JPET Fast Forward. Published on October 20, 2003 as DOI:10.1124/jpet.103.058495

Effects of YM928, an orally active AMPA receptor antagonist, in models of generalized epileptic seizure in mice and rats

HIROSHI YAMASHITA, KAZUSHIGE OHNO, YOKO AMADA, HANAE HATTORI, YUKIKO OZAWA-FUNATSU, TAKASHI TOYA, HIROSHI INAMI, JUN-ICHI SHISHIKURA, SHUICHI SAKAMOTO, MASAMICHI OKADA, TOKIO YAMAGUCHI

Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd.

21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan.

Running Title: Effects of YM928 in generalized seizure

Correspondence:

Hiroshi Yamashita

Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd.

21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan.

Tel: +81-29-863-6469

Fax: +81-29-852-2925

E-mail: yamasita@yamanouchi.co.jp

Number of text pages: 33

Number of tables: 2

Number of figures: 4

Number of references: 38

Number of words in the Abstract: 229

Number of words in the Introduction: 517

Number of words in the Discussion: 1112

Recommended section: Neuropharmacology

JPET Fast Forward. Published on October 20, 2003 as DOI: 10.1124/jpet.103.058495
This article has not been copyedited and formatted. The final version may differ from this version.

Abbreviations:

AEDs, antiepileptics

AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

GYKI52466,

1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-[5*H*-2,3]-benzodiazepine

talampanel(LY300164), (R)-7-acetyl-5-(4-aminophenyl)-8,9-dihydro-8-methyl-7*H*-1,3

-dioxolo[4,5-*H*][2,3] benzodiazepine

MES, maximal electroshock seizure

NBQX, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide

NMDA, *N*-methyl-D-aspartate

PTZ, pentylenetetrazol

STR, strychnine

YM90K, 6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione hydrochloride

YM928, 2-[N-(4-chlorophenyl)-N-methylamino]-4H-pyrido[3.2-e]-1,3-thiazin-4-one

JPET Fast Forward. Published on October 20, 2003 as DOI: 10.1124/jpet.103.058495
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

The anticonvulsant activity of YM928 (2-[N-(4-chlorophenyl)-N-methylamino]-4H-pyrido[3.2-e]-1,3-thiazin-4-one), a novel α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist, was studied in animal models of generalized seizure. YM928 exerted significant anticonvulsant effects in the maximal electroshock seizure (MES) test (ED_{50} = 7.4 mg/kg p.o.), pentylenetetrazol (PTZ)-induced seizure test (ED_{50} = 9.6 mg/kg p.o.), AMPA-induced seizure test (ED_{50} = 5.5 mg/kg p.o.) and strychnine-induced seizure test (ED_{50} = 14.0 mg/kg p.o.) in mice. Effects in rats were detected in the MES test (ED_{50} = 4.0 mg/kg p.o.) and PTZ-induced seizure test (ED_{50} = 6.2 mg/kg p.o.). The profile of YM928 was compared with that of established antiepileptics. Valproate showed beneficial effects in all tests used. In contrast, carbamazepine, phenytoin, lamotrigine, phenobarbital, diazepam, ethosuximide, and gabapentin were not active against seizures induced by at least one stimulant. In the rotarod test, YM928 impaired motor coordination (TD_{50} = 22.5 mg/kg p.o.). The protective index (TD_{50} value of the rotarod test/ED_{50} value of MES) was 3.0, suggesting that YM928 can exert antiepileptic effects with only minor motor disturbances. YM928 at doses of 2, 4, and 8 mg/kg p.o. did not significantly affect the threshold of electroshock seizure in rats after 16 days' repeated

administration. These data indicate that YM928 does not induce tolerance after subchronic administration. These results indicate that YM928 is a broad-spectrum anticonvulsant which would prove useful for the treatment of generalized seizure in human epileptic patients.

JPET Fast Forward. Published on October 20, 2003 as DOI: 10.1124/jpet.103.058495
This article has not been copyedited and formatted. The final version may differ from this version.

Epilepsy is one of the most common neurological diseases, affecting approximately 1% of the population (Sander and Shorvon, 1987). It is commonly accepted that a significant proportion of patients suffer from seizures resistant to drug treatment (Dam, 1986). The ratio of intractable patients has not been reduced despite the marketing of several new antiepileptics (AEDs) in recent years (Cramet et al., 1999). Thus, the development of new AEDs is expected.

Epilepsy is characterized by epileptic seizure caused by excessive neuronal discharge. Possible mechanisms of AEDs are the enhancement of inhibitory neuronal transmission and suppression of excitatory neuronal transmission. The mechanisms of most AEDs presently available can be categorized as blockade of voltage-dependent Na^+ channels, potentiation of GABA-ergic transmission, and blockade of T-type Ca^{2+} channels (Löscher, 1998; Macdonald and Kelly 1995). Compounds that selectively block excitatory neurotransmitter receptors are not utilized as AEDs.

Glutamate is a major excitatory neurotransmitter in the vertebrate central nervous system. Excessive activation of glutamate receptors is thought to be involved in the generation and propagation of epileptic seizures (Chapman, 1998), and glutamate

JPET Fast Forward. Published on October 20, 2003 as DOI: 10.1124/jpet.103.058495
This article has not been copyedited and formatted. The final version may differ from this version.

receptor antagonists have been proposed as potential new AEDs. Glutamatergic receptors consist of ionotropic subtypes (*N*-methyl-D-aspartate (NMDA) receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and kainate receptors) and metabotropic receptors. Competitive (Croucher et al., 1982) and non-competitive (Troupin, et al., 1986) antagonists for NMDA receptors have been reported to possess anticonvulsant action in preclinical seizure models. However, adverse effects such as psychosis hamper their clinical use (Rogawski and Porter, 1990; Sveinbjornsdottir et al., 1993). Selective antagonists of AMPA/kainate receptors are also candidates for new AEDs. AMPA receptors mediate fast excitatory neuronal transmission, and the anticonvulsant effects of selective antagonists such as NBQX (Ikonomidou and Turski, 1997), YM90K (Shimizu-Sasamata et al., 1996), GYKI52466 (Smith et al., 1991; Yamaguchi et al., 1993) and talampanel (Czuczwar et al., 1998) have been reported. However, the properties of these AMPA receptor antagonists have not been optimized for the treatment of epilepsy. For example, the short half-life of NBQX restricts its use in chronic diseases like epilepsy (Gill et al., 1992: Chizh et al., 1994); a decrease in plasma concentration of talampanel is seen on concomitant use with enzyme-inducing AEDs (Langan et al., 2003); and nephrotoxicity is reported for NBQX (Xue et al., 1994). Thus, the development of novel AMPA antagonists has been

sought.

