Jpn. J. Pharmacol. 82, 247 – 260 (2000)

The Chinese Herbal Medicine *Chai-Hu-Long-Ku-Mu-Li-Tan* (TW-001) Exerts Anticonvulsant Effects Against Different Experimental Models of Seizure in Rats

Hung-Ming Wu[1], Chiung-Chun Huang[1], Li-Hsin Li[1], Jing-Jane Tsai[2] and Kuei-Sen Hsu[1,*]

[1]*Department of Pharmacology and* [2]*Department of Neurology, College of Medicine, National Cheng-Kung University, Tainan City, Taiwan 70101*

Received October 27, 1999 *Accepted December 16, 1999*

ABSTRACT—We evaluated the anticonvulsant effect of *Chai-Hu-Long-Ku-Mu-Li-Tan* (TW-001), a Chinese herbal medicine, and its mechanisms in several standard rodent models of generalized seizure. TW-001 (4 g/kg, p.o.) significantly increased the threshold for tonic electroconvulsions and the threshold for tonic seizures in response to i.v. infusion of pentylenetetrazole (PTZ). In the s.c. PTZ seizure test, both the incidence and severity of seizures were decreased by TW-001. TW-001 (1 – 10 mg/ml) did not alter resting membrane potential or input resistance of the hippocampal CA1 neurons, but elicited a reversible suppression of stimulus-triggered epileptiform activity in area CA1 and spontaneously occuring epileptiform burst discharges in area CA3 elicited by picrotoxin. Both field excitatory postsynaptic potentials and population spikes were reversibly depressed by TW-001 (0.5 – 15 mg/ml) in a concentration-dependent manner. The sensitivity of postsynaptic neurons to a glutamate-receptor agonist, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid or *N*-methyl-D-aspartate, was not altered by TW-001 (10 mg/ml). However, TW-001 (5 mg/ml) clearly increased the magnitude of paired-pulse facilitation. TW-001 (5 – 10 mg/ml) reversibly limited the repetitive firing and reduced the maximal rate of rise of action potentials elicited by injection of depolarizing current pulses (0.4 nA, 200 ms) into the pyramidal cells. TW-001 (1 – 10 mg/ml) exerted a concentration-dependent reduction of the tetrodotoxin-sensitive sodium currents and high voltage-activated calcium currents. These results suggest that TW-001 is an interesting new anticonvulsant agent that exerts its anticonvulsant activity through inhibition of sodium and calcium channels, stabilizing neuronal membrane excitability and inhibiting glutamate release.

Keywords: *Chai-Hu-Long-Ku-Mu-Li-Tan* (TW-001), Epileptiform activity, Paired-pulse facilitation, Sodium channel, Calcium channel

Epilepsy, a common neurological disorder, is characterized by abnormal discharges of cerebral neurons manifested as various types of seizures. Different types of seizures may have different neurobiological conditions and are controlled with different medications. Extensive research during the past decades has elucidated the neural system involved in seizures, including the biochemical processes underlying seizures and the mechanism of action of antiepileptic drugs (AEDs) (1 – 3). Although judicious use of presently available AEDs allows 70 – 75% of epileptic patients to be seizure-free, as many as 25 – 30% of patients who develop partial and secondary generalized seizures are refractory to treatment with the current AEDs (4, 5). In addition, severe adverse effects are common with most of the presently available AEDs. For example, chronic administration of carbamazepine can cause stupor, coma and respiratory depression, along with drowsiness, vertigo, ataxia and blurred vision (6). As a consequence, there still remains an urgent need for the development of new AEDs with high efficacy and less adverse effects.

Recently, the alternative management of epilepsy in terms of Chinese medicine becomes attractive to patients as well as medical professionals both in Taiwan and Western countries. Some Chinese herbals and mixtures were shown to have potential antiepileptic effects in both experimental studies and clinical use (7). The Chinese herbal medicine *Chai-Hu-Long-Ku-Mu-Li-Tan* (TW-001) is a mixture of 13 herbal drugs: *Chai-Hu* (*Bupleuri radix*), *Long-Gu* (*Fossllia ossis mastodi*), *Huang-Qin* (*Scutellariae radix*), *Sheng-Jiang* (*Zingiberis rhizoma*), *Dang-Shen* (*Codonopsitis*

* To whom correspondence should be addressed.

radix), *Gui-Zhi* (*Cinnamomi ramulus*), *Fu-Ling* (*Hoelen*), *Ban-Xia* (*Pinelliae rhizoma*), *Mou-Li* (*Ostrease testa*), *Tian-Ma* (*Gastrodiae rhizoma*), *Da-Huang* (*Rhei rhizoma*), *Da-Zao* (*Zizyhpi fructus*) and *Gou-Teng* (*Uncaria thorn*). It has been used as a sedative and an anticonvulsant drug from ancient times according to the description in the Chinese medicine book *Shang-Han-Lun*. However, there has not been any report regarding the pharmacological evaluation of the cellular mechanism underlying the anticonvulsant action of TW-001. In the present study, we examined the anticonvulsant profile of TW-001 in several types of in vivo seizure models previously proposed for the evaluation of new anticonvulsant drugs in mice and rats (8). Furthermore, the possible cellular mechanism responsible for the anticonvulsant action of TW-001 was also investigated. Because an abnormality of glutamate-mediated neurotransmission in the brain was thought to serve as a possible trigger for initiating the epileptic phenomena in various animal and human syndromes (9, 10), this prompted us to evaluate whether TW-001 exerts its anticonvulsant effect via interfering with the glutamatergic synaptic transmission in the brain. To test this possibility, we examined the effect of TW-001 on the excitatory synaptic transmission in the CA1 region of rat hippocampal slices by using intracellular and extracellular recording techniques. We found that TW-001, p.o. elevates the seizure threshold for tonic electroconvulsions and the threshold for tonic seizures in response to i.v. infusion of pentylenetetrazole (PTZ). This anticonvulsant activity of TW-001 is likely to be mediated by the inhibition of sodium and calcium channels. These effects are functionally related to stabilize neuronal membrane excitability and inhibit neurotransmitter release, particularly that of glutamate.

MATERIALS AND METHODS

Animals

Animal care was consistent with the guidelines set by the Laboratory Animal Center of National Cheng-Kung University. All experimental procedures were approved by the Animal Research Committee of Medical College of the National Cheng-Kung University. Adult male Sprague-Dawley rats were obtained from a commercial breeder (Laboratory Animal Center NCKU, Tainan, Taiwan) at ages of 8 – 10 weeks (body weight 180 – 200 g) and were used after 1 week of adaptation to the laboratory. Animals were kept in groups of 5 in plastic cages at controlled temperature (25°C) and were housed in a vivarium with a 12-h light/dark cycle (light on at 7:00 a.m.), 50 – 60% humidity, and free access of food and drinking water. To minimize the bias of circadian rhythms, all drug injection were done in the forenoon at an ambient temperature of 24 – 25°C.

Maximal electroshock seizure threshold (MEST) test

The threshold for seizures induced by maximal (tonic hindlimb extension) electroshock was determined via transauricular electrodes (i.e., copper electrodes introduced bilaterally into the ears) by means of a stimulator (Stoelting Physiology Research Instruments, Wood Dale, IL, USA) that delivered a constant current (1-s duration, 50 Hz, sinewave form, fully adjustable between 1 – 99 mA). The stimulus intensity was varied (a baseline current of 15 mA) by an up-and-down method shock titration (8) whereby the current was lowered or raised (in 3- to 5-mA steps) if the preceding animal did or did not show hindlimb extension, respectively. The data thus generated in groups of 20 rats were used to calculate the threshold current for inducing hindlimb extension in 50% of the rats [medium convulsive current (CC_{50}) with confidence limits for 95% probability] using the method of Kimball et al. (11). Each group of animals was used for only one threshold determined. The effects of TW-001 (2 and 4 g/kg, p.o., 0.5 – 2 h pretest) on seizure threshold were compared to those of phenytoin (10 and 20 mg/kg, i.p., 1 h pretest), valproate (100 and 200 mg/kg, i.p., 1 h pretest) and lamotrigine (2.5 and 5 mg/kg, i.p., 3 h pretest).

PTZ seizure test

The threshold for different types of PTZ-induced seizures was determined by infusing of a 1% solution of PTZ (0.5 ml/min) into the tail vein. Groups of 20 – 25 were used per threshold determined. Both the threshold for myoclonic (defined as one or more isolated whole body jerks) and tonic hindlimb extension seizures were quantitated.

For the s.c. PTZ seizure test, before TW-001 evaluation, the dose response of PTZ was determined in rats by s.c. injection of different PTZ doses in groups of 20 – 30 rats. PTZ was injected s.c. in the back of the neck of the rats. The rats were then observed for 30 min after injection and the first generalized clonic seizure with loss of righting reflexes was used as the endpoint. The dose inducing this seizure type in 97% of the rats was calculated in rats (60 mg/kg) by the method of Litchfield and Wilcoxon (12). Seizure severity was classified behaviorally into five stages according to a modification of Löscher and Hönack (13): stage 1, one or more generalized myoclonic twitches of the whole body; stage 2, repeated clonic seizures of fore- and/or hindlimbs without loss of righting reflexes; stage 3, a generalized clonus with repeated clonic seizures of fore- and hindlimbs and exhibited loss of righting reflexes; stage 4, clonic seizures with loss of righting reflexes followed by backward extension of forelimbs; stage 5, tonic hindlimb extension.

