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Effects of T-type calcium channel blockers on a parkinsonian tremor model in rats

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ABSTRACT

T-type calcium channels are strongly associated with the generation of rhythmic firing patterns in the CNS. Blockers of these channels may have therapeutic potential for treating various types of tremor. The present study aimed to study the effects of a range of T-type calcium channel blockers in a parkinsonian tremor model in rats. We tested the effects of several T-type calcium channel blockers, including zonisamide (ZNS), ethosuximide, lomerizine, amiloride, mibefradil, and NCC 55-0396, a mibefradil derivative, on tacrine-induced tremulous jaw movements (TJMs), an animal model of parkinsonian tremor. Among the tested drugs, only ZNS and NCC 55-0396 significantly suppressed TJMs when given at a non-sedating dose. The transitivity of drugs to the central nervous system (CNS) may at least partially explain their differential anti-TJM effects. However, further studies are necessary to reveal other factors, since ethosuximide failed to show anti-TJM effects despite being known to cross the blood brain barrier. The present results suggest that T-type calcium channels in the CNS may be a suitable target for developing new therapeutic strategies for treating parkinsonian tremor.

1. Introduction

Voltage-gated calcium channels (VGCCs) mediate the entry of calcium ions into cells, and are involved in a variety of calcium-dependent processes such as muscle contraction, neurotransmitter release, gene expression, and cell division or death (Lory and Chemin, 2007). VGCCs are divided into two major functional classes: high-voltage-activated and low-voltage-activated channels (Catterall, 2000). Among these, 'T-type calcium channels' refer to transient or low-voltage-activated channels. T-type calcium channels are reported to be strongly associated with the generation of rhythmic firing patterns in the CNS (Llinás and Yarom, 1981; Gutnick and Yarom, 1989), raising the possibility that blockers of these channels may have therapeutic potential for tremors. It was recently reported that T-type calcium channel blockers exhibited anti-tremor effects on harmaline-induced tremor, an experimental model of essential tremor (Yang et al., 2008; Handforth et al., 2010). The olivocerebellar system has been shown to play a key role in the tremor-generating mechanisms underlying harmaline-induced tremor (Llinás and Yarom, 1981; Wilms et al., 1999; Cavelier et al., 2008; Miwa et al., 2008). In addition, experimental studies have revealed that Purkinje cell complex spikes are mediated by T-type calcium channels (Cavelier et al., 2008), and that $Ca_V3.1$ T-type calcium channels in the inferior olive nucleus are involved in the tremor-rhythm pacemaker in harmaline-induced tremor (Park et al., 2010). These findings suggest that T-type calcium channel blockers may directly inhibit tremor-rhythm generation in the olivocerebellar system, resulting in an improvement of tremors. In accord with this notion, recent clinical studies demonstrated that zonisamide (ZNS), a widely used antiepileptic drug with a T-type calcium channel blocking action, successfully ameliorated essential tremor (Morita et al., 2005; Song et al., 2008; Handforth et al., 2009). Since it could be speculated that tremor-generating rhythmic firing is present in not only the olivocerebellar system but also other functional systems in the CNS such as the basal ganglia, T-type calcium channel blockers may have therapeutic potential for treating parkinsonian tremor, for which tremor-generators exist in the basal ganglia.

Thus, it is important to determine whether or not currently available T-type calcium channel blockers suppress experimental parkinsonian tremors. Unfortunately, there has been limited research into the anti-tremor effects of T-type calcium channel blockers, in terms of both experimental tremors in animals and human tremor disorders. The primary purpose of the present study was to investigate the effects of several currently available T-type calcium channel blockers, including ethosuximide, ZNS, lomerizine, amiloride, mibefradil, and the mibefradil derivative NCC 55-0396 (Huang et al., 2004), on tacrine-induced tremulous jaw movements (TJMs) in rats (Salamone et al., 1998; Salamone et al., 2005). Most of these drugs are already used in clinical settings. Tacrine-induced TJMs are considered a rat model of parkinsonian tremor (Miwa, 2007b; Salamone et al., 1998; Salamone et al., 2005). Clinically, the development of new therapeutics that specifically treat parkinsonian tremor is important, because it is one of the most important factors associated with the quality of life impairment in patients with Parkinson's disease (PD), and is often residual even after dopamine

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replacement therapy has been initiated. Unfortunately, no previous studies have examined the effects of T-type calcium channel blockers other than ZNS using the parkinsonian tremor model (Miwa et al., 2008). The present study sought to provide information helpful for the development of new therapeutic strategies for the treatment of parkinsonian tremor.

