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Suppression of fully kindled seizure and retardation of kindling acquisition by YM928 in the rat kindling model of epilepsy

Hiroshi Yamashita*, Kazushige Ohno, 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

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Abstract

We investigated the effects of 2-[*N*-(4-chlorophenyl)-*N*-methylamino]-4*H*-pyrido[3.2-e]-1,3-thiazin-4-one (YM928), a selective α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor antagonist, in the rat kindling model of complex partial seizures. YM928 (10 and 30 mg/kg p.o.) markedly suppressed the motor seizures and afterdischarge induced by electrical stimulation of the amygdala at generalized seizure-triggering threshold intensity. YM928 (10 mg/kg p.o.) did not induce apparent abnormal behavior, but did induce sedation at a dose of 30 mg/kg p.o. YM928 (30 mg/kg p.o.) showed a similar anticonvulsant effect at twice the threshold intensity as it did at threshold intensity. Diazepam (10 mg/kg p.o.) and phenobarbital (60 mg/kg p.o.) also exerted anticonvulsant activities. Diazepam (10 mg/kg) showed a similar effect at twice the threshold as at threshold, but the anticonvulsant effect of phenobarbital (60 mg/kg p.o.) was reversed when the stimulus was doubled. When YM928 (10 mg/kg p.o.) was administered 60 min before daily stimulation of the amygdala, the development of kindling seizure was significantly retarded. These results indicate that YM928 has anticonvulsant effects and suppresses kindling acquisition without sedative effects, and may be suitable as an antiepileptic drug for the treatment of complex partial seizures in humans.

Keywords: Epilepsy; Anticonvulsant; Kindling; AMPA receptor antagonist; YM928

1. Introduction

Several new antiepileptics have been introduced during the past decade for the treatment of epilepsy (Sabers and Gram, 1996; Bialer et al., 2002). These antiepileptics are reported to reduce the number of seizures in some refractory patients (Cramer et al., 1999). However, few such patients have been shown to be seizure-free, indicating an ongoing need for the development of novel antiepileptic agents. Given that the majority of refractory adult patients suffer from complex partial seizures (Mumford and Dulac, 1991), the development of new drugs for this seizure type has been particularly sought.

Kindling is a widely used rat model of human complex partial seizures with secondary generalization. It is produced by daily stimulation of the limbic brain area, resulting in the progressive development of seizure (Sato et al., 1990). The amygdala is the most sensitive structure for the induction of kindled seizures. The seizures are similar to those observed in complex partial seizure patients. Paroxysmal alteration of the electroencephalogram is also detected when these seizures are induced. Furthermore, the pharmacologic profile of amygdala kindling corresponds to that of human complex partial seizures (Albertson et al., 1980; Albright and Burnham, 1980). Compounds that suppress the seizure of fully kindled rats are thought to have potential as new antiepileptics. Further, the antiepileptogenic effects of a drug can be assessed by studying its effects on the progressive intensification of kindled seizure (Sato et al., 1990).

Glutamate is widely considered to be the principal excitatory neurotransmitter in the brain. Excessive activation of ionotropic glutamate receptors, namely *N*-methyl-D-aspartate (NMDA) receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and kainate receptors, has been thought to be involved in the induction and

* Corresponding author. Tel.: +81-29-863-6469; fax: +81-29-852-2925.

E-mail address: yamasita@yamanouchi.co.jp (H. Yamashita).

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propagation of seizures and pathological changes after kindling (Meldrum, 1994; Meldrum et al., 1999). The effects of selective NMDA receptor antagonists and AMPA/kainate receptor antagonists have been evaluated using amygdala kindling (Löscher, 1998). Results showed that NMDA receptor antagonists have anticonvulsant effects but generally weak efficacy (Löscher and Hönack, 1991), whereas competitive AMPA/kainate antagonists such as 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(F)-quinoxaline (NBQX) (Meldrum et al., 1992; Löscher and Hönack, 1994; Namba et al., 1994) and 6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione (YM90K) (Kodama et al., 1999) have anticonvulsant effects against kindled seizure but also nephrotoxicity (Xue et al., 1994). Further, the short half-life of NBQX could hamper its use as an antiepileptic drug in humans (Gill et al., 1992; Chizh et al., 1994). The noncompetitive AMPA antagonist 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (GYKI52466) (Meldrum et al., 1992; Dürmüller et al., 1994) showed an anticonvulsant effect but, to our knowledge, this compound is not under development for the treatment of epilepsy, and its analog 7-acetyl-5-(4-aminophenyl)-8,9-dihydro-8-methyl-7*H*-1,3-dioxolo(4,5*H*)-2,3-benzodiazepine (LY300164) produced only a moderate reduction in seizure duration without significantly affecting seizure score (Borowicz et al., 2001). Assessment of clinical efficacy against complex partial seizure therefore requires the development of novel AMPA receptor antagonists.

