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Increased corticotropin-releasing factor concentrations in the bed nucleus of the stria terminalis of anhedonic rats

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Abstract

Chronic mild stress in rats is an antidepressant-responsive model for anhedonic symptoms of major depression. Many patients with depression exhibit alterations in hypothalamic–pituitary–adrenal axis activity, and corticotropin-releasing factor (CRF) neuronal function. This study investigated the potential involvement of CRF and CRF receptors in the development of chronic mild stress-induced anhedonia in rats. Rats were subjected to 19 days of chronic mild stress, during which time anhedonia was periodically assessed by determining the threshold for self-stimulation of the ventral tegmental area. Anhedonic rats exhibited a 50% increase in CRF concentrations in the bed nucleus of the stria terminalis compared to control rats. There were no significant changes in hypothalamic–pituitary–adrenal axis activity, CRF or CRF_1 receptor mRNA expression, or CRF receptor binding in the brain regions analyzed. Though preliminary, these results are consistent with the hypothesis that chronic stress-induced modulation of CRF function in specific brain structures such as the bed nucleus of the stria terminalis may contribute to the pathophysiology of depression.

Keywords: Animal model; Depression; CRF (corticotropin-releasing factor); Bed nucleus of the stria terminalis

1. Introduction

One of the two core symptoms required for diagnosis of a major depressive episode is anhedonia, the loss of interest or pleasure in all or most activities (DSM-IV, 1994). An analogous state of anhedonia or subsensitivity to reward can be induced in rats by a regimen of chronic, mild and unpredictable stressors (Willner, 1997). This chronic mild stress regimen alters behavioral parameters consistent with a loss of responsiveness to reward, such as decreased sucrose consumption (Willner et al., 1987), decreased place preference conditioning (Papp et al., 1991) and decreased intracranial self-stimulation performance (Moreau et al., 1992). The stress-induced anhedonic-like state in rats gradually develops over several days and can be prevented or reversed by chronic, but not acute, administration of a variety of antidepressant drugs or electroconvulsive shock (Moreau et al., 1998). Moreover, similar electroencephalographic changes during the sleep cycle (reduced latency to the first rapid eye movement (REM) sleep episode and increased time spent in REM sleep) are found in depressed patients and in rats exposed to chronic mild stress (Cheeta et al., 1995; Moreau et al., 1995). Finally, the chronic mild stress model has construct validity in that adverse life events and chronic stress have been recognized as predisposing factors in the etiology of depression (Billings et al., 1983; Kendler et al., 1993; Lloyd, 1980a,b). For these reasons, the chronic mild stress model may provide a means to study pathophysiological mechanisms underlying major depression.

Corticotropin-releasing factor (CRF) is the primary hypothalamic regulator of the stress neuroendocrine axis in many mammalian species, including rats and humans. In addition to its endocrine function at the pituitary level,

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CRF functions as a neurotransmitter in the brain to integrate behavioral and autonomic responses to stress. Substantial clinical evidence is concordant with the hypothesis that CRF is involved in stress-related disorders including major depression (see Heit et al., 1997 for review). Patients with major depression exhibit endocrine changes including hypercortisolemia and a blunted adrenocorticotropic hormone (ACTH) response to CRF administration (Plotsky et al., 1998). Furthermore, increased cerebrospinal fluid CRF concentrations and decreased CRF binding sites in the frontal cortex have been demonstrated in depressed patients and/or suicide victims (Banki et al., 1987; Nemeroff et al., 1988); the latter finding is hypothesized to reflect receptor downregulation in response to increased synaptic CRF release. Precise neuroanatomic and neuropharmacologic characterization of CRF hypersecretion in depression has not been achieved, with the exception of Raadsheer et al.'s (1994, 1995) findings that CRF concentration and CRF mRNA expression is increased in postmortem tissue of depressed patients compared to matched controls. The present study was designed to investigate whether changes in CRF and/or CRF receptor function occur in chronically stressed, anhedonic rats.

2. Materials and methods

2.1. Animals

Male, albino Wistar rats (Ibm:RoRo; Biological Research Institute, Füllinsdorf, Switzerland) weighing approximately 350 g at the start of the experiment were used. After surgery, rats were maintained individually in Macrolon type III containers under standard laboratory conditions (12 h light/dark cycle, 21–23°C, relative humidity of 55–65%) with free access to food (Kliba Mhhlen, Kaiseraugst, Switzerland) and tap water. Stressed and control animals were housed in the same quarters, except as otherwise indicated. All procedures described here are in compliance with ethical principles and guidelines for scientific experiments on animals (Swiss Academies of Sciences and Medical Sciences, 1995).

