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Brain Research 850 1999. 136–143

BRAIN RESEARCH

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Research report

Psychological stress increased corticotropin-releasing hormone mRNA and content in the central nucleus of the amygdala but not in the hypothalamic paraventricular nucleus in the rat

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Accepted 14 September 1999

Abstract

The central administration of corticotropin-releasing hormone CRH. to experimental animals sets into motion a coordinated series of physiological and behavioral events that promote survival during threatening situation. A large body of evidence suggest that CRH in the central nucleus of the amygdala CEA. induces fear-related behaviors and is essential to fear conditioning; however, evidence of CRH-mediated activation of the amygdala under physiological situation is still limited. We report here a study of the impact of a psychological stressor on hypothalamic and amygdala CRH systems in the rat. Non-footshocked rats placed in a floored compartment surrounded by footshocked rats were defined as the psychological stress group. Rats were exposed to psychological stress for 15 min, and then sacrificed 1.5 and 3 h after cessation of stress. We found that our psychological stressor induced an increase in both CRH mRNA levels, as assessed by in situ hybridization histochemistry, and CRH content, as assessed by micropunch RIA, in the CEA. Exposure to the psychological stressor also caused a significant increase in CRH mRNA levels with a trend for an increase in CRH content in the dorsolateral subdivision of the bed nucleus of the stria terminalis BNST. which is anatomically associated with the CEA. In contrast, psychological stress induced a small, but significant increase in type-1 CRH receptor CRHR-1. mRNA in the hypothalamic paraventricular nucleus PVN., while it failed to elevate either PVN CRH mRNA levels or content, CRH content in the median eminence ME., or levels of plasma ACTH or corticosterone CORT.. Thus, in the context of a psychological stressor, the activation of the amygdala CRH system can occur without robust activation of the hypothalamic CRH system. In the light of previous data that the psychological stress-induced loss of sleep was reversed by the central administration of a CRH antagonist, these data suggest that CRH in the CEA may contribute to the psychological stress-evoked fear-related behavior such as hyperarousal. These data also indicate that in response to a psychological stressor, the amygdala CRH system is much more sensitive than is the CRH system emanating from the PVN.

Keywords: Corticotropin-releasing hormone; Amygdala; Bed nucleus of the stria terminalis; Paraventricular nucleus; Psychological stress

1. Introduction

Corticotropin-releasing hormone CRH. is a 41 amino acid neuropeptide that acts in discrete but widespread areas of the brain to coordinate physiological and behavioral responses to threatening situations [3,31,44]. CRH pathways emanating from the paraventricular nucleus of the hypothalamus PVN. activate the pituitary–adrenal axis and sympathetic nervous system while inhibiting so-called vegetative functions whose inhibition is adaptive during stress e.g., feeding, sexual behavior, growth, reproduction. [6,9]. On the other hand, a growing body of evidence suggests that activation of CRH receptors in the central nucleus of the amygdala CEA. and/or CRH pathways emanating from the CEA play an important role in the fear-related behaviors. Electrical lesions of the CEA, but not of the PVN, abolished many of the behavioral effects of centrally administered CRH such as conditioned startle [24], whereas chemical lesion of the CEA blocked fear-

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0006-8993/99/$ - see front matter
PII: S0006-8993 99.02114-9

potentiated startle [23]. The direct injection of CRH antagonist into the CEA also diminished the stress-evoked freezing [41] or reduced emotionality in socially defeated rats [13].

Although both hypothalamic and amygdala-derived CRH are thought to interact in the activation of brainstem arousal centers such as the locus coeruleus (LC) [10], they do not necessarily respond in a similar fashion to the same stimuli. We and others have previously reported that while sustained glucocorticoid administration inhibits the hypothalamic CRH system, it causes a subtle but significant increase in the levels of CRH mRNA in the amygdala [26,40,43]. On the other hand, although restraint or footshock stress which gives both physical and psychological threats to the rats have been shown to activate the hypothalamic CRH system [43,44], evidence for the activation of amygdala CRH system under these mixed stressors is still limited. Thus, while two other groups demonstrated activation of the amygdala CRH system following mild restraint stress [15,19,35], we have shown that immobilization stress that increases PVN CRH mRNA fails to increase CRH mRNA levels in the CEA [32].

