A Novel Cognitive Enhancer, ZSET1446/ST101, Promotes Hippocampal Neurogenesis and Ameliorates Depressive Behavior in Olfactory Bulbectomized Mice

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ABSTRACT

In the adult brain, neurogenesis persistently occurs in the subgranular zone of the hippocampal dentate gyrus (DG), and impaired neurogenesis is implicated in depressive behaviors and poor learning memory. Here, we investigated the effects of oral administration of spiro[imidazo[1,2-a]pyridine-3,2-indan]-2(3H)-one (ZSET1446/ST101), a novel cognitive enhancer stimulating acetylcholine release, on adult neurogenesis in olfactory bulbectomized (OBX) mice. OBX mice showed significant decreases in the number of newborn cells in the DG by immunohistochemical analysis of 5-bromo-2-deoxyuridine incorporation. Impaired neurogenesis observed in OBX mice was significantly improved by chronic administration with ZSET1446. We confirmed that administration with mecamylamine, a nicotinic acetylcholine receptor antagonist, inhibits ZSET1446-enhanced neurogenesis in the DG. ZSET1446 administration also restored decreased phosphorylation of Akt and extracellular signal-regulated kinase in the DG of OBX mice. Consistent with restored neurogenesis, chronic but not single ZSET1446 administration promoted significant decreases in immobility in tail suspension tests and improved cognitive behaviors in OBX mice. Taken together, chronic ZSET1446 administration antagonized impaired neurogenesis seen in OBX mice, an effect closely associated with improvement of depressive behavior.

Neurogenesis in the dentate gyrus (DG) has been specifically implicated in learning tasks that involve the hippocampus (Gould et al., 1999). Suppression of neurogenesis in the DG by X-irradiation impairs hippocampus-dependent learning and memory formation in adult rats (Madsen et al., 2003) and mice (Rola et al., 2004). In pathological conditions, exposure to chronic stresses and aging cause a reduction in cell proliferation in SGZ (Tanapat et al., 1998; Cameron and McKay, 1999). It is noteworthy that impaired neurogenesis in SGZ partially accounts for depressive behaviors. For example, administrations with antidepressants such as fluoxetine (Malberg and Duman, 2003) and imipramine (Santarelli et al., 2003) restore impaired neurogenesis in the hippocampus of rodents exposed to stress.

Conversely, X-irradiation of a restricted region of mouse brain containing the hippocampus prevents adult neurogenesis and antagonizes behavioral improvement mediated by antidepressants resulting in depressive behaviors (Santarelli et al., 2003), indicating that hippocampal neurogenesis is required for antidepressant-like effects.

Loss of cholinergic neurons or blockade of acetylcholine (ACh) receptors in the central nervous system causes learning impairment in experimental and clinical situations in humans (Drachman and Leavitt, 1974; Rasmusson and Dardar, 1979) and rhesus monkeys (Ogura and Aigner, 1993). Furthermore, the cholinergic system is also implicated in depressive behaviors. Behaviors in genetically depressive rats (Flinders Sensitive Line rats), which are hyper-responsive to cholinergic stimulation, are improved by nicotine administration in a forced swim test (Tizabi et al., 1999).

Cholinergic neuronal activity is also implicated in adult neurogenesis. Innervation of newborn neurons by cholinergic fibers has been demonstrated in the DG (Frotscher and Le-
ranth, 1985; Kaneko et al., 2006). Kaneko et al. (2006) reported that cholinergic fibers innervate both the olfactory bulb and the DG, where neuronal progenitors and immature neurons express various nicotinic acetylcholine receptor (nAChR) subunits, such as α7 and β2, in the rodent DG. Experimental lesion of cholinergic neurons projecting to the hippocampus suppresses neurogenesis in rats (Cooper-Kuhn et al., 2004). In vitro cholinergic stimulation also modifies proliferation and survival of neural precursor cells in cultured rat olfactory bulb cells (Coronas et al., 2000) and in cortical precursor cells (Ma et al., 2000). Taken together, the development of progenitor cells and immature neurons is probably controlled by cholinergic neurons.