2.2. Surgery

Animals were anesthetized with i.p. injection of 90 mg/kg ketamine hydrochloride and 10 mg/kg Xylazin 2% in solution in physiological saline and administered atropine sulfate (0.125 mg i.p.) to prevent excessive bronchopulmonary secretion. Properly-insulated stainless-steel bipolar electrodes (MS 303/1, Plastic Products, Roanoke, VA, USA) were stereotaxically implanted unilaterally in the ventral tegmental area of the midbrain (2 mm anterior from lambda, 0.3 mm lateral from the midline suture, and 8.5 mm ventral from the skull surface). The electrode assembly was secured to the skull by three stainless-steel screws and an autopolymerizing resin. A histophilic and antiseptic plastic film (Nobecutan) was sprayed to close the wound. Animals were maintained post-operatively in a warm environment until fully awake and were given post-operative analgesic treatment (0.05 mg/kg buprenorphine, s.c.). They were allowed at least 5 days recovery before starting training.

2.3. Ventral tegmentum self-stimulation procedure

The test chambers consisted of Plexiglas boxes ($30 \times 25 \times 25$ cm) with a hole (2.5 cm in diameter) located in a sidewall 5 cm above the floor. The rat could interrupt a convergent light beam with a nose poke to trigger electrical brain stimulation. Bipolar stimulation (0.5 s trains of monophasic square pulses of 0.1 ms duration) was delivered from a constant-current stimulator controlled by a PDP 11 computer, which also recorded the number of nose poke responses per test session. In the training phase, each rat was placed into a test chamber and trained to make a nose poke response for rewarding intracranial electrical stimulation. The frequency was kept at 70 Hz and the current intensity was made available which, for each individual rat, maintained the highest response rate without observable motor impairment (range: 260–480 μA). Training continued until stable responding was achieved. Subsequently, the threshold frequency for ventral tegmentum self-stimulation behavior was determined, with stimulation intensity maintained constant for each rat. Briefly, the frequency of stimulation was varied between 20 and 80 Hz in a stepwise descending and ascending fashion, in steps of 10 Hz, with 2-min testing periods at each level. The ventral tegmentum self-stimulation threshold was defined as the mean of the ascending and descending frequencies eliciting 15 nose pokes/min. In the absence of brain stimulation, response rate was usually lower than 10 nose pokes/min and never exceeded 15. This procedure has been described in greater detail elsewhere (Moreau et al., 1992).

2.4. Stress procedure

The chronic mild stress procedure was applied for 19 days. The stress regimen consisted of various mild physical, psychological and circadian stressors as previously described (Moreau et al., 1992), and similar to another model (Willner et al., 1992) in which the combination of stressors but not any of the individual stressors is sufficient to produce anhedonia. The stressors included repeated periods of confinement to small ($24 \times 10 \times 9$ cm) cages, one period of continuous overnight illumination, one overnight period of food and water deprivation immediately followed by 2 h of access to restricted food, one overnight period of water deprivation immediately fol-

lowed by 1 h exposure to an empty bottle, one overnight period of group housing in a soiled cage, and three consecutive reversed light/dark cycles. The rats were removed to a separate facility for all stressors, and were returned to the main animal quarters following a recovery period of several hours. All rats within the chronic mild stress group underwent the same schedule of stressors.

2.5. Test procedure

The experiment was started when the self-stimulation threshold of individual rats varied by less than 10% over three consecutive daily test sessions. For a total of 19 days, one group of 10 rats was subjected to the chronic mild stress regimen whereas the control group ($n = 10$) was maintained under standard laboratory conditions. In both groups, ventral tegmentum self-stimulation threshold was determined twice weekly and the threshold value for each test day was compared to average pretest baseline threshold values. All ventral tegmentum self-stimulation testing was done in the morning prior to any acute stressors. Results are expressed as percentage change in ventral tegmentum self-stimulation threshold (anhedonia index). Sacrifices took place between 1300 and 1600 h, 6–9 h after the final stressor. Rats were decapitated, and trunk blood collected in cooled tubes. Brains and anterior pituitary glands were quickly removed from the skull and dropped in isopentane -20°C for about 1 min before being stored in deep freezer at -80°C until assayed.

