6 nmol/L. Brain regions were dissected out of 300-μm frozen tissue slices by a modification of the techniques of Glowinski and Iversen (20) and Palkovits and Brownstein (21), as previously described (19). Samples were homogenized in 4 ml buffer (50 mmol/liter Tris-HCl, 10 mmol/liter $MgCl_2$, and 2 mmol/liter EDTA, pH 7.2, at 22 C) containing the peptidase inhibitors aprotinin (0.1%) and bacitracin (0.1 mmol/L) and 0.1% BSA using a Brinkmann Polytron model 3100 (Brinkmann Instruments, Westbury, NY) at 20,000 rpm for 10 sec, followed by centrifugation at 32,000 × *g* for 10 min at 4 C. This procedure was repeated, and the sample pellets were washed, capped, and frozen at −70 C. On the day of the assay, samples were resuspended and homogenized in buffer to yield a final concentration of approximately 150 μg protein/100 μl. One hundred-microliter aliquots of membrane homogenate were incubated for 2 h with 100 μl [^{125}I]ovine CRF (0.1 nmol/liter final concentration; New England Nuclear, Boston, MA), 50 μl ovine CRF (0.9 nmol/liter final concentration), and 50 μl of either ovine CRF (1 μmol/liter final concentration) to define nonspecific binding or incubation buffer (total binding). Specific binding as a percentage of total binding varies slightly among brain regions and represents 60–70% of total binding. Aliquots of the tissue homogenate were used to determine total protein content using BSA as the standard (22). After incubation, samples were microcentrifuged for 3 min at 12,000 × *g*, aspirated, then washed in ice-cold PBS (8.0 g/liter NaCl, 0.2 g/liter KCl, 1.44 g/liter Na_2HPO_4, 0.24 g/liter KH_2PO_4, pH 7.4 with HCl) containing 0.01% Triton X-100. The supernatant was removed, and the pellet was counted in an LKB γ-counter (LKB, Rockville, MD; 76% efficiency).

CRF RIA

Tissue for CRF RIA was micropunched from 300-μm slices thaw-mounted onto polarized slides and stored with dessicant at −70 C until dissection. The dry weight of each sample was not measured; immunoreactivity is expressed as picograms per mg protein. Immunoreactive CRF (irCRF) concentrations were determined by RIA as described by Vale *et al.* (23) with minor modifications (24). Briefly, after extraction in 1 M HCl and lyophilization, duplicate aliquots from each sample were reconstituted in 200 μl RIA buffer (SPEAB buffer = 100 mmol/liter NaCl, 50 mmol/liter Na_2HPO_4, 25 mmol/liter EDTA, 0.1% sodium azide, 0.1% BSA, and 0.1% Triton X-100, pH 7.3) and incubated at 4 C for 24 h with 100 μl oC33 Salk Institute antiserum (generously provided by W. Vale) at a final dilution of 1:26,250 in SPEAB buffer containing 1.5% normal rabbit serum. After dilution in SPEAB buffer, 50 μl (~20,000 cpm) radiolabeled [^{125}I]Tyr^0-rat/human CRF (New England Nuclear, Boston, MA) were added to each tube. After incubation with radiolabeled CRF for 24 h at 4 C, 10 μl of a second antibody (goat antirabbit serum; Arnel Products, New York, NY) were added to precipitate bound CRF.

A standard curve was prepared using rat/human CRF (Bachem, Torrance, CA) from 0.625–5120 pg/tube. The sensitivity of the assay was 1.25 pg/tube, with 50% displacement of radiolabeled CRF (IC_{50}) at 30 pg/tube. The inter- and intraassay coefficients of variation are 10–13% and 2–8%, respectively. CRF immunoreactivity measured by the antiserum used in this assay from brain extracts subjected to HPLC has been shown to cochromatograph with synthetic CRF (25).

All data are expressed as the mean ± SE. Statistical analysis was

performed using a two-way ANOVA, followed by the Student-Newman-Keuls test.

Results

Plasma ACTH

Measurement of plasma ACTH concentrations (Fig. 1) revealed that DEP/CON rats exhibited a significant increase in basal ACTH concentrations compared to NDEP/CON rats. Moreover, the DEP/STRESS group exhibited an augmented ACTH response to stress compared to the NDEP/STRESS group.