In vitro hippocampal slice studies

Hippocampal slices (400-μm-thick) were obtained from

5- to 6-week-old male Sprague-Dawley rats for intracellular and extracellular synaptic recordings by the procedures described previously (14, 15). In brief, the rats were decapitated, and transverse slices were cut from a tissue block of the brain using Vibroslice (Campden Instruments, Silbey, UK). The slices were placed in a storage chamber of artificial cerebral spinal fluid (ACSF) oxygenated with 95% O_2 – 5% CO_2 and kept at room temperature for at least 1 h before recording. The composition of the ACSF solution was as follows: 117 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM $NaHCO_3$, 1.2 mM NaH_2PO_4 and 11 mM glucose at pH 7.3 – 7.4 and equilibrated with 95% O_2 – 5% CO_2.

A single slice was then transferred to the recording chamber, in which it was held submerged between two nylon nets and maintained at 32.0 ± 0.5°C. The chamber consisted of a circular well of a low volume (1 – 2 ml) and was perfused constantly at a rate of 2 – 3 ml/min. Standard extracellular field recording techniques were used. Extracellular recordings of fEPSPs and PSs were obtained from the stratum radiatum and stratum pyramidal of CA1, respectively, using microelectrodes filled with 1 M NaCl (resistance 2 – 3 MΩ). A bipolar stainless steel stimulating electrode was placed in stratum radiatum to activate Schaffer collateral/commissural afferents. In some experiments, the extracellular recordings were made in stratum pyramidal of CA3 with microelectrodes containing 1 M NaCl (resistance 2 – 3 MΩ) and the bipolar stimulating electrode was placed in stratum radiatum of CA3 to activate recurrent collaterals. Stimulus-response curves were performed at the beginning of each experiment. Pulses of an intensity that gave 30 – 40% of the maximal fEPSP or PS were given at a frequency of 0.033 Hz for the remainder of the experiment. In all experiments, baseline synaptic transmission was monitored for 30 min before drug administration. The strength of synaptic transmission was quantified by measuring the slope of fEPSP and the amplitude of PS. The fEPSP slope was measured from approximately 20 – 70% of the rising phase using a least-squares regression. The PS amplitude was measured from the peak negativity to a tangent line drawn between the first and second maximum positivities. Intracellular recordings were made from CA1 pyramidal neurons using glass microelectrodes filled with 4 M potassium acetate (80 – 100 MΩ). Microelectrodes were pulled from microfiber 1.0-mm capillary tubing on a Brown-Flaming electrode puller (Sutter Instruments, San Rafael, CA, USA). Electrical signals were collected with an Axoclamp-2B (Axon Instruments, Foster, CA, USA) filtered at 1 kHz, sampled at 10 kHz; and an IBM 586-based computer with pCLAMP software (Versions 7.0, Axon Instruments) was used to on-line acquire and analyze the data.

Acutely dissociated hippocampal CA1 neurons and patch clamp whole cell recordings

Hippocampal neurons from 12- to 14-day-old Sprague-Dawley rats were acutely dissociated using procedures similar to those that we have described previously (16, 17). Briefly, after decapitation, the brain was removed, and 200- to 400-μm-thick transverse hippocampal slices were cut at room temperature by use of a McIlwain tissue chopper. Then, they were incubated for 30 min with oxygenated piperazine-*N*-*N*′-bis(2-ethanesulfonic acid) (PIPES) saline solution containing: 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 25 mM glucose, 20 mM PIPES and 0.5% pronase (protease type XIV) at 34 – 35°C (pH 7.4). The CA1 region was microdissected and subsequently triturated using a fire-polished Pasteur pipette in a test tube containing 2 ml cold (4 – 8°C) PIPES saline solution. The neuronal suspension was then transferred into a recording chamber mounted on a Olympus IX-70 inverted microscope. Before recording, the neurons were thoroughly washed with external recording solution (see below). Experiments were performed between 30 min and 8 h after isolation of neurons.

Recordings were carried out using the whole cell voltage-clamp techniques as previously described (16, 17). Patch pipettes for the whole cell recordings were pulled from borosilicate glass and fire polished before use. Direct current resistances were 2 – 5 MΩ. For the calcium current recordings, the patch pipettes were filled with internal solution of the following composition: 100 mM cesium methanesulfonate, 2 mM $MgCl_2$, 20 mM tetraethylammonium (TEA) Cl, 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 10 mM 1,2-bis(2-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), 5 mM MgATP, 0.3 mM NaGTP and 0.2 mM leupeptin. The ATP regeneration system [tris(hydroxy-methyl)aminomethane-phosphocreatine (20 mM) and creatine kinase (20 U/ml)] was added to the internal solution to minimize rundown of the calcium currents. The pH was adjusted with 1 M CsOH to 7.2 after the addition of ATP, and the osmolarity was 280 – 290 mosmol. The external recording solution for calcium currents contained: 120 mM TEA Cl, 3 mM $CaCl_2$, 10 mM HEPES, 5 mM 4-aminopyridine (4-AP), 10 mM CsCl and 10 mM glucose. Tetrodotoxin (TTX, 1 μM) was added to the external recording solution to inhibit voltage-dependent sodium channels, and the solution was adjusted to pH 7.4 with 1 M HCl solution and to an osmolarity of 320 mosmol. For the sodium current recordings, the patch pipettes were filled with internal solution of the following composition: 100 mM cesium methanesulfonate, 1 mM $CaCl_2$, 20 mM TEA Cl, 10 mM HEPES, 10 mM BAPTA, 10 mM glucose, 5 mM MgATP, 0.3 mM NaGTP, 0.2 mM leupeptin, 20 mM tris(hydroxymethyl)aminomethane-phosphocreatine and 20 U/ml crea-

tine kinase. The pH was adjusted with 1 M CsOH to 7.2 after the addition of ATP, and the osmolarity was 280 – 290 mosmol. The external recording solution for sodium currents contained: 120 mM NaCl, 20 mM TEA Cl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 2 mM 4-AP, 0.3 mM $CdCl_2$ and 10 mM glucose. The external solution was adjusted to pH 7.4 with 1 M HCl solution and to an osmolarity of 320 mosmol.

Recordings were carried out at room temperature using a patch-clamp amplifier (Axopatch 200A, Axon Instruments). After seal formation and before entering the whole cell mode, electrode capacitance was neutralized by using the capacitance compensation circuitry of the Axopatch 200 A. The series resistance was 80 – 90% compensated; only recordings with access resistance below 8 MΩ were included in the analysis. Current recordings were filtered at 2 kHz and digitized at sampling rates of 200 μs using a 12-bit (333 kHz) analog-to-digital converter (Digidata 1200B, Axon Instruments) interfaced with an IBM 586 computer. Stimulation and acquisition were made using pCLAMP software (version 6.0.3, Axon Instruments). Capacitive and leakage currents were digitally subtracted from all recordings.

Drug applications

The composition of TW-001 used in this study was as follows: 8.0 g *Chai-Hu* (*Bupleuri radix*), 3.0 g *Long-Gu* (*Fossllia ossis mastodi*), 3.0 g *Huang-Qin* (*Scutellariae radix*), 3.0 g *Sheng-Jiang* (*Zingiberis rhizoma*), 3.0 g *Dang-Shen* (*Codonopsitis radix*), 3.0 g *Gui-Zhi* (*Cinnamomi ramulus*), 3.0 g *Fu-Ling* (*Hoelen*), 3.0 g *Ban-Xia* (*Pinelliae rhizoma*), 3.0 g *Mou-Li* (*Ostrease testa*), 2.0 g *Tian-Ma* (*Gastrodiae rhizoma*), 1.5 g *Da-Huang* (*Rhei rhizoma*), 3.0 g *Da-Zao* (*Zizyhpi fructus*) and 6.0 g *Gou-Teng* (*Uncaria thorn*). These 13 herbal drugs were mixed, added to 600 ml of water and then boiled down to 300 ml, filtered, and spray-dried. TW-001 was prepared by the local Chinese pharmaceutical factory Yung-Shin (Taichung, Taiwan). For control groups, distilled water was administered instead of TW-001.

In the in vitro hippocampal slice recordings, the drugs were applied by dissolving them to the desired final concentrations in the ACSF and by switching the perfusion from control ACSF to drug-containing ACSF. Appropriate stock solutions of drugs were made and diluted with ACSF just before application. In the in vitro whole cell voltage-clamp recordings, TW-001 was dissolved in the external solutions and its superfusate was filtered with a 0.45-μm microfilter before application. Picrotoxin, phenytoin, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), *N*-methyl-D-aspartate (NMDA), valproate, TTX, pronase and nimodipine were purchased from Sigma (St. Louis, MO, USA); ω-conotoxin GVIA, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D-2-amino-5-phosphonovalerate (D-APV) were obtained from Research Biochemicals Inc. (Natick, MA, USA). Lamotrigine was a kind gift from Welcome Foundation (Kent, England).

Statistical analyses

The data for each experiment were normalized relative to baseline. All figures show the mean ± S.E.M. The significant difference was evaluated by Student's two-tailed unpaired *t*-test; multiple comparisons were made with one-way represented measure analysis of variance (ANOVA). Numbers of experiments are indicated by n. A probability value (P) of less than 0.05 was considered to represent significant differences.

