Short communication

Effects of MK-801, dantrolene, and FK506 on convulsive seizures and brain nitric oxide production in seizure-susceptible EL mice

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Accepted 10 October 2000

Abstract

To clarify the role of nitric oxide (NO) in the pathogenesis of seizures in susceptible EL mice, we investigated effects of three drugs potentially related to NO production, MK-801, dantrolene, and FK506, on convulsive seizures and brain NO metabolites (NOx). MK-801 or dantrolene, but not FK506, suppressed convulsive seizures in EL mice; only MK-801 reduced NOx in the brain. Our results suggested involvement of the N-methyl-D-aspartate receptor–channel complex and intracellular calcium mobilization, but not calcineurin, in the convulsions of EL mice.

Keywords: Nitric oxide; EL mouse; Convulsion; MK-801; Dantrolene; FK506

The EL mouse is an inbred mutant strain of ddY mouse that is susceptible to convulsive seizures. It was developed in 1954 [8], registered internationally in 1964, and established as an authentic model of epilepsy by electroencephalography in 1976 [20]. This strain exhibits seizures in response to rhythmic vestibular stimuli such as tossing, rocking, to-and-fro horizontal swinging, pendular movement, or alternating rotation. Repetition of such stimulation induces generalized convulsions. The EL mouse is an excellent model of complex partial seizures, or temporal lobe epilepsy.

Nitric oxide (NO) has been regarded as a neuronal messenger in the central nervous system (CNS) and a modulator of several brain functions [4]. Activation of excitatory amino acid (EAA) receptors, particularly the N-methyl-D-aspartate (NMDA) subtypes, causes an influx of Ca\(^{2+}\) into neurons leading to calmodulin-dependent activation of NO synthase (NOS) [3,7]. NO in the CNS is involved in the pathogenesis of convulsive seizures [5,15,17], and we have shown that lower NO production in the brain may be related to the susceptibility of the EL mouse to convulsive seizures [15,25]. However, the role of NO in epileptic convulsion in the EL mouse is still unclear.

MK-801, a potent and selective noncompetitive antagonist at the NMDA receptor, dose-dependently suppresses generalized tonic–clonic convulsions and increases levels of glycine in the hippocampus [19]. FK506, a contrasting drug originally developed as a potential immunosuppressant, was found to inhibit a Ca\(^{2+}\)- and calmodulin-dependent protein phosphatase, calcineurin [13]. Dawson et al. [6] demonstrated that FK506 enhanced phosphorylation of neuronal NOS (nNOS), which reduced NO production, and thus attenuated glutamate neurotoxicity in cultured neurons. To clarify the role of NO in the pathogenesis of seizures in EL mice, we investigated the effects of MK-801, FK506, and dantrolene (a compound that prevents mobilization of Ca\(^{2+}\) from intracellular stores) on convulsive seizures and NO metabolites in mouse brain.

This study was carried out after receiving permission from the Committee of Animal Experimentation, Faculty of Medicine, Kagoshima University. EL mice were inbred at our animal center. They housed in individual cages in a room at 23±2°C, constant humidity (50±10%) and a 12

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The severity of seizures was scored according to our previous study [16] as: grade 0, no seizures with 50 tossing stimulations; grade 1, abortive seizures within 50 such stimulations; grade 2, abortive seizures within 25 such stimulations; grade 3, tonic–clonic seizures within 50 such stimulations; grade 4, tonic–clonic seizures within 25 such stimulations. Abortive seizures were characterized by immobility with fixed staring. Mice with grade 4 seizures were used in this study. Since the EL mouse shows a refractory period between convulsions, we evaluated the time-course of the convulsive seizure. No seizures occurred 15 min following the first stimulation. The seizure scores at 15 and 30 min after the first stimulation were significantly lower than the grade 4, while the scores at 1 and 2 h after the first stimulation approached grade 4. We therefore evaluated seizure scores at 1 h after the first stimulation. Details of the time-course of convulsive seizures in the EL mouse have been described elsewhere [6].

MK-801 and dantrolene were dissolved in saline. FK506 was dissolved in mannitol and polyoxyethylene hydroxylated castor oil 60. With the mouse gently restrained by the experimenter’s hands, an injection needle attached by a polyethylene tube to a microsyringe was inserted intraperitoneally for administration of MK-801 (0.05, 0.1, 0.25, or 0.5 mg/kg), dantrolene (20, 40, or 80 mg/kg) and FK506 (5, 10, or 20 mg/kg). One hour after administration, the mouse was subjected to tossing stimulation and the severity of the resulting seizure was scored.

