Neuroscience 250 (2013) 300–308

CRH RECEPTOR ANTAGONISM REVERSES THE EFFECT OF SOCIAL SUBORDINATION UPON CENTRAL $GABA_A$ RECEPTOR BINDING IN ESTRADIOL-TREATED OVARIECTOMIZED FEMALE RHESUS MONKEYS

V. MICHOPOULOS, [a,b*] **M. EMBREE,** [b] **K. REDING,** [b] **M. M. SANCHEZ,** [a,b] **D. TOUFEXIS,** [b,c] **J. R. VOTAW,** [d,e] **R. J. VOLL,** [d,e] **M. M. GOODMAN,** [b,d,e] **J. RIVIER,** [f] **M. E. WILSON** [b] **AND S. L. BERGA** [g]

[a] *Department of Psychiatry & Behavioral Sciences, School of Medicine, Emory University, Atlanta, GA, United States*

[b] *Division of Developmental & Cognitive Neuroscience, Yerkes National Primate Research Center, Emory University, Atlanta, GA, USA*

[c] *Department of Psychology, University of Vermont, Burlington, VT, USA*

[d] *Department of Radiology and Imaging Sciences, School of Medicine, Emory University, Atlanta, GA, USA*

[e] *Imaging Core, Yerkes National Primate Research Center, Emory University, Atlanta, GA, USA*

[f] *The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA, USA*

[g] *Department of Obstetrics & Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, USA*

Abstract—Persistent exposure to environmental stressors causes dysregulation of the limbic–hypothalamic–pituitary–adrenal (LHPA) axis and alters $GABA_A$ receptor ($GABA_AR$) levels throughout the brain. Social subordination in socially housed female rhesus results in distinctive stress-related physiological and behavioral phenotypes that are dependent on the ovarian hormone estradiol (E2). In the present study, we utilized ovariectomized adult female rhesus monkeys undergoing hormone replacement with E2 to test the hypothesis that the chronic psychosocial stress of subordination alters $GABA_AR$ binding potential ($GABA_AR$ BP_{ND}) in limbic regions implicated in emotional processing including the prefrontal cortex, temporal lobe (amygdala and hippocampus), and hypothalamus. Furthermore, we tested the hypothesis that peripheral administration of a corticotropin-releasing hormone (CRH) receptor antagonist (astressin B) would reverse the alterations in $GABA_AR$ binding within these regions in subordinate females. After subjects received astressin B or saline for three consecutive days, $GABA_AR$ BP_{ND} was determined by positron emission tomography (PET) using ^{18}F-flumazenil as a radioligand. T1-weighted structural magnetic resonance imaging scans were also acquired for PET scan co-registration, in order to perform a region of interest analysis using the pons as a reference region. Compared to socially dominant females, subordinate females exhibited increased $GABA_AR$ BP_{ND} in the prefrontal cortex but not in the temporal lobe or the hypothalamus. Administration of astressin B eliminated the status difference in $GABA_AR$ BP_{ND} in the prefrontal cortex, suggesting that the chronic stressor of social subordination modulates GABAergic tone via effects on CRH and the LHPA axis, at least in prefrontal regions.

Key words: social subordination, stress, flumazenil, astressin B, GABAA receptor, monkeys.

