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NMDA receptor antagonist memantine promotes cell proliferation and production of mature granule neurons in the adult hippocampus

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ABSTRACT

Memantine, which is used clinically for the treatment of Alzheimer's disease (AD), is classified as an *N*-methyl-D-aspartate (NMDA) receptor antagonist. Since previous studies have shown that NMDA receptor antagonists promote neurogenesis in the adult brain, we examined the effect of memantine on neurogenesis in the adult mouse hippocampus. We intraperitoneally injected 3-month-old mice with memantine (at 10 or 50 mg/kg body weight) followed by 5-bromo-2-deoxyuridine (BrdU) injections (3×) after 3 days. We then examined the number of BrdU+ cells in the dentate gyrus (DG) of the hippocampus at different time points. The number of BrdU+ cells in the 50 mg/kg memantine-injected group increased by 2.1-fold (1 day after BrdU-injection), 3.4-fold (after 7 days), and 6.8-fold (after 28 days), whereas the 10 mg/kg dose of memantine had little effect on labeling compared to the control group. Immunohistochemical staining at 28 days after BrdU-injection revealed that the newly generated cells in the 50 mg/kg memantine-group had differentiated into mature granule neurons. Moreover, when 12-month-old mice were injected with memantine, cell proliferation was promoted in the DG (3.7-fold). These findings demonstrate that memantine promotes the proliferation of neural progenitor cells and the production of mature granule neurons in the adult hippocampus.

1. Introduction

Neurogenesis occurs throughout life in various mammalian brains, including the human brain (Altman and Das, 1965; Seki and Arai, 1993; Eriksson et al., 1998; Alvarez-Buylla and Lim, 2004; Maekawa et al., 2005; Namba et al., 2005; Zhao et al., 2008). Neural progenitor cells divide and give rise to new neurons in at least two regions of the adult brain: the dentate gyrus (DG) of the hippocampus and the subventricular zone (SVZ) of the lateral ventricle (Doetsch et al., 1997; Seri et al., 2001). In the hippocampus, neural progenitor cells arising from the subgranular zone (SGZ) migrate into the granule cell layer, where they differentiate into mature neurons that are electrically active and receive synaptic input; these neurons ultimately integrate into the local neural network (van Praag et al., 2002; Schmidt-Hieber et al., 2004). Cumulative evidence has shown that hippocampal neurogenesis plays an important role in hippocampal cognitive functions, including spatial learning and memory (Shors et al., 2001, 2002; Madsen et al., 2003; Snyder et al., 2005; Saxe et al., 2006, 2007; Winocur et al., 2006; Kee et al., 2007).

Interestingly, Jin et al. (2004a,b) observed an increase in the proliferation of neural progenitor cells in the DG of patients with Alzheimer's disease (AD). However, since recent studies have shown that the survival of newly generated cells and the subsequent production of mature neurons are impaired in AD patients (Li et al., 2008) and an AD animal model (Verret et al., 2007), one recent treatment strategy for AD is to replace dead, dying, and dysfunctional neurons with new functional neurons by promoting neurogenesis with effective agents, e.g., growth factors, neurotransmitters, and antidepressants (Kelleher-Andersson, 2006; Chen et al., 2008).

Memantine is one such drug that has been found to be clinically useful for the treatment of AD, but the neurobiological basis of its therapeutic effect is not fully understood. Memantine is known to have an antagonistic effect on *N*-methyl-D-aspartate (NMDA) receptors at therapeutic concentrations and to display a neuroprotective effect both in vivo and in vitro (Bormann, 1989; Seif el

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Nasr et al., 1990; Volbracht et al., 2006). Jin et al. (2006) recently showed that memantine increases cell proliferation by 26% in the DG of adult mice and by 27% in the SVZ, similar to the effects of other NMDA receptor antagonists like MK-801, D(−)-2-amino-5-phosphonopentanoic acid (D-APV), and CGP-43487, which all increase neurogenesis in the developing and adult brain (Cameron et al., 1995, 1998; Gould et al., 1997; Nacher et al., 2001, 2003; Hirasawa et al., 2003; Okuyama et al., 2004). However, since it was unknown whether the newly generated cells in the memantine-treated mice survived and subsequently differentiated into mature neurons, we investigated the proliferation, survival, and differentiation of the newly generated cells in the DG of memantine-treated mice in the present study. Our results clearly showed that memantine promotes the proliferation of progenitor cells and the subsequent production of mature neurons in the SGZ. Based on the above findings, memantine's therapeutic efficacy likely results from both its promotion of neurogenesis and its neuroprotective effect.

2. Materials and methods

2.1. Animals and drug treatment

The animals used in this study were 3-month-old and 12-month-old male C57BL6/J mice (Clea Japan Inc., Tokyo, Japan). All experimental procedures were approved by The Animal Care and Use Committee of the National Institute of Neuroscience.

The 3-month-old mice were injected intraperitoneally (i.p.) with memantine (Sigma, St. Louis, MO) at a dose of 10 or 50 mg/kg body weight, and the 12-month-old mice were injected i.p. with memantine at a dose of 50 mg/kg body weight. Control mice were injected with the same volume of 0.9% saline (Ohtsuka Pharmaceuticals, Tokyo, Japan). Three days later, the mice were injected i.p. with 75 mg/kg body weight of 5-bromo-2-deoxyuridine (BrdU; Sigma) three times at intervals of 2 h. The mice were then sacrificed at 1 day, 7 days, or 28 days after BrdU-injection.