In contrast, whether the PVN and amygdala CRH systems are differentially responsive to psychological stressors has not yet been determined. Given the fact that the amygdala CRH system seems to play a preferential role in fear-related behaviors, we sought to test whether the amygdala CRH system was more sensitive than the hypothalamic CRH system to a psychological stressor. To test the impact of well characterized psychological stressor on the hypothalamic CRH system, we assessed CRH mRNA levels and content in the PVN, CRH content in the median eminence (ME), and the secretion of plasma ACTH and corticosterone (CORT). In the amygdala, we measured CRH mRNA and content in the CEA, as well as the bed nucleus of the stria terminalis (BNST), thought to represent extended amygdala. CRH content in the LC was also measured, because it has been suggested that the LC is one of the important target areas of CRH neurons emanating from the CEA [21,42]. In addition, as an index of tissue-specific effects of CRH, we measured mRNA levels of type-1 CRH receptor (CRHR-1) in the PVN, amygdala and BNST [16,28].

2. Materials and methods

Male Wistar rats, weighing 250–280 g, were individually housed under conditions of controlled temperature and illumination (0800–2000 h) and were allowed ad libitum access to food and water.

2.1. Experiment 1 (in situ hybridization study)

A communication box (height: 19.5 cm) divided into nine compartments (each compartment: $21 \times 21 \times 19.5$ cm) was used for this study. The details of the box was reported elsewhere [38]. On the day of the experiment, one rat was placed in each compartment, and the animals were divided into two groups, a footshock and a psychological stress group. After a period of 60 min for adaptation, the five rats of the footshock group were given electrical footshock through grids with electrical currents (1.5 mA, 1 s duration) delivered randomly on an average of two per minute for 15 min. The four rats of the psychological group were placed in compartments in which the grids were covered by an insulating acrylic floor. These animals were surrounded by rats in the footshock group on three sides, which they can see, hear, and smell via transparent acrylic panels. At the end of footshock psychologically stressed rats were placed back to their home cage and were then sacrificed 1.5 and 3 h after cessation of stress. This duration (15 min) of psychological stress was chosen according to the previous report showing that 15 min is long enough to shorten pentobarbital-induced sleeping time [38]. Rats which had never been exposed to the communication box were used as a control, because there have been confirmed to be no significant differences of the pentobarbital-induced sleeping time between rats which had never been exposed to the communication box and rats which were placed in the communication box for 60 min [38]. The number of rats for each group was eight.

2.2. Experiment 2 (measurement of CRH content in discrete brain areas)

Another set of male Wistar rats were used. The design of the experiment was exactly the same as experiment 1. Rats with psychological stress for 15 min were sacrificed 1.5 and 3 h after cessation of stress. Rats which had never been exposed to the communication box were used as a control. The number of rats for each group was eight.

For all experiments, the rats were decapitated between 1200 and 1400 h, corresponding to 6–8 h after lights-on, and their brains were quickly removed and frozen by immersion in 2-methyl butane at -30°C, then stored at -70°C until sectioning the tissue on the cryostat. The trunk blood was also collected on ice at the decapitation, centrifuged and stored at -70°C until assay. Plasma CORT and ACTH were measured by commercially available kit (CORT: radioimmunoassay kit, ICN biomedicals, Cleveland, OH; ACTH: immunoradiometric assay kit, Yuka Medias, Tokyo, Japan). The intra-assay and inter-assay coefficients of variance were $< 10\%$, and the detection limits of the assay (ED_{90}) for CORT or ACTH were 15 ng/ml or 10 pg/ml, respectively.

2.2.1. In situ hybridization

Frozen tissue was cut coronally in 15-μm thick sections for in situ hybridization histochemistry in the PVN, CEA and BNST. The sections were taken from the following sites: PVN, 1.60–1.90 mm; CEA, 2.60–2.80 mm; BNST, 0.26–0.40 mm posterior from the bregma. The sections

were thaw-mounted and air dried on gelatin-coated slides, and were stored at −70°C prior to in situ hybridization histochemistry.

We used a prepro-CRH complementary RNA probe that was kindly donated by Dr. K. Mayo. The pGEM4 construct contained an approximately 1-kb insert complementary to the rat CRH cDNA [18]. CRHR-1 cRNA probe was generously given to us by Dr. W. Vale. The pBluescript contains a 1.3 kb corresponding to the full length of rat CRHR-1 protein coding region [34]. The template was linearized, then antisense probe labeled with [α−35S]UTP DuPont/NEN, Wilmington, DE. was generated by transcription of the linearized plasmid DNA using the Riboprobe System Promega Biotech, Madison, WI.. We modified the standard Promega labeling method and increased the concentration of [α−35S]UTP from 62.5 to 187.5 μCi for 1 μg of linearized DNA labeling. This improved the sensitivity for detecting CRH and CRHR-1 mRNAs. The cRNA probe was purified by extraction with Nuctrap push columns Stratagene, La Jolla, CA..