RESULTS

Effect of TW-001 on MEST test

As shown in Table 1, TW-001 at 4 g/kg, p.o. significantly increased the threshold for tonic hindlimb extension seizures induced by electroshock. One hour was the time of the peak effect after administration of 4 g/kg of TW-001, because a significant seizure threshold increase of 32.4% was seen after 1 h and no anticonvulsant effect was observed after 0.5 h. The level of anticonvulsant efficacy attained by TW-001 in the MEST test was also compared with that

Table 1. Effect of TW-001, phenytoin, valproate and lamotrigine on the threshold for maximal (tonic hindlimb extension) electroshock seizures in rats

Treatment	Time (h)	Dose	Seizure threshold (mA)
Vehicle	1	4 g/kg (p.o.)	24.6 ± 1.9
TW-001	1	2 g/kg (p.o.)	28.7 ± 1.5
TW-001	1	4 g/kg (p.o.)	32.4 ± 1.5*
TW-001	0.5	4 g/kg (p.o.)	28.6 ± 1.3
TW-001	2	4 g/kg (p.o.)	31.4 ± 1.8*
Vehicle	1	— (i.p.)	26.2 ± 2.1
Phenytoin	1	10 mg/kg (i.p.)	34.1 ± 1.5**
Phenytoin	1	20 mg/kg (i.p.)	41.1 ± 1.4***
Vehicle	1	— (i.p.)	27.3 ± 1.5
Valproate	1	100 mg/kg (i.p.)	34.3 ± 1.7**
Valproate	1	200 mg/kg (i.p.)	42.3 ± 1.7***
Vehicle	3	— (i.p.)	29.2 ± 2.1
Lamotrigine	3	2.5 mg/kg (i.p.)	43.8 ± 1.7***
Lamotrigine	3	5 mg/kg (i.p.)	53.7 ± 1.5***

The drugs were administered p.o. or injected i.p. in groups of rats, and the seizure threshold was determined after 0.5, 1, 2 and 3 h, respectively. The seizure thresholds were evaluated as medium convulsive current (CC_{50}) with confidence limits for 95% probability in groups of 20 rats. Significant difference between vehicle and drug-treated groups is indicated (*P<0.05, **P<0.01, ***P<0.001).

Table 2. Effect of TW-001 on the threshold for different seizure types induced by i.v. infusion of pentylenetetrazole (PTZ) in rats

Treatment	Time (h)	Dose (g/kg, p.o.)	Seizure threshold with PTZ (mg/kg)	
			Initial myoclonic twitch	Hindlimb extension
Vehicle	1	4	42.6 ± 4.7	70.3 ± 5.5
TW-001	1	2	43.8 ± 5.3	76.7 ± 3.6
TW-001	1	4	44.4 ± 4.5	91.6 ± 5.3**
TW-001	0.5	4	43.6 ± 5.7	77.4 ± 5.8
TW-001	2	4	44.1 ± 4.8	89.4 ± 4.8*

TW-001 was administered p.o. in groups of rats, and the seizure threshold was determined after 0.5, 1 or 2 h, respectively. The seizure thresholds were calculated as the dose of PTZ (mean ± S.E.M.) inducing the respective seizure types in all animals of a group, using 20 – 25 rats per group. Significant difference between vehicle and drug-treated groups is indicated (*P<0.05, **P<0.01).

generated by the standard AEDs, phenytoin (10 and 20 mg/kg, i.p.), valproate (100 and 200 mg/kg, i.p.) and lamotrigine (2.5 and 5 mg/kg, i.p.). The threshold increase obtained with TW-001 (4 g/kg, p.o.) was equal to both phenytoin (10 mg/kg, i.p.) and valproate (100 mg/kg, i.p.), but was clearly less than lamotrigine (2.5 mg/kg, i.p.).

Effect of TW-001 on i.v. and s.c. PTZ test

TW-001 was found to markedly increase the threshold for PTZ-induced tonic hindlimb extension seizures at a dose of 4 g/kg, p.o., but had no significant effect on myoclonic twitches induced by this chemical convulsant. The peak effect of TW-001 to increase the threshold for PTZ-induced hindlimb extension seizure was reached at 1 h after oral administration.

Based on the time-course studies with TW-001 on the MEST test (Table 1) and i.v. PTZ test (Table 2), we performed the s.c. PTZ test by pretreating the animals with TW-001 at 1 h before the PTZ administration (Table 3). TW-001 exerted anticonvulsant effect against seizures in the traditional s.c. PTZ seizure test, in which animals were observed for the occurrence of seizures for 30 min after s.c. injection of PTZ (60 mg/kg). TW-001 significantly decreased the incidence, the severity and the mortality of PTZ seizures in a dose-dependent manner. In addition, the latency of myoclonic twitches after PTZ s.c. infusion was markedly prolonged by TW-001 at a dose of 4 g/kg pretreatment.

Adverse effects of TW-001

For rats treated with high doses of TW-001 (8 and 16 g/kg, p.o.), no overt behavioral changes were observed. For example, it did not induce any significant changes in the walking, rearing and grooming behaviors of rats. In addition, no sedation or loss of righting reflex was evident. Oral administration of TW-001 to rats at 4 g/kg twice daily for three weeks also did not result in any toxic symptoms. Histological analysis of the hippocampus of chronic TW-001 treatment rats also revealed no abnormalities (data not shown). It was impossible to determine the medium lethal dose (LD_{50}) of TW-001, since no intoxication or death was observed in rats even at a dose as large as 16 g/kg, p.o. These results suggest that TW-001 is safer than presently available AEDs.

Lack of effect of TW-001 on resting membrane potential and input resistance

To characterize the molecular mechanisms underlying the TW-001-induced anticonvulsant effect, the isolated rat hippocampal slices were used to address this issue by using intracellular and extracellular recording techniques. Firstly, we examined the effect TW-001 on passive electrophysiological properties of hippocampal CA1 neurons, including resting membrane potential (RMP) and input resistance. As shown in Table 4, TW-001 (1 – 10 mg/ml) produced no significant changes on the level of either RMP or input resistance of the recording neurons.

Effect of TW-001 on the Schaffer collateral-commissural synaptic transmission

Because the decrease of glutamatergic synaptic transmission has been claimed as part of the mechanism of action of some new AEDs (3, 18), we next examined whether TW-001 exerts its anticonvulsant activity via the modula-

Table 3. Effect of TW-001 on seizure parameters induced by s.c. injection of pentylentetrazole (PTZ) in rats

Treatment	Dose (g/kg, p.o.)	n	Seizure parameters			
			Incidence (%)	Latency(s) (± S.E.M.)	Severity (± S.E.M.)	Mortality (%)
Vehicle	4	30	87	392.6 ± 24.7	3.8 ± 0.3	17
TW-001	2	26	57*	447.8 ± 31.3	2.8 ± 0.2**	4*
TW-001	4	20	35*	508.4 ± 34.5**	2.3 ± 0.2**	0*

PTZ (60 mg/kg) was applied by s.c. injection at 1 h after orally administration of placebo or TW-001. The latency is for the appearance of the hindlimb extension. Significant difference between vehicle and drug-treated groups is indicated (*P<0.05, **P<0.01). The number of animals tested was represented by n.

tion of glutamatergic synaptic transmission in the brain. As shown in Fig. 1 (A–E), superfusion of TW-001 (0.5–15 mg/ml) reversibly decreased fEPSP and PS in a concentration-dependent manner. At a concentration of 5 mg/ml, it decreased the slope of fEPSP by 30.2 ± 5.3% (n = 6, $P<0.05$) and the amplitude of PS by 41.4 ± 5.8% (n = 7, $P<0.05$) of baseline, respectively. The apparent IC_{50} of TW-001 against fEPSP and PS was 11.7 and 8.6 mg/ml, respectively. The maximum effect of TW-001 was obtained in 10 to 15 min, and completely recovery from its effects required a 10–15 min washout with ACSF. Furthermore, the presynaptic fiber volley, which proceeds the fEPSP, was not significantly affected by TW-001. Application of TW-001 (5 and 10 mg/ml) produced a 6.8 ± 1.5% (n = 6) and 9.4 ± 3.5% (n = 6) reduction of the amplitude of the presynaptic fiber volley, respectively (Fig. 1F).

To determine whether the blockade of glutamatergic synaptic transmission induced by TW-001 was mediated by a presynaptic or postsynaptic mechanism, we examined the effect of TW-001 (10 mg/ml) on the postsynaptic response to exogenously applied glutamatergic receptor agonists, AMPA and NMDA, by intracellular recordings. These experiments were done in the presence of TTX (0.5 μM). As shown in Fig. 2, application of AMPA (1 μM) and NMDA (1 μM) produced a profound membrane depolarization on the hippocampal CA1 neurons. TW-001 pretreatment for 20 min did not affect the AMPA- or NMDA-induced membrane depolarization. These data indicate that the blockade of glutamatergic synaptic transmission induced by TW-001 in the hippocampal CA1 neurons is not mediated by a postsynaptic modulation of AMPA or NMDA glutamatergic receptors.

