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FULL-LENGTH ORIGINAL RESEARCH

Perampanel: A novel, orally active, noncompetitive AMPA-receptor antagonist that reduces seizure activity in rodent models of epilepsy

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Summary

Purpose: To assess the pharmacology of perampanel and its antiseizure activity in preclinical models. Perampanel [2-(2-oxo-1-phenyl-5-pyridin-2-yl-1,2-dihydropyridin-3-yl) benzonitrile] is a novel, orally active, prospective antiepileptic agent currently in development for refractory partial-onset seizures.

Methods: Perampanel pharmacology was assessed by examining changes in intracellular free Ca^{2+} ion concentration ($[Ca^{2+}]_i$) in primary rat cortical neurones, and [^{3}H]perampanel binding to rat forebrain membranes. Antiseizure activity of orally administered perampanel was examined in amygdala-kindled rats and in mice exhibiting audiogenic, maximal electroshock (MES)–induced, pentylenetetrazole (PTZ) –induced, or 6 Hz-induced seizures.

Key Findings: In cultured rat cortical neurones, perampanel inhibited α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)–induced increases in $[Ca^{2+}]_i$ (IC_{50} 93 nM vs. 2 μM AMPA). Perampanel had a minimal effect on *N*-methyl-D-aspartate (NMDA)–induced increases in $[Ca^{2+}]_i$, and only at a high concentration (30 μM). [^{3}H]Perampanel binding to rat forebrain membranes was not significantly displaced by glutamate or AMPA but was displaced by the noncompetitive AMPA receptor antagonists CP465022 (K_i 11.2 ± 0.8 nM) and GYKI52466 (K_i 12.4 ± 1 μM). In mice, perampanel showed protective effects against audiogenic, MES-induced, and PTZ-induced seizures (ED_{50}s 0.47, 1.6, and 0.94 mg/kg, respectively). Perampanel also inhibited 6 Hz electroshock-induced seizures when administered alone or in combination with other antiepileptic drugs (AEDs). In amygdala-kindled rats, perampanel significantly increased afterdischarge threshold ($p < 0.05$ vs. vehicle), and significantly reduced motor seizure duration, afterdischarge duration, and seizure severity recorded at 50% higher intensity than afterdischarge threshold current ($p < 0.05$ for all measures vs. vehicle). Perampanel caused dose-dependent motor impairment in both mice (TD_{50} 1.8 mg/kg) and rats (TD_{50} 9.14 mg/kg), as determined by rotarod tests. In mice, the protective index (TD_{50} in rotarod test/ED_{50} in seizure test) was 1.1, 3.8, and 1.9 for MES-induced, audiogenic, and PTZ-induced seizures, respectively. In rat, dog, and monkey, perampanel had a half-life of 1.67, 5.34, and 7.55 h and bioavailability of 46.1%, 53.5%, and 74.5%, respectively.

Significance: These data suggest that perampanel is an orally active, noncompetitive, selective AMPA receptor antagonist with potential as a broad spectrum antiepileptic agent.

KEY WORDS: Antiseizure, Broad spectrum, Antiepileptic agent.

Epilepsy is a common neurologic disorder that is estimated to affect 1–2% of the world population (Browne & Holmes, 2001). In recent years, the number of new antiepileptic drugs (AEDs) gaining regulatory approval has increased markedly. However, despite the introduction of agents with novel mechanisms of action, it is estimated that more than one third of patients continue to experience partial seizures that are refractory to current treatments (Perucca et al., 2007).

Adjunctive therapy is widely used in the treatment of epilepsy. Selecting appropriate AED combinations is difficult, however, and quantitative techniques measuring efficacy and side effects of different AED combinations in preclinical studies have not proven useful in predicting clinical benefits (Stafstrom, 2010). Currently, AED combinations are chosen based on avoidance of drug–drug interactions and unwanted side effects rather than on evidence of improved efficacy (French & Faught, 2009). Many AEDs target similar pathways [such as voltage-gated ion channels or γ-aminobutyric acid (GABA)–mediated neurotransmission) or have multiple mechanisms of action that

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may contribute to the difficulty in predicting clinical efficacy of drug combinations. As new data on drug combinations and novel AEDs emerge, successful rational polytherapy may evolve based on synergistic effects of AEDs, and drugs with different and discrete mechanisms of action may potentially be the most effective in this regard.

Encouragingly, the development pipeline for AEDs contains a number of novel compounds with discrete mechanisms of action that have largely been identified using mechanism-independent preclinical seizure models (Rogawski, 2006). Several of these developmental compounds target the AMPA receptor subfamily of ionotropic glutamate receptors. AMPA receptors, named after the selective agonist α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), are activated by glutamate, the major excitatory neurotransmitter, which is thought to play a key role in inducing seizures by initiating and synchronizing glutamatergic transmission (Scharfman, 2007). AMPA itself, administered systemically or by cerebroventricular infusion, is able to elicit seizures in preclinical models, thereby supporting a role for AMPA receptors in the development of seizures (Meldrum & Rogawski, 2007).

AMPA receptor antagonists have been investigated for antiseizure activity both preclinically and clinically, with mixed success. The prototypical competitive AMPA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f] quinoxaline (NBQX) showed activity in maximal electroshock (MES) and pentylenetetrazole (PTZ)–induced seizure models (Yamaguchi et al., 1993), but has poor solubility, resulting in precipitation in the kidney at therapeutic plasma levels. Derivatives of NBQX with polar constituents have shown improved solubility, but these molecules exhibit reduced blood–brain barrier (BBB) penetration (Weiser, 2005). Prototypical noncompetitive AMPA receptor antagonists, such as 2,3-benzodiazepine-type compounds, have shown weak in vitro efficacy compared with competitive antagonists, but good in vivo efficacy, indicating substantial BBB penetration (Weiser, 2005). Talampanel, a recently developed noncompetitive AMPA receptor antagonist, has been evaluated in a number of clinical trials (Howes & Bell, 2007), but has a relatively short half-life, which may limit its utility in the clinical setting (Langan et al., 2003).

