ELSEVIER

Pharmacology, Biochemistry and Behavior 72 (2002) 213–220

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Elevated extracellular CRF levels in the bed nucleus of the stria terminalis during ethanol withdrawal and reduction by subsequent ethanol intake

M. Foster Olive*, Heather N. Koenig, Michelle A. Nannini, Clyde W. Hodge[1]

Ernest Gallo Clinic and Research Center, Department of Neurology, University of California, San Francisco, 5858 Horton Street, Suite 200, Emeryville, CA 94608, USA

Received 31 July 2001; received in revised form 24 October 2001; accepted 6 November 2001

Abstract

Corticotropin-releasing factor (CRF) is widely distributed throughout the brain and has been shown to mediate numerous endocrine and behavioral responses to stressors. During acute ethanol withdrawal, CRF release is increased in the central nucleus of the amygdala (CeA), and there is evidence to suggest that this activation of amygdala CRF systems may mediate the anxiogenic properties of the ethanol withdrawal syndrome. The present study was conducted to determine if another CRF-containing limbic structure, the bed nucleus of the stria terminalis (BNST), we would exhibit similar increases in CRF neurotransmission during ethanol withdrawal. Rats were administered an ethanol-containing (6.7% v/v) or control liquid diet for 2 weeks and subsequently implanted with microdialysis probes into the lateral BNST. A 50–75% increase in dialysate CRF levels was observed following removal of the ethanol-containing diet, while no changes were observed in control animals. When ethanol-withdrawn animals were given subsequent access to the ethanol-containing diet, dialysate CRF levels returned to basal levels. However, when ethanol-withdrawn animals were given subsequent access to the control diet, dialysate CRF levels increased further to 101% above basal levels. These data demonstrate that extracellular CRF levels are increased in the BNST during ethanol withdrawal, and that these increases are reduced by subsequent ethanol intake.

Keywords: Microdialysis; Extended amygdala; Ethanol; Withdrawal; Bed nucleus of stria terminalis; Liquid diet

1. Introduction

The neuropeptide corticotropin-releasing factor (CRF) plays an integral role in the behavioral and neuroendocrine responses to physiological or psychological stressors (Koob and Heinrichs, 1999; Smagin et al., 2001). CRF is widely distributed throughout the brain, with highest concentrations found in the hypothalamus and subcortical limbic structures (Cummings et al., 1983; Morin et al., 1999; Olschowka et al., 1982; Swanson et al., 1983). While hypothalamic CRF is the primary initiator of the hypothalamic–pituitary–adrenal (HPA) axis response (Rivier and Plotsky, 1986; Vale et al., 1981), extrahypothalamic CRF systems appear to mediate the behavioral and autonomic responses to stress (Dunn and Berridge, 1990; Koob and Heinrichs, 1999; Koob et al., 1994; Sutton et al., 1982).

Withdrawal from chronic intake of alcohol and other drugs of abuse is often associated with severe physiological and psychological manifestations of stress and anxiety. Studies have demonstrated that these withdrawal symptoms are largely mediated by limbic CRF-containing structures. For example, antagonism of central CRF neurotransmission can attenuate behavioral signs of drug and alcohol withdrawal (Baldwin et al., 1991; Brugger et al., 1998; Sarnyai et al., 1995). Other studies have shown that neuronal CRF release is increased in the central nucleus of the amygdala (CeA) during acute withdrawal from ethanol (Merlo-Pich et al., 1995), cocaine (Richter and Weiss, 1999) and cannabinoids (Rodríguez de Fonseca et al., 1997), and that antagonism of CRF neurotransmission in the CeA attenuates the behavioral signs of drug and alcohol withdrawal (Heinrichs et al., 1995; Rassnick et al., 1993). Thus, amygdalar

* Corresponding author. Tel.: +1-510-985-3922; fax: +1-510-985-3101.

E-mail address: folive@itsa.ucsf.edu (M.F. Olive).

[1] Present address: Department of Psychiatry, Bowles Center for Alcohol Studies, School of Medicine CB No. 7178, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7178, USA.

0091-3057/02/$ – see front matter
PII: S0091-3057(01)00748-1

CRF systems appear to contribute largely to the behavioral signs of drug withdrawal.

The bed nucleus of the stria terminalis (BNST) is considered to be an integral part of the extended amygdala complex and shares various neuroanatomical and neurochemical homologies with the CeA (Alheid et al., 1995, 1998; de Olmos and Heimer, 1999). The BNST contains numerous CRF-immunopositive neuronal cell bodies (Cummings et al., 1983; Morin et al., 1999; Olschowka et al., 1982; Phelix and Paull, 1990; Swanson et al., 1983) and also receives CRF-containing projections from the CeA (Sakanaka et al., 1986). The BNST has been implicated in neuronal (Bonaz and Tache, 1994) and behavioral (Gewirtz et al., 1998; Walker and Davis, 1997) responses to stress. In particular, CRF systems in this region appear to mediate behavioral responses to stressors (Lee and Davis, 1997), as well as stress-induced relapse to drug-seeking behaviors (Erb and Stewart, 1999). The goal of the present study was to use microdialysis to assess changes in extracellular CRF levels in the BNST during acute ethanol withdrawal. In addition, we sought to determine if extracellular CRF levels in this region could be modulated by voluntary ethanol consumption following the acute withdrawal phase.