The mouse was killed by cervical dislocation, and the brain was rapidly excised. Since the half-life of NO is very short, NOS activities were determined by measuring levels of nitrite plus nitrate (NOx). The brain with twice amount Doses of the three drugs used in the present study were stored at −80°C for 1 to 3 days until assay.

NOx concentrations in brain-derived supernatants were determined using an NO-analyzing system (ENO-20, EICOM). Nitrite and nitrate in the brain was separated by a reverse-phase separation column packed with polystyrene polymer (NO-PAK, 4.6×50 mm, EICOM), and nitrate was reduced to nitrite in a reduction column packed with copper-plated cadmium fillings (NO-RED, EICOM). Nitrite was mixed with a Griess reagent to form a purple azo dye in a reaction coil. The separation and reduction columns and the reaction coil were placed in a column oven that was set at 35°C. The absorbance of the color of the product dye at 540 nm was measured by a flow-through spectrophotometer (NOD-10, EICOM). The mobile phase, which was delivered by a pump at a rate 0.33 ml/min, was 10% methanol containing 0.15 M NaCl/NH₄Cl and 0.5 g/l 4Na-EDTA. The Griess reagent, which was 1.25% HCl containing 5 g/l sulfanilamide with 0.25 g/l N-naphthyl-ethylenediamine, was delivered at a rate of 0.1 ml/min.

Differences were tested for statistical significance using the Kruskal–Wallis test followed by the Mann–Whitney test and one-way analysis of variance (ANOVA) followed by Fisher’s PLSD. A P-value of <0.05 was accepted as statistically significant.

MK-801 suppressed seizure scores in an essentially dose-dependent manner, while all doses of dantrolene had this effect. On the other hand, FK506 did not inhibit seizure significantly (Fig. 1). Brain NOx levels of mice treated with MK-801 at doses of 0.25 and 0.5 mg/kg were significantly lower than in controls, but dantrolene and FK506 did not influence brain NOx levels at any dose (Fig. 2).

Recent advances in understanding epileptic phenomena have emphasized the importance of EAAs. The EAA receptor agonist NMDA is known to be a potent convulant, and NMDA receptor activation has been implicated in development of epilepsy. NO can regulate NMDA receptor activity and protects neurons from effects of excessive receptor stimulation [12].

Although MK-801, dantrolene, and FK506 all might be related to NO production, the present results of these drugs differed concerning convulsive seizures in EL mice and in effects on brain NO metabolites; both MK-801 and dantrolene, but not FK506, suppressed convulsive seizures in EL mice, while only MK-801 reduced brain NOx levels. Doses of the three drugs used in the present study were comparable to those in previous experiments [2, 14, 19, 22, 23].

MK-801 is a potent, selective noncompetitive antagonist at the NMDA receptor, and has been reported to possess anticonvulsant properties. Labeled MK-801 also has been shown to occupy a high-affinity site within the opened ion channel of the NMDA-receptor channel complex, which is distinct from the NMDA-recognition site [11]. MK-801 has well-known actions against seizures in models of epilepsy [18, 22, 23]. In EL mice, Sato et al. [19] found that...
Fig. 1. Dose-dependent effects of MK-801, dantrolene, and FK506 on seizure scores in EL mice. Each point is the mean±S.E.M. (Left) MK-801 (saline, n=14; 0.05 mg/kg, n=10; 0.1 mg/kg, n=10; 0.25 mg/kg, n=10; 0.5 mg/kg, n=10). (Middle) Dantrolene (saline, n=14; 20 mg/kg, n=8; 40 mg/kg, n=8; 80 mg/kg, n=8). (Right) FK506 (vehicle, n=8; 5 mg/kg, n=8; 10 mg/kg, n=12; 20 mg/kg, n=9). Seizure scores of EL mice treated with MK-801 at four doses were significantly lower than the control (all, P<0.01). Seizure scores of mice treated with MK-801 at doses of 0.25 and 0.5 mg/kg were significantly lower than those with MK-801 at doses of 0.05 and 0.1 mg/kg (0.5 mg/kg vs. 0.1 mg/kg, P<0.05; others, P<0.01). Seizure scores of mice treated with dantrolene at all doses were significantly lower than the control (all, P<0.01). There were no significant differences among seizure scores of mice treated with FK506.

