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The antihyperalgesic effects of the T-type calcium channel blockers ethosuximide, trimethadione, and mibefradil

Matthew E. Barton, Elizabeth L. Eberle, Harlan E. Shannon *

Neuroscience Research Division, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, United States

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Abstract

The purpose of the present study was to explore the analgesic effects of the low voltage-activated T-type Ca^{2+} channel blockers ethosuximide, trimethadione, and mibefradil in persistent and acute nociceptive tests. The anticonvulsant effects of the compounds were also determined in the intravenous pentylenetetrazol seizure model. Following intraperitoneal administration, ethosuximide and trimethadione dose-dependently reversed capsaicin-induced mechanical hyperalgesia. Similarly, the highest dose of mibefradil tested (30 μg, intracisternal) reversed capsaicin-induced mechanical hyperalgesia. Ethosuximide and mibefradil produced statistically significant analgesic effects in both early and late phase formalin-induced behaviors and trimethadione reduced late phase behaviors. Additionally, ethosuximide and trimethadione produced antinociceptive effects in the rat-tail flick reflex test. In contrast, following intracisternal administration, mibefradil had no effect in the tail flick reflex test. In addition, the anticonvulsants ethosuximide and trimethadione increased the doses of pentylenetetrazol required to produce both first twitch and clonic seizures. In contrast however, mibefradil had no anticonvulsant effect. The present results demonstrate that the clinically used anticonvulsants ethosuximide and trimethadione provide analgesic effects at doses, which are anticonvulsant. Furthermore, the data further supports the idea that T-type Ca^{2+} channels may be important targets for treating persistent pain syndromes.

Keywords: Ethosuximide; T-type Ca^{2+} channel; Central sensitization; Pentylenetetrazol; Capsaicin; Formalin

1. Introduction

A hallmark of epilepsy and neuropathic as well as inflammatory pain is hyperexcitable neurons. From a pharmacological perspective then, treating persistent pain conditions with antiepileptic drugs may not be entirely unexpected as the primary goal of antiepileptic therapy is to decrease or limit neuronal network excitability. A number of antiepileptic drugs are in fact efficacious in treating some pain syndromes. For instance, the sodium channel blocker carbamazepine is useful in treating trigeminal neuralgia (e.g., Campbell et al., 1966) and diabetic neuropathy (e.g., Gomez-Perez et al., 1996). In addition, the anticonvulsant gabapentin has been reported to be effective in treating diabetic neuropathy (Backonja et al., 1998) and post herpetic neuralgia (e.g., Rowbotham et al., 1998).

Evidence has been accumulating that the anticonvulsant ethosuximide, a low voltage-activated T-type Ca^{2+} channel blocker (Coulter et al., 1989a,b), may also be efficacious in the treatment of persistent pain. For instance, studies have shown that ethosuximide produced dose-dependent inhibition of mechanical and thermal evoked responses (Matthews and Dickenson, 2001; Doğrul et al., 2003) in the spinal nerve ligation model of Kim and Chung (1992). Moreover, Flatters and Bennett (2004) have shown that ethosuximide reversed paclitaxel-induced cold allodynia and vincristine-induced mechanical hyperalgesia. Additionally, Shannon et al. (2005) have recently shown that ethosuximide produced analgesic effects in the formalin-induced model of persistent pain.

T-type channels were originally characterized in sensory neurons and described as small conductance channels that were activated by weak depolarization (Carbone and Lux, 1984; Nowycky et al., 1985). T-type channels have been reported in

* Corresponding author. Tel.: +1 317 276 4360; fax: +1 317 276 5546.
E-mail address: H.Shannon@Lilly.com (H.E. Shannon).

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dorsal root ganglion and dorsal horn neurons (Ryu and Randic, 1990; Talley et al., 1999; Ikeda et al., 2003; Shin et al., 2003) and T-channel mRNA transcripts have been reported in small and medium but not large sized neurons of the dorsal root ganglion (Talley et al., 1999). Correspondingly, large T-type currents have been recorded in medium-sized dorsal root ganglion neurons (Scroggs and Fox, 1992). Collectively, the data suggest that T-type currents are expressed in smaller sensory neurons, which transmit thermal and nociceptive information.

