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European Journal of Pharmacology 531 (2006) 59–65
www.elsevier.com/locate/ejphar

Effect of YM872, a selective and highly water-soluble AMPA receptor antagonist, in the rat kindling and rekindling model of epilepsy

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Received 25 August 2005; received in revised form 17 November 2005; accepted 22 November 2005
Available online 3 January 2006

Abstract

We examined antiepileptogenic and anticonvulsant effects of [2,3-dioxo-7-(1H-imidazol-1-yl)-6-nitro-1,2,3,4-tetrahydro-1-quinoxalinyl]-acetic acid monohydrate (YM872), a potent and highly water-soluble *alpha*-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) receptor antagonist, in the rat amygdala kindling model of epilepsy. Administration of YM872 significantly suppressed fully kindled seizures. Daily pretreatment with YM872 markedly retarded development of kindling during drug sessions. We also used the rekindling method to investigate the antiepileptogenic effect of YM872 in an attempt to differentiate between true and false effects in the conventional method of daily administration. The results using the rekindling method suggested that the effect of YM872 was truly antiepileptogenic, indicating its possible clinical usefulness as an antiepileptogenic drug. We also affirmed the importance of AMPA receptors in the seizure expression mechanism and development of kindling-induced epileptogenesis.

Keywords: Kindling; AMPA receptor antagonists; YM872; Epileptogenesis; (Rat)

1. Introduction

Kindling is an animal model of epilepsy that has been used as a model of secondary generalization of temporal lobe epilepsy. It is also a useful model for the development of a novel antiepileptic drug. A number of studies have reported that glutamate receptors play important roles in the generation of seizures and development of epilepsy (Meldrum, 1994; Löscher, 1998). In spite of several issues such as low solubility in water and neurotoxic side effects (Auberson, 2001), glutamate receptor antagonists may still be attractive candidates for antiepileptic drugs because they are potentially antiepileptogenic, not simply anticonvulsant. A number of anticonvulsants are already available, but patients and clinicians need a drug that can inhibit the development of epileptogenesis.

There are two major subtypes of ionotropic glutamate receptors: *N*-Methyl-d-Aspartate (NMDA) subtypes and non-NMDA subtypes. Non-NMDA receptors are further classified as AMPA or kainate receptors. Although NMDA receptor antagonists significantly suppress kindling development, data from our lab has demonstrated that these substances are not potent enough to suppress fully kindled seizures (Sato et al., 1988; Morimoto et al., 1991; Namba et al., 1993). We have reported that 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(*f*)-quinoxaline (NBQX), an AMPA receptor antagonist, not only retarded the development of kindling, but also suppressed fully kindled seizures (Namba et al., 1994). We have also reported that 6-(1*H*-imidazol-1-yl)-7-nitro-2,3-(1*H*,4*H*)-quinoxalinedione hydrochloride (YM90K) exhibited potent antiepileptogenic and anticonvulsant effects in the kindling model of epilepsy (Kodama et al., 1999). Recently, another novel AMPA antagonist, 2-[*N*-(4-chlorophenyl)-*N*-methylamino]-4*H*-pyrido[3.2-e]-1,3-thiazin-4-one (YM928), which suppresses kindling acquisition without significant sedative effects (Yamashita et al., 2004), has been introduced.

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doi:10.1016/j.ejphar.2005.11.044

However, the antiepileptogenic effect of NBQX is controversial (Dürmüller et al., 1994). In our previous investigation of YM90K, kindling developed rapidly as soon as drug administration ceased. Thus, the antiepileptogenic effect of AMPA antagonists may have been more apparent than real. In other words, we suspect that the antagonists may suppress only the intensity of each seizure during kindling, but not inhibit development of kindling-induced plastic changes. There may have also been a rebound effect from drug administration as a result of chronic treatment, which complicates the interpretation of results from kindling development.

YM872 is a selective and competitive AMPA receptor antagonist. YM872 has approximately 700–900 times the water solubility of other AMPA receptor antagonists such as YM90K or NBQX (Kohara et al., 1998; Takahashi et al., 1998). This is important because one problem with other antagonists in the past was that they are hardly soluble in water. The neuroprotective effects of YM872 have already been demonstrated in various brain ischemic models (Takahashi et al., 1998; Kawasaki-Yatsugi et al., 1998; Shimizu-Sasamata et al., 1998; Häberg et al., 1998). These findings suggest that this compound is a good candidate for a new antiepileptic drug.