We recently discovered a novel cognitive enhancer, ZSET1446 (ST101) that is a new azaindolizinone derivative and does not inhibit acetylcholine esterase (AChE). ZSET1446 (ST101) improved learning and memory by potentiating nicotinic ACh release in the hippocampus of amyloid-(1-40)-infused rats (Yamaguchi et al., 2006). In addition, ZSET1446 restored methamphetamine-induced memory impairment in rats (Ito et al., 2007) and olfactory bulbectomized (OBX)-induced cognitive deficits in mice (Han et al., 2008a).

Using OBX mice as an animal model showing depressive behaviors (Harkin et al., 2003), we observed significant reduction of neurogenesis in the DG. The OBX-induced reduction of neurogenesis was restored by chronic administration of ZSET1446 in a dose-dependent manner. Consistent with enhanced neurogenesis, chronic but not single administration with ZSET1446 elicited antidepressive-like effects on OBX mice. We also confirmed that ZSET1446-enhanced neurogenesis is partially mediated by nAChRs based on an in-fusion experiment using mecamylamine, a potent nAChR blocker. These results suggest that the antidepressive-like action of ZSET1446 could benefit treatment of depression and improve cognitive impairment observed in patients with Alzheimer’s disease.

**Materials and Methods**

**Animals.** Adult male DDY mice weighing 23 to 26 g were obtained from Nippon SLC (Hamamatsu, Japan), housed in polycarbonate cages at 23 ± 1°C in a humidity-controlled environment, and maintained on 12-h light/dark schedules (lights on from 8:00 AM to 8:00 PM). Mice were provided food and water ad libitum. Experiments were performed according to the *Guide for Care and Use of Laboratory Animals* at Tohoku University and were also conducted according to the *Guide for the Care and Use of Laboratory Animals* (National Research Council, revised 1996). All efforts were made to reduce animal suffering and minimize the total number of animals used.

**Bilateral Olfactory Bulbectomy Preparation and ZSET1446 Administration.** After an acclimatization period of 1 week, bilateral olfactory bulbectomy was performed at day 0. OBX mice were prepared as described by Han et al. (2008a). In brief, mice anesthetized with sodium pentobarbital (50 mg/kg i.p.; Dainippon, Osaka, Japan) were placed in a stereotaxic instrument. After exposure of the skull, 1-mm-diameter holes were drilled on both sides of the olfactory bulbs. Olfactory bulbs were removed through the hole by gentle aspiration with a suction pump, and care was taken not to damage the frontal cortex. Holes were filled with a homeostatic sponge to avoid further bleeding, and skin was closed. Sham-operated mice were treated similarly but bulbs were left intact.

A diagram of the experimental schedule is given as Fig. 1. The experiments were conducted with three different groups: group 1, chronic administration of ZSET1446 for 5-bromo-2-deoxyuridine (BrdU) incorporation examination (n = 6); group 2, single administration of ZSET1446 for behavioral studies (behavioral tests were performed at 29 days after OBX (n = 9)); group 3, chronic administration of ZSET1446 for behavioral tests (n = 9). Behavioral studies were performed at 29 days after OBX. At 30 days, mice in group 3 were separated randomly into two groups for immunohistochemical analyses (n = 4) and immunoblot analysis (n = 5).

BrdU (Sigma-Aldrich, St Louis, MO; 50 mg/kg i.p.) was administered once daily for 7 consecutive days for 15 to 21 days after OBX operation (group 1). Mice were then perfused transcardially with 4% paraformaldehyde (1% for BrdU incorporation examination). Mice were perfused at 29 days after OBX and killed at day 30. The frontal cortex and the olfactory bulb were dissected, immersed in 4% paraformaldehyde, and transferred to 30% sucrose in PBS. After 48 h, the olfactory bulbs were sectioned at 20 μm and mounted on gelatin-coated slides. Thin sections were processed for staining with hematoxylin and eosin (H&E). The positions of BrdU+ neurons were determined by confocal microscopy (Olympus FluoView 500, Tokyo, Japan).

