Levetiracetam induction of theta frequency oscillations in rodent hippocampus in vitro

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Abstract

Background Levetiracetam (LEV), an antiepileptic drug, has been recently demonstrated to improve the cognitive function. Hippocampal theta rhythm (4–12 Hz) is associated with a variety of cognitively related behaviors, such as exploration, locomotion and spatial memory in both humans and animal models. We investigated the effects of LEV on the theta rhythm in the rat hippocampal CA3 area. **Results** We found that LEV increased the power of theta oscillation in a dose-dependent manner. The increase in theta power can be blocked by GABAA receptor or NMDA receptor antagonists but not by AMPA receptor antagonist, indicating the involvement of GABAA receptor and NMDA receptor in the induction of theta activity. Interestingly, LEV enhancement of theta power can be also blocked by taurine, indicating that LEV induction of theta may be related to the indirect boosting of GABA action via reduction of extrasynaptic GABAA receptor activation. Furthermore, the increased theta power can be partially reduced by mACh receptor antagonist atropine but not by nACh receptor antagonists, suggesting that mACh receptor activation provides excitatory input into local network responsible for LEV induction of theta. **Conclusions** Our study demonstrated that induction of a synchronized network oscillation, a novel role of LEV may especially benefit for the treatment of the neuronal disorders with impaired theta oscillation and cognitive function.

Background

Hippocampal theta rhythm (4–12 Hz) plays a key role in cognitive processes in both humans and animals [1, 2] and is associated with a variety of cognitively related behaviors, including spatial coding, memory and sniffing exploratory locomotion[3]. The decreased theta activity is correlated with cognitive deficit seen in neurodegenerative disease such as epilepsy and Alzheimer's disease (AD)[4, 5, 6, 7].

The pharmacological induction of theta rhythm is determined by local neuronal circuits activated by specific receptor agonists including both metabotropic acetylcholine receptor (mAChR) and nicotinic acetylcholine receptor (nAChR) agonists in hippocampus[8, 9], suggesting that cholinergic neurotransmission play a role in hippocampal theta generation.

Levetiracetam (LEV) is an anti-epileptic drug for the treatment of partial onset and generalized seizures [10, 11]. Recently, LEV has been demonstrated to improve cognitive impairment of an Alzheimer's disease model [12] and to have a neuroprotective effects on brain injury [13, 14, 15] at its therapeutic plasma concentration (35–118μM) [16].

Accumulated evidence indicates that LEV improves the impairment in patients with amnesic mild cognitive impairment by reducing the hyperactivity of the hippocampus [17] and spatial memory impairment in a mouse kindling model and rat pilocarpine model of temporal lobe epilepsy(TLE) [18, 19]. It has also been reported that LEV can improve the memory function defects of a ketamine-induced schizophrenia model [20].

GABA_A receptor (GABA_AR) appears to be critical for pharmacologically induced theta oscillations [8, 9, 21, 22, 23]. Taurine, rich in the brain, is an inhibitory amino acid, potently activates GABA_AR in rat hippocampal CA1 area and consequently modulates theta via activating GABA_AR [24, 25]. LEV has been reported to enhance GABA_AR function [26], it is likely LEV modulates theta activity, which maybe affected by taurine, as there were negative effects of LEV on taurine production[27], which could indirectly boost GABA action via extrasynaptic GABA_AR activation and increase network activity.

Whether LEV affects theta oscillation has not yet been reported. In this study, we found that LEV dramatically enhanced theta oscillations of the rat hippocampal CA3 area.