The primary purpose of the present study was to further investigate the analgesic effects of several T-type calcium channel blockers. Dose–response curves were determined for ethosuximide (Coulter et al., 1989a,b), trimethadione (Coulter et al., 1990; Zhang et al., 1996), and mibefradil (Clozel et al., 1997) using the formalin, capsaicin, and tail flick tests in rats. Ethosuximide, trimethadione, and mibefradil also were tested in the intravenous pentylenetetrazol seizure model to determine whether doses producing antinociception were also anticonvulsant. In addition, dose–response curves were determined for the opioid agonist morphine in each test for purposes of comparison.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN) were allowed free access to food and water and were housed in a temperature- and light-controlled environment (12-h on/12-h off). All experiments were conducted in accordance with NIH regulations of animal care and were approved by the Eli Lilly Institutional Animal Care and Use Committee.

2.2. Capsaicin test

Rats (250–300 g) were administered vehicle or drug and 15 min later administered capsaicin (8-methyl-*N*-vanillyl-*trans*-6-nonenamide; 30 μg in 25 μl olive oil, Sigma, St. Louis, MO) subcutaneously (s.c.) into the plantar surface of the right hind paw. Fifteen minutes after the administration of capsaicin, tactile allodynia was determined by an up–down method with a calibrated series of von Frey filaments, as previously described in detail by Chaplan et al. (1994). Briefly, animals were placed into clear plastic cages (17.5 × 15 × 15 cm) fitted with a wire floor for a 5-min acclimation period. Each filament was applied to the mid-plantar region of each hind paw from below the wire floor. Von Frey filaments with a bending force above 15 g lifted the hind paws of uninjected animals without bending and were not used; consequently the maximum withdrawal threshold was 15 g.

2.3. Formalin test

Animals weighing 180–220 g were administered vehicle or drug and individually placed in restraint cylinders (i.d. 8.5 cm; length 16 cm), which were positioned in startle behavior chambers (Model SR-Lab, San Diego Instruments, San Diego, CA). Following a 30 min pretreatment and acclimation period, rats were removed, injected s.c. with formalin (50 μl of a 5% solution in saline) into the plantar surface of the right hind paw, and immediately placed back into the restraint cylinders. The magnitude of movements was recorded continuously for 60 min in 1-s bins, as previously described in detail (Shannon and Lutz, 2000). "Agitation" events, defined as the number of 1-s bins with a change in force that exceeded a predetermined threshold (20 arbitrary units), were totaled into 5-min bins and included licking and flinching the affected paw, hopping, and turning.

2.4. Tail flick reflex

Tail flick latency was measured using the Ugo Basile Tail Flick Unit (Ugo Basile, Comerio (VA), 21025, Italy), as previously described (D'Amour and Smith, 1941; Simmons et al., 2002). Briefly, rats (180–220 g) were gently restrained and tested prior to drug administration to establish a baseline latency to tail flick. Drug or vehicle was then administered and tail flick latencies were determined 30, 60, and 120 min following treatment. The heat (50 W, I.R. = 40 U) was focused

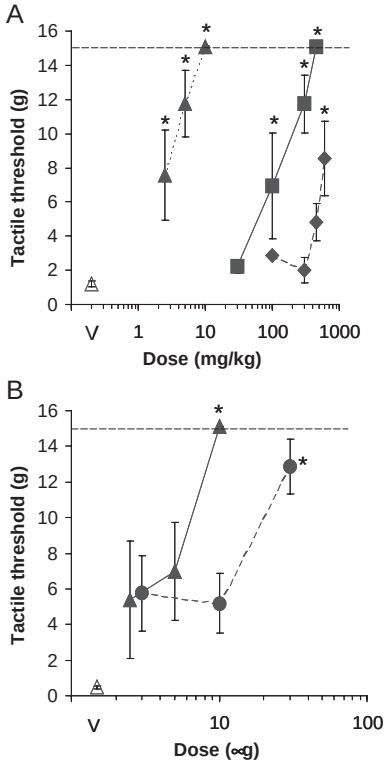

Fig. 1. A) Dose-related effects following administration of morphine (▲,s.c.), ethosuximide (■, i.p.), and trimethadione (♦, i.p.) in reversing capsaicin-induced mechanical allodynia. B) Dose-related effects following i.c. administration of morphine (▲) and mibefradil (●) in reversing capsaicin-induced mechanical allodynia. Values represent the mean ± S.E.M. Points above V represent the effects of vehicle alone. *$P<0.05$ versus vehicle control, Dunnett's *t*-test.