**Group 1: Chronic ZSET1446 administration for BrdU incorporation study**

**Group 2: Single ZSET1446 administration for behavioral tests**

**Group 3: Chronic ZSET1446 administration for behavioral tests**

![Fig. 1. Diagrams of the experimental schedule. Group 1, chronic ZSET1446 administration (by mouth) for BrdU incorporation study. Vehicle (water) or ZSET1446 were administered for 14 days once daily on days 15 to 28 after OBX. In the separate experiment, intracerebroventricular mecamylamine administration with a micro-osmotic pump carried out on days 15 to 28. These mice were sacrificed at 30 days (n = 6). Group 2, single ZSET1446 administration (by mouth) for behavioral tests. Vehicle (water) or ZSET1446 were administered once at 28 days after OBX (n = 9). Group 3, chronic ZSET1446 administration (by mouth) for behavioral tests (n = 9) and neurochemical studies. Vehicle (water) or ZSET1446 were administered for 14 days once daily on days 15 to 28 after OBX. These mice were sacrificed at 30 days after OBX, and Western blot analysis (WB) (n = 5) or immunohistochemical analysis (IHC) (n = 4) were used. In the separate experiment, mice were injected intracerebroventricularly with mecamylamine during days 16 to 28 with a micro-osmotic pump and performed a behavioral test on day 29 (n = 7).**
paraformaldehyde in 0.1 M phosphate buffer (4°C, pH 7.4) 30 days after OBX for immunohistochemical examination. Chronic administration began after a 14-day recovery period. ZSET1446/ST101 was prepared by Zenyaku Kogyo Co. Ltd. (Tokyo, Japan). For repeated administration, ZSET1446 (0.01–1 mg/kg, dissolved in distilled water) or vehicle was administered by mouth daily for 14 consecutive days (15–28 days) after OBX operation (group 1 and group 3). For the single administration, ZSET1446 was admin- istered once a day before behavioral tests at 28 days after OBX (group 2). In each experiment, brains were confirmed by complete removal of olfactory bulbs and lack of cortical damage.

**Chronic Mecamylamine Administration.** For BrdU incorpora- tion and behavioral experiments in groups 1 and 3, mice were treated with mecamylamine (Sigma-Aldrich) dissolved in physiological saline and injected via intracerebroventricular administration. Specif- ically, mecamylamine (30 nmol in total per mouse) or saline vehicle alone was infused into the right brain lateral ventricle for 14 days (15–28 days after OBX operation) with a micro-osmotic pump (model 1004; flow rate, 0.11 μl/h; Alzet Osmotic Pumps, Cupertino, CA, http://www.alzet.com). The cannula was implanted stereotaxically at the following coordinates: anterior, 0 mm; lateral, 1 mm; depth, 2 mm (relative to the bregma and the surface of the brain). Thirty days after surgery, brains were fixed for immunohistochemistry.

**Behavioral Analyses.** The tail suspension test (TST) is a widely used mouse model for assessing antidepressant-like activity. The test is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by the tail will develop an immobile posture. The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. (1985). In brief, acoustically and visually isolated mice were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 10-min period. Mice were considered immobile only when they hung passively and remained completely motionless.

Spontaneous alternation behavior in a Y-maze task was also recorded as a spatial memory task as described by Han et al. (2008a). The apparatus consisted of three identical black Plexiglas arms (length × width × height, 50 × 16 × 32 cm). Each mouse was placed at the end of one fixed arm and allowed to move freely through the maze during an 8-min session. The sequence of arm entries was recorded visually, and three consecutive choices of different arms were defined as an alternation. The percentage of alternation was calculated as (actual alternations/maximal alternations) × 100. In addition, the total number of arms entered during the session was determined as a measure of locomotor activity.