Plasma corticosterone

No significant differences in basal serum corticosterone concentrations were observed between the DEP/CON animals and the NDEP/CON animals (Fig. 2). There were no significant differences in stress-induced increases in plasma corticosterone concentrations between the two differentially reared groups. As expected, an increase in corticosterone concentrations was observed after the mild foot shock stress in both DEP and NDEP rats.

[^{125}I]Ovine CRF receptor binding

Compared to the NDEP group, a significant decrease in the number of [^{125}I]ovine CRF-binding sites was observed in the adenohypophysis from the DEP rats (Fig. 3). CRF binding in the DEP group as a whole was 3.86 ± 0.21 fmol/mg protein, compared to 4.54 ± 0.25 fmol/mg protein in the NDEP group as a whole. No significant differences in [^{125}I]ovine CRF binding were observed between the STRESS and CON experimental groups.

In the raphe nucleus (Fig. 4), a significant increase (59%) in [^{125}I]ovine CRF-binding sites was observed in the DEP rats (n = 21) compared with the NDEP group (n = 16). There were no significant differences in the density of CRF-binding sites between the STRESS and CON animals. [^{125}I]Ovine CRF binding in DEP rats was 0.38 ± 0.045 fmol/mg protein, whereas their NDEP counterparts exhibited a mean CRF-binding site concentration of 0.24 ± 0.053 fmol/mg protein.

There were no other group-related differences in CRF

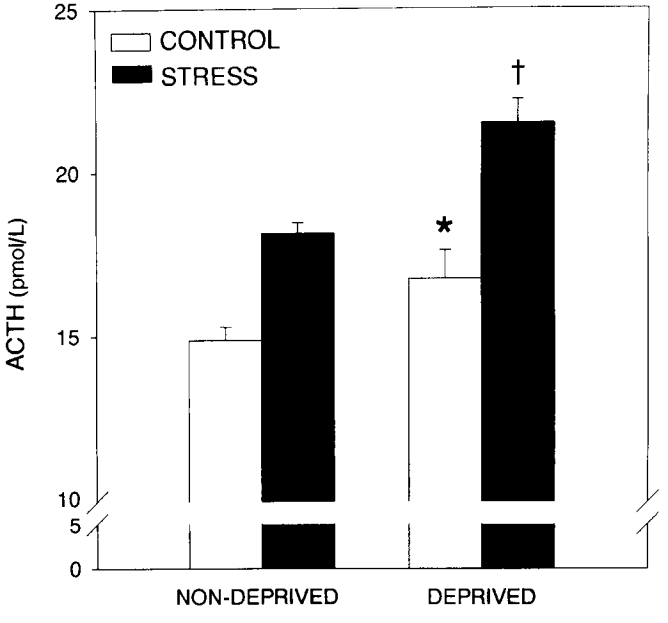

FIG. 1. Plasma ACTH concentrations (mean ± SEM) in four experimental groups: NDEP/CON (n = 12), NDEP/STRESS (n = 12), DEP/CON (n = 15), and DEP/STRESS (n = 15). *, $P < 0.05$ *vs.* NDEP/CON counterparts; †, $P < 0.05$ *vs.* NON/STR.