We used the hybridization procedures described previously [29]. In brief, sections were fixed in 4% formaldehyde, subsequently treated with 0.25% acetic anhydride in 0.1 M triethanolamine/0.9% saline pH 8.0. over a 10-min period to reduce non-specific hybridization of the probe. Then the sections were dehydrated in increasing concentrations of ethanol, and dilapidated with chloroform for 5 min, rinsed in ethanol and air-dried. Sections were hybridized overnight at 54°C with 5×10^5 c.p.m. of labeled probe per section. Then non-specifically hybridized probe was removed through washing procedures as following; the sections were rinsed with 4× SSC (1× = 0.15 M NaCl/0.015 M sodium citrate, pH 7.2. for 5 min on four separate occasions. They were incubated with the RNase A Boehringer-Mannheim Biochemicals, Indianapolis, IN; 20 μg/ml in RNase buffer. solution at room temperature for 30 min, followed by wash with 0.1× SSC/0.1 mM DTT solution at 65°C for 60 min. Finally, the slides were dehydrated for 1 min in ascending alcohols 50%, 70%, 90% and 95% ethanol containing 300 mM ammonium acetate, and 100% ethanol..

2.2.2. Analysis and quantification

For analysis of CRH and CRHR-1 mRNAs, the slides and 14C-standards of known radioactivity American Radiochemicals, St. Louis, MO. were placed in X-ray cassettes, apposed to 35S-sensitive film Hyperfilm-βMax, Amersham. for the following durations: 1 day for CRH in the PVN, 7–8 days for CRH in the CEA, 14 days for CRH in the BNST, 28 days for CRHR-1 in the PVN and BNST, and 24 days for CRHR-1 in the amygdala. Films were then developed Rendol, Fuji Photo, Tokyo, Japan. for 5 min at 20°C. To determine the anatomical localization of probe at the cellular level in these nuclei, sections were dipped in NR-M2 autoradiographic emulsion Konica, Tokyo, Japan., exposed for 1–6 weeks, developed Rendol. for 2 min at 16°C, and counterstained with thionine. The amounts of probe hybridized in the PVN, CEA and BNST were measured as regional optical densities of autoradiographic film images with a computerized image analysis system composed of a light box, a solid state video camera, and Macintosh II-based IMAGE software developed by Wayne Rasband, Research Service Branch, National Institute of Mental Health. Optical densities were obtained in 2 consecutive sections per rat in the PVN and BNST. In the CEA, optical densities were determined from six sections showing the highest CRH mRNA levels within this region, or from two consecutive sections for CRHR-1 mRNA. Values were converted to disintegrations per minute per milligram dpm/mg. of rat brain tissue using a standard curve generated by 14C standards which had been matched with [α−35S]dATP-impregnated rat brain paste standards. The average value for each rat was used to calculate group means. Statistical significance between the control and experimental groups was determined by ANOVA, followed by Student–Newman–Keuls test.

Table 1
Hormonal responses to psychological stress
Values are means ± S.E.M.

	Control $n=8$.	1.5 h $n=8$.	3 h $n=8$.
Plasma CORT ng/ml.	43.9 ± 13.3	65.7 ± 15.7	36.5 ± 13.7
Plasma ACTH pg/ml.	153.7 ± 47.5	149.0 ± 25.5	110.0 ± 32.4

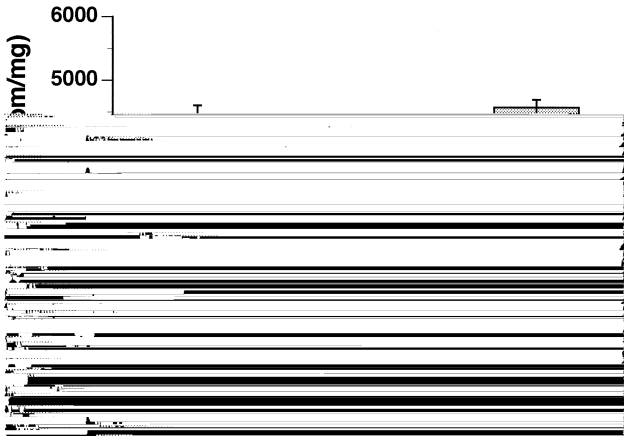

Fig. 1. CRH mRNA hybridization levels in the hypothalamic PVN following psychological stress. Values are means ± S.E.M.