**Western Blotting Analysis.** Hippocampal DG samples were homogenized in 70 μl of buffer containing 50 mM Tris-HCl, pH 7.4, 0.5% Triton X-100, 4 mM EGTA, 10 mM EDTA, 1 mM Na3VO4, 40 mM sodium pyrophosphate, 50 mM NaF, 100 mM calyculin A, 50 μg/ml leupeptin, 25 μg/ml pepstatin A, 50 μg/ml trypsin inhibitor, and 1 mM dithiothreitol. Insoluble material was removed by a 10-min centrifugation (15,000 rpm). After determining supernatant protein concentration using Bradford’s solution, samples were boiled 3 min in Laemmli’s sample buffer. Samples containing equivalent amounts of protein were subjected to SDS-polyacrylamide gel elec- trophoresis. Proteins were transferred to an Immobilon polyvinylidene difluoride membrane for 2 h at 70 V. After blocking with TTBS (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.1% Tween 20) containing 2.5% bovine serum albumin for 1 h at room temperature, membranes were incubated overnight at 4°C with antiphosphory- lated Akt (Thr-308) antibody (1:1000) (Cell Signaling, Billerica, MA), anti-Akt antibody (1:1000) (Cell Signaling, Woburn, MA), antiphosphorylated ERK (Thr-202/Tyr-204) antibody (1:1000) (Cell Signaling), and anti-GAD67 antibody (1:1000) (Millipore). Bound antibodies were visualized using the enhanced chemiluminescence detection system (GE Healthcare, Chalfont St. Giles, UK) and analyzed semiquantitatively using ImageJ software (National Institutes of Health, Bethesda, MD).

**Immunohistochemistry.** Immunohistochemical study was performed as reported by Shioda et al. (2008). Thirty days after OBX operation, mice were anesthetized with pentobarbital and perfused via the ascending aorta with phosphate-buffered saline (PBS; pH 7.4) until the outflow became clear, followed by phosphate buffer (pH 7.4) containing 4% paraformaldehyde for 15 min. The brain was removed, postfixed in the same solution for 24 h at 4°C, and sliced at 50 μm using a vibratome (Dosaka EM Co. Ltd., Kyoto, Japan). Coronal brain sections were incubated as follows: 30 min in PBS; 30 min 2 N HCl; 1 h in PBS with 3% bovine serum albumin (blocking solution); overnight with mouse anti-NeuN monoclonal antibody (1:500) (Millipor, a rat anti-BrdU monoclonal antibody (1:500) (Accu- rate Chemical and Scientific, Oxford Biotechnology, Oxfordshire, UK), a rabbit antiphosphorylated ERK (Thr-202/Tyr-204) polyclonal antibody (1:1000) (Cell Signaling) or a rabbit antiphosphorylated Akt (Thr-308) polyclonal antibody (1:1000) (Millipore) in blocking solu- tion at 4°C. After thorough washing in PBS, sections were incubated 3 h in Alexa 488-labeled anti-rat or -rabbit IgG, or Alexa 594-labeled anti-mouse or -rabbit IgG. After several PBS washes, sections were mounted on slides with Vectashield (Vector Laboratories, Burlin- game, CA). Immunofluorescent images were analyzed using a confocal laser scanning microscope (Leica TCS, Olympus, Tokyo, Japan).

**Cell Counting.** Cell counting was performed as reported by Shioda et al. (2008). In brief, to count BrdU and NeuN double- positive cells after immunohistochemistry, six hippocampal sections were cut every 50 μm beginning at 1.7 to 2.2 mm caudal to the bregma. The number of BrdU/NeuN double-positive cells was deter- mined in a 300 × 300 μm area per section in the DG region. In the DG, the granular cell layer (GCL) (approximately 50 μm in width) and the SGZ, which is defined as a zone that is two cell bodies wide (5 μm) along the border of the GCL and hilus, were quantified together. The number of BrdU/NeuN double-positive cells counted in each mouse was expressed as the number of the double-positive cells per a 300 × 300 μm area. Six sections per mouse and six mice per condition were used. The person responsible for cell counts was blind to the experimental conditions.