Results

LEV-induced theta oscillations in hippocampal slices

The basal activity of field potentials were recorded at CA3 area of rat hippocampal slices, perfusion of aCSF for 80 min had no effect on basal activity of field potentials (n = 4, Fig. 1A-B). The application of LEV (3–100 μM) induced persistent theta oscillation (4–12Hz) in CA3 area of rat hippocampal slices. Examples of LEV-induced theta oscillation were shown in figure 1C-F. The peak frequency of LEV-induced theta oscillation was 8.1 ± 1.6 Hz (vs control 5.0 ± 1.3 Hz, paired t-test, $p < 0.05$, n = 6), 9.7 ± 0.9 Hz (vs control 5.4 ± 1.1 Hz, paired t-test, $p < 0.01$, n = 6), 8.8 ± 0.6 Hz (vs control 5.4 ± 1.0 Hz, paired t-test, $p < 0.01$, n = 6) and 9.5 ± 1.6 Hz (vs control 5.8 ± 1.0 Hz, paired t-test, $p < 0.01$, n = 11) for 3μM, 10μM, 30μM and 100μM, respectively (figure 1F). Compared with the control, there was a significant difference in the mean peak frequency after applying LEV and there was no significant difference in the mean peak frequency among the various concentrations of LEV treatments (ANOVA, $p > 0.05$). The area power of LEV-induced theta oscillation was 9.6 ± 1.6 μV² (vs control 7.7 ± 1.2 μV²), 6.9 ± 1.0 μV² (vs control 5.0 ± 0.8 μV²), 23.9 ± 9.1 μV² (vs control 15.5 ± 6.0 μV²) and 24.9 ± 6.3 μV² (vs control 8.0 ± 1.2 μV²) for 3μM, 10μM, 30μM and 100μM, respectively. Compared with the control, LEV dose-dependently increased the area power by 23% ($p > 0.05$, n = 6), 44% ($p > 0.05$, n = 6), 70% ($p < 0.05$, n = 6) and 174% ($p < 0.01$, n = 11) for 3μM, 10μM, 30μM and 100μM, respectively (ANOVA, *post hoc* Tukey test, $p < 0.01$, figure 1G).

LEV-induced theta oscillations were mediated by NMDA receptors and GABA_A receptors

In order to determine the mechanisms of LEV-induced theta oscillation, we examined the effects of the ionotropic glutamate receptor antagonist D-AP5 or NBQX or the ionotropic GABA_AR antagonist bicuculline on LEV-induced theta oscillation. In a set of experiments (n = 6), pretreatment of hippocampal slices with D-AP5 (50 μM) had no effect on baseline area power, further application of LEV (100 μM) caused a small increase without statistically significant difference (paired t-test, $p > 0.05$, n = 6, figure 2 A1–3). In a different set of experiments (n = 8), we pretreated slices with NBQX (20 μM), further application of LEV

(100 μ M) caused a dramatic increase ($383 \pm 111\%$ vs NBQX 100%, paired t-test, $p < 0.01$, $n = 8$, figure 2 B1–3) in area power. Compared with LEV alone, there was no significant difference (Student t-test, $p > 0.05$). In another set of experiments ($n = 6$), pretreatment of hippocampal slices with bicuculline (2 μ M) had no effect on baseline area power, further application of LEV (100 μ M) failed to induce any oscillatory activity (paired t-test, $p > 0.05$, $n = 6$, figure 2 C1–3). The results therefore indicated that LEV-induced theta oscillation is mediated by both NMDA receptor and GABA_A receptor.

Taurine was involved in LEV-induced theta oscillations

Taurine is an inhibitory amino acid, potentially acts on GABA_A receptors located at both synaptic and non-synaptic sites, is functionally similar to the role of GABA[24]. LEV was reported to significantly reduce taurine level in the hippocampus [27]. To determine whether taurine is involved in LEV-induced theta oscillations, we studied the effects of taurine on LEV-induced oscillation. We pretreated hippocampal slices with taurine (100 μ M) for 20min and further application of LEV (100 μ M) caused little change on baseline area power ($107 \pm 8.4\%$ vs taurine 100%, paired t-test, $p > 0.05$, $n = 6$, figure 3). The results showed that taurine pretreatment blocked LEV-induced theta oscillations.

mACh receptors but not nACh receptors or L-type Ca²⁺ channel mediate LEV-induced theta oscillations

Previous study indicates that, we here determined whether LEV-induced theta oscillations are mediated by mACh receptors. Atropine pretreatment (50 μ M) had no role on baseline, further application of LEV (100 μ M) caused a small but significant increase in area power ($150 \pm 13.7\%$ vs atropine 100%, paired t-test, $p < 0.01$, $n = 10$, figure 4 A1–3). There was significant difference in area power between LEV and LEV+atropine (Student t-test, $p < 0.05$), indicating that LEV-induced theta oscillation was partially blocked by atropine.