2. Materials and methods

2.1. Animals

Male Long–Evans rats (250–400 g, Harlan, Madison, WI) were housed individually in cylindrical Plexiglas microdialysis cages (30 cm diameter, Instech Laboratories, Plymouth Meeting, PA) under a 12:12 light–dark cycle with lights on at 06:00 h. All experiments were performed during the light portion of the light–dark cycle and were performed in accordance with approved institutional protocols and the National Institutes of Health Guide for Care and Use and Laboratory Animals (NIH Publication No. 85-23, revised 1985).

2.2. Surgical procedures

Animals were anesthetized with 2% halothane vaporized in a 1:1 mixture of O_2 and N_2O, implanted with guide cannulae (SciPro, North Tonawanda, NY) aimed at the lateral region of the BNST (stereotaxic coordinates AP −0.3 mm, ML ±1.6 mm from bregma, DV −6.0 mm from the skull surface, according to the atlas of Paxinos and Watson, 1997) and secured with skull screws and dental cement. The wound was treated with 2% bacitracin and 2% xylocaine topical ointments, sutured closed with 3-0 vicryl sutures, and animals were allowed to recover in home microdialysis cages for at least 5 days prior to the administration of the liquid diet. Food and water were available ad libitum during recovery from surgical procedures.

2.3. Administration of liquid diet

Following recovery from surgery, rats were placed on a Lieber–DeCarli liquid ethanol diet (No. 710260, Dyets, Bethlehem, PA) or control diet (No. 710027, Dyets) (Lieber and DeCarli, 1982) in the home microdialysis cage as the sole source of nutrients for 2 weeks. The ethanol diet contained 6.7% (v/v) ethanol, while the control diet contained an equicaloric amount of maltose dextrin (both diets = 1 kcal/ml). Body weight and amount of diet consumed were recorded daily during diet administration. During microdialysis procedures, diets were removed from home cages following 90 min of baseline sample collection and were replaced 7.5 h later followed by an additional 90 min of postwithdrawal sample collection. Following the withdrawal period, control diet-fed animals were fed the control diet (CTRL–CTRL), while ethanol-fed animals were fed either the ethanol-containing diet (ETOH–ETOH) or control diet (ETOH–CTRL).

2.4. Microdialysis procedures

Following 2 weeks of diet consumption, animals were lightly reanesthetized as described above and implanted with microdialysis probes with 2 mm polyethylsulfone membranes (15 kDa cut-off, 0.6 mm o.d., SciPro) to a final depth of −8.0 mm from the skull surface. These probes have an in vitro recovery rate of 13.5% for CRF (Olive and Hodge, 2001). Probes were continuously perfused with artificial cerebrospinal fluid (aCSF), containing 125 mM NaCl, 2.5 mM KCl, 0.5 mM $NaH_2PO_4 \cdot H_2O$, 5 mM Na_2HPO_4, 1 mM $MgCl_2 \cdot 6H_2O$, 1.2 mM $CaCl_2 \cdot 2H_2O$, 5 mM D-glucose, 0.2 mM L-ascorbic acid and 0.025% (w/v) bovine serum albumin, pH = 7.3–7.5. Probes were attached to dual channel liquid swivels (Instech Laboratories) with FEP tubing (0.005 in. i.d., CMA/Microdialysis, North Chelmsford, MA) for freely moving microdialysis procedures. Animals were allowed to recover from probe implantation overnight prior to withdrawal experiments. On the following day, the aCSF flow rate was set at 2.0 μl/min, and microdialysis samples were collected into polypropylene microcentrifuge tubes in a refrigerated microsampler (SciPro) at 30-min intervals. Samples were immediately stored on dry ice following collection and later frozen at −70 °C until analysis by radioimmunoassay (RIA).

2.5. Brain histology

Following microdialysis procedures, animals were deeply anesthetized with Nembutal (150 mg/kg ip) and perfused transcardially with 100 ml of 0.9% NaCl followed by 250 ml of Streck Tissue Fixative (Streck Laboratories, La Vista, NE). Brains were then removed and placed in the same fixative for at least 48 h at 4 °C. Coronal brain sections (30 μm thickness) were cut on a cryostat (Leica, Deerfield,

IL), placed onto gelatin-coated slides and coverslipped. Probe placement was verified under light microscopy, and data from animals with probe placements outside of the target region were discarded.