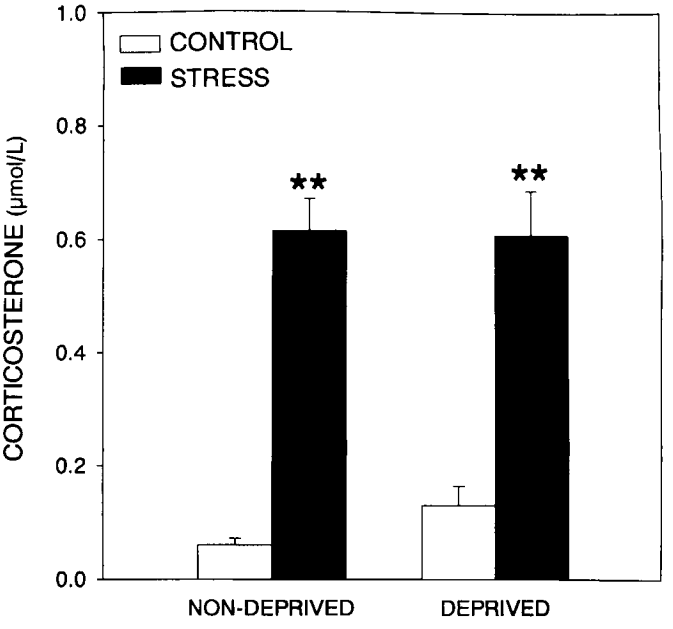

FIG. 2. Serum corticosterone concentrations (mean ± SEM) in four experimental groups: NDEP/CON (n = 12), DEP/CON (n = 12), NDEP/STRESS (n = 15), and DEP/STRESS (n = 15). **, $P < 0.001$ *vs.* no shock.

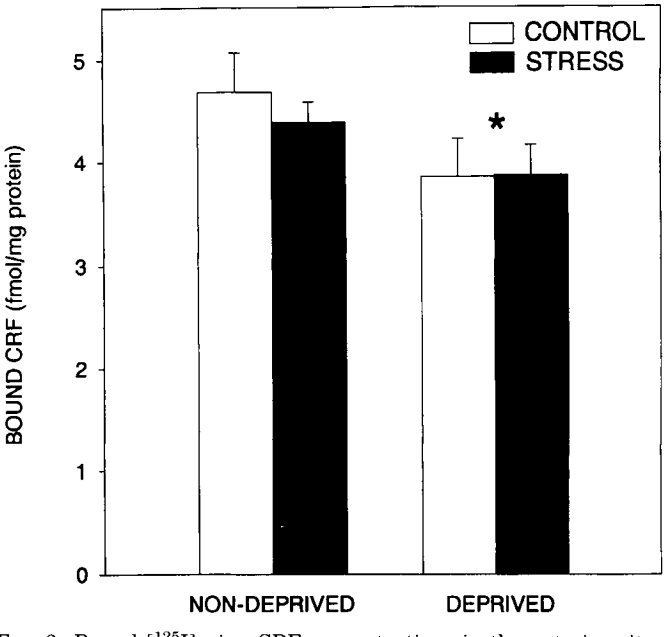

FIG. 3. Bound [^{125}I]ovine CRF concentrations in the anterior pituitary (mean ± SEM) in four experimental groups: NDEP/CON (n = 12), NDEP/STRESS (n = 11), DEP/CON (n = 15), and DEP/STRESS (n = 15). *, $P < 0.05$ *vs.* all NDEP rats.

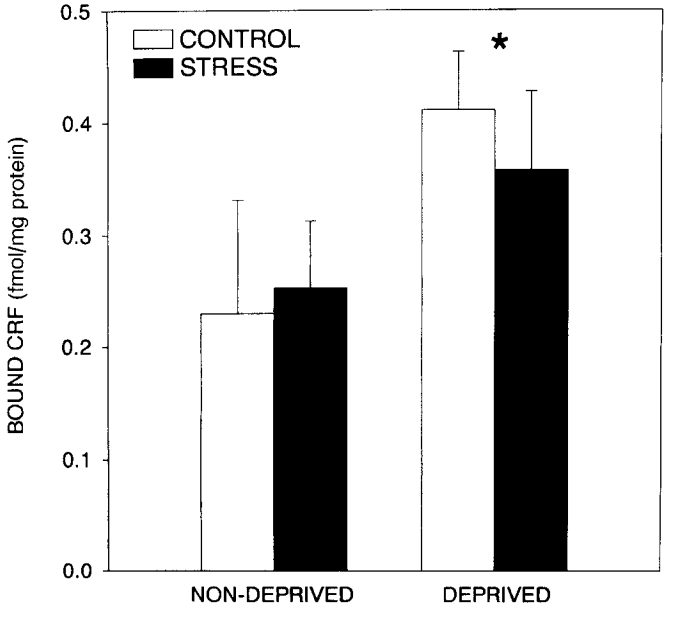

FIG. 4. Bound [^{125}I]ovine CRF concentrations in the raphe nuclei (mean ± SEM) in four experimental groups: NDEP/CON (n = 5), NDEP/STRESS (n = 10), DEP/CON (n = 11), and DEP/STRESS (n = 10). *, $P < 0.05$ *vs.* all NDEP rats.

