Psychoneuroendocrinology (2013) 38, 1349—1361

Available online at www.sciencedirect.com

SciVerse ScienceDirect

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

Escitalopram alters gene expression and HPA axis reactivity in rats following chronic overexpression of corticotropin-releasing factor from the central amygdala

Elizabeth I. Flandreau [a,*], Chase H. Bourke [b], Kerry J. Ressler [c,1], Wylie W. Vale [d], Charles B. Nemeroff [e,2], Michael J. Owens [b]

[a] *Psychiatry Department, University of California San Diego, 9500 Gilman Drive MC 0804, La Jolla, CA 92093-0804, United States*
[b] *Laboratory of Neuropsychopharmacology, Department of Psychiatry and Behavioral Sciences, Emory University, Woodruff Memorial Research Building, Suite 4000, 101 Woodruff Circle, Atlanta, GA 30322, United States*
[c] *Department of Psychiatry and Behavioral Sciences, Yerkes Research Center, Emory University, 954 Gatewood Dr, Atlanta, GA 30329, United States*
[d] *Peptide Biology Laboratory, Salk Institute for Biological Studies, 10010 North Torrey Pines Rd., La Jolla, CA 92037, United States*
[e] *Department of Psychiatry & Behavioral Sciences, University of Miami Miller School of Medicine, Clinical Research Building, 1120 NW 14th Street, Room 1455 (D-21), Miami, FL 33136, United States*

Received 10 July 2012; received in revised form 29 November 2012; accepted 30 November 2012

KEYWORDS
Corticotropin-releasing factor;
Amygdala;
Escitalopram;
Anxiety;
Hypothalamic-pituitary-adrenal (HPA) axis

Summary We have previously demonstrated that viral-mediated overexpression of corticotropin-releasing factor (CRF) within the central nucleus of the amygdala (CeA) reproduces many of the behavioral and endocrine consequences of chronic stress. The present experiment sought to determine whether administration of the selective serotonin reuptake inhibitor (SSRI) escitalopram reverses the adverse effects of CeA CRF overexpression. In a 2 × 2 design, adult male rats received bilateral infusions of a control lentivirus or a lentivirus in which a portion of the CRF promoter is used to drive increased expression of CRF peptide. Four weeks later, rats were then implanted with an Alzet minipump to deliver vehicle or 10 mg/kg/day escitalopram for a 4-week period of time. The defensive withdrawal (DW) test of anxiety and the sucrose-preference test (SPT) of anhedonia were performed both before and after pump implantation. Additional post-implant behavioral tests included the elevated plus maze (EPM) and social interaction (SI) test. Following completion of behavioral testing, the dexamethasone/CRF test was performed to assess HPA axis reactivity. Brains were collected and expression of HPA axis-relevant transcripts

* Corresponding author. Tel.: +1 404 3949147; fax: +1 619 543 2493.
E-mail addresses: eimartin@ucsd.edu (E.I. Flandreau), cbourke@emory.edu (C.H. Bourke), kressle@emory.edu (K.J. Ressler), cnemeroff@med.miami.edu (C.B. Nemeroff), mowens@emory.edu (M.J. Owens).
[1] Tel.: +1 404 727 7739; fax: +1 404 727 8070.
[2] Tel.: +1 305 243 3740; fax: +1 305 243 1619.

0306-4530/$ — see front matter
http://dx.doi.org/10.1016/j.psyneuen.2012.11.020

were measured using *in situ* hybridization. Amygdalar CRF overexpression increased anxiety-like behavior in the DW test at week eight, which was only partially prevented by escitalopram. In both CRF-overexpressing and control groups, escitalopram decreased hippocampal CRF expression while increasing hypothalamic and hippocampal expression of the glucocorticoid receptor (GR). These gene expression changes were associated with a significant decrease in HPA axis reactivity in rats treated with escitalopram. Interestingly, escitalopram increased the rate of weight gain only in rats overexpressing CRF. Overall these data support our hypothesis that amygdalar CRF is critical in anxiety-like behavior; because the antidepressant was unable to reverse behavioral manifestations of CeA CRF-OE. This may be a potential animal model to study treatment-resistant psychopathologies.

1. Introduction

Mood and anxiety disorders are a leading cause of disability and increasing burden on society in terms of direct medical costs and lost productivity (Alonso et al., 2011; Birnbaum et al., 2010; Levinson et al., 2010). In the United States, the lifetime prevalence rate is 28.8% for anxiety disorders and 20.8% for mood disorders (Kessler et al., 2005a). The 12-month prevalence for anxiety and mood disorders is 18.1% and 9.5%, respectively, with comorbid cases having the greatest severity (Kessler et al., 2005b).