2.6. CRF radioimmunoassay

CRF content in microdialysates was measured using a commercially available RIA kit (RK-019-06, Phoenix Pharmaceuticals, Mountain View, CA) adapted to solid-phase procedures (Olive and Hodge, 2001). Briefly, 96-well microtiter plates (Dynex Microlite 2+, Dynex Technologies, Chantilly, VA) were incubated with a protein A solution (0.4 μg/50 μl, in 0.1 M $NaHCO_3$, pH = 9.0) for at least 24 h at 4 °C to facilitate binding of the antisera to the plate wells. Plates were then washed with assay buffer (0.15 M K_2HPO_4, 0.2 mM ascorbic acid, 0.1% Tween-20, 0.1% gelatin, pH = 7.4, with phenol red added for enhanced visualization), blotted dry on a paper towel and incubated with 50 μl/well of rabbit antisera to rat/human CRF (diluted 1:25 from stock in assay buffer) for 24 h at 4 °C. According to the manufacturer, this antisera crossreact 100% with rat/human CRF and 0% with urocortin, adrenocorticotropic hormone, Arg^8-vasopressin, pituitary adenylate cyclase activating polypeptide and luteinizing hormone-releasing hormone. Following incubation with the antisera, plates were washed and incubated with 0–50 fmol/50 μl (in quadruplicate) of synthetic rat CRF standards diluted in aCSF. Microdialysis samples (50 μl) were also added at this time. Standards and samples were incubated at 4 °C for 24 h. Next, approximately 5000 cpm/50 μl of ^{125}I-labelled rat/human Tyr^0-CRF (diluted in assay buffer) was added to each well, and the plates were incubated at 4 °C for 48 h. Finally, plates were washed with assay buffer and blotted dry on a paper towel, and 100 μl of Microscint 40 scintillation fluid (Packard Instrument, Meriden, CT) was added to all wells. The plates were covered with TopSeal film, agitated for 1 min on an orbital shaker and counted on a TopCount Microplate Scintillation Counter (Packard Instrument). Data from microdialysis samples falling outside of the linear range for this assay (1.5–50 fmol/50 μl) were discarded.

2.7. Data analysis

Femtomole values of CRF content for each 30-min sample were transformed to percentage of basal CRF release, assigning a value of 100% to the average CRF level in the three 30-min baseline samples collected prior to diet removal. Percent baseline data were then collapsed into 90-min time blocks. All data are presented as mean ± S.E.M. and were analyzed using a two-way repeated-measures analysis of variance (ANOVA) followed by a Neuman–Keuls post hoc test (SigmaStat, SPSS Science, Chicago, IL).

3. Results

3.1. Placement of microdialysis probes

As shown in Fig. 1, the majority of dialysis probes were placed in the rostrolateral portion of the BNST. Probes often extended ventrally beyond the anterior commissure into the ventral portions of the BNST as well.

3.2. Diet consumption

Rats placed on the ethanol-containing diet consumed an average of 10.3 ± 0.8 g/kg/day of ethanol (65.8 ± 4.9 ml of

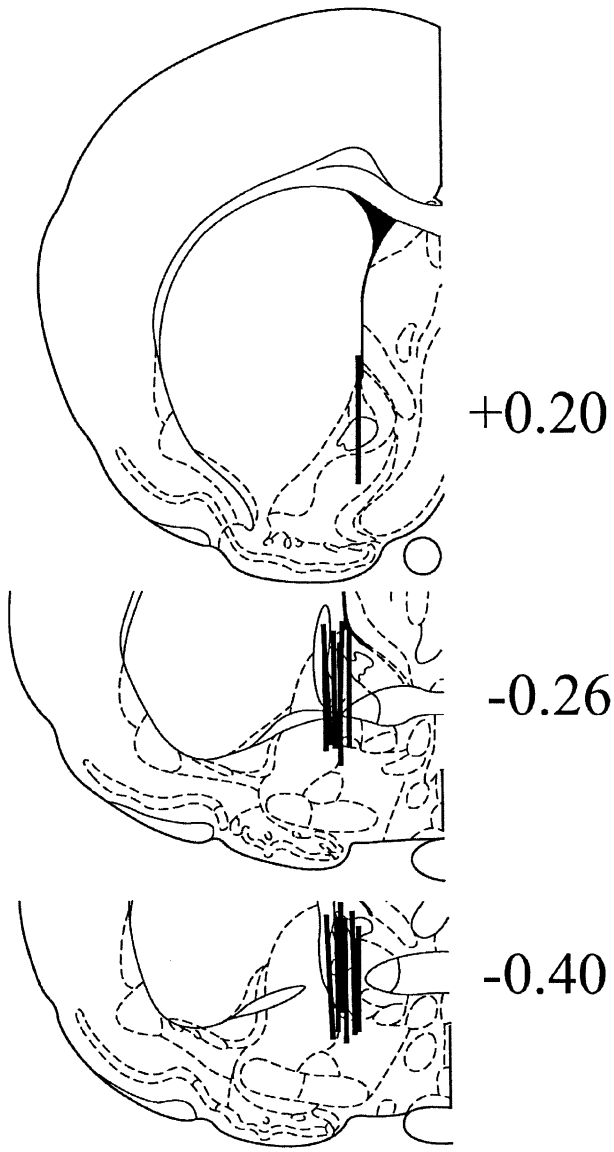

Fig. 1. Diagram of coronal sections of the rat brain indicating location of dialysis probe placements in the lateral BNST. Vertical lines indicate approximate location of probe membrane derived from histological sections. Numbers indicate distance (in mm) from bregma. Figure adapted from Paxinos and Watson (1997).

