Neuroscience Letters 518 (2012) 69–74

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

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

Levetiracetam inhibits neurotransmitter release associated with CICR

Kouji Fukuyama[a,b,1], Shunsuke Tanahashi[a,b], Masanori Nakagawa[a,b], Satoshi Yamamura[a,b], Eishi Motomura[a,b], Takashi Shiroyama[a,b], Hisashi Tanii[a,b], Motohiro Okada[a,b,*,1]

[a] Department of Psychiatry, Division of Neuroscience, Graduate School of Medicine, Mie University, Tsu, Mie, Japan
[b] Brain Science and Animal Model Research Center (BSAM), Mie University, Tsu, Mie, Japan

ARTICLE INFO

Article history:
Received 25 January 2012
Received in revised form 19 March 2012
Accepted 20 March 2012

Keywords:
Epilepsy
GABA
Glutamate
Levetiracetam
CICR
Monoamine

ABSTRACT

To define the antiepileptic mechanisms of levetiracetam (LEV), the present study determined the concentration-dependent effects of locally perfused LEV on the releases of norepinephrine, dopamine, serotonin, L-glutamate and GABA induced by 50 mM K^+-evoked stimulation and agonists of ryanodine receptor (RyR) and inositol-triphosphate receptor (IP3R) in the median prefrontal cortex (mPFC) using in vivo microdialysis. Local perfusion with LEV (10, 30 and 100 μM) alone did not affect the extracellular levels of all neurotransmitters in the mPFC. The release of neurotransmitters induced by K^+-evoked stimulation was inhibited by perfusion with LEV in a concentration-dependent manner, and those induced by agonists of RyR and IP3R were also inhibited by LEV. Specifically, the RyR-induced release was inhibited by 10 μM LEV, whereas the IP3R-induced release was inhibited by 100 μM LEV, but not by 10 or 30 μM LEV. The above results suggest that LEV has little effect on the components of normal synaptic transmission but selectively inhibits transmission induced by neuronal hyperactivation. Thus, the mechanisms of the antiepileptic and neuroprotective actions of LEV seem to be mediated, at least in part, through the combination of these two inhibitory effects on depolarization-induced and CICR-associated neurotransmitter releases.

The antiepileptic drug, (S)-α-ethyl-2-oxo-pyrrolidine (levetiracetam: LEV), has a wide antiepileptic spectrum against partial and generalized epilepsies [4,7]. It has a unique anticonvulsive profile in animal models of epilepsies; it is ineffective in traditional convulsion screening tests; the maximal electroshock (MES) and pentylenetetrazol (PTZ) tests [9,11], whereas it provides protection in several spontaneous epileptic animal models, e.g., Strasbourg and Wistar albino Glaxo rats of Rijswijk [21]. The main molecular mechanism of LEV is the binding to synaptic vesicle protein 2A (SV2A) [12], although other pharmacological actions have also been demonstrated such as inhibition of N-type voltage-sensitive Ca^{2+} and K^+ channels [13,16] and Ca^{2+}-induced Ca^{2+} releasing system (CICR) [2,15].

Synaptic vesicles are the key organelles in neurotransmitter release from nerve terminals and seem to be so far underappreciated factor in the neurobiology of epilepsy [14,17]. On the other hand, Ca^{2+} mobilization plays important roles in various neuronal events, including neurotransmitter exocytosis [5], and is composed of two processes, Ca^{2+} influx from the extracellular space and output from intracellular Ca^{2+} stores associated with the endoplasmic-reticulum, namely the CICR system [5]. The CICR is comprised of the ryanodine receptor (RyR) and inositol 1,4,5-trisphosphate receptors (IP3R) [5]. Evidence suggests that hyperactivation of both RyR and IP3R contributes to neuronal cell damage in acute and chronic pathological states [5]; however, IP3R is considered the primary molecule of glial excitability [18]. Furthermore, we demonstrated previously that several antiepileptic drugs, e.g., carbamazepine, topiramate, valproate and zonisamide, inhibit neurotransmitter release associated with CICR [15,17,29–31]. Previous microdialysis study demonstrated that LEV decreased the extracellular taurine level without affecting those of other amino acids, including glutamate or GABA [23]. However, subsequent studies demonstrated that LEV inhibits both the basal and convulsion-induced glutamate release [24,25]. To date, however, the effects of LEV on the release of other neurotransmitters have not been studied.

The present study was designed to evaluate the effects of LEV on neurotransmission by measuring the release of L-glutamate, GABA, norepinephrine, dopamine and serotonin in the median prefrontal cortex (mPFC) using in vivo microdialysis.

All experiments described in this report were performed in accordance with the specifications of the Ethical Guidelines established by Institutional Animal Care and Use Committee at Mie

* Corresponding author at: Department of Psychiatry, Division of Neuroscience, Graduate School of Medicine, Mie University, Tsu, Mie, Japan. Tel.: +81 59 231 5018; fax: +81 59 2315208.
E-mail address: okadamot@clin.medic.mie-u.ac.jp (M. Okada).
[1] Kouji Fukuyama and Motohiro Okada contributed equally to this work.

