Pharmacology, Biochemistry and Behavior 183 (2019) 6–13

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Memantine ameliorates learning and memory disturbance and the behavioral and psychological symptoms of dementia in thiamine-deficient mice

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ARTICLE INFO

Keywords:
Behavioral and psychological symptoms of dementia (BPSD)
Memantine
Memory
Thiamine

ABSTRACT

Several studies have reported on the beneficial effects of memantine on behavioral and psychological symptoms of dementia (BPSD) in patients with Alzheimer's disease. However, the effects of memantine on BPSD-like behaviors in animals have not been well addressed. Here, the effects of memantine on memory disturbance and BPSD-like behaviors were evaluated in thiamine-deficient (TD) mice. Memantine (3 and 10 mg/kg, b.i.d.) was orally administered to ddY mice fed a TD diet for 22 days. During the treatment period, the forced swimming test, elevated plus-maze test, passive avoidance test, and locomotor activity test were performed. Neurotransmitter levels in the brain were analyzed after the treatment period. Daily oral administration of memantine ameliorated the memory disturbances, anxiety-like behavior, and depression-like behavior observed in TD mice. Memantine did not have a significant effect on monoamine levels, but increased glutamate levels in the hippocampus in TD mice. These results suggest that memantine prevents or suppresses the progression of BPSD-like behaviors that develop due to TD. This effect may be mediated in part by the enhancement of glutamatergic neuron activity in the hippocampus.

1. Introduction

Alzheimer's disease (AD) is the most common cause of progressive dementia, and is characterized by extracellular amyloid plaques consisting of amyloid beta (Aβ) and intracellular neurofibrillary tangles comprising hyperphosphorylated tau protein (Gotz et al., 2001; Hardy and Selkoe, 2002; Holtzman et al., 2011). During the course of the disease, behavioral and psychological symptoms of dementia (BPSD) such as apathy, agitation, aggression, anxiety, depression, and delusions are observed, along with the core symptoms of the condition, namely, cognitive impairment and memory dysfunction (McKeith and Cummings, 2005). BPSD increase the burden on the caregivers of patients with dementia; therefore, reducing behavioral disturbance is important to alleviate the caregivers' burden and psychological distress.

Memantine is widely used as medication for patients with moderate to severe AD and exerts a unique mechanism of action; it is a moderate-affinity, uncompetitive, voltage-dependent, *N*-methyl-D-aspartate (NMDA) receptor antagonist with fast on–off kinetics (Parsons et al., 1993, 2005), leading to selective blockage of the excitotoxic effects in neurons without affecting the physiological transmission required for normal neuronal function (Paoletti et al., 2013; Parsons and Raymond, 2014). Animal studies have indicated that memantine can ameliorate impaired cognition in parallel with a neuroprotective effect or reduction of the brain Aβ burden (Nakamura et al., 2006; Scholtzova et al., 2008). Recent clinical analyses have suggested that memantine provides beneficial effects in terms of behavioral outcomes in patients with AD (Corbett et al., 2012; Huang et al., 2012; Matsuzono et al., 2015); however, little research has been performed on animal models to support the clinical benefits of memantine on BPSD.

Thiamine (vitamin B1) is an essential nutrient involved in glucose and amino acid metabolism, and thiamine deficiency (TD) is associated with neurodegenerative disorders, such as Wernicke-Korsakoff syndrome and AD, in which both neurological and psychiatric symptoms are observed (Harper et al., 1995; Gibson et al., 2016; Lu'o'ng and

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https://doi.org/10.1016/j.pbb.2019.05.008
Received 29 January 2019; Received in revised form 22 May 2019; Accepted 23 May 2019
Available online 05 June 2019

Nguyen, 2011; Thomson, 2000). TD in rodents results in AD-like abnormalities, including neuronal loss, plaque formation, and hyperphosphorylation of tau (Calingasan et al., 1999; Karuppagounder et al., 2009; Zhang et al., 2011), and these animals exhibit memory impairment and BPSD-like behaviors, such as anxiety, depressive behavior, and startle responses (Nakagawasai et al., 2000). Therefore, TD rodents might be a useful model for evaluating pharmacotherapeutic effects on both BPSD and learning and memory dysfunction.

In the present study, we investigated the effects of memantine on memory disturbance and BPSD-like behaviors observed in TD mice, produced by feeding on a TD diet for 22 days, using the passive avoidance test, forced swimming test, and elevated plus-maze test. The neurotransmitter levels in the brain were analyzed on the day after the treatment period.

2. Materials and methods

2.1. Animals

Three-week-old male ddY mice, weighing 11–15 g, were obtained from Japan SLC Inc. (Hamamatsu, Japan) and housed under conditions of constant temperature (23 ± 3 °C) and humidity (55 ± 8%), and a light/dark cycle (12 h/12 h). The mice were housed individually in plastic cages (width: 175 × depth: 245 × height: 125 mm). All experimental procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals approved by the Laboratory Animal Committee of Nihon Bioresearch Inc.

2.2. Drug and diets

Memantine hydrochloride was obtained from Daiichi Sankyo Propharma Co., Ltd. (Tokyo, Japan). Memantine was dissolved in distilled water (DW; Otsuka Pharmaceutical, Tokyo, Japan) for oral administration. The TD and AIN-93G control diets were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). The TD diet was the same as the AIN-93G control diet, but without thiamine.

