Psychoneuroendocrinology (2015) 53, 16—28

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/psyneuen

Cell type-specific modifications of corticotropin-releasing factor (CRF) and its type 1 receptor (CRF_1) on startle behavior and sensorimotor gating

Elizabeth Flandreau[a,1], Victoria Risbrough[b,*,1], Ailing Lu[c], Martin Ableitner[d], Mark A. Geyer[a], Florian Holsboer[d], Jan M. Deussing[e]

[a] *Department of Psychiatry, University of California San Diego, 9500 Gilman Drive MC 0804, La Jolla, CA 92093-0804, USA*
[b] *Veterans Administration Center of Excellence for Stress and Mental Health, 3350 La Jolla Village Drive San Diego, CA 92161, USA*
[c] *Unit of Innate Immunity, Key Laboratory of Molecular Virology and Immunology, Institut Pasteur of Shanghai, Chinese Academy of Sciences, 320 Yue Yang Road, Shanghai, 200031, China*
[d] *Max Planck Institute of Psychiatry, Kraepelinstrasse 2-10, D-80804, Munich, Germany*
[e] *Department Stress Neurobiology and Neurogenetics, Max Planck Institute of Psychiatry, Kraepelinstrasse 2-10, D-80804, Munich, Germany*

Received 16 September 2014; received in revised form 25 November 2014; accepted 3 December 2014

KEYWORDS
CRF;
CRH;
Acoustic startle;
Prepulse inhibition;
PTSD

Abstract The corticotropin-releasing factor (CRF) family of peptides and receptors coordinates the mammalian endocrine, autonomic, and behavioral responses to stress. Excessive CRF production has been implicated in the etiology of stress-sensitive psychiatric disorders such as posttraumatic stress disorder (PTSD), which is associated with alterations in startle plasticity. The CRF family of peptides and receptors mediate acute startle response changes during stress, and chronic CRF activation can induce startle abnormalities. To determine what neural circuits modulate startle in response to chronic CRF activation, transgenic mice overexpressing CRF throughout the central nervous system (CNS; CRF-COE^{CNS}) or restricted to inhibitory GABAergic neurons (CRF-COE^{GABA}) were compared across multiple domains of startle plasticity. CRF overexpression throughout the CNS increased startle magnitude and reduced ability to inhibit startle (decreased habituation and decreased prepulse inhibition (PPI)),

* Corresponding author. Tel.: +1 619 543 3582; fax: +1 619 543 2493.
E-mail address: vrisbrough@ucsd.edu (V. Risbrough).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.psyneuen.2014.12.005
0306-4530/Published by Elsevier Ltd.

similar to previous reports of exogenous effects of CRF. Conversely, CRF overexpression confined to inhibitory neurons decreased startle magnitude but had no effect on inhibitory measures. Acute CRF receptor 1 (CRF_1) antagonist treatment attenuated only the effects on startle induced by CNS-specific CRF overexpression. Specific deletion of CRF_1 receptors from forebrain principal neurons failed to alter the effects of exogenous CRF or stress on startle, suggesting that these CRF_1 expressing neurons are not required for CRF-induced changes in startle behaviors. These data indicate that the effects of CRF activation on startle behavior utilize an extensive neural circuit that includes both forebrain and non-forebrain regions. Furthermore, these findings suggest that the neural source of increased CRF release determines the startle phenotype elicited. It is conceivable that this may explain why disorders characterized by increased CRF in cerebrospinal fluid (e.g. PTSD and major depressive disorder) have distinct symptom profiles in terms of startle reactivity.
Published by Elsevier Ltd.

1. Introduction

CRF is a 41 amino acid peptide, discovered for its role in activating the hypothalamic—pituitary—adrenal (HPA) axis, a primary endocrine response to disruption of body homeostasis and perceived threat (Vale et al., 1981). CRF acts centrally to coordinate autonomic and behavioral reactions to stress via extrahypothalamic actions in the brainstem and limbic system, respectively (e.g. Hauger et al., 2009; Nemeroff and Vale, 2005). While critical in the face of real threat, overactive or inappropriate activation of CRF can have severe consequences for mental and physical health (Mitchell, 1998; Risbrough and Stein, 2006; Tache and Brunnhuber, 2008).