Herein, we report on the in vitro and in vivo activity of perampanel, a novel, orally active, prospective antiepileptic agent currently in phase III clinical studies for refractory partial-onset seizures. We provide evidence to suggest that perampanel is a potent, noncompetitive and selective AMPA receptor antagonist with broad spectrum anticonvulsant activity in preclinical models. Perampanel [2-(2-oxo-1-phenyl-5-pyridin-2-yl-1,2-dihydropyridin-3-yl) benzonitrile] is structurally dissimilar to other AMPA receptor ligands that show antiseizure activity (Fig. 1).

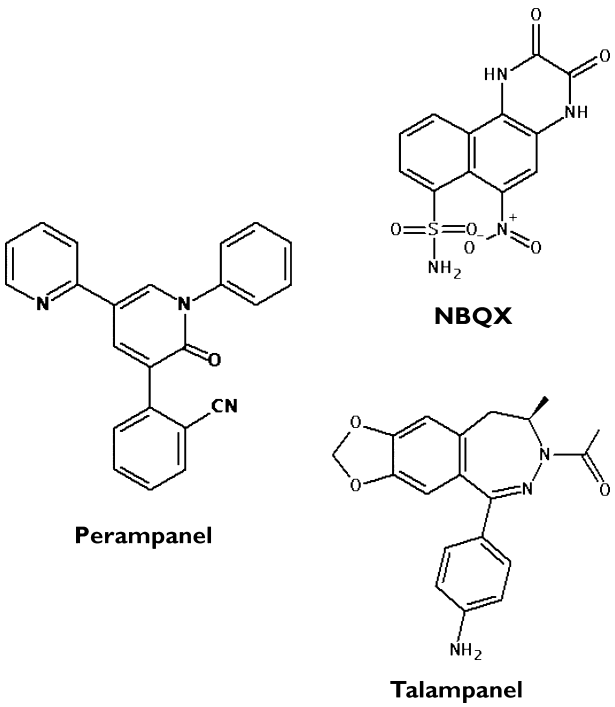

Figure 1.
Structure of perampanel compared with other AMPA antagonists.
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Methods

All animal experiments were performed in compliance with the regulations of the Animal Ethical Committee of Eisai Co., Ltd. Animals were kept at approximately 23°C (permitted range 20–26°C) in 55% relative humidity (permitted range 40–70%) with a 12-h dark/light cycle (lighting between 07:00 and 19:00).

Materials

Reagents were purchased from the following sources: GYKI52466, N-methyl-D-aspartate (NMDA), and MK-801 from Sigma/RBI (St. Louis, MO, U.S.A.); (*RS*)-AMPA from Tocris Cookson Inc. (Ellisville, MO, U.S.A.); tetrodotoxin (TTX) from Sankyo (Tokyo, Japan); and fura-2-AM from Dojin Chemical (Tokyo, Japan). Perampanel was synthesized by Eisai Co., Ltd. (Tokyo, Japan).

Rat cortical neuron cultures

Rat cortical neuron cultures were prepared based on the method described by Abe et al. (1990). Briefly, the cerebral cortex was excised from embryonic day 18 (E18) Wistar rats (Charles River Japan, Kanagawa, Japan) and dissociated by incubation for 30 min at 37°C in Ca^{2+}/Mg^{2+}-free Hanks' balanced salt solution containing 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES),

3.3 mM D-glucose, 0.25% trypsin, 0.2 mg/ml DNase I, 50 units/ml penicillin, and 50 μg/ml streptomycin. Cells were resuspended in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and plated at a density of 1×10^5 cells/well on 96-well test plates on an astrocyte feeder layer. Cultures were maintained without exchange of medium for 7–17 days at 37°C in a 5% CO_2/95% air incubator.

Rat forebrain neuronal membranes

Rat forebrain membranes were prepared as described previously (Balannik et al., 2005). Briefly, forebrains of Sprague-Dawley rats were homogenized in ice-cold 0.32 M sucrose containing 0.1 mM EGTA (pH 7.4) and centrifuged at 1,000 ***g*** for 10 min. The supernatant was centrifuged at 30,000 ***g*** for 20 min and the resulting pellet was lysed in 1 mM EGTA/Tris–HCl (pH 8.0) and centrifuged at 30,000 ***g*** for 20 min to collect the membranes. This lysis and centrifugation step was repeated, and the pellet was resuspended in 50 mM Tris–HCl buffer (pH 7.4). Membranes were sonicated and washed three times, resuspended in 50 mM Tris–HCl buffer (pH 7.4), and stored at −80°C. Before use, aliquots were thawed and sonicated, washed twice in 50 mM Tris–HCl buffer, and resuspended at 0.5 mg/ml with 50 mM Tris–HCl buffer (pH 7.4).

Measurements of intracellular free calcium concentration

Changes in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) were measured in rat cortical neurons using the fluorescent Ca^{2+} indicator dye fura-2, as described previously (Fischer et al., 2000). Cells were incubated with fura-2 AM (10 μM) in a 5% CO_2/95% air incubator at 37°C for 2 h and washed with Ca^{2+} assay buffer (140 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 24 mM D(+)-glucose, 10 mM HEPES, 1 μM MK801; pH 7.4 adjusted with NaOH for AMPA). Changes in $[Ca^{2+}]_i$ were determined by fluorimetry (Fluorescence Drug Screening System, Hamamatsu Photonics, Shizuoka, Japan) by measuring changes in the fluorescence emission ratio of fura-2 after consecutive excitations at 340 and 380 nM wavelengths. AMPA (2 μM) and NMDA (100 μM) were used to stimulate specific receptor subtypes, and the inhibitory effects of perampanel, GYKI52466, and MK-801 on these responses were assessed. Ca^{2+} assay buffer without $MgCl_2$ or MK-801 was used in assays involving NMDA.