2.3. Behavioral assessments

2.3.1. Experimental design

TD mice were produced by feeding mice a formulated TD diet. Mice were divided into four groups: the pair feeding control (PF, n = 10), TD (n = 10), memantine (3 mg/kg)-treated TD (n = 10), and memantine (10 mg/kg)-treated TD (n = 10) groups. The animals in the TD and memantine (3 and 10 mg/kg)-treated TD groups were given a TD diet ad lib. In the PF group, the amount of AIN-93G control diet given to the animals was determined on the basis of the average amount eaten by animals on the TD diet because the food intake of animals fed a TD diet is known to decrease significantly.

The experimental schedule was determined in reference to previous reports (Nakagawasai et al., 2004; Nakagawasai, 2005) and our own preliminary studies. In our preliminary studies, a number of animals in the TD feeding group died after day 25; therefore, we conducted this study until day 23 (Fig. 1). DW (10 mL/kg) was orally administered twice daily (9–11 AM and 3–4 PM) for 22 days in the PF group and TD group. Memantine (3 and 10 mg/kg) was orally administered twice daily (9–11 AM and 3–4 PM) for 22 days in the memantine-treated TD groups. The elevated plus-maze test, forced swimming test, and open-field test were performed on days 15, 17, and 20, respectively. Passive avoidance tests were performed on days 21 and 22, and mice were then sacrificed on day 23 for pathological examination and analysis of monoamine and amino acid levels in the brain.

2.3.2. Elevated plus maze test

The elevated plus-maze test was performed on day 15 after the start of TD feeding. The plus-maze was composed of two open arms (8 × 25 cm) and two enclosed arms (8 × 25 × 24 cm), extending from a central platform (8 × 8 cm), facing each other. The entire apparatus was set 60 cm above the floor. The animals were administered either DW or memantine and tested 30 min thereafter. At the beginning of the 5-min test session, each mouse was placed in the central platform, facing one of the open arms. The total number of visits to the closed arms, open arms, and platform, and the cumulative time spent in the closed arms, open arms, and platform, were determined automatically on a monitor using a CCD video camera system. An arm visit was recorded when the center of gravity of the mouse's body entered the arm. All data were analyzed using a video-tracking system (SMART; Panlab, MA, USA). The apparatus was wiped clean and dried after testing each subject.

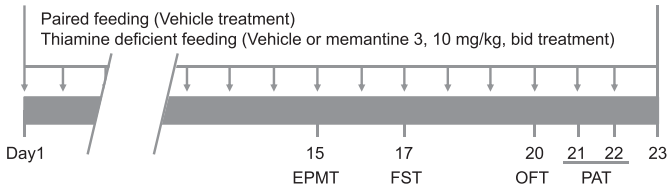

Fig. 1. Treatment schedule and test order.
Thiamine-deficient diet or paired feeding was performed throughout the study. Memantine or vehicle was administered twice daily for 22 days. Behavioral tests were carried out from day 15 to day 22, and the animals were sacrificed on day 23. EPMT, elevated plus-maze test; FST, forced swimming test; OFT, open-field test; PAT, passive avoidance test.

2.3.3. Forced swimming test

The forced swimming test was performed on day 17 after starting TD feeding, in accordance with the method of Porsolt et al. (1977), with some modifications (Makino et al., 1998). The animals were administered either DW or memantine and tested 30 min later. Immediately before the test, a small magnet (1 mm in diameter, 3 mm in length) was attached to both of the hind paws, and then each mouse was placed in a vertical cylinder (height 19 cm; diameter 14.5 cm) containing 10 cm of water that was maintained at 24 ± 2 °C. The total duration of immobility was measured during a 10-min test using an automated behavior analysis system, MicroAct™ (Neuroscience, Tokyo, Japan).

2.3.4. Measurement of locomotor activity

The test was performed on day 20 after the start of TD feeding. Locomotor activity was measured by EthoVision (Noldus Information Technology, Wageningen, Netherlands). The animals were administered either DW or memantine and tested 30 min thereafter. The mice were individually placed in the center of the open-field apparatus (50 × 50 × 30 cm), the floor of which was divided into 25 equal squares (central area: 9 squares, wall area: 16 squares). The total distance moved in the fields and the time spent in the central area and wall area were recorded for 5 min.

2.3.5. Step-through passive avoidance test

Acquisition and retention trials of the passive avoidance task were conducted on days 21 and 22 of TD feeding, respectively. The apparatus consisted of a dark area (width: 240 × depth: 245 × height: 300 mm) with a stainless-steel grid floor and a light area (width: 100 × depth: 100 × height: 300 mm) with an electronic stimulator (Shock Scrambler; Unicom, Kochi, Japan). The acquisition trial was started by placing the mouse in the light compartment for the passive avoidance task. When the mouse had completely entered the dark compartment, an electric shock (0.2 mA for 2 s) was applied. The next day, the mouse was placed in the light compartment again and the time taken to move into the dark compartment was measured as the step-through latency, with a cut-off time of 300 s as a retention trial.

