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Increased protein and mRNA expression of corticotropin-releasing factor (CRF), decreased CRF receptors and CRF binding protein in specific postmortem brain areas of teenage suicide subjects

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ABSTRACT

Overactivity of hypothalamic-pituitary-adrenal (HPA) axis function has been implicated in depression and suicidal behavior. This is based on the observation of an abnormal dexamethasone (DEX) and DEX-adrenocorticotropic hormone (ACTH) test in patients with depression and suicidal behavior. Recently, some studies have also found abnormalities of glucocorticoid receptors (GR), mineralocorticoid receptors (MR), corticotropin releasing factor (CRF), CRF receptors (CRF-R) and CRF binding protein (CRF-BP) in depressed and suicidal patients. Some investigators have also observed increased levels of CRF in the cerebrospinal fluid (CSF) and altered levels of MR, GR and CRF in the postmortem brain of depressed and suicidal subjects. We have earlier reported decreased protein and mRNA expression of GR and GILZ, a chaperone protein, in the postmortem brain of teenage suicide subjects. We have further studied CRF and its receptors in different areas of the postmortem brain of suicide subjects, i.e., the prefrontal cortex (PFC), hippocampus (HIPPO), subiculum and amygdala (AMY) from teenage suicide subjects. The CRF and its receptors were determined in the PFC (Brodmann area 9), HIPPO, subiculum and different amygdaloid nuclei from 24 normal control subjects and 24 teenage suicide subjects. Protein expression of CRF, its receptors and CRF-BP was determined by immunolabeling using the Western blot technique and mRNA expression was determined by real-time PCR (qPCR) technique. We found that the mRNA levels of CRF were significantly increased in the PFC, in the central amygdaloid nucleus (CeAMY) and in the subiculum. mRNA levels of CRF-R1 and CRF-BP were significantly decreased in the PFC. We did not find any changes in the HIPPO of any of the CRF components we studied. When we compared the protein expression of CRF components we found that CRF was significantly increased and CRF-R1, CRF-R2 and CRF-BP significantly decreased in the PFC. On the other hand, there were no changes in the protein expression of CRF components in the HIPPO. Our results in the postmortem brain suggest that, as found by clinical studies in the CSF, there are significant alterations of CRF and its receptors in the postmortem brain of teenage suicide subjects. These alterations of CRF and its components were region-specific, as changes were not generally observed in the HIPPO.

1. Introduction

Hyperactive hypothalamic-pituitary-adrenal (HPA) axis has been implicated in the stress-related disorders, such as depression, post-traumatic stress disorder (PTSD) and suicide (Gao and Bao, 2011; Holsboer, 2001), and is probably one of the most consistent biological psychiatry findings in depression. In the HPA axis system corticotropin releasing factor (CRF) is released from the paraventricular nucleus (PVN) of the hypothalamus and activates the HPA axis by causing the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary. The release of ACTH from the pituitary stimulates the synthesis and secretion of glucocorticoids, such as cortisol in humans and corticosterone in rats, from the adrenal glands (Aguilera et al., 2004). Glucocorticoids regulate the HPA axis through a negative feedback mechanism by binding to soluble glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) in the pituitary and the hypothalamus, and thus inhibit the release of CRF and ACTH (Binder and Nemeroff, 2010; Gao and Bao, 2011).

The hyperactive HPA axis function in depression is evidenced by the observation that most patients with depression have increased concentrations of cortisol in plasma and cerebrospinal fluid (CSF) [see review by Gao and Bao (2011)], and a deficient feedback mechanism, as

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Table 1
Demographic characteristics of teenage suicide and normal control subjects.

Patient No/Sex/Age,y/Race	PMI, h	Brain pH	Cause of Death	Psychotropic Drugs (at the time of death)	Psychiatric Diagnosis
CONTROL GROUP[*]					
1/M/19/B	6	6.14	GSW	None	Normal
2/M/16/B	6	6.54	GSW	None	Normal
3/M/16/B	8	5.64	GSW	None	Normal
4/M/19/B	12	5.9	GSW	None	Normal
5/M/13/W	NA	5.19	Accident	None	Normal
6/M/17/B	11	6.52	GSW	None	Normal
7/M/16/W	10	5.42	Stabbing	None	Normal
8/M/17/B	10	5.9	GSW	None	Normal
9/M/13/B	22	6.07	GSW	None	Normal
10/M/14/B	18	5.73	GSW	None	Normal
11/M/18/B	27	6.09	Drowning	None	Normal
12/M/16/W	21	5.97	Accidental hanging	None	Normal
13/F/18/W	35	5.99	Multiple injuries	None	Normal
14/F/17/B	26	6.23	Multiple injuries	None	Normal
15/F/19/W	30	6.2	Cardiac arrhythmia	None	Normal
16/F/18/B	16	6.6	MVA	None	Normal
17/M/13/B	20	6.6	Drowning	None	Normal
18/M/19/B	16	6.6	Congenital heart disease	None	Normal
19/F/16/W	24	6.71	Myocarditis	None	Normal
20/M/15/W	16	6.5	Cardiac arrhythmia	None	Normal
21/M/15/W	21	6.37	PE/DVT	None	Normal
22/F/16/W	20	6.89	MVA	None	Normal
23/F/13/B	23	6	Hanging	None	
24/M/18/W	19	5.80	Complications of Morbid Obesity	None	Normal
SUICIDE GROUP[+]					
1/F/15/W	7	5.48	GSW	Ethanol	Alcohol abuse
2/M/20/W	32	6.41	Hanging	Ethanol	Alcohol abuse
3/M/12/B	10	5.91	Hanging	None	Major depression
4/M/15/W	11	5.33	Asphyxia	None	Major depression
5/F/15/W	17	5.58	Drug overdose	Imipramine, Desipramine	Major depression, Hyperactivity attention deficit disorder
6/M/15/W	27	6.08	GSW	None	Adjustment disorder, Major depression
7/M/18/W	17	6.3	Hanging	None	Major depression - single episode
8/M/19/W	18	6.2	CO intoxication	Ethanol	Major depression, Ethanol abuse, Polysubstance abuse
9/F/15/W	20	6.59	Hanging	None	Major depression - single episode, Ethanol abuse
10/M/17/W	23	6.66	Hanging	Ethanol	Major depression - single episode, Ethanol abuse
11/M/13/W	18	6	Hanging	Ritalin	Hyperactivity attention deficit disorder
12/F/17/W	25	5.55	Drug overdose	None	Adjustment disorder
13/F/16/W	33	6.61	GSW	None	Adjustment disorder
14/M/16/W	24	6.81	Hanging	None	Adjustment, Conduct disorders
15/F/15/W	21	6.48	Hanging	None	Adjustment disorder with depressed mood
16/F/15/W	20	6.1	Hanging	None	Borderline personality disorder
17/M/19/W	15	6.9	GSW to chest	Fluoxetine	Dissociative disorder, Substance abuse (kind unclear), PTSD
18/F/15/W	11	6.44	Drug overdose	None	No mental disorder
19/M/17/A	7	5.9	GSW	Ethanol	No mental disorder
20/M/16/H	20	6.17	Hanging	None	No mental disorder
21/F/16/W	18	6.31	GSW	Amitriptyline	No mental disorder
22/M/15/W	16	6.18	Hanging	None	No mental disorder
23/M/14/B	22	6.44	Hanging	None	No mental disorder
24/F/17/W	24	6.49	Diphenhydramine overdose	Citalopram	Dx (not enough info)

Abbreviations: M male; F female; B black; W white; AAsian; H Hispanic; ACSVD atherosclerotic cardiovascular disease; GSW gunshot wound; MVA motor vehicle accident; NA not available; PE/DVT pulmonary embolism deep vein thrombosis; PMIPMI postmortem inter.