2. Methods

2.1. Subjects

The subjects were adult male Sprague–Dawley rats (N = 36), weighing 250–380 g at the time of the experiments. The animals were housed in cages and kept in a temperature-controlled room under a 12 h/12 h light/dark cycle. Food and tap water were freely accessible. The experimental protocols were approved by Wakayama Medical University's Animal Care and Use Committee.

2.2. Drugs

Ethosuximide, amiloride HCl, lomerizine HCl, and mibefradil are all known to have T-type calcium channel blocking actions, and have all been used widely in clinical settings. NCC 55-0396, a derivative of mibefradil, is a potent and T-type calcium channel blocker that is more selective than mibefradil (Huang et al., 2004). These drugs were purchased from Sigma-Aldrich (USA). Zonisamide HCl was generously provided by Dainippon-Sumitomo Pharmaceuticals (Japan). All drugs were prepared immediately prior to administration.

2.3. Procedures

Cholinomimetic-induced TJMs have been proposed as a pharmacological model of Parkinsonian tremor in rodents (Salamone et al., 1998; Salamone et al., 2005). To examine the effects of T-type calcium channel blockers on tacrine-induced TJMs, a T-type calcium channel blocker or the same amount of a control vehicle, was administered intraperitoneally. Subsequently, 30 min later, 9-amino-1,2,3-tetrahydroaminoacridine HCl (tacrine; Sigma-Aldrich, St Louis, MO, USA; 5 mg/kg, dissolved in saline) was administered intraperitoneally. Ten minutes after the administration of tacrine, tacrine-induced TJMs were measured for 5 min by two experienced observers (J.K. and Y. K.) who were unaware of the treatment procedures. TJMs were defined as vertical deflections of the lower jaw that resemble chewing, in accord with previous reports (Salamone et al., 1998; Salamone et al., 2005). In addition, sedation was semi-quantified with a sedation score determined by the response to external stimulation including sound, and whisker touching (0 = awake and active; 1 = awake, but composed, with preserved response to external stimulation; 2 = sedated, with preserved response only to intense external stimulation, and slow response; and 3 = overly sedated and unresponsive to external stimulation). Inter-rater variability was found to be significant by linear regression analysis ($r = 0.88$, $P < 0.05$). The median sedation scores obtained were analyzed statistically. Rats were tested for a period of at least one week. Consistent with earlier studies, vehicle levels of TJM activities were consistent across the repeated weeks of the study. Over the course of the study, pharmacobehavioral studies were performed either two or three times in each rat.

2.4. Data analysis

TJMs and sedation scores were represented as mean ± SE and median, respectively. Differences between groups were examined with a non-parametric Kruskal–Wallis test, and a Mann–Whitney test was used for comparisons between the two groups. The level of statistical significance was set at $P < 0.05$.

3. Results

Tacrine-induced TJMs that were measured following the administration of each drug and vehicle in each experiment were demonstrated (Fig. 1). As shown in Fig. 1, ZNS significantly suppressed tacrine-induced TJMs in a dose-dependent manner (Fig. 1A).