2.6. Neurochemical experiments

Plasma concentrations of ACTH were determined using a two-site immunoradiometric assay (Nichols). Serum concentrations of corticosterone were determined using a commercial radioimmunoassay kit (ICN Biochemicals). Intra- and inter-assay variability for these assays are less than 8%.

Brains were thawed on a chilled glass plate and dissected into brain regions according to anatomic landmarks (Paxinos and Watson, 1986). Right and left amygdalae were dissected off of the diencephalon, and the remaining tissue block was cryosectioned at 20 μm for in situ hybridization histochemistry. Dissected brain regions and mounted sections were stored at -80°C until the day of the assay. Brain regions were assayed for CRF or CRF receptor mRNA, peptide, or receptor binding primarily on the basis of relative signal densities (e.g., the bed nucleus of the stria terminalis contains a high CRF concentration), as well as preliminary reports from the literature (Bissette et al., 1997). It was not possible in this single experiment to assay multiple variables in the same brain region.

CRF was extracted from tissue dissections in 1 M HCl with protease inhibitors as previously described (Ladd et al., 1996). CRF concentration was determined in duplicate by radioimmunoassay (RIA), as previously described except a CRF antiserum obtained from Peninsula Laboratories was used (final dilution 1:23,000). Synthetic peptides were used for the CRF standard (Bachem), and [^{125}I]-^{0}Tyr-rat/human-CRF tracer (NEN Dupont). The limit of sensitivity was 2.5 pg/tube; with few exceptions the sample tubes contained at least 10 pg CRF.

CRF receptor binding in membrane homogenates was determined as previously described (Ladd et al., 1996). Tissue samples were washed twice to remove endogenous ligand. Single-point binding of 1.0 nM oCRF (0.1 nM [^{125}I]-^{0}Tyr-oCRF (NEN Dupont) + 0.9 nM unlabeled oCRF) was determined. Non-specific binding was determined in the presence of 1.0 μM rat/human CRF. Under these assay conditions, we have obtained K_d values of 0.3–0.5 nM in tissue homogenates from various rat brain regions; therefore, we consider binding of 1.0 nM oCRF equivalent to B_{max}. Due to cost and tissue limitations, and our hypothesis that changes in receptor number rather than affinity would be more likely to occur, full Scatchard analysis was not performed.

RIA and receptor binding data were normalized with respect to protein content according to the method of Lowry (Lowry et al., 1951) using bovine serum albumin as the standard.

The CRF and CRF_1 receptor plasmids were obtained from K. Mayo (Northwestern University, Evanston, IL) and W. Vale (Salk Institute, La Jolla, CA), respectively. The CRF plasmid was linearized with *Pvu*II and transcribed with SP6 polymerase to generate a 593-base antisense probe. The sense-strand probe was generated using *Fsp*I and T7 enzymes. The CRF_1 receptor plasmid was linearized with *Bsa*HI and transcribed with T7 polymerase to generate a 189-base probe. A commercial actin template (Ambion, Austin, TX) was transcribed with T7 polymerase to generate a 125-base probe. Buffers and nucleotides for in vitro transcription were obtained from Ambion (Maxiscript); [^{35}S]- or [^{32}P]-UTP (NEN Dupont) was included in the CRF, or CRF_1 and actin reactions, respectively. The CRF RNA probe was purified by ethanol precipitation, and the probes for ribonuclease protection assay were gel-purified.

Every fourth coronal section through the paraventricular nucleus of the hypothalamus was processed for in situ hybridization histochemistry as described previously (Simmons et al., 1989), with minor modifications. The fresh-frozen brain sections were post-fixed in 4% paraformaldehyde and washed in phosphate-buffered saline (Sigma) prior to the proteinase K step. Approximately 1,000,000 cpm (~10 ng) of CRF probe were added to each slide prior to overnight hybridization at 60°C. The high-stringency wash step was conducted in 0.1× saline-sodium citrate/1 mM dithiothreitol at 60°C for 30 min. Slides were apposed to X-ray film (Kodak Biomax) for approximately 8 h, and coded images were digitized and analyzed