Radiolabeled perampanel binding assay

Specific and nonspecific binding of [^{3}H]perampanel to rat forebrain neuronal membranes was measured in the presence and absence of various glutamate receptor agonists and antagonists. Briefly, membranes and compounds were mixed with 50 nM [^{3}H]perampanel (specific activity 1.92 TBq/mM) and incubated for 1.5 h at 4°C. Samples were filtered onto Whatman GF/B glass-fiber filters presoaked in 0.3% polyethyleneimine and washed three times with 2 ml ice-cold 50 mM Tris–HCl buffer (pH 7.4) using a Brandel M-30R cell harvester. Radioactivity was quantified by liquid scintillation counting. Nonspecific binding was determined by incubation with 15 μM perampanel. Specific binding was determined by subtraction of nonspecific binding from total binding.

Mouse seizure models

The effects of perampanel on audiogenic, MES-induced, and PTZ-induced seizures were determined in mice. Drug doses and pretreatment times were chosen based on results from preliminary experiments. Pretreatment time in all rodent models was within the range of T_{max} values for perampanel in mice (0.5–1 h) and rats (0.5–1 h).

MES-induced seizures

Electroshock seizures were induced in 4-week-old male ddY mice obtained from Japan SLC Inc. (Shizuoka, Japan). Animals were dosed orally with perampanel (0.75, 1.06, 1.50, or 2.12 mg/kg), carbamazepine (9.2, 13, 18.4, or 26 mg/kg), sodium valproate (284, 400, 567, or 800 mg/kg), or vehicle 1 h prior to electrical stimulation. An electrical stimulus (80 V) was applied to both corneas for 0.4 s. An occurrence of electroshock-induced tonic extension (TE) was defined as a hindlimb TE lasting >1 s following stimulation. Fifty percent effective dose (ED_{50}) and 95% confidence intervals (CIs) for TE inhibition were calculated by regression analyses.

Audiogenic seizures

Audiogenic seizures were induced in 3-week-old male DBA/2J mice obtained from CLEA Japan, Inc. (Tokyo, Japan). Mice were dosed orally with perampanel (0.3, 1, or 3 mg/kg), carbamazepine (1, 3, or 10 mg/kg), sodium valproate (30, 100, or 300 mg/kg), or vehicle 1 h prior to initiation of seizures. Mice were then habituated for 1 min in an observation box and exposed to sound stimulation (11 kHz, 115 dB) for 1 min or until TE occurred. An occurrence of sound-induced seizure was defined as a hindlimb TE lasting >1 s during sound stimulation.

PTZ-induced seizures

PTZ-induced seizures were generated in 9-week-old male ICR mice obtained from CRJ Inc. (Kanagawa, Japan). Animals were dosed orally with perampanel (0.75, 1.5, or 3 mg/kg), carbamazepine (100 mg/kg), sodium valproate (150, 300, or 600 mg/kg), or vehicle 30 min prior to initiation of seizures. A 30 min pretreatment time was chosen in this model to allow for PTZ-induced seizures to be measured within the range of T_{max} (0.5–1 h). Seizures were then induced by subcutaneous injection of PTZ (90 mg/kg). An occurrence of PTZ-induced seizure was defined as a seizure of at least 3 s duration within 30 min of PTZ administration.

Six-hertz electroshock-induced seizures

Perampanel (0.5, 1, 2 or 4 mg/kg) was administered orally to 9-week-old male ICR mice 1 h prior to test. An electrical stimulus (6 Hz, 0.2 ms rectangular pulse, 3 s duration, 32 or 44 mA) was applied to both corneas and an occurrence of seizure was defined as appearance of immobility or stun, forelimb clonus, twitching of the vibrissae, and an elevated (or Straub) tail (Barton et al., 2001).

Rat amygdala-kindling model

Eight-week-old male Sprague-Dawley rats obtained from SLC Inc. (Shizuoka, Japan) were anesthetized with pentobarbital (50 mg/kg), and a tripolar electrode was inserted into the amygdala complex using stereotaxic surgery. After a recovery period of 1 week, threshold current of afterdischarge was determined using a 25% ascending stimulation paradigm. Rats were stimulated at the threshold current once daily until three or more consecutive stage 5 seizures were observed. Animals that showed stable threshold current of afterdischarge for at least three consecutive stimulations were selected for further experiments.

Threshold current of afterdischarge was determined using an ascending stimulation schedule prior to administration of either perampanel or vehicle. A second threshold determination was performed 1 h following oral administration of perampanel (1, 1.25, 2.5, 5, or 10 mg/kg) or vehicle. Afterdischarge duration, motor seizure duration (time with stage 4 and 5 seizure), and seizure severity (Racine severity scale) were then determined in each rat using a stimulus that was two steps (or 50%) stronger than the afterdischarge threshold current.

Rotarod test

Six-week-old male Sprague-Dawley rats and 8-week-old male ICR mice were obtained from Charles River Japan Inc. Animals were dosed orally with perampanel (2, 4, 8, or 16 mg/kg for rats; 0.5, 1, 2, or 4 mg/kg for mice) or vehicle, 1 h prior to being placed on a rotating rod (rotation speed 6 rpm for rats, 8 rpm for mice), and the time animals were able to remain on the rod was recorded. Experiments were conducted twice for each animal. Animals that failed to remain on the rotarod for 120 s in both trials were classified as motor-uncoordinated.