0304-3940/$ – see front matter © 2012 Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.neulet.2012.03.056

University. Male Sprague-Dawley rats (SLC, Shizuoka, Japan, body weight: 250–300 g), were housed under conditions of constant temperature at 22 ± 2 °C with 12–12 h light–dark cycle.

UCB Pharma (Chemin du Foriest, Belgium) kindly supplied LEV, while inositol 1,4,5-trisphosphate receptor (IP3R) agonist, adenophostin-A (AdA) was purchased from Sigma-Aldrich (St. Louis, MO) and ryanodine receptor (RyR) agonist, ryanodine from Calbiochem (San Diego, CA). All agents were diluted directly in the perfusion medium.

Each rat was placed in a stereotaxic frame and kept under 1.8% isoflurane anesthesia, then a guide cannula was implanted in the mPFC (A = +3.2 mm, L = −0.8 mm, V = −6.0 mm relative to bregma) [26]. At 3 days after the implantation, the I-type microdialysis probes (0.22 mm diameter, 2 mm exposed membrane: Eicom, Kyoto, Japan) were implanted. Perfusion experiments commenced 18 h after recovery from anesthesia [26]. The perfusion rate was set at 2 μL/min, using either modified Ringer's solution (MRS) composed of (in mM) 145 Na^+, 2.7 K^+, 1.2 Ca^{2+}, 1.0 Mg^{2+}, and 154.4 Cl^-, and buffered with 2 mM phosphate buffer and 1.1 mM Tris buffer to adjust to pH 7.4, or 50 mM K^+-containing MRS (HKMRS). The ionic composition of these two perfusate solutions were modified and isotonicity was maintained by equimolar change of Na^+ [26]. Perfusion commenced using MRS alone. Extracellular neurotransmitter levels were measured at 6 h after starting the perfusion. After collection, each sample (40 μL/20 min) was immediately injected into liquid chromatography. When the coefficient of variation of the level of each neurotransmitter was less than 5% over 60 min stabilization, control data were obtained over another 60-min period. To study the effects of LEV on neurotransmitter release, the perfusate was switched to MRS containing LEV (10, 30 or 100 μM) [24,25]. After the above experiment, we investigated the effects of LEV on depolarization-induced neurotransmitter release by switching the perfusate to HKMRS containing the same LEV (10, 30 or 100 μM). To study the effects of LEV on CICR-induced neurotransmitter release, the perfusate was switched to MRS containing LEV (10, 30 or 100 μM) with 100 μM ryanodine or 1 μM AdA [17,29–31]. At the end of each experiment, the brain was fixed with 4% paraformaldehyde. Horizontal sections (200 μm thick) were prepared, and the site of the dialysis probe was localized according to the atlas of Paxinos and Watson [19].

The levels of L-glutamate and GABA in the perfusate were measured by extreme liquid chromatography (dual xLC 3185PU, Jasco, Tokyo) with fluorescence detection (xLC3120FP, Jasco), after derivatization. The derivatizing reagent solutions were prepared by dissolving isobutyryl-L-cysteine (2 mg) and o-phthalaldehyde (1 mg) in 0.1 mL ethanol followed by the addition of 0.9 mL 0.2 M sodium borate buffer (pH 9.0). Automated pre-column derivatization was carried out by drawing up a 5-μL aliquot sample, standard or blank solution and 5 μL of derivatizing reagent solution, and holding in the reaction vials 5 min prior to injection (xLC3059AS, Jasco). Five microliters derivatized samples were injected by auto sampler (xLC3059AS, Jasco). The excitation/emission wavelengths were 345/455 nm. The analytical column (Triart C18, particle 1.9 μm, 50 mm × 2.1 mm, YMC, Kyoto, Japan) was maintained at 50 °C and the flow rate of the mobile phase was set at 500 μL/min. A linear gradient elution program was performed over 10 min with mobile phase A (5 mM citrate buffer, pH 8.0) and B (5 mM citrate buffer containing 30% acetonitrile and 30% methanol, pH 3.0) [27,28].

The extracellular levels of norepinephrine, dopamine and serotonin were determined by ion-exchange high performance liquid chromatography with an electrochemical detector (HITEC-500, Eicom) and a graphite carbon electrode set at +450 mV (vs. Ag/AgCl reference electrode). The ion-exchange column (EicomPack CAX, 200 mm × 2.0 mm, Eicom) was maintained at 25 °C and the flow rate of the mobile phase was set at 250 μL/min. The mobile phase comprised 0.1 M ammonium acetate buffer (pH 6.0) containing 0.04 M sodium sulfate, 0.3 mM EDTA-2Na, and 30% (v/v) methanol [26].

The concentration-dependent effects of locally perfused LEV in mPFC on the extracellular levels of norepinephrine, dopamine, serotonin, GABA and L-glutamate were analyzed by multivariate analysis of variance (MANOVA) with Tukey's multiple comparison. The interaction between LEV and several stimuli (perfusion with ryanodine, AdA or HKMRS) on the extracellular neurotransmitter levels was analyzed by MANOVA with Tukey's multiple comparisons.