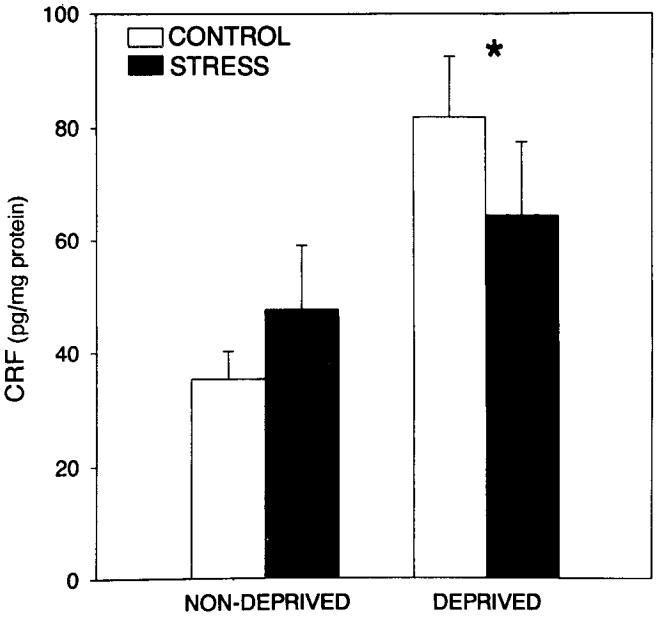

FIG. 5. Immunoreactive CRF (mean ± SEM) in the parabrachial nucleus in four experimental groups: NDEP/CON (n = 8), NDEP/STRESS (n = 8), DEP/CON (n = 8), and DEP/STRESS (n = 9). *, $P < 0.005$ *vs.* all NDEP rats.

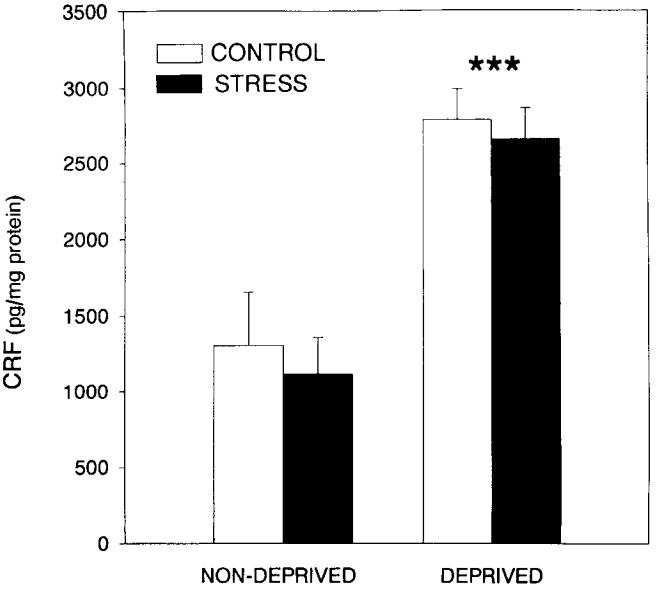

FIG. 6. Immunoreactive CRF (mean ± SEM) in the median eminence in four experimental groups: NDEP/CON (n = 10), NDEP/STRESS (n = 11), DEP/CON (n = 15), and DEP/STRESS (n = 15). ***, $P < 0.0001$ *vs.* all NDEP rats.

receptor binding in any of the other five regions examined (Table 1).

CRF RIA

irCRF concentrations in both the parabrachial nucleus (Fig. 5) and median eminence (Fig. 6) were markedly elevated in the DEP rats, with 86% and 125% increases in irCRF concentrations, respectively. irCRF concentrations in the parabrachial nucleus were 67.9 ± 5.6 pg/mg protein for all DEP rats (n = 18) and 36.4 ± 5.8 pg/mg protein for all NDEP rats (n = 17). In the median eminence, irCRF averaged 2723.4 ± 146.4 pg/mg protein in the DEP (n = 30) group as a whole and 1209.8 ± 175.2 pg/mg protein in the NDEP (n = 21) group. A trend, not statistically significant, toward decreased irCRF concentrations in the median eminence between CON and STRESS groups after the foot shock stressor was observed (Fig. 6).