YM928 (2-[N-(4-chlorophenyl)-N-methylamino]-4H-pyrido[3.2-e]-1,3-thiazin-4-one) is a selective, noncompetitive AMPA receptor antagonist. It inhibited kainate-induced neurotoxicity, AMPA-induced intracellular Ca^{2+} increase and AMPA-induced current in rat hippocampal cultures. Oral administration of YM928 was effective against sound-induced seizure in DBA/2 mice (Ohno et al., 2003). However, because genetically epilepsy-prone rodents sometimes exhibit false-positive responses to non-anticonvulsant compounds, in the present study we examined the effect of YM928 in the maximal electroshock seizure (MES) test, pentylenetetrazol (PTZ)-induced seizure test, and strychnine (STR)-induced seizure test, all widely used animal models in the screening of potential AEDs, and in the AMPA-induced seizure test. The anticonvulsant effect of YM928 was compared with those of established AEDs. Effect on motor coordination was examined using the rotarod test. To assess the development of tolerance, effects of subchronic administration were assessed in the electroshock seizure threshold test.

Materials and methods

All experiments were performed in accordance with the guidelines of the Animal Ethical Committee of Yamanouchi Pharmaceutical Co., Ltd.

Animals

Male Wistar rats (Japan SLC, Hamamatsu, Japan) weighing 180 - 220 g were used for the MES and PTZ-induced seizure tests. Male Fisher rats (F344/DuCrj, Japan Charles River, Yokohama, Japan) weighing 190 - 260 g were used for the electroshock seizure threshold test. Male ICR mice (Japan SLC) weighing 27 - 43 g were used for the MES and PTZ-, STR- and AMPA-induced seizure tests. The animals were given free access to standard diet (CE-2, CLEA Japan, Tokyo, Japan) and tap water. Rats were kept in groups of 3-5 and mice in groups of 10 at a controlled temperature (23±3°C) and humidity (55±10%) with a 13-h light cycle (lights on 0730 - 2030).

Seizures induced by electroshock in mice and rats

Mice were stimulated with corneal electrodes from a stimulator (MK-800, Muromachi Kikai, Tokyo, Japan) using a suprathreshold current (50 Hz, 50 mA, 0.2 sec). Rats were stimulated with auricular electrodes from a stimulator (MK-810, Muromachi Kikai)

using a suprathreshold current (50 Hz, 40 mA, 0.2 sec). The electrodes were placed in 0.9 % sodium chloride solution before application. Tonic hindlimb extension (limb extension exceeding a 90° angle with the plane of the body) was used as the criterion of convulsion. Drugs were administered orally 60 min prior to the stimulus. ED_{50} values, the dose at which tonic hind limb seizures were prevented in 50% of animals, and 95% confidence interval were calculated (n = 10/group).

Seizures induced by PTZ in mice and rats

PTZ at a dose of 100 mg/kg in mice and 70 mg/kg in rats was injected subcutaneously 60 min after the oral administration of test compounds. The animals were observed for 30 min after injection and wild running, clonic seizures, tonic seizures and respiratory arrest were monitored. ED_{50} values and 95% confidence interval of clonic seizure were calculated (n = 9-10/group).

Seizures induced by STR in mice

STR at a dose of 0.8 mg/kg was injected subcutaneously 60 min after the oral administration of test compounds. The animals were then observed for 30 min after injection and wild running, clonic seizures, tonic seizures and respiratory arrest were

JPET Fast Forward. Published on October 20, 2003 as DOI: 10.1124/jpet.103.058495
This article has not been copyedited and formatted. The final version may differ from this version.

monitored. ED_{50} values and 95% confidence interval of tonic extension seizure were calculated (n = 10/group).

Seizures induced by AMPA in mice

For the intracerebroventricular (i.c.v.) injection of AMPA, canulae were prepared according to a previously reported method (Nakajima et al. 1994). Briefly, mice were anesthetized with pentobarbital (2.5 - 3.0 mg/mouse i.p.) and the skull was exposed. The tip of the injection apparatus was placed at 0.9 mm lateral and 0.7 mm posterior to the bregma, at a depth 3.0 mm below the surface of skull. It was held in place with dental cement applied to the exposed skull surface. After the operation, mice were housed individually to avoid damage to the injection apparatus. After a recovery period of at least 6 days, test compounds were injected orally. 60 min later, AMPA at a dose of 1 μg/mouse was injected at a volume of 4 μl. Wild running, clonic seizures, tonic seizures and respiratory arrest were monitored for 10 min. ED_{50} values and 95% confidence interval of clonic seizure were calculated (n = 8/group).

Rotarod performance in mice

Animals that were able to remain on a rotarod apparatus revolving at 5 rpm for 120 sec

were initially selected for the evaluation. Test compounds were then administered orally and rotarod performance was retested 60 min later. Mice that were not able to remain on the apparatus for 60 sec in 3 trial sessions were termed to have motor impairment. The number of mice having motor impairment was counted and TD_{50} values and 95% confidence interval were calculated (n = 10/group).

Electroshock seizure threshold test, tolerance study in rats

On day 1, the threshold for seizures induced by electroshock in rats was determined via auricular electrodes by means of a current stimulator with a sine wave stimulus (50 Hz, 0.2 sec). YM928 at doses of 2, 4, and 8 mg/kg p.o. was administered orally 120 min before stimulation (n = 16-20/group). Stimulus intensity was varied by an up-and-down method in which the intensity of current was lowered or raised if the preceding animal did or did not show hindlimb extension, respectively. From days 2 to 15, YM928 was administered once a day, and on day 16 the threshold was determined 120 min after the injection of YM928. On days 17 and 23, the threshold was determined without drug administration. The same animals were repeatedly used throughout the study.