Pharmacokinetic parameters

For pharmacokinetic experiments, perampanel was administered as follows: to fasted male Sprague-Dawley rats (n = 4), intravenously (bolus in 0.25 M HCl in saline) or orally (in 0.33 M HCl) at 1 mg/kg; to fasted male beagle dogs (n = 3), intravenously (bolus in 0.1 M HCl in saline) or orally (in 0.1 M HCl) at 0.1 mg/kg; and to fasted male cynomolgus monkeys (n = 4), intravenously (bolus in 0.1 M HCl in saline) or orally (in 0.1 M HCl) at 0.03 mg/kg. Blood samples were collected from rats via the jugular vein (0.25 ml), from dogs via the cephalic vein (1 ml), and from monkeys via the femoral vein (0.5 ml) using heparinized syringes. For all species, time points for blood collection were predose, 5, 15, 30 min, 1, 2, 4, 6, 8, and 24 h after intravenous dosing, and 15, 30 min, 1, 2, 4, 6, 8, and 24 h after oral dosing. Samples from monkey were also taken at 12 h. Plasma was obtained by centrifugation and deproteinized using methanol: 60% perchloric solution (500:1 v/v). The plasma concentration of perampanel was determined by high-performance liquid chromatography with fluorescence (HPLC–FL). Pharmacokinetic parameters for perampanel were calculated by model independent analysis (WinNonlin; Pharsight, Cary, NC, U.S.A.).

In vitro metabolic stability of perampanel using liver microsomes

Liver microsomes from human, rat, dog, and monkey were purchased from Xenotech, LLC (Lenexa, KS, U.S.A.). Microsomes (0.5 mg/ml microsomal protein) were incubated with perampanel (0.03 μg/ml), EDTA (0.1 mM), an NADPH-generating system (0.33 mM β-$NADP^+$, 0.8 mM glucose 6-phosphate, 0.1 unit/ml glucose 6-phosphate dehydrogenase, 6 mM $MgCl_2$), and phosphate buffer (100 mM; pH 7.4) for 20 min at 37°C, and reactions were terminated by addition of acetonitrile. The residual concentration of perampanel was determined by liquid chromatography–tandem mass spectrometry (LC–MS/MS) with multiple reaction monitoring operated in the positive ionization mode.

Statistical analysis

For in vitro studies, values for Ca^{2+} response and radioligand binding were expressed as mean ± SEM (standard error of the mean). IC_{50} values were determined by regression analyses. In animal studies, ED_{50} (effective dose causing 50% reduction in seizures), TD_{50} (dose causing 50% reduction in motor coordination), and 95% CIs were calculated by regression analyses. Two-way ANCOVA (analysis of covariance) was used to assess overall effect of treatment, and Dunnett's post test was used to compare differences between treatment groups. Differences between treatment groups were considered significant if the p-value was <0.05.

RESULTS

Effects of perampanel on agonist-induced increases in $[Ca^{2+}]_i$

Rat cortical neurons were used to determine the effects of perampanel on AMPA receptor function, as measured by AMPA-induced increases in $[Ca^{2+}]_i$. Perampanel inhibited AMPA-induced increases in $[Ca^{2+}]_i$ in a concentration-dependent manner (IC_{50} 93 nM vs. 2 μM AMPA; 95% CI, 40–150 nM) (Fig. 2A). The well-characterized, 2,3-benzodiazepine-type, noncompetitive AMPA receptor antagonist GYKI52466 (Donevan & Rogawski, 1993) inhibited AMPA-induced increases in $[Ca^{2+}]_i$ in cultured rat cortical neurons with an IC_{50} of 12.5 μM (data not shown).

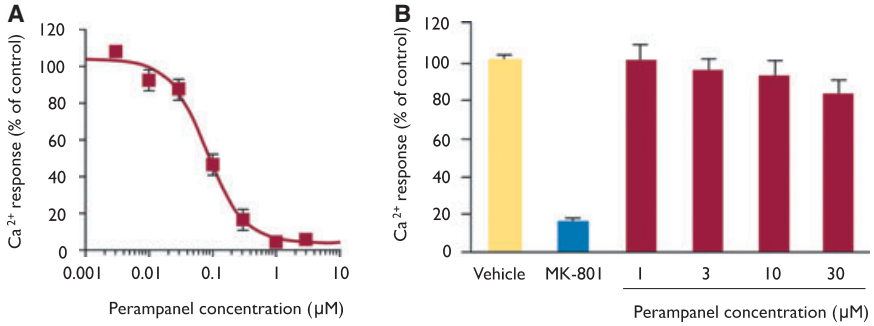

Figure 2.
Effect of perampanel on (**A**) AMPA- and (**B**) NMDA-induced increases in $[Ca^{2+}]_i$ in rat cortical neurons. (**A**) Rat cortical neurons were exposed to AMPA (2 μM) in the absence (control) and presence of increasing concentrations of perampanel and changes in $[Ca^{2+}]_i$ were determined as described in Methods. Data points represent mean ± SEM (n = 3) for AMPA-induced increases in $[Ca^{2+}]_i$ relative to agonist responses in the absence of antagonist. (**B**) Rat cortical neurons were pretreated with vehicle (control; open bar), MK-801 (1 μM; black bar), or perampanel (1, 3, 10, 30 μM; red bars) prior to addition of NMDA (100 μM). Bars represent mean ± SEM (n = 10) for agonist-induced increases in $[Ca^{2+}]_i$ relative to agonist-induced responses with vehicle.
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The effects of perampanel on NMDA receptor activity induced by NMDA (100 μM) were determined in rat cortical neurons by measuring changes in $[Ca^{2+}]_i$. Perampanel inhibited NMDA-induced increases in $[Ca^{2+}]_i$ by 18%, but only at the highest concentration tested (30 μM) (Fig. 2B). In contrast, the noncompetitive NMDA receptor antagonist MK801 (1 μM) markedly inhibited NMDA-induced increases in $[Ca^{2+}]_i$ by approximately 85% (Fig. 2B).