[*] Mean ± SD age was 16.29 ± 2.03 years; PMI, 18.13 ± 7.67 h; and brain pH, 6.15 ± 0.43, 17 male, 7 female.

[+] Mean ± SD age was 15.92 ± 1.86 years; PMI, 19.00 ± 6.85 h; and brain pH, 6.21 ± 0.44, 14 male, 10 female.

evidenced by an abnormal dexamethasone suppression test (DST) (Bale, 2005). The hyperactive HPA axis in depression has been attributed to increased release of CRF, mostly as part of the stress system, and a deficient feedback mechanism, mediated by GR in the brain (Gao and Bao, 2011; Naughton et al., 2014).

Thus, CRF is one of the major components of the HPA axis. The CRF system is thought to play an important role in the pathophysiology of depression (Waters et al., 2015) and suicidal behavior. The CRF system consists of the peptide CRF and two receptors for CRF known as CRF-R1 and CRF-R2. In addition, a protein known as CRF binding protein (CRF-BP) is also a member of the CRF system and is involved in the deactivation of CRF functions in the brain and the periphery. In addition to CRF, the urocortin (URC) family URC1 and URC2 also activate the CRF receptors in the brain (Gallagher et al., 2008; Waters et al., 2015). The role of CRF, CRF-R1, CRF-R2 and CRF-BP in depression and suicide is supported by both animal and human studies. Abnormality of CRF is also reported by a dexamethasone (DEX)-CRF blood test, which has become a very sensitive method for the detection of hyperactive HPA axis in depression. CRF regulates both basal and stress-related release of the pituitary ACTH (Nemeroff and Owens, 2002). Because of the important role CRF plays in stress and depression several investigators have therefore proposed a corticosteroid hypothesis of depression (Holsboer, 2000; Nemeroff, 1996; Nemeroff and Owens, 2002). This hypothesis proposes that corticosteroid receptor signaling is defective in depression (Keck and Holsboer, 2001). Markers of hyperactive brain CRF neurotransmission include increased levels of CRF in the CSF of depressed patients. CRF levels are highest in depressed suicide victims indicating that higher levels of CRF may be associated with greater illness severity. This is also based on increased levels of CRF mRNA and CRF immunoreactive neurons in the hypothalamic PVN as well as in the prefrontal cortex (PFC), locus coeruleus (LC) and median (MRN) and dorsal (DRN) raphe nuclei in major depression. The involvement of CRF in depression is suggested by the observation that specific microinjections of CRF produce behavioral responses reminiscent of major depression in human beings, including increased anxiety, reduced slow-wave sleep, psychomotor alterations, anhedonia, decreased appetite and libido [see (Waters et al., 2015)]. This is also based on the observation obtained from transgenic animals, either lacking or overexpressing CRF or CRF receptors (Bale and Vale, 2004; Muller et al., 2003). It is found that mice overexpressing CRF in the forebrain areas have anxiety and depression related phenotypes which are specific for activation of the CRF1 receptor. Evidence from human studies also suggests hyperactive CRF in depression and suicide. Human postmortem studies suggest CRF protein and mRNA overexpression in the hypothalamus, PFC and LC (Gao and Bao, 2011). Behavioral and functional effects of CRF are mediated by two CRF receptors, CRF-R1 and CRF-R2, which have also been observed to be decreased in the postmortem brain of suicide subjects.

Abnormalities of CRF-BP, although not many, have been observed in patients with depression and suicidal behavior (Herringa et al., 2006). Taken together, the evidence from the knockout mice and human studies suggest that CRF and its receptors may play an important role in the pathophysiology of depression and suicide (Bale and Vale, 2004; Muller et al., 2003).

Teenage suicide is a major public health problem and among the teenagers it is the second leading cause of death (CDC, 2019; Moscicki et al., 1988). Stress and early life trauma may play an important role and are greater risk factors in teenage than in adult suicide (Binder and Nemeroff, 2010). Also, some studies suggest a difference in the neuroendocrine response between teenage and adult depression (Ryan et al., 1992). It is therefore important to study the factors associated with teenage suicide postmortem brain samples separately from the adults. In this context, we earlier examined if the abnormal HPA axis system in suicide may be related to abnormalities of GR and MR in different postmortem brain areas of teenage suicide subjects and found that the protein and mRNA expression of GR, but not the MR, were significantly decreased in the PFC and amygdala (AMY), but not the hippocampus (HIPPO) of teenage suicide subjects (Pandey et al., 2013).

In light of these observations we have further studied the abnormalities of HPA axis in the brain by determining the protein and mRNA expression of CRF system (CRF, CRF receptors and CRF-BP) in different brain regions, including PFC, HIPPO and different AMY nuclei in the postmortem brain of teenage suicide subjects and normal controls.

2. Method

2.1. Acquisition of human postmortem brain samples

Brain tissue was obtained from the Maryland Brain Collection at the Maryland Psychiatric Research Center, Baltimore, Maryland, in collaboration with the Office of the Chief Medical Examiner of the State of Maryland. Tissue samples were obtained from 24 teenage suicide subjects and 24 teenage control subjects (Table 1). AMY tissue was available only from 10 suicide subjects and 10 normal control subjects. Toxicological data were obtained by analysis of urine and blood samples from these subjects. All procedures were approved by the University of Maryland Institutional Review Board and written consents were obtained from family members. None of the subjects included in this study had any gunshot wounds (GSW) to the head.

2.2. Diagnostic method

At least one family member and/or a friend, after giving written informed consent, underwent an interview based on the Structured Clinical Interview for DSM-IV (SCID) (First et al., 1997). The interviews were done by a trained psychiatric social worker. Two psychiatrists independently reviewed the write-up from this interview, as well as the SCID that was completed from it, as part of their diagnostic assessment of the case. Diagnoses were made from the data obtained in this interview, medical records (if available) from the case, and records obtained from the Medical Examiner's office. This was done for all subjects (i.e., normal controls and suicide cases). The collection of clinical data for the control and suicide subjects was identical, and the procedure for the diagnosis of normal control subjects was the same as for the suicide subjects. The diagnoses from two psychiatrists were compared and discrepancies were resolved by means of a consensus conference. There was a consistent reliability of diagnosis with kappa > 0.9.This was found to be a very accurate way to make diagnoses (Ramirez Basco et al., 2000). Control subjects were verified as free from mental illness using these consensus diagnostic procedures.

2.3. Dissection of postmortem brain tissue

The brain samples were received from the Maryland Brain Collection at the Maryland Psychiatric Research Center already dissected in different areas. All frozen brains were dissected with a Stryker autopsy saw. The brain was hemisected and each side was stored whole in the freezer. To keep the samples frozen, the dissections were performed on a metal plate over a container filled with dry ice, and different brain areas were dissected very quickly with minimum defrosting. The PFC samples were cut out of the coronal sections by a fine microdissecting (Graefe) knife under a stereomicroscope with low magnification. The PFC (Brodmann area 9, BA9) was taken just dorsal to the fronto-polar area, including the most polar portion of the superior and partly the middle frontal gyrus between the superior and intermediate frontal sulci. The HIPPO and AMY were rolled out of the temporal lobe and removed together with the underlying entorhinal cortex, with the AMY and HIPPO indicated, as well as the 5 rostrocaudal divisions of the HIPPO (Supplemental Fig. S1). Levels (2–4) of the HIPPO were usually sampled. The tissues were stored at −80 °C until use.