Fig. 2. NOx levels in the brain of EL mouse treated with MK-801, dantrolene, and FK506. Each value is the mean±S.E.M. (Left) MK-801 (saline, n=9; 0.05 mg/kg, n=10; 0.1 mg/kg, n=10; 0.25 mg/kg, n=10; 0.5 mg/kg, n=10). (Middle) Dantrolene (saline, n=9; 20 mg/kg, n=8; 40 mg/kg, n=8; 80 mg/kg, n=8). (Right) FK506 (vehicle, n=8; 5 mg/kg, n=8; 10 mg/kg, n=8; 20 mg/kg, n=9). Brain NOx levels of mice treated with MK-801 at a dose of 0.25 mg/kg was significantly lower than those with MK-801 at doses of 0.05 and 0.1 mg/kg (0.5 mg/kg vs. 0.1 mg/kg, P<0.05; others, P<0.01). Those of mice treated with MK-801 at a dose of 0.5 mg/kg was significantly lower than those of the control and treated at a dose of 0.1 mg/kg (all, P<0.01). Brain NOx levels of mice treated with dantrolene and FK506 at any dose did not differ from those of the control.
systemic injection of MK-801 potently suppressed generalized tonic–clonic convulsions in a dose-dependent manner, and suggested that the NMDA system may be essential in triggering seizures in EL mice. Present results, like those of Sato et al. [19], indicated that MK-801 reduced brain NO metabolite levels as well as seizure scores. This suggests that the NMDA system is central to both convulsive seizures and changes in NO production, since MK-801 suppressed brain NO production.

Reported studies concerning the effects of dantrolene on convulsive seizures have had conflicting results. Tizzano and colleagues [22,23] reported that limbic seizures induced by 3,5-dihydroxyphenylglycine or by (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid, a selective metabotropic glutamate receptor agonist, were attenuated by injection of dantrolene (62.5 to 500 mg/kg, i.p.). On the other hand, Borowicz et al. [2] reported that dantrolene (5 to 20 mg/kg, i.p.) did not influence the electroconvulsive threshold. Our results supported those of Tizzano and colleagues [22,23]; furthermore, even smaller doses (20 and 40 mg/kg) were effective in suppressing seizures in EL mice. Doses of dantrolene that were effective in suppressing seizures did not alter brain NOx levels. Dantrolene prevents intracellular calcium mobilization subsequent to metabotropic glutamate receptor agonist activation in primary neuronal cultures [23]. In this context, our results suggested that the anticonvulsive effect of dantrolene in EL mice may occur via a pathway not involving NO metabolites.

FK506 previously has shown to have significant neuroprotective properties. In primary cortical cultures, this agent strongly reduced NMDA, but not non-NMDA, neurotoxicity [6]. Moia et al. [14] reported that FK506 reversibly inhibited the progression of the kindling stage, and suggested that calcineurin may be essential to epileptogenesis in kindling. FK506 inhibits the function of calcineurin, thereby inhibiting Ca⁺⁺-dependent dephosphorylation and activation of nNOS [24]. Despite the use of a larger dose of FK506 than that required to block progression of the kindling stage [14], we found that FK506 neither suppressed convulsive seizures of EL mice nor reduced brain NOx levels.

The present results supported the suggestions of Kawasaki et al. [11] and Sato et al. [19] that functional changes of the NMDA receptor–channel complex are important to mechanisms of convulsions in EL mice. However, Kawasaki et al. [11], who investigated [¹³C]MK-801 binding activities in the forebrains of EL mice, concluded that seizure susceptibility of EL mice could not be completely explained by changes in affinity of the NMDA receptor–channel complex. Our results concerning the effects of dantrolene and FK506 on convulsive seizures and NO production suggested that intracellular calcium mobilization, but not calcineurin, may be important in the occurrence of convulsions in EL mice.

Acknowledgements

We thank the Yamanouchi Pharmaceutical Co. Ltd. (Tokyo, Japan) and the Fujisawa Pharmaceutical Co. Ltd. (Tokyo, Japan) for providing dantrolene and FK506, respectively.

References