2-[*N*-(4-chlorophenyl)-*N*-methylamino]-4*H*-pyrido[3.2-e]-1,3-thiazin-4-one(YM928) is an AMPA receptor antagonist with no structural similarity to known AMPA receptor antagonists (Ohno et al., 2003). YM928 inhibited AMPA-induced intracellular Ca^{2+} increase and AMPA-induced inward current in rat hippocampal neuronal cell cultures. YM928 also inhibited kainate-induced toxicity noncompetitively in rat hippocampal cultures. YM928 showed anticonvulsant effects in animal models of generalized seizure such as the maximal electroshock seizure test, pentylenetetrazol-induced seizure test, strychnine-induced seizure test and AMPA-induced seizure test when administered orally (Yamashita et al., 2004). These studies indicate that YM928 may be useful in study of the therapeutic utility of AMPA receptor antagonists in chronic diseases including epilepsy.

In the present study, we evaluated the anticonvulsant effect of YM928 on fully kindled rats in comparison with phenobarbital and diazepam, as well as the effect of YM928 on seizure acquisition, to evaluate the possibility of its use in the treatment of partial seizures.

2. Materials and methods

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

2.1. Animals

Male Wistar rats (Japan SLC, Hamamatsu, Japan) weighing 250–370 g were used. The animals were given free access to standard diet (Japan Clea, Tokyo, Japan, CE-2) and tap water. They were kept in groups of three to five rats at a controlled temperature (23 ± 3 °C) and humidity (about 55 ± 10%) under a 13-h light cycle (lights on 0730–2030 h).

2.2. Electrode implantation

Rats were anesthetized with sodium pentobarbital (55 mg/kg i.p.) for stereotaxic implantation. The bipolar electrodes consisted of two twisted 0.2-mm-diameter stainless steel wires separated by 0.5 mm at the tip chronically implanted at the basolateral amygdala (A 6.4, L 4.3, H 1.8 from the interaural line) according to the stereotaxic coordinates of Paxinos and Watson (1986). Bipolar electrodes with the coating removed for 1 mm at the tip and then bent were implanted on the surface of the parietal cortex to monitor the secondary propagation of seizure activity. The electrodes were connected to plugs and anchor screws were placed in skull. The plug was held in place with dental cement applied to the exposed skull surface.

2.3. Effects on fully kindled seizure

Electrical stimulation of the amygdala was initiated after a postoperative period of at least 7 days. The stimulus consisted of a 1-ms duration, monophasic square-wave pulse of 60 Hz delivered for 1 s (SEN-7203, Nihon-Koden, Tokyo, Japan). Initial stimulus intensity was set at 50 μA and increased by 30-μA steps every 10 min until an afterdischarge of more than 3 s duration was monitored (EEG-4314, Nihon-Koden). Rats that showed afterdischarge with a stimulus less than 200 μA were used for drug evaluation. The stimulus intensity that first produced afterdischarge was designated as the afterdischarge threshold. When afterdischarge was generated at 50 μA on the first day, the effect of 30 μA was tested on the second day. In the present study, all rats had an afterdischarge threshold greater than 50 μA. Assessment of seizure stage was conducted according to the following six classes reported by Racine (1972): stage 0, no behavioral response to amygdaloid stimulation; stage 1, mouth movement; stage 2, head nodding; stage 3, forepaw up and/or forelimb clonus; stage 4, rearing; and stage 5, generalized convulsive seizure with falling. From the second day, the stimulus at the afterdischarge threshold was applied once daily until the rats showed a generalized convulsion for 5 consecutive days. Intensity was then reduced by 30-μA steps daily until rats no longer showed stage 5 seizure. The generalized seizure-triggering threshold, defined as the minimal current that elicited stage 5 seizure, was determined for each rat. Drug evaluation was initiated after three consecutive stage 5 seizures had been

induced by stimulation at the generalized seizure-triggering threshold intensity.

Initially, vehicle solution was administered orally 60 min before stimulation and seizures and afterdischarge were monitored. The test session was then started. At 60 min after the administration of test drugs, rats were placed in the test cage and the sedation was monitored. The loss of exploratory behavior such as rearing or sniffing was termed sedative. Next, stimulation at the generalized seizure-triggering threshold intensity was applied and seizure stage and afterdischarge were monitored. Between each test session, rats received generalized seizure-triggering threshold stimulation without drug administration to confirm the reproducibility of stage 5 seizure. At least 4 days were allowed between test sessions to avoid drug accumulation or the development of tolerance. Each rat was used to test one compound only, given in ascending dosages. After the test session, rats received stimulation at twice the generalized seizure-triggering threshold 60 min after administration at the dose that markedly suppressed the seizure and afterdischarge.