Table 1
Body weight and basal dialysate levels of CRF in each treatment group[a]

Treatment group	Prediet body weight (g)	Postdiet body weight (g)	Basal dialysate CRF levels (fmol/50 μl)
CTRL–CTRL	360.12 ± 4.53	400.25 ± 4.09	9.92 ± 1.17
ETOH–ETOH	344.12 ± 3.68	339.38 ± 4.39	7.14 ± 1.06
ETOH–CTRL	334.71 ± 5.58	341.43 ± 5.44	7.26 ± 0.50

Data are presented as means ± S.E.M.
[a] See Section 2.3 for description of treatment groups.

diet/day). Rats administered the control diet consumed 102.5 ± 4.8 ml of diet/day. The body weights of animals in the three different treatment groups before and after diet administration are shown in Table 1. Control diet-fed animals gained approximately 40 g during the 2 weeks of diet administration, while the body weights of ethanol-fed animals did not change.

When ethanol-fed rats were given access to the ethanol-containing diet during the 90-min postwithdrawal period (ETOH–ETOH group), 1.3 ± 0.3 g/kg ethanol was consumed. When ethanol-fed rats were given access to the control diet during the 90-min postwithdrawal period (ETOH–CTRL group), 15.9 ± 4.6 ml of diet was consumed. When control-fed rats were given access to the control diet during the 90-min postwithdrawal period (CTRL–CTRL group), 12.7 ± 1.6 ml of diet was consumed.

3.3. Radioimmunoassay of CRF

The IC_{50} of the CRF RIA ranged from 8 to 12 fmol/50 μl, and the limit of detection was approximately 1.5 fmol/50 μl (Olive and Hodge, 2001). Absolute basal levels of dialysate CRF content in each of the three treatment groups are shown in Table 1 and did not differ across treatment groups. Microdialysis data from one animal in each treatment group had to be discarded as the dialysate CRF concentrations were outside of the linear range for this assay (1.5–50 fmol/50 μl).

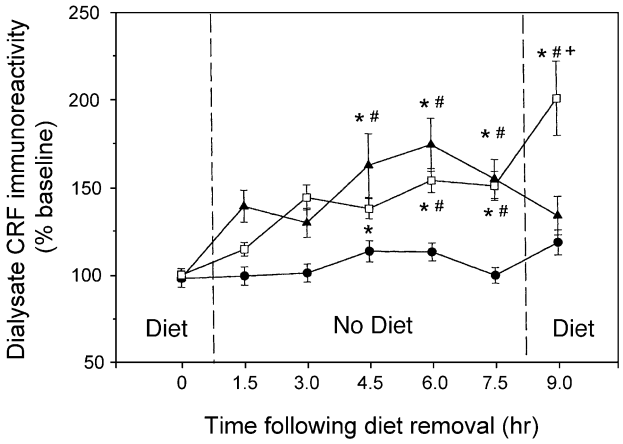

Fig. 2. Effect of acute ethanol withdrawal and subsequent access to ethanol-containing or control liquid diet on extracellular CRF levels in the BNST. Each data point represents the mean ± S.E.M. dialysate level of CRF (expressed as a percent of basal levels) in three 30-min microdialysis samples for each animal. Treatment groups are designated as control-fed rats with subsequent access to the control diet (●, $n=7$), ethanol-fed rats with subsequent access to the ethanol-containing diet (▲, $n=7$), and ethanol-fed rats with subsequent access to the control diet (□, $n=7$). * $P<.05$ vs. baseline. # $P<.05$ vs. control-fed animals at the same time point. + $P<.05$ vs. ethanol-fed animals at the same time point.

3.4. Effects of removal and replacement of liquid diet on extracellular CRF levels in the BNST

Two-way ANOVA tests revealed significant main effects of time [$F(6,403)=5.62$, $P<.001$] and treatment group [$F(2,403)=18.83$, $P<.001$]. A significant interaction between time and treatment was also found [$F(12,403)=2.17$, $P<.05$]. Pairwise multiple comparison procedures showed that dialysate CRF levels were increased during withdrawal only in ethanol-fed animals ($P<.001$). As seen in Fig. 2, dialysate CRF levels in ethanol-withdrawn rats were increased approximately 50–75% above baseline and control-fed animals starting at 4.5 h following diet removal. When ethanol-withdrawn animals were given subsequent access to the ethanol-containing diet, dialysate CRF levels declined to basal values and were not significantly different from that of control-fed animals. However, when ethanol-withdrawn animals were given access to the control diet, dialysate CRF levels increased to 101 ± 21% above baseline. These values were significantly higher than those at the same time point of control-fed animals and those of ethanol-withdrawn animals given access to the ethanol diet ($P<.001$).

4. Discussion

In the present study, we demonstrated an increase in extracellular CRF levels in the BNST during the acute withdrawal phase following chronic ethanol ingestion. These data parallel the results of an earlier study demonstrating increases in extracellular CRF levels in the CeA during acute ethanol withdrawal (Merlo-Pich et al., 1995). These investigators demonstrated that extracellular CRF levels in the CeA begin to increase approximately at 6–8 h following diet removal and peak at 10–12 h postwithdrawal. Yet, in the present study, we observed significant increases in extracellular CRF levels starting at 4.5 h following diet removal and apparently peaking at 6 h postwithdrawal (although we did not measure CRF

release at 8–12 h after diet removal). Thus, possible differences in the temporal dynamics of CRF release during ethanol withdrawal may exist between different regions of the extended amygdala, with CRF systems in the BNST being activated earlier in the withdrawal phase than in the CeA.

