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T-type calcium channel antagonists suppress tremor in two mouse models of essential tremor

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ABSTRACT

Essential tremor is a common disorder that lacks molecular targets for therapeutic development. T-type calcium channel activation has been postulated to underlie rhythmicity in the olivo-cerebellar system that is implicated in essential tremor. We therefore tested whether compounds that antagonize T-type calcium channel currents suppress tremor in two mouse models that possess an essential tremor-like pharmacological response profile. Tremor was measured using digitized spectral motion power analysis with harmaline-induced tremor and in the $GABA_A$ receptor α1 subunit-null model. Mice were given ethosuximide, zonisamide, the neuroactive steroid (3β,5α,17β)-17-hydroxyestrane-3-carbonitrile (ECN), the 3,4-dihydroquinazoline derivative KYS05064, the mibefradil derivative NNC 55-0396, or vehicle. In non-sedating doses, each compound reduced harmaline-induced tremor by at least 50% (range of maximal suppression: 53–81%), and in the $GABA_A$ α1-null model by at least 70% (range 70–93%). Because the T-type calcium channel Cav3.1 is the dominant subtype expressed in the inferior olive, we assessed the tremor response of Cav3.1-deficient mice to harmaline, and found that null and heterozygote mice exhibit as much tremor as wild-type mice. In addition, ECN and NNC 55-0396 suppressed harmaline tremor as well in Cav3.1-null mice as in wild-type mice. The finding that five T-type calcium antagonists suppress tremor in two animal tremor models suggests that T-type calcium channels may be an appropriate target for essential tremor therapy development. It is uncertain whether medications developed to block only the Cav3.1 subtype would exhibit efficacy.

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1. Introduction

In 1982, Llinás and Jahnsen described the T-type calcium channel as responsible for post-inhibitory rebound in thalamocortical neurons, whereby a vigorous depolarization with an action potential burst occurs after hyperpolarization (Llinás and Jahnsen, 1982). The T-type calcium channel became recognized as critical to the thalamic oscillation, in which excitatory thalamocortical collaterals to the reticular thalamic nucleus evoke inhibitory outflow back to thalamic projection neurons, triggering a post-inhibitory rebound, with repetition of the cycle. It became appreciated that various processes affect this oscillatory dynamic and synchrony, so that absence seizures may occur (Huguenard and McCormick, 2007). Deletion of the T-type channel gene subtype Cav3.1 eliminates absence seizures in animal models (Kim et al., 2001); and ethosuximide, which reduces T-type calcium currents (Gomora et al., 2001), suppresses absence seizures clinically and in animal models (Aizawa et al., 1997).

At the same time Llinás' group studied the thalamic T-type calcium conductance, they described this current in inferior olive neurons as part of a voltage oscillation that depends on low- and high-threshold calcium conductances and on potassium currents (Llinás and Yarom, 1981).

On the basis of physiological, neuropathological, and other studies (Köster et al., 2002; Axelrad et al., 2008), essential tremor (ET) is recognized as a cerebellar disorder. An important influence

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on cerebellar functioning is exerted by the inferior olive. In animals, harmaline hyperpolarizes olivary neurons and induces burst-firing that is transmitted through the cerebellum, manifested behaviorally as action tremor (Bernard et al., 1984). Harmaline tremor shares several similarities with ET, including suppression by anti-ET drugs (Martin et al., 2005). Llinás' group demonstrated that 1-octanol, a low-threshold calcium channel antagonist, suppresses harmaline-induced tremor (Sinton et al., 1989), and predicted that T-type current antagonists would be effective for ET. However 1-octanol has other actions at concentrations below that of half-maximal T-type calcium channel blockade, 122 μM (Todorovic and Lingle, 1998), offering alternative explanations for its tremor suppression, such as GABA current potentiation at 50 μM (Dildy-Mayfield et al., 1996).