2.2.3. Microdissection and CRH radioimmunoassay

The brain microdissection method has been described elsewhere [5,33,39]. In brief, frozen section was sliced into 400–600-μm thick coronal sections in a cryostat at −10°C. The PVN, CEA, ME, LC and the dorsolateral or ventral part of the BNST were then removed bilaterally by using

thin-walled stainless steel tubing (i.d. 0.97 mm). Samples were blown in polypropylene tube and placed on dry ice and stored at −70°C until assay.

Micropunched samples were homogenized by sonication in 2 ml of 0.1 N hydrochloric acid. Three hundred microliter aliquots of each tissue homogenate were dried for protein measurement. Tissue protein was measured with a protein assay kit (Bio-Rad Laboratories, CA, USA). The rest of homogenate was centrifuged for 10 min at 10000 rpm at 4°C. The supernatant was transferred to another tube and then evaporated and dried in a vacuum centrifuge. The dried extracts were stored at −70°C until assay.

Immunoreactive CRH in the lypophilized tissue sample was measured by a radioimmunoassay using anti-CRH serum provided by Mitsubishi-Petrochemicals (Tokyo, Japan) and [^{125}I-Tyr^{0}]-CRH (New England Nuclear Research Products, USA). The lyophilized samples was reconstituted in 1 ml of assay buffer (0.1 M phosphate buffer containing 0.14 M NaCl, 0.1% bovine serum albumin, 0.05% Tween 20, 0.01% NaN_3; pH 7.4), and duplicate 50 μl or 200 μl samples were used for CRH measurement in the ME or other areas, respectively. The anti-CRH serum recognized the C-terminal portion of human/rat CRH. Samples were incubated at 4°C for 24 h with 100 μl anti-CRH serum at a dilution of $1{:}3 \times 10^5$. Approximately 10^4 c.p.m. of rat/human [^{125}I-Tyr^{0}]-CRH was added in a volume of 100 μl RIA buffer to each tube and incubated for an additional 24 h. Thereafter, 150 μl goat anti-rabbit gamma globulin was added and incubated for 24 h. The precipitates were collected by centrifugation at 3500 rpm for 30 min. The supernatant were removed by aspiration and the pellet counted for 2 min each in a gamma counter. The recovery rates for CRH in tissue CRH radioimmunoassay were 73.3 ± 2.4%. The detection limit of this assay was 1 pg/tube. The intra-assay variations for CRH was 9.4%. Result for CRH content in each area were analyzed by ANOVA, followed by Student–Newman–Keuls test.

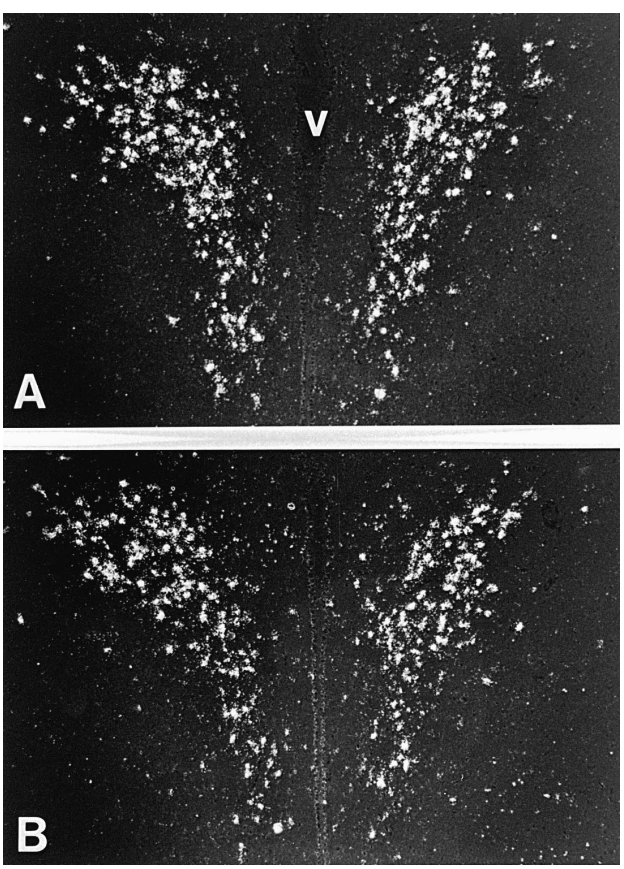

Fig. 2. Effects of psychological stress on CRH mRNA levels in the hypothalamic PVN. Darkfield photomicrographs show the autoradiographic distribution of CRH mRNA in control rats (A) and rats sacrificed 1.5 h after cessation of stress (B). Autoradiographic silver grains appear white. Note that CRH mRNA levels in the PVN were unchanged.