Previous study showed that nAChR agonist induced theta oscillation in hippocampus [9], we further determined whether nACh receptors is involved in LEV-induced theta oscillations by using $\alpha 7$ nAChR antagonist MLA (100 nM) and $\alpha 4\beta 2$ nAChR antagonist DH β E (0.4 μ M). Pretreatment of a combined MLA and DH β E, had no effect on baseline activity, further application of LEV (100 μ M) caused a significant increase in area power ($261 \pm 36.9\%$ vs MLA and DH β E 100%, paired t-test, $p < 0.01$, $n = 6$, figure 4 B1–3). Our results indicated that nACh receptors don't contribute to LEV-induced oscillatory activity.

In another set of experiments ($n = 6$), L-type Ca²⁺ channel antagonist nifedipine (10 μ M) was applied to pretreat hippocampal slices, further application of LEV (100 μ M) caused a significant increase in area power ($221 \pm 38\%$ vs nifedipine 100%, paired t-test, $p < 0.01$, $n = 6$, figure 4 C 1–3). Thus, these results indicate that L-type Ca²⁺ channel did not involve in the regulation of LEV-induced theta oscillations.

Discussion

In this study, we demonstrate that LEV is able to elicit persistent oscillatory activity at theta frequency bands in hippocampal CA3 area, which are mediated by NMDA receptors and GABA_A receptors as well as partially by mAChR.

Hippocampal theta rhythm is crucial for spatial memory and is thought to be generated by extrinsic synaptic transmissions. Ionotropic glutamatergic receptor agonist kainate or nAChR agonist nicotine can both induce theta oscillation in medial septal diagonal band (MSDB), which was blocked by GABA_AR antagonist and was partially blocked by the AMPA/kainate or NMDA receptor (NMDAR) blocker [21, 22]. Carbachol (a mAChR agonist) or nicotine induced theta oscillations in hippocampal CA3 area, which was also blocked by GABA_A receptor antagonist and was partially blocked by the AMPA/kainate or NMDA receptor blocker [8, 9, 23]. Thus, it appears that these pharmacologically-induced oscillations were reduced by both GABA_AR and ionotropic glutamate receptor antagonists, indicating the involvement of local GABAergic and glutamatergic neurons in the production of the rhythmic theta activity.

In this study, LEV-induced theta oscillation was also involved in the activation of local GABA_AR and NMDAR. Surprisingly, we found that pretreatment with NBQX did not affect LEV-induced theta oscillations, suggesting that AMPA receptor (AMPA) was not involved in LEV-induction of theta oscillation. This is in agreement with previous findings that GABA_A receptors and ionotropic glutamatergic receptors (NMDAR) are critical for the induction of theta oscillation [3]. AMPAR appears to be not involved in LEV induced oscillation, which is sort of similar to the theta oscillation induced by DHPG+ NBQX [28]. In our case, LEV+NBQX appears to induced a higher theta relative to LEV alone, suggesting that in DHPG+NBQX and LEV induced theta did not require AMPAR activation.

LEV had no effect on AMPA-induced glutamate release [29], which may explain that AMPAR was not involved in LEV-induced theta activity. Whereas others reported that LEV reduced the EPSC amplitude via affecting the presynaptic voltage-dependent calcium channel [30]. Thus, we currently have no clear explanation about why LEV-induced theta was not involved in AMPAR. NMDAR involvement of LEV-induced theta may be explained by the observation that LEV increased selectively NMDA-induced glutamate release [29].

LEV specifically binds to synaptic vesicle protein 2A (SV2A), which is widely expressed in different brain regions. Interestingly, SV2A is a only subtype of the SV2 family expressed in GABAergic neurons [31] and is implicated in the control of GABA release [32]. Acute application of LEV reversed the negative allosteric regulation of Zn²⁺ on the GABA_AR in cultured hippocampal neurons [26]. These studies suggest that LEV may enhance GABA release and GABA_AR function and thus theta oscillation.