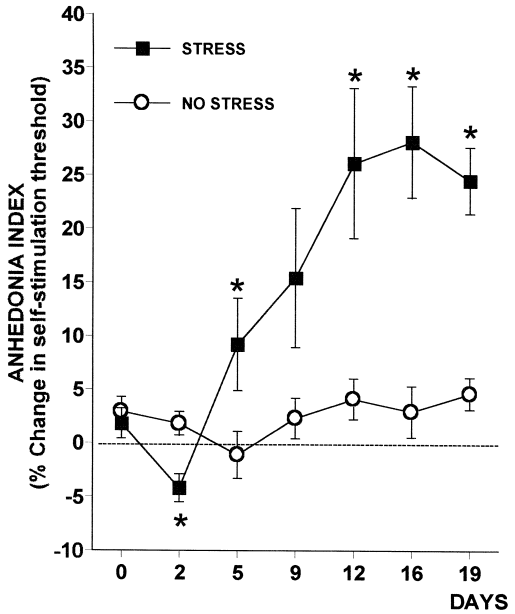

Fig. 1. Anhedonia induced by a chronic mild stress procedure in rats. Variations in self-stimulation threshold in stressed and non-stressed animals are shown as a function of stress exposure time. Asterisks indicate statistically significant difference (Student's *t*-test, $P < 0.05$) between stressed and control animals.

for grain density in the circled paraventricular nucleus region (NIH Image). Density measurements were converted to tissue-equivalent nCi/g using a ^{14}C standard curve (Amersham). Background nCi/g was measured in the thalamus of each section and subtracted from paraventricular nucleus signal. A single number for each brain was obtained by averaging the three paraventricular nucleus sections containing the highest CRF mRNA density. No detectable signal was observed when the sense-strand probe was used.

Total RNA was extracted from frontal cortex and cerebellum samples using a modified phenol/guanidinium thiocyonate procedure (Sigma, TriReagent). RNA concentrations were determined by optical density at 260 nm. Tissue RNA (20 μg/tube) was hybridized with 80,000 cpms actin and 200,000 cpms CRF_1 receptor probes; the specific activity of the actin probe was roughly 1000 times lower than of CRF_1 to correct for the relative abundance of signal. The protocol and reagents for the RPA were obtained from Ambion (HybSpeed). A 1:1600 dilution of the supplied RNAse A/T1 mixture was used in the reaction. The hybrids were separated using 8 M urea, 4% polyacrylamide gel electrophoresis, and the gels apposed to X-ray film (Kodak X-omat) with intensifying screens for 3–5 days. Background and band densities were determined using NIH Image. Doubling the amount of tissue RNA in the assay produced 2.0- and 2.8-fold increases in the actin and CRF_1 signals. Therefore, the signal was quantitative if not directly proportionate to RNA amount.

2.7. Data analysis

Behavioral data were analyzed by repeated-measures analysis of variance followed by post-hoc Dunnett's *t*-test. Neurochemical data were analyzed by individual *t*-tests ($\alpha = 0.05$) following Kolmogorov–Smirnov testing for normality ($P > 0.1$); exceptions are noted in the text. Correlations between pairs of variables were evaluated using Pearson product moment correlation.

3. Results

3.1. Behavioral evaluation of stress-induced anhedonia

The typical effects of a chronic unpredictable mild stress regimen on ventral tegmentum self-stimulation behavior are shown in Fig. 1. Before stress, ventral tegmentum self-stimulation frequency thresholds (mean ± S.E.M.) for control and stressed groups were 46.4 ± 0.4 and 47.1 ± 0.5 Hz, respectively. Comparisons of data obtained in