**Statistical Evaluation.** All values were expressed as means ± S.E.M. Comparison between two experimental groups was made using the unpaired Student’s t test. Statistical significance for differences among groups was tested by one-way analysis of variance (ANOVA), followed by multiple comparisons between control and other groups using Dunnett’s multiple comparison test. P < 0.05 was considered significant.

**Results**

**Decreased Hippocampal Neurogenesis after OBX Is Prevented by Repeated ZSET1446 Administration.** Hippocampal neurogenesis was analyzed in animals injected with BrdU 15 to 21 days after OBX or sham surgery and analyzed 30 days later as shown in Fig. 1. To identify BrdU-positive cells, slices were double-stained with anti-NeuN ant- ibody, a neuronal marker. BrdU/NeuN double-positive cells (Fig. 2A) were observed in some hippocampal cells in DG in sham-operated animals, but the number was significantly decreased 30 days after OBX (OBX, 174 ± 1.1 cells versus sham, 508 ± 2.6 cells, P < 0.01; Fig. 2, B and C). Chronic administration of ZSET1446 (15–28 days) after OBX dose- dependently and partially restored the number of BrdU/ NeuN double-positive cells (0.01 mg/kg drug, 220 ± 1.6 cells; 0.1 mg/kg drug, 321 ± 1.7 cells; 0.5 mg/kg drug, 405 ± 3.3 cells; 1.0 mg/kg drug, 396 ± 2.8 cells) compared with OBX alone (Fig. 2, B and C). Chronic ZSET1446 administration in sham- operated animals had no effect on the number of BrdU/NeuN double-positive cells (0.5 mg/kg, ZSET1446-treated sham; 548 ± 1.4 cells) compared with sham-operated animals.
ERK (Thr-202/Tyr-204) and anti-BrdU antibodies. Phospho-ERK immunoreactivity was localized in cell bodies of granule cells including BrdU-positive cells (Fig. 4A) and axons in the hilus region (Fig. 4B). Phospho-ERK immunoreactivity was markedly reduced after OBX without changes in GAD67 immunoreactivity (Fig. 4C). It is noteworthy that phospho-ERK immunoreactivity markedly recovered after ZSET1446 administration compared with that seen in OBX (Fig. 4C). To quantify changes in ERK phosphorylation in DG after OBX with or without ZSET1446 administration, immunoblotting analysis using cell extracts from the DG region was performed. Consistent with reduced immunoreactivity of phospho-ERK, OBX caused significant reduction of phospho-ERK levels (37.3 ± 4.7%, P < 0.05), and ZSET1446 administration partially restored ERK phosphorylation dose-dependently compared with OBX mice (0.01 mg/kg, 31.2 ± 5.8%; 0.1 mg/kg, 61.9 ± 3.6%; 0.5 mg/kg, 72.3 ± 4.3%; and 1.0 mg/kg, 75.6 ± 3.7%; Fig. 4D).

We also confirmed increased numbers of activated Akt (phospho-Akt)/BrdU double-positive cells in the DG. Phospho-Akt immunoreactivity was localized in cell bodies of granule cells including BrdU-positive cells (Fig. 5A). Consistent with the decreased number of BrdU-positive cells after OBX, phospho-Akt immunoreactivity in DG significantly decreased after OBX.
Chronic Administration of ZSET1446 Reduces Duration of Immobility in a Tail Suspension Test in OBX Mice. We previously reported that chronic ZSET1446 administration for 3 to 12 days after OBX ameliorated spatial working memory in a Y-maze task (Han et al., 2008a). In the present study, we administered ZSET1446 to OBX mice for 15 to 28 days after OBX to eliminate the possibility of neuroprotective effects during the first 2 weeks. It is noteworthy that locomotor activity assessed by number of arm entries in a Y-maze task dramatically increased in OBX mice at 29 days, and chronic ZSET1446 administration (0.01–1 mg/kg p.o.) or vehicle was administered by mouth daily for 14 consecutive days (15–28 days) after OBX operation. Each bar represents the mean ± S.E.M. *P < 0.05 versus sham-operated animals; #, P < 0.05 versus OBX group. Dunnett’s multiple comparison test. n = 5 in each group. ZSET, ZSET1446.