2.4. Biochemical analysis

2.4.1. Sample collection

The sample collection was performed on day 23 of TD feeding. The mice were anesthetized with isoflurane inhalation, exsanguinated, decapitated, and then the brain was dissected out. To analyze the monoamine and amino acid contents, the frontal cortex, striatum, hippocampus, and thalamus were divided from the brain slices using a Rodent Brain Matrix (ASI Instruments Inc., Warren, MI, USA) and each sample was immediately frozen in liquid nitrogen and stored at −80 °C until use.

2.4.2. Measurement of monoamine and amino acid content

To assess the monoamine and amino acid contents, 0.2 N perchloric acid and 1/30 volume of internal standard solution were added to each brain sample; the brain sample was then homogenized and placed on ice for 60 min. The supernatant was separated by centrifugation (20,227 × *g*, 4 °C, 5 min). The levels of monoamines, their metabolites, glutamate, and gamma-aminobutyric acid (GABA) in each sample were measured using a high-performance liquid chromatography apparatus coupled to electrochemical detection (ECD300; Eicom Corporation, Kyoto, Japan).

For the analysis of monoamines and their metabolites, samples were separated using a C18 reverse-phase column (Eicompak SC-5ODS, 150 × 3.0 mm ϕ; Eicom Corporation) with a guard column (PC-04; Eicom Corporation). The electrochemical detector (ECD-300; Eicom Corporation) was equipped with a graphite electrode (WE-3G) that was used at a voltage setting of +750 mV vs. an Ag/AgCl reference electrode (RE-100). The mobile phase consisted of 0.1 M acetic acid/citric acid (pH 3.5)/methanol/sodium 1-octanesulfonate/5 mg/mL ethylenediaminetetraacetic acid disodium salt, 2-hydrate (EDTA-2Na) (820 mL/180 mL/2.2 mL/1 mL). The mobile phase flow rate was maintained at 0.5 mL/min, with a column temperature of 25 °C.

For the analysis of glutamate levels, samples were separated using an ion-exchange column (Eicompak E-GEL, 150 mm × 4.6 mm ϕ; Eicom Corporation) and enzyme column (E-ENZYMPAK, 4 mm × 3.0 mm ϕ; Eicom Corporation) with a guard column (PC-03; Eicom Corporation). The electrochemical detector (ECD-300; Eicom Corporation) was equipped with a platinum electrode (WE-PT) that was used at a voltage setting of +450 mV vs. an Ag/AgCl reference electrode (RE-100). The mobile phase consisted of 60 mM ammonium chloride-ammonia solution (pH 7.2)/hexadecyltrimethylammonium bromide/5 mg/mL EDTA-2Na (1000 mL/250 mg/0.01 mL). The mobile phase flow rate was maintained at 0.37 mL/min, with a column temperature of 33 °C.

For the analysis of GABA levels, samples were separated using a reverse-phase column (Eicompak FA-3ODS, 50 mm × 3.0 mm ϕ; Eicom Corporation) with a guard column (PC-03; Eicom Corporation). The electrochemical detector (ECD-300; Eicom Corporation) was equipped with a graphite electrode (WE-3G) that was used at a voltage setting of +600 mV vs. an Ag/AgCl reference electrode (RE-100). The mobile phase consisted of 0.1 M phosphate buffer (pH 6.0)/acetonitrile/5 mg/mL EDTA-2Na (500 mL/500 mL/1 mL). The mobile phase flow rate was maintained at 0.5 mL/min, with a column temperature of 40 °C.

2.5. Statistical analysis

Data are expressed as mean ± standard error (S.E.M.). Comparisons between the PF and TD groups for all data, and between the TD and memantine (TD) groups for monoamine and amino acid levels were performed using the unpaired Student's *t*-test. Data of behavioral tests were analyzed using one-way analysis of variance and Dunnett's multiple comparison test. SAS System Release 8.2 (SAS Institute, Inc.) was used to perform all of the analyses and *P* values of < 0.05 were considered to indicate statistical significance.