Drugs

JPET Fast Forward. Published on October 20, 2003 as DOI: 10.1124/jpet.103.058495
This article has not been copyedited and formatted. The final version may differ from this version.

YM928 and diazepam were synthesized by Yamanouchi Pharmaceutical Co., Ltd. Lamotrigine was obtained from Glaxo Wellcome Research and Development Ltd. (Hertfordshire, UK). Gabapentin was obtained from Parke-Davis (Ann Arbor, MI). Carbamazepine and valproate were purchased from Wako Pure Chemical Industries (Osaka, Japan). Phenytoin was purchased from Dainippon Pharmaceutical (Osaka, Japan). Phenobarbital was purchased from Sanko Seiyaku Kogyo (Tokyo, Japan). Ethosuximide, STR and AMPA were purchased from Sigma (St. Louis, MO). PTZ was purchased from Tokyo Kasei (Tokyo, Japan). PTZ, STR, AMPA were dissolved in saline. Valproate and ethosuximide were dissolved in distilled water. All other test compounds were suspended in 0.5% methylcellulose solution. Drugs were administered at a dosing volume of 10 ml/kg in mice and 2 ml/kg in rats. Doses are expressed in terms of base.

Statistical analysis

ED_{50} values for anticonvulsant tests and TD_{50} values for the rotarod test were calculated by the method of probit analysis. In the subchronic administration test, seizure threshold and standard deviation were calculated by the reported method (Kimball et al., 1957). Students t-test was used to determine the difference in threshold on the first and last days of administration. Differences were considered significant when $p<0.05$.

Results

Anticonvulsant activity in mice

The electroshock produced hind limb extension in all animals that received vehicle solution. YM928 at doses of 2-30 mg/kg p.o. dose-dependently suppressed the tonic extensions, with complete inhibition observed at doses of 15-30 mg/kg (Fig. 1A). ED_{50} value of YM928 was 7.4 mg/kg. Carbamazepine, phenytoin, lamotrigine, valproate, phenobarbital, diazepam, and gabapentin showed anticonvulsant activity (Table 1). Ethosuximide, which blocks T-type Ca^{2+} channels, was ineffective at the dose range tested.

PTZ (100 mg/kg) induced clonic seizures in all mice treated with vehicle. YM928 at doses of 3 - 30 mg/kg p.o. dose-dependently suppressed the clonic seizures, with complete suppression observed at 20-30 mg/kg (Fig. 1B). ED_{50} value was 9.6 mg/kg. Valproate, phenobarbital, diazepam and ethosuximide were effective (Table 1). ED_{50} value of YM928 in the MES test did not differ significantly from that in the PTZ-induced seizure test. Valproate and phenobarbital inhibited MES and PTZ-induced seizures with similar potencies. In contrast, the effect of diazepam against PTZ-induced seizure was much more potent than that in MES. Carbamazepine, phenytoin, and

lamotrigine, which exert a blocking action on voltage-dependent Na^+ channels, and gabapentin were not active.

STR (0.8 mg/kg) induced seizures in all mice treated with vehicle. YM928 at doses of 5–50 mg/kg p.o. dose-dependently suppressed the tonic extension, with complete suppression observed at 50 mg/kg (Fig. 1C). ED_{50} value was 14.0 mg/kg. Valproate, carbamazepine, phenytoin, lamotrigine, diazepam, ethosuximide and gabapentin showed anticonvulsive activity (Table 1). No anticonvulsant activity was detected for phenobarbital.

In the AMPA-induced seizure model, mice showed wild running, clonic seizures and tonic seizures immediately after injection. YM928 at doses of 0.5 - 20 mg/kg p.o. dose-dependently suppressed the clonic seizures (Fig. 1D). ED_{50} value was 5.5 mg/kg. Valproate, carbamazepine, phenytoin, and lamotrigine showed anticonvulsant activities (Table 1). Under the present experimental condition, the potencies of YM928 in MES and AMPA-induced seizure tests were similar. In contrast, carbamazepine, phenytoin and lamotrigine were less potent in the AMPA-induced seizure test. No anticonvulsant activity was detected for phenobarbital, diazepam and ethosuximide.

Anticonvulsant activity in rats

The Antiepileptic Drug Development Program proposed that anticonvulsant action be confirmed in another rodent species (Porter et al., 1984). The effects of YM928 and reference compounds were therefore examined in rats. Electroshock induced tonic extension in all rats treated with vehicle. YM928 at doses of 1-10 mg/kg p.o. dose-dependently suppressed the tonic extension induced by electroshock (Fig. 2A). ED_{50} value was 4.0 mg/kg. PTZ (70 mg/kg) induced clonic seizures in all rats treated with vehicle. YM928 at doses of 2-20 mg/kg p.o. suppressed the clonic seizure induced by PTZ (Fig. 2B). ED_{50} value was 6.2 mg/kg. The effects of YM928 were compared with those of AEDs. Carbamazepine, phenytoin, lamotrigine, valproate, phenobarbital, and diazepam showed anticonvulsant activity in electroshock seizure test (Table 2). Valproate, phenobarbital, diazepam and ethosuximide were effective in the PTZ-induces seizure test (Table 2). The anticonvulsive profiles of AEDs were similar to those in mice.

Rotarod performance in mice

YM928 significantly impaired rotarod performance in mice (TD_{50} = 22.5 mg/kg, Fig. 3).

JPET Fast Forward. Published on October 20, 2003 as DOI: 10.1124/jpet.103.058495
This article has not been copyedited and formatted. The final version may differ from this version.

The protective index (P.I., rotarod TD_{50}/MES ED_{50}) was calculated to be 3.0. TD_{50} values of reference compounds were: carbamazepine = 120 mg/kg, phenytoin = 240 mg/kg, lamotrigine = 63.7 mg/kg, valproate = 1120 mg/kg, phenobarbital = 49.9, diazepam = 9.1 mg/kg, and ethosuximide = 721 mg/kg (Table 1). The P.I. of these compounds were 0.7 - 17.2. Gabapentin did not induce motor disturbances at a dose of 800 mg/kg (Table 1).