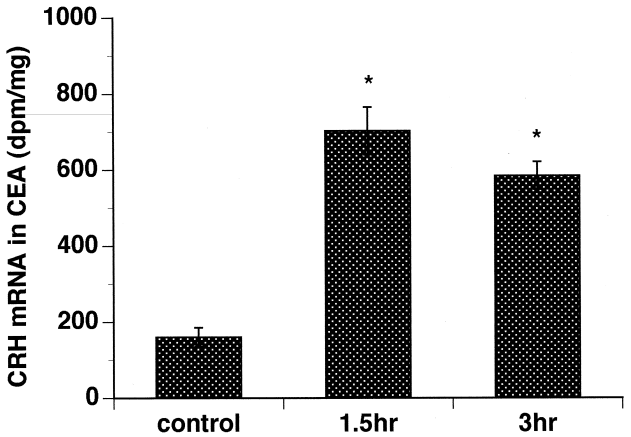

Fig. 3. CRH mRNA hybridization levels in the CEA following psychological stress. Values are means ± S.E.M. $^{*}P < 0.01$ vs. control group.

3. Results

3.1. Plasma CORT and ACTH responses to psychological stress

As shown in Table 1, plasma CORT and ACTH were unaltered at both 1.5 and 3 h following psychological stress. This is consistent with a previous report [38] demonstrating that psychological stress identical in the present study did not evoke robust pituitary–adrenocortical responses.

3.2. CRH mRNA changes in the PVN, CEA and BNST

Consistent with plasma ACTH and CORT responses, CRH mRNA in the PVN did not show any significant changes following psychological stress (Figs. 1 and 2). In contrast, CRH mRNA in the CEA significantly increased at both 1.5 and 3 h after cessation of stress (Figs. 3 and 5; F (2, 21) = 41.06, $P < 0.001$). In the BNST, CRH mRNA

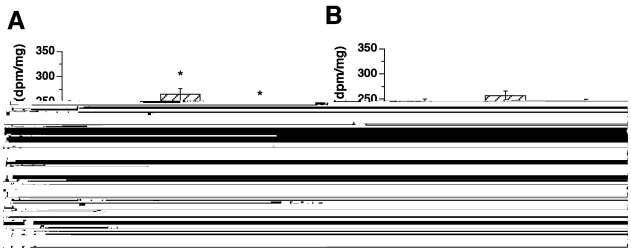

Fig. 4. CRH mRNA hybridization levels in the dorsolateral subdivision A: BSTLD· and ventral subdivision B: BSTV· of the BNST following psychological stress. Values are means ± S.E.M. $^{*}P < 0.01$ vs. control group.

was evaluated in the two distinct subdivisions, namely dorsolateral and ventral subdivisions. CRH mRNA in the dorsolateral BNST showed an increase F 2, 18· = 47.40, $P < 0.001$·, while CRH mRNA in the ventral BNST was unaltered Figs. 4 and 5·.

3.3. CRH content in the various brain regions

CRH content in the various brain regions is shown in Fig. 6. CRH content in the CEA was significantly increased at 1.5 h after cessation of stress F 2, 21· = 3.70, $P < 0.05$·, but returned to the baseline levels by 3 h. CRH contents in the dorsolateral BNST showed a tendency for an increase at 1.5 h F 2, 21· = 1.87, $P = 0.0853$·, but it was not statistically significant. CRH contents in the PVN, ME and LC were unaltered.