In the present study, we first examined the effect of YM872 on fully-kindled seizures and kindling development using a conventional kindling technique. In the latter half of the study, we used a rekindling protocol to assess the true antiepileptogenic effect. In the rekindling protocol, there were two electrical stimulation periods with an 8-week interval. We administered the drug only during the initial kindling period, and in the second kindling period, rats were stimulated without the drug.

2. Materials and methods

2.1. General procedure

Male Sprague–Dawley rats, weighing 280–370 g at the time of surgery, were used. The rats were housed in an environmentally controlled animal facility under a 12 h / 12 h light/dark cycle and allowed free access to food and water, except during the experimental sessions. All animals were treated in accordance with the Guidelines for Animal Experimentation of Okayama University. The rats were anesthetized with sodium pentobarbital (50 mg/kg administered i.p.), and a tripolar stimulating and recording electrode was stereotaxically implanted in the left amygdala complex (2.8 mm posterior and 4.8 mm lateral to the bregma, and 7.4 mm below the dura). The stereotaxic coordinates were determined using an incisor bar placed 3.3 mm below the interaural plane. The tripolar electrode consisted of three twisted Diamel-insulated Nichrome wires (0.18 mm in diameter). A screw electrode was placed into the right frontal skull to serve as a recording indifferent.

After a recovery period of 1–2 weeks, each rat was subjected to daily kindling stimulation sessions, each consisting of a 2 s train of 50 Hz biphasic square pulses at the stimulus intensity described below. The development of kindled seizures was assessed using Racine's classification (1972). EEGs between the remaining pole of the tripolar electrode and the skull screw electrode were recorded during all tests. YM872 was dissolved in saline. The final pH of the YM872 solution was approximately 7.2–7.6.

2.2. Experiment 1: anticonvulsant effect of YM872 on previously fully kindled amygdala seizures

Eight rats with tripolar electrodes implanted in the amygdala were used. After a recovery period, they were subjected to kindling stimulation. The afterdischarge threshold was determined by increasing the intensity of the stimulatory current by 25 μA at 20 min intervals until an afterdischarge duration of longer than 5 s was obtained. Each rat received kindling stimulation once daily at an intensity that was determined by adding 25 μA to the afterdischarge threshold value. Kindling stimulation was continued until the animals experienced at least five consecutive generalized convulsions (stage 5 seizures) over five successive days. The generalized seizure-triggering threshold was then determined in each rat by increasing the intensity of the stimulatory current by 25 μA at 20 min intervals until a stage 5 seizure occurred. After stable stage 5 seizures had been induced by stimulation at the predetermined generalized seizure-triggering threshold intensity, drug experiments were performed as follows.

Eight fully kindled rats were stimulated 30 min after i.p. administration of YM872 (5, 25, 50 mg/kg) or saline randomly. The stimulus intensity for each rat was the same as the generalized seizure-triggering threshold value. There was a 24-h interval between each drug session. One week after the last drug session, rats were stimulated at the generalized seizure-triggering threshold intensity with neither YM872 nor the vehicle (post-drug test). The anticonvulsant effects of YM872 were assessed according to the kindled seizure stage scores and afterdischarge durations.

2.3. Experiment 2: antiepileptogenic effect of YM872 on amygdala seizure kindling development

For investigation of an antiepileptogenic effect on kindling development, the rats received daily administration of either 25 mg/kg YM872 ($n=5$, i.p.) or saline ($n=6$, i.p.) before electrical stimulation. The afterdischarge threshold was determined as in Experiment 1 (day 1). Then the rats received daily administration of either 25 mg/kg YM872 or saline 30 min prior to electrical stimulations from days 2 to 8. Kindling stimulation was given at a stimulus intensity of afterdischarge threshold +25 μA. After day 9, kindling stimulations without drug administration were continued until animals reached stage 4 or 5 seizures. We assessed seizure stage scores during kindling development and the number of stimulations and cumulative afterdischarge durations required to reach the first stage 4 or 5 seizure, and compared the YM872-treated groups with the control groups.