Electroshock seizure threshold test, tolerance study in rats

To examine the effect of YM928 after repeated administration, seizure threshold was measured on the first and last days of subchronic administration in rats. Based on the ED_{50} value of YM928 in the rat MES test, YM928 at doses of 2-8 mg/kg were used. Seizure threshold was not significantly altered after repeated administration of YM928 (Fig. 4). Seizure threshold was reexamined one and seven days after the subchronic administration to assess the possibility of rebound (a decrease in the threshold of convulsions compared to that of the control group). Seizure threshold in rats injected with YM928 was not different from those receiving vehicle (Fig. 4, Day 17, 23).

JPET Fast Forward. Published on October 20, 2003 as DOI: 10.1124/jpet.103.058495
This article has not been copyedited and formatted. The final version may differ from this version.

Discussion

The present study clearly demonstrates that YM928 exerts anticonvulsant actions in models of generalized seizure. Oral administration of YM928 protected against seizures induced by electroshock in mice and rats. Anticonvulsants that block voltage-dependent Na^+ channels show potent effects in the MES test, and the test is generally thought to be a model of generalized tonic-clonic seizure in human (Macdonald and Kelly, 1995; Löscher and Schmidt, 1988). YM928 is therefore expected to be valuable in the treatment of generalized tonic-clonic seizures. The electric current used in the mice experiments (50 mA, 0.2sec) was of the same intensity as that commonly reported by other researchers, whereas that for rats (40 mA, 0.2 sec) was weaker: here, intensity was set just above threshold so that YM928 dosage in the subchronic seizure test could be adjusted according to results. ED_{50} values for carbamazepine and phenobarbital were comparable to those in a previous study employing stimulation at 150 mA (Porter et al., 1984). In contrast, ED_{50} for diazepam was markedly lower in our study, suggesting that the efficacy of diazepam in the MES test critically depends on experimental conditions.

YM928 showed anticonvulsant effects in the PTZ-induced seizure test also. Anticonvulsant efficacy against seizures induced by PTZ is thought to correlate with that against human generalized absence/myoclonic seizures (Macdonald and Kelly,

1995; Löscher and Schmidt, 1988). Considering the efficacy of YM928 against PTZ-induced seizures, a beneficial effect of YM928 in these seizure types is also expected. It was recently pointed out that results in the MES and PTZ tests does not always predict clinical efficacy of novel AEDs against generalized tonic-clonic and absence seizures, respectively (Löscher, 1998). Conclusive characterization is therefore dependent on clinical evaluation. Lamotrigine and gabapentin were not active against clonic seizure induced by PTZ. Previous study reported that lamotrigine and gabapentin had no effect against PTZ-induced clonic convulsions, but dose-dependently antagonized tonic convulsions induced by PTZ (Dalby and Nielsen, 1997). Therefore it should be stressed that profile of test compounds against PTZ-induced seizure depends on seizure type and our results should be compared to those monitoring clonic seizure.

Most AEDs tested in this study showed preferential efficacy in either the MES or PTZ-induced seizure test, but the potency of YM928 against the MES did not significantly differ from that against PTZ-induced seizure, suggesting that various seizures could be treated by single dosage of YM928. In the STR-induced seizure test, YM928 showed an anticonvulsant effect. All compounds except phenobarbital suppressed seizure induced by STR. YM928 had anticonvulsant effects in all tests

examined, suggesting that it is a broad-spectrum antiepileptic compound. Effects on the AMPA-induced seizure test were investigated to assess anti-AMPA activity in vivo. YM928 significantly reduced seizure induced by AMPA. Carbamazepine, phenytoin, and lamotrigine, which are not thought to inhibit neuronal transmission by acting selectively at AMPA receptors, also showed marked effects. In the present study, all AEDs were tested 60 min after administration. ED_{50} values of these AEDs may not show the peak effects.

In our previous study, YM928 blocked AMPA-induced increases in intracellular Ca^{2+} and AMPA-induced inward currents in rat hippocampal cultures. YM928 also blocked kainate-induced toxicity noncompetitively, and showed little effect on NMDA- and veratridine-induced increases in intracellular Ca^{2+} in rat hippocampal culture (Ohno et al., 2003). These findings indicate that the mechanism of YM928's anticonvulsant effect is most likely its antagonistic action at AMPA receptors. AMPA receptor antagonists have been reported to show anticonvulsant effects in generalized seizure models. Both quinoxalinedione derivatives such as NBQX (Ikonomidou and Turski, 1997) and YM90K (Shimizu-Sasamata et al., 1996) as well as 2,3 benzodiazepines such as GYKI-52466 (Smith et al., 1991; Yamaguchi et al., 1993) and talampanel (Czuczwar et

al., 1998) have been shown to be anticonvulsive when administered intraperitoneally. The present results are consistent with these previous studies and reveal that YM928 is well absorbed orally.

The effect of YM928 on motor coordination was examined in the rotarod test. The protective index of YM928 was 3.0, suggesting that YM928 can exert antiepileptic effects with only minor motor disturbances. In contrast, NBQX and GYKI showed anticonvulsant effects in MES and PTZ threshold tests (Löscher and Hönack, 1994) and fixed intensity tests (Yamaguchi et al., 1993) only at doses inducing ataxia or muscle relaxation. Contrary to these previous studies, our present study indicates that AMPA antagonists can exert antiepileptic effects with only minor motor disturbances. Antagonists of the NMDA receptor-channel complex also induce motor disturbances at anticonvulsant doses (Carter, 1994). AMPA antagonists may thus have an advantage in terms of safety profile when studied clinically. To confirm this conclusion, NBQX, GYKI52466 and YM928 should be examined under the same experimental conditions.