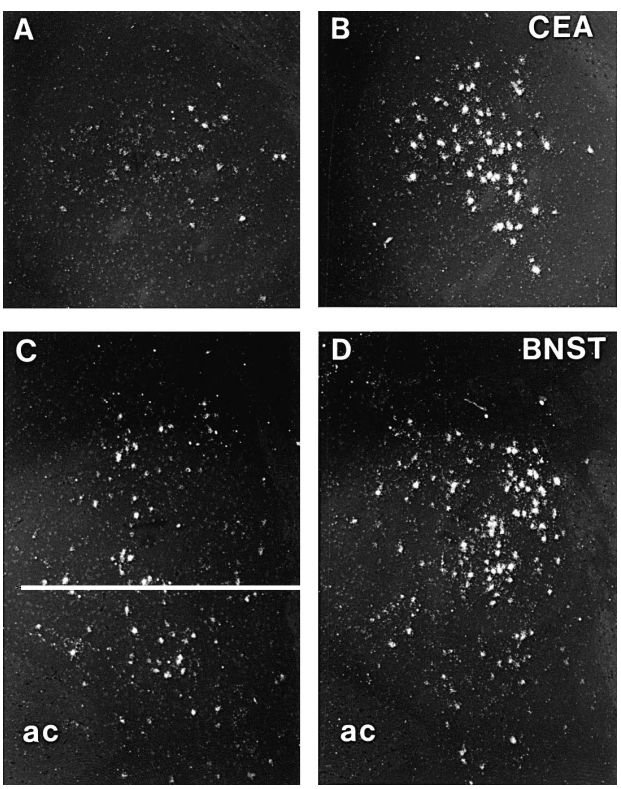

Fig. 5. Effects of psychological stress on CRH mRNA levels in the CEA A, B· and the dorsolateral subdivision of the BNST BSTLD; C, D·. Darkfield photomicrographs show the autoradiographic distribution of CRH mRNA in control rats A, C· and rats sacrificed 1.5 h after cessation of stress B, D·. Autoradiographic silver grains appear white. Note that CRH mRNA levels in the CEA and BSTLD robustly increased following psychological stress.

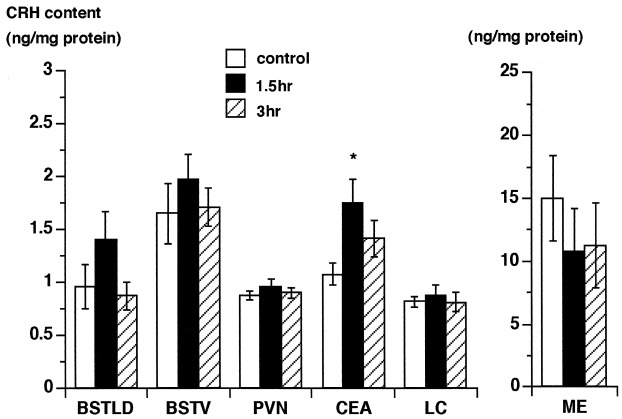

Fig. 6. Effect of psychological stress on CRH content in the various brain regions. Values are means ± S.E.M. $^{*}P < 0.05$ vs. control values. BSTLD; dorsolateral subdivision of the bed nucleus of the stria terminalis, BSTV; ventral subdivision of the bed nucleus of the stria terminalis, PVN; hypothalamic paraventricular nucleus, CEA; central nucleus of the amygdala, LC; locus coeruleus, ME; median eminence.

3.4. CRHR-1 mRNA changes in the PVN, amygdala and BNST

Despite the lack of changes in PVN CRH mRNA and CRH content in the PVN and ME, CRHR-1 mRNA in the PVN showed a slight, but significant increase at both 1.5 and 3 h after cessation of stress F 2, 21· = 14.44, $P <$ 0.01, Table 2·. We evaluated CRHR-1 mRNA levels in the discrete subnuclei in the amygdala i.e., lateral or medial subdivision of the CEA, lateral and basolateral amygdala BLA·, or medial amygdala· as previously described [28]. There were no significant alterations in CRHR-1 mRNA levels in any of subnuclei in the amygdala Table 2·. Psychological stress did not affect CRHR-1 mRNA levels in the BNST Table 2·.

Table 2
Changes in CRHR-1 mRNA
Values are the mean disintegrations per minute per milligram ± S.E.M.

	Control $n = 8$·	1.5 h $n = 8$·	3 h $n = 8$·
PVN	110.1 ± 3.21	145.1 ± 6.9*	165.0 ± 10.1*
CeL	64.6 ± 0.6	66.2 ± 0.5	63.8 ± 0.5
CeM	111.2 ± 3.2	117.3 ± 4.5	115.1 ± 4.1
BLA	200.5 ± 5.0	201.6 ± 10.1	193.5 ± 9.4
Med	206.7 ± 5.0	205.7 ± 6.3	213.8 ± 7.6
BNST	95.1 ± 1.4	94.1 ± 1.6	94.2 ± 1.2

$^{*}P < 0.01$ vs. control. CeM; medial subdivision of the central amygdala, CeL; lateral subdivision of the central amygdala, BLA; lateral and basolateral amygdala, Med; medial amygdala.