In order to adequately compare the present results with those of Merlo-Pich et al. (1995), a few minor procedural differences should be noted and addressed. First, although both studies administered the liquid diet for at least 2 weeks, a Lieber–DeCarli liquid diet containing 6.7% (v/v) ethanol was used in the present study, whereas a Sustacal diet containing 8.5% (v/v) ethanol was used by Merlo-Pich et al. (1995). Second, the present study used Long–Evans rats as subjects while Merlo-Pich et al. used Wistar rats. Third, control diet-fed animals were not pair-fed in the present study; that is, the volume of control diet consumed was not yoked to the volume consumed by ethanol-fed animals. Other procedural variations include minor differences in aCSF composition and flow rate, probe membrane type and relative CRF recovery, and RIA procedures. Thus, any of these procedural disparities may have contributed to the slight temporal differences in CRF release in the BNST during ethanol withdrawal observed here versus those observed in the CeA by Merlo-Pich et al. (1995). Future studies examining CRF release in both regions using the exact same experimental paradigms will shed light on whether the BNST CRF systems are indeed activated prior to those in the CeA during acute ethanol withdrawal.

The present study did not quantify physical withdrawal symptoms during the 7.5-h period following diet removal so as to minimize disturbance of the animals, which might lead to confounding alterations in CRF release. However, numerous studies have shown that administration of an ethanol-containing liquid diet for at least 2 weeks produces overt physical signs of withdrawal such as anxiety (Baldwin et al., 1991; Rassnick et al., 1993), decreased locomotor activity (Merlo-Pich et al., 1995), body tremor (Merlo-Pich et al., 1995), acoustic startle (Rassnick et al., 1992), ultrasonic vocalizations (Knapp et al., 1998) and audiogenic or handling-induced seizures (Frye et al., 1983; Olive et al., 2001) at 6–8 h following diet removal. Thus, it is highly likely that the animals in the present study were experiencing one or more symptoms of ethanol withdrawal during this period, when peak increases in CRF release were observed.

Likely sources of extracellular CRF in the BNST are the CRF-containing projections from the CeA (Sakanaka et al., 1986). Thus, the BNST–CeA pathway may be activated during ethanol withdrawal. However, given that numerous CRF-immunopositive neuronal cell bodies have been observed in the BNST (Cummings et al., 1983; Morin et al., 1999; Olschowka et al., 1982; Phelix and Paull, 1990; Swanson et al., 1983), it is possible that extracellular CRF could arise from local somatodendritic release of this peptide. Thus, the precise source of basal and withdrawal-induced increases in extracellular CRF levels in the BNST remains to be determined.

The neurochemical mechanism(s) governing limbic CRF release also need to be assessed. It has been demonstrated that stress increases norepineprhine (NE) release in the BNST (Pacak et al., 1995), and numerous studies have demonstrated reciprocal interactions between NE, CRF and stress (for review, see Koob, 1999). In addition, acute ethanol administration was recently demonstrated to increase dopamine release in the BNST (Carboni et al., 2000). Thus, catecholaminergic mechanisms may contribute to withdrawal-induced release of CRF in the BNST.

The present study also demonstrated that acute ethanol intake, but not control diet intake, following the withdrawal period reduced withdrawal-induced increases in extracellular CRF levels in the BNST. Thus, endogenous CRF release can be modulated by acute ethanol intake. The mechanisms by which ethanol suppresses withdrawal-induced increases in extracellular CRF in the BNST are currently unknown. To our knowledge, the current study is the first in vivo determination of CRF release in the BNST. However, other studies have implicated numerous neurotransmitter systems in the secretion of hypothalamic CRF in vitro (Grossman and Costa, 1993; Grossman et al., 1993), including inhibition of CRF secretion by GABAergic mechanisms (Calogero, 1995; Calogero et al., 1988; Grossman et al., 1993). Thus, if similar regulatory mechanisms govern CRF release in the BNST, it could be postulated that acute ethanol could inhibit CRF release via facilitation of $GABA_A$ receptor function. Exploration of this possibility is clearly warranted.

The precise physiological and behavioral ramifications of the observed increased extracellular levels of CRF in the BNST during ethanol withdrawal are unknown. Given the intricate connections of the BNST with other limbic brain regions, it is tempting to speculate that the increased CRF neurotransmission in the BNST contributes to the anxiogenic and negative emotional aspects of the acute ethanol withdrawal phase. While other studies have suggested that the anxiogenic properties of ethanol withdrawal are mediated by CRF systems in the CeA (Rassnick et al., 1993), contributions of CRF systems in the BNST cannot be ruled out at this point. Indeed, it has been demonstrated that intra-BNST infusions of CRF enhance fear-potentiated acoustic startle reflexes, and that these effects are specifically mediated by CRF receptors in this region (Lee and Davis, 1997). Thus, increased CRF release in the BNST may mediate anxiety-like behaviors during ethanol withdrawal. Other neurotransmitters in this region such as NE may also contribute to the aversive nature of the acute withdrawal phase (Delfs et al., 2000).