The development of tolerance to the anticonvulsant action of some of AEDs has been reported in animal studies (File, 1983; De Sarro et al., 1992; Rundfeldt et al, 1995) and

clinical investigations (Oxley, 1986). In the subchronic administration test, YM928 did not alter the threshold of electronic seizure, suggesting that YM928 does not induce tolerance. In this test, Fischer rats were used and the threshold was measured 120 min after the administration of YM928. The plasma concentrations of YM928 at a dose of 10 mg/kg were not significantly changed after 28 days of treatment under these conditions (Day1, 2.59±0.40 μg/ml, Day28, 2.80±0.16 μg/ml, (mean±SD, n=4)). On this basis, these conditions were employed to cancel the effect of pharmacokinetic alteration. Seizure thresholds were monitored one and seven days after repeated administration. Seizure threshold in the YM928-treated group was not significantly different from that of the control group. These results indicate that seizure susceptibility is not changed after the withdrawal of YM928.

The effects of AMPA antagonists on animal models of stroke are well documented. NBQX (Gill et al., 1992: Sheardown et al., 1990), YM90K (Shimizu-Sasamata et al., 1996; Kawasaki-Yatsugi et al., 1998), and GYKI-52466 (Le Peillet et al., 1992) have been shown to be neuroprotective in global and focal ischemia models in rats. The AMPA receptor is also thought to be involved in the pathogenesis of amyotrophic lateral sclerosis (Plaitakis, 1990; Rothstein et al., 1993). Recently, it was reported that NBQX

ameliorated the oligodendrocyte damage in experimental autoimmune encephalomyelitis, a model of multiple sclerosis (Smith et al., 2000; Pitt et al., 2000). YM928 may be effective in these acute and chronic neurodegenerative disorders.

In summary, oral administration of YM928 showed anticonvulsant actions in the MES and PTZ-induced seizure, STR-induced seizure and AMPA-induced seizure tests. The broad action of YM928 was similar to that of valproate. These effects were produced without apparent motor impairment, and no diminution of anticonvulsant effect was seen on subchronic administration. The findings of this study show that YM928 has potential value as a novel antiepileptic drug.

References

Carter AJ (1994) Many agents that antagonize the NMDA receptor-channel complex in vivo also cause disturbances of motor coordination. *J Pharmacol Exp Ther* **269**:573-580.

Chapman AG (1998) Glutamate receptors in epilepsy. *Prog Brain Res* **116**:371-383.

Chizh BA, Cumberbatch MJ and Headley PM (1994) A comparison of intravenous NBQX and GYKI53655 as AMPA antagonists in the rat spinal cord. *Br J Pharmacol* **112**:843-846.

Cramer JA, Fisher R, Ben-Menachem E, French J and Mattson RH (1999) New antiepileptic drugs: comparison of key clinical trials. *Epilepsia* **40**:590-600.

Croucher MJ, Collins JF and Meldrum BS (1982) Anticonvulsant action of excitatory amino acid antagonists. *Science* **216**:899-901.

Czuczwar SJ, Swiader M, Kuzniar H, Gasior M and Kleinrok Z (1998) LY300164, a novel antagonist of AMPA/kainate receptors, potentates the anticonvulsive activity of antiepileptic drugs. *Eur J Pharmacol* **359**:103-109.

Dalby NO and Nielsen EB (1997) Comparison of the preclinical anticonvulsant profiles of tiagabine, lamotrigine, gabapentin and vigabatrin. *Epilepsy Res* **28**:63-72.

Dam M (1986) Intractable epilepsy: Introduction, in *Intractable Epilepsy: Experimental and Clinical Aspects*(Schmidt D and Morselli PL eds) pp 1-3, Raven Press, New York.

De Sarro GB, Rotiroti D, Gratteri S, Sinpoli S, Juliano M and De Sarro A (1992) Tolerance to anticonvulsant effects of clobazam, diazepam and clonazepam in genetically epilepsy-prone rats, in *GABA-ergic synaptic transmission. Adv. in Biochemical Pharmacology Vol. 47* (Biggio G, Concas A and Costa E eds)pp 249-254, Raven Press, New York.

File SE (1983) Tolerance to the anti-pentylenetetrazol effects of diazepam in the mouse. *Psychopharmacology* **79**:284-286.

Gill R, Nordholm L and Lodge D (1992) The neuroprotective actions of 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX) in a rat focal ischemia model. *Brain Res* **580**:35-43.

Ikonomidou C and Turski L (1997) Pharmacology of the AMPA antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo-(F)-quinoxaline. *Ann NY Acad Sci* **825**:394-402.

Kawasaki-Yatsugi S, Ichiki C, Yatsugi S, Shimizu-Sasamata M and Yamaguchi T (1998)

YM90K, an AMPA receptor antagonist, protects against ischemic damage caused by permanent and transient middle cerebral artery occlusion in rats. *Naunyn-Schmiedeberg's Arch Pharmacol* **358**:586-591.

Kimball AW, Burnett WT and Doherty DG (1957) Chemical protection against ionizing radiation. *Radiat Res* **7**:1-12.

Langan YM, Lucas R, Jewell H, Toublanc N, Schaefer H, Sander JW and Patsalos PN (2003) Talampanel, a new antiepileptic drug: single- and multiple-dose pharmacokinetics and initial 1-week experience in patients with chronic intractable epilepsy. *Epilepsia* **44**:46-53.

Le Peillet E, Arvin B, Moncada C and Meldrum BS (1992) The non-NMDA antagonists, NBQX and GYKI52466, protect against cortical and striatal cell loss following transient global ischemia in the rat. *Brain Res* **571**:115-120.

Löscher W (1998) New visions in the pharmacology of anticonvulsion. *Eur J Pharmacol* **342**:1-13.

Löscher W and Hönack D (1994) Effects of the non-NMDA antagonists NBQX and the 2,3-benzodiazepine GYKI 52466 on different seizure types in mice: comparison with diazepam and interactions with flumazenil. *Br J Pharmacol* **113**:1349-1357.

Löscher W and Schmidt D (1988) 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.

Macdonald RL and Kelly KM (1995) Antiepileptic drug mechanisms of action. *Epilepsia* **36 (Suppl 2)**:S2-S12.