2.3.1. Dissection of the amygdaloid nuclei

The section profiles of the AMY appeared in coronal sections throughout the rostral pole of the temporal lobe. The central nucleus (CeAMY) was removed at the dorsal part of the AMY, just ventral to the optic tract, with a punch needle with 1.5 mm inside diameter. Samples were taken from the medial nucleus (MeAMY) with a same size needle, just at the most medial parts of the AMY, ventromedial to the central nucleus. Subiculum was punched out of the HIPPO.

2.4. Determination of protein levels of CRF, CRF-R1, CRF-R2, and CRF-BP using Western blot technique

The protein samples (30 μg) were separated by sodium SDS-polyacrylamide gel electrophoresis (PAGE) and then electrophoretically transferred to nitrocellulose membranes (Amersham, Arlington Heights, IL) using transfer buffer (25 mM Tris base; 192 mM glycine; 20% v/v methanol, pH = 8.4). The membrane was blocked for 1 h at room temperature with 5% non-fat milk in phosphate-buffered saline (pH 7.4) and then incubated overnight at 4 °C with primary polyclonal anti-CRH-R1, anti-CRH-R2 and anti-CRH-BP antibodies (Thermo Scientific Pierce, Fisher Scientific Company, LLC, Hanover Park, Illinois) at a dilution of 1:1000 or anti-CRF monoclonal antibody (Abcam) at a dilution of 1:1000. The membranes were extensively washed with TBST and exposed to ECL autoradiography film. For immunoblotting of β-

Table 2
Uncorrected and False Discovery Rate Corrected p Values.

mRNA Expression of CRF-Related Peptides in Different Brain Regions

	PFC		HIPPO		SUBICULUM		CeAMY		MeAMY	
	Raw	Corrected	Raw	Corrected	Raw	Corrected	Raw	Corrected	Raw	Corrected
CRF	0.0054	0.0108	0.1146	0.4584	0.0389	0.0848	0.0326	0.0652	0.82	0.82
CRF-R1	0.0383	0.047	0.63	0.84	0.4808	0.4808	0.0332	0.0997	0.5908	0.82
CRF-R2	0.7388	0.7388	0.578	0.84	0.4808	0.4808	0.8351	0.8351	0.6849	0.82
CRF-BP	0.0054	0.0108	0.9997	0.9997	0.0424	0.0848	0.021	0.0652	0.2703	0.82

Protein Expression of CRF-Related Peptides in Different Brain Regions

	PFC		HIPPO		AMYGDALA	
	Raw	Corrected	Raw	Corrected	Raw	Corrected
CRF	0.0294	0.0294	0.245	0.3267	0.2596	0.5192
CRF-R1	0.0003	0.0012	0.4491	0.4491	0.0391	0.1564
CRF-R2	0.03	0.0294	0.1233	0.3267	0.4958	0.6611
CRF-BP	0.0006	0.0012	0.2446	0.3267	0.9642	0.9642

actin, the blots were stripped with stripping buffer and then blocked and incubated with β-actin primary antibody (Sigma Chemical Co., St. Louis, Missouri, 1:5000 dilution) and then secondary anti-mouse antibody (1:5000 dilution) according to the procedure described above. The bands on the autoradiogram were quantified using the Loats Image Analysis System (Loats Associates, Inc., Westminster, MD), and the optical density of each sample was corrected by the optical density of the corresponding β-actin band. The values are represented as a percent of the control

2.5. Determination of mRNA levels of CRF, CRF-R1, CRF-R2 and CRF-BP using real-time PCR

2.5.1. RNA extraction and reverse transcription

Total RNAwas extracted from 100 mg of tissue using the TRIZOL reagent as per the manufacturer's instructions (Invitrogen, USA). RNA yield and quality was determined by absorbance at 260 nm using NanoDrop®ND-1000 and using Agilent Bioanalyzer 2100. Only samples showing 28S/18Sratios > 1.2 and RIN ≥7.0 were included.

2.5.2. Relative real-time PCR

Expression levels of mRNA were determined using a two-step real-time RT-PCR (qPCR) method. One ug of total RNA was reverse transcribed using 50 ng random hexamers, 2 mM dNTP mix, 10 units ribonuclease inhibitor, 50 mM Tris–HCl (pH 8·3), 75 mM KCl, 3 mM MgCl2, 10 mM DTT, and 200 units MMLV-reverse transcriptase (Invitrogen) in a final reaction volume of 20 µl. Reverse transcription was performed at 37 °C for 60 min, and enzyme was denatured at 70 °C for 15 min. The cDNA was stored at −20 °C.

Real-time PCR was performedwith MX3005p sequence detection system (Agilent) using pre-designed Taqman gene expression assays (Applied Biosystems, Foster City, CA) targeting CRF, CRF-BP, CRF-R1 and CRF-R2 along with two housekeeping genes β-actin (ACTB), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), shown in below (section 2.5.3). The stability and optimal number of housekeeping genes was determined using geNORM version 3.4 (PrimerDesign Ltd, UK) according to the manufacturer's instructions (Vandesompele et al., 2002). This comparison identified ACTB and GAPDH as the most stable housekeeping genes for this cohort. PCR efficiency after 5-log dilution series of pooled cDNA was similar for all housekeeping and target genes. For each primer/probe set, qPCR reaction is carried out using 10 µl of cDNA (diluted 1:10) in 1X TaqMan Universal PCR MasterMix (Applied Biosystems) as per manufacturer'sinstructions. Each qPCR plate included a "no reverse transcriptase" and "no template" control to eliminate non-specific amplification and each sample is assayed in triplicate.

For qPCR gene expression analysis, raw expression data (C_t) are normalized to the geometric mean of the two housekeeping genes. Outliers were excluded if the normalized (delta C_t) values were greater than 2 standard deviations from the group mean. Relative expression levels, reported as fold change, were determined by $2^{-(\Delta\Delta Ct)}$ method, as described in Applied Biosystems User Bulletin No. 2 (P/N 4303859) and ΔC_t values are used for further statistical analysis.

2.5.3. TaqMan Primer/Probes used for qPCR assays

	TaqMan Accession	Probe Location (exon boundary)	Assay Function
ACTB	Hs99999903_m1	1-1	House Keeping (HK)
GAPDH	Hs99999905_m1	3-3	HK
CRF	Hs00174941_m1	1-2	Target gene
CRF-R1	Hs00366363_m1	8-9	Target gene
CRF-R2	Hs00266401_m1	2-3	Target gene
CRF-BP	Hs00181810_m1	6-7	Target gene

2.6. Statistical analysis

The data analyses were performed using the SAS 9.4 statistical software package. A generalized linear model (GLM) was fitted to compare the effects of two groups — teenage suicide and teenage normal control subjects — on protein and mRNA expression of CRF, CRF-R1, CRF-R2 and CRF-BP by adjusting the effects of the covariates age, sex, postmortem interval (PMI) and brain pH. We used MANCOVA (Wilks' Lambda statistic) to compare the two groups for protein and mRNA levels (CRF, CRF-R1, CRF-R2 and CRF-BP) across each brain region (PFC, HIPPO, subiculum, CeAMY and MeAMY) by taking into account the inter-correlation of the dependent variables and adjusting the above covariates. Final model excluded the non-significant covariates. After this, we performed multiple univariate ANCOVAs as a follow-up analysis to test the group differences on individual outcome measures per region. The significant results (corrected p-values) are reported based on False Discovery Rates (FDR) approach for each region. Both corrected and uncorrected p-values for each measure and region are shown in Table 2.

