Positive impact of levetiracetam on emotional learning and memory in naive mice

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A B S T R A C T

Aims: The effect of an antiepileptic drug on cognitive function is of primary importance with respect to the patient’s quality of life. Levetiracetam (LEV) is a novel antiepileptic drug used to treat epilepsy, but its effects on spatial and emotional learning and memory are not yet well understood. The goal of our study was to establish the effects of LEV (17 and 54 mg/kg, intraperitoneally (IP)) on spatial memory retrieval in the Morris water maze test and on acquisition and memory formation in the passive avoidance (PA) test in naive mice.

Main methods: The subjects were adult male BALB/c mice. Spatial learning and memory was established with the Morris water maze (MWM) test. The ‘time spent in escape platforms quadrant’ and the ‘distance to platform’ analyses were measured using a video tracking system to determine spatial memory function. Emotional learning and memory were determined with a one-trial, step-through passive avoidance test.

Key findings: In the MWM test, LEV (17 and 54 mg/kg) neither affected the time spent in the target quadrant nor altered the distance to platform. Moreover, LEV had no effect on swim speed. In the PA task, LEV (17 and 54 mg/kg) significantly prolonged retention latency.

Significance: Our results indicate that LEV did not alter spatial memory retrieval in the MWM test, but it did show some ameliorating effects on acquisition and memory formation in the PA test in naive mice.

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Introduction

Impaired memory is among the most common complaints of epileptic patients. Multiple factors may contribute to memory impairment in epileptic patients. There is evidence from animal and healthy volunteer studies supporting an independent potential for antiepileptics to impair memory (Motamedi and Meador, 2004; Shannon and Love, 2007).

Levetiracetam (Keppra) (LEV), a novel antiepileptic drug, is a pyrrolidine derivative characterized by a unique mechanism of action that involves binding to the synaptic vesicle protein 2A (SV2A) (Lynch et al., 2004).

The effects of LEV on cognitive functions were evaluated in previous studies in different animal models. In one study, the effects of LEV on cognitive function were studied in normal and amygdala-kindled rats with the Morris water maze (MWM) test. It was reported that doses of LEV known to suppress motor seizures did not alter cognitive performance. It is suggested that LEV may be devoid of any negative impact on cognition in epileptic patients (Lamberty et al., 2000).

Additionally, the effects of LEV on visual spatial memory following status epilepticus were investigated. It was concluded that LEV treatment resulted in less histological damage in the hippocampus and had no effect on visual–spatial function or place cell physiology in either control or status epilepticus rats (Zhou et al., 2007).

A delayed spatial alternation behavior method was used to evaluate the effects of new antiepileptic drugs (AEDs) on working memory in nonepileptic rats, and it has been reported that LEV had no effect on working memory function (Shannon and Love, 2004).

A recent study reported that brivaracetam, a novel high affinity SV2A ligand, does not alter spatial learning and memory in both normal and amygdala-kindled rats in the place learning version of the MWM test (Detrait et al., 2010).

In a study of scopolamine-induced amnesia, in which a one-trial passive avoidance (PA) step-through type test was used, mice received scopolamine either alone, in combination with LEV or in combination with a nootropic agent. Unlike oxiracetam and rolziracetam, both piracetam and LEV significantly increased retention latencies, and therefore actively antagonized the amnesic effect of scopolamine. LEV was reported to be effective only at higher doses (Verloes et al., 1988).

It was reported that behavioral alterations, such as the behavioral hypereexcitability and learning deficits in epileptic rats, were not affected by treatment with LEV after status epilepticus (Brandt et al., 2007).
Physical, motor, cognitive and teratogenic effects of LEV exposure throughout pregnancy in rats have been established. It has been reported that LEV had only a transient impact on reflex maturation and no impact on physical and cognitive function in the offspring of rats exposed to the drug during pregnancy (Ozyurek et al., 2010).

The results of a few short-term clinical studies are also available. A preliminary study with chronic epilepsy patients reported no significant cognitive effects during levetiracetam treatment (Neyens et al., 1995). AEDs have also been shown to induce cognitive deficits in healthy individuals. Carbamazepine, phenytoin, and valproate have been reported to adversely affect cognition to a similar extent (Meador et al., 1995).

In general, memory processes are divided into three parts: learning acquisition, memory consolidation and retrieval. This study was designed to investigate the effects of LEV on spatial memory retrieval in the MWM test and on acquisition and memory formation in the PA test.

