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The postnatal 5-HT$_{1A}$ receptor regulates adult anxiety and depression differently via multiple molecules

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ABSTRACT

Serotonin (5-HT) and the 5-HT$_{1A}$ receptor during development are known to modulate anxiety and depression in later life. However, the brain mechanisms linking the postnatal 5-HT system and adult behavior remain unknown. Here, we examined the effects of pharmacological 5-HT$_{1A}$ receptor activation during the postnatal period on anxiety and depression-like behavior in adult BALB/c male mice. To elucidate the underlying mechanisms, we measured mRNA expression of the 5-HT$_{1A}$ receptor, brain-derived neurotrophic factor (BDNF), GABA$_A$ receptor subunits, and AMPA receptor subunits in the medial prefrontal cortex (mPFC), amygdala, and hippocampus. Treatment with the selective 5-HT reuptake inhibitor (fluoxetine) and 5-HT$_{1A}$ receptor agonist (8-OH-DPAT) during the postnatal period decreased anxiety-like behavior in adulthood, whereas only 8-OH-DPAT treatment increased depression-like behavior. Concomitantly with the behavioral effects, postnatal treatment with fluoxetine and 8-OH-DPAT decreased the mRNA expression of the GABA$_A$ receptor α3 subunit in the mPFC and ventral hippocampus in adulthood, while 8-OH-DPAT, but not fluoxetine, decreased the mRNA expression of the 5-HT$_{1A}$ receptor and BDNF in the mPFC and the GABA$_A$ receptor α2 subunit in the mPFC and ventral hippocampus. On the basis of the correlative changes between behavior and mRNA expression, these results suggest that the GABA$_A$ receptor α3 subunit in the mPFC and ventral hippocampus may regulate anxiety-like behavior. In contrast, depression-like behavior may be regulated by the 5-HT$_{1A}$ receptor and BDNF in the mPFC and by the GABA$_A$ receptor α2 subunit in the mPFC and ventral hippocampus. In summary, activation of the 5-HT$_{1A}$ receptor during the postnatal period may reduce anxiety levels, but increase depression levels during adulthood via different multiple molecules in the mPFC and ventral hippocampus.

1. Introduction

Early-life experiences are known to alter brain development and behaviors in later life. For example, many studies have shown that prolonged maternal separation during the postnatal period increases both anxiety and depression levels in adulthood and impairs brain development including hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis and low neurotrophin levels (Nishi et al., 2014; Tractenberg et al., 2016). In addition, early-life chronic stress induces disabilities in learning and memory in adulthood together with structural changes, including loss of dendrites, dendritic spines, and excitatory synapses (McClelland et al., 2011). In contrast to early-life adverse experiences, postnatal brief maternal separation reduces anxiety-like behavior and enhances spatial learning and memory in adulthood, which is accompanied by lower HPA axis responsiveness (Nishi et al., 2014; Raineki et al., 2014). Thus, alterations in brain structures and functions may cause behavioral changes in later life. However, the brain mechanisms (molecules and brain regions), that link early-life experiences to behavioral changes in later lifer are not well known.

Serotonin (5-hydroxytryptamine; 5-HT) regulates various brain functions, and malfunction of the 5-HT system during development is closely related to mood disorders in adulthood (Daubert and Condron, 2010). In addition to effects on behavior, 5-HT has neurotrophic activity during development to regulate the formation of neural circuits (Whitaker-Azmitia, 2001). Thus, the roles of 5-HT during development are important for the development of brain and behavior.

There are at least 14 5-HT receptors, among which the 5-HT$_{1A}$ receptor is well characterized. Similar to the action of 5-HT, the 5-HT$_{1A}$ receptor modulates dendrite formation and synaptogenesis (Wilson et al., 1998; Yoshida et al., 2011; Mogha et al., 2012) and affects behavior in later life. Interestingly, previous studies suggested that the 5-HT$_{1A}$ receptor during the postnatal period has different effects on anxiety and depression-like behavior in adulthood. By use of conditional knockout of the 5-HT$_{1A}$ receptor and treatment with a 5-HT$_{1A}$

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receptor antagonist, it has been shown that blockade of the 5-HT_{1A} receptor during the postnatal period increases anxiety-like behavior in adulthood (Gross et al., 2002; Vinkers et al., 2010). In contrast, blockade of the 5-HT_{1A} receptor during the postnatal period normalizes depression-like behavior in adult 5-HT transporter knockout mice (Alexandre et al., 2006). However, the brain mechanisms linking the 5-HT_{1A} receptor during the postnatal period to anxiety or depression in adulthood are not known.

One of the candidate molecules regulating anxiety in response to 5-HT is the $GABA_A$ receptor. The $GABA_A$ receptor is a target of benzodiazepines, anxiolytics. Benzodiazepines have acute effects in the treatment of generalized anxiety disorder, social anxiety disorder, and panic disorder (Griebel and Holmes, 2013), whereas selective 5-HT reuptake inhibitors (SSRIs) show their effects after several weeks of treatment (Vaswani et al., 2003). Among the 19 $GABA_A$ receptor subunits, the α2 and α3 subunits modulate anxiety-like behavior. The diazepam-induced anxiolytic effect is absent in mice with point mutation of the α2 subunit, suggesting that the α2 subunit has an anxiolytic effect (Low et al., 2000). In addition, the selective agonist for the α3 subunit shows an anxiolytic effect (Dias et al., 2005) and the α3 subunit inverse agonist shows an anxiogenic effect in rats (Attack et al., 2005).