Fig. 4. ZSET1446 stimulates ERK phosphorylation in newborn cells and surrounding DG areas, including the hilus region. A, confocal microscopy images of double staining in the DG for BrdU (green), phospho (p)-ERK (red), and merged images. Samples were obtained in hippocampal slices of ZSET1446-treated (0.5 mg/kg p.o.) OBX mice. Strong p-ERK immunoreactivity was observed in granular cells in the hilus and in BrdU-positive neurons (arrows) in the DG. Scale bar, 50 μm. B and C, in the hilus region, p-ERK immunoreactivity (green) was reduced after ischemia and rescued by ZSET1446 (0.5 mg/kg p.o.) administration without changing GAD67 immunoreactivity (red). C, magnification of the areas showing by box in B. D, representative images of immunoblots using antibodies against p-ERK (Thr-202/Tyr-204) and GAD67. The bar graphs summarized the quantitative analyses of p-ERK and GAD67 levels analyzed by densitometry. Data are expressed as the percentage of the value of sham-operated animals. ZSET1446 (0.01–1 mg/kg) or vehicle was administered by mouth daily for 14 consecutive days (15–28 days) after OBX operation. Each bar represents the mean ± S.E.M. *P < 0.05 versus sham-operated animals. **P < 0.01 versus sham-operated animals. #, P < 0.05 versus OBX group. Dunnett’s multiple comparison test. n = 5 in each group. ZSET, ZSET1446.

Fig. 5. ZSET1446 stimulates Akt phosphorylation in newborn cells and surrounding DG areas. A, confocal microscopy images of double staining in the DG for BrdU (green), p-Akt (red), and merged images. Samples were obtained in hippocampal slices in ZSET1446-treated mice (0.5 mg/kg p.o.). Strong p-Akt immunoreactivity was observed in the granular cell layer and in BrdU-positive neurons (arrows) in the subgranular zone. Scale bar, 40 μm. B, p-Akt immunoreactivity was reduced after OBX and rescued by ZSET1446 administration (0.5 mg/kg p.o.). C, representative image of immunoblot using antibodies against p-Akt (Thr-308). The bar graph summarizes quantitative analyses of p-Akt levels analyzed by densitometry. Data are expressed as the percentage of the value of sham-operated animals. ZSET1446 (0.01–1 mg/kg) or vehicle was administered by mouth daily for 14 consecutive days (15–28 days) after OBX operation. Each bar represents the mean ± S.E.M. *P < 0.05 versus sham-operated animals; #, P < 0.05 versus OBX group. Dunnett’s multiple comparison test. n = 5 in each group. ZSET, ZSET1446.
memory deficits assessed by Y-maze alternation was improved by chronic ZSET1446 administration at 0.5 and 1.0 mg/kg. OBX mice exhibited a significant decrease in alternation behaviors compared with sham-operated animals (sham, 77.9 ± 4.4%; OBX, 48.3 ± 5.6%, P < 0.01; Fig. 7A, right). Chronic administration with ZSET1446 (0.01–1 mg/kg p.o., 14 days) significantly improved alternation behavior in a Y-maze (0.01 mg/kg, 46 ± 2.9%; 0.1 mg/kg, 60.2 ± 4.2%; 0.5 mg/kg, 71.8 ± 1.6%; and 1 mg/kg, 72.6 ± 4.1%) compared with OBX mice.

