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Adolescence fluoxetine increases serotonergic activity in the raphe-hippocampus axis and improves depression-like behaviors in female rats that experienced neonatal maternal separation

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Summary This study was conducted to examine if fluoxetine, a selective 5-hydroxytryptamine (5-HT) reuptake inhibitor, would reverse adverse behavioral effects of neonatal maternal separation in female rats. Sprague—Dawley pups were separated from dam daily for 3 h during postnatal day (PND) 1—14 (maternal separation; MS) or left undisturbed (non-handled; NH). Female NH and MS pups received intraperitoneal injection of fluoxetine (10 mg/kg) or vehicle daily from PND 35 until the end of the whole experimental period. Rats were either subjected to behavioral tests during PND 44—54, or sacrificed for neurochemical analyses during PND 43—45. Daily food intake and weight gain of both NH and MS pups were suppressed by fluoxetine, with greater effects in MS pups. MS experience increased immobility and decrease swimming in forced swim test. Swimming was increased, although immobility was not significantly decreased, in MS females by adolescence fluoxetine. However, adolescence fluoxetine increased immobility during forced swim test and decreased time spent in open arms during elevated plus maze test in NH females. Fluoxetine normalized MS-induced decrease of the raphe 5-HT levels and increased 5-HT metabolism in the hippocampus in MS females, and increased the hypothalamic 5-HT both in NH and MS. Fluoxetine decreased the raphe 5-HT and increased the plasma corticosterone in NH females. Results suggest that decreased 5-HTergic activity in the raphe nucleus is implicated in the pathophysiology of depression-like behaviors, and increased 5-HTergic activities in the raphe-hippocampus axis may be a part of anti-depressant efficacy of fluoxetine, in MS females. Also, an

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extra-hypothalamic 5-HTergic activity may contribute to the increased anorectic efficacy of fluoxetine in MS females. Additionally, decreased 5-HT in the raphe and elevated plasma corticosterone may be related with fluoxetine-induced depression- and/or anxiety-like behaviors in NH females.

1. Introduction

Neonatal maternal separation (MS) is considered as an animal model of stressful experience early in life, leading to a permanent alterations in the characteristics of the hypothalamic–pituitary–adrenal (HPA) axis responding to stress (Vazquez et al., 2000) and the development of depression- (Khoury et al., 2006) and anxiety-like behaviors (Daniels et al., 2004) later in life. We have previously demonstrated that rats experienced 3 h of daily maternal separation during the first 2 weeks of birth (MS) exhibit depression- and anxiety-like behaviors (Lee et al., 2007; Ryu et al., 2009) with altered response of the HPA axis to stress challenges later in life (Kim et al., 2005; Ryu et al., 2008). Dysfunction of the HPA axis (Putignano et al., 2001; Gluck et al., 2004) and symptoms of anxiety and depression (Goossens et al., 2009; see for review) are associated with the pathophysiology of eating disorders, especially with binge-like eating disorders (Javaras et al., 2008). Our MS rats showed binge-like eating with increased activity of the HPA axis, when they were challenged with repeated fasting/refeeding cycles during adolescent period (Ryu et al., 2008).

Dysfunction in the brain serotonin (5-hydroxytryptamine; 5-HT) system is implicated in a variety of psychiatric disorders, including major depression (Bhagwagar et al., 2002) and anxiety (Nutt, 2001). MS-induced anxiety-like behaviors were prevented by treatment with 5-$HT_{2A/2C}$ receptor antagonist during the MS period (Benekareddy et al., 2011). 5-HT neurotransmission in the hippocampus is believed to be involved in the regulation of the HPA axis activity throughout life. 5-HT_{1A} receptor expression was decreased in the hippocampus (Maniam and Morris, 2010a), and 5-HT_{2A} increased in the prefrontal cortex (Benekareddy et al., 2011), in MS rats. Our previous study suggested that decreased 5-HTergic activity in the raphe-hippocampus axis is implicated in the pathogenesis of depression-like behaviors by MS experience, likely in relation with dysfunctions of the HPA axis activities (Lee et al., 2007).

It has been reported that fluoxetine, a widely used selective 5-HT reuptake inhibitor, increases brain-derived neurotrophic factor (BDNF) and rescues impaired neurogenesis in the hippocampus (Kohl et al., 2012), and that the hippocampal neurogenesis is necessary for the antidepressant behavioral effect of fluoxetine (Santarelli et al., 2003). BDNF expression (Maniam and Morris, 2010a) and neurogenesis in the hippocampus (Lajud et al., 2012) were decreased in MS rats subjected to a similar MS protocol that we used. Also, fluoxetine is known to have anorectic properties, suppresses food intake in rats (Placidi et al., 2004; Myung et al., 2005) and human (Ward et al., 1999), and an animal model of binge-like eating was hypersensitive to the satiety effect of fluoxetine (Placidi et al., 2004). Taken all together, we hypothesized that fluoxetine can be a tentative candidate to improve depression-like behaviors of our MS rats, possibly in relation with its anorectic property.

Early life stress is a major risk factor for major depressive disorder and posttraumatic stress disorder, especially in women (MacMillan et al., 2001; Shea et al., 2005). Women are roughly twice as likely as men to experience depression (Halbreich and Kahn, 2007), and the incidence of eating disorders is known to be higher in females than in males. Despite the predominance of affective disorders in females and the significant influence of gender on long-term effects of early life stress (Lehmann et al., 1999; Mclntosh et al., 1999; Barna et al., 2003), the vast majority of studies have been conducted in males. There is a growing body of literature showing a sexually dimorphic effect of neonatal maternal separation on its long-term behavioral and neuroendocrine outcome, such as the brain monoamine levels (Matthews et al., 2001), the HPA axis activity (Slotten et al., 2006; Renard et al., 2007; Desbonnet et al., 2008) and anxiety-like behavior (Slotten et al., 2006; Renard et al., 2007). Although we have previously demonstrated that our MS rats can be used as an animal model system to study the pathophysiology of eating disorders accompanied by symptoms of depression and/or anxiety, results were mostly limited to males (Jahng, 2011; see for review). In this study, we have examined if treatment with a selective 5-HT reuptake inhibitor fluoxetine would reverse the adverse behavioral effects of neonatal maternal separation in young female rats. Fluoxetine was administrated during adolescence, especially because the incidence of eating disorders is higher among adolescents and youth (Fairburn and Harrison, 2003) and our MS male model showed binge-like eating when they were on a metabolic challenge during adolescence (Ryu et al., 2008).

2. Materials and methods

2.1. Animals

Sprague–Dawley rats were purchased (Samtako Bio, Osan, Republic of Korea), and cared in a specific pathogen-free barrier area with constant control of temperature (22 ± 1 °C), humidity (55%) and a 12–12 h light/dark cycle (lights-on at 0700 h). Standard laboratory food (Purina Rodent Chow; Purina Co., Seoul, Republic of Korea) and membrane-filtered purified water were available *ad libitum*. Animals were cared according to the Guideline for Animal Experiments, 2000, edited by the Korean Academy of Medical Sciences, which is consistent with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals, revised 1996. All animal experiments were

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