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

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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

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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

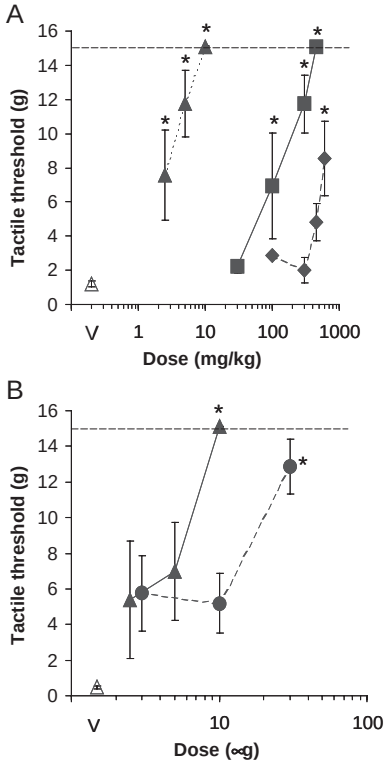

Fig. 1. A) Dose-related effects following administration of morphine (▲,s.c.), ethosuximide (■, i.p.), and trimethadione (♦, i.p.) in reversing capsaicin-induced mechanical allodynia. B) Dose-related effects following i.c. administration of morphine (▲) and mibefradil (●) in reversing capsaicin-induced 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.

approximately midway the length of the tail and was set so that baseline response latencies were 2–4 s in naïve animals, with a 10 s time limit to prevent tissue damage. The percent of maximal possible effect (% MPE) was calculated based on the recorded tail flick latencies: % MPE = [(Post test drug latency − baseline latency)/(10 s − baseline latency)] * 100.

2.5. Intravenous pentylenetetrazol seizure threshold

Separate groups of animals weighing 55–75 g were pretreated with vehicle or drug. Following a 30 min pretreatment period, pentylenetetrazol (1% PTZ, in 0.9% saline, 10 U.S.P. U/ml heparin sulfate, Sigma, St. Louis, MO) was infused into the lateral tail vein at a constant rate (0.34 ml/min; White, 1998). Time in seconds from the start of the infusion to the appearance of the first twitch and the onset of clonic activity were recorded for each animal. Each time was then converted to mg/kg PTZ for each animal.

2.6. Drugs

Morphine SO_4, ethosuximide, trimethadione and mibefradil were purchased from Sigma (St. Louis, MO), dissolved in water and administered subcutaneous (s.c.; morphine, 1.25–10 mg/kg), intraperitoneally (i.p.; ethosuximide, 30–600 mg/kg and trimethadione, 100–600 mg/kg), or intracisternally (i.c.; morphine, 2.5–10 μg and mibefradil, 3–100 μg).

2.7. Statistics

Dose–response curves in the formalin test were constructed by totaling the number of events during the first 5-min bin after formalin administration (early phase) and the total number of events from the third to ninth 5-min bins (late phase). For capsaicin and tail flick data, median effective doses (ED_{50}) and corresponding 95% confidence intervals were calculated using a Litchfield–Wilcoxon regression. Treatment groups were compared to appropriate vehicle control groups by analysis of variance and Dunnett's *t*-test.

3. Results

3.1. Capsaicin test

Paw withdrawal threshold to a mechanical stimulus was reduced to approximately 1.2 g when determined 30 min after vehicle administration and 15 min following capsaicin administration (Fig. 1, points above "V"). Pretreatment with ethosuximide (i.p.), trimethadione (i.p.), or morphine (s.c.) resulted in a dose-dependent reversal of capsaicin-induced mechanical allodynia (Fig. 1A) with a rank order of potency: morphine (ED_{50} = 2.5 mg/kg) > ethosuximide (ED_{50} = 108 mg/kg) > trimethadione (ED_{50} = 567 mg/kg). In addition, ethosuximide and morphine produced complete reversal of the allodynia. Following systemic administration (i.p.) however,