The BNST gives rise to extensive projections to the paraventricular nucleus of the hypothalamus (Alheid et al., 1995; Herman et al., 1994). Thus, increases in extracellular CRF levels in the BNST may contribute to the HPA axis

activation commonly observed during ethanol withdrawal (Gallant and Pena, 1992; Rasmussen et al., 2000; Tabakoff et al., 1978). The BNST also sends projections to various brainstem regions known to regulate autonomic function (Alheid et al., 1995; Moga et al., 1989). Indeed, a recent study demonstrated that CRF signaling in the BNST mediates stress-induced activation of cardiovascular function (Nijsen et al., 2001). Thus, the observed increases in extracellular CRF in the BNST may contribute to the cardiovascular activation and dysregulation commonly observed during ethanol withdrawal (Mehta and Sereny, 1979; Smile, 1984; Weise et al., 1985).

In animal models of drug dependence, exposure to stressors, drug-paired environmental stimuli and priming doses of the drug induce reinstatement of drug and ethanol self-administration following extinction (Katner et al., 1999; Le et al., 1998; Koob, 2000; Shaham et al., 2000; Stewart, 2000). Stress-induced "relapse" behavior can be attenuated by administration CRF antagonists (Lê et al., 2000; Sarnyai et al., 2001; Shaham et al., 2000; Stewart, 2000), even when microinjected into the BNST (Erb and Stewart, 1999). Thus, the increased CRF release in the BNST observed in the present study may play a role in the ability of stress to induce relapse to ethanol-seeking behavior following detoxification. However, other neuropeptide systems may also be involved in stress-induced relapse to drug-seeking behaviors (Martin-Fardon et al., 2000).

A particularly interesting aspect of the present study was the finding that when animals that were previously fed the ethanol-containing diet were exposed to the control diet following withdrawal, CRF release in the BNST increased to levels above those seen during the withdrawal period. It is possible that this effect could be a form of conditioned withdrawal or cue reactivity (for review, see Drummond, 2001). For instance, conditioning theories suggest that neutral stimuli, such as the sensory cues associated with the liquid diet, can elicit unconditioned responses after repeated with a drug (i.e., ethanol). Disruption of this pairing in ethanol-fed animals by the presentation and intake of the control diet might have produced a stress response, as reflected in increased CRF release. Indeed, there is ample evidence that reactivity to learned ethanol-associated stimuli (i.e., "cue reactivity") indeed can influence craving and relapse to ethanol consumption during acute withdrawal, as well as protracted abstinence (for reviews, see Drummond, 2000, 2001). Additional studies measuring CRF release following explicit pairing of ethanol exposure and environmental stimuli are required to further address this issue.

In conclusion, the present study demonstrates that extracellular CRF levels are elevated in the BNST during acute ethanol withdrawal, and that this activation can, in turn, be reduced by subsequent ethanol consumption or further increased by the presentation of a nonalcohol containing diet. Further investigations into the motivational, affective and autonomic consequences of these increases in CRF release in the BNST are clearly needed.

Acknowledgments

This research was supported by funds from the State of California for medical research on alcohol and substance abuse through the University of California at San Francisco. The authors wish to thank Nigel Maidment and Hoa Lam for their technical advice on solid-phase CRF RIA procedures.

References

Alheid GF, de Olmos JS, Beltramino CA. Amygdala and extended amygdala. In: Paxinos G, editor. The rat nervous system 2nd ed. San Diego: Academic Press, 1995. pp. 495–578.

Alheid GF, Beltramino CA, De Olmos JS, Forbes MS, Swanson DJ, Heimer L. The neuronal organization of the supracapsular part of the stria terminalis in the rat: the dorsal component of the extended amygdala. Neuroscience 1998;84:967–96.

Baldwin HA, Rassnick S, Rivier J, Koob GF, Britton KT. CRF antagonist reverses the "anxiogenic" response to ethanol withdrawal in the rat. Psychopharmacology 1991;103:227–32.

Bonaz B, Tache Y. Induction of Fos immunoreactivity in the rat brain after cold-restraint induced gastric lesions and fecal excretion. Brain Res 1994;652:56–64.

Brugger S, Sanchez R, Brugger AJ, Martinez JA. ICV administration of CRF blocker (CRF_{9-41} δ helical) reduces morphine withdrawal in rats. Prog Neuropsychopharmacol Biol Psychiatry 1998;22:775–85.

Calogero AE. Neurotransmitter regulation of the hypothalamic corticotropin-releasing hormone neuron. Ann NY Acad Sci 1995;771:31–40.

Calogero AE, Gallucci WT, Chrousos GP, Gold PW. Interaction between GABAergic neurotransmission and rat hypothalamic corticotropin-releasing hormone secretion in vitro. Brain Res 1988;463:28–36.

Carboni E, Silvagni A, Rolando MTP, Di Chiara G. Stimulation of in vivo dopamine transmission in the bed nucleus of stria terminalis by reinforcing drugs. J Neurosci 2000;20(RC102):1–5.

Cummings S, Elde R, Ells J, Lindall A. Corticotropin-releasing factor immunoreactivity is widely distributed within the central nervous system of the rat: an immunohistochemical study. J Neurosci 1983;3:1355–68.

