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Behavioral and gene expression analyses in heterozygous *XBP1* knockout mice: Possible contribution of chromosome 11qA1 locus to prepulse inhibition

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ABSTRACT

The *Xbp1* gene, located on chromosome 11qA1 in *Mus musculus*, encodes a key transcription factor in the endoplasmic reticulum stress response pathway. *XBP1* play a role in brain development and implicated in pathogenesis of neurodegenerative and psychiatric diseases. To evaluate the role of Xbp1 in behavioral phenotypes, we subjected heterozygous *Xbp1* knockout (*Xbp1*+/−) mice to a battery of behavioral tests. *Xbp1*+/− mice showed enhanced prepulse inhibition (PPI). We also examined gene expression profiles in frontal cortex and hippocampus of *Xbp1*+/− mice to investigate the molecular basis that could underlie behavioral phenotypes. Gene expression analysis showed that several genes located on chromosome 11qA1 were differentially expressed. Among them, *Uqcr10* and *Nipsnap1* were strongly up-regulated. Significant up-regulation of these genes in 129S compared with BALB/c as well as higher PPI in 129S than BALB/c was previously reported. The ES cells used to generation of *XBP1* knockout mice were derived from 129S and the founder was backcrossed with BALB/c. Thus, these findings would be accounted for by 129S-derived chromosomal region flanking *Xbp1*. These results support the contribution of chromosome 11qA1 locus to the amount of PPI. *Uqcr10* and *Nipsnap1* are good candidate genes that could impact PPI.

1. Introduction

Accumulation of unfolded proteins in the endoplasmic reticulum (ER) activates intracellular signaling, referred to as the unfolded protein response (UPR). UPR is a conserved cellular homeostatic mechanism and involved in normal development and pathogenesis of diseases (Schroder and Kaufman, 2005). X-box binding protein 1 (XBP1) is a key transcription factor in UPR pathway. Non-conventional splicing of *XBP1* mRNA on ER membrane by inositol-requiring 1 (IRE1) causes a frame shift and the spliced mRNA encodes active-form of XBP1, which induces expression of UPR-related genes (Yoshida et al., 2001).

Previous studies suggested a potential role of reduced induction of XBP1 in pathophysiology of bipolar disorder (Kakiuchi et al., 2003; So et al., 2007; Hayashi et al., 2009) and depression (Grunebaum et al., 2009) as well as mechanism of action of mood stabilizers (Kakiuchi et al., 2003; Kakiuchi and Kato, 2005; Masui et al., 2006; Kim et al., 2009). Genetic association of a functional polymorphism of *XBP1* promoter with bipolar disorder (Kakiuchi et al., 2003; Cichon et al., 2004; Hou et al., 2004) or schizophrenia (Chen et al., 2004; Kakiuchi et al., 2004; Jonsson et al., 2006; Watanabe et al., 2006) was tested, but the initial association was not replicated in larger sample sets.

XBP1 up-regulates *WFS1*, a causative gene for Wolfram disease that accompanies mental disorders (Kakiuchi et al., 2006). In *XBP1* knockout neurons, BDNF-induced up-regulation of GABAergic markers such as somatostatin, neuropeptide Y, and calbindin was attenuated (Hayashi et al., 2008). Brain-derived neurotrophic factor (BDNF) induced splicing of *XBP1* pre-mRNA in neurites of cultured hippocampal neurons, and BDNF-induced neurite extension was impaired in *XBP1* knockout neurons. Splicing of *XBP1* by BDNF was inhibited by rapamycin, which inhibits BDNF-dependent protein synthesis. These findings suggested that BDNF-induced protein synthesis caused endoplasmic reticulum stress like condition, and lead to activation of *XBP1* splicing (Hayashi et al., 2007).

These findings collectively suggest that XBP1 affects neural development and plasticity, and thereby might play a role in pathophysiology of mental disorders. However, the consequence of attenuated *XBP1* expression in the brain is not well understood yet.

To elucidate the effect of *XBP1* haploinsufficiency on the brain function, we conducted behavioral analysis of heterozygous *Xbp1*

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knockout (*Xbp1+/−*) mice and performed gene expression analysis in the frontal cortex and hippocampus.

Though it was difficult to clarify the role of XBP1 in the brain in the present experiments, we came across a finding suggesting the roles of two mitochondria-related genes, *Uqcr10* and *Nipsnap1*, at chromosome 11qA1 flanking Xbp1, on prepulse inhibition (PPI).

2. Materials and methods

2.1. Animals

The *Xbp1* knockout mice were kindly provided by Dr. L.H. Glimcher (Harvard School of Public Health, Cambridge, MA). We used heterozygous *Xbp1* knockout (*Xbp1+/−*) mice for analyses, because homozygous *XBP1* knockout mice are embryonic lethal. *Xbp1+/−* mice are originally derived from 129S strain and congenic mice with BALB/c background were generated by repeated backcrossing. The Animal Experiment Committee of RIKEN approved all experimental procedures. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Behavioral analysis

This analysis was performed at BMSRC (Akashi, Japan). Fifteen *Xbp1+/−* mice, and 15 wild-type (WT) littermates were analyzed. All were males aged 14 weeks at the initiation of the behavioral analysis. The analyses were performed in the order of open-field test, startle response and prepulse inhibition test, elevated plus maze, Morris water maze, passive avoidance learning, active avoidance learning, and forced swimming test.