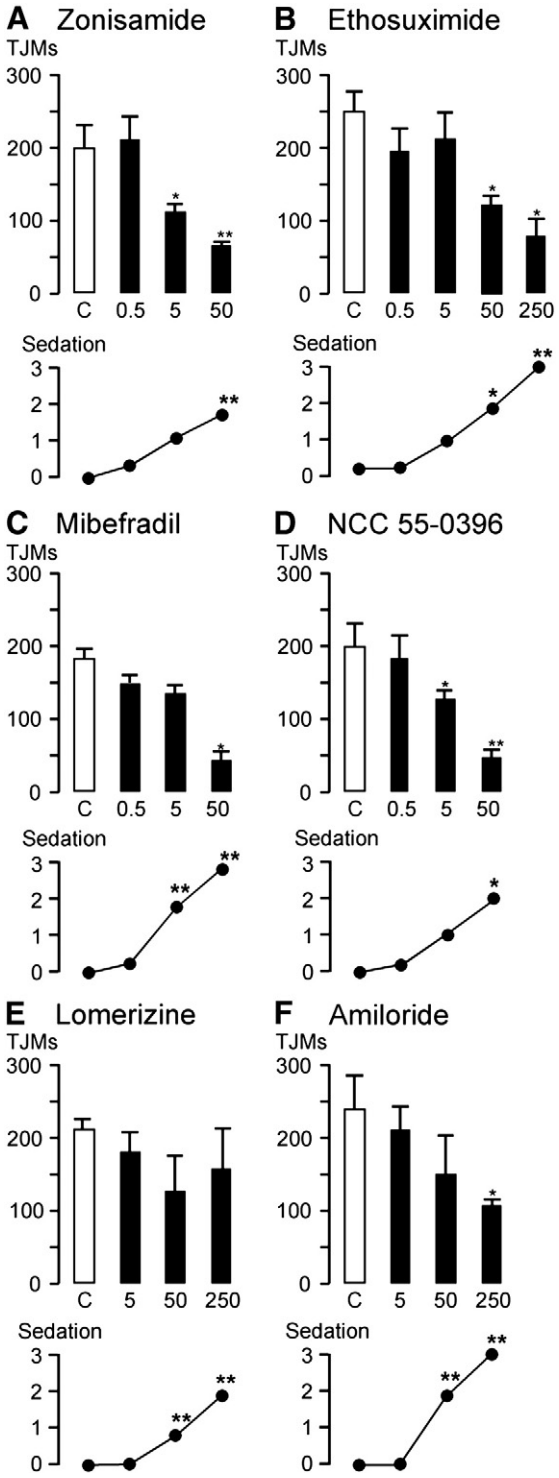

Fig. 1. Anti-tremor effects of T-type calcium blockers on tacrine-induced tremulous jaw movements (TJMs) per 5 min. Data are shown as mean (columns) and S.E. (bars). The results of experiments performed with each drug treatment. Either four or five rats were tested with each drug dose. Sedation scores were simultaneously obtained. Asterisks in the TJM data indicate $P < 0.05$ (* vs. control; ** vs. 5 mg/kg). Asterisks in data of sedation score indicate $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control.

Treatment with a higher dose of ZNS (50 mg/kg) but not a lower dose (5 mg/kg) caused a sedative effect. Ethosuximide failed to suppress tacrine-induced TJMs at non-sedating doses (Fig. 1B), and significant suppressive effects were found only at the higher doses (50 and 250 mg/kg), which induced marked sedative effects. Mibefradil did not suppress tacrine-induced TJMs (Fig. 1C), except at an extremely high dose (50 mg/kg) that caused strong sedation. On the other hand, NNC 55-0396 significantly suppressed tacrine-induced TJMs (Fig. 1D) in a dose-dependent fashion that was observable at non-sedating doses. Lomerizine did not show any significant anti-TJM effects at any dose (Fig. 1E). Similarly, amiloride failed to show any anti-tremor effects (Fig. 1F), and significant suppressive effects on TJMs were found only at the highest dose (250 mg/kg), which induced a marked sedative sate.

4. Discussion

Currently, the T-type calcium channel is a promising therapeutic target for the treatment of a number of neurological disorders including epilepsy, sleep disorders, and neuropathic pain (Shin et al., 2008). In addition, the notion that T-type calcium channel blockers may possess anti-tremor potential for treating various types of tremors is becoming increasingly accepted. This is because T-type calcium channel blockers have been found to successfully suppress essential tremor (Morita et al., 2005; Song et al., 2008; Handforth et al., 2009), harmaline-induced tremor in rodents (Miwa et al., 2008; Handforth et al., 2010), and genetic tremor in $GABA_A$ receptor alpha1 subunit-null mice (Handforth et al., 2010). The present findings provide additional data in favor of the notion that T-type calcium channel blockers may have an anti-tremor action on various types of tremor, including parkinsonian tremor.