Effect of TW-001 on paired-pulse facilitation

We furthermore examined whether the blockade of glutamatergic synaptic transmission by TW-001 involves a presynaptic mechanism that could be detected with the phenomenon of paired-pulse facilitation (PPF). When the excitatory afferents to the hippocampal CA1 neurons are activated twice with a short interval between each stimulus, the response to the second stimulus is generally facilitated in relation to the initial stimulus (16, 19). This phenomenon is called a PPF and is attributed to an increase in the amount of transmitter release in response to the second stimulus (20, 21). On the other hand, the manipulations of presynaptic transmitter release may result in the change in the magnitude of PPF. If the TW-001-induced depression of fEPSP involved a presynaptic mechanism of action, it will be associated with an alteration of PPF magnitude. Alternatively, if TW-001 reduced synaptic transmission by another type of mechanism (e.g., reducing the sensitivity of postsynaptic receptors), then the PPF magnitude should be relatively unaffected. To test this hypothesis, the magnitude of PPF was determined at the control period prior to the application of TW-001 and 20 min after starting the application of 5 mg/ml TW-001. Synaptic responses to a paired stimuli were recorded with an interstimulus interval of 40 ms. Figure 3 shows that the reduction of fEPSP slope induced by TW-001 was accompanied by an increase in the magnitude of PPF. The magnitude of PPF was 136.8 ± 5.9% before and 169.6 ± 8.5% (n = 6) during the application of TW-001. The increase of the magnitude of PPF by TW-001 was reversible during washout with ACSF. These results suggest that TW-001 may act at a presynaptic site to modulate the transmitter release mechanisms in the CA1 region of rat hippocampus.

Table 4. Effect of TW-001 on passive membrane properties of rat hippocampal CA1 neurons

Treatment	E_m (mV)	E_{in} (MΩ)	n
Control	−63.4 ± 1.9	44.6 ± 2.1	10
TW-001			
1 mg/ml	−64.8 ± 2.1	43.1 ± 1.9	6
5 mg/ml	−65.3 ± 1.7	41.9 ± 2.3	10
10 mg/ml	−64.4 ± 2.2	40.8 ± 1.7	8

E_m, resting membrane potential; E_{in}, membrane input resistance; n, number of recording neurons. During the recording, 0.2 nA hyperpolarizing current pulses were intracellularly injected to monitor the membrane input resistance. Data are presented as the mean ± S.E.M.

Effect of TW-001 on epileptiform activity in the hippocampal CA1 and CA3 areas

If TW-001 could be used as an anticonvulsant agent, it could not only reduce the evoked subthreshold fEPSP and PS but also the evoked epileptiform activity induced by convulsant agents in vitro. To test this possibility, we examined the effect of TW-001 on the epileptiform burst discharges evoked by picrotoxin in the hippocampal areas CA1 and CA3. As shown in Fig. 4, after superfusion of picrotoxin (50 μM) to block γ-aminobutyric acid (GABA) type A receptors, the same stimulus intensity evoked epileptiform burst discharges that consisted a train of several spikes riding on a large depolarizing potential. Application of TW-001 (5 and 10 mg/ml) reduced the later spikes riding on the depolarizing potential in a concentration-dependent manner. The effect of TW-001 was reversible within 20 min of washout (Fig. 4E).

In area CA3, picrotoxin caused recurrent epileptiform burst discharges with a regular repetition rates about 15–20 min after starting perfusion, which occurred in the absence of electrical stimulation. The spontaneously occurring recurrent discharges were monophasic or biphasic and occurred with a regular repetition rate of 4.2 ± 0.8 min^{-1} (n = 10). As shown in Fig. 5, TW-001 at a concentration of

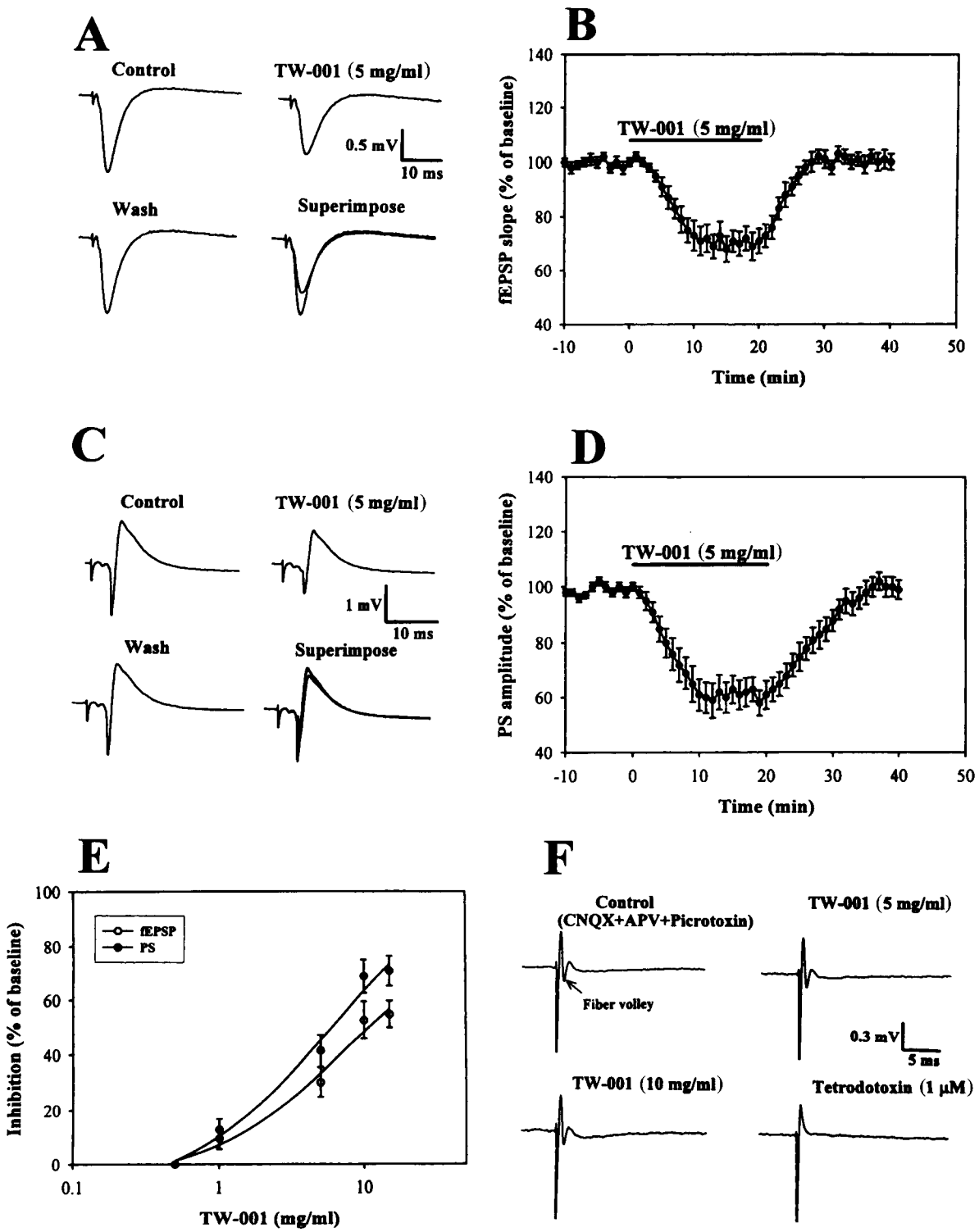

Fig. 1. Effect of TW-001 on the glutamatergic synaptic transmission in the hippocampal CA1 area. A and C: Typical fEPSPs and PSs recorded before, during and after wash out of 5 mg/ml TW-001. Note that TW-001 reversibly decreased the slope of fEPSP and the amplitude of PSs. B and D: Time course of the action of TW-001 on the fEPSP and PS. Bars denote the period of delivery of TW-001. E: Concentration-dependent inhibition of the fEPSP and PS by TW-001. Data are presented as the mean ± S.E.M. (n = 4 – 7 experiments). F: Representative traces showing TW-001 has no significant effect on the pharmacologically isolated presynaptic fiber volley. The presynaptic fiber volley was isolated by recording the fEPSP in the presence of 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX, 20 μM), D-2-amino-5-phosphonovalerate (D-APV, 50 μM) and picrotoxin (50 μM). Similar results were also observed in another five experiments.

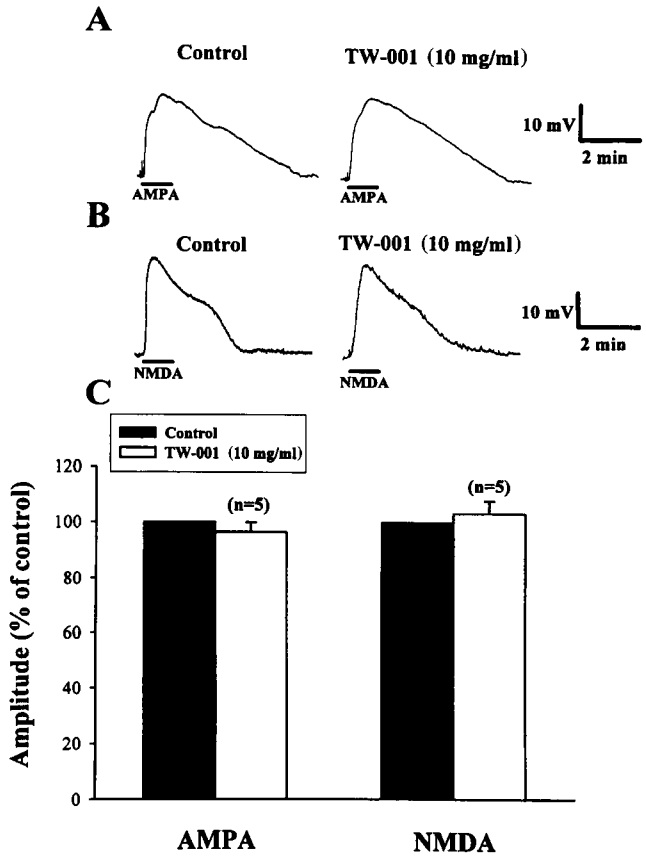

Fig. 2. Effect of TW-001 on the AMPA- and NMDA-induced membrane responses. A and B: Superfusion of either AMPA (1 μM, 1 min) or NMDA (1 μM, 1 min) evoked a membrane depolarization. Pretreatment of the hippocampal slices with TW-001 (10 mg/ml) for 20 min did not significantly affect either the AMPA- or NMDA-induced membrane depolarization. The experiments were performed in the presence of TTX (0.5 μM). Bars denote the periods of drug applications. C: The average percentage changes of either AMPA- or NMDA-induced membrane depolarization before and during TW-001 application. Data are presented as the mean ± S.E.M.