2.3. Open-field test

A transparent cubic box without a ceiling (30 cm × 30 cm × 30 cm) was placed in a ventilated sound-attenuating chamber. A 40-W white lamp provided room lighting, which was approximately 110 lx on the floor of the chamber. A fan attached on the upper part of the wall at one end of the chamber presented a masking noise of 45 dB. Two infrared beams were set on each wall 2 cm above the floor with an interval of 10 cm. The total number of successive interceptions of two adjoining beams on each bank was scored as locomotion behavior. The other 12 infrared ray beams were attached 4.5 cm above the floor in 2.6 cm intervals, and the total number of vertical beam interceptions was scored as rearing behavior. Each mouse was allowed to explore freely in the open-field area for 20 min. Total scores of locomotor activity and rearing in 20 min session were statistical analyzed using Student's *t*-test.

2.4. Startle response and prepulse inhibition (PPI)

Each mouse was enclosed in a transparent acrylic box (7 cm × 7 cm × 10 cm). Startle response was detected as vibration of the box, using an accelerometer (GH-313A, Keyence, Osaka, Japan). The acoustic startle pulse of broadband burst (115 dB, 50 ms) and tone prepulse (85 dB, 30 ms) were presented via a speaker located in front of the box. Light prepulse (30 ms) was applied by LED (Light Emitting Diode). At the beginning of the session, 40 startle pulses were presented to test for basal startle responsiveness and its habituation. The average values of eight blocks, consisting of five startle pulses each, were used for the statistical analysis. After that, three different types of trials were performed: startle pulse alone ($n = 12$), startle pulse preceded by a tone prepulse ($n = 12$), and startle pulse preceded by a light prepulse ($n = 12$). Prepulses were presented 50, 100, or 200 ms before the startle pulse. In total, six types of prepulse ($n = 4$, each) were applied. The mean interval averaged 25 s (15–45 s) throughout the session. The startle response was recorded for 200 ms with the sampling frequency of 1000 Hz. The PPI was assessed by the ratio of the mean response of trials with one type of prepulse ($n = 4$) divided by the mean response of trials without prepulse ($n = 12$). We excluded the values exceeding mean + 2 SD as outliers. This is because the data with large values were possibly artifacts by free moving of mice rather than startle responses. By this process, total 27.6% of the data obtained from *Xbp1+/−* mice and 28.2% of the data obtained from WT mice were excluded. We used a box to measure startle response, and thus spontaneous movement could not be completely prevented. This might have caused the relatively large number of outliers. This is the limitation of the present study. Usage of a cylinder could have improved the reliability of the data.

Because light prepulse did not attenuate the startle response at all in this study, only the data of tone prepulse were presented. Results were statistically analyzed using repeated measures analysis of variance (RMANOVA) for magnitude startle response in session for habituation and Student's *t*-test for PPI.

2.5. Elevated plus maze

The maze consisted of four arms, two open arms and two closed arms, 5 cm wide and 30 cm long with a gray acrylic floor, that met at a 10 cm × 10 cm center zone. Two closed arms had the transparent walls of 15 cm height on both sides, and the open arms had the low walls of 3 mm height on the both sides. The apparatus was mounted 75 cm above the floor of the room. The room lighting was approximately 20 lx on the maze. The video camera was placed 80 cm above the maze. A fan generated a masking noise of 45 dB. The animal was placed gently onto the center of the maze and was allowed to explore the maze freely for 10 min. Number of entries into each arm and time spent in each arm were recorded from videotapes. For statistical analysis, Student's *t*-test was applied.

2.6. Morris water maze

A round pool, with the diameter of 95 cm and the depth of 21.5 cm, was placed in the center of a 140 cm × 130 cm room. A platform with the diameter of 11 cm was set in one of quadrants and 5 mm beneath the surface of black water maintained at 21 ± 1 °C. On the first to fifth days, five trials per day were performed for learning phase. The mouse was released on one of three quadrants of the pool without the platform, and the time to reach the platform was measured. When the mouse could not reach the platform within 60 s, the experimenter placed the mouse on the platform. On the sixth day, a probe test was performed to examine whether the mouse remembered the place of the platform. The mouse was released in the quadrant on the opposite side of the platform and its behavior for 60 s was videotaped. The time staying in the target quadrant, where the platform had been placed, and immobility time were measured. For statistical analysis, Student's *t*-test was applied.

2.7. Passive avoidance learning

A mouse was placed in a box, consisting of two rooms separated by a shutter, that is, light and dark compartments (10 cm × 10 cm each). In the acquisition trial, the mouse was kept in the light compartment. Five seconds later, the door to the dark compartment was opened. When the mouse moved into the dark compartment, the shutter was closed, and 10 s later, an electrical shock (160 V, 3 s) was delivered through the grid floor. Twenty-four hours later, each mouse was placed again in the light compartment and the latency