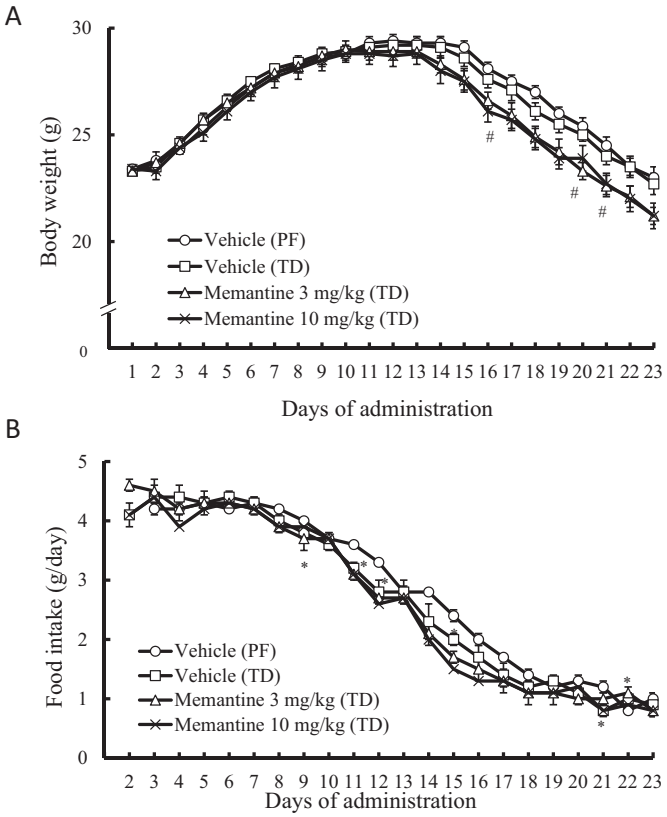

Fig. 2. Changes in body weight and food intake.
Changes in body weight (a) and food intake (b) of mice for 23 days after feeding. Thiamine-deficient (TD) groups were provided a TD diet and water. The PF group was provided an amount of AIN-93G control diet identical to that of the TD mice. Vertical bars represent S.E.M. $N = 10$ per group. $^*P < 0.05$ compared with the vehicle (PF) group (Student's *t*-test). #P < 0.05 compared with the vehicle (TD) group (one-way ANOVA followed by Dunnett's test).

3. Results

3.1. Effects of memantine on physiological changes in TD mice

The effects of memantine on food intake (A) and body weight (B) in TD mice are shown in Fig. 2. The body weight of the PF, TD, and memantine (3 and 10 mg/kg)-treated TD groups gradually increased and reached a plateau around days 10–13, and then gradually decreased. Compared to the TD group, both doses of memantine-treated TD groups showed no significant difference in body weight, except for decreases on days 20 and 21 (3 mg/kg) and on day 16 (10 mg/kg). The food intake of all groups gradually decreased from about 4 g at day 1 to about 1 g on day 23. Compared to the PF group, TD and memantine-treated TD groups showed statistically significant decreases in food intake on days 9, 11, 12, 15, and 21, and an increase on day 22. Compared to the TD group, both doses of memantine-treated TD groups showed no significant changes in food intake. All animals had unkempt fur on day 23, which was not affected by memantine treatment (data not shown).

3.2. Elevated plus-maze task

Compared to the PF group, the TD group demonstrated statistically significant decreases in the number of entries into the open arm and the platform, and the time spent in the open arm and the platform, and a significantly increased number of entries into the closed arm, as well as increased time spent in the closed arm (Fig. 3), suggesting the existence of an anxious state in mice fed a TD diet. Compared to the TD group,

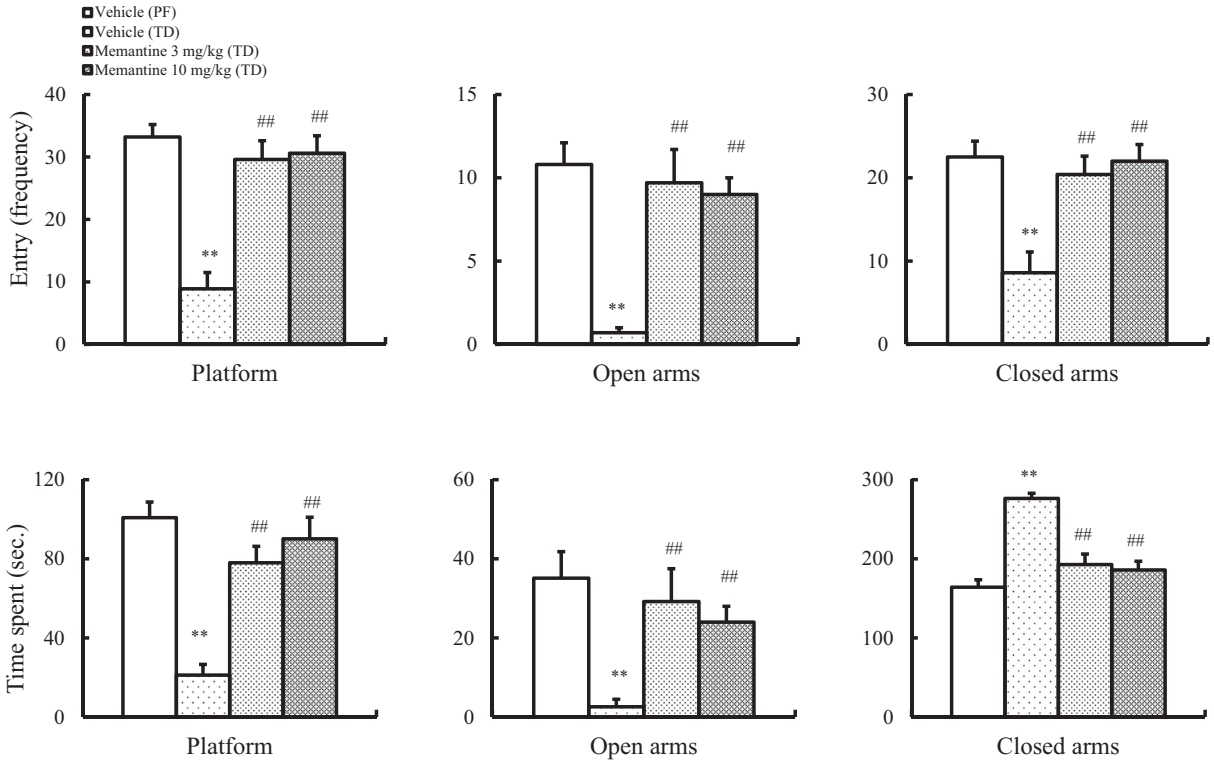

Fig. 3. Effect of memantine on behaviors in the elevated plus-maze test in thiamine-deficient (TD) mice.
The test was conducted on day 15 of TD feeding. The number of entries into platform, open arms, and closed arms (a) and the time spent in the platform, open arms, and closed arms (b) were measured. $N = 10$ per group. Vertical bars represent S.E.M. $^{**}P < 0.01$ compared with the vehicle (PF) group (Student's *t*-test). ##P < 0.01 compared with the vehicle (TD) group (one-way ANOVA followed by Dunnett's test).