2.2. Tissue preparation

After deeply anesthetizing the mice with sodium pentobarbital (Kyoritsu Pharmaceuticals, Tokyo, Japan), the mice were transcardially perfused with 4% paraformaldehyde containing 0.5% picric acid in 0.1 M phosphate-buffered saline (PBS). The brains were then removed and immersion-fixed for 24 h at 4 °C in the same fixative. After washing in PBS, the brains were successively equilibrated in 5, 10, and 20% sucrose in PBS, embedded in Tissue-Tek optimal cutting temperature compound (Sakura, Tokyo, Japan), and frozen in dry ice. The frozen brains were coronally sliced into 14-μm sections using a cryostat (CM-3000; Leica, Nussloch, Germany) and were mounted on a MAS-coated glass slide (SUPERFROST; Matsunami, Osaka, Japan).

2.3. Immunohistochemistry

The sections were washed with Tris-buffered saline (pH 7.4) containing 0.1% Tween 20 (TBST) and then incubated at 4 °C for 18 h in TBST with one of the primary antibodies shown in Table 1 (mouse monoclonal anti-poly-sialylated neural cell adhesion molecule, PSA-NCAM antibody was provided by Dr. T. Seki (Seki and Arai, 1991)). After washing in PBS, the sections were incubated at 4 °C for 2 h in TBST with an appropriate secondary antibody, shown in Table 1. For immunostaining with anti-BrdU antibody, the sections were boiled in 0.01 M citric acid for 10 min, incubated in 2N HCl at 37 °C for 10 min, and washed in 0.1 M PBS. The sections were examined for fluorescent signals using a confocal laser-scanning microscope (LSM5 Pascal; Zeiss or FV1000; Olympus) or a fluorescence microscope (Axioplan-2; Zeiss).

2.4. Quantitative and statistical analysis of BrdU+ cells

BrdU+ cells throughout the rostro-caudal extent of the DG were counted in every sixth section, and the total number of BrdU+ cells was calculated by multiplying the count in each section by 6 and then totaling the values (Maekawa et al., 2005). The statistical analysis was performed using Microsoft Excel (Office 98) software; a, one-way analysis of variance followed by the Scheffé *post hoc F*-test or Student's *t*-test was used to evaluate the data. All values are expressed as the mean ± S.E.M., and *P*-values less than 0.05 were considered significant.

3. Results

3.1. Memantine promotes cell proliferation and survival in the DG

To investigate the effect of memantine on cell proliferation in the DG, we intraperitoneally injected 3-month-old mice with either a 10 mg/kg or a 50 mg/kg dose of memantine, followed by the injections of BrdU 3 days later. The brains were fixed 1 day after BrdU-injections (Fig. 1A), sectioned, and immunostained with anti-BrdU antibody. As shown in Fig. 1B and C, the total number of BrdU+ cells in the 50 mg/kg memantine-injected group increased dramatically, but no significant difference was seen between the 10 mg/kg memantine-injected group and the control group (Fig. 1C; control, 3742 ± 144 (mean ± S.E.M.), $n = 8$; 10 mg/kg memantine, 3658 ± 243, $n = 8$; 50 mg/kg memantine, 8103 ± 321, $n = 4$). Almost all the BrdU+ cells in the memantine-injected groups and the control group were located in the SGZ (control and 50 mg/kg memantine, Fig. 1B; 10 mg/kg memantine, data not shown). We also confirmed that the proliferating cells in the

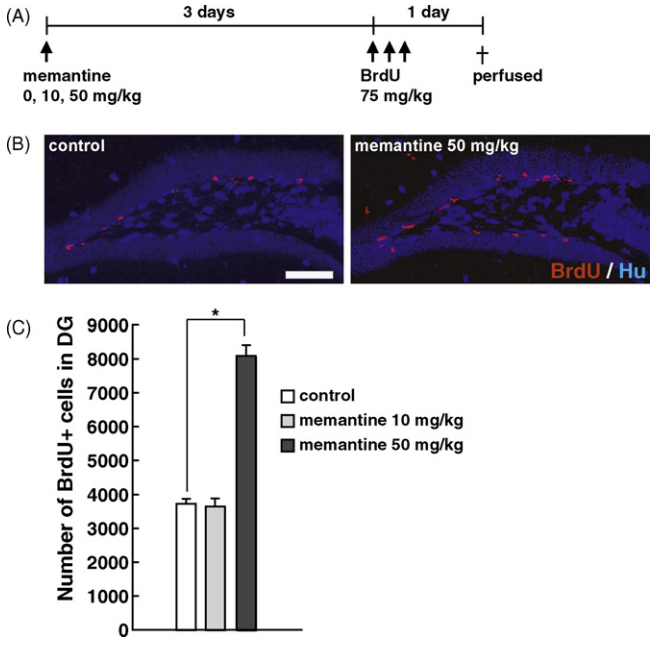

Fig. 1. Effect of memantine on cell proliferation in the hippocampus of 3-month-old mice: (A) Schematic illustration of the experimental design. (B) Representative immunohistochemical staining with anti-BrdU antibody (red) and anti-Hu antibody (blue) in the control group (left panel) and the 50 mg/kg memantine-injected group (right panel). Scale bar = 100 μm. (C) Quantitative analysis of the number of BrdU+ cells in the DG 1 day after BrdU-injection. $^{*}P < 0.1 \times 10^{-9}$, compared with the control group. A Scheffé's *post hoc F*-test was used, and the error bars show the mean ± S.E.M.