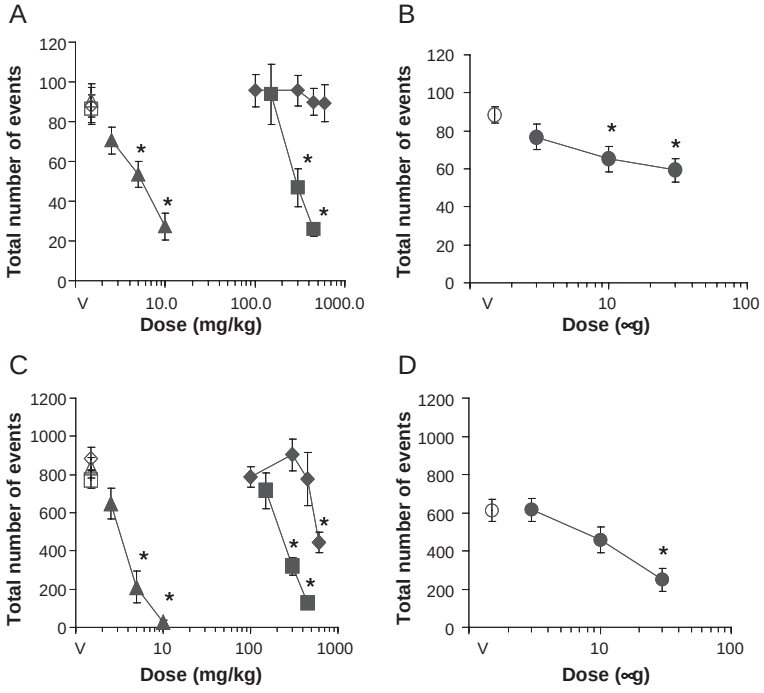

Fig. 2. A) Dose-related effects following administration of morphine (▲, s.c.), ethosuximide (■, i.p.), and trimethadione (♦, i.p.) in reducing early phase behaviors (first 5 min block) produced by the intraplantar injection of formalin in rats. B) Dose-related effects following i.c. administration of mibefradil (●) in reducing formalin-induced early phase behaviors. C) Following administration, morphine (▲, s.c.), ethosuximide (■, i.p.), and trimethadione (♦, i.p.) dose-dependently reduced the number of formalin-induced late phase behaviors (third to ninth 5-min block). D) Mibefradil (●) dose-dependently reduced the number of formalin-induced late phase behaviors following i.c. administration. 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.

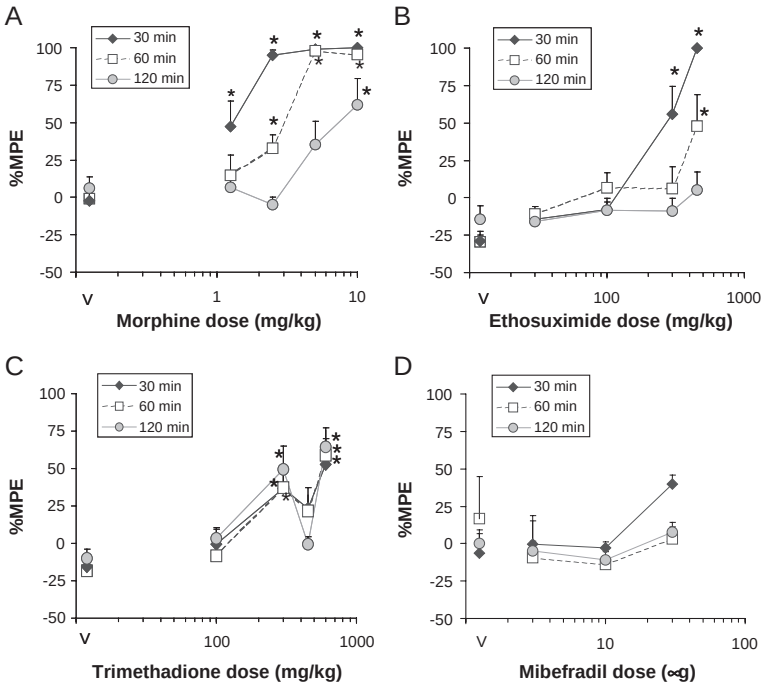

Fig. 3. A) Morphine s.c., B) ethosuximide i.p., and C) trimethadione i.p. produced dose- and time-dependent increases in tail flick latency. Values represent the mean±S.E.M. Points above V represent the effects of vehicle alone. *$P<0.05$ versus vehicle control, Dunnett's *t*-test. % MPE=percentage of maximum possible effect.