both doses of memantine (3 and 10 mg/kg) statistically significantly increased the number of entries into the open arm (3 mg/kg, $p = 0.0003$, 10 mg/kg, $p = 0.0002$; one-way ANOVA) and the platform (3 mg/kg, $p < 0.0001$, 10 mg/kg, $p < 0.0001$; one-way ANOVA), and the time spent in the open arm (3 mg/kg, $p = 0.0015$, 10 mg/kg, $p = 0.0020$; one-way ANOVA) and the platform (3 mg/kg, $p < 0.0001$, 10 mg/kg, $p < 0.0001$; one-way ANOVA), and significantly decreased the number of entries into the closed arm (3 mg/kg, $p = 0.0017$, 10 mg/kg, $p = 0.0004$; one-way ANOVA), as well as the time spent in the closed arm (3 mg/kg, $p < 0.0001$, 10 mg/kg, $p < 0.0001$; one-way ANOVA). These data suggest that memantine prevented the anxious state induced by feeding on a TD diet or exerted an anxiolytic effect.

3.3. Forced swimming test and open-field test

Compared with the PF group, the TD group had statistically significantly increased immobility time in the forced swimming test (Fig. 4), suggesting that a depression-like state was induced in mice fed a TD diet. The TD group also showed statistically significantly reduced spontaneous locomotor activity in the open-field test (Fig. 5A). This might have been due to the restricted feeding-induced increase in locomotor activity in the PF group. There was no significant difference among groups in terms of the time spent in the central area and wall area (Fig. 5B). Both doses of memantine (3 and 10 mg/kg) statistically significantly reduced the immobility time (0–5 min: 3 mg/kg, $p = 0.0005$, 10 mg/kg, $p = 0.0021$; 5–10 min: 3 mg/kg, $p = 0.0032$, 10 mg/kg, $p = 0.0052$; 0–10 min: 3 mg/kg, $p = 0.0008$, 10 mg/kg, $p = 0.0013$; one-way ANOVA), without affecting locomotor activity (3 mg/kg, $p = 0.435$, 10 mg/kg, $p = 0.664$; one-way ANOVA). These results suggest that memantine prevented the depression-like behavior induced by a TD diet or exerted an antidepressant-like effect.

3.4. Passive avoidance test

Compared to the PF group, the latency to enter the dark area was statistically significantly shortened in the TD group, suggesting that TD

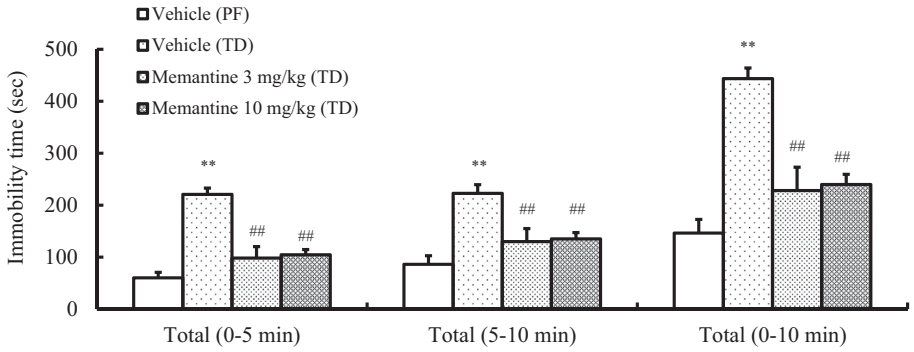

Fig. 4. Effect of memantine on the immobility time in the forced swimming test in thiamine-deficient (TD) mice.
The test was conducted on day 17 of TD feeding. The immobility time was measured during a 10-min test and analyzed during 0–5 min, 5–10 min, and 0–10 min. $N = 10$ per group. Vertical bars represent S.E.M. $^{**}P < 0.01$ compared with the vehicle (PF) group (Student's *t*-test). ##P < 0.01 compared with the vehicle (TD) group (one-way ANOVA followed by Dunnett's test).