Taurine is a potent activator of extrasynaptic GABA_A receptors [33, 34]. Extrasynaptic GABA_AR may down-regulate theta oscillation, as extrasynaptic GABA_AR-mediated tonic inhibition counteracts the excitation of interneurons and down-regulate network oscillations [35]. This may explain taurine blockage of LEV

induction of theta oscillation. Thus it can infer that LEV may inhibit extrasynaptic GABA_AR activation via reducing taurine level in the hippocampus [27], indirectly boost GABA action and contribute to the theta oscillations.

In this study LEV-induced theta oscillation was partially blocked by atropine but not affected by nAChR antagonist, indicating that mAChR but not nAChR was involved in the regulation of LEV-induced theta, although nAChR agonist nicotine induced theta oscillations in hippocampal CA3 area[22]. Our results were supported by the observation that LEV facilitated cholinergic function via mAChR activation [36].

It is likely that LEV may activate G-protein related signaling molecules including mAChR activation and NMDAR, leading to the excitation of GABAergic neurons and GABA release as well as activation of GABA_A receptors within local neuronal network. Thus LEV-induced oscillation in hippocampal CA3 area is mediated by GABA_AR, NMDAR and mAChR.

Animal model of TLE exhibits a decrease in the power of theta oscillations correlated with spatial memory deficits [37]. LEV can improve the impaired spatial memory in the TLE rats [19], LEV induction of theta oscillation may contribute to the improvement of cognition. Thus our findings provide additional evidence to support clinical application of LEV in the neuropsychological disorders associated with impairment of theta activity.

Conclusions

In summary, it is the first study to demonstrate that LEV is able to elicit persistent oscillatory activity at theta frequency bands in hippocampal CA3 area, which are mediated by NMDA receptors and GABA_A receptors as well as partially by mAChR. LEV may especially benefit for the treatment of the neuronal disorders with impaired theta oscillation and cognitive function.

Materials And Methods

Experimental animals

Adult male Sprague–Dawley rats weighing 100–150 g were used in the current study. The rats were purchased from Zhengzhou University (Zhengzhou, China). All the animals were maintained under a 12 h/12 h light/dark cycle with a constant room temperature of 23 ± 1 °C. This study was carried out in accordance with the principles of the Basel Declaration and recommendations of the guidelines for animal experiments, Ethics Committees at Xinxiang Medical University. The protocol was approved by the Ethics Committees at Xinxiang Medical University. Furthermore, this manuscript reporting adheres to the ARRIVE guidelines for the reporting of animal experiments. In addition, we have made every effort to minimize the number of animals used.

Drugs and agents

(2S)-(2-Oxopyrrolidin-1-yl)butyramide (Levetiracetam), 2-Aminoethylsulfonic acid (taurine) and 1,4-Dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester (nifedipine) were purchased from Sigma-Aldrich (St Louis, MO, USA); D-(-)-2-Amino-5-phosphonopentanoic acid (D-AP5), 2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX), [R-(R*,S*)]-6-(5,6,7,8-Tetrahydro-6-methyl-1,3-dioxolo[4,5-g]isoquinolin-5-yl)furo[3,4-e]-1,3-benzodioxol-8(6H)-one (bicuculline), [1 α ,4(S),6 β ,14 α ,16 β]-20-Ethyl-1,6,14,16-tetramethoxy-4-[[[2-(3-methyl-2,5-dioxo-1-pyrrolidinyl)benzoyl]oxy]methyl]aconitane-7,8-diol citrate (MLA) and (2S,13bS)-2-Methoxy-2,3,5,6,8,9,10,13-octahydro-1H,12H-benzo[i]pyrano[3,4-g]indolizin-12-one hydrobromide (DH β E) were purchased from Tocris Bioscience (Bristol, UK); endo-($\pm$)- α -(Hydroxymethyl)benzeneacetic acid 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester (atropine) was purchased from MedChemexpress (Monmouth Junction, NJ, USA). All agents were dissolved in water, except nifedipine was dissolved in DMSO.