[^{3}H]perampanel binding

The K_d for [^{3}H]perampanel binding to rat forebrain membranes was 59.8 ± 5.2 nM and the B_{max} was 3.2 ± 0.1 pM/mg (n = 4). The glutamate receptor agonists glutamate (1 mM) and AMPA (0.1 mM), and the competitive glutamate receptor antagonist NBQX (0.1 mM) did not significantly reduce [^{3}H]perampanel binding to rat forebrain membranes (Fig. 3A). In contrast, [^{3}H]perampanel binding was displaced by the noncompetitive AMPA receptor antagonists CP465022 (IC_{50} 21.1 ± 1.4 nM; K_i 11.2 ± 0.8 nM; mean ± SEM; n = 4) and GYKI52466 (IC_{50} 23.3 ± 1.9 μM; K_i 12.4 ± 1 μM; mean ± SEM; n = 4) (Fig. 3B).

Effect of perampanel on audiogenic, MES-induced, and PTZ-induced seizures in mice

Perampanel protected mice from tonic–clonic generalized seizures in audiogenic and MES-induced seizure tests, and from absence or myoclonic seizures in PTZ-induced seizure tests (Table 1). Perampanel ED_{50} values for seizure protection were lower than corresponding values for the traditional AEDs carbamazepine and sodium valproate (Table 1).

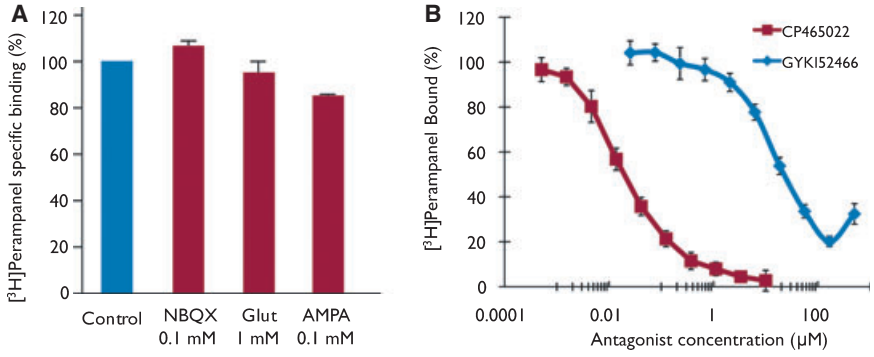

Figure 3.
Effect of glutamate receptor agonists and antagonists on [^{3}H]perampanel binding to rat forebrain neuronal membranes. Binding of [^{3}H]perampanel to rat forebrain neuronal membranes was determined in the presence of (**A**) 0.1 mM NBQX, 1 mM glutamate, or 0.1 mM AMPA (bars represent mean ± SE; n = 3 individual experiments) or (**B**) various concentrations of the noncompetitive AMPA receptor antagonists CP465022 or GYKI52466 (points represent mean ± SE; n = 4 individual experiments), as described in Methods.
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Table 1. Effect of perampanel on audiogenic, MES-, and PTZ-induced seizures, and on motor coordination (rotarod test) in mice

Drug	Seizure test ED_{50} mg/kg, (95% CI)			Rotarod test TD_{50} mg/kg, (95% CI)
	MES	Audiogenic	PTZ	
Carbamazepine	21 (16–45)	6.1 (4.1–9.0)	>100	ND
Sodium valproate	460 (290–600)	160 (93–280)	350 (260–470)	ND
Perampanel	1.6 (1.3–1.9)	0.47	0.94 (ND)	1.8 (1.4–2.8)
Perampanel protective index (TD_{50}/ED_{50})	1.1	3.8	1.9	

MES, maximal electroshock; ND, not determined; PTZ, pentylenetetrazole.
Seizure tests (n = 10 animals per group) and rotarod tests (n = 9 animals per group) were conducted as described in Methods.

6 Hz electroshock-induced seizure model

Perampanel, given orally 1 h prior to test, protected mice from 6 Hz electroshock-induced seizures in a dose-dependent manner (Fig. 4). The ED_{50} value for perampanel was similar at 32 mA (ED_{50} 2.1 mg/kg; 95% CI, 1.4–2.9) and 44 mA (ED_{50} 2.8 mg/kg; 95% CI, 2.0–4.2) stimulus intensities (Fig. 4A). Carbamazepine (20 mg/kg), phenytoin (10 mg/kg), and valproate (100 mg/kg) each further reduced the incidence of seizures in the presence of perampanel (Fig. 4B).

Antiseizure effects of perampanel in amygdala-kindled rats

Perampanel, given orally 1 h prior to test, significantly increased afterdischarge threshold in amygdala-kindled rats at 10 mg/kg (Fig. 5). At 5 mg/kg or higher, perampanel significantly decreased motor seizure duration recorded at 50% higher intensity than afterdischarge threshold current (Fig. 6A). At 10 mg/kg, perampanel significantly decreased afterdischarge duration and seizure severity at 50% higher intensity than afterdischarge threshold current (Fig. 6B–D).

Rotarod model

The effect of perampanel on motor coordination was determined using the rotarod test. Perampanel caused dose-dependent motor impairment in both mice (TD_{50} 1.8 mg/kg; 95% CI, 1.4–2.8; n = 9 per group) and rats (TD_{50} 9.14 mg/kg; n = 8 per group). In mice, the protective index, defined as TD_{50} in rotarod test/ED_{50} in individual seizure tests, was 1.1, 3.8, and 1.9 for MES-induced, audiogenic, and PTZ-induced seizures, respectively (Table 1).