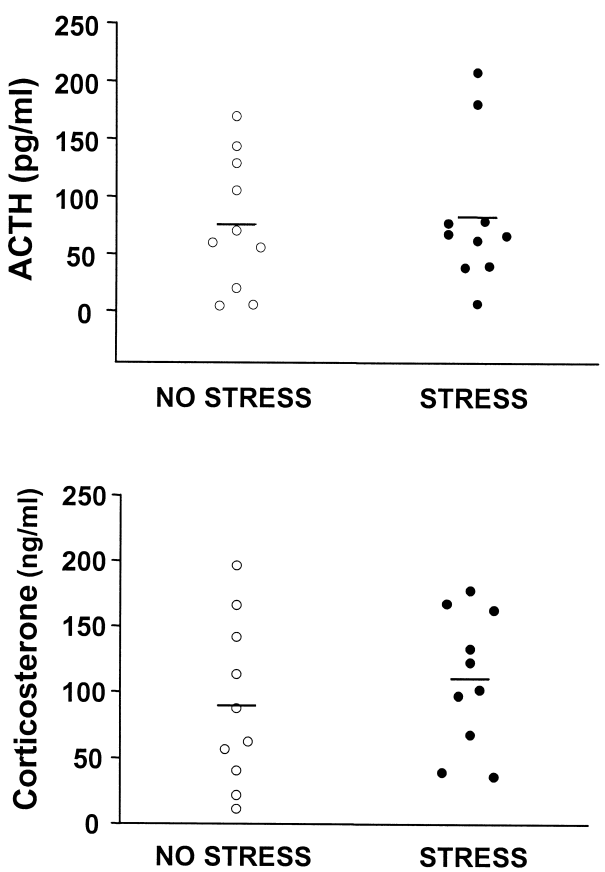

Fig. 2. Hypothalamic–pituitary–adrenal axis activity in rats exposed to chronic mild stress. Plasma ACTH and corticosterone concentrations were determined in trunk blood of stressed and non-stressed rats. There was no statistically significant effect of chronic mild stress on either measure.

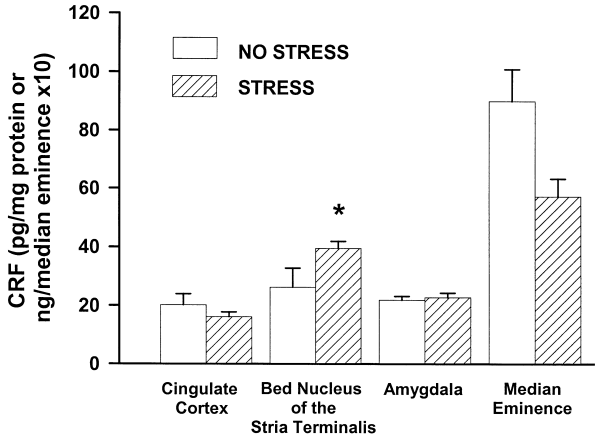

Fig. 3. CRF immunoreactivity in dissected brain regions. CRF concentration was determined in stressed and non-stressed rats (mean ± S.E.M.). There was a statistically significant effect of chronic mild stress on CRF concentration in the bed nucleus of the stria terminalis ($P < 0.01$). $N = 10$ per group, except median eminence $n = 4$–5 per group (see text).

stressed and non-stressed animals revealed a significant stress effect [$F(6,108) = 9.99$, $P < 0.001$] from day 5 to day 19 of the experiment. In stressed animals, ventral tegmentum self-stimulation threshold progressively increased (i.e., reward sensitivity progressively decreased) over a period of about 2 weeks of mild stress and then remain consistently high throughout the remainder of the stress period. At the 19th day of the stress regimen, ventral tegmentum self-stimulation frequency thresholds for control and stressed animals were 48.5 ± 0.8 and 58.6 ± 1.4 Hz, respectively. In non-stressed animals, ventral tegmentum self-stimulation threshold did not appreciably vary throughout the entire experiment [$F(6,63) = 1.04$, $P > 0.05$)

3.2. ACTH and corticosterone

ACTH and corticosterone concentrations were determined in trunk blood obtained from rats at the time of sacrifice. There was no significant effect of chronic mild stress on serum ACTH or corticosterone concentration (Fig. 2). Deletion of the two high outliers (1.6 and 2.1 × S.D. from the mean) within the stress ACTH data set did not change the outcome of statistical testing.

3.3. CRF peptide concentration

CRF RIAs were performed in the following dissected brain regions: cingulate gyrus, bed nucleus of the stria terminalis, amygdala (unilateral, hemispheres randomized between RIA and receptor assay), and median eminence. There was no effect of chronic mild stress on CRF content in the amygdala or cingulate gyrus (Fig. 3). There was a 50% increase in mean CRF concentration in the bed nucleus of the stria terminalis of stressed compared to non-stressed rats (39.5 vs. 26.3 pg/mg; $P < 0.01$, Mann–Whitney rank-sum test). The sample distribution in the non-stress group was non-Gaussian due to a high and a low outlier (82.3 and 2.3 pg/mg); statistical significance is preserved after deletion of these two data points ($P < 0.001$, *t*-test). Mean CRF concentration was lower in the median eminence of stressed rats relative to controls (570 vs. 900 pg), but this association was not statistically significant ($P = 0.07$, *t*-test). Sample sizes were reduced to five and four in control and chronic mild stress groups, respectively, due to the fact that some median eminence samples were damaged during brain collection.

