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Effects of levetiracetam, an antiepileptic drug, on memory impairments associated with aging and Alzheimer's disease in mice

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ABSTRACT

Emerging evidence suggests that elevated hippocampal activation may be important for disrupting cognitive functions in aged subjects as well as patients with Alzheimer's disease (AD). Therefore, reducing deleterious overactivity of the hippocampus may have therapeutic benefits. This study was designed to compare the effects of levetiracetam, an antiepileptic drug, on memory deficits associated with normal aging and AD in mouse models. Pretraining administration of levetiracetam ameliorated memory impairments of aged C57BL/6 mice (17–20 months of age) in the contextual fear conditioning paradigm. Acute levetiracetam immediately after training was also efficacious in rescuing contextual memory decline in aged mice, whereas administration at a later posttraining interval (3 h) had no effect. These results suggest that suppressing overexcitation with acute levetiracetam around the time of acquisition or early consolidation may be sufficient to reverse memory decline associated with aging. In contrast, pretraining administration of levetiracetam was not able to rescue memory deficits in 5XFAD transgenic mice harboring amyloid plaque pathologies at moderate (6–8 months old) or massive (12–15 months old) levels, differentiating between normal aging- and AD-related memory impairments in the responsiveness to acute levetiracetam treatment.

1. Introduction

Normal aging is often accompanied by a decline in memory functions that depend on the hippocampus, in the absence of age-associated diseases such as Alzheimer's disease (AD). Although the underlying neurobiological mechanisms have not been fully understood, recent evidence suggests that excess neural activity in the CA3 region of the hippocampus occurs in aged rodents with memory impairment when these neurons are unable to encode new information (Wilson, Gallagher, Eichenbaum, & Tanila, 2006; Wilson, Ikonen, Gallagher, Eichenbaum, & Tanila, 2005). Consistent with these findings, studies with functional magnetic resonance imaging (fMRI) have successfully detected increased hippocampal activation such as a specific pattern of hyperactivity in the CA3/dentate gyrus (DG) region in elderly humans with mild cognitive impairment (MCI), a transient phase between normal aging and AD (Ewers, Sperling, Klunk, Weiner, & Hampel, 2011; Yassa et al., 2010). Moreover, it is demonstrated that treatments targeting elevated neuron activity, including administration of low-dose antiepileptic agents, can improve cognition in aged rats (Koh, Haberman, Foti, McCown, & Gallagher, 2010; Koh, Rosenzweig-Lipson, & Gallagher, 2013) and patients with MCI (Bakker et al., 2012), supporting the idea that the overactive hippocampal system may represent a dysfunctional condition leading to memory impairments associated with aging or MCI.

Intriguingly, the neuronal overexcitation is also hypothesized to play a role in progression on the path to AD conditions (Gallagher & Koh, 2011). For example, hippocampal hyperactivation during memory testing in individuals with MCI has been reported to predict greater subsequent cognitive decline and conversion to AD-related neurodegeneration (Miller et al., 2008; Putcha et al., 2011). Recent investigations of transgenic mice overexpressing amyloid precursor protein (APP) have revealed amyloid-β (Aβ)-associated hyperactive neurons and aberrant excitatory network activity or nonconvulsive seizures in the hippocampus and cerebral cortex (Busche et al., 2008; Minkeviciene et al., 2009; Palop & Mucke, 2010; Palop et al., 2007; Ziyatdinova et al., 2011), although a causal link between the hyperexcitability and memory deficits in these AD models remains unclear. In this study, we examined the effects of pretraining or posttraining administration of levetiracetam, an antiepileptic drug, on memory impairments associated with normal aging in C57BL/6 mice. Furthermore, the effects were compared with those on memory deficits in 5XFAD transgenic mice, which provide a rapid-onset and aggressive amyloid model based on a combination of five familial AD (FAD) mutations and

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the consequent acceleration of Aβ42 production (Oakley et al., 2006; Ohno et al., 2006, 2007). We found that an acute injection of levetiracetam around the time of acquisition or early consolidation is efficacious in rescuing memory decline in aged C57BL/6 mice in the contextual fear conditioning task, while it has no effects on memory deficits in 5XFAD mice at pathological stages developing moderate (6–8 months of age) to massive (12–15 months of age) levels of Aβ plaque deposition.

2. Materials and methods

2.1. Animals

We used a similar number of male and female C57BL/6 mice (Taconic, Hudson, NY, USA) to test memory decline associated with normal aging. As a transgenic mouse model of AD, we used 5XFAD mice (Tg6799 line) that co-overexpress FAD mutant forms of human APP (the Swedish mutation: K670N, M671L; the Florida mutation: I716V; the London mutation: V717I) and presenilin 1 (PS1) (M146L; L286V) transgenes under transcriptional control of the neuron-specific mouse Thy-1 promoter (Oakley et al., 2006; Ohno et al., 2006, 2007). 5XFAD lines were maintained by crossing hemizygous transgenic mice to C57BL/6 breeders (Taconic), and 5XFAD hemizygotes were used for the experiments with non-transgenic wild-type littermate mice served as controls. Genotyping was performed by PCR analysis of tail DNA. Since our previous study shows that there is no sex difference in cerebral Aβ levels in 5XFAD mice except for the younger age (⩽3 months) (Oakley et al., 2006), both males and females were used to examine the effects of levetiracetam in this model at 6–8 and 12–15 months of age. All experiments were conducted according to National Institutes of Health guidelines and approved by the Nathan Kline Institute Animal Care and Use Committee.