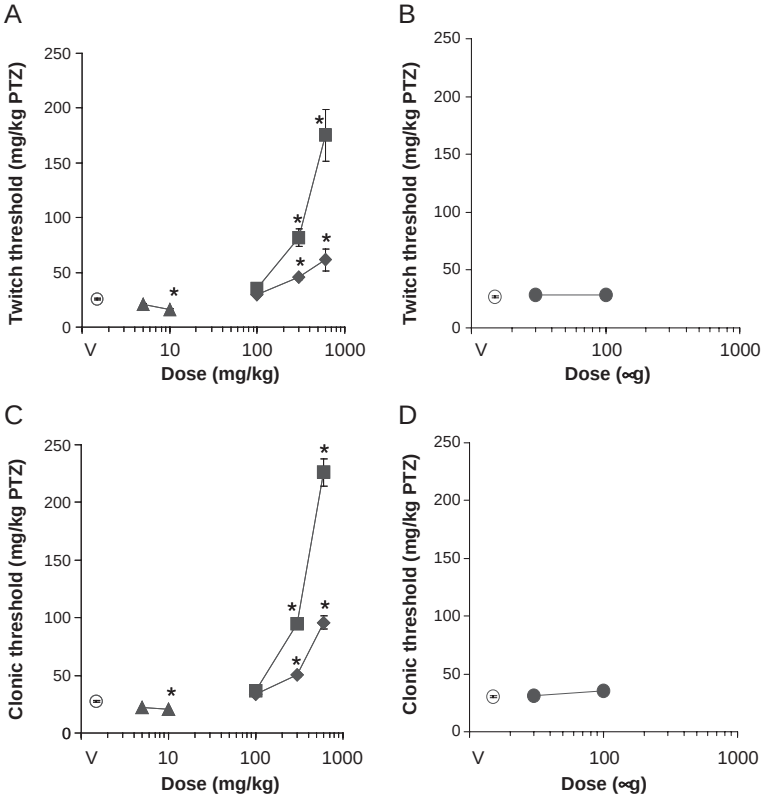

Fig. 4. A) Ethosuximide (■) and trimethadione (♦) dose-dependently increased the dose of PTZ required to produce the first twitch. Morphine (▲; 10 mg/kg) decreased the amount of PTZ required to produce the first twitch. B) Dose-related effects of mibefradil (●) on PTZ-induced first twitch. C) Ethosuximide (■) and trimethadione (♦) dose-dependently increased the dose of PTZ required to produce clonic seizures. In contrast, morphine (▲; 10 mg/kg) decreased the amount of PTZ required to produce clonic seizures. D) Mibefradil (●) had no effect on the dose of PTZ required to produce clonic seizures. 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.

mibefradil had no effect on capsaicin-induced allodynia (data not shown). However, pretreatment with mibefradil i.c. or morphine i.c. resulted in dose-dependent reversal of capsaicin-induced mechanical allodynia with ED_{50} values of 9.2 and 4.1 μg for mibefradil and morphine, respectively (Fig. 1B).

3.2. Formalin test

Administered 30 min before formalin, morphine (2.5–10 mg/kg, s.c.) and ethosuximide (100–450 mg/kg, i.p.) produced dose-dependent analgesic effects in the early phase (the initial 5 min block; Fig. 2A) of the formalin response. The effects of the 5 and 10 mg/kg doses of morphine and 300 and 450 mg/kg of ethosuximide were statistically different from vehicle controls. Similarly, pretreatment with mibefradil (i.c.) produced dose-dependent analgesic effects in the early phase (Fig. 2B) of the formalin response with doses of 10 and 30 μg providing modest but statistically significant decreases when compared to vehicle controls. In contrast, however, pretreatment with trimethadione up to 600 mg/kg did not alter the early phase response (Fig. 2A). In addition, morphine, ethosuximide, and mibefradil also produced dose-dependent analgesic effects in the late phase (5 min blocks 3–9; Fig. 2C and D) of the formalin response. Moreover, trimethadione (i.p.) produced analgesia in the late phase at the highest dose tested (600 mg/kg; Fig. 2C).

3.3. Tail flick reflex

Tail flick latencies were time- and dose-dependently increased following pretreatment with morphine (ED_{50}=1.2 mg/kg; Fig. 3A), ethosuximide (ED_{50}=132 mg/kg; Fig. 3B), and trimethadione (ED_{50}=232 mg/kg; Fig. 3C). Additionally, ethosuximide and morphine produced the maximum possible effect at the highest doses tested (450 mg/kg, i.p. and 10 mg/kg, s.c., respectively). In contrast, mibefradil (3–30 μg/rat) was without significant effect on tail flick latencies after i.c. administration (Fig. 3D).