3.4. CRF mRNA expression

CRF mRNA expression in the paraventricular nucleus of the hypothalamus was not significantly different between stressed and non-stressed rats. The mean signal densities were 289 nCi/g ± 27 (mean ± S.E.M., $n = 7$) in the control group, and 320 ± 21 ($n = 9$) in the stressed group (seven and nine samples were inadvertently sampled rather than eight in each group).

3.5. CRF receptor binding

Specific binding of [^{125}I]-^{0}Tyr-oCRF was determined in the following, homogenized tissue regions: anterior pituitary gland, prefrontal cortex, amygdala and raphe nuclei. There was no significant effect of chronic mild stress on CRF receptor binding in any of these regions (Table 1).

3.6. CRF_1 receptor expression

For practical reasons related to the number of electrophoresis wells, 2 of 10 frontoparietal cortex and cerebel-

Table 1
CRF receptor binding

Group	Prefrontal cortex	Amygdala	Raphe nuclei	Anterior pituitary
Non-stressed	90.8 ± 4.3 (10)	14.6 ± 0.8 (9)	17.2 ± 0.9 (10)	30.3 ± 3.5 (10)
Stressed	82.6 ± 3.2 (10)	17.8 ± 1.2 (10)	16.1 ± 1.1 (10)	25.1 ± 1.8 (10)

Mean fmol/mg protein specific [^{125}I]-^{0}Tyr-oCRF binding ± S.E.M. (n).

Table 2
CRF_1 receptor mRNA expression

Treatment group	Frontoparietal cortex	Cerebellum
Non-stressed	100 ± 14	100 ± 18
Stressed	92 ± 12	81 ± 6

Mean band density relative to actin (percent control) $\pm$ S.E.M ($n = 8$ per group).

lum samples from each group were randomly discarded prior to determination of relative CRF_1 receptor/actin mRNA expression. There was no statistically significant effect of chronic mild stress on CRF_1 receptor expression in either brain region (Table 2).

3.7. Correlations

There were no significant correlations between the percent increase in ventral tegmentum self-stimulation thresholds of stressed rats on day 19 and any of the neurochemical or endocrine measurements, or between endocrine and neurochemical measurements for either group of rats ($|r| < 0.6$, $P > 0.10$ for each pair of observations). Correlations between ventral tegmentum self-stimulation threshold in non-stressed rats and other variables were not evaluated due to the lack of variance within this behavioral data set.

4. Discussion

The behavioral data generated in this study confirm and extend previous findings that repeated exposure of rats to chronic mild unpredictable stressors leads to the gradual development of an anhedonic state, as assessed by attenuated self-stimulation behavior (Moreau et al., 1992). As predicted, rats in the chronic mild stress group gradually required higher frequency stimulation in order to exhibit ventral tegmentum self-stimulation behavior, as defined by 15 nose poke responses/min.

In the second part of this study, we tested the hypothesis that CRF neuronal function would be altered in anhedonic rats. Selected measures (CRF concentration, gene expression, CRF_1 receptor density and gene expression) were obtained from different regions of the brain which were chosen on the basis of relative signal intensity and preliminary reports in the literature (Bissette et al., 1997). From these determinations, a notable positive result was that CRF peptide concentrations were elevated in the bed nucleus of the stria terminalis of rats in the chronic mild stress group. This effect is preserved when the Bonferonni correction is made for the four brain regions analyzed for CRF content. A similar effect of chronic mild stress was noted recently in a preliminary report by Bissette et al. (1997), and a trend towards increased bed nucleus of the stria terminalis CRF content was also observed after exposure to a different chronic stress procedure (Chappell et al., 1986).

The precise origin of the increased CRF concentration within the bed nucleus of the stria terminalis is not known, nor whether this effect corresponded with an increased rate of release. The bed nucleus of the stria terminalis contains a dense collection of CRF-expressing cells (Young, 1990), and also receives CRF-immunopositive projections from the central nucleus of the amygdala (CeA) (Sakanaka et al., 1986). The bed nucleus of the stria terminalis contains a moderate density of CRF receptors (De Souza et al., 1985). Further elucidation of the role of changes in CRF peptide concentrations in the bed nucleus of the stria terminalis will be obtained by measurement of CRF mRNA and CRF receptor mRNA and density in future experiments using rats exposed to chronic mild stress.