Preparation of hippocampal slices

Experimental animals were anaesthetised by intraperitoneal injection of chloral hydrate (400 mg/kg). When all pedal reflexes were abolished, the animals underwent intracardiac perfusion with chilled (5°C), oxygenated artificial cerebrospinal fluid (aCSF) in which the sodium chloride had been replaced by iso-osmotic sucrose. This aCSF solution (305 mmol/L) contained the following (in mmol/L): 225 sucrose, 3 KCl, 1.25 NaH₂PO₄, 24 NaHCO₃, 6 MgSO₄, 0.5 CaCl₂, and 10 glucose. Horizontal hippocampal slices were cut into 400- μ m sections using a Leica VT1000S vibratome (Leica Microsystems UK, Milton Keynes, UK). The slices were then transferred to an incubation chamber, where they remained submerged in oxygenated aCSF, which consisted of (in mmol/L) 126 NaCl, 3 KCl, 1.25 NaH₂PO₄, 2 MgSO₄, 24 NaHCO₃, 2CaCl₂, and 10 glucose, pH 7.35–7.45 at room temperature until used for recording.

Field potential recording

For local field potential (LFP) recordings, slices were placed in a recording chamber at the interface between aCSF (2–3 ml/min) at 32 °C and warm moist carbogen that maintained a thin film of aCSF covering the slice to ensure applied substances could diffuse into the area recorded. LFP were recorded from hippocampal area CA3 with aCSF-filled glass pipette recording electrodes (2–4 M Ω).

Field potentials were amplified with Neurolog NL106 AC-coupled amplifiers (Digitimer, Welwyn Garden City, UK) and band-pass filtered at 2–200 Hz with Neurolog NL125 filters (Digitimer). After mains line noise was removed with Humbug noise eliminators (Digitimer), the signal was digitized and sampled at 2 kHz using a CED-1401 Plus (Cambridge Electronic Design, Cambridge, UK) and Spike-2 software (Cambridge Electronic Design).

Data were analyzed off-line using software from Spike 2 (CED, Cambridge, UK). Power spectra were generated to provide a quantitative measure of the frequency components in a stretch of recording, where power, a quantitative measure of the oscillation strength, was plotted against the respective frequency.

Power spectra were constructed for 60 s epochs of field potential recordings (1Hz bin size, Hanning window, FFT size 2048) using a fast Fourier transform algorithm provided by Spike2. The parameters used for measuring the oscillatory activity in the slice were peak frequency (Hz) and area power (μV^2). In the current study, area power was equivalent to the computed area under the power spectrum between the frequencies of 4 and 14 Hz[38]. All statistical tests were performed using SPSS (SPSS Inc, USA).

Statistical analysis

All data are expressed as mean $\pm$ standard error of mean (SEM) or medians $\pm$ min-max for non-normally distributed data. Statistical significance for comparison between two groups was performed using a student *t*-test if data appeared to be normally distributed or a Wilcoxon signed-rank test if the data were not normally distributed (nonparametric data). Multiple comparisons among groups were analyzed using one-way repeated measures Analysis of Variance (ANOVA, *post hoc Tukey* test). If $P < 0.05$, the treatments were considered to have a statistically significant difference.

Abbreviations

AMPA AMPA receptor

LEV Levetiracetam

mAChR metabotropic acetylcholine receptor

nAChR nicotinic acetylcholine receptor

NMDAR NMDA receptor

TLE temporal lobe epilepsy

GABAAR GABAA receptor

SV2A synaptic vesicle protein 2A

Declarations

Ethics approval and consent to participate

This study was carried out in accordance with the principles of the Basel Declaration and recommendations of the guidelines for animal experiments, Ethics Committees at Xinxiang Medical University. The protocol was approved by the Ethics Committees at Xinxiang Medical University.

Consent for publication

Not applicable.

Availability of data and material

The datasets of this study are available from the corresponding author on request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Study design: CBL and XH. Study conduct: HX, XEX and YW. Data collection: HX, XEX and YW. Data analysis: HX and CBL. Data interpretation: HX and CBL. Drafting manuscript: HX and CBL. Revising manuscript: CBL, XH and HX. All authors read and approved the final manuscript.