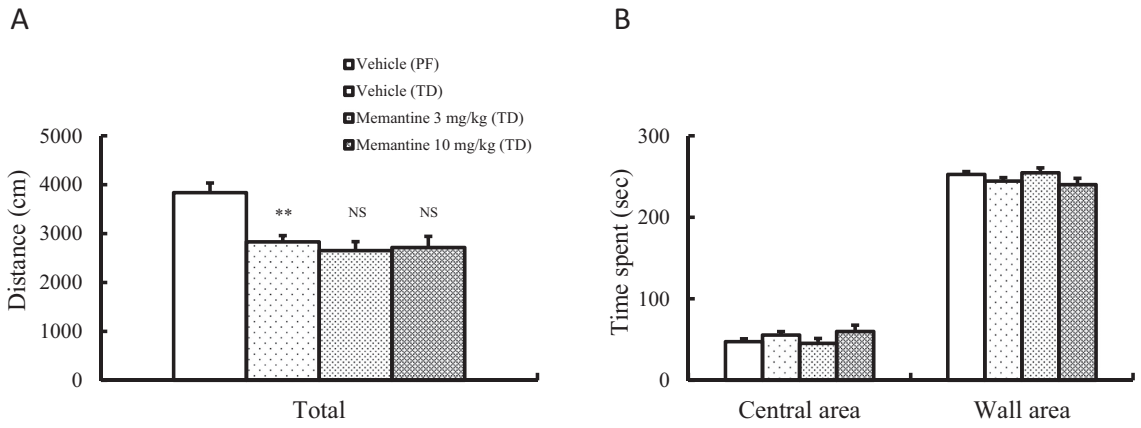

Fig. 5. Effect of memantine on behaviors in the open-field test in thiamine-deficient (TD) mice.
The test was conducted on day 20 of TD feeding. Total distance (a) and the time spent in the central area and wall area (b) were measured. $N = 10$ per group. Vertical bars represent S.E.M. $^{**}P < 0.01$ compared with the vehicle (PF) group (Student's *t*-test). NS: No significant change compared with the vehicle (TD) group (one-way ANOVA followed by Dunnett's test).

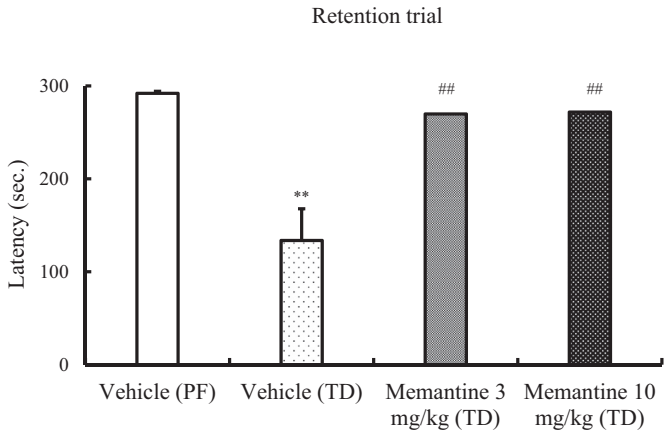

Fig. 6. Effect of memantine on the latency time of the step-through passive avoidance task in the retention trial in thiamine-deficient (TD) mice.
Acquisition and retention trials were conducted on days 21 and 22 of TD feeding, respectively. N = 10 per group. Vertical bars represent S.E.M. **P < 0.01 compared with the vehicle (PF) group (Student's *t*-test). ##P < 0.01 compared with the vehicle (TD) group (one-way ANOVA followed by Dunnett's test).

feeding induced learning and memory impairment (Fig. 6). Both doses of memantine (3 and 10 mg/kg) statistically significantly prolonged the latency for entering the dark area (3 mg/kg, $p = 0.0010$, 10 mg/kg, $p = 0.0008$; one-way ANOVA). This suggests that memantine prevented the learning and memory impairment induced by feeding on a TD diet or alternatively improved learning and memory function.

3.5. Levels of monoamines, their metabolites, glutamate, and GABA in the brain

The levels of monoamines, their metabolites, glutamate, and GABA in the frontal cortex, striatum, hippocampus, and thalamus are indicated in Table 1. Compared to the PF group, the TD group showed a significant reduction of homovanillic acid (HVA, metabolite of dopamine) level in the frontal cortex; reductions of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC, metabolite of dopamine), HVA, and 5-hydroxyindole acetic acid (5-HIAA, metabolite of serotonin) levels in the striatum; and reductions of HVA, 5-HIAA, glutamate, and GABA in the hippocampus and thalamus. Memantine (3 mg/kg) statistically significantly reduced the change in glutamate levels in the hippocampus, and showed a slight or no significant effect against the changes of other monoamines and amino acids that were induced by TD diet feeding.

4. Discussion

In the present study, we demonstrated that memantine exerted a preventive effect or suppressed the progression of the development of memory/learning impairment, depressive behavior, and anxiety resulting from feeding on a TD diet, which might have been mediated in part by the enhancement of glutamatergic neuron activity in the hippocampus.