INTRODUCTION

Exposure to psychosocial stressors is implicated in the etiology of psychopathologies in humans. These illnesses, including depression and anxiety, are often associated with alterations in the regulation and function of the limbic–hypothalamic–pituitary–adrenal (LHPA) axis (Juster et al., 2010). Furthermore, stress-induced psychopathologies occur in women twice as often as they do in men (Weissman and Olfson, 1995), implicating a role for gonadal steroid hormones in vulnerability to stress-induced adverse health outcomes. Indeed, the major ovarian hormone, estradiol (E2), plays a key role not only in the control of reproductive function in females, but also in emotional reactivity and the expression of prosocial behavior (Pfaff et al., 2000). E2 modulates both cognitive and affective behavior (McEwen et al., 1997; Bodo et al., 2006) and influences the activity of the LHPA axis under both basal and stress-induced conditions throughout the course of the menstrual cycle across species (Roy et al., 1999; Giussani et al., 2000; Altemus et al., 2001; Wilson et al., 2005). E2 also increases the expression of corticotropin-releasing hormone (CRH) in the hypothalamus in female rhesus monkeys (Roy et al., 1999). Importantly, exposure to stressors in female rodents and monkeys alters both behavioral and physiological sensitivity to E2 (White and Uphouse, 2004; Uphouse et al., 2005; Michopoulos et al., 2009) but the mechanism responsible for this stress-induced change in sensitivity is poorly understood.

*Correspondence to: V. Michopoulos, Yerkes National Primate Research Center, Emory University, 954 Gatewood Road, Atlanta, GA 30329, USA. Tel: +1-404-727-9058; fax: +1-404-727-8088.
E-mail address: vmichop@emory.edu (V. Michopoulos).

Abbreviations: BNST, bed nucleus of the stria terminalis; BP_{ND}, binding potential; CRH, corticotropin-releasing hormone; CRHR1/2, CRH receptor type 1 and type 2; E2, estradiol; FOV, field of view; $GABA_AR$, $GABA_A$ receptor; GAD, glutamic acid decarboxylase; GR, glucocorticoid; LH, luteinizing hormone; LHPA, limbic–hypothalamic–pituitary–adrenal; MR, mineralocorticoid; MRI, magnetic resonance imaging; PET, positron emission tomography; PVN, paraventricular nucleus; ROIs, regions of interest; YNPRC, Yerkes National Primate Research Center.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.07.002

The GABA neurotransmitter system has widespread regulatory function on systems that regulate physiology and behavior, and is significantly modulated by E2. For example, it has been shown that E2 increases the expression of GABA and the GABA synthesizing enzyme, glutamic acid decarboxylase (GAD), in the cortex and hippocampus (Tan et al., 2012). Additionally, E2 treatment in rodents increases $GABA_A$ receptor levels ($GABA_AR$) in the olfactory bulb (Guerra-Araiza et al., 2008) as well as alters subunit organization of $GABA_ARs$ in the hypothalamus and bed nucleus of the stria terminalis (BNST) (Herbison and Fenelon, 1995). The GABAergic system is also regulated by the activity of the LHPA axis (Bowers et al., 1998; Cullinan et al., 2008) and $GABA_AR$ levels throughout the brain are altered following stress exposure (Skerritt et al., 1981; Serra et al., 2000; Skilbeck et al., 2010). Recent studies in humans have shown $GABA_AR$ binding is decreased in brain regions involved in emotional regulation and the control of the LHPA axis in individuals with posttraumatic stress disorder and depression (Cameron et al., 2007; Geuze et al., 2008; Klumpers et al., 2010).

Social subordination in subordinate female rhesus monkeys results in altered physiological responses to E2, including enhanced E2 negative feedback inhibition of luteinizing hormone (LH) (Michopoulos et al., 2009) and an attenuated ability of E2 to decrease body weight (Michopoulos and Wilson, 2011). Furthermore, social subordination also impairs the ability of E2 to produce socio-sexual (Reding et al., 2012) and anxiolytic behavior (Michopoulos et al., 2011b), changes E2-regulated modulation of the central serotonergic system (Asher et al., 2012), and alters E2-induced activation in the prefrontal cortex (unpublished observation) in adult female rhesus monkeys. Thus, the goal of the current study was to assess whether the psychosocial stressor of social subordination in ovariectomized adult female rhesus monkeys alters E2's ability to modify $GABA_AR$ levels in the medial and dorsolateral prefrontal cortex, the anterior cingulate cortex, the orbitofrontal cortex, the amygdala and the hippocampus, and the hypothalamus, all of which are brain regions that have been implicated in the regulation of emotional and stress-related behavior and the LHPA axis and express $GABA_AR$ (Skerritt et al., 1981; Serra et al., 2000; Herman et al., 2004; Skilbeck et al., 2010; Mody and Maguire, 2011; Sarkar et al., 2011).