Nakajima M, Inui A, Teranishi A, Miura M, Hirosue Y, Okita M, Hiromi N, Baba S and Kasuga M (1994) Effects of pancreatic polypeptide family peptides on feeding and learning behavior in mice. *J Pharmacol Exp Ther* **268**:1010-1014.

Ohno K, Tsutsumi R, Matsumoto N, Yamashita H, Amada Y, Shishikura J, Inami H, Yatsugi S, Okada M, Sakamoto S and Yamaguchi T (2003) Functional characterization of YM928, a novel noncompetitive AMPA receptor antagonist. *J Pharmacol Exp Ther* **306**:66-72.

Oxley J (1986) Tolerance to the antiepileptic effect of clobazam in patients with severe epilepsy, in *Tolerance to beneficial and Adverse Effects of Antiepileptic Drugs* (Koella WP, Frey HH, Froscher W and Meinardi H eds)pp 119-125, Raven Press, New York.

Pitt D, Werner P and Raine CS (2000) Glutamate excitotoxicity in a model of multiple

sclerosis. *Nat Med* **6**:67-70.

Plaitakis A (1990) Glutamate dysfunction and selective motor neuron degeneration in amyotrophic lateral sclerosis: a hypothesis. *Ann. Neurol* **28**:3-8.

Porter RJ, Cereghino JJ, Gladding GD, Hessie BJ, Kupferberg HJ, Scoville B and White BG (1984) Antiepileptic Drug Development Program. *Cleve Clin Q* **51**:293-305.

Rogawski MA and Porter RJ (1990) Antiepileptic drugs: pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds. *Pharmacol Rev* **42**:223-286.

Rothstein JD, Jin L, Dykes-Hoberg M and Kuncl RW (1993) Chronic inhibition of glutamate uptake produces a model of slow neurotoxicity. *Proc Natl Acad Sci USA* **90**:6591-6595.

Rundfeldt C, Wlaz P, Hönack D and Löscher W (1995) Anticonvulsant tolerance and withdrawal characteristics of benzodiazepine receptor ligands in different seizure models in mice. Comparison of diazepam, bretazenil and abecarnil. *J Pharmacol Exp Ther* **275**:693-702.

Sander JW, Shorvon SD (1987) Incidence and prevalence studies in epilepsy and their methodological problems: a review. *J Neurol Neurosurg Psychiatry* **50**:829-839.

Sheardown MJ, Nielsen EO, Hansen AJ, Jacobsen P and Honoré T, (1990) 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline: a neuro-protectant for cerebral ischemia. *Science* **247**:571-574.

Shimizu-Sasamata M, Kawasaki-Yatsugi S, Okada M, Sakamoto S, Yatsugi S, Togami J, Hatanaka K, Ohmori J, Koshiya K, Usuda S and Murase K (1996) YM90K: Pharmacological characterization as a selective and potent α-amino-3-hydroxy-5-methylisoxazole-4-propionate/kainate receptor antagonist. *J Pharmacol Exp Ther* **276**:84-92.

Smith SE, Dürmüller N and Meldrum BS (1991) The non-N-methyl-D-aspartate receptor antagonists, GYKI52466 and NBQX are anticonvulsant in two animal models of reflex epilepsy. *Eur J Pharmacol* **201**:179-183.

Smith T, Groom A, Zhu B and Turski L (2000) Autoimmune encephalomyelitis ameliorated by AMPA antagonists. *Nat Med* **6**:62-66.

Sveinbjornsdottir S, Sander JW, Upton D, Thompson PJ, Patsalos PN, Hirt D, Emre M, Lowe D and Duncan JS (1993) The excitatory amino acid antagonist D-CPP-ene (SDZ EAA-494) in patients with epilepsy. *Epilepsy Res* **16**:165-174.

Troupin AS, Mendius JR, Cheng F and Risinger MW (1986) MK-801, in *New Anticonvulsant Drugs* (Meldrum BS and Porter RJ eds) pp 191-202, John Libbey, London.

Xue D, Huang Z-G, Barnes K, Lesiuk HJ, Smith KE and Buchan AM (1994) Delayed treatment with AMPA, but not NMDA, antagonists reduces neocortical infarction. *J Cereb Blood Flow Metab* **14**:251-261.

Yamaguchi S, Donevan SD and Rogawski MA (1993) Anticonvulsant activity of AMPA/kainate antagonists: comparison of GYKI52466 and NBQX in maximal electroshock and chemoconvulsant seizure models. *Epilepsy Res* **15**:179-184.

Footnotes

Address correspondence to: Hiroshi Yamashita, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan. E-mail: yamasita@yamanouchi.co.jp

Legends

Fig. 1. Effects of YM928 on seizures induced by electroshock, PTZ, STR and AMPA in mice A, MES test. Mice were stimulated with corneal electrodes (50 Hz, 50 mA, 0.2 sec), 60 min after the oral administration of YM928. (n=10/group). B, PTZ-induced seizure test. PTZ (100 mg/kg s.c.) was injected 60 min after the oral administration of YM928 (n=10/group). C, STR-induced seizure test. STR (0.8 mg/kg s.c.) was injected 60 min after the oral administration of YM928 (n=10/group). D, AMPA-induced seizure test. AMPA (1 μg/mouse i.c.v.) was injected in a volume of 4 μl/mouse 60 min after the injection of YM928 (n=8/group).

Fig. 2. Effects of YM928 on seizure induced by electroshock and PTZ in rats. A, MES test. Rats were stimulated with auricular electrodes (50 Hz, 40 mA, 0.2 sec) 60 min after the oral administration of YM928. B, PTZ-induced seizure test. PTZ (70 mg/kg) was injected subcutaneously 60 min after the administration of YM928 (n=10/group).

Fig. 3. Effect of YM928 on motor coordination. Animals that were able to remain on a rotarod apparatus revolving at 5 rpm for 120 sec were selected for evaluation. YM928

was administered 60 min before the test. Mice that were not able to remain on the apparatus for 60 sec in 3 trial sessions were termed to have motor impairment (n=10/group).