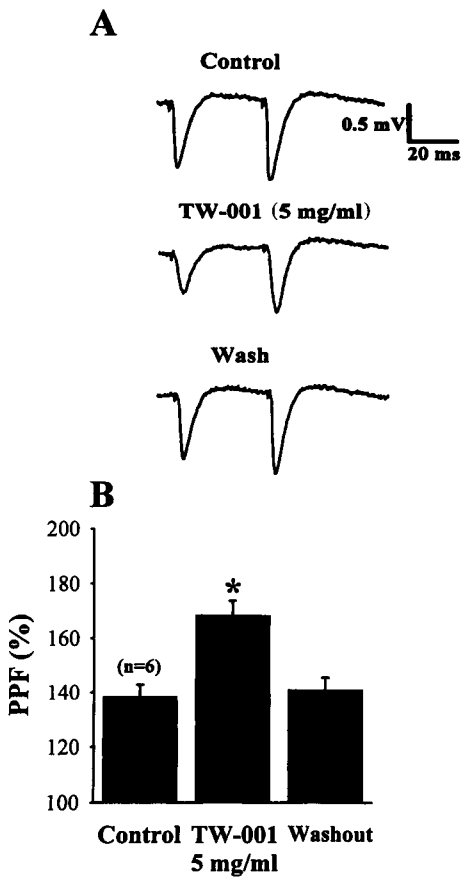

Fig. 3. TW-001 increases paired-pulse facilitation (PPF). A: Sample records of fEPSP evoked by paired pulse stimuli with a 40-ms interstimulus interval under before, during and 20 min after washout of TW-001 (5 mg/ml). B: Bar graph showing the average of similar experiments as shown in panel A. The PPF ratio was calculated according to the formula PPF (%) = $P_2 / P_1 \times 100\%$, with P_1 being the response to the first stimulus and P_2 being the response to the second stimulus. Data are presented as the mean ± S.E.M. *P<0.05 as compared with the control.

10 mg/ml reduced the amplitude, the duration and the repetition rate of the spontaneously occurring discharges (right panel). The rate of the spontaneously occurring discharges was decreased to 2.5 ± 0.6 min^{-1} after application of TW-001. Furthermore, TW-001 also reversibly attenuated the stimulus-triggered epileptiform burst discharges elicited by picrotoxin in the hippocampal area CA3 (left panel).

Effect of TW-001 on depolarizing current-induced repetitive firing (RF) of action potentials and the maximal rate of rise (V_{max}) of action potentials

The effect of TW-001 on the RF of action potentials elicited by depolarizing current pulses applied through the recording microelectrodes into the hippocampal CA1 neurons were examined. As shown in Fig. 6, a depolarizing current pulse of 0.4 nA with 200-ms duration evoked RF in the control condition. Superfusion of TW-001 (5 and 10 mg/ml) reversibly limited RF in a concentration-dependent manner (left panel). The effect of 5 and 10 mg/ml TW-001 on RF were observed in 8 out of 10 and 9 out of 10 neurons tested, respectively. During the application of TW-001 at 5 and 10 mg/ml, the mean number of action potential spikes was significantly reduced from 7.2 ± 0.8 to 5.8 ± 0.4 ($n = 10$, $P<0.05$) and 4.1 ± 0.6 ($n = 10$, $P<0.05$), respectively.

Because the V_{max} of the action potential is generally used as an index of inward sodium currents, we therefore used this parameter to determine whether the limitation of the depolarizing current pulse-induced RF by TW-001 is due to the retardation of inward sodium currents. A typical example of TW-001 on V_{max} is shown in Fig. 6. During a depolarizing current pulse applied into the cell in the

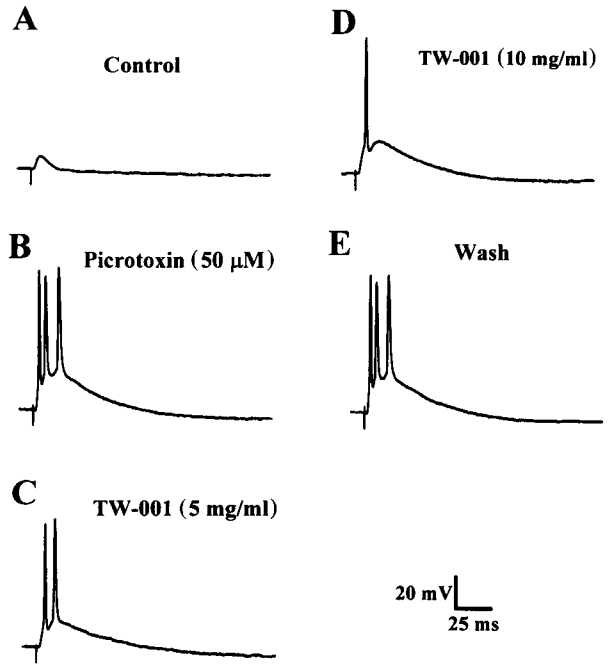

Fig. 4. Effect of TW-001 on the epileptiform burst discharges induced by picrotoxin in hippocampal area CA1. A: Control EPSP. B: Evoked epileptiform burst recorded in the hippocampal CA1 neuron in the presence of picrotoxin (50 μM). C and D: Superfusion of TW-001 (5 and 10 mg/ml) effectively suppressed the epileptiform burst discharges in a concentration-dependent manner. E: The effect of TW-001 was reversible within 20 min washing. The RMP of this cell was -62 mV. Similar results were also found in another tested five cells. Closed triangles represent the point of synaptic stimulation. The stimulus intensity was 25 V with 0.02-ms duration and kept constant throughout the experiment.

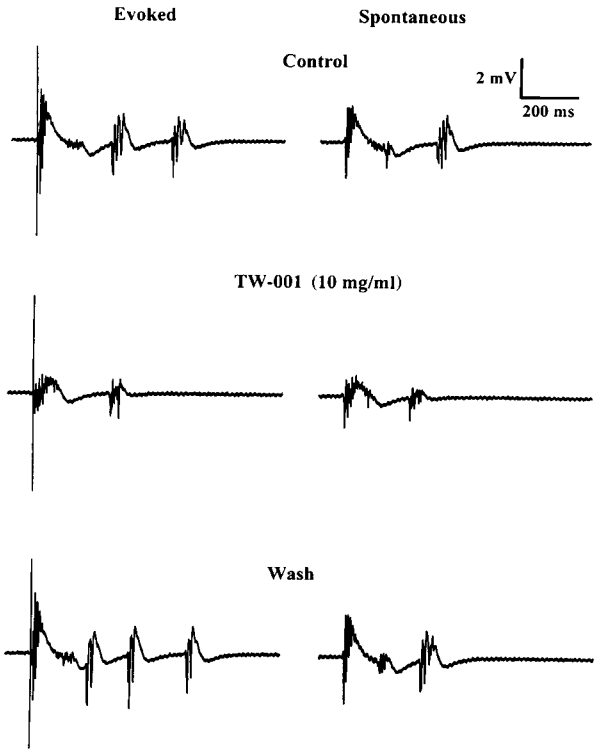

Fig. 5. Effect of TW-001 on evoked and spontaneously occuring epileptiform activity induced by picrotoxin in hippocampal area CA3. On the left, superfusion of TW-001 (10 mg/ml) effectively suppressed the evoked epileptiform burst discharges induced by picrotoxin (50 μM). On the right, TW-001 reversibly reduced the duration and the repetition rate of the spontaneously occuring epileptiform burst discharges. Similar results were also observed in another nine experiments.

control condition, the values of V_{max} of successive action potentials sustained throughout the pulse. Upon superfusion of TW-001 (5 and 10 mg/ml), V_{max} declined steadily until the action potential failed (right panel). The V_{max} of the first action potential after adding 5 or 10 mg/ml TW-001 to the bathing solution was the same amplitude as the control condition. In addition, it can be seen that the V_{max} values of latter action potentials were proportionally more reduced than the first action potential. Similar results were also observed in another 9 cells. These data imply the possibility that TW-001 exerts a use-dependent blockade of sodium channels on the hippocampal CA1 neurons.