3. Results

We determined the mRNA levels of CRF, CRF-R1, CRF-R2 and CRF-BP in the PFC (BA9), and HIPPO obtained form 24 teenage suicide

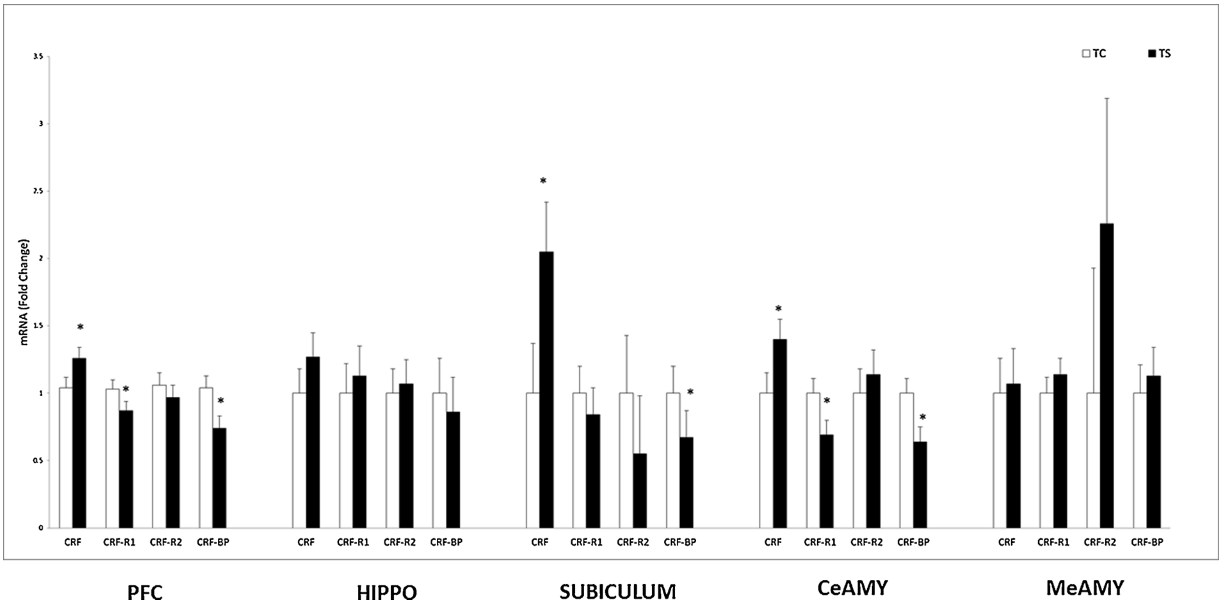

Fig. 1. Mean mRNA expression levels of CRF, CRF-R1, CRF-R2, and CRF-BP in the prefrontal cortex (PFC), hippocampus (HIPPO), subiculum, central (CeAMY) and medial (MeAMY) amygdala nuclei of teenage suicide (TS) subjects and teenage normal control subjects (TC). The data are shown as fold change in mRNA levels. Values are fold change ± standard error of the mean (SEM).$^*p < 0.05$.

subjects and 24 matched normal control subjects. We also determined the mRNA expression of these CRF-related peptides in the subiculum, CeAMY and MeAMY nuclei of the AMY obtained from normal control ($n = 10$) and suicide subjects ($n = 10$). The results are described below.

3.1. mRNA levels of CRF, CRF-R1, CRF-R2 and CRF-BP in the PFC of teenage suicide and normal control subjects

We examined if mRNA levels of the above CRF-related peptides are correlated with each other in the PFC, and the results are shown in supplemental table S1a and S1b. We found that the mRNA levels of CRF-BP are significantly correlated with CRF ($r = -0.32$, $p = 0.02$) and CRF-R1 ($r = 0.57$, $p < 0.0001$). Also the mRNA levels of CRF-R1 are correlated significantly with CRF-R2 ($r = 0.46$, $p = 0.0008$). MANCOVA analysis showed that the two groups are significantly different [F (4, 41) = 4.82, $p = 0.0025$] in the PFC. Univariate analysis showed that the mRNA levels of CRF were significantly increased in the PFC ($p = 0.0108$) of teenage suicide compared with control subjects (Fig. 1, Table S2a). In contrast, we found that the mRNA levels of CRF-R1 ($p = 0.047$) and CRF-BP ($p = 0.0108$) were significantly decreased in the PFC of suicide subjects compared with normal control subjects (Fig. 1, Table S2a). There was no significant difference in the PFC mRNA levels of CRF-R2 between teenage suicide and control subjects (Fig. 1, Table S2a). The supplemental figure S1 shows all the data points for the mRNA expression of CRF components in the PFC.

3.2. mRNA levels of CRF, CRF-R1, CRF-R2 and CRF-BP in the HIPPO of teenage suicide and normal control subjects

The mRNA levels of CRF are significantly correlated (Table S1a) with CRF-R1($r = -0.47$, $p = 0.026$), MANCOVA analysis showed that the two groups are not significantly different [F (4, 16) = 1.60, $p = 0.45$] in the HIPPO. In univariate ANCOVA analysis, when we compared the HIPPO mRNA levels, we found no significant differences in CRF, CRF-R1, CRF-R2 or CRF-BP between the teenage suicide and normal control subjects.

3.3. mRNA levels of CRF, CRF-R1, CRF-R2 and CRF-BP in the subiculum, CeAMY and MeAMY of teenage suicide and normal control subjects

The levels of *CRF, CRF-R1, CRF-R2 and CRF-BP* are uncorrelated (Table S1a) in the subiculum and MANCOVA analysis showed that there is a significant group difference [F (4, 7) = 8.91, $p = 0.007$] in this brain region.

We found that the mRNA levels of CRF ($p = 0.0389$, uncorrected) were significantly increased and those of CRF-BP ($p = 0.0424$, uncorrected) significantly decreased in the subiculum of teenage suicide compared with normal control subjects (Fig. 1, Table S2a). However, these significant differences are lost when the FDR correction is applied (see Table 2), probably due to the small sample size. There was no significant difference in subiculum mRNA levels of CRF-R1 or CRF-R2 between the teenage suicide and normal control subjects (Fig. 1, Table S2a).

As for the AMY nuclei, we found that the mRNA levels of CRF ($p = 0.0326$, uncorrected) were significantly increased and those of CRF-R1 ($p = 0.0332$) and CRF-BP ($p = 0.021$, uncorrected) significantly decreased in the CeAMY of teenage suicide subjects compared with normal control subjects (Fig. 1, Table S2a). Again, due to the small sample size, these significant differences disappeared after the FDR correction (Table 2). There was no significant difference in CeAMY mRNA levels of CRF-R2 between the teenage suicide and normal control subjects (Fig. 1, Table S2a).