Davis and colleagues have recently provided evidence that the bed nucleus of the stria terminalis is necessary for the potentiating effects of intracerebrally administered CRF and unconditioned fear stimuli on the startle reaction in rats (Lee and Davis, 1997; Walker and Davis, 1997). Although the relationship between experimental animal responses and human symptoms is far from clear, one may hypothesize that CRF release in the bed nucleus of the stria terminalis contributes to symptoms of both depression and anxiety disorders. On the other hand, chronic mild stress was not shown to produce anxiety as measured in the social interaction test or the elevated plus maze (D'Aquila et al., 1994). Many further experiments are required to clarify the role of bed nucleus of the stria terminalis CRF in behavioral models, including the determination of the effects of bed nucleus of the stria terminalis CRF administration on pharmacologically validated anxiety models such as the elevated plus maze procedure, the effects of CRF_1-selective and nonselective receptor antagonist administration on these behavioral responses, and further verification of our own data and those of Bissette et al. (1997).

There was no alteration in basal hypothalamic–pituitary–adrenal axis activity at the time of sacrifice in stressed rats. This may be due to the fact that the chronic mild stress procedure was not long and/or intense enough to generate sustained elevations of plasma ACTH or corticosterone concentrations. In a preliminary report, Bissette et al. showed that plasma corticosterone concentrations became significantly elevated only after 7 weeks of chronic mild stress as compared to 3 weeks (Bissette et al., 1997). In our study, ACTH and corticosterone concentrations were anomalously high within both groups, and the distributions of data points were quite variable. A possible explanation for the high baseline levels is that our animals were not habituated to the euthanasia procedure and this may have interfered with observation of ''basal'' hypothalamic–pituitary–adrenal axis activity in this group of animals. It is also possible that acute stress at the time of sacrifice may have influenced CRF peptide concentrations,

but unlikely that any other variables such as CRF mRNA were affected. Other forms of variable stress have differed in their capacity to produce elevations in resting ACTH or corticosterone concentrations (Chappell et al., 1986; García-Marquez and Armario, 1987; Herman et al., 1995). Based on these other reports, it appears that more prolonged or severe forms of stress than 3-week chronic mild stress may be required to mimic hypothalamic CRF mRNA or hypothalamic–pituitary–adrenal axis changes in depression.

There was a statistically non-significant decrease in mean CRF immunoreactivity within the median eminence of stressed rats. Though no conclusion may be drawn from this association (in part, due to sample losses during brain removal), it is worth noting that the diminished median eminence CRF concentration has been demonstrated in a previous study in which a more rigorous, chronic stress regimen involving more physical stressors such as immobilization was used (Chappell et al., 1986). This result was presumed to reflect depletion of nerve terminal peptide content due to a prolonged increase in the rate of CRF release, which exceeds the rate of synthesis and axonal transport.

Other indices of CRF or CRF receptor function in various brain regions were not significantly altered in response to chronic mild stress. One pertinent negative was the lack of difference in CRF mRNA expression in the paraventricular nucleus between control and stressed rats. In contrast, increased CRF mRNA expression in the paraventricular nucleus was demonstrated in postmortem brains of depressed individuals (Raadsheer et al., 1994), and following a more severe, 4-week variable stress procedure in rats (Herman et al., 1995). Decreased CRF receptor density has been observed in the prefrontal cortex of suicide victims (Nemeroff et al., 1988) or in the anterior pituitary gland of rats subjected to prolonged footshock stress (Anderson et al., 1993), but we found no changes in [^{125}I]-^{0}Tyr-oCRF binding in the present study. A possible explanation for these negative results is unchanged basal hypothalamic–pituitary–adrenal axis activity in this study. Enhanced corticosteroid feed-back regulation may be a necessary mechanism for some effects of chronic stress on CRF neurotransmission.

In summary, we have found that a chronic mild stress procedure generating an anhedonic state in rats resulted in increased CRF concentrations in the bed nucleus of the stria terminalis. CRF is known to be released during periods of stress or anxiety. Sustained release of CRF into or from bed nucleus of the stria terminalis neurons could lead to long-term changes in the activity of this structure and/or its outputs, regions long implicated in the control/modulation of the functioning of the limbic system. Prolonged changes in CRF neurotransmission in this region may underlie the development of certain symptoms observed in depressed individuals. Further preclinical and clinical experiments are required to clarify the role of CRF neurotransmission in the bed nucleus of the stria terminalis and other brain regions in contributing to symptoms of anxiety and/or depressive disorders.

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