People with epilepsy may have different types of learning and memory problems. Memory impairment may depend on the seizure frequency, seizure type, duration of the disorder or adverse effects of the antiepileptic medication. However, the question persists concerning the mechanism by which novel antiepileptic drugs (AEDs) affect learning and memory functions that are unrelated to changes caused by epilepsy. In a previous study from our laboratory in which we used naïve (nonepileptic) animals, we showed that gabapentin enhances cognitive performance in mice based on the MWM test, the PA test and the modified elevated plus–maze test (Celikyurt et al., 2011).

In this study, we tried to determine the effects solely due to LEV on learning and memory in naïve (non-epileptic) animals, which were preferred to distinguish these effects from those associated with epilepsy. The purpose of this study was to investigate the effects of LEV (17 mg/kg and 54 mg/kg) on spatial memory and emotional learning and memory in the MWM and PA tests in naïve (non-epileptic) mice.

Materials and methods

Drugs

LEV was purchased from Sigma Chemical Company (Sigma, St. Louis, MO) and dissolved in saline. LEV was freshly prepared and administered via the intraperitoneal (IP) route in a volume of 1 ml per 10 g of mouse body weight. The selected doses did not cause any significant change in the swim speed of mice (which reflects locomotor activity) and were selected based on previously reported literature data (Lamberty et al., 2000). The control group received saline. LEV (17 and 54 mg/kg) was administered IP 60 min before the probe trial of the MWM test to evaluate the effects of LEV on spatial memory retrieval function. In addition, LEV (17 and 54 mg/kg) was administered IP 60 min before the acquisition session of the PA test to determine the effects of LEV on acquisition and memory formation.

Animals

Male BALB/c mice (Istanbul University Research Center, DETAM, Turkey) weighing 35–45 g were housed four to five per cage (L30 × W20 × H12.5 cm) in an animal colony facility for 2 weeks prior to the start of the experiment. The animals were maintained at a constant room temperature (22 ± 2 °C) under a 12-h light/dark cycle (light onset at 0700 h). Tap water and food pellets were provided ad libitum. All animals were naïve to the tests. Each mouse was tested individually and only once. A total of 55 mice were used in the study. Experiments were conducted between 1000 and 1400 h. All procedures complied with the European Community Council Directive of 24 November 1986, and the Ethics Committee of Kocaeli University granted ethical approval (Number: KOU/HADYEK-6/1-13, Kocaeli, Turkey).

Behavioral Studies

Morris water maze test

The MWM is a circular pool (90 cm in diameter and 30 cm in height) that is filled to a depth of 14 cm with water (22 °C) and rendered opaque by the addition of small black balls. The pool was located in a semi-soundproof, dimmed test room that was illuminated with a table lamp (80 lx); a number of extra-maze visual cues, including a white and black colored poster on the wall, a camera and the experimenter were also present. The maze was divided into four quadrants; three equally spaced points were used as starting positions around the edge of the pool. The order of release positions varied systematically throughout the experiment. An escape platform (6 cm in diameter and 12 cm in height) was located in one quadrant 1 cm above the water surface during the familiarization session and 1 cm below the water surface during other sessions. Video tracking was conducted with a video camera focused on the entire pool. Navigation parameters were analyzed using the ETHOVISION 3.1 video analysis system (Noldus). Mice were trained during 5 daily sessions (S1, S2, S3, S4, and S5). One familiarization and four acquisition sessions were performed. During the familiarization session and acquisition phase, each mouse was given three trials. The delay between trials was 60 s, and a 1-day interval was used between sessions. For each daily trial, mice were taken from the home cage and placed into the maze at one of 3 randomly determined locations with its head facing the center of the maze. After the mouse had found and climbed onto the platform, the trial was stopped and the escape latency was recorded. If the mouse had not climbed onto the platform within 60 s, the trial ended; in these cases, the experimenter guided the mouse to the platform, and an escape latency of 60 s was recorded (Cachard-Chastel et al., 2008). Twenty-four hours after the last acquisition session, a probe trial was used to assess the mouse’s spatial retention of the location of the hidden platform. During this trial, the platform was removed from the maze and the mouse was allowed to search the pool for 60 s. The percentage of time spent in each quadrant was recorded. To determine spatial cognitive function, the time spent in the escape platform’s quadrant and the distance to platform analyses were measured; the swim speed parameter was used to assess motor function.