In contrast to CeAMY, univariate ANCOVA analysis of mRNA levels in MeAMY showed no significant differences in CRF, CRF-R1, CRF-R2 or CRF-BP between the teenage suicide and normal control subjects.

3.4. Protein expression of CRF, CRF-R1, CRF-R1 and CRF-BP in the PFC, HIPPO and AMY of teenage suicide and normal control subjects

In order to examine if the protein expression of CRF-related peptides is similar or dissimilar from their mRNA expression we also determined the protein expression of CRF, CRF-R1, CRF-R2 and CRF-BP in the PFC, HIPPO, and AMY of teenage suicide subjects and teenage normal control subjects. We did not determine protein levels in the subiculum, CeAMY or MeAMY because of the paucity of tissue samples. The immunoblots showing the protein expression of CRF and related peptides in the PFC of two normal control and two suicide subjects are shown in

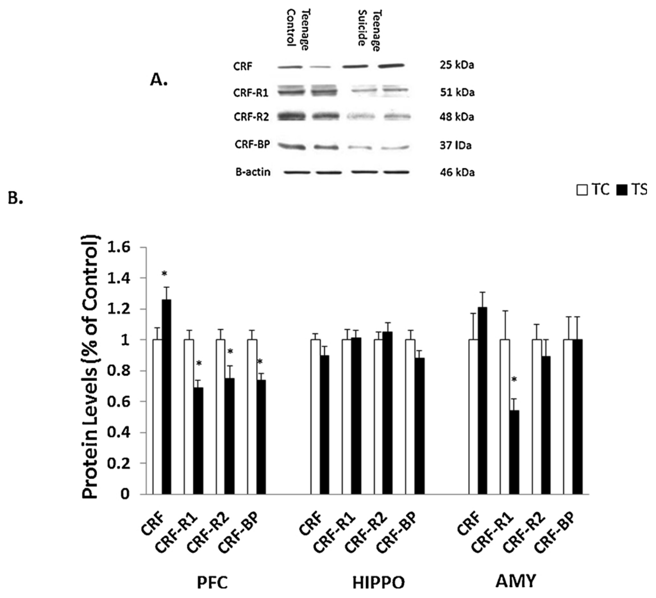

Fig. 2. ARepresentative Western blots showing the immunolabeling of CRF, CRF-R1, CRF-R2, CRF-BP and β-actin in the PFC membrane fraction of two teenage suicide and two teenage normal control subjects.
BMean protein expression levels of CRF, CRF-R1, CRF-R2, and CRF-BP in the PFC, HIPPO and AMY of TS and TC subjects. The data are shown as percent of controls. Values are mean ± SEM.*$p < 0.05$.

Fig. 2A. As can be seen, the protein expression of CRF appears to be increased and that of CRF-R1, CRF-R2 and CRF-BP decreased in the PFC of teenage suicide compared with normal control subjects (Fig. 2A).

The correlation analysis (Table S1b) showed that there is a correlation between CRF and CRF-R1 (r = - 0.338, p = 0.0151) in the PFC. Significant correlation also exists between CRF-BP and CRF-R2 in HIPPO (r = - 0.33, p = 0.01) and AMY(r = - 0.63, p = 0.002). The MANCOVA results showed that the two groups are significantly different [F (4, 44) = 8.88, $p < 0.0001$]. Using independent ANCOVA analysis, we then compared the CRF protein expression between teenage suicide and teenage control subjects and found that CRF protein levels were significantly increased (p = 0.0294) in the PFC of teenage suicide compared with normal control subjects (Fig. 2B, Table S2c). However, there was no significant difference in the protein expression levels of CRF between normal control and suicide subjects in the HIPPO or AMY (Fig. 2B, Table S2c). We also found that the protein expression levels of CRF-R1 (p = 0.0012), CRF-R2 (p = 0.0294) and CRF-BP (p = 0.0012) were significantly decreased in the PFC of teenage suicide subjects compared with normal control subjects (Fig. 2B, Table S2c). There was no significant difference in the protein expression levels of CRF-R1, CRF-R2 and CRF-BP in the HIPPO or AMY, except that CRF-R1 was significantly (p = 0.0391, uncorrected) decreased in AMY of teenage suicide subjects compared with normal control subjects (Fig. 2B, Table S2c), but again this significance was lost after FDR correction (Table 2).

The supplemental figure S2 shows all the data points for the protein expression of CRF components in the PFC.

3.5. *Effect of confounding variables, antidepressants, ethanol and mode of death on CRF measures*

To examine whether the protein or mRNA expression of CRF, CRF-R1, CRF-R2 and CRF-BP was affected by confounding variables, such as age, sex, postmortem interval (PMI) and brain pH, we included them as covariates in the generalized linear model. MANCOVA results showed that only mRNA values in PFC area are affected by gender.

Using univariate ANCOVA, we did not observe any significant effect of confounding variables, such as age, sex, PMI and brain pH on protein expression of the main outcome measures between the teenage suicide and teenage control groups, except for the effect of sex on CRF in the PFC (p = 0.0317). There was a significant effect of sex on mRNA expression of CRF-R2 in the PFC (p = 0.0006), of age on that of CRF-R2 in the HIPPO (p = 0.0465), of brain pH on that of CRF-BP in the subiculum (p = 0.0243) and of brain pH (p = 0.0002) and PMI (p = 0.0044) on that of CRF in the MeAMY.

When we compared the mRNA and protein expression of CRF measures between all male (control + suicide) and all female (control + suicide) subjects, only the mRNA expression of CRF-R2 is significantly decreased in the PFC of female compared to male subjects.

Several suicide subjects were probably treated for depression since they had the presence of antidepressants in their blood at the time of death. We examined if antidepressant presence or treatment affected the expression of CRF and related genes. Out of 24 suicide subjects, 4 subjects had the presence of antidepressants, and 5 subjects had the presence of ethanol at the time of death.

We ran our analysis for the suicide group only to see the effect of antidepressants and ethanol over all outcome measures individually adjusted for other covariates, such as brain pH, PMI, age and gender. The antidepressants had a significant effect on protein expression (i.e., tended to increase protein levels) of CRF-BP (t = - 2.21; p = 0.0386) and CRF-R2 (t = - 3.0; p = 0.0077) in the PFC. Ethanol had a significant effect on mRNA expression (i.e., tended to increase mRNA levels) of CRF-BP in the HIPPO (t = - 2.13; p = 0.045).

The exclusion of 4 subjects with antidepressants did not change any of our findings that the protein expression of CRF was significantly increased (p = 0.033), and protein expression of CRF-R1 (p = 0.0004), CRF-R2 (p = 0.014) and CRF-BP (p = 0.0003) was significantly decreased in the PFC. Similarly, after the exclusion of the 4 subjects with antidepressants, mRNA expression of CRF was still significantly increased (p = 0.008), and mRNA expression of CRF-R1 (p = 0.088) and CRF-BP (p = 0.019) was decreased in the PFC, although it did not reach statistical significance for CRF-R1.

Also, there was no significant difference in the PFC of either mRNA or protein expression of any of the outcome measures when we compared the group of suicide subjects without antidepressants (n = 20) with the suicide subjects who had antidepressants at the time of death (n = 4) for any of the CRF components.

