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SHORT COMMUNICATION

Antiepileptogenic and anticonvulsive actions of levetiracetam in a pentylenetetrazole kindling model

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Summary Levetiracetam (LEV) is a unique antiepileptic drug that preferentially interacts with synaptic vesicle protein 2A (SV2A). To evaluate the antiepileptogenic action of LEV, we studied its effects on the development and acquisition of pentylenetetrazole (PTZ) kindling and compared them to those of sodium valproate (VPA). Anticonvulsive actions of LEV in PTZ-kindled animals were also determined. LEV did not affect PTZ seizures in naïve animals even at high doses (≈300 mg/kg, i.p.). However, combined treatment of LEV (30 and 100 mg/kg, i.p.) with PTZ significantly suppressed the development and acquisition of PTZ kindling. In addition, LEV at relatively low doses (3—30 mg/kg, i.p.) inhibited PTZ-evoked seizures in fully kindled animals. In contrast to LEV, VPA at sub-anticonvulsive doses (30 and 100 mg/kg, i.p.) failed to prevent the development of PTZ kindling and its anticonvulsive potency was similar in PTZ-kindled and naïve mice. The present study shows that LEV contrasts VPA by preventing the development of PTZ kindling and inhibiting seizures selectively in kindled animals.

Introduction

Levetiracetam (LEV) is a unique antiepileptic drug (AED) which preferentially interacts with synaptic vesicle protein 2A (SV2A) without affecting activities of neurotransmitter receptors or ion channels (Lynch et al., 2004; Sasa, 2006; Kaminski et al., 2008). Unlike conventional AEDs, LEV is not active in the classical convulsion tests (i.e., maximal electroshock- and maximal pentylenetetrazole (PTZ)-evoked seizures) (Löscher and Hönack, 1993; Klitgaard et al., 1998; Bastlund et al., 2005), but it inhibits seizures in various animal models including kindled animals (e.g., corneal- and amygdala kindling) (Löscher and Hönack, 1993; Klitgaard et al., 1998; Löscher et al., 1998) and genetically defined animal models of epilepsy (Gower et al., 1995; Bouwman and van Rijn, 2004; Yan et al., 2005; Jiqun et al., 2005). In addition, previous studies demonstrated that LEV can inhibit the development of amygdala kindling in rats, suggesting that LEV has antiepileptogenic activi-

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Table 1 Anticonvulsive effects of LEV and VPA in PTZ-kindled and naïve mice.

	Naïve animals				PTZ-kindled animals			
	Test dose (mg/kg, i.p.)	Inhibition of PTZ seizures		ED_{50} (mg/kg, i.p.)	Test dose (mg/kg, i.p.)	Inhibition of PTZ seizures		ED_{50} (mg/kg, i.p.)
		No. of animals	% Inhibition			No. of animals	% Inhibition	
LEV	30	0/6*	0	>300	3	0/16	0	27.3
	100	0/6	0		10	6/15	40	(18.7—58.7)
	300	0/6	0		30	8/16	50	
VPA	100	1/6	17	229	100	2/8	25	227
	200	1/6	17	(151.5—300.4)	300	5/8	62	(46.6—389.3)
	300	5/6	83		600	7/7	100	
	400	6/6	100					

Seizures were evoked by 70 mg/kg (i.p.) PTZ in naïve mice and by 40 mg/kg (i.p.) PTZ in PTZ-kindled mice. VPAs in parentheses under the ED_{50} values indicate 95% confidence limits.
* Number of animals in which PTZ-evoked seizures were inhibited/total number of animals examined.

ties (Löscher et al., 1998; Husum et al., 2004; Gu et al., 2004). Nonetheless, a recent study by Matveeva et al. (2008) showed that LEV retarded the development of amygdala kindling, but could not prevent kindling acquisition. Studies using animals with spontaneous recurrent seizures after status epilepticus also demonstrated the lack of effectiveness of LEV against the epileptogenesis (Brandt et al., 2007). Thus, the antiepileptogenic potential of LEV is still unclear and remains to be verified using different animal models.

In order to address this question further, we studied the effects of LEV on the development and acquisition of PTZ-induced kindling in mice, and compared them with those of the typical AED sodium valproate (VPA).

Methods

Male ddY mice (Japan SLC, Shizuoka, Japan) weighing 20—25 g were used. Animals were housed in air-conditioned rooms under a 12-h light/dark cycle (light on: 7:00 a.m.) and allowed *ad libitum* access to food and water. The housing conditions of the mice and animal care methods complied with NIH guide for the care and use of laboratory animals. The experimental protocols of this study were approved by the Experimental Animal Research Committee at Osaka University of Pharmaceutical Sciences.

In order to set the test doses of LEV and VPA for PTZ kindling, we first determined their anticonvulsive actions against maximal PTZ seizures using naïve mice (Bastlund et al., 2005). Namely, animals were first given with different doses of LEV or VPA, and 30 min later, PTZ (70 mg/kg, i.p.) was injected. The incidence and severity of seizures were evaluated over 15 min immediately after the PTZ injection, using a 4-point ranked scale (0: none; 1: occasional head twitches; 2: myoclonic jerk or partial clonic seizure of forepaws and/or upper body trunk; 3: generalized clonic seizures). The observers were kept blind to the drug treatment, and the incidence of PTZ-induced seizures was judged as positive when the animal showed a score 2 or more.

PTZ kindling was induced as published previously (Ohno et al., 2009). Briefly, animals were given a sub-convulsive dose of PTZ (40 mg/kg, i.p.) every weekday for 12 days. For the evaluation of antiepileptogenic activity, LEV or VPA at the doses which negligibly affect PTZ seizures by themselves was repeatedly administered to the animals 30 min before PTZ injection for 12 days. The incidence and severity of the PTZ-evoked seizures were evaluated over 15 min immediately after the PTZ injection in the same manner as described above.

We also determined the anticonvulsive actions of LEV and VPA in PTZ-kindled animals. The mice were treated with PTZ (40 mg/kg, i.p.) every weekday for 15 days, and only PTZ-kindled mice (exhibiting seizures at least 3 successive days) were subjected to the anticonvulsive test for LEV and VPA. On the day of experiments, animals were first given with different doses of LEV, VPA or the vehicle, and 30 min later, PTZ (40 mg/kg, i.p.) was injected. Incidence and severity of PTZ-evoked seizures were evaluated over 15 min immediately after the PTZ injection in the same manner as described previously.

PTZ hydrochloride and VPA hydrochloride were purchased from Sigma—Aldrich. LEV was a gift from UCB Japan (Tokyo, Japan). All drugs were dissolved in saline and injected at a volume of 5 ml/kg.

Results

In naïve animals, LEV did not affect PTZ seizures even at high doses up to 300 mg/kg (i.p.) while VPA inhibited the seizures with an ED_{50} value of 229 mg/kg (i.p.) (Table 1). We therefore set the test doses of LEV and VPA at 30 and 100 mg/kg