Effect of TW-001 on the membrane sodium and calcium currents

Since the blockade of sodium and calcium currents contributes to the mechanism of action of many of the classic AEDs such as phenytoin (22, 23), we therefore directly examined whether TW-001 exerted its anticonvulsant activity via the inhibition of these two types of membrane currents. Stable whole cell voltage clamp recordings of sodium and calcium currents were obtained from neurons acutely dissociated from 12- to 14-day-old rat hippocampal area CA1. Recordings were taken from cells with pyramidal cell bodies. We first examined the effect of TW-001 (1 – 10 mg/ml) on the sodium currents. Step depolarization from a holding potential of -80 to -10 mV elicited a fast pronounced inward current that was blocked by 1 μM TTX (% of inhibition, $97.6 \pm 2.3\%$, $n=6$, $P<0.05$), indicating that it was carried by sodium channels. Bath application of TW-001 (1 – 10 mg/ml) reversibly inhibited the whole cell sodium currents in a concentration-dependent manner. The mean TW-001 depression of peak whole cell sodium currents (% of inhibition, ± S.E.M.) was as follows: 1 mg/ml, $2.5 \pm 1.2\%$ ($n=5$); 5 mg/ml, $21.3 \pm 3.4\%$ ($n=8$); and 10 mg/ml, $36.7 \pm 3.8\%$ ($n=7$) (Fig. 7). TW-001 (10 mg/ml) produced a comparable effect to phenytoin (200 μM) and lamotrigine (200 μM) on the blockade of TTX-sensitive fast sodium inward currents.

In subsequent experiments, we examined the effect of TW-001, phenytoin and lamotrigine on the high-voltage activated whole cell calcium currents. To obtain the cal-

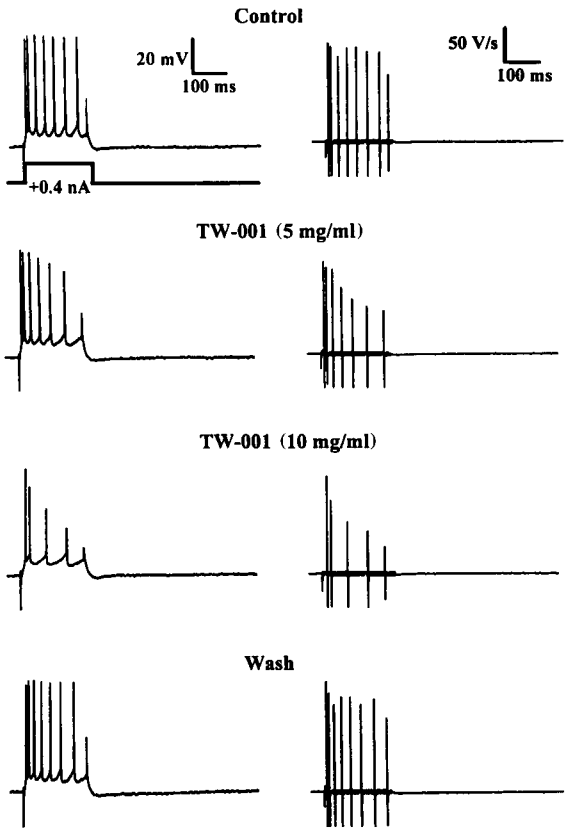

Fig. 6. Effect of TW-001 on the RF and the V_{max} of action potentials. The RF of action potentials was elicited by injection of a 200-ms depolarizing current pulse (0.4 nA) into the cell through the recording microelectrode. Note that application of TW-001 (5 and 10 mg/ml) produced a concentration-dependent and reversible limitation of RF (left panel). TW-001 also decreased the V_{max} of action potentials (right panel). The V_{max} of latter action potentials was proportionally more reduced than that the first action potential. The RMP of this cell was −61 mV.

cium currents, the external solution was added with TTX to block sodium currents and with cesium replacing potassium in the recording pipette to block potassium outward currents. Step depolarization from a holding potential of −80 to −10 mV elicited a pronounced inward current that was blocked by 100 μM cadmium chloride (% of inhibition, 96.8 ± 2.5%, n = 8, $P<0.05$), indicating that it was carried by calcium channels. As shown in Fig. 8, the calcium currents evoked by this voltage protocol displayed a transient and a sustained phase. Bath application of TW-001 (1 − 10 mg/ml) reversibly depressed the whole cell calcium currents in a concentration-dependent manner. The mean TW-001-induced depression of peak whole cell calcium currents (% of inhibition, ± S.E.M.) was as follows: 1 mg/ml, 4.5 ± 2.1% (n = 4); 5 mg/ml, 22.3 ± 3.6% (n = 6); and 10 mg/ml, 43.5 ± 4.3% (n = 6) (Fig. 8). Phenytoin and lamotrigine inhibited calcium currents by less than 10%, even at the higher concentration of 200 μM tested. These results suggest that TW-001 could inhibit both the sodium and high-voltage activated calcium channel activity on the hippocampal CA1 neurons.

To identify which subtypes of high-voltage activated calcium channels in rat hippocampal CA1 neurons are sensitive to TW-001, we examined the ability of TW-001 to reduce whole cell calcium currents before and after selective blockade of N- and L-type calcium current components. The L-type calcium channel blocker nimoidipine only partially blocked the TW-001-mediated inhibition. In the presence of 20 μM nimodipine, there was a 21.3 ± 2.8% (n = 4) inhibition of calcium currents for TW-001 (10 mg/ml). After the inhibition of the N-type calcium channels by bath application of ω-conotoxin GVIA (3 μM), TW-001 continued to inhibit a fraction of the calcium currents. The inhibition of calcium currents caused by TW-001 (10 mg/ml) was 20.3 ± 3.2% (n = 4). However, coapplication of nimodipine (20 μM) and ω-conotoxin GVIA (3 μM) almostly completely blocked the TW-001 inhibition of calcium currents. These results suggest that TW-001 may inhibit both N- and L-type high-voltage activated calcium channels on the acutely dissociated hippocampal CA1 neurons.

DISCUSSION

Four principal findings emerge from this work. First, oral administration of TW-001 markedly elevated the threshold to tonic hindlimb extension seizures induced by electroshock or i.v. infusion of PTZ. In addition, TW-001 also reduced the incidence and severity and prolonged the latency of the s.c. PTZ seizure test. Second, TW-001 produced a concentration-dependent depression of glutamatergic synaptic transmission in the hippocampal CA1 area. Furthermore, both the stimulus-triggered epileptiform activities evoked by picrotoxin in area CA1 and spontaneously occuring epileptiform burst discharges in area CA3 elicited by picrotoxin were reversibly suppressed by TW-001. Third, TW-001 limited the depolarizing current pulse-induced RF and the V_{max} of the action potentials in the hippocampal CA1 neurons. Forth, TW-001 exerted a reduction of the TTX-sensitive sodium currents and high voltage-activated calcium currents under voltage-clamp conditions in a concentration-dependent manner.

To our knowledge, the present study is the first to systematically and scientifically investigate the anticonvulsant effect of the Chinese herbal medicine *Chai-Hu-Long-Ku-Mu-Li-Tan* (TW-001). The experimental results confirmed previous non-systematically clinical observations, which were described in the ancient Chinese medicine book *Shang-Han Lun*. We further provided evidence that the depressant effect of TW-001 on the glutamatergic synaptic transmission could probably be responsible for its anticon-

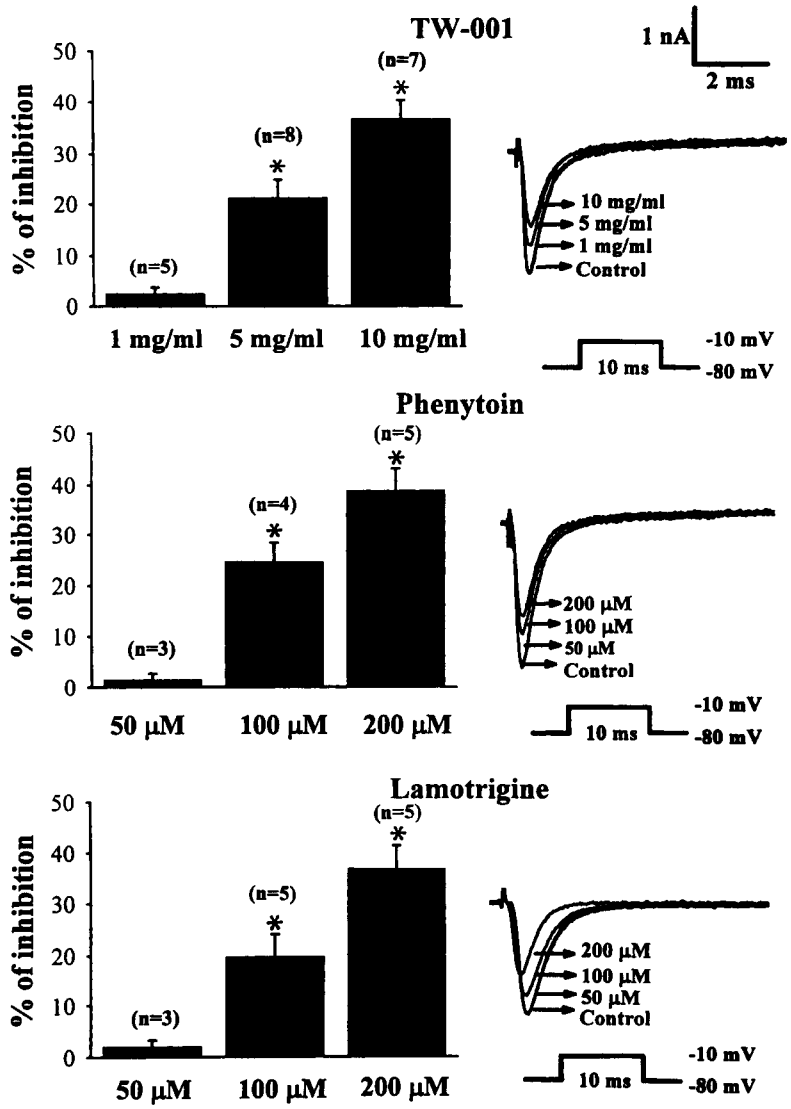

Fig. 7. Effect of TW-001, phenytoin and lamotrigine on whole cell sodium currents recorded from acutely dissociated hippocampal CA1 neurons. Whole cell sodium currents were elicited by a 10-ms depolarizing step from −80 to −10 mV. On the left, bar graphs show the inhibitory effect of different concentration of TW-001, phenytoin and lamotrigine on peak sodium currents. On the right, typical sodium current traces recorded before and during drug application as indicated. Data are presented as the mean ± S.E.M. *P<0.05 as compared with the control.