The exclusion of 5 subjects with ethanol at the time of death did not change our finding that the expression of CRF was increased (protein: p = 0.0229; mRNA: p = 0.0097) and that of CRF-R1 (protein: p = 0.0012; mRNA: p = 0.0754), CRF-R2 (protein: p = 0.0048; mRNA: p = 0.4657) and CRF-BP (protein: p = 0.0018; mRNA: p = 0.0100) was decreased in the PFC. Thus, these observations suggested that the presence of antidepressants or ethanol had no significant effect on the mRNA or protein expression of CRF, CRF-R1, CRF-R1 and CRF-BP.

In the normal control group, 8 subjects died of gunshot wounds (GSW) and one of stabbing, and in the suicide group, 5 died of GSW. There was no significant difference between violent death (n = 9) and the rest (n = 15) of normal control subjects, and also no difference between GSW (n = 5) and the rest (n = 19) of suicide subjects relative to mRNA and protein expression of any of the CRF measures.

4. Discussion

In this study we found that the protein and mRNA expression levels of CRF were increased and CRF-R1 and CRF-BP were decreased in the PFC, but not in the HIPPO, of teenage suicide subjects compared with controls. The mRNA expression of CRF was increased in CeAMY and subiculum of teenage suicide subjects, CRF-R1 was decreased in the CeAMY and CRF-BP was decreased in the subiculum and CeAMY, and CRF-R2 was unchanged in subiculum, CeAMY or MeAMY of teenage suicide compared with normal control subjects. We also found that the protein but not mRNA expression of CRF-R2 was significantly decreased in the PFC of teenage suicide subjects compared with controls.

The reasons for the dissociation between protein and mRNA levels of CRF-R2 are not clear. There may be several reasons why changes in mRNA levels do not match changes in protein levels. For example, steady state mRNA levels are regulated by a balance between the rate of synthesis and the rate of degradation. The same is also true of protein levels. Changes in mRNA levels for a particular gene reflect changes in transcription of mRNA and its half-life. Additional processes, including RNA splicing, polyadenylation, length of polyA tail, miRNA expression and the expression of other noncoding RNAs, all influence the mRNA half-life. Some of the long non-coding RNAs being discovered can act as endogenous antisense transcripts. Collectively these factors impact RNA stability. Any changes in mRNA or protein levels reflect the balance of these factors acting to affect mRNA half-life.

Several teenage suicide subjects had the presence of antidepressants ($n = 4$) or ethanol ($n = 5$) at the time of death. Although we found that the exclusion of those subjects from the analysis had no significant effect on our findings, we cannot rule out the effect of antidepressants or ethanol on the protein or mRNA expression of CRF, CRF-BP or CRF receptors, because the number of such subjects in our teenage suicide group was small.

4.1. CRF and the effect of sex

In our studies we found an effect of sex on CRF expression levels in teenage suicide, such that CRF-R2 mRNA expression was decreased in female compared with male subjects. The effect of sex, especially age-related has been observed in both human and animal studies. Seeman et al. (2001) studied cortisol response to a cognitive challenge and found that cortisol response in young women (22–28 years) was less than in old women (63–88 years).

Kudielka et al. (1998) found sex-related changes in cortisol in response to a stress test. Weathington et al. (2014) have reported sex- and age-related changes in CRF receptors in rats. Sterrenburg et al. (2011) studies the effect of stress on CRF in male and female rats and found sex-related changes in CRF after stress. Our finding that mRNA expression of CRF-R2 in the PFC of female subjects is lower than in male subjects appears to be consistent with previous studies.

4.2. CRF in depression and suicide

CRF is a major hormone mediating the HPA axis system. Abnormalities of HPA axis in depression is one of the most consistent findings in biological psychiatry. CRF is also a mediator of certain stressful events and the stress causes a rapid expression of CRF gene and local CRF release (Beurel and Nemeroff, 2014; Holsboer, 2001). Both depression and suicidal behavior have been related to an abnormality of HPA axis system (Coryell and Schlesser, 2007; Holsboer, 2001). This is based on the observation that dexamethasone suppression test (DST) is abnormal in depressed and suicidal patients and it may even be a predictor of completed suicide (Coryell and Schlesser, 2007). A specific test of HPA axis system, i.e., the DEX-CRF test was also observed to be abnormal in depression (Holsboer, 2000).

CRF is an important member of the HPA axis which, as mentioned earlier, is released in response to stressors and acts on the pituitary to cause the release of ACTH into the circulation. Neurons in extra-hypothalamic regions, such as PFC, HIPPO and AMY also express CRF. CRF in these areas plays an important role in the autonomic, immune and behavioral effects of stress (Chen et al., 2004). Although CRF is the main activator of the HPA axis, there are two main sources of the CRF in the brain: i) within the HPA axis sites, and ii) in the non-HPA axis sites. The production of anxiety-like behaviors and autonomic effects after centrally administered CRF has been associated with non-HPA axis CRF (Dunn and File, 1987; Koob and Bloom, 1985). Also, extra-HPA axis abnormalities of CRF have been associated with neuropsychiatric disorders, such as anxiety disorders or depression (Reul and Holsboer, 2002).

4.3. Role of extra-hypothalamic CRF in HPA axis regulation

CRF in PFC, HIPPO and AMY is involved in psychiatric disorders. These areas are also involved in HPA axis activation, although they have little or no direct interactions with the PVN neurons. They thus require intermediate neurons to relay their influence on CRF release (Sawchenko et al., 2000; Ulrich-Lai and Herman, 2009). The role of PFC, HIPPO and AMY in activating HPA axis is primarily derived from stress paradigms and lesion studies.

4.3.1. Role of HIPPO in HPA axis regulation

The HIPPO inhibits the activity of HPA axis. This is based on the observation that hippocampal lesions promote basal hypo-secretion of glucocorticoids and enhanced basal CRF and AVP mRNA expression and elevate basal AVP, but not CRF in total blood. The HIPPO is also involved in terminating HPA axis response to stress. The inhibition of the HPA axis by HIPPO is mediated by a restricted set of neurons in the ventral subiculum. The work of Herman et al. (1998) shows that ventral subiculum is the principal source of HIPPO effects and is involved in the coordination of the HPA axis and behavioral responses to cognitively processed information (Herman et al., 1998; Herman and Mueller, 2006).

4.3.2. Role of Amygdala in HPA axis regulation

Several lines of evidence suggest that the AMY is involved in the activation of the HPA axis (Herman et al., 2003). Electrical stimulation of AMYa causes corticosterone biosynthesis (Saito et al., 1989) and secretion in rats, rabbits, and monkeys (Matheson et al., 1971) and elicits ACTH secretion in humans (Gallagher et al., 1987). Localized stimulation or lesion paradigms implicate CeAMY, MeAMY, and basolateral amygdaloid (BlAMY) nuclei in the control of ACTH release. The CeAMY is involved in the integration of behavioral responses to fear and/or anxiety provoking behaviors. It appears to regulate HPA activation in several paradigms. Selective damage of CeAMY decreases ACTH and corticosterone release following immobilization stress, photic or acoustic stimuli (Feldman et al., 1994) and fear conditioning (Van de Kar et al., 1991). The MeAMY also plays a role in HPA axis integration as selective stimulation of neurons in this nucleus increases corticosterone release in anesthetized rats (Feldman et al., 1994) and also increases adrenal sensitivity to ACTH (Saito et al., 1992). Lesions of medial amygdala cause a decrease in ACTH and corticosterone secretion and inhibit CRF depletion following photic or acoustic stimulation (Feldman et al., 1994). The BlAMY functions as an integrator of sensory input from the thalamus and cognitive information from the PFC and HIPPO (Van de Kar and Blair, 1999).