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Figures

Figure 1

LEV-induced theta oscillations in the CA3 area of rat hippocampal slices. A, Examples of local field potentials recorded in the CA3 area of a hippocampal slice for the control at 15min (a) and 60 min (b) perfusion of aCSF. B, The time-effect curve for area power of the theta oscillation (theta power) under aCSF perfusion. C, Examples of local field potentials recorded in the CA3 area of a hippocampal slice for the control (15min, a) and the presence of 100 μM LEV (60 min, b). D, The time-effect curve for the theta power before and after application of LEV (100 μM). E, The power spectra of the recordings corresponding to that shown in C. F, The effects of different concentrations of LEV on the peak frequency of oscillations. G, Normalized area powers of LEV-induced theta oscillations for 3 μM (n=6), 10 μM (n=6), 30 μM (n=6) and 100 μM (n=13), respectively. Error bars indicate SEM. * indicates $P < 0.05$, ** indicates $P < 0.01$, in comparison with control, paired t-test or ANOVA with post hoc Tukey test.

Figure 2

GABAA receptors and NMDA receptors mediate LEV-induced theta oscillations in the hippocampal CA3 area. A1, Time-effect curve for the area power of the oscillations in the application of 50 μM D-AP5, followed by 100 μM LEV. A2, The corresponding power spectra of of the oscillations shown in A1. A3, Bar graph summarized the changes in normalized area power of oscillations for 50 μM D-AP5 and D-AP5+100 μM LEV ($n = 8$). B1, Time-effect curve for the area power of the oscillations in the application of 2 μM bicuculline, followed by 100 μM LEV. B2, The corresponding power spectra of of the oscillations shown in B1. B3, Bar graph summarized the changes in normalized area power of oscillations for 2 μM bicuculline and bicuculline+100 μM LEV ($n = 6$). C1, Time-effect curve for the area power of the oscillations in the application of 20 μM NBQX, followed by 100 μM LEV. C2, The corresponding power spectra of of the oscillations shown in C1. C3, Bar graph summarized the changes in normalized area power of oscillations for 20 μM NBQX and NBQX+100 μM LEV ($n = 8$). Error bars indicate SEM. * indicates $P < 0.05$, ** indicates $P < 0.01$, in comparison with antagonist, paired t-test.

Figure 3

Taurine mediates LEV-induced theta oscillations in the hippocampal CA3 area. A, Time-effect curve for the area power of the oscillations in the application of 100 μM taurine, followed by 100 μM LEV. B, The corresponding power spectra of the oscillations shown in A. C, Bar graph summarized the changes in normalized area power of oscillations for 100 μM taurine and taurine+100 μM LEV ($n = 6$). Error bars indicate SEM. * indicates $P < 0.05$, ** indicates $P < 0.01$, in comparison with antagonist, paired t-test.

Figure 4

mACh receptors but not nACh receptors or L-type Ca^{2+} channel mediate LEV-induced theta oscillations in the hippocampal CA3 area. A1, Time-effect curve for the area power of the oscillations in the application of 50 μM atropine, followed by 100 μM LEV. A2, The corresponding power spectra of of the oscillations shown in A1. A3, Bar graph summarized the changes in normalized area power of oscillations for 50 μM atropine and atropine+100 μM LEV ($n = 10$). B1, Time-effect curve for the area power of the oscillations in the application of 100nM MLA and 0.4 μM DH β E, followed by 100 μM LEV. B2, The corresponding power spectra of of the oscillations shown in B1. B3, Bar graph summarized the changes in normalized area power of oscillations for 100nM MLA+0.4 μM DH β E and MLA+DH β E+100 μM LEV ($n = 6$). C1, Time-effect curve for the area power of the oscillations in the application of 10 μM nifedipine, followed by 100 μM LEV. C2, The corresponding power spectra of of the oscillations shown in C1. C3, Bar graph summarized the changes in normalized area power of oscillations for 10 μM nifedipine and nifedipine +100 μM LEV ($n = 6$). Error bars indicate SEM. * indicates $P < 0.05$, ** indicates $P < 0.01$, in comparison with antagonist, paired t-test.

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