We also examined effect of a single ZSET1446 administration at 28 days after OBX operation in the Y-maze task. Consistent with previous observations (Han et al., 2008a), single ZSET1446 administration did not ameliorate impaired memory-related behaviors and hyperlocomotion in OBX mice (Fig. 7B). The chronic and single ZSET1446 administration in sham-operated animals also had no effect on the memory-related behaviors and hyperlocomotion compared with sham-operated animals (Fig. 7).

**Chronic Mecamylamine Administration Blocks ZSET1446-Ameliorated Depressive and Memory-Related Behavior in OBX Mice.** We finally determined whether ZSET1446-ameliorated depressive and memory-related behaviors were prevented by administration of mecamylamine (15–28 days after OBX operation). In tail suspension test, mecamylamine infusion alone did not affect the duration of immobility (190.3 ± 19.1 s) compared with the saline-treated OBX mice (177.4 ± 21.5 s; Fig. 8A), whereas mecamylamine administration significantly inhibited ZSET1446-induced improvement of duration of immobility (153.3 ± 24.2 s) compared with ZSET1446-treated OBX mice (96.6 ± 18.9 s, P < 0.05; Fig. 8A).

Likewise, mecamylamine infusion alone did not affect OBX-induced reduced alternation behavior (OBX, 46.3 ± 4.0%; OBX + mecamylamine, 44.8 ± 8.0%) (Fig. 8B, right). However, mecamylamine administration significantly blocked the improvement of alternation behavior by ZSET1446 (ZSET1446 in OBX, 72.5 ± 5.6%; ZSET1446 + mecamylamine in OBX, 56.7 ± 9.1%, P < 0.05) (Fig. 8B, right). These results suggest that ZSET1446-induced improvement of depressive and memory-related behaviors is probably mediated by nAChR stimulation.

**Discussion**

Here, we showed that chronic ZSET1446 administration significantly rescues decreased neuronal precursor cell proliferation seen in the DG after OBX. Consistent with enhanced neurogenesis, chronic ZSET1446 administration reduced immobility in a TST in OBX mice, which indicates an antidepressive-like effect. The ZSET1446 effect was mediated by nAChRs, because chronic intracerebroventricular mecamylamine administration inhibited ZSET1446-enhanced cell proliferation and ZSET1446-improved depressive behavior in OBX. PI3K/Akt and ERK pathways might mediate ZSET1446-induced neurogenesis. These results suggest that ZSET1446-induced neurogenesis, in part, contributes to amelioration of depressive-like behaviors in OBX mice.

Our findings regarding decreased neurogenesis in the hippocampal DG in OBX mice are consistent with previous studies using different animal models of depression, including...
intruder stress in marmosets (Gould et al., 1998), psychosocial stress in tree shrews (Czeh et al., 2001), social defeat in rats (Czeh et al., 2002) and footshock stress in rats (Malberg and Duman, 2003). Jaako-Movits and Zharkovsky (2005) demonstrated that OBX rats show reduced adult hippocampal neurogenesis. However, the mechanisms underlying impaired OBX-induced neurogenesis are unknown. OBX is known to cause retrograde degeneration of cholinergic neurons in the septum, thereby eliciting anterograde degeneration of cholinergic neurons from the septum to the hippocampus (Yamamoto et al., 1997). It is noteworthy that antidepressants induce increased proliferation of neuronal progenitors and enhance their maturation into neurons in the DG of the hippocampus after chronic but not acute administration. For example, fluoxetine (Malberg and Duman, 2003) and imipramine (Santarelli et al., 2003) increase neurogenesis in hippocampus of rats exposed to stress. In normal rodents, fluoxetine, rolipram, and lithium also enhance neurogenesis after chronic, repeated administration, whereas short-term or single administration has no effect on neurogenesis (Son et al., 2003; Malberg and Duman, 2003). In contrast to the effects of these antidepressant drugs, chronic ZSET1446 administration did not affect cell proliferation in sham-operated mice. However, administrations of 4, but not 2 weeks with donepezil, an AChE inhibitor, enhanced neurogenesis in normal rats (Kotani et al., 2006). Thus, further prolonged administration with ZSET1446 may be required to stimulate hippocampal neurogenesis in normal animals.