4.3.3. Role of PFC in HPA axis regulation

The PFC appears to have a prominent negative effect upon HPA activity (Diorio et al., 1993; Herman and Cullinan, 1997; Herman et al., 2003). Direct evidence for the involvement of PFC in HPA axis regulation is derived from lesion studies which suggest that the damage to PFC result in altered HPA axis responses to stress (Diorio et al., 1993). Bilateral lesions of the anterior cingulate, prelimbic components of the medial prefrontal cortex, enhanced ACTH and corticosterone responses and stimulated PVN CRF secretion following restraint stress but not ether inhalation (Diorio et al., 1993). The PFC is a target site for the negative effects of glucocorticoids. The PFC (especially the orbital and medial prefrontal cortex) is rich in both MR and GR (higher GR than MR levels). It has been shown by Feldman and Conforti (1985) that electrical stimulation of the frontal cortex altered plasma levels of corticosterone in the rat. Herman et al.(2003) have reported that cortical lesions result in increased CRF and AVP mRNA levels in the PVN. Implants of corticosterone into the PFC produced a significant decrease in plasma levels of both ACTH and corticosterone in rats with restraint stress (Diorio et al., 1993). These results also suggest that PFC glucocorticoid receptors regulate the HPA axis by inhibiting corticosterone levels. On the other hand, Feldman and Conforti (1985) have shown that electrical stimulation of the PFC causes significant increase in plasma corticosterone levels in rats.

These areas are also involved in the regulation of mood and behavior and their abnormalities have been reported and implicated in mood disorders and suicide (Drevets, 2000; Sheline et al., 2003). Also, PFC abnormalities have been implicated in mood disorders based on several studies. Abnormalities of HIPPO, such as volume reduction (Sheline et al., 2003) have also been reported. Hippocampus is the major site of stress and it is also involved in learning and memory. Amygdala is involved in stress response, in fear and anxiety [see review by (Berretta, 2005)]. These areas are also indirectly involved in the regulation of HPA axis (Herman et al., 2003). CRF in these areas may represent both HPA axis and non-HPA axis CRF.

4.4. Rationale for CRF studies of teenage suicide

Neurobiological studies of teenage suicide, specifically of HPA axis studies are important as differences in neuroendocrine profile have been reported between teenage and adult depression. Also, adolescence is a period of substantial neuroplasticity in stress-regulatory neurocircuits (Wulsin et al., 2016). Chronic stress exposure during this period leads to long-lasting changes in neuroendocrine function and emotional behaviors, suggesting that adolescence may be a crucial period for the development of stress vulnerability and neuroendocrine alterations. Depression and stress-related psychopathology generally occur during late adolescence (Kessler et al., 2002). Because of these reasons it was important to study the CRF system in the postmortem brain of teenage suicide.

Although there are no studies of CRF in teenage suicide, CRF system has been studied in adult suicide and depression in both CSF and the postmortem brain. Nemeroff et al. (1984) found elevated CSF CRF levels in depressed patients, most of them were DST non-suppressors (Nemeroff et al., 1984). They also found an increase in the levels of CSF CRF concentrations in depressed patients many of whom were melancholic or psychotic (Banki et al., 1987).

CRF levels have also been studied in the postmortem brain of depressed/suicide subjects. Nemeroff et al. (1988) found decreased number of CRF binding sites in the frontal cortex of suicide subjects compared with control subjects (Nemeroff et al., 1988). Raadsheer et al. (1994) found that the total number of CRF expressing neurons in depressed subjects was higher than in the control group. Wang et al (2008) found significantly increased levels of CRF mRNA in the PVN of depressed subjects compared with normal control subjects. Merali et al. (2004) found that CRF levels were elevated in fronto-polar and dorsomedial PFC of suicide subjects compared with normal controls. Bissette et al. (2003) found increased concentrations of CRF-like immunoreactivity in the micropunches of postmortem locus coeruleus from subjects with major depressive disorder (MDD) compared to non-depressed subjects.

Thus, several studies suggest increased CRF levels in different brain areas, but primarily in the PVN and the pituitary of suicide subjects compared with normal control subjects. In our studies we determined the CRF protein and mRNA levels in the PFC, HIPPO and AMY of teenage suicide subjects and our observation of increased CRF in PFC and AMY appears to be similar to those reported in the PFC and PVN of adult suicide subjects, although we did not find any changes in the CRF in the HIPPO of teenage suicide subjects.

4.5. CRF receptors

The effects of CRF and its signaling are mediated through specific receptors, known as CRF receptors, and two different subtypes have been identified, known as CRF-R1 and CRF-R2 (Chalmers et al., 1995; Reul and de Kloet, 1985). Both receptor subtypes couple to the same G protein and signal through the same second messenger system. Both CRF-R1 and CRF-R2 subtypes are coupled to adenylyl cyclase (AC) and phosphoinositide (PI) signaling pathways (Hauger et al., 2006).

The two types of CRF receptors, CRF-R1 and CRF-R2, are present in many brain areas of the rat and non-human primates (Hauger et al., 2003). The highest density of CRF-R1 mRNA has been observed in anterior pituitary, cerebral cortex, cerebellum, AMY, HIPPO, olfactory bulb and LC (Chalmers et al., 1995; Sanchez et al., 1999).

Both CRF and URC activate CRF receptors. However, CRF has a greater affinity for CRF-R1 than for the CRF-R2 (Hauger et al., 2009). Thus, low concentrations of CRF activate CRF-R1, but not CRF-R2, in brain regions. High concentrations of CRF can activate both CRF-R1 and CRF-R2 receptors. CRF-R1 has been proposed to mediate the effects of CRF on HPA system function and anxiety-related behaviors, whereas CRF-R2 may be predominantly involved in feeding behavior (Keck, 2006) as well as cardiovascular functions and recovery of HPA responses. CRF-R2 may also mediate anxiety-related behaviors (Coste et al., 2000).

Some investigators have determined the levels of CRF-R1 and CRF-R2 in the postmortem brain of depressed and suicide subjects. Although there are some studies of CRF levels in the postmortem brain of suicide subjects, the studies of CRF receptors in the postmortem brain are very sparse. Merali et al. (2004), determined the levels of CRF-R1 and CRF-R2 in the postmortem brain of suicide subjects. They observed that the mRNA levels of CRF-R1 in fronto-polar cortex were decreased compared with normal control subjects. They found a modest reduction of CRF-R1 mRNA expression in the suicide brain, but this difference was just shy of statistical significance. The expression of CRF-R2 or CRF-BP did not differ between suicide subjects and normal control subjects. There was no difference in the levels of CRF receptors mRNA in the ventro-lateral PFC between suicide subjects and normal control subjects. Our results are generally similar to those of Merali et al. (2004). Similar to Merali's results, we also found increased protein and mRNA CRF and decreased CRF-R1, but not CRF-R2, in the PFC area. However, we also found a decrease in CRF-BP protein and mRNA expression in the PFC as opposed to Merali et al. (2004), who did not find any difference in CRF-BP between suicide and control subjects. This difference may be due to specific PFC area (dorsolateral PFC used by them, and BA9 used by us). In our study of teenage suicide subjects, we found a significant decrease in the expression of CRF-R1 receptors in the PFC and AMY, but not in the HIPPO of suicide subjects compared with normal control subjects.