In contrast to the growth in understanding the role of T-type channels in absence seizures and other thalamocortical rhythms, their potential role in ET has not been adequately studied. In this study we re-visited the prediction made 20 years ago that T-type calcium antagonists may effectively suppress tremor (Sinton et al., 1989). We tested whether five compounds with T-type calcium channel antagonist activity can suppress tremor in the harmaline and the $GABA_A$ receptor α1 subunit knockout mouse models. The latter model is genetic and also responds to anti-ET drugs (Kralic et al., 2005). We tested two drugs with clinical anti-absence activity, ethosuximide and zonisamide (Wilfong and Schultz, 2005), as well as the neuroactive steroid ECN, the 3,4-dihydroquinazoline derivative KYS05064, and the mibefradil derivative NNC 55-0396, three compounds with in vitro activity against T-type calcium channels (Todorovic et al., 1998; Park et al., 2006; Huang et al., 2004). Despite the structural dissimilarities among these five compounds (Fig. 1), all demonstrated anti-tremor efficacy in both models, suggesting that T-type calcium channels represent a credible therapeutic target for ET.

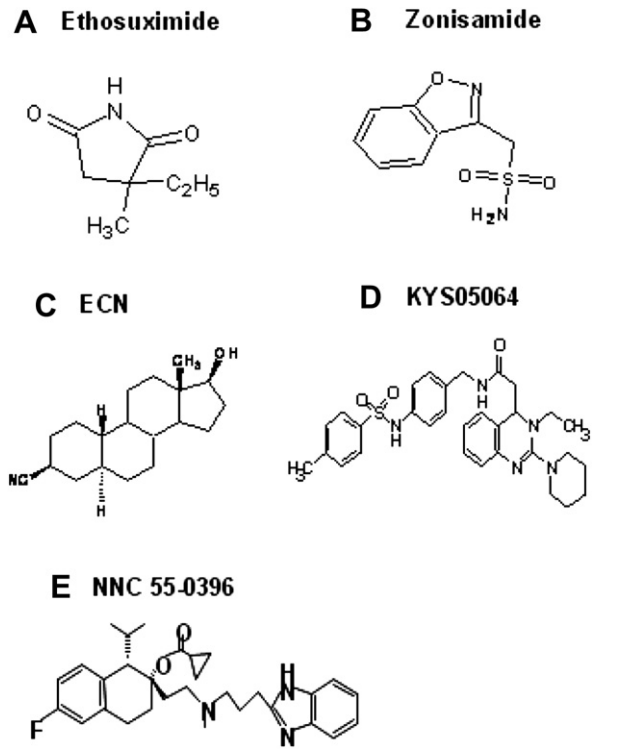

Fig. 1. Structures of T-type calcium channel antagonists tested for anti-tremor efficacy.

2. Materials and methods

2.1. Animals

Male ICR mice (20–24 g, Harlan, Indianapolis, Indiana, USA) were used in harmaline experiments. Cav3.1-null mice on a C57BL/6 background, created by K. Sakimura by deleting exon 5 of the cacna1g gene as previously described (Petrenko et al., 2007), were obtained from Riken as embryos, and bred with C57BL/6 wild-type mice and genotyped at Jackson Labs before delivery to the Veterans Affairs animal facility where they were used in harmaline experiments. Genotyping was repeated at our facility on animals utilized in this paper to confirm the genotype. $GABA_A$ receptor α1 heterozygous mice (+/−) of the F10+ generation on a mixed genetic background (~25% C57BL/6J, ~25% strain 129/Sv/SvJ, and ~50% FVB/N) were generated in Pittsburgh as previously described (Vicini et al., 2001). Heterozygotes were shipped to the VA Greater Los Angeles and subsequently interbred to produce knockout littermates for the current studies. Mice were housed in groups with free access to rodent diet and water. Genotyping was performed using previously described primers and methods (Ortinski et al., 2004). Experiments were approved by the Institutional Animal Care and Use Committee, in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health. All efforts were made to reduce animal suffering, and the number used was kept to a minimum.