Memory and/or learning deficiency in TD model animals has been demonstrated using several approaches, including the Morris water-maze test (Inaba et al., 2016), the passive avoidance test, and the elevated plus-maze task (Nakagawasai et al., 2004). In our study, memory/learning impairment induced by TD feeding was detected in the passive avoidance test, against which memantine was confirmed to be effective. The mechanisms of pathogenesis in the TD model are thought to be excitotoxicity involved in hippocampal cell loss (Inaba et al., 2016), astrocytic activation (Hazell, 2009), and increased blood-brain barrier permeability due to impaired endothelial cells (Beauchesne et al., 2010). The neuroprotective effect of the NMDA receptor antagonist MK-801 in TD has previously been suggested (Langlais and Mair, 1990). Memantine has been shown to have a neuroprotective effect that is mediated through NMDA receptor inhibition in neurodegenerative models induced by Aβ and/or excitotoxicity (Nakamura et al., 2006; Nyakas et al., 2011). Furthermore, reduction of brain Aβ plaque by treatment with memantine in several AD animal models has been reported (Alley et al., 2010; Ito et al., 2017; Martinez-Coria et al., 2010). Recently, we proposed an explanation of the mechanisms underlying the actions of memantine: inhibition of Aβ production and Aβ oligomer formation via the regulation of intracellular trafficking of amyloid precursor protein, the inhibition of Aβ aggregation, and the acceleration of Aβ degradation through KLK-7 (Ito et al., 2017; Kidana et al., 2018; Takahashi-Ito et al., 2017). Increased Aβ production and its accumulation in the brain have been reported in the TD model (Mouton-Liger et al., 2015; Zhang et al., 2011), suggesting that Aβ might be involved in the pathogenesis in TD animals. In our study, we fed mice a TD diet for 22 days, which is shorter than the periods previously reported (Ikarashi et al., 2009; Nakagawasai et al., 2004); in addition to brain Aβ plaque and microglial activation, constitutional abnormalities were not observed in histopathological analysis (data not shown). However, the involvement of neurological damage induced by soluble Aβ oligomers in the TD model and the beneficial effects of memantine still remain to be clarified.

Table 1
Effects of memantine on contents of monoamines, monoamine metabolites, glutamate, and GABA in thiamine-deficient mice.

Group	Contents (ng/g wet weight)							Contents (μg/g wet weight)	
	NE	MHPG	DA	DOPAC	HVA	5-HT	5-HIAA	Glutamate	GABA
Frontal cortex									
Vehicle (PF)	617.40 ± 68.76	76.96 ± 5.00	49.14 ± 9.31	26.97 ± 5.71	111.91 ± 16.24	533.50 ± 39.85	247.96 ± 27.30	1632.01 ± 184.51	211.93 ± 41.62
Vehicle (TD)	689.02 ± 62.78	68.04 ± 3.95	32.96 ± 4.57	20.93 ± 3.71	55.47* ± 3.71	552.86 ± 23.48	212.37 ± 18.40	4152.66 ± 2024.88	120.78 ± 11.54
Memantine 3 mg/kg (TD)	614.44 ± 38.29	77.95 ± 16.49	35.52 ± 5.89	21.78 ± 2.63	65.38 ± 3.95	479.10 ± 20.03	192.46 ± 23.72	1552.67 ± 145.05	183.70 ± 65.88
Striatum									
Vehicle (PF)	617.97 ± 114.16	87.58 ± 9.70	12,371.73 ± 349.44	1331.61 ± 146.15	1275.49 ± 106.52	556.98 ± 23.83	531.81 ± 40.19	1175.33 ± 41.86	273.40 ± 19.29
Vehicle (TD)	716.42 ± 99.91	81.34 ± 10.13	9351.90* ± 1010.87	710.90** ± 53.22	605.99** ± 50.15	543.70 ± 44.10	369.51* ± 36.78	998.81 ± 69.49	256.84 ± 20.76
Memantine 3 mg/kg (TD)	565.25 ± 73.74	84.81 ± 19.54	10,365.60 ± 1377.83	893.10 ± 190.44	830.78 ± 108.35	493.93 ± 33.80	429.34 ± 71.42	910.97 ± 38.27	212.58 ± 13.99
Hippocampus									
Vehicle (PF)	868.06 ± 99.69	75.64 ± 10.04	51.19 ± 30.18	11.78 ± 3.59	40.93 ± 5.41	408.63 ± 24.16	401.59 ± 24.83	1283.32 ± 63.58	211.95 ± 21.13
Vehicle (TD)	982.37 ± 57.66	73.10 ± 5.36	20.56 ± 2.54	7.61 ± 0.63	22.40* ± 1.67	477.58 ± 24.82	303.90** ± 12.60	990.76** ± 32.34	152.43* ± 9.84
Memantine 3 mg/kg (TD)	953.75 ± 40.31	86.71 ± 15.69	21.30 ± 2.14	8.29 ± 0.76	27.66 ± 3.94	434.76 ± 40.35	339.40 ± 24.33	1141.39# ± 25.06	164.02 ± 3.14
Thalamus									
Vehicle (PF)	1251.91 ± 82.61	129.54 ± 14.94	211.80 ± 48.39	84.22 ± 8.73	229.08 ± 27.46	612.46 ± 104.05	543.61 ± 18.13	1036.47 ± 30.79	233.83 ± 14.82
Vehicle (TD)	1325.25 ± 85.17	130.69 ± 12.62	109.56 ± 10.39	64.55 ± 4.32	125.56* ± 4.76	553.20 ± 27.55	433.37** ± 18.45	870.59* ± 38.64	188.57* ± 12.27
Memantine 3 mg/kg (TD)	1364.98 ± 71.81	137.71 ± 22.43	138.87 ± 10.19	69.80 ± 6.29	146.18 ± 14.64	611.60 ± 23.41	505.77 ± 55.76	934.75 ± 86.31	230.33 ± 10.13

Data are presented as mean ± S.E.M.
* $p < 0.05$,
** $p < 0.01$ compared with the vehicle (PF) group (Student's *t*-test).
# $p < 0.05$ compared with the vehicle (TD) group (Student's *t*-test).