Passive-avoidance test

Animals were trained in a one-trial, step-through PA apparatus for evaluating memory based on contextual fear conditioning and instrumental learning (Phillips and LeDoux, 1992; Yan et al., 2001). The apparatus consisted of a box with an illuminated part (L7 × W12.5 × H14 cm) and a dark part (L24 × W12.5 × H14 cm), both equipped with a grid floor composed of steel bars (0.3 cm diameter) spaced 0.9 cm apart. The inhibitory avoidance task consisted of two trials. On the first day of training, mice were individually placed in the light compartment and allowed to explore the boxes. The inter-compartment door was opened after a 60 s acclimation period. In the acquisition trial, each mouse was placed in the illuminated compartment, which was lighted by a bright bulb (2000 lx). The animals received levetiracetam doses prior to acquisition training. If the mouse stepped into the dark compartment (defined as 2/3 of the tail in the dark compartment), the door closed automatically, and an inescapable foot shock (0.25 mA/1 s) was delivered through the grid floor of the dark compartment. A cutoff time of 5 min for the mouse to enter the dark compartment was selected. The time taken to enter the dark compartment (training latency) was recorded. Immediately after the shock, the mouse was returned to the home cage.

The retention trial (second day) started 24 h after the end of the acquisition trial. Each mouse was placed in the illuminated compartment as in the training trial. The door was opened after a 30 s acclimation period. The step-through latency in the retention trial (with a maximum 300 s cutoff time) was used as the index of retention of
the learned experience. The electric shock was not applied in the retention trial. A decrease in retention latency indicates memory impairment in the PA task.

Statistical analyses

Statistical analyses were performed using one-way analysis of variance (ANOVA) with a post-hoc Tukey for the PA and MWM tests. Data are expressed as the mean values±SEM. Differences were considered to be statistically significant when \( p < 0.05 \).

Results

Effects of LEV in the Morris water maze test

a. Effects of LEV on the time spent in target quadrant

No significant difference was observed between the LEV administered groups in the time spent in the target quadrant (one way ANOVA, post hoc Tukey; \( n = 9–10, F(2,27) = 1.99, p > 0.05 \)). LEV (17 and 54 mg/kg) did not have any significant effect on the time spent in the target quadrant compared to the control group (Fig. 1).

b. Effects of LEV on the distance to platform

The mean distance to platform in the probe trial of the MWM test was not significantly different between the groups in the LEV treated group (one way ANOVA, post hoc Tukey test; \( n = 9–10, F(2,27) = 0.096, p > 0.05 \)). LEV (17 and 54 mg/kg) did not alter the distance to the platform of mice compared with the control group (Fig. 2).

c. Effects of LEV on motor function

Each treatment did not result in significant differences in the swim speed in the LEV treated group (one way ANOVA, post hoc Tukey; \( n = 9–10, F(2,27) = 2.78, p > 0.05 \)). LEV (17 and 54 mg/kg) had no effect on the swim speed of the animals compared with the control group (Fig. 3).

Effects of LEV on the passive avoidance test

Although LEV (17 and 54 mg/kg) did not affect the acquisition (1 day) latency (ANOVA followed by post-hoc Tukey; \( n = 8–10, F(2,27) = 0.76, p > 0.05 \)) (Fig. 4), it significantly prolonged the retention latency compared with the control group (\( n = 8–10, F(2,27) = 5.95, p < 0.05 \)) (Fig. 5).

Discussion

In this study, LEV (17 and 54 mg/kg) neither affected the time spent in the target quadrant nor altered the distance to platform compared to the control group. These findings indicate that LEV, at the doses we used in our study, does not have an effect on spatial memory retrieval in the MWM test.

The first test we used in this study was the Morris water maze (MWM) test. This is a widely used experimental procedure in behavioral neuroscience to study spatial learning and memory functions.
in rats and mice (D’Hooge and De Deyn, 2001). In this test, mice can use three different strategies (Brandeis et al., 1989) to locate the escape platform. Firstly, the animal learns the sequence of movements needed to reach the platform. Secondly, the animal uses cues or visual proximal guides to reach the platform. Thirdly, the animal reaches the target using a spatial strategy involving information about the spatial location of the platform according to the spatial configuration of a distal cue. Spatial memory is conceptualized as a subtype of episodic memory because it stores information within the spatiotemporal frame and depends on the hippocampus (Sharma et al., 2010).

Our findings indicate that LEV did not affect the spatial memory retrieval in the MWM test. The effects of LEV (when administered following status epilepticus) on cognitive function and the place cell firing patterns of adult male rats were studied (Zhou et al., 2007). It has been reported that LEV had no major effects on water-maze performance, which supports our findings. Place cell function had no effect on visual–spatial function or place cell physiology in rats. The effect of LEV and other AEDs on the spatial learning performance of rats was investigated in a maze. At doses known to suppress motor seizures (17 and 54 mg/kg), LEV did not alter cognitive performance in fully amygdala-kindled rats; higher doses of LEV (170 mg/kg) also did not produce this effect. In contrast, valproate, clonazepam, phenobarbital and carbamazepine all induced cognitive impairment (Lamberty and Klitgaard, 1998).