Pharmacokinetic parameters and metabolic stability of perampanel

Pharmacokinetic parameters for perampanel in rat, dog, and monkey are summarized in Table 2. Values for half-life, area under the curve (AUC), and bioavailability of perampanel were higher in dog and monkey than in rat; clearance values were lower in dog and monkey than in rat. Metabolic stability of perampanel in the presence of rat, dog, monkey, or human liver microsomes was examined in vitro. The residual amount of perampanel remaining after

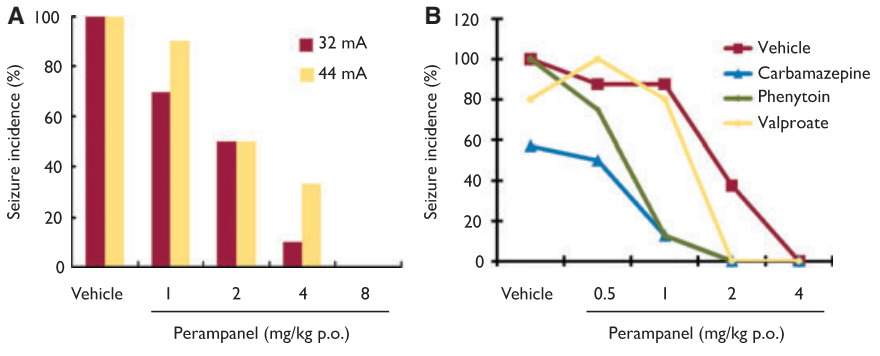
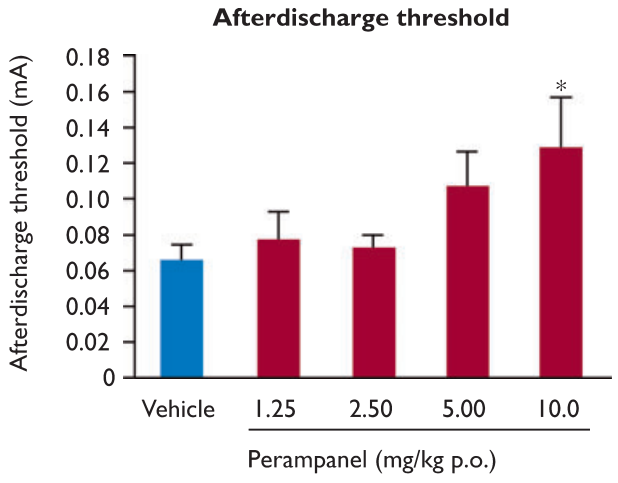


Figure 4.
Effect of orally administered perampanel on 6 Hz electroshock-induced seizures in mice. Perampanel was orally administered 1 h prior to test. 6 Hz electroshock-induced seizures were measured (**A**) with different stimulus intensities (32 mA or 44 mA; n = 9–10) and (**B**) at 32 mA stimulus intensity in the presence of other orally administered AEDs (carbamazepine 20 mg/kg, n = 7–8; phenytoin 10 mg/kg, n = 7–8; or sodium valproate 100 mg/kg, n = 5), as described in Methods.

20 min of incubation with rat, dog, monkey, or human microsomes was 69.9%, 54.6%, 85.3%, and 100.2%, respectively (n = 2).



*p < 0.05 (ANCOVA followed by Dunnett's post test)

Figure 5.
Effects of orally administered perampanel on afterdischarge threshold in amygdala-kindled rats. Threshold current of afterdischarge was determined in amygdala-kindled rats as described in Methods. Bars show mean afterdischarge threshold ± SEM (n = 18 animals per group).
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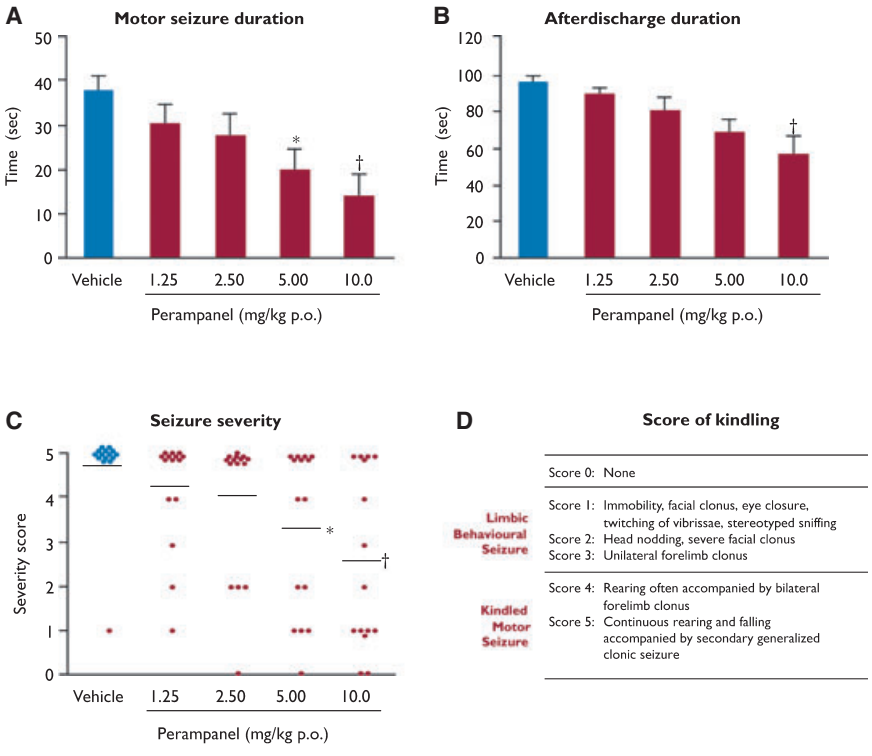
Discussion

Data presented in this study suggest that perampanel is an orally active, noncompetitive, selective AMPA receptor antagonist with broad spectrum antiseizure activity in preclinical epilepsy models.