Symptoms of depression and anxiety are most commonly treated with selective serotonin reuptake inhibitors (SSRIs) and SSRI use is increasing worldwide (Lockhart and Guthrie, 2011). Although the precise mechanisms of action of the many therapeutic effects of SSRIs are unclear, one postulated pathway to symptom improvement in a significant subgroup of individuals is *via* normalization of dysregulated central corticotropin-releasing factor (CRF) circuits.

CRF is a 41 amino acid peptide discovered for its role in regulating hypothalamic-pituitary-adrenal (HPA) axis activity (Vale et al., 1981) and it has since been identified as a key mediator of the endocrine, autonomic, and behavioral response to stress. Dysregulation of CRF circuits and the stress response systems they coordinate have been implicated in the pathophysiology of mood and anxiety disorders, most notably major depressive disorder (MDD) and post-traumatic stress disorder (PTSD). For example, many MDD and PTSD patients exhibit state-dependent disruptions in HPA axis reactivity, perhaps in part from altered expression of CRF within the paraventricular nucleus of the hypothalamus (PVN), and exhibit elevated CRF concentrations within cerebrospinal fluid (CSF), attributable to overexpression of CRF from extrahypothalamic sources such as the central amygdala (CeA). The role of CRF in the pathogenesis of mood and anxiety disorders has been extensively reviewed (Claes, 2004; Gold et al., 1995; Holsboer and Ising, 2008; Kasckow et al., 2001; Lloyd and Nemeroff, 2011; Owens and Nemeroff, 1991; Reul and Holsboer, 2002; Risbrough and Stein, 2006; von Bardeleben and Holsboer, 1988).

SSRIs have previously been shown to normalize HPA axis reactivity in patients with depressive and anxiety disorders (Lenze et al., 2011; Manthey et al., 2011; Nikisch et al., 2005b); this normalization is associated with improved clinical outcome (Hinkelmann et al., 2012; Paslakis et al., 2010). Similarly, in depressed patients, normalization of CSF CRF concentration is associated with improvements in the Hamilton Depression Rating Scale (HAM-D) following treatment with the SSRI escitalopram (Nikisch et al., 2005a) or fluoxetine (De Bellis et al., 1993). These changes also roughly follow the time course of symptom resolution, supporting the hypothesis that normalization of CRF neurotransmission plays a causal role in the mechanism of action of antidepressant drugs (Brothers et al., 2012). In animal models, antidepressants, anxiolytics, and mood stabilizers have been shown to reduce the overall responsiveness of the HPA axis, the activity of hypothalamic and extrahypothalamic CRF neurons, and to alter CRF mRNA expression and CRF concentrations as well as type-1 CRF receptor (CRF_1) mRNA expression and binding (Gilmor et al., 2003; Grigoriadis et al., 1989; Skelton et al., 2000; Stout et al., 2001; Valentino and Curtis, 1991).

We have previously shown that lentiviral vector-mediated chronic overexpression of CRF from the CeA (CeA CRF-OE) increases anxiety-like behavior and HPA axis reactivity in rats (Flandreau et al., 2012). The following experiment was designed to assess whether chronic administration of the SSRI escitalopram can reverse the behavioral effects of CeA CRF-OE and prevent CeA CRF-OE-induced HPA axis hyperactivity. To better understand the mechanism of any escitalopram and CRF-OE interactions, we also examined central expression patterns of HPA axis-relevant transcripts. Given that amygdalar CRF is more closely associated with the behavioral stress response and hypothalamic CRF more closely associated with the endocrine stress response, we hypothesized that escitalopram would reverse CeA CRF-OE-induced HPA axis hyperreactivity but may have limited efficacy on behavioral measures because it is unable to decrease virally mediated overexpression of CeA CRF. Alternatively, escitalopram attenuation of CeA CRF-OE behavioral effects may occur if SSRI effects are downstream of or at the same level as postsynaptic CRF receptors.

2. Materials and methods

2.1. Animals

Animal protocols were approved by the Emory University Institutional Animal Care and Use Committee (IACUC) and carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Resources, 1996). All efforts were made to minimize animal suffering and to reduce the number of animals used. Adult male Wistar rats (approximately PND70) were purchased from Charles River Laboratories (Burlington, MA) and pair housed. In compliance with Emory University biosafety requirements for lentiviral