vulsant effect. How does TW-001 decrease glutamatergic synaptic transmission? Data from the present study show that TW-001 decreases the glutamatergic synaptic transmission by reducing glutamate release from presynaptic terminals. We have provided three lines of evidence to support this conclusion. First, TW-001 inhibited synaptic transmission with negligible changes in the RMP or the membrane input resistance of the postsynaptic neurons (Table 4). Second, TW-001 decreased the fEPSP and PS without altering the sensitivity of postsynaptic neurons to the glutamatergic receptor agonists, AMPA and NMDA (Fig. 2). Third, the depression of fEPSP was accompanied by an increase in the magnitude of PPF, a phenomenon generally accepted to be presynaptic (Fig. 3). The depressant effect of TW-001 was greater on the first response of a pair of stimuli rather than affecting both response equally, so that the magnitude of PPF was increased by TW-001. These results are consistent with previous studies; we showed that when transmitter release is reduced by agents (e.g., adenosine, L-AP4 and dopamine), an increase in the magnitude of PPF is observed (16, 19, 24, 25).

The observed limitation of RF caused by TW-001 is likely to be the result of its reduction of the V_{max} of action potentials. Because sodium is the predominant charge car-

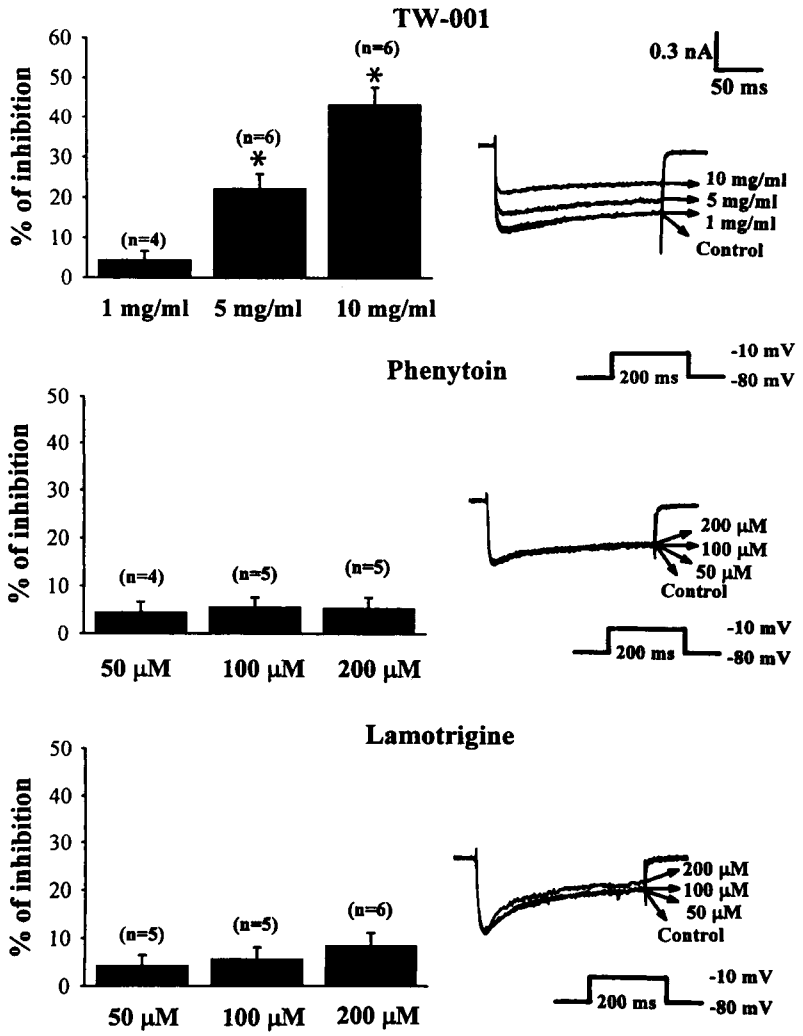

Fig. 8. Effect of TW-001, phenytoin and lamotrigine on high voltage-activated whole cell calcium currents recorded from acutely dissociated hippocampal CA1 neurons. Whole cell calcium currents were elicited by a 200-ms depolarizing step from −80 to −10 mV. On the left, bar graphs show the inhibitory effect of different concentrations of TW-001, phenytoin and lamotrigine on peak calcium currents. On the right, typical high voltage-activated whole cell calcium current traces recorded before and during drug application as indicated. Note that TW-001 but not phenytoin or lamotrigine effectively reduced the high voltage-activated whole cell calcium currents under our experimental conditions. Data are presented as the mean ± S.E.M. *P<0.05 as compared with the control.

rier responsible for the generation of the upstroke of action potential in the hippocampal neurons (26), the maximal rate of rise (V_{max}) of action potential has been widely used as an index to reflect the peak inward sodium currents. Thus, the present study supports the possibility that the blockade of voltage-dependent sodium channels is involved in TW-001's inhibition of RF. In agreement with this suggestion, we have also observed that TW-001 produced both tonic and concentration-dependent depression of TTX-sensitive sodium inward currents in the acutely dissociated hippocampal CA1 neurons under voltage-clamp conditions. For comparison, TW-001 produced an inhibition of sodium channels in a similar manner to those of the established AEDs phenytoin and carbamazepine. Evidence to support this suggestion is that the reduction of V_{max} by TW-001 is similar to the reduction of V_{max} by phenytoin and carbamazepine (27−29). They all produced a slight reduction of V_{max} of the first action potential but evolved with progressive reduction of V_{max} of successive action potentials during a long step depolarization (27−29). The present findings, therefore, suggest that TW-001 exerts its inhibitory effect on sodium inward current is in a use-dependent manner.

In this study, we also demonstrate that TW-001 possesses antiepileptic activity against an in vitro picrotoxin-induced model of epilepsy. Although many mechanisms

could contribute to this effect, our results show that the blockade of voltage-dependent sodium and high-voltage activated calcium channels is relevant. Indeed, both the sodium and calcium channels have been shown to play an important role in controlling the firing activity in hippocampal neuron (2). Blockade of these two types of ion channels may stabilize neuronal membrane excitability and inhibit neurotransmitter release. Direct support for an involvement of high-voltage activated calcium channels in picrotoxin-induced epileptiform activity is provided by current electrophysiological studies using both hippocampal and neocortical slices in vitro. Straub et al. (30) showed that organic calcium channel blockers, verapamil and flunarizine, reduced the amplitude and duration of paroxysmal depolarization shift and epileptic field potentials induced by picrotoxin in hippocampal and neocortical regions of rats. Based on these findings, it seems likely that a calcium-influx through voltage-activated calcium channels is involved in the convulsant action of the antagonist of GABA receptors. Although previous studies have shown that NMDA-receptor antagonist D-APV as well as non-NMDA-receptor antagonist CNQX may also reduce the picrotoxin-induced epileptiform activity (31, 32), TW-001 is unlikely to act on the postsynaptic NMDA or AMPA receptors to inhibit the picrotoxin-induced epileptiform activity. Evidence to support this suggestion is that the sensitivity of postsynaptic neurons to exogenous application of NMDA or AMPA was not significantly altered by prior TW-001 application (Fig. 2).

Hippocampal pyramidal neurons have been shown to possess multiple types of high-voltage activated calcium channels according to their electrophysiological and pharmacological properties (17, 33). For example, we have previously shown that nimodipine (L-type calcium channel blocker, 20 μM) reversibly blocked approx. 40%, ω-conotoxin GVIA (N-type calcium channel blocker, 3 μM) irreversibly suppressed 25% and ω-agatoxin IVA (P/Q-type calcium channel blocker, 0.2 μM) irreversibly blocked 29% of the total high-voltage activated calcium currents in 2-week-old rat hippocampal CA1 neurons (17). In this study, we found that TW-001 (1 – 10 mg/ml) strongly blocked both L- and N-type calcium channels in a concentration-dependent manner. Since N- and L-type calcium channels have been identified to be involved in the regulation of presynaptic neurotransmitter release and postsynaptic neuronal excitability in the hippocampus (34), the inhibitory effect of TW-001 on these two types of calcium channels could be used to explain the profile of its anticonvulsant activity in both in vivo and in vitro models of epilepsy. Under our experimental conditions, classic AEDs phenytoin and lamotrigine did not exert any significant effect on the high-voltage activated calcium channels. These results are consistent with the current findings of Salvati et al. (35).