4.6. CRF binding protein

A recently discovered protein known as CRF-BP is another major regulator of the levels of CRF in the brain. CRF-BP binds to CRF with high affinity almost equal to that of CRF receptors (Lombardo et al., 2001). CRF-BP is expressed in many regions of the brain, including the cortex, HIPPO, and AMY (Potter et al., 1992).

CRF-BP reduces bioavailability of CRF-like peptide by binding CRF and dimerizing and thus preventing CRF from binding to its receptors (Behan et al., 1995a). More broadly, the potential role for CRF-BP is for buffering and inhibiting or enhancing the effects of CRF family of peptides binding to CRF-R1 or CRF-R2 receptors. Neurons and glia of all CRF-related pathways in the brain express membranal CRF-BP, sometimes co-localized with CRF or CRF receptors (Behan et al., 1995b).

Multiple roles for CRF-BP have been suggested. CRF-BP may bind CRF with high affinity and sequester ligand away from the receptors thereby decreasing the effect of CRF. It may also act to stimulate the activity of CRF and may increase the half-life of CRF by protecting it from degradation and delivering the ligand to receptors in the target tissue (Seasholtz et al., 2002).

Although there are some studies of CRF and its receptors in the postmortem brain, the studies of human CRF-BP are limited. Claes et al.

(2003) studied MDD population from Northern Sweden and found that the CRF-BP gene is significantly associated with MDD. Herringa et al. (2006) examined AMY CRF-BP gene expression in postmortem brain from subjects with major depression, bipolar disorder, schizophrenia and controls. They found a significant decrease in CRF-BP mRNA levels in the basolateral AMY of male bipolar and male schizophrenic subjects and the lateral AMY of bipolar subjects.

In our studies, we find a consistent and strong decrease in CRF-BP mRNA expression in the PFC and CeAMY of teenage suicide subjects, suggesting an important role for this protein in the pathophysiology of suicide. Decreased CRF-BP levels in the PFC and CeAMY together with increased CRF levels in these brain regions may further enhance the effect of CRF.

4.7. Stress-related CRF changes: possible risk factors for suicide

Several animal studies show that acute and chronic stress causes changes in CRF. Imaki et al. (1991) found that acute and chronic stress (electrical foot shock) causes changes in CRF. Chronic foot shock causes 84% increase in CRF mRNA in the hypothalamus. A single exposure to foot shock did not cause any changes in CRF. Becker et al. (2007) studied the effect of neonatal separation stress on CRF and found that higher numbers of CRF immunoreactivity neurons in basolateral amygdaloid complex of stressed compared with non-stressed rats, while no differences were observed in other regions, such as HIPPO and central amygdala. There are some studies which show that CRF-BP is involved in stress response. After acute stress, the expression of CRF-BP gene in rats is increased in the pituitary and AMY (Herringa et al., 2004). Hsu et al. (1998) found that acute restraint stress significantly increased CRF mRNA levels in the PVN and in rostral CeAMY region, providing evidence that CRF neurons in the CeAMY and PVN exhibit rapid increases in CRF mRNA.

A variety of studies have shown that CRF induces depressive-like behavior in animals, which include loss of excitement (anhedonia), loss of motivation, sleep disturbances, deficient sociability skills, anxiety, change in appetitive behavior, cognitive deficits, which have all been associated with chronic stress exposure. These behaviors in animals are induced by a variety of stressors, such as chronic restraint stress. One of the biological consequences of chronic stress is increased CRF or decreased CRF-R1. This suggests that stress-induced CRF may lead to depression.

Based on human and animal studies, CRF is thought to mediate acute and chronic responses to stress. In rodents, many symptoms in response to stress resemble symptoms exhibited by patient with PTSD and depression (Beurel and Nemeroff, 2014). Recent studies also show that chronic stress is one of the causal factors for the development of depression [see review by Waters et al. (2015)]. Stress and depression are major risk factors for suicidal behavior and completed suicide. Thus, increased CRF and/or decreased CRF-BP or CRF-R1 may be associated and/or are risk factors for suicide.

Our findings of increased CRF and decreased CRF-R1 and CRF-BP may suggest that teenage suicide subjects may be suffering with acute or chronic stress causing changes in the CRF parameters, thus suggesting CRF as a potential risk factor for suicide.

5. Conclusion

Increased CRF in depression and suicidal behavior is primarily derived from studies of DEX-CRF test in these patients. GR and MR receptors, CRF, CRF-BP and CRF receptors may be altered in these patients. This is also based on some studies of CRF in the postmortem brain of suicide subjects. However, there are no such studies in the postmortem brain of teenage suicides. In this study we determined the CRF, CRF receptors and CRF-BP in three different brain areas of teenage suicide and found that the mRNA and protein expression levels of CRF are increased in the PFC of suicide subjects. The mRNA expression of CRF is increased in the PFC, subiculum and CeAMY of teenage suicide subjects. However, no changes in CRF protein or mRNA expression were observed in the HIPPO. We also observed generally a decrease in CRF-R1 receptor in the PFC and AMY of teenage suicide victims. Whereas the CRF system has been studied to some extent in the hypothalamus, the contribution of extra-hypothalamic areas, such as PFC, HIPPO and AMY is not known, and also these areas are involved both in the pathophysiology of depression and suicide and in the regulation of HPA axis. CRF is the major mediator of stress and our results show that CRF gene and protein expression is not only increased but it also causes the down-regulation of CRF receptors, and most likely a decrease in CRF-BP binding. These studies thus indicate that increased secretion of CRF and a decrease in CRF-BP binding may be involved in the pathophysiology of teenage suicide. Also, the hyperactive CRF may be causing a decrease in the CRF-R1 receptor, which is involved in anxiety-related behaviors.

Our findings of increased CRF and decreased CRF-R1 and CRF-BP in the PFC, subiculum and CeAMY may have significant clinical relevance and implications. Hyperactive HPA axis in depression or suicide is primarily based on DEX-CRF test, suggesting increased CRF release in the PVN and probably input from extra-hypothalamic areas. But it is not clearly demonstrated by the postmortem brain studies if the abnormalities of CRF exist in the relevant areas implicated in the pathophysiology of depression and suicide. Our studies indicate increased CRF and decreased CRF-R1 and CRF-BP in areas known to be involved in the pathophysiology of depression and suicide and their indirect role in HPA axis activity. This finding may thus suggest a role of extra-hypothalamic CRF function in depression and suicide and its role in the pathophysiology of depression and suicide.

The observation of decreased CRF-R1 and possibly CRF-R2 in teenage suicide brain may suggest an opportunity to develop newer therapies for suicidal behavior. CRF-R1 antagonists have been tried in the treatment of depression but not of suicidal behavior. Our studies may suggest that a clinical trial of a CRF-R1 antagonist in the treatment of suicidal behavior should be considered. In addition, CRF-R2 may be another potential target for treatment of suicidal behavior.

6. Study limitations

There are some limitations of the study. Although the total number of subjects for this study is reasonable, the suicide cohort consists of subjects with different diagnoses. When broken by different diagnoses, the number in each diagnostic group is small. So it is hard to evaluate the effect of diagnosis on the CRF measures. Also, some of the subjects were on treatment with psychoactive drugs.

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Statement of interest

All authors declare that they have no financial interests or potential conflicts of interest related directly or indirectly to this work.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2019.04.015.

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