4. Discussion

We report here that CRH mRNA and content are both increased in the CEA following the psychological stress generated in the communication box. Psychological stress also increased CRH mRNA in the dorsolateral subdivision of the BNST, whereas CRH content in that region showed a tendency towards an increase. In contrast, we found that psychological stress failed to elevate any components of the hypothalamic–pituitary–adrenocortical HPA· axis i.e., CRH mRNA and content in the PVN, CRH content in the ME, or levels of plasma ACTH and CORT· except a small but significant increase in CRHR-1 mRNA in the PVN.

Although three previous studies have focused on the effect of psychophysical stressors such as acute restraint on the amygdala CRH system [15,19,35], we have failed to show a significant change in CEA CRH mRNA following acute immobilization [32]. Thus, the possible differential impacts of the physical and psychological components of these stressors on the amygdala CRH system is not known. In the present study, pure psychological stress showed a highly significant impact on the CEA CRH mRNA levels. These data indicate that psychological component of the stressor could activate the amygdala CRH system. It should be noted that a relatively pure physical stressors such as salt-loading [43] or cold stress [25] can actually lead to decreased CRH mRNA levels in the CEA, so that under some circumstances the physical component of a mixed stressor could counteract the psychological component. Thus, the balance between psychological and physical component of the stressor may determine the responsivity of the amygdala CRH system. Our data indicating a greater responses of the amygdala CRH system to a psychological stressor supports the hypothesis provided by Herman and Cullinan [14] that limbic stress pathways are sensitive to stressors involving higher-order sensory processing, but are insensitive to simple physical threats.

In the present study, we showed that the increase in CRH mRNA levels in the CEA were accompanied by an increase in CRH content in the CEA, as measured by the Palkovits' punch technique [33]. Utilizing the microdialysis technique, Pich et al. [35] had previously reported that CRH levels were increased in the extracellular fluid of the CEA following restraint or ethanol withdrawal. These authors also noted that pharmacological blockade of K-channels in CEA neurons increased both CRH levels in extracellular fluid and several behavioral indices of arousal. This finding is of great interest in the light of the fact that the identical psychological stressor paradigm utilized here reduced pentobarbital-induced sleeping time, an index of arousal, and that the impact of the stressor upon pentobarbital-induced sleeping was antagonized by the i.c.v. administration of the CRH antagonist, alpha-helical CRH [38]. The present study extends these previous findings and suggests that psychological stress-evoked activation of amygdala CRH system is linked to the fear-related behavior/hyperarousal.

The increased CRH content in the CEA may reflect that locally released CRH binds to CRHR-1 in the BLA to promote fear-related behaviors. Thus, the CEA contains few CRHR-1, which are present in abundance in the BLA [8,45]. Anatomically, most sensory information enters the amygdala through the BLA, which in turn projects to the CEA. Both LeDoux and Davis have shown that the connection between the BLA and the CEA play a critical role in conditioned fear [22] and fear-potentiated startle [7]. We have previously shown that glucocorticoid administration to rats for 7 or 14 days caused an increase in CEA CRH mRNA levels that were associated with a significant decrease in CRHR-1 mRNA levels in the BLA [26,28], suggesting ligand-induced downregulation of CRHR-1 in the BLA. This finding is contrasted with no impact here of psychological stress upon CRHR-1 mRNA levels in any amygdaloid nuclei tested, including the BLA. The lack of such an effect could reflect the fact that the duration of changes in CEA CRH were too short to affect CRHR-1 in the BLA. Alternatively, high plasma concentration of CORT directly affect CRHR-1 in the BLA.

In addition to its possible activation of CRHR-1 in the BLA, CRH may promote fear-related behaviors via CEA CRH projections to many sites e.g., the lateral hypothalamus, mesencephalic reticular formation, medial and lateral parabrachial nuclei, the nucleus tractus solitarius, as well as the midbrain central gray, and in and around the LC· [12]. Nemeroff's group has been accumulating evidence that a stress-induced increase in CRH content in the LC could potentially reflect increased CRH release from nerve terminals emanating from the CEA [4,5,21]. In the present study, however, this seems to be unlikely, because we could not detect any changes in CRH content in and around the LC following the psychological stressor.