On the other hand, brain-derived neurotrophic factor (BDNF) and the glutamate receptor have been shown to be involved in depression. Similar to 5-HT, BDNF contributes to various functions in the developing and mature brain, including survival and differentiation of neurons, dendrite formation, synaptogenesis, and synaptic plasticity (Park and Poo, 2013). Moreover, dysfunction of BDNF is related to depression (Nestler et al., 2002). For example, both acute and chronic stresses decrease the expression of BDNF in the hippocampus, and antidepressants increase the expression of BDNF to recover stress-induced reduction of BDNF (Nestler et al., 2002).

Besides the 5-HT system, glutamate receptors are known as a target of antidepressants. Recently, it was shown that ketamine has acute antidepressant activity (Murrough, 2012). Ketamine shows antidepressant effects in a few hours to several days, whereas SSRI requires a few weeks to show antidepressant effects. In addition to NMDA receptor antagonism, ketamine potentiates AMPA receptors, which have a crucial role in the antidepressant effect (Koike and Chaki, 2014). Most AMPA receptors are composed of the GluR1 and GluR2 subunits (Derkach et al., 2007), and these subunits are involved in long-term potentiation (LTP) and long-term depression (LTD) in synaptic plasticity (Huganir and Nicoll, 2013).

In the present study, we first examined the effects of postnatal treatment with SSRI and the 5-HT_{1A} receptor agonist on adult behaviors such as anxiety and depression. Next, to elucidate the underlying brain mechanisms of anxiety and depression, we measured the mRNA expression of the 5-HT_{1A} receptor, BDNF, the $GABA_A$ receptor α2 and α3 subunits, and the AMPA GluR1, and GluR2 subunits in the mPFC, amygdala, and dorsal and ventral hippocampi during the postnatal period and adulthood. By comparing the changes in behaviors with those in the mRNA expressions induced by SSRI and the 5-HT_{1A} receptor agonist, we found some candidate genes, that may regulate adult behaviors.

2. Materials and methods

2.1. Animals

Pregnant female BALB/c mice (Japan SLC Inc., Shizuoka, Japan) were individually housed and maintained under a 12:12 h light/dark cycle (lights on 8:00 AM) at 24 °C, with food and water available ad libitum. The date of birth was considered as postnatal day 0 (P0). Only male offspring were used. All the experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (USA) and were approved by the Animal Experimentation Committee of the University of Tsukuba.

2.2. Drug treatment

Offspring were randomly assigned to 3 groups; control group, treatment with the selective 5-HT reuptake inhibitor (SSRI) group, and treatment with the 5-HT_{1A} receptor agonist group. Pups from each group received an oral administration of 5% sucrose (control), 5 mg/kg fluoxetine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) (SSRI), or 5 mg/kg (R)-(+)-8-hydroxy-DPAT hydrobromide (8-OH-DPAT) (Sigma-Aldrich) (5-HT_{1A} receptor agonist), using a pipettor (Pipetman, Gilson, Middleton, WI, USA) with a small tip at its end, from P1 to P21. The dose was decided on the basis of a previous study (Ishiwata et al., 2005).

2.3. Elevated plus maze (EPM)

All behavioral tests were conducted from postnatal week 9 to 10. To investigate the effects on anxiety-like behavior, the offspring of each experimental group were tested using the EPM (Ohara & Co., Ltd., Tokyo, Japan). The apparatus was elevated 60 cm from the floor and consisted of two opposing open arms (25 cm length × 5 cm - width × 0.3 cm height) and two opposing closed arms (25 cm × 5 cm × 15 cm) that were connected by a central platform (6 cm × 6 cm). Each animal was placed in the central platform, facing a closed arm. All animals were tested once between 10:00 and 13:00, under regular room light (360 lx). The behavior was recorded for 5 min by use of an overhead color CCD camera. The time spent in the open arms and the number of entries into the open arms were calculated as indices of anxiety-like behavior.

2.4. Forced swim test (FST)

Depression-like behavior was assessed as previously described (Porsolt et al., 1977) with slight modifications. The offspring of each experimental group were tested using the FST. All animals were tested between 12:00 and 14:00, under regular room light (360 lx). Each animal was placed into the water (23 ± 1 °C) (7-cm height) in a 4 L beaker (20-cm diameter). The behavior was analyzed during the last 4 min of the 6-min testing period. The first 2 min were considered as habituation. The time spent floating on the water surface without any movement except for minimal activity that the mouse kept them from drowning was measured as an index of depression-like behavior. The time spent climbing was recorded when vigorous vertical movements with the forepaws against the wall of the beaker were displayed. Swimming time was calculated by subtracting the floating and the climbing times from the total testing period.

2.5. Sucrose preference test (SPT)

For the SPT, the offspring were acclimatized to two identical drinking bottles, one filled with 2% sucrose solution and the other with water, for 4 h/day for 4 days. After overnight water deprivation, the SPT was conducted for 4 h on the day 5. To avoid the side preference, the positions of the bottles were switched after 2 h and interchanged daily. Sucrose and water consumption were determined by measuring the change in the volume of fluid consumed. Sucrose preference was defined as the ratio of the volume of sucrose versus the total volume of sucrose and water consumed during the 4-h test.

2.6. Brain dissection

Under anesthesia by isoflurane, the mice were decapitated and their brains were quickly removed at P22 and P71. 2 mm thick coronal slices were cut on ice from the frontal pole of the cerebral cortex using a