2.2. Drugs

Harmaline, pentylenetetrazole, ethosuximide, zonisamide, NNC 55-0396 [(1*S*,2*S*)-2-(2-(N-[(3-benzimidazol-2-yl)propyl]-*N*-methylamino)ethyl)-6-fluoro-1,2, 3,4-tetrahydro-1-isopropyl-2-naphtyl cyclopropanecarboxylate dihydrochloride], and (2-hydroxypropyl)-β-cyclodextrin were purchased from Sigma–Aldrich (St Louis, Missouri, USA). Harmaline, pentylenetetrazole, ethosuximide, zonisamide, and NNC 55-0396 were dissolved in sterile physiological saline. KYS05064 (3-Ethyl-2-(piperidin-1-yl)-4-[N-4-(p-toluenesulfonamido)benzylacetamido]-3,4-dihydroquinazoline) was synthesized by author J. Y. Lee and dissolved in 10% DMSO/saline. ECN ((3β,5α,17β)-17-hydroxyestrane-3-carbonitrile) was synthesized by authors K. Krishnan and D. F. Covey and dissolved in (2-hydroxypropyl)-β-cyclodextrin 45% w/v in water. Harmaline was injected s.c., other drugs i.p., in a volume of 4 ml/kg. When available, the literature guided the choice of dose. In addition we utilized the "horizontal wire test" to assess for potential sedation or ataxia (Vanover et al., 1999). In this test, mice are held by the base of the tail, their forepaws brought in contact with a 2-mm diameter, 25-cm long horizontal wire, then released. In order to be scored a pass, mice had to bring at least one hindpaw in contact with the wire and not to fall within 10 s. With each compound the dose was determined at which all 6/6 ICR mice passed on all tests at 10-min intervals over 1.5 h, and the dose at which 3/6 failed the test. In each model, pilot experiments were performed to find the dosages capable of suppressing tremor by approximately 50% and 80%. Some α1-null mice were used in more than one experiment; at least 3 days separated experiments.

2.3. Tremor measurement in the harmaline model

Motion activity was measured with a Convuls-1 Replacement Sensing Platform model 1335-1A (Columbus Instruments, Columbus, Ohio, USA), a metal platform with a load sensor beneath it, connected to a Grass model P511 AC amplifier (Grass Instruments, West Warwick, Rhode Island, USA) with 1 and 70 Hz filter settings. Digitally recorded motion power was analyzed using Spike2 software (Cambridge Electronic Design, United Kingdom) to perform Fourier transformation of the data into frequency spectra. Data were sampled at 128 Hz. The *motion power percentage* (*MPP*) is the tremor bandwidth divided by overall motion power: (10–16 Hz power)/(0–34 Hz power) × 100. Baseline motion data were collected for 20 min, then harmaline, 20 mg/kg, administered, followed by test drug or vehicle 15 min later (time 0), when tremor had fully developed. Motion power was recorded for five successive 20-min epochs.

2.4. Tremor measurement in the $GABA_A$ receptor subunit α1-null model

Tremor was measured using the above apparatus while the mouse was suspended by the base of the tail with a padded clip from the ceiling of a plexiglass box resting on the motion detector platform. Tremor-associated motion power data (usually 22–27 Hz) were sampled at 454 Hz for 30 s, then the mouse returned to its cage. Each mouse had tremor sampled four times with 1-min rest periods in between during baseline, and again four times at a specified time after drug treatment. The mean 22–27 Hz motion power from each quartet of measures was used for data analysis.

2.5. Pentylenetetrazole seizures

Mice received ethosuximide, 100 mg/kg, ECN, 50 mg/kg, NNC 55-0396, 40 mg/kg, or vehicle, followed 30 min later by pentylenetetrazole, 70 mg/kg i.p. Each mouse was then placed in a plexiglass chamber and videotaped for 600 s. Each videotape was later viewed by an examiner blinded to treatments. Analyzed