Delfs JM, Zhu Y, Druhan JP, Aston-Jones G. Noradrenaline in the ventral forebrain is critical for opiate withdrawal-induced aversion. Nature 2000;403:430–4.

de Olmos JS, Heimer L. The concepts of the ventral striatopallidal system and extended amygdala. Ann NY Acad Sci 1999;877:1–32.

Drummond DC. What does cue-reactivity have to offer clinical research? Addiction 2000;95(Suppl 2):S129–44.

Drummond DC. Theories of drug craving, ancient and modern. Addiction 2001;96:33–46.

Dunn AJ, Berridge CW. Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? Brain Res Rev 1990;15:71–100.

Erb S, Stewart J. A role for the bed nucleus of the stria terminalis, but not the amygdala, in the effects of corticotropin-releasing factor on stress-induced reinstatement of cocaine seeking. J Neurosci 1999;19(RC35):1–6.

Frye GD, McCown TJ, Breese GR. Characterization of susceptibility to audiogenic seizures in ethanol-dependent rats after microinjection of gamma-aminobutyric acid (GABA) agonists into the inferior colliculus, substantia nigra or medial septum. J Pharmacol Exp Ther 1983;227:663–70.

Gallant DM, Pena JM. Plasma cortisol concentrations during ethanol withdrawal. Alcohol: Clin Exp Res 1992;16:139–40.

Gewirtz JC, McNish KA, Davis M. Lesions of the bed nucleus of the stria

terminalis block sensitization of the acoustic startle reflex produced by repeated stress, but not fear-potentiated startle. Prog Neuropsychopharmacol Biol Psychiatry 1998;22:625–48.

Grossman A, Costa A. The regulation of hypothalamic CRH: impact of in vitro studies on the central control of the stress response. Funct Neurol 1993;8:325–34.

Grossman A, Costa A, Navarra P, Tsagarakis S. The regulation of hypothalamic corticotropin-releasing factor release: in vitro studies. Ciba Found Symp 1993;172:129–43.

Heinrichs SC, Menzaghi F, Schulteis GF, Koob GF, Stinus L. Suppression of corticotropin releasing factor in the amygdala attenuated aversive consequences of morphine withdrawal. Behav Pharmacol 1995;6: 74–80.

Herman JP, Cullinan WE, Watson SJ. Involvement of the bed nucleus of the stria terminalis in tonic regulation of paraventricular hypothalamic CRH and AVP mRNA expression. J Neuroendocrinol 1994;6:433–42.

Katner SN, Magalong JG, Weiss F. Reinstatement of alcohol-seeking behavior by drug-associated discriminative stimuli after prolonged extinction in the rat. Neuropsychopharmacology 1999;20:471–9.

Knapp DJ, Duncan GE, Crews FT, Breese GR. Induction of Fos-like proteins and ultrasonic vocalizations during ethanol withdrawal: further evidence for withdrawal-induced anxiety. Alcohol: Clin Exp Res 1998; 22:481–93.

Koob GF. Corticotropin-releasing factor, norepinephrine, and stress. Biol Psychiatry 1999;46:1167–70.

Koob GF. Animal models of craving for ethanol. Addiction 2000;95 (Suppl 2):S73–81.

Koob GF, Heinrichs SC. A role for corticotropin releasing factor and urocortin in behavioral responses to stressors. Brain Res 1999;848: 141–52.

Koob GF, Heinrichs SC, Menzaghi F, Pich EM, Britton KT. Corticotropin releasing factor, stress and behavior. Semin Neurosci 1994;6:221–9.

Le AD, Quan B, Juzytch W, Fletcher PJ, Joharchi N, Shaham Y. Reinstatement of alcohol-seeking by priming injections of alcohol and exposure to stress in rats. Psychopharmacology 1998;135:169–74.

Lê AD, Harding S, Juzytsch W, Watchus J, Shalev U, Shaham Y. The role of corticotrophin-releasing factor in stress-induced relapse to alcohol-seeking behavior in rats. Psychopharmacology 2000;150:317–24.

Lee Y, Davis M. Role of the hippocampus, the bed nucleus of the stria terminalis, and the amygdala in the excitatory effect of corticotropin-releasing hormone on the acoustic startle reflex. J Neurosci 1997;17: 6434–46.

Lieber CS, DeCarli LM. The feeding of alcohol in liquid diets: two decades of applications and 1982 update. Alcohol: Clin Exp Res 1982;6: 523–31.

Martin-Fardon R, Ciccocioppo R, Massi M, Weiss F. Nociceptin prevents stress-induced ethanol- but not cocaine-seeking behavior in rats. NeuroReport 2000;26:1939–43.

Mehta B, Sereny G. Cardiovascular manifestations during alcohol withdrawal. Mt Sinai J Med 1979;46:484–5.

Merlo-Pich E, Lorang M, Yeganeh M, Rodriguez de Fonseca F, Raber J, Koob GF, Weiss F. Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. J Neurosci 1995;15:5439–47.

Moga MM, Saper CB, Gray TS. Bed nucleus of the stria terminalis: cytoarchitecture, immunohistochemistry, and projection to the parabrachial nucleus in the rat. J Comp Neurol 1989;283:315–32.