3.4. i.v. PTZ seizure threshold

Pretreatment with ethosuximide (100–600 mg/kg, i.p.) produced approximately a 7-fold increase in the threshold dose of PTZ required to produce the first twitch (Fig. 4A). Trimethadione (100–600 mg/kg, i.p.) increased first twitch threshold approximately 3-fold (Fig. 4A). Mibefradil (30–100 μg, i.c.) pretreatment however had no significant effect on PTZ-induced twitch threshold (Fig. 4B). In contrast, the dose of PTZ required to produce the first twitch was reduced following the administration of morphine (5–10 mg/kg, s.c.; Fig. 4A).

As observed with first twitch thresholds, pretreatment with ethosuximide (100–600 mg/kg, i.p.) and trimethadione (100–600 mg/kg, i.p.) produced approximately 8- and 4-fold increases in the dose of PTZ required to produce clonus when compared to vehicle controls (Fig. 4C), respectively. Mibefradil (30–100 μg, i.c.) had no significant effect on PTZ-induced clonic seizure thresholds (Fig. 4D). Morphine (5–10 mg/kg, s.c.) however produced a significant decrease in the clonic seizure threshold for PTZ (Fig. 4C).

4. Discussion

The goal of the present study was to investigate the antihyperalgesic properties of several low voltage-activated T-type Ca^{2+} channel blockers. The results demonstrate that the antiepileptic drugs and T-type Ca^{2+} channel blockers ethosuximide and trimethadione are effective in attenuating the response to formalin and capsaicin, and produce antinociception at doses that are anticonvulsant. Mibefradil (i.c.) was similarly effective in decreasing the response to formalin and capsaicin but unlike ethosuximide and trimethadione did not produce antinociception nor was mibefradil anticonvulsant.

Ethosuximide, trimethadione and mibefradil all produced dose-related reversals of capsaicin-induced mechanical allodynia. The subcutaneous injection of capsaicin selectively activates primary afferent C-fibers (Baumann et al., 1991) and sensitizes spinothalamic tract neurons (Simone et al., 1991; Dougherty and Willis, 1992), resulting in thermal hyperalgesia and mechanical allodynia (Gilchrist et al., 1996). T-type Ca^{2+} channels were originally characterized in sensory neurons (Carbone and Lux, 1984; Nowycky et al., 1985) and dorsal root ganglion as well as in dorsal horn neurons in the spinal cord (Ryu and Randic, 1990; Talley et al., 1999; Ikeda et al., 2003; Shin et al., 2003). Activation of T-type Ca^{2+} channels is thought to lower the threshold for action potentials and promote bursting activity (e.g., Huguenard, 1996; Matthews and Dickenson, 2001), factors which could contribute to central sensitization and mechanical allodynia. The present findings that ethosuximide, trimethadione and mibefradil are efficacious in reversing capsaicin-induced mechanical allodynia thus provide further support for the hypothesis that low voltage-activated T-type Ca^{2+} channels are important in modulating sensory transmission, including pain-related information.

The s.c. injection of formalin produces biphasic behavioral and electrophysiological effects (Dubuisson and Dennis, 1977; Puig and Sorkin, 1996; Dickenson and Sullivan, 1987a,b) with the early phase thought to represent an acute nociceptive injury and the late phase a model of central sensitization (e.g., Dubuisson and Dennis, 1977). While ethosuximide, trimethadione, and mibefradil all reduced the formalin-induced late phase behaviors, only ethosuximide and mibefradil produced dose-related reductions in the formalin-induced early phase behaviors. Ethosuximide (Matthews and Dickenson, 2001; Doğrul et al., 2003) and mibefradil (Doğrul et al., 2003) have been shown to reverse both tactile allodynia and thermal hyperalgesia in animals with ligation of the L_5/L_6 branches of the sciatic nerve. Additionally, Flatters and Bennett (2004) demonstrated that ethosuximide produced analgesia in paclitaxil- and vincristine-induced peripheral neuropathy. The present investigation complements the findings of these previous reports, which demonstrated that ethosuximide and mibefradil were efficacious in neuropathic pain models and extends these findings to include the T-type channel blocker

trimethadione. Taken together, the data indicate that blockade of T-type Ca^{2+} channels may provide a novel treatment of persistent pain conditions.