ZSET1446 administration enhances nicotine-stimulated ACh release in the hippocampus in amyloid-β(1-40)-infused rats and rescued decreased choline acetyltransferase activity seen in the medial septum and hippocampus of the same model (Yamaguchi et al., 2006). In the support of involvement of the ACh-stimulating action of the drug, the nonselective nAChR antagonist mecamylamine inhibited ZSET1446-induced neurogenesis, suggesting that enhanced neurogenesis requires stimulation by nAChRs. Because the precise targets of ZSET1446 underlying the enhancement of ACh release remain unclear, further efforts to define the target in the ACh synaptic transmission in the central nervous system are required. The chronic administration of ZSET1446 is required for the improvement of depressive-like behaviors of OBX mice. We will define in future experiments the targets to improve the neurogenesis and the reason why ZSET1446 effect requires chronic administration rather than single administration.

The nAChR stimulation can activate numerous signaling pathways. In vitro studies using SH-SY5Y cells and cultured hippocampal neurons reveal that activation of δ7-containing nAChRs induces ERK activation (Dajas-Bailador et al., 2002). In primary cultures of rat cortical neurons, neuroprotective effects of AChE inhibitors are mediated by α7 and δ7-containing nAChRs via activation of Janus kinase 2 and PI3K/Akt (Shaw et al., 2002; Takada-Takatori et al., 2006). For example, carbachol-induced activation of both PI3K/Akt and ERK pathways stimulates DNA synthesis in basic fibroblast growth factor-treated neural progenitors isolated from rat cortical neuroepithelium. PI3K inhibitors (LY294002 and wortmannin) and the mitogen-activated protein kinase/ERK kinase inhibitor PD98059 inhibit carbachol-induced increases in DNA synthesis in progenitor cells (Li et al., 2001). We recently demonstrated that activators of PI3K/Akt and ERK pathways promote brain ischemia-induced neurogenesis in the DG (Shioda et al., 2008). Taken together, activation of PI3K/Akt and ERK pathways through ACh receptors in neural precursors probably plays a central role in induction of adult neurogenesis.

In conclusion, we documented that chronic oral administration of ZSET1446 improves decreased neurogenesis seen in

and/or serotonergic pathways play important roles to restore neurogenesis mediated by ZSET1446.

We also demonstrated that ZSET1446 has antidepressant-like effects with concomitant enhanced neurogenesis in OBX mice. It is noteworthy that antidepressants induce increased proliferation of neuronal progenitors and enhance their maturation into neurons in the DG of the hippocampus after chronic but not acute administration. For example, fluoxetine (Malberg and Duman, 2003) and imipramine (Santarelli et al., 2003) increase neurogenesis in hippocampus of rats exposed to stress. In normal rodents, fluoxetine, rolipram, and lithium also enhance neurogenesis after chronic, repeated administration, whereas short-term or single administration has no effect on neurogenesis (Son et al., 2003; Malberg and Duman, 2003). In contrast to the effects of these antidepressant drugs, chronic ZSET1446 administration did not affect cell proliferation in sham-operated mice. However, administrations of 4, but not 2 weeks with donepezil, an AChE inhibitor, enhanced neurogenesis in normal rats (Kotani et al., 2006). Thus, further prolonged administration with ZSET1446 may be required to stimulate hippocampal neurogenesis in normal animals.
OBX mice. Consistent with restored neurogenesis, ZSET1446-treated mice show improvements in OBX-induced depressive behavior. After ACh release in the hippocampus mediated by ZSET1446 administration, activation of Pi3K/Akt and ERK pathways via nAChR stimulation probably mediates improvement of neurogenesis and depressive behavior. In this context, ZSET1446 is an attractive drug that may improve not only cognitive deficits but also depressive behaviors seen in patients with Alzheimer’s disease in the clinic.

References


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