Morin SM, Ling N, Liu X-J, Kahl SD, Gehlert DR. Differential distribution of urocortin- and corticotropin-releasing factor-like immunoreactivities in the rat brain. Neuroscience 1999;92:281–91.

Nijsen MJ, Croiset G, Diamant M, De Wied D, Wiegant VM. CRH signalling in the bed nucleus of the stria terminalis is involved in stress-induced cardiac vagal activation in conscious rats. Neuropsychopharmacology 2001;24:1–10.

Olive MF, Hodge CW. Sensitive radioimmunoassays for β-endorphin, CRH and substance P suitable for use with microdialysis. In: O'Connor WT, Lowry JP, O'Connor JP, O'Neill RD, editors. Monitoring molecules in neuroscience. Proceedings of the 9th international conference on in vivo methods. Dublin: University College Dublin, 2001. pp. 29–30.

Olive MF, Mehmert KK, Nannini MA, Camarini R, Messing RO, Hodge CW. Reduced ethanol withdrawal severity and altered withdrawal-induced c-fos expression in various brain regions of mice lacking protein kinase C-epsilon. Neuroscience 2001;103:171–9.

Olschowka JA, O'Donohue TL, Mueller GP, Jacobowitz DM. The distribution of corticotropin releasing factor-like immunoreactive neurons in rat brain. Peptides 1982;3:995–1015.

Pacak K, McCarty R, Palkovits M, Kopin IJ, Goldstein DS. Effects of immobilization on in vivo release of norepinephrine in the bed nucleus of the stria terminalis in conscious rats. Brain Res 1995;688: 242–6.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates 3rd ed. San Diego: Academic Press, 1997.

Phelix CF, Paull WK. Demonstration of distinct corticotropin releasing factor-containing neuron populations in the bed nucleus of the stria terminalis. A light and electron microscopic immunocytochemical study in the rat. Histochemistry 1990;94:345–64.

Rasmussen DD, Boldt BM, Bryant CA, Mitton DR, Larsen SA, Wilkinson CW. Chronic daily ethanol and withdrawal: 1. Long-term changes in the hypothalamo-pituitary–adrenal axis. Alcohol: Clin Exp Res 2000;24: 1836–49.

Rassnick S, Koob GF, Geyer MA. Responding to acoustic startle during chronic ethanol intoxication and withdrawal. Psychopharmacology 1992;106:351–8.

Rassnick S, Heinrichs SC, Britton KT, Koob GF. Microinjection of a corticotropin-releasing factor antagonist into the central nucleus of the amygdala reverses anxiogenic-like effects of ethanol withdrawal. Brain Res 1993;605:25–32.

Richter RM, Weiss F. In vivo CRF release in rat amygdala is increased during cocaine withdrawal in self-administering rats. Synapse 1999;32: 254–61.

Rivier CL, Plotsky PM. Mediation by corticotropin releasing factor (CRF) of adenohypophyseal hormone secretion. Annu Rev Physiol 1986;48: 475–94.

Rodríguez de Fonseca F, Carrera MRA, Navarro M, Koob GF, Weiss F. Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. Science 1997;276:2050–4.

Sakanaka M, Shibasaki T, Lederis K. Distribution and efferent projections of corticotropin-releasing factor-like immunoreactivity in the rat amygdaloid complex. Brain Res 1986;382:213–38.

Sarnyai Z, Bíró E, Gardi J, Vecsernyés M, Julesz J, Telegdy G. Brain corticotropin-releasing factor mediates 'anxiety-like' behavior induced by cocaine withdrawal in rats. Brain Res 1995;675:89–97.

Sarnyai Z, Shaham Y, Heinrichs SC. The role of corticotropin-releasing factor in drug addiction. Pharmacol Rev 2001;53:209–44.

Shaham Y, Erb S, Stewart J. Stress-induced relapse to heroin and cocaine seeking in rats: a review. Brain Res Rev 2000;33:13–33.

Smagin GN, Heinrichs SC, Dunn AJ. The role of CRH in behavioral responses to stress. Peptides 2001;22:713–24.

Smile DH. Acute alcohol withdrawal complicated by supraventricular tachycardia: treatment with intravenous propranolol. Ann Emerg Med 1984;13:53–5.

Stewart J. Pathways to relapse: the neurobiology of drug- and stress-induced relapse to drug-taking. J Psychiatry Neurosci 2000;25:125–36.

Sutton RE, Koob GF, Le Moal M, Rivier J, Vale W. Corticotropin releasing factor produces behavioural activation in rats. Nature 1982;297:331–3.

Swanson LW, Sawchenko PE, Rivier J, Vale WW. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology 1983;36: 165–86.

Tabakoff B, Jafee RC, Ritzmann RF. Corticosterone concentrations in mice during ethanol drinking and withdrawal. J Pharm Pharmacol 1978;30: 371–4.

Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 1981;213:1394–7.

Walker DL, Davis M. Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. J Neurosci 1997;17:9375–83.

Weise F, Muller D, Krell D, Kielstein V, Koch RD. Heart rate variability in withdrawing alcoholic patients. Drug Alcohol Depend 1985;16: 85–8.