Ethosuximide and mibefradil were analgesic not only in the early phase of the formalin test, but also, along with trimehadione, produced antinociception in the tail flick test, suggesting that T-type channel blockers may be efficacious in treating acute pain as well as persistent pain. The present findings support those of Todorovic et al. (2001) which reported that mibefradil produced antinociception in thermal and mechanical nociceptive tests following systemic administration. In contrast, Doğrul et al. (2001) reported that mibefradil, administered systemically, had no effect in the tail flick test. Furthermore, neither ethosuximide nor mibefradil produced thermal antinociception in either the uninjured limb of sciatic nerve ligated rats or in sham operated rats (Doğrul et al., 2003). The reasons for these differences in the acute antinociceptive efficacy of T-type channel blockers are not entirely apparent, but may be related to the differences in the pain models, doses, or routes of administration used in each study. Alternatively, T-type channels may only be activated in states of hyperexcitability, such as after the administration of capsaicin or formalin, or when an acute noxious stimulus is more intense. Further studies are needed to more clearly define the potential acute antinociceptive properties of T-type Ca^{2+} channel blockers following systemic and central administration.

Both central and peripheral T-type channels may be important in modulating pain-related sensory information. In the present studies, i.c. but not systemically administered mibefradil reversed capsaicin-induced mechanical allodynia as well as decreased both early and late phase formalin behaviors, suggesting that supraspinal T-type channels may be important in modulating pain-related information. However, in the sciatic nerve ligation model (Doğrul et al., 2003), both mibefradil and ethosuximide blocked tactile and thermal hypersensitivities after i.p. administration. Following local intraplantar administration however, only mibefradil, and not ethosuximide, produced antinociception (Doğrul et al., 2003). Because mibefradil does not cross the blood–brain barrier, the efficacy of mibefradil after i.p. and intraplantar administration suggests that peripheral T-type channels are important in modulating pain-related information, at least in neuropathic pain. Moreover, neither mibefradil nor ethosuximide were efficacious in the sciatic nerve ligation model after intrathecal administration at the doses tested (Doğrul et al., 2003), thereby suggesting that spinal T-type channels may be less important. However, Matthews and Dickenson (2001) demonstrated the importance of T-type channels in the spinal cord in the sciatic nerve ligation model. While further experiments are needed, the findings to date suggest that T-type channels in the periphery, spinal cord and brain may play different roles in different pain states.

Of particular clinical relevance from the present investigation is the demonstration that ethosuximide and trimethadione provided analgesia at doses that were also anticonvulsant. Thus, our results suggest the possibility that ethosuximide and trimethadione may be useful in the treatment of pain at clinically relevant (i.e., anticonvulsant) doses. Although mibefradil produced analgesia, it was not anticonvulsant after i.c. administration. The reason(s) for the lack of anticonvulsant activity of mibefradil are unclear; however, mibefradil (i.c.) may not have reached sufficient concentrations in higher brain centers such as the thalamus to produce anticonvulsant effects. Alternatively, three subtypes of T-type channels have been cloned (e.g., see Gomora et al., 2001) and it is possible that the different subtypes of T-type channels may be differentially involved in producing anticonvulsant, acute antinociceptive, antihyperalgesic and/or antiallodynic effects.

T-type calcium channels enhance neuronal excitability by allowing Ca^{2+} entry near resting membrane potentials (Magee and Johnston, 1995). This Ca^{2+} entry strengthens synaptic inputs and decreases the activation threshold required for action potential generation (Huguenard, 1996). Thus, blockade of T-type channels may be expected to lead to a decrease in overall neuronal excitability. It has been suggested that block of T-type channels with ethosuximide may decrease excitability through a number of mechanisms. Matthews and Dickenson (2001) have suggested that block of T-type channels may lead to decreased neurotransmitter release thereby preventing depolarization. Interestingly, NMDA-mediated post-discharge spikes, which are a hallmark of central sensitization and hyperexcitability, were especially sensitive to the effects of ethosuximide (Matthews and Dickenson, 2001). However, ethosuximide had a similar effect both before and after neuropathic injury (Matthews and Dickenson, 2001). In contrast, the T-type channel antagonist α-methyl-α-phenylsuccimide was more effective in reducing T-currents in normal rats than axotomised rats (Chung et al., 1993). Taken together, the data suggest that T-type channel modulation of neuronal excitability may be more prominent in hyperexcitable and/or neuropathic states.

In summary, the analgesic activity of ethosuximide, trimethadione, and mibefradil further supports the proposed role of T-type channels in nociception and processing of pain-related information. Moreover, the observation that dose ranges providing analgesia and anticonvulsant activity overlap suggests that the T-type channel blockers such as ethosuximide and trimethadione may be a potentially novel therapy for some pain syndromes.

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