In addition to memory/cognitive dysfunction, BPSD are a major contributor to a poor quality of life in patients with dementia and also place a burden on their caregivers. In a clinical context, BPSD include four domains: behaviors, such as aggression and agitation; mood, such as depression and anxiety; thought content, such as delusions and illogical thoughts; and perception, such as hallucinations and misidentification (Huang et al., 2012). Although the predominant symptoms of BPSD may vary with different types of dementia, mood changes including anxiety, depression, emotional labiality, and apathy are more common in dementia (Huang et al., 2012; O'Brien, 2003). Cumulative events indicate that memantine has potentially beneficial effects on BPSD based on the Neuropsychiatric Inventory (Cummings et al., 2006; Fox et al., 2012; Gauthier et al., 2008; Tariot et al., 2004). In this study using a TD model, we evaluated the effect of memantine on depressive behavior and anxiety, since these behaviors were reproducibly observed in our preliminary studies. We confirmed that memantine has the potential to show antidepressant-like effects and anxiolytic effects in this model. Nakagawasai et al., (2005) reported that TD model animals exhibit many behavioral changes other than depression and anxiety, such as aggression and convulsions. However, the effects of memantine on these behaviors are unknown because we could not produce these behaviors in this TD model; thus, further experiments will be required to evaluate these behaviors.

In this study, mice were housed individually in cages to control the amount of food intake, since breeding in groups increased fighting behavior among animals, which resulted in variation in animal size and behaviors, especially in the PF group, in the preliminary study. Ikarashi et al. (2009) also evaluated the effects of yokukansan, a traditional Japanese medicine, in this TD model with individual breeding. Taking the clear effects of memantine into consideration, although it is possible that isolation can impact on animal behaviors, we think that it did not influence the effect of memantine in this model. In this context, the question can be raised about whether the effects of memantine in this study were acute or chronic, since each experiment was conducted 30 min after dosing. In our experience, single oral dosing of memantine did not produce a significant anxiolytic effect in the elevated plus-maze test in normal mice (data not shown). Although it is difficult to compare the results between normal mice and mice fed TD, it appears that the effect of memantine in this study might have been produced by a chronic dosing effect rather than an acute effect.

Regarding neurotransmitters, it has been reported that TD feeding reduced brain levels of norepinephrine, serotonin, acetylcholine, glutamate, and GABA, which might have been related to the behavioral changes (Anzalone et al., 2010; Langlais and Mair, 1990; Nakagawasai et al., 2004; Vigil et al., 2010). Since the levels of efficacy of memantine against memory/learning impairment, and depressive and anxiety behaviors were almost the same between the doses of 3 and 10 mg/kg, we considered that the maximum effect was observed at 3 mg/kg and analyzed the levels of neurotransmitters in the brains of the group receiving memantine at 3 mg/kg. In this study, we confirmed that TD feeding induced a dopaminergic deficit in the frontal cortex, striatum, hippocampus, and thalamus; a serotonergic deficit in the striatum, hippocampus, and thalamus; and a glutamatergic deficit in the hippocampus and thalamus. These results suggest that TD feeding induced serotonergic and dopaminergic neuronal dysfunction, and an imbalance of glutamatergic and GABAergic neuronal function. These changes could be related to both memory/learning impairment and BPSD-like behaviors. Memantine reduced the change in glutamate content in the hippocampus, suggesting that it improves imbalanced glutamate/GABA neuronal function. Memantine had no statistically significant effect, but tended to reverse the changes in HVA and 5-HIAA, suggesting that it has the potential to improve serotonergic and dopaminergic neuron function in this model. It has been reported that reduction of NMDA receptor activity combined with $GABA_A$ activation could produce an anxiolytic-like effect in rats (Bertoglio and Carobrez, 2003), and both serotonergic and dopaminergic neuron activation could contribute to antidepressant-like behavior in rats (Rogóz et al., 2002). Ketamine, an NMDA receptor antagonist that has been indicated to confer clinical benefit for patients with major depressive disease, is thought to exert its antidepressant-like effect by glutamatergic activation via the disinhibition of GABAergic function by the inhibition of NMDA receptor on GABA interneurons (Zanos and Gould, 2017). Collectively, these findings suggest that the neurotransmitter changes might be related to improved learning and memory, anxiolytic behavior, and antidepressant-like behavior in the memantine-treated mice.

In conclusion, the results of this study suggest that memantine exerts a preventive effect or suppresses the progression of memory/learning dysfunction and BPSD-like behaviors caused by TD, which might be mediated in part by the enhancement of glutamatergic neuron activity in the hippocampus. These animal study results support the clinical benefit of memantine in the treatment of BPSD.

Declaration of Competing Interest

The authors declared that they have no conflicts of interest.

Acknowledgements

We would like to thank Chikako Nishimoto for her assistance in conducting the study.This study was supported by Daiichi Sankyo Co., Ltd.

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