In addition, because CRH release from the paraventricular nucleus (PVN) of the hypothalamus is modulated, in part, by projections for the prefrontal cortex (Sullivan and Gratton, 2002), and because it has been shown that E2 can increase CRH release in brain regions that mediate emotional behavior (Lunga and Herbert, 2004; Jasnow et al., 2006), we assessed whether acute treatment with astressin B, a mixed CRH receptor type 1 and type 2 (CRHR1/2) antagonist (Broadbear et al., 2004), would eliminate any status differences in E2's ability to modulate $GABA_AR$ binding within these brain regions in subordinate females. Positron emission tomography (PET) using a ^{18}F-flumazenil (benzodiazepine antagonist) (Geuze et al., 2008) was undertaken to test the hypothesis that subordinate female monkeys would have decreased $GABA_AR$ binding compared to dominant females in the prefrontal cortex, temporal lobe and hypothalamus in response to E2 administration, and that administration of astressin B would abolish status differences in E2-induced changes in $GABA_AR$ binding. The data from this study will elucidate whether exposure to psychosocial stressors change $GABA_AR$ binding potential in response to E2 replacement and whether these changes are corrected by the administration of a CRH receptor antagonist.

EXPERIMENTAL PROCEDURES

Subjects

Adult ovariectomized female rhesus macaques (n = 17) receiving hormone replacement via E2 benzoate injections and living in indoor/outdoor enclosures, measuring 3.8 by 3.8 by 3.8 m, at the Yerkes National Primate Research Center (YNPRC) Field Station were subjects for the current study. Subjects were members of small social groups of four and five females each. Animals were fed Purina monkey chow (diet 5038, PMI, St Louis, MO, USA) *ad libitum* twice daily and had continuous access to water. In addition, seasonal fruits and vegetables were provided daily as a nutritional supplement. The Emory University Institutional Animal Care and Use Committee in accordance with the Animal Welfare Act and the U.S. Department of Health and Human Services ''Guide for Care and Use of Laboratory Animals'' approved all procedures.

Female rhesus monkeys represent an appropriate translational model to investigate the effects of psychosocial stress exposure and changes in behavior and physiology (Shively and Kaplan, 1984; Michopoulos et al., 2012a,b). Female macaques, when housed socially, form linear dominance hierarchies wherein dominant females constantly harass lower ranking females (Bernstein and Gordon, 1974; Bernstein et al., 1974). Subordinate female macaques show dysregulation of the LHPA axis (Wilson et al., 2008; Collura et al., 2009; Arce et al., 2010; Michopoulos et al., 2012b) and alterations in behavior and physiology (Abbott et al., 2003; Michopoulos et al., 2012a), including reproductive dysfunction (Michopoulos et al., 2009; Kaplan et al., 2010), immune compromise (Paiardini et al., 2009; Tung et al., 2012), emotional feeding (Michopoulos et al., 2012c), impaired cardiovascular function (Kaplan and Manuck, 1999) and altered reward pathways (Grant et al., 1998; Morgan et al., 2002). Importantly for the purposes of this study, subordinate female rhesus monkeys show enhanced sensitivity to E2 negative feedback inhibition of the reproductive axis (Michopoulos et al., 2009) and altered sensitivity to E2's anxiolytic (Michopoulos et al., 2011b) and affiliative effects (Reding et al., 2012).

The formation of the small social groups, as previously described (Jarrell et al., 2008), occurred three years previous to the initiation of the current study. Females were ovariectomized (Michopoulos et al., 2011a) prior to new group formation (Jarrell et al., 2008) as they were