2.2. Drug treatments

Levetiracetam was purchased from Sigma–Aldrich Corporation (St. Louis, MO, USA) and dissolved in saline. Mice received a single intraperitoneal (i.p.) injection of levetiracetam or saline vehicle in a volume of 1 ml per 100 g of body weight. The doses of levetiracetam (10–20 mg/kg) were chosen on the basis of a previous behavioral study (Koh et al., 2010). Mice were treated with levetiracetam or saline 30 min before training for the contextual fear conditioning. In a separate set of experiments, levetiracetam or saline was administered immediately or 3 h after training to determine a critical time window in which this drug may exert beneficial effects on memory consolidation processes. Experiments were done blind with respect to the drug treatments as well as the genotype of mice.

2.3. Contextual fear conditioning

Contextual fear conditioning was tested as described previously (Kimura, Devi, & Ohno, 2010; Kimura & Ohno, 2009; Ohno, Frankland, Chen, Costa, & Silva, 2001). The experiments were performed using four standard conditioning chambers, each of which was housed in a soundproof isolation cubicle and equipped with a stainless-steel grid floor connected to a solid-state shock scrambler. Each scrambler was connected to an electronic constant-current shock source that was controlled via an interface connected to a Windows XP computer running FreezeFrame software (Coulbourn Instruments, Allentown, PA, USA). A digital camera was mounted on the steel ceiling of each chamber, and video signals were sent to the same computer for analysis. During training, mice were placed in the conditioning chamber for 3 min and then received two unsignaled footshocks (1.0 mA, 2 s) at 1-min intervals. After the last shock delivery, mice were left in the chamber for another 30 s. Contextual fear memory was evaluated by scoring freezing behavior (the absence of all movement except for that needed for breathing) for 3 min when the mice were placed back into the same conditioning chamber 24 h after training. The automated FreezeFrame system (Coulbourn Instruments), which digitizes the video signal at 4 Hz and compares movement frame by frame, was used to score the amount of freezing.

2.4. Aβ immunohistochemistry

Mice were transcardially perfused with 0.1 M phosphate buffered saline (PBS, pH7.4), followed by 4% paraformaldehyde in PBS under deep isoflurane anesthesia. Brains were postfixed for 24 h in 4% paraformaldehyde in PBS at 4 °C, and transferred to PBS. The brain was sectioned coronally at 30 μm using a vibratome (VT1200, Leica Microsystems, Wetzlar, Germany), and successive sections were stored in PBS containing 0.01% sodium azide at 4 °C. The sections collected from four mice per group, at levels between −1.7 and −1.9 mm to bregma according to the mouse brain atlas of Franklin and Paxinos (2008), were stained by the avidin–biotin peroxidase complex method as described (Devi, Alldred, Ginsberg, & Ohno, 2010; Devi & Ohno, 2010; Kimura et al., 2010). Briefly, the sections were incubated overnight at 4 °C with monoclonal anti-Aβ 1–16 antibody (1: 200, 6E10; Signet, Dedham, MA, USA). The ABC kit (PK-2200; Vector Laboratories, Burlingame, CA, USA) was utilized with 3,3′-diaminobenzidine tetrahydrochloride as a chromogen to visualize the reaction product. The sections were then mounted on charged slides, dehydrated in a series of alcohol, cleared in xylene and covered with a coverslip. Light microscopy was conducted on an Axioskop 2 microscope equipped with an AxioCaM HRc digital camera (Zeiss, Munich, Germany) for capturing images.

2.5. Statistical analysis

The significance of differences between the groups was determined by a one-way or two-way ANOVA, and *post-hoc* Fisher's PLSD tests were performed when appropriate. Data were presented as mean ± SEM and the level of significance was set for p value less than 0.05.

3. Results

3.1. Effects of levetiracetam on memory deficits associated with aging in C57BL/6 mice

We first characterized aging-related memory decline in C57BL/6 mice in the contextual fear conditioning paradigm (Fig. 1), in which mice learn to associate a distinct context (CS: conditioned stimuli) with aversive footshocks (US: unconditioned stimuli) through hippocampus-dependent mechanisms (Fanselow, 2000; Maren, 2001). Young-adult mice (3–5 months of age) exhibited a robust conditioned fear response as assessed by freezing (the absence of all but respiratory movements) when placed back into the conditioning chamber 24 h after training with two CS–US parings (Fig. 1A). A one-way ANOVA revealed age-dependent reductions of freezing levels in C57BL/6 mice ($F(3,43) = 5.08$, $p < 0.05$), representing significant impairments in contextual fear memory at ⩾17 months of age ($p < 0.05$).

We next examined the effects of pretraining administration of levetiracetam, an antiepileptic drug, on memory decline in C57BL/6 mice at 17–20 months of age (Fig. 1B). A two-way ANOVA for contextual freezing levels revealed significant main effects of