Discussion

Maternal deprivation was chosen as the stressor in this experiment for several reasons. As a paradigm for early neglect/trauma, it removes a social zeitgeber of the mother that is believed to suppress the stress response in neonatal rats (26). Secondly, maternal deprivation is considered the most potent naturalistic stressor to which rats can be exposed during the stress hyporesponsive period on postnatal days 3–16 (18, 27, 28). Thirdly, early loss has been demonstrated to contribute to the development of affective disorders in adults (17).

Studies using immunohistochemistry and RIA have localized CRF-containing neurons in cortical, limbic, and brain stem regions (29). Several of these regions have been implicated in the pathophysiology of various psychiatric illnesses, specifically affective and anxiety disorders (30, 31). The locus

TABLE 1. Regional brain CRF receptor concentrations in deprived rats *vs.* controls

Brain region	Nondeprived	Maternally deprived
Prefrontal cortex	4.09 ± 0.16	4.14 ± 0.16
Piriform cortex	2.20 ± 0.32	2.41 ± 0.26
Amygdala	1.31 ± 0.14	1.34 ± 0.14
Hippocampus	1.49 ± 0.10	1.60 ± 0.09
Cerebellum	3.76 ± 0.16	3.76 ± 0.15

ceruleus (LC), the source of the vast majority of the noradrenergic (NE) projections to the forebrain, is a fundamental region of interest in the hypothesis linking alterations of NE neurotransmission with stress, anxiety, and affective disorders (32–34); these LC NE neurons exhibit an increase in firing rate after the application of CRF. Furthermore, exposure to both acute and chronic stress results in a 2-fold increase in irCRF concentrations in the LC (35); interestingly, acute or chronic administration of the anxiolytic triazolobenzodiazepine alprazolam decreases irCRF concentrations in the same region (24, 44). Additionally, several investigators have examined the role of the central nucleus of the amygdala as a primary mediator of behavioral and emotional responses to stress (36); these responses are attenuated in rats after intraamygdaloid injections of the CRF antagonist α-helical CRF-(9–41) (37, 38). Thirdly, numerous recent studies suggest considerable plasticity in both hypothalamic and extrahypothalamic CRF systems modulated by CRF itself (39), psychosocial stress (40), early maternal deprivation (41), and several psychoactive drugs (24, 42–44). Thus, both extrahypothalamic and hypothalamic CRF neurons may play a substantial role in the development of and/or vulnerability to stress and affective disorders and in the mechanisms of action of antidepressants and anxiolytics.

Maternal deprivation stress appears to cause persistent alterations in regional CNS CRF systems without measurably hindering the pup's growth, as no significant differences in body weight were observed on day 108 between DEP and NDEP rats (NDEP, 562 ± 12 g; DEP, 557 ± 10 g).

The mild foot shock used was sufficient to provoke a submaximal stress response, as indicated by elevated plasma ACTH and corticosterone concentrations in the stressed rats of both DEP and NDEP groups. Of particular interest is our observation that the DEP animals exhibited augmentation of both basal and stress-induced plasma ACTH concentrations compared to NDEP controls. A similar trend in basal corticosterone concentrations between the DEP and NDEP rats was noted, although the differences did not attain statistical significance. Indeed, there were no significant differences in plasma corticosterone concentrations (stress induced or basal) between the differently reared animals. This apparent discrepancy between plasma ACTH and corticosterone responses in the deprived animals might be corrected if corticosterone concentrations were measured at a slightly later time period or if the area under the duration-response curve were examined. Alternatively, measurement of adrenocortical function and structure might reveal differential ACTH sensitivity and/or adrenal weight between the DEP and NDEP animals.

The 125% increase in irCRF concentrations in the median eminence in DEP rats in conjunction with the observed 23% decrease in CRF receptor binding in the anterior pituitary of this same group suggests that CRF secretion from nerve terminals in the median eminence may be chronically elevated, resulting in CRF receptor down-regulation. This hypothesis of increased CRF secretion is further strengthened by the augmented ACTH release in deprived animals compared to the controls. Another view of these findings is that of increased synthesis and storage of CRF in the median eminence and little or no increase in secretion. This consideration seems unlikely, however, in light of the apparent hyperactivity of the HPA axis downstream from the median eminence and evidence of anterior pituitary CRF receptor down-regulation. Alternatively, the DEP rats might be desensitized to negative feedback signals of basal corticosterone compared to their NDEP counterparts, a hypothesis explored extensively by Plotsky and Meaney (41), who reported similar findings in irCRF concentrations and HPA axis activity in adult rats previously exposed to either maternal separation or handling as pups *vs.* those in a nonhandled control group. In addition, Maccari *et al.* (45) observed decreased glucocorticoid receptor concentrations in rats exposed to prenatal stress. Still another possible explanation for these findings is that anterior pituitary CRF receptors of the deprived group may become more sensitive after early trauma, such that, when subjected to later stress, these rats exhibit an augmented ACTH response. This phenomenon is currently being studied by measuring second messenger responsivity to CRF in pituitary and brain tissue from DEP *vs.* NDEP rats.