In this study, we evaluated LEV’s effect on memory retrieval by administering the drug before testing each group on the probe trial of the MWM test. During this trial, the platform was removed from the maze and the mouse was allowed to search the pool. We found that LEV does not alter spatial memory retrieval in the MWM test.

The involvement of different neurotransmitters and modulator systems in spatial learning was reviewed and it was suggested that only the cholinergic, glutamatergic and some peptidergic systems may be required for this type of learning (McNamara and Skelton, 1993). Unlike other AEDs, LEV is devoid of any negative impact on memory performance in preclinical models (Lamberty et al., 2000). The particular neuropharmacological interactions that form the basis of LEV’s effects on different types of learning and memory should be further investigated.

The second test we used in this study is the PA test. This is an emotional learning and memory test. Emotional memory is a special category of memory involving the implicit learning and storage of information about the emotional significance of events; it is studied in rodent experiments using aversive classical conditioning techniques. The thalamo-amygdala pathway contains and uses glutamate for synaptic transmission, suggesting the possibility that an amino acid mediated form of synaptic plasticity is involved in the emotional learning functions (LeDoux, 1993).

In the PA task, a decrease in retention latency indicates memory impairment (Raghavendra and Kulkarni, 2001). In the present study, in the PA task, LEV (17 and 54 mg/kg) significantly prolonged the retention latency of the animals, indicating that the mice remembered the electrical shock after exposure.

The regulation of several neurotransmitters is thought to control emotional learning and memory. The cholinergic system has been shown to influence cognitive and behavioral functions in a variety of ways, and cholinergic innervation of the amygdala is involved in the modulation of emotional memory storage (McGaugh, 2004).

Behavioral experiments have shown that the N-methyl D-aspartate (NMDA) subclass of glutamate receptors plays an important role in the acquisition of emotional memory (Yagi et al., 1998). In the modulation of memory storage, catecholamines, epinephrine, and corticosteroids play an important role (Cahill and McGaugh, 1996).

It is known that LEV is different in its mechanism of action from other AEDs. Levetiracetam reduces high voltage Ca\(^{2+}\) currents in vitro (Niespodziany et al., 2001), and it also appears to be devoid of direct GABAergic (GABA) effects (Margineanu and Klitgaard, 2003). It was reported that LEV attenuates hippocampal expression of synaptic plasticity-related early and late response genes in amygdala-kindled rats (Christensen et al., 2010).

It was recently established that the most relevant mechanism of action of LEV is its binding to the synaptic vesicle protein SV2A. The SV2A binding affinity of LEV derivatives correlated strongly with their binding affinity in the brain, as well as with their ability to protect against seizures in the audiogenic mouse model (Lynch et al., 2004).

The specific effect of LEV binding to SV2A appears to be a reduction in the rate of vesicle release (Yang et al., 2007). LEV has other mechanisms of action that likely play a smaller role. Examples include reversing the inhibition of neuronal GABA and glycine-gated currents by the negative allosteric modulators zinc and β-carbolines (Rigo et al., 2002) and partial depression of the calcium current (Niespodziany et al., 2001). Several AEDs were tested for their effects on LTP formation and maintenance. Reports indicate that phenobarbital or valproic acid is able to block NMDA-dependent LTP, but the SV2A ligand levetiracetam did not share such effects (Lamberty et al., 2000).

In this study, the animals received the drugs prior to training for the PA test; consequently, the acquisition and memory formation were ameliorated in the PA test. The promising effects of LEV on emotional learning and memory in the PA test could be explained by a different mechanism of action of LEV, but this speculation should be confirmed by further studies.

In the present study, we evaluated the effects of LEV on spatial memory retrieval and emotional learning and memory in naive mice, which is independent of a pathology such as epilepsy.

In conclusion, LEV does not alter spatial memory retrieval in the MWM test but has some ameliorating effects on acquisition and memory formation in the PA test in naive mice. Additional studies should be performed to further delineate relative effects of novel AEDs on learning and memory functions. An investigation of the effects of chronic LEV treatment and a comparison of LEV with at least one conventional antiepileptic drug, employing the same tasks both in naive animals and in epilepsy models are needed.

Conflict of interest statement

The authors declare no conflict of interest.

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