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Epigenetic regulation of dorsal raphe $GABA_{B1a}$ associated with isolation-induced abnormal responses to social stimulation in mice

Ryota Araki [a, *], Yosuke Hiraki [a], Shoji Nishida [a], Nobuyuki Kuramoto [b], Kinzo Matsumoto [c], Takeshi Yabe [a, **]

[a] Laboratory of Functional Biomolecules and Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotoge-cho, Hirakata, Osaka 573-0101, Japan
[b] Laboratory of Toxicology, Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotoge-cho, Hirakata, Osaka 573-0101, Japan
[c] Division of Medicinal Pharmacology Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan

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ABSTRACT

In isolation-reared mice, social encounter stimulation induces locomotor hyperactivity and activation of the dorsal raphe nucleus (DRN), suggesting that dysregulation of dorsal raphe function may be involved in abnormal behaviors. In this study, we examined the involvement of dorsal raphe GABAergic dysregulation in the abnormal behaviors of isolation-reared mice. We also studied an epigenetic mechanism underlying abnormalities of the dorsal raphe GABAergic system. Both mRNA and protein levels of $GABA_{B1a}$, a $GABA_B$ receptor subunit, were increased in the DRN of isolation-reared mice, compared with these levels in group-reared mice. In contrast, mRNA levels for other GABAergic system-related genes ($GABA_A$ receptor α1, β2 and γ2 subunits, $GABA_B$ receptor 1b and 2 subunits, and glutamate decarboxylase 67 and 65) were unchanged. Intra-DRN microinjection of 0.06 nmol baclofen (a $GABA_B$ receptor agonist) exacerbated encounter-induced hyperactivity and aggressive behavior, while microinjection of 0.3 nmol phaclofen (a $GABA_B$ receptor antagonist) attenuated encounter-induced hyperactivity and aggressive behavior in isolation-reared mice. Furthermore, microinjection of 0.06 nmol baclofen elicited encounter-induced hyperactivity in group-reared mice. Neither baclofen nor phaclofen affected immobility time in the forced swim test and hyperactivity in a novel environment of isolation reared mice. Bisulfite sequence analyses revealed that the DNA methylation level of the CpG island around the transcription start site (TSS) of $GABA_{B1a}$ was decreased in the DRN of isolation-reared mice. Chromatin immunoprecipitation analysis showed that histone H3 was hyperacetylated around the TSS of $GABA_{B1a}$ in the DRN of isolation-reared mice. These findings indicate that an increase in dorsal raphe $GABA_{B1a}$ expression via epigenetic regulation is associated with abnormal responses to social stimulation such as encounter-induced hyperactivity and aggressive behavior in isolation-reared mice.

1. Introduction

Rearing in social isolation from early life causes abnormal behaviors such as aggression, hyper-locomotion in a novel environment, deficits of pre-pulse inhibition, cognitive impairment, decreased social behaviors, depression-like behavior and anxiety-like behavior in rodents (Ago et al., 2008, 2007; Fone and Porkess, 2008; Lukkes et al., 2009a, 2009b; Zhao et al., 2009). Recently, we found that an encounter with a novel mouse causes locomotor hyperactivity and activation of the dorsal raphe nucleus (DRN), including increases in c-Fos positive cells and serotonin (5-HT) release. Furthermore, these behavioral and neurochemical abnormalities were attenuated by $GABA_A$ receptor positive allosteric modulator, diazepam; a $5\text{-}HT_{1A}$ receptor agonist, osemozotan; and a metabotropic $glutamate_{2/3}$ receptor agonist, LY379268; all of which have anxiolytic properties (Ago et al., 2013). These findings raise the possibility that dysregulation of dorsal raphe function may be involved in abnormal behaviors such as encounter-induced hyperactivity in isolation-reared mice.

The DRN contains the largest accumulation of cell bodies of 5-

* Corresponding author. Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotoge-cho, Hirakata, Osaka 573-0101, Japan.
** Corresponding author.
E-mail addresses: r-araki@pharm.setsunan.ac.jp (R. Araki), yabe@pharm.setsunan.ac.jp (T. Yabe).

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HT neurons that project to several brain regions. Pharmacological, electrophysiological and lesion studies have shown that the function of the DRN is associated with emotional behavior such as aggressive and defensive behaviors in rodents (Bannai et al., 2007; Faccidomo et al., 2008; Jacobs and Cohen, 1976; Mos et al., 1993; Sijbesma et al., 1991; van der Vegt et al., 2003; Vergnes et al., 1986). The function of the DRN is predominantly regulated by local GABA neurons that are thought to provide an inhibitory relay for converging inputs (Amat et al., 2005; Celada et al., 2001; Chiba et al., 2001; Freedman et al., 2000; Tao and Auerbach, 2000; Varga et al., 2003, 2001).

Environmental factors during early life such as isolation rearing are thought to contribute to development of responses to stress and the etiology of psychiatric disorders. The mechanisms through which environmental factors influence development of brain function are unclear; however, several studies have shown involvement of epigenetic regulation, such as DNA methylation and histone modification, in environmental factor-induced behavioral and neuronal abnormalities in rodents (Boku et al., 2015; Golden et al., 2013; Matrisciano et al., 2013, 2012; Murgatroyd et al., 2009; Niwa et al., 2013; Tsankova et al., 2006; Weaver et al., 2004). Several human studies have also reported epigenetic alterations in development of responses to stress and the etiology of psychiatric disorders (Abdolmaleky et al., 2005; Golden et al., 2013; Grayson et al., 2005; McGowan et al., 2009). Thus, epigenetics is of a considerable interest in environmental factor-based psychiatric abnormalities.