With respect to the anticonvulsant effect of TW-001, it may be also interesting to know which constituent(s) contained in TW-001 underlies its anticonvulsant efficacy. Recently, the effect of two component herbal drugs of TW-001, *Tian-Ma* (*Gastrodiae rhizoma*) and *Gou-Teng* (*Uncaria thorn*), on the seizure threshold for tonic electroconvulsion and the in vitro picrotoxin-induced epileptiform activity in the hippocampal area CA1 were also examined. We found that the aqueous extract of *Tian-Ma* but not *Gou-Teng* effectively elevated the threshold for tonic electroconvulsion and showed almost the similar inhibitory action of TW-001 on picrotoxin-induced epileptiform activity, although the concentration of *Tian-Ma* had to be higher than that of TW-001 in the component ratio (H.-M. Wu et al., unpublished observations). Furthermore, behavioral studies have also shown that the aqueous extract of *Tian-Ma* or vanillin that was purified from the tuber of *Gastrodia elata* has sedative and anticonvulsant properties (36). However, we could not exclude the possibility that other component herbal drugs of TW-001 may also possess anticonvulsant activity. Further studies using chemically identified substances of TW-001 and examining their anticonvulsant activity are in progress to clarify the anticonvulsant effect of TW-001.

In conclusion, both the in vivo behavioral tests and the in vitro electrophysiological recordings reported here indicate that the Chinese herbal medicine TW-001 is a potential anticonvulsant agent. The inhibitory effect on both neuronal excitability and glutamate release due to blockade of sodium and calcium channels may account largely for the anticonvulsant profile of TW-001. Because TW-001 is orally active and possesses no overt adverse effects, these results indicate that this agent may have great potential for clinical antiepileptic therapy. However, further preclinical evaluation should be provided, including the adverse effect of long-term administration of this drug, before this agent is used as an anticonvulsant drug in humans. Furthermore, because the experimental seizure models used here are widely used to evaluate the therapeutic efficacy of new AEDs against generalized tonic-clonic seizures, it can be predicted that TW-001 will be an effective treatment for generalized tonic-clonic seizures in view of its overall anticonvulsant profile observed in the present study.

Acknowledgments

This work was financially supported by a research grant from the Committee of Chinese Medicine and Pharmacy of the Department of Health, Taiwan (CCMP88-RD-037) to J.J.T.

REFERENCES

1 Delgado-Escueta AV, Ward AA JR, Woodbury DM and Porter RJ: New wave of research in the epilepsies. Adv Neurol **44**, 3 – 55

(1986)
2 Rogawski MA and Porter RJ: Antiepileptic drugs: pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds. Pharmacol Rev **42**, 223 - 286 (1990)
3 Macdonald RL and Kelly K: New antiepileptic drug mechanisms of action. *In* An Appraisal of Some New Anticonvulsants - A Clinical Perspective, Edited by Trimble M, pp 35 - 50, Wiley, New York (1994)
4 Juul-Jenson P: Epidemiology of intractable epilepsy. *In* Intractable Epilepsy: Experimental and Clinical Aspects, Edited by Schmidt D and Morselli PL, pp 5 - 11, Raven Press, New York (1986)
5 Regesta G and Tanganelli P: Clinical aspects and biological bases of drug-resistant epilepsies. Epilepsy Res **34**, 109 - 122 (1999)
6 Harvey RA and Champe PC: Drugs used to treat epilepsy. *In* Lippincott's Illustrated Reviews: Pharmacology, Edited by Harvey RA and Champe PC, Vol **1**, pp 143 - 150, JB Lippincott Co, New York (1992)
7 Sugaya E and Sugaya A: Cellular physiology of epileptogenic phenomena and its application to therapy against intractable epilepsy. Comp Biochem Physiol **98**, 249 - 270 (1991)
8 Löscher W and Schmidt D: Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. Epilepsy Res **2**, 145 - 181 (1988)
9 Dingledine R, Mcbain CJ and McNamara JO: Excitatory amino acid receptors in epilepsy. Trends Pharmacol Sci **11**, 334 - 338 (1990)
10 Löscher W: Basic aspects of epilepsy. Curr Opin Neurol Neurosurg **6**, 223 - 232 (1993)
11 Kimball AW, Burnett WT and Doherty DG: Chemical protection against ionizing radiation. I. Sampling methods for screening compounds in radiation protection studies with mice. Radiation Res **7**, 1 - 12 (1957)
12 Litchfield JT and Wilcoxon F: A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther **96**, 99 - 113 (1949)
13 Löscher W and Hönack D: Profile of ucb L059, a novel anticonvulsant drug, in models of partial and generalized epilepsy in mice and rats. Eur J Pharmacol **232**, 147 - 158 (1993)
14 Hsu KS and Huang CC: Characterization of the anoxia-induced long-term synaptic potentiation in area CA1 of the rat hippocampus. Br J Pharmacol **122**, 671 - 681 (1997)
15 Huang CC and Hsu KS: Nitric oxide signaling is required for the generation of anoxia-induced long-term potentiation in the hippocampus. Eur J Neurosci **9**, 2202 - 2206 (1997)
16 Hsu KS: Characterization of dopamine receptors mediating inhibition of excitatory synaptic transmission in the rat hippocampal slices. J Neurophysiol **76**, 1887 - 1895 (1996)
17 Hsu KS, Huang CC, Kan WM and Gean PW: TXA_2 agonists inhibit high-voltage-activated calcium channels in rat hippocampal CA1 neurons. Am J Physiol **271**, C1269 - C1277 (1996)
18 Walker MC and Sander JW: Developments in anti-epileptic drug therapy. Curr Opin Neurol **7**, 131 - 139 (1994)
19 Dunwiddie TV and Hass HL: Adenosine increases synaptic facilitation in the in vitro rat hippocampus: Evidence for a presynaptic site of action. J Physiol (Lond) **369**, 365 - 377 (1985)
20 Manabe T, Wyllie DJA, Perkel DJ and Nicoll RA: Modulation of synaptic transmission and long-term potentiation: Effects on paired pulse facilitation and EPSC variance in the CA1 region of the hippocampus. J Neurophysiol **70**, 1451 - 1459 (1993)
21 Schulz PE, Cook EP and Johnston D: Changes in paired-pulse facilitation suggest presynaptic involvement in long-term potentiation. J Neurosci **14**, 5325 - 5337 (1994)
22 Lang DG, Wang CM and Cooper BR: Lamotrigine, phenytoin and carbamazepine interactions on the sodium current present in N4TG1 mouse neuroblastoma cells. J Pharmacol Exp Ther **266**, 829 - 835 (1993)
23 Twombly DA, Yoshii M and Narahashi T: Mechanisms of calcium channel block by phenytoin. J Pharmacol Exp Ther **246**, 189 - 195 (1988)
24 Harris EW and Cotman CW: Effects of acidic amino acid antagonists on paired-pulse potentiation at the lateral perforant path. Exp Brain Res **52**, 455 - 460 (1983)
25 Kable JS and Cotman CW: Adenosine, L-AP4, and baclofen modulation of paired-pulse potentiation in the dentate gyrus: Interstimulus interval-dependent pharmacology. Exp Brain Res **94**, 97 - 104 (1993)
26 Peacock JH and Walker CR: Development of calcium action potentials in mouse hippocampal cell cultures. Dev Brain Res **8**, 39 - 52 (1983)
27 Mclean MJ and Macdonald RL: Sodium valproate, but not ethosuximide, produces use- and voltage-dependent limitation of high frequency repetitive firing action potentials of mouse central neurons in cell culture. J Pharmacol Exp Ther **237**, 1001 - 1011 (1986)
28 Mclean MJ and Macdonald RL: Carbamazepine and 10,11-epoxycarbamazepine produce use- and voltage-dependent limitation of rapidly firing action potentials of mouse central neurons in cell culture. J Pharmacol Exp Ther **238**, 727 - 738 (1986)
29 Willow M, Gonoi T and Catterall WA: Voltage clamp analysis of the inhibitory actions of diphenylhydantoin and carbamazepine on voltage-sensitive sodium channels in neuroblastoma cells. Mol Pharmacol **27**, 549 - 558 (1985)
30 Straub H, Kohling R and Speckmann EJ: Picrotoxin-induced epileptic activity in hippocampal and neocortical slices (guinea pig): suppression by organic calcium channel blockers. Brain Res **658**, 119 - 126 (1994)
31 Lee WL and Hablitz JJ: Involvement of non-NMDA receptors in picrotoxin-induced epileptiform activity in the hippocampus. Neurosci Lett **107**, 129 - 134 (1989)
32 Lee WL and Hablitz JJ: Initiation of epileptiform activity by excitatory amino acid receptors in the disinhibited rat neocortex. J Neurophysiol **65**, 87 - 95 (1991)
33 Thompson SM and Wong RKS: Development of calcium current subtypes in isolated rat hippocampal cells. J Physiol (Lond) **439**, 671 - 689 (1991)
34 Reuther H:Calcium channel modulation by neurotransmitters, enzymes and drugs. Nature **301**, 569 - 574 (1983)
35 Salvati P, Maj R, Caccia C, Cervini MA, Fornaretto MG, Lamberti E, Pevarello P, Skeen GA, White HS, Wolf HH, Faravelli L, Mazzanti M, Mancinelli E, Varasi M and Fariello RG: Biochemical and electrophysiological studies on the mechanism of action of PNU-151774E, a novel antiepileptic compound. J Pharmacol Exp Ther **288**, 1151 - 1159 (1999)
36 Shen D and Chang H: The anticonvulsant and analgesic activities of *Tianma* (Gastrodia elata Bl). Acta Physiol Sin **10**, 242 - 245 (1963)