Extrahypothalamic CRF neuronal systems also appear to be influenced by early maternal deprivation, as indicated by the observed 59% increase in CRF-binding sites in the raphe nucleus and the 86% increase in irCRF concentrations in the parabrachial nucleus in DEP *vs.* NDEP animals. The increase in CRF receptor binding in the raphe nucleus of the DEP animals suggests an up-regulation of CRF receptors in this area, which is known to contain serotonergic perikarya. Potential neuroanatomical and functional connections between these two neurotransmitter systems is especially intriguing in view of the considerable evidence that alterations in serotonin neural systems play an important role in the pathophysiology of depression (46). This observation marks the first of its kind regarding alterations in CRF receptor binding in the raphe nucleus. Although Valentino and colleagues (32) studied the effects of CRF on LC firing rates, we know of no study that has examined the electrophysiological effects of CRF on serotonergic perikarya in the raphe nuclei.

The parabrachial nucleus (PBN) receives CRF projections from the central nucleus of the amygdala (47). Moreover, the parabrachial nucleus has been implicated in behavioral and autonomic manifestations of stress. We (31) recently reported that CRF infused bilaterally into the PBN induces fear, anxiety, and decreased motor activity in rats, responses that are blocked by α-helical CRF-(9–41). In our study, an 86% increase in irCRF in DEP *vs.* NDEP rats was observed in the PBN, a finding that further supports the hypothesis that DEP rats experience a persistent heightened sensitivity toward stress and/or a chronically stressed state.

As opposed to the aforementioned brain regions, no significant differences in CRF receptor density concentrations were found between the differently reared groups in the prefrontal cortex, piriform cortex, amygdala, cerebellum, or hippocampus. The brain regions chosen for investigation in the present study were based on previous observations in both our laboratory and others. We recently observed changes in CRF receptor density in the frontal cortex, amygdala, hippocampus, cerebellum, and anterior pituitary of 12-day-old rats maternally deprived for 24 h before death (48). These rats also exhibited heightened basal and aug-

mented stress-induced ACTH concentrations compared to NDEP controls. Future studies will examine other brain regions, as modulation of either CRF immunoreactivity or CRF receptor concentrations in the LC, bed nucleus of the striata terminalis, or amygdala, for example, would not be unexpected in light of the suspected CRF pathways linking these regions in the rat brain (see above). Moreover, examination of the PVN of the hypothalamus for changes in CRF messenger RNA and *c-fos* expression before, during, and at close intervals after a mild foot shock stressor and/or maternal deprivation might be revealing.

As a result of early maternal deprivation, both hypothalamic and extrahypothalamic CRF systems in the neonatal rat develop long term neurophysiological modifications that may contribute to pathological basal and stress-induced behavior in the adult compared to that in NDEP controls. Our results suggest that in the DEP group, CRF may be chronically hypersecreted from hypothalamic neurons via the median eminence, leading to down-regulation of CRF receptors in the anterior pituitary and a concomitant augmentation of both basal and stress-induced ACTH concentrations in the rat. Additionally, we provided the first evidence that chronic early maternal deprivation before weaning in male rats can have profound long term effects on developing CRF neurons in extrahypothalamic brain regions, possibly further modifying the HPA axis and potentially leading to behavioral manifestations as well. In conjunction with recently published studies (see above), these data provide increasing evidence that early traumatic environmental stressors can lead to long lasting alterations in an organism's neural systems and its functional responses to the environment. If further supported by more extensive examination, these results could have a profound impact on our understanding of the etiology of affective and other psychiatric disorders.

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