In in vitro studies, perampanel potently inhibited AMPA-induced increases in intracellular $[Ca^{2+}]_i$ in cultured rat cortical neurons, but did not significantly inhibit MK801-sensitive NMDA-induced Ca^{2+} responses, suggesting that perampanel is a selective AMPA receptor antagonist. Selectivity of perampanel for AMPA receptors over NMDA receptors may be an important feature clinically, as NMDA receptor antagonists are known to produce psychoactive effects, including schizophrenia-like symptoms and cognitive impairment (Meldrum & Rogawski, 2007).

Although perampanel inhibited AMPA-induced functional responses, [^{3}H]perampanel binding to rat forebrain membranes was only slightly reduced by high concentrations of AMPA or glutamate, and was unaffected by the competitive AMPA receptor antagonist NBQX. Furthermore, perampanel at a concentration of 1.25 μM did not inhibit [^{3}H]AMPA binding (data not shown). These data suggest a noncompetitive interaction of perampanel with the AMPA receptor. In addition, perampanel binding was reduced in a concentration-dependent manner by the selective noncompetitive AMPA receptor antagonists GYKI52466 and CP465022, suggesting a common binding



*p < 0.05 and †p < 0.01 compared with vehicle (ANCOVA followed by Dunnett's test)

Figure 6.
Effects of orally administered perampanel on (**A**) motor seizure duration, (**B**) afterdischarge duration, and (**C**) seizure severity in amygdala-kindled rats. Motor seizure duration, afterdischarge duration, and seizure score were measured as described in Methods. Data are represented as mean ± SEM (n = 15 animals per group).
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Table 2. Pharmacokinetic parameters for perampanel in rat, dog, and monkey, determined as described in Methods

	Rat		Dog		Monkey	
Route	IV	PO	IV	PO	IV	PO
Dose (mg/kg)	1	1	0.1	0.1	0.03	0.03
T_{max} (h)	–	1.00 (0.50–1.00)	–	0.50 (0.25–0.50)	–	1.50 (1.00–2.00)
C_{max} (ng/ml)	–	100 ± 17	–	26 ± 4	–	13 ± 1
$t_{1/2}$ (h)	1.36 ± 0.15	1.67 ± 0.13	6.87 ± 0.72	5.34 ± 0.73	6.94 ± 0.59	7.55 ± 0.46
MRT (h)	1.29 ± 0.14	3.19 ± 0.20	5.78 ± 0.75	4.88 ± 0.34	7.66 ± 1.87	7.69 ± 0.39
AUC (ng h/ml)	836 ± 95	386 ± 25	132 ± 15	70 ± 5	175 ± 28	118 ± 20
AUC normalized to 1 mg/kg dose (ng h/ml)	836 ± 96	386 ± 25	1,317 ± 152	696 ± 53	5,829 ± 945	3,939 ± 674
CL (ml/h/kg)	1,239 ± 125	–	780 ± 90	–	185 ± 28	–
Vss (ml/kg)	1,560 ± 122	–	4,424 ± 461	–	1,813 ± 256	–
Bioavailability (%)	–	46.1	–	53.5	–	74.5

CL, total clearance; C_{max}, maximum concentration; MRT, mean residence time; T_{max}, time of occurrence for peak concentration; Vss, volume of distribution at steady state.

Values of C_{max}, $T_{1/2}$, MRT, AUC, CL, and Vss are shown as mean ± SEM; T_{max} is shown as median (range).

site between perampanel and these compounds. In line with these findings, other studies have demonstrated common binding sites between noncompetitive AMPA receptor antagonists on the AMPA receptor. CP465022 and GYKI53655 are reported to share a binding site at the interface between the glutamate binding core and the channel region of the AMPA receptor (Balannik et al., 2005). Binding to this site is thought to stabilize the resting state of the channel and disrupt channel opening in response to agonist binding (Balannik et al., 2005).

The potential benefits of noncompetitive antagonists over competitive antagonists with respect to antiseizure activity are unclear, although noncompetitive antagonists would be expected to retain their antagonist effects in the presence of high agonist concentrations, and, therefore, may be more effective under conditions of increased excitation (Yamaguchi et al., 1993). Indeed, Fritsch et al. (2010) reported superior efficacy of GYKI52466 compared with diazepam in an animal model of status epilepticus (SE). Similarly, the noncompetitive NMDA antagonist MK-801 was more effective than the competitive antagonist CPP in terminating prolonged experimental SE (Yen et al., 2004). These results suggest superiority of noncompetitive antagonists to competitive antagonists in treating severe seizure conditions.

Perampanel displayed a broad spectrum of activity across preclinical seizure models (Table 1). In mouse models of tonic–clonic generalized seizures (audiogenic and MES-induced seizure tests) and absence/myoclonic seizures (PTZ-induced seizure tests), the potency of perampanel was higher than the potency of the traditional AEDs carbamazepine and sodium valproate. Perampanel also showed efficacy in the rat amygdala-kindling model of temporal lobe epilepsy, but did not show activity in genetic absence epilepsy rats from Strasbourg (Danober et al., 1998). Therefore, perampanel shows activity in preclinical models of both partial and generalized seizures. In the search for novel AEDs, drug effects observed in chronic models of epilepsy, such as the amygdala-kindling model, are considered to be more predictive of clinical efficacy and toxicity than effects observed in acute models (Löscher, 2002), potentially leading to fewer false positives. In the current study, broad spectrum activity of perampanel is suggested by activity in both chronic and acute epilepsy models.