Fig. 4. Effect of subchronic administration of YM928 on electroshock seizure threshold in rats. From days 1 to 16, YM928 (2, 4, 8 mg/kg p.o.) was administered once a day. On the first and last days of administration, the threshold current inducing tonic seizures was determined 120 min after the administration of YM928. On days 17 and 23, the threshold was determined without drug administration. Data are shown as mean ± S.E. (n=16-20/group).

JPET Fast Forward. Published on October 20, 2003 as DOI: 10.1124/jpet.103.058495
This article has not been copyedited and formatted. The final version may differ from this version.

Table 1 Summary of anticonvulsant and ataxic effects in mice

	MES ED_{50} (mg/kg)	PTZ ED_{50} (mg/kg)	STR ED_{50} (mg/kg)	AMPA ED_{50} (mg/kg)	Rotarod TD_{50} (mg/kg)	PI
YM928	7.4 (5.9-9.1)	9.6 (7.6-12.0)	14.0 (12.0-14.0)	5.5 (2.9-12.6)	22.5 (19.5-26.1)	3.0
carbamazepine	17.0 (14.6-18.6)	>200 (-)	4.1 (0.28-13.3)	62.3 (31.5-81.5)	120 (96.5-163)	7.1
phenytoin	15.8 (13.1-18.4)	>300 (-)	102 (35.1-284)	93.5 (53.5-137)	240 (205-298)	15.2
lamotrigine	3.7 (3.1-4.4)	>100 (-)	54.5 (32.4-112)	32.5 (17.6-45.1)	63.7 (51.6-72.8)	17.2
valproate	406 (362-434)	530 (481-611)	454 (252-727)	695 (358-840)	1120 (981-1260)	2.8
phenobarbital	15.6 (12.8-16.8)	19.0 (15.3-23.4)	>100 (-)	>60 (-)	49.9 (41.7-57.3)	3.2
diazepam	13.0 (10.7-15.0)	1.0 (0.80-1.3)	8.1 (4.8-15.1)	>12 (-)	9.1 (5.8-12.6)	0.7
ethosuximide	>1000 (-)	381 (334-445)	280 (152-383)	>800 (-)	721 (509-926)	-
gabapentin	124 (110-137)	>300 (-)	153 (109-216)	>300 (-)	>800 (-)	>4.0

ED_{50} values and TD_{50} values were calculated by the probit method (95% confidence limits are shown in parentheses). Protective index (PI; TD_{50} of rotarod/ED_{50} of MES) was also calculated. MES, electroshock seizure test; PTZ, pentylenetetrazol-induced

seizure test; STR, strychnine-induced seizure test; AMPA, AMPA-induced seizure test.

JPET Fast Forward. Published on October 20, 2003 as DOI: 10.1124/jpet.103.058495
This article has not been copyedited and formatted. The final version may differ from this version.

Table 2 Summary of anticonvulsant effects in rats

	MES ED_{50} (mg/kg)	PTZ ED_{50} (mg/kg)
YM928	4.0 (3.1-5.0)	6.2 (4.4-8.5)
carbamazepine	8.1 (5.2-12.0)	>400 (-)
phenytoin	9.1 (6.3-13.6)	>600 (-)
lamotrigine	5.2 (4.1-6.7)	>200 (-)
valproate	212 (164-257)	255 (215-304)
phenobarbital	8.8 (7.0-10.8)	13.7 (10.0-19.1)
diazepam	4.7 (2.3-7.0)	0.83 (0.53-1.3)
ethosuximide	>1000 (-)	267 (194-350)

ED_{50} values were calculated by the probit method (95% confidence limits are shown in parentheses). MES, electroshock seizure test; PTZ, pentylenetetrazol-induced seizure test.

JPET Fast Forward. Published on October 20, 2003 as DOI: 10.1124/jpet.103.058495
This article has not been copyedited and formatted. The final version may differ from this version.