The present study provides new evidence that psychological stress also increased CRH mRNA levels in the dorsolateral BNST. The lack of CRH mRNA changes in the ventral part of the BNST further highlights that CRH in the dorsolateral BNST is sensitive to psychological threats. This may not be surprising, because this region is anatomically associated with the CEA [11]. The parallel changes in CRH mRNA in the CEA and dorsolateral BNST have been reported in response to CORT treatment [26,27], cold stress [25] and salt-loading [43]. In addition to its role in the regulation of the HPA axis [14], the role of the BNST in the fear-related behavior has now received attention as well [23,37]. Thus, Lee and Davis reported that a microinfusion of CRH into the BNST enhanced the acoustic startle reflex, while an infusion of CRH antagonist into the BNST blocked CRH-enhanced startle [23]. They postulated that CRH in the CSF can activate the BNST which leads to activation of brainstem and hypothalamic BNST target areas involved in anxiety and in the stress response. Although we found only a tendency towards

increase in CRH content in the dorsolateral BNST, a significant increase in CRH mRNA suggest that psychological stressor activated CRH neurons in the dorsolateral BNST leading to the activation of the target areas similar to those of the CEA CRH neurons.

We and others have also previously shown that the administration of glucocorticoids in pharmacological doses to the rat causes a subtle, but significant increase in CRH mRNA levels in the CEA and dorsolateral BNST while significantly reducing CRH mRNA in the PVN [26,27,40,43]. The present results indicate that the magnitude of the stress-induced increases in CRH mRNA levels and content in the CEA and dorsolateral BNST do not require activation of hypothalamic CRH secretion nor do they depend solely upon glucocorticoid secretion. Recently, Albeck et al. [1] reported a similar dissociation between the levels of CRH mRNA in the CEA and in the PVN in non-responsive subordinates in the visible burrow system model of chronic social stress. Anatomical evidence indicates that CEA CRH neurons receive afferents conveying a variety of neurotransmitters and neuropeptides i.e., CRH, noradrenaline, calcitonin gene-related peptide, substance P, etc.· [12]. The cytokines interleukin-2 [36] and interleukin-6 [20] are also capable of inducing CRH release from amygdala slices in vitro. However, the functional significance of these neuropeptides or neuromodulators in the regulation of CEA CRH during psychological stress remains to be elucidated. The mechanism underlying the regulation of CRH in the dorsolateral vs. ventral BNST also awaits clarification.

In contrast to the robust increase in amygdala CRH system, psychological stress had less of an impact upon the hypothalamic CRH system and pituitary–adrenocortical axis. This finding is consistent with a previous report showing that the identical psychological stressor paradigm utilized here was of sufficient intensity to reduce pentobarbital-induced sleeping time, an index of arousal, without causing evidence of HPA activation at 30 and 60 min during the stressor [38]. Ishikawa et al. [17] also demonstrated that the plasma CORT response in the rats acutely exposed to the sociopsychological stress in the communication box was not evident and much less than that of footshocked group. As previously noted, the impact of the stressor upon pentobarbital-induced sleeping was antagonized by the i.c.v. administration of the CRH antagonist, alpha-helical CRH [38]. Thus, the absence of indices of CRH-mediated HPA activation in the present study cannot be due to our utilization of a stressor paradigm that was insufficient to exert tangible effects upon the CNS via the CRH system. However, we cannot rule out the possibility that indices of CRH-mediated HPA activation had come and gone by the time the animals were sacrificed in the present experiment 1.5 and 3 h after stressor exposure·. The subtle but significant rise in CRHR-1 mRNA levels in the PVN may be index of a transient rise in HPA function that was missed in our studies and those of others [30]. Nevertheless, the lack of a robust activation of hypothalamic CRH system during psychological stress highlights the importance of amygdala CRH system for the psychological stress-evoked hyperarousal. In agreement with this, Britton et al. [2] have reported that CRH-induced behavioral changes could occur in the absence of pituitary–adrenocortical activation.

Acknowledgements

We are extremely grateful to Dr. K. Mayo and Dr. W. Vale for providing the ribonucleotide probes for CRH and CRHR-1. We also wish to thank Drs. K. Asaba and M. Nishiyama for plasma CORT measurement, and Miss M. Nakatsukasa for her technical assistance.

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