Increased release of CRF, as measured by elevated CRF concentration in cerebrospinal fluid (CSF), is observed in some patients with mood and anxiety disorders, most notably major-depressive disorder (MDD) and post-traumatic stress disorder (PTSD) (Kasckow et al., 2001). However the source of increased CRF is not clear, nor is it clear if different sources of CRF might be linked to specific symptom domains. Understanding the effects and mechanisms of CRF over-activation in the brain across differential neural circuits may provide a better understanding of its potential role in these disorders and their symptoms. A primary symptom of PTSD that is not common in depression is hyperarousal, which can manifest as increased acoustic startle reactivity at baseline (e.g. Butler et al., 1990) and greater startle responses in aversive contexts, (reviewed in Grillon and Baas, 2003; Risbrough, 2010). PTSD may also be associated with decreased sensorimotor gating as measured by reduced habituation to repeated stimuli and reduced inhibition of startle as measured by prepulse inhibition (PPI) (reviewed in Clark et al., 2009).

The acoustic startle reflex (ASR) consists of a series of involuntary reflexes elicited by a sudden, intense auditory stimulus and the pathways mediating this reflex are analogous in rodent models and humans (Graham, 1975; Yeomans et al., 2002). The simple startle circuit begins in the auditory nerve and cochlear nuclei, continues through the caudal pontine reticular formation and on to motor neurons that elicit the physical startle response. Startle reactivity is modulated by forebrain limbic regions such as the hippocampus, amygdala, and bed nucleus of the stria terminalis (BNST), and by brainstem autonomic centers such as the locus coeruleus (LC; reviewed in Koch, 1999; Swerdlow et al., 2001).

In rodent models, stress or exogenous administration of CRF increases startle magnitude; this effect is mediated by activation of CRF_1 and CRF_2 receptors and can be attenuated by anxiolytics (Risbrough et al., 2003, 2004, 2009; Swerdlow et al., 1986). The CRF_1 and CRF_2 receptors are located throughout the neocortex, extended amygdala, and brainstem (Perrin and Vale, 2002; Risbrough and Stein, 2006). CRF peptide is produced in a variety of cell types including GABAergic interneurons and glutamatergic projection neurons, and is colocalized with other neurotransmitters and neuropeptides (Chen et al., 2004; Gallopin et al., 2006; Kubota et al., 2011; Sawchenko and Swanson, 1985). CRFergic circuits overlap with startle modulatory circuits at several points including the BNST, central nucleus of the amygdala (CeA), dorsal raphe, and LC (reviewed in Koch, 1999; Swerdlow et al., 2001).

The goal of the present experiment was to determine what neural circuits and cell types are sensitive to excessive CRF signaling effects on startle reactivity using selective genetic tools. We examined transgenic mice with differential CRF overexpression, i.e. within the entire central nervous system (CNS; CRF-COE^{CNS}) or confined to forebrain GABAergic neurons (CRF-COE^{GABA}). To determine the selective influence of forebrain CRF_1 receptors in acoustic startle we also used a mouse in which this receptor is deleted from CamKIIα-producing neurons in the forebrain (forebrain CRF_1-KO). This strategy avoids the glucocorticoid confound of the traditional CRF_1-KO in that the receptor is still present in hypothalamus and pituitary. Loss of CRF_1 receptor expression is restricted to principal neurons of the anterior forebrain (Minichiello et al., 1999).

In the rodent brain, CRF is endogenously expressed in the paraventricular nucleus of the hypothalamus (PVN), CeA, BNST, olfactory bulb, cortex and brain stem nuclei. CRF-COE^{CNS} mice exhibit substantial elevations of CRF expression in neurons and glia throughout the CNS, in particular in the cortex, and hippocampus (Lu et al., 2008). The increased expression is gene-dose dependent; mice heterozygous for the conditional CRF expression unit at the ROSA26 (R26) locus show half expression compared to mice homozygous for the modified R26 allele following Cre