A total of six drugs known to exhibit low-voltage-activated T-type calcium channel blocking actions were examined in the present study: ZNS, ethosuximide, mibefradil, NCC 55-0396, lomerizine, and amiloride. Apart from NCC 55-0396, all of these drugs are currently used in clinical settings or have been in the past. Their pharmacological actions are heterogeneous. First, ZNS inhibits T-type calcium currents without affecting L-type channels, but also enhances sodium channel inactivation (Biton, 2007; Miwa, 2007a). This drug also modulates the monoaminergic system (Okada et al., 1992; Okada et al., 1995; Gluck et al., 2004; Yabe et al., 2009). Second, Ethosuximide also is an antiepileptic drug that acts on T-type calcium channels. Its anti-epileptic mechanisms remain unclear, but it has been proposed that ethosuximide may inhibit the pacemaker oscillatory activity of thalamocortical circuitry (Gören and Onat, 2007). Ethosuximide also decreases persistent Na^+ and Ca^{2+}-activated K^+ currents in neurons (Gören and Onat, 2007). Third, mibefradil was the first known selective T-type calcium channel blocker (Lory and Chemin, 2007). It was used for the treatment of hypertension and angina pectoris, but is currently withdrawn from the market because of unfavorable drug interactions. Mibefradil is regarded as a suitable pharmacological tool for influencing T-type calcium channels, and also inhibits L-type calcium channels (Lory and Chemin, 2007). Unfortunately, it cannot cross the blood brain barrier. NCC 55-0396 is a structural analog of mibefradil, and has been shown more selective to T-type calcium channels than mibefradil (Huang et al., 2004; Li et al., 2005). In addition, lomerizine, 1-[bis(4-fluorophenyl)methyl]-4-(2,3,4-trimethoxybenzyl) piperazine dihydrochloride, is a L- and T-type calcium channel blocker that is clinically used as a prophylactic for treating migraines. It inhibits not only voltage-dependent calcium channels but also serotonin receptors (Ishii et al., 2009). It does not affect the central dopaminergic system (Ozaki et al., 1991). Amiloride is a potassium-conserving diuretic drug that inhibits epithelial sodium channels, and has a T-type calcium channel blocking action (Tang et al., 1988). It has been used clinically for the treatment of hypertension and heart failure.

Among these drugs, only ZNS and NCC 55-0396 were found effective in suppressing tacrine-induced TJMs in a dose-dependent fashion, in non-sedating doses. The lack of an anti-tremor effect of mibefradil suggests that T-type calcium channel blockers that are unable to cross the blood brain barrier do not have anti-tremor properties. It has been previously suggested experimentally that M4 receptors in the ventrolateral neostriatum play a critical role in the generation of cholinomimetic-induced TJMs (Cousins et al., 1999), supporting the speculation that the anti-TJM effect may result from central rather than peripheral mechanisms. Although there is currently no available data clearly demonstrating that NCC 55-0396 actually enters the brain, it was reported that NNC 55-0396 effectively suppress both harmaline-induced tremor and genetic tremor in mice (Handforth et al., 2010), suggesting that the NNC 55-0396 exerts pharmacological effects in the CNS. Thus, it would be expected that T-type calcium channel blockers that can reach the CNS would contribute to tremor suppression. Indeed, neither lomerizine nor amiloride are able to cross the blood brain barrier, and neither showed anti-tremor effects. However, although ethosuximide shows high transitivity to the brain, it failed to suppress tacrine-induced TJMs in non-sedating doses. This finding may be related to the conflicting results of previous studies regarding the anti-parkinsonian tremor action of ethosuximide. It was reported that ethosuximide suppressed tremor in a primate model of parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Gomez-Mancilla et al., 1992); however, the anti-tremor effect of ethosuximide was not clinically replicated in patients with PD (Pourcher et al., 1992). The present results do not support a therapeutic potential of ethosuximide for treating anti-parkinsonian tremor. In contrast, we observed a significant anti-TJM effect of ZNS. In accord with our finding, it has been clinically reported previously that ZNS can suppress parkinsonian tremor (Murata et al., 2001; Nakanishi et al., 2003; Murata and Hasegawa, 2007). Although ZNS may have a monoamine oxidase-B inhibitory action and improves not only tremor but also motor fluctuations (Murata et al., 2001; Nakanishi et al., 2003), it was experimentally demonstrated that the anti-TJM effect of ZNS remained under conditions of monoamine-depletion or dopamine blockade, suggesting that its anti-tremor effect is not mediated via dopaminergic neurotransmission (Miwa et al., 2008). The cause of the differences in the anti-tremor effects between ZNS and ethosuximide is currently unclear. However, the potent anti-TJM potential of zonisamide, which was demonstrated in the present study and has been suggested clinically (Nakanishi et al., 2003), is particularly noteworthy.

To fully elucidate the anti-tremor efficacy of T-type calcium channel blockers, it is essential to develop a drug that selectively blocks T-type calcium channels and easily crosses blood brain barrier. However, based on the present results as well as previously reported findings that T-type calcium channel blockers effectively suppress harmaline-induced tremor (Miwa et al., 2008; Handforth et al., 2009), we propose that T-type calcium channels may be an appropriate therapeutic target for the treatment of various types of tremors, including parkinsonian tremor as well as essential tremor.

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