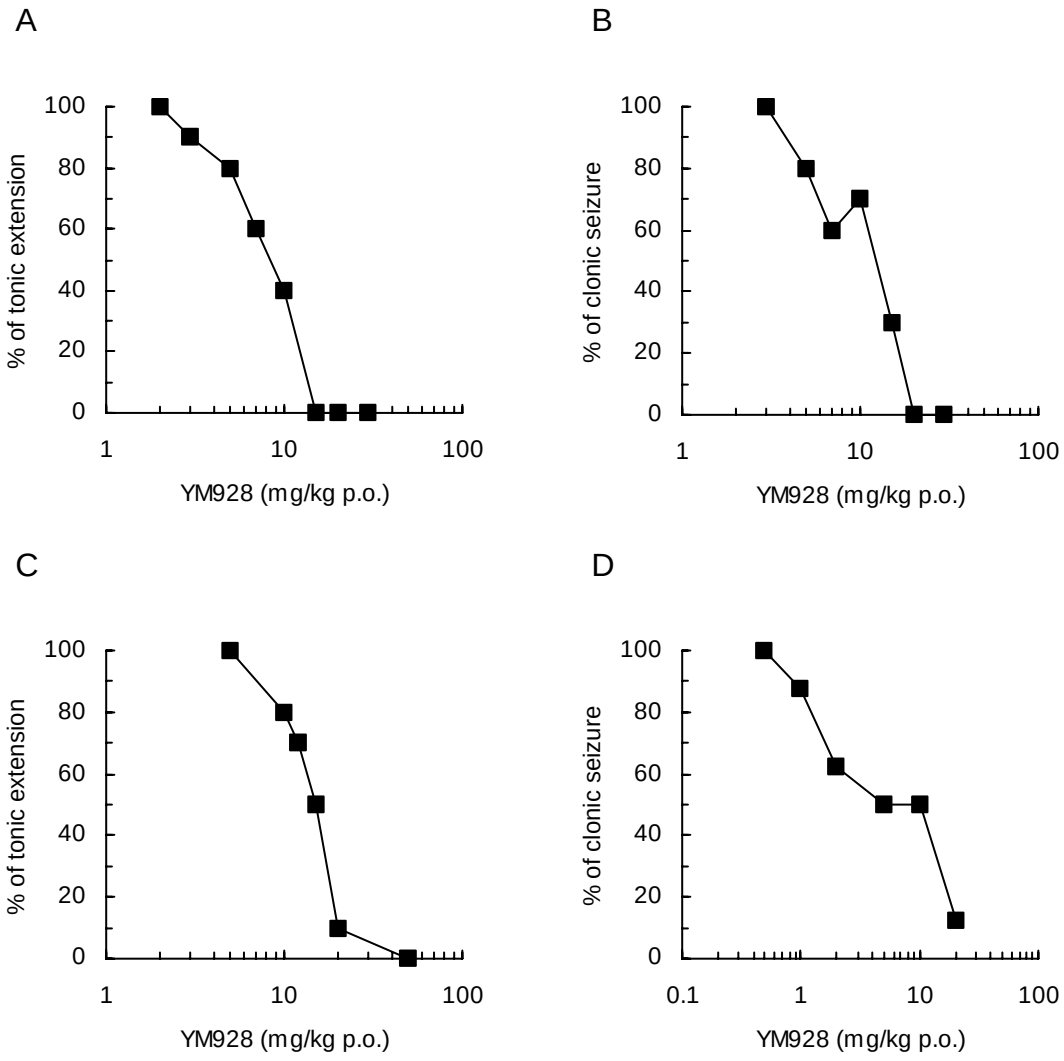

Fig. 1

JPET Fast Forward. Published on October 20, 2003 as DOI: 10.1124/jpet.103.058495
This article has not been copyedited and formatted. The final version may differ from this version.

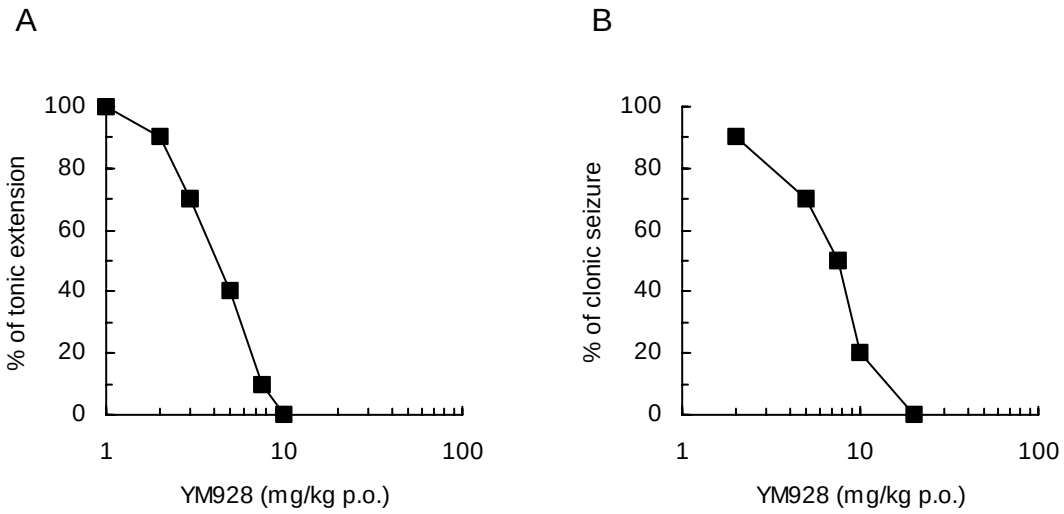

Fig. 2

JPET Fast Forward. Published on October 20, 2003 as DOI: 10.1124/jpet.103.058495
This article has not been copyedited and formatted. The final version may differ from this version.

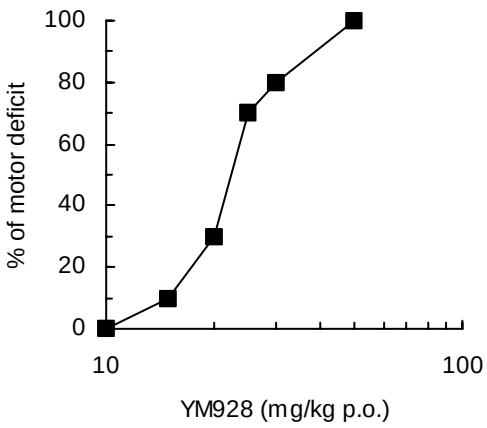

Fig. 3

JPET Fast Forward. Published on October 20, 2003 as DOI: 10.1124/jpet.103.058495
This article has not been copyedited and formatted. The final version may differ from this version.

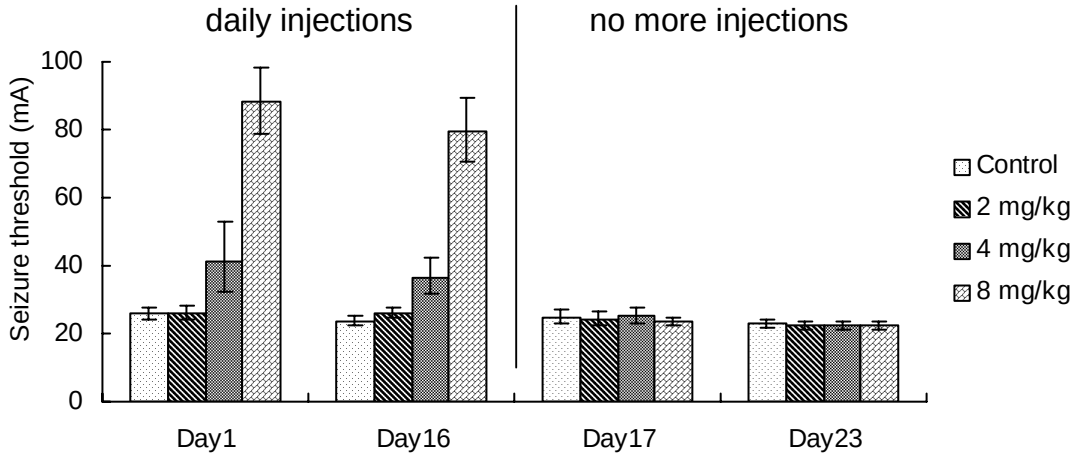

Fig. 4