In the rat amygdala-kindling model, perampanel increased afterdischarge threshold and significantly reduced motor seizure duration, afterdischarge duration, and seizure severity. Therefore, perampanel inhibited both secondary generalized seizures (seizure score ≥4) and focal seizures (score ≤3). Although most AEDs are reported to inhibit amygdala-kindled seizures, their effects differ significantly. Phenytoin, for example, appears to suppress focal seizures, but does not inhibit the spread of afterdischarges and the development of secondary generalized seizures (Ebert et al., 1997). In contrast, some NMDA receptor antagonists have been shown to block only generalized seizures in this model (Barton & White, 2004). AMPA antagonists, on the other hand, block both the focal and generalized components (Hara et al., 2006), consistent with characteristic broad spectrum antiseizure effects of these compounds. AMPA receptors are thought to play a critical role in the propagation of seizures (Namba et al., 1994; Rogawski & Donevan, 1999), and are also likely to be involved in their initial triggering (Tortorella et al., 1997). Interestingly, afterdischarge persisted in some perampanel-treated animals despite complete inhibition of behavioral seizures, suggesting that perampanel may have a greater inhibitory effect on propagation of seizures than on their initiation.

Seizures induced by 6 Hz electroshock are classically considered resistant to phenytoin and other inhibitors of voltage-dependent ion channels, with AED activity being

closely linked to the stimulation intensity (Barton et al., 2001). Phenytoin and lamotrigine show only a partial inhibitory response when stimulation intensity is increased from 22 to 32 mA in this model, whereas many other AEDs lose effectiveness when stimulation intensity increases further to 44 mA (Barton et al., 2001). In a test of seven established AEDs (phenytoin, carbamazepine, clonazepam, phenobarbital, ethosuximide, trimethadione, and valproic acid) and five new generation AEDs (lamotrigine, levetiracetam, felbamate, tiagabine, and topiramate), only levetiracetam and valproate showed activity in the 6 Hz model at 44 mA stimulation intensity, and in both cases, efficacy of the compounds was lower at 44 mA than at 32 mA stimulation intensity, as shown by higher ED_{50} values (Barton et al., 2001). In contrast, perampanel showed similar effectiveness at 32 and 44 mA stimulation intensities in our studies. Activity in the 6 Hz seizure model has been observed for some newer AEDs (Bialer & White, 2010), including lacosamide, carisbamate, and retigabine, and also appears to be a characteristic of both competitive and noncompetitive AMPA receptor antagonists (Barton et al., 2003). As yet, the clinical significance of activity in the 6 Hz seizure model is unknown.

Data from the 6 Hz electroshock model were also consistent with perampanel acting synergistically with other AEDs. Individually, phenytoin (10 mg/kg) and a low dose of perampanel (1 mg/kg) had little effect on 6 Hz electroshock seizures, but they almost completely inhibited these seizures when applied in combination. Seizure inhibition was also enhanced by coadministration of perampanel with carbamazepine or valproate. Consistent with these observations, synergism between AEDs has been described more frequently in preclinical studies involving AMPA receptor antagonists than in studies with other AEDs (Jonker et al., 2007), suggesting that AMPA receptor antagonists may be promising candidates for combination therapy. Concomitant AEDs did not appear to alter behavioral side effects of perampanel in our studies, although side effects were not evaluated using objective methodology.

The therapeutic window for antiseizure effects of perampanel in rodents appears small due to significant effects on motor coordination. In both the mouse and rat rotarod tests, perampanel showed significant effects on motor coordination close to the concentrations required to reduce seizure activity. Similar effects on motor coordination have been reported for both noncompetitive (e.g. GYKI52466) and competitive (e.g. NBQX) AMPA receptor antagonists, suggesting a mechanism associated with AMPA receptor blockade (Yamaguchi et al., 1993). The window between antiseizure effects and motor dysfunction, however, is different across preclinical seizure models, making predictions of clinical therapeutic index difficult. Furthermore, AEDs such as gabapentin and pregabalin cause central nervous system (CNS) depressant effects in patients (Arroyo & Lesser, 1993; Arroyo et al., 2004) despite preclinical studies that indicated a very wide therapeutic margin (Dalby & Nielsen, 1997; Vartanian et al., 2006), whereas valproate shows a narrow therapeutic margin in rodent models (Barton et al., 2001) but does not show strong CNS depressant side effects (Mattson et al., 1992). Therefore, clinical therapeutic index of AEDs cannot reliably be inferred from preclinical studies.

The noncompetitive AMPA antagonist talampanel has already demonstrated efficacy in patients with refractory partial-onset seizures (Chappell et al., 2002), although with associated adverse events including mild-to-moderate ataxia and dizziness occurring around plasma peak concentration. Pharmacokinetic studies of talampanel revealed a short terminal half-life (5.6 h) at steady state (Langan et al., 2003), which led the investigators to adopt a three-times-daily dosing regimen in the efficacy studies. This regimen caused repeated plasma peaks of talampanel, which may have contributed to the adverse event profile. Hence, AMPA antagonists with a longer half-life potentially may be beneficial in reducing the incidence and severity of adverse events. In preclinical animal studies, perampanel was shown to have good oral bioavailability across species, and notably a half-life of 7.55 h in primates. Furthermore, perampanel was much more stable in the presence of human liver microsomes than in the presence of microsomes from nonhuman animal species, suggesting that the half-life of perampanel may be even longer in humans. This may offer advantages for perampanel over talampanel, since a less frequent dosing regimen may be possible, with a consequently smoother drug concentration profile and potentially fewer adverse events.

In conclusion, we report here on a novel anticonvulsant, perampanel, which demonstrates potent, noncompetitive AMPA receptor antagonist activity with selectivity over NMDA receptors in vitro. The antiseizure activity of perampanel in various preclinical models of epilepsy is consistent with its activity as an AMPA receptor antagonist, and supports its further development as an orally active, broad spectrum antiepileptic agent. Perampanel has potential as a treatment in patients whose seizures are refractory to other AEDs, and may be effective either alone or as add-on therapy with other AEDs.

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