In this study, we examined the involvement of dorsal raphe GABAergic dysregulation in abnormal behaviors of isolation-reared mice. We also studied an underlying epigenetic mechanism in the dorsal raphe GABAergic system, with the goal of understanding the mechanisms of isolation-induced abnormal behaviors.

2. Materials and methods

2.1. Animals and drug treatments

Experimental procedures concerning the use of animals were conducted according to the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society and the committee for Ethical Use of Experimental Animals at Setsunan University. Every effort was made to minimize animal suffering and to reduce the number of animals used. Three-week-old male ddY mice (Shimizu Laboratory Supplies Co., Ltd., Kyoto, Japan) were allocated to isolation- or group-housed conditions. In the isolation group, mice were individually housed for 4 or 6 weeks in wire-topped opaque polypropylene cages (24 × 17 × 12 cm), while those in the control group continued to be housed under normal group conditions (5 animals per cage) in wire-topped clear plastic cages of the same size (Ago et al., 2013; Araki et al., 2014). All mice were housed under a standard 12-h light/dark cycle (lights on at 8:00 a.m.) at a constant temperature of 23 ± 1 °C, with free access to food and water throughout the study. A total of 208 mice were used, with different mice used in each experiment. All chemicals were of the highest purity. Baclofen and phaclofen (Sigma, St. Louis, MO, USA) were dissolved in Ringer's solution (147.2 mM NaCl, 4.0 mM KCl, 2.2 mM $CaCl_2$; pH 6.0) and administered as described elsewhere (Takahashi et al., 2010).

2.2. Dissection of the DRN

Serial 1 mm thick coronal sections containing the DRN (stereotaxic coordinates −4.2 to −4.5 mm from bregma according to a brain atlas) were cut using brain matrices (Ted Pella, Inc., Redding, CA, USA). The DRN (1 × 2 mm) was dissected from the sections.

2.3. Total RNA isolation, reverse transcription and quantitative real-time PCR

Total RNA was isolated from the DRN and PFC with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA (1 μg) was used in reverse transcription with ReverTra Ace (Toyobo, Osaka, Japan). Quantitative real-time PCR was performed using Thunderbird qPCR Mix (Toyobo) and the primers indicated in Table 1, using a Thermal Cycler Dice Real Time System Single (Takara Bio Inc., Shiga, Japan). Changes in gene expression were calculated relative to the endogenous β-actin standard.

2.4. Western blotting

The DRN was dissected from the mouse brain and immediately homogenized in Homogenizing (H) buffer at 4 °C (Kuramoto et al., 2003). The H buffer contained 10 mM Tris–HCl (pH 7.5), 0.32 M sucrose, 1 mM EDTA, 1 mM EGTA, 10 mM sodium β-glycerophosphate, 10 mM sodium pyrophosphate, and 50 mM sodium fluoride. Prior to use, the buffer was supplemented with 1 μM dithiothreitol (DTT) and 1 μg/ml of each protease inhibitor (PMSF, benzamidine, leupeptin and antipain). Homogenization was performed with a glass-Teflon grinder. To obtain subcellular fractions, the DRN was homogenized in H buffer using a glass-Teflon homogenizer. The whole homogenate was centrifuged at 1000 rcf for 10 min and the resultant supernatant was further centrifuged at 20000 rcf for 20 min. The supernatant was removed and the pellet was resuspended in a half volume of H buffer. Protein samples were boiled for 10 min in 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, and 0.01% bromophenol blue immediately after preparation and then stored at −80 °C until used for immunoblot analysis (Kuramoto et al., 2003). Briefly, aliquots (10 μg total protein) were subjected to SDS-PAGE in 7.5% polyacrylamide gels and transferred to PVDF membranes. The membranes were blocked with 5% skimmed milk in 0.05% Tween-20 containing Tris-buffered saline for 1 h, and consecutively incubated at room temperature with primary antibodies: chicken IgYs against the intracellular C-terminal region of $GABA_{B1}$ (1:10000; (Kuramoto et al., 2007); the specificity was shown in Fig. S1) or rabbit IgGs against GAPDH (1:3000; Santa Cruz Biotechnology, Santa Cruz, CA. USA) for 2 h; and horseradish peroxidase-conjugated secondary antibodies: donkey anti-chicken IgY (1:10000; Jackson Immuno Research, West Grove, PA, USA) or swine anti-rabbit IgG (1:3000; DakoCytomation, Glostrup, Denmark) for 1 h. Membranes were developed using Western Lightning Chemiluminescence Reagent Plus and exposed to X-ray films.

2.5. Intracerebral drug administration

Each mouse was anaesthetised with sodium pentobarbital (40 mg/kg, i.p.) and a stainless-steel guide cannula (26-gauge, 4.0 mm in length) was implanted in the DRN. The cannula was fixed at stereotaxic coordinates of A −4.4 mm, L −1.5 mm, and V −3.8 mm from the bregma and skull; angled 26° to the vertical. Following a recovery period of a week, mice were lightly restrained and a 28-gauge injection cannula was inserted into the guide cannula. Baclofen at 0.06 and 0.12 nmol/0.2 μL/mouse, or phaclofen at 0.1 and 0.3 nmol/0.2 μL/mouse was infused into the DRN in a 0.2-μL volume over 5 min using a Hamilton microsyringe connected to the injection cannula via a Teflon tube. The injector remained in place for an additional 1 min (before slow removal) to allow for maximum drug diffusion. Drugs were administered 10 min before behavioral tests. After the experiments, mice were deeply anesthetized with pentobarbital and perfused transcardially with saline, followed by 4% paraformaldehyde in PBS. The brain was fixed with