The basal extracellular levels of norepinephrine, dopamine, serotonin, GABA and L-glutamate in the mPFC were 0.39± 0.05 nM, 0.54 ± 0.12 nM, 0.23 ± 0.05 nM, 0.19 ± 0.03 μM and 1.63 ± 0.28 μM, respectively (not corrected for in vitro dialysis probe recovery). The basal extracellular levels of norepinephrine, dopamine, serotonin and GABA were tetrodotoxin-sensitive, Ca^{2+}-dependent and K^+-sensitive, whereas the basal extracellular L-glutamate level was tetrodotoxin-insensitive, Ca^{2+}-independent and K^+-sensitive (data not shown). These data indicate that the levels of norepinephrine, dopamine, serotonin and GABA in frontal perfusate (basal release) were primarily neuronal in origin, whereas those of L-glutamate (basal glutamate release) were not.

To study the concentration-dependent effects of local perfusion of LEV into mPFC on the release of norepinephrine, dopamine, serotonin, GABA and L-glutamate, the perfusate was switched from MRS to MRS containing LEV (10, 30 or 100 μM) for 120 min. Perfusion with 10, 30 and 100 μM LEV did not affect the extracellular levels of norepinephrine, dopamine, serotonin, GABA or L-glutamate (data not shown).

After the above study, the perfusate was switched to HKMRS containing the same concentrations of LEV for 20 min. Perfusion with 10, 30 and 100 μM LEV concentration-dependently decreased K^+-evoked releases of norepinephrine [MANOVA: $F_{Dose}(3,20) = 5.4$ ($P < 0.01$), $F_{Time}(9,12) = 95.7$ ($P < 0.01$), $F_{DosexTime}(27,180) = 16.7$ ($P < 0.01$)], dopamine [MANOVA: $F_{Dose}(3,20) = 7.3$ ($P < 0.01$), $F_{Time}(9,12) = 64.8$ ($P < 0.01$), $F_{DosexTime}(27, 180) = 57.1$ ($P < 0.01$)], serotonin [MANOVA: $F_{Dose}(3,20) = 5.9$ ($P < 0.01$), $F_{Time}(9,12) = 31.9$ ($P < 0.01$), $F_{DosexTime}(27,180) = 19.4$ ($P < 0.01$)], GABA [MANOVA: $F_{Dose}(3,20) = 3.6$ ($P < 0.05$), $F_{Time}(9,12) = 27.6$ ($P < 0.01$), $F_{DosexTime}(27,180) = 12.7$ ($P < 0.01$)] and L-glutamate [MANOVA: $F_{Dose}(3,20) = 9.9$ ($P < 0.01$), $F_{Time}(9,12) = 36.5$ ($P < 0.01$), $F_{DosexTime}(27, 180) = 16.4$ ($P < 0.01$)] (Fig. 1).

To study the effects of ryanodine-induced release, the perfusate was switched from LEV to MRS containing LEV with 100 μM ryanodeine for 120 min [17,29–31]. Perfusion with 100 μM ryanodine increased the extracellular levels of norepinephrine, dopamine, serotonin, GABA and L-glutamate (Fig. 2). Perfusion with 10, 30 and 100 μM LEV decreased ryanodine-induced release of norepinephrine [MANOVA: $F_{Dose}(3,20) = 6.5$ ($P < 0.01$), $F_{Time}(9,12) = 15.2$ ($P < 0.01$), $F_{DosexTime}(27,180) = 5.0$ ($P < 0.01$)], dopamine [MANOVA: $F_{Dose}(3,20) = 13.7$ ($P < 0.01$), $F_{Time}(9,12) = 15.2$ ($P < 0.01$), $F_{DosexTime}(27,180) = 2.7$ ($P < 0.01$)], serotonin [MANOVA: $F_{Dose}(3,20) = 4.5$ ($P < 0.05$), $F_{Time}(9,12) = 22.4$ ($P < 0.01$), $F_{DosexTime}(27,180) = 5.8$ ($P < 0.01$)], GABA [MANOVA: $F_{Dose}(3,20) = 6.7$($P < 0.01$), $F_{Time}(9,12) = 11.6$ ($P < 0.01$), $F_{DosexTime}(27,180) = 10.7$ ($P < 0.01$)] and L-glutamate [MANOVA: $F_{Dose}(3,20) = 3.7$ ($P < 0.05$), $F_{Time}(9,12) = 13.6$ ($P < 0.01$), $F_{DosexTime}(27,180) = 1.6$ ($P < 0.01$)]; however, the inhibitory effects of LEV on ryanodine-induced release were not concentration-dependent (Fig. 2).

Finally, we studied the effects of AdA-induced release. The perfusate was switched from LEV to MRS containing LEV with 1 μM AdA for 120 min [17,28]. Perfusion with 1 μM AdA increased the extracellular levels of norepinephrine, dopamine, serotonin, GABA or L-glutamate (Fig. 3). Perfusion with LEV decreased