After the experiments, the brains were removed and the part housing the electrode was stained with cresyl violet. Only animals with the electrode correctly situated in the basolateral amygdala were used for evaluation.

2.4. Effects on seizure acquisition

After a postoperative period of at least 7 days, afterdischarge threshold was determined for each rat as described above (day 0). From day 1 to day 20, the rats received single daily oral administration of YM928 3 mg/kg, YM928 10 mg/kg, or vehicle. At 60 min after administration, electrical stimulation was applied at 125% of the afterdischarge threshold intensity and kindled seizure and afterdischarge were monitored. After the drug session, the rats that were administered 3 mg/kg of YM928 received stimulation without drug administration from day 21 to day 30, while those that were administered 10 mg/kg received stimulation without drug from day 21 to day 35.

The brains were removed after the experiments and histologically assessed as described above.

2.5. Drugs

YM928 and diazepam were synthesized in Yamanouchi Pharmaceutical. Phenobarbital (Sanko Seiyaku Kogyo, Tokyo, Japan) and sodium pentobarbital (Dainippon Pharmaceutical, Osaka, Japan) were obtained commercially. YM928 and diazepam were suspended in 0.5% methylcellulose solution. Phenobarbital was dissolved in a solution of propylene glycol/polyethylene glycol/physiological saline (1:1:4). All drug solutions and suspensions were freshly prepared before administration.

2.6. Statistics

The significance of differences between the different dosing groups was calculated by the Steel test (seizure score) or by one-way analysis of variance (ANOVA) followed by post hoc comparison using Dunnett's test (afterdischarge). When comparing seizures between groups of animals receiving the generalized seizure-triggering threshold current and those receiving current at twice the threshold, the Wilcoxon signed-rank test was used for seizure score and Student's *t*-test for afterdischarge. Two-way ANOVA was used to evaluate the development of afterdischarge. The number of seizures required to induce stages 2 and 5 was compared using one-way ANOVA followed by post hoc comparison using Dunnett's test. A P value less than 0.05 was considered statistically significant.

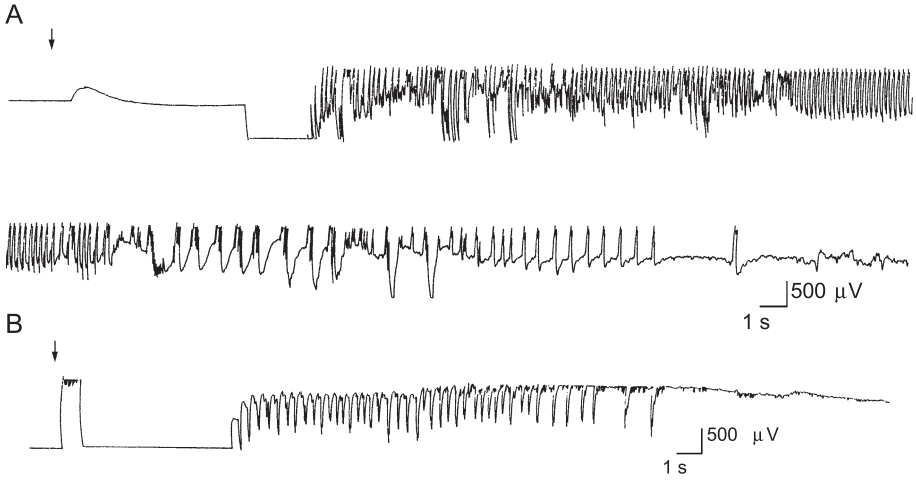

Fig. 1. EEG recordings from electrodes inserted at the amygdala in fully kindled rats. Vehicle (A) or YM928 (30 mg/kg) (B) was administered orally, followed 60 min later by electrical stimulation via the amygdala electrode. The arrows indicate the termination of 1-s stimulation. Stage 5 seizure was monitored with trace (A) and stage 1 with trace (B).

3. Results

3.1. Dose response for fully kindled seizure

Animals received daily electrical stimulation ranging from 50 to 200 μA. Seizures and afterdischarge were progressively intensified, and eventually stage 5 seizures with rhythmic polyspikes at the amygdala (Fig. 1A) occurred in all animals except those with connector detachment during experiments. After confirming the stable expression of stage 5 seizures, the generalized seizure-triggering threshold was determined and the range found to be 50–110 μA.

Administration of YM928 at doses of 1–10 mg/kg p.o. induced no obvious behavioral change. YM928 at 30 mg/kg caused sedation in all rats tested ($n = 8$). Pretreatment with YM928 significantly suppressed motor seizures in fully kindled rats at a dose of 10 mg/kg p.o. (Fig. 2A) Administration of 30 mg/kg YM928 further suppressed the motor seizures. YM928 also reduced the afterdischarge duration as illustrated in the electroencephalogram records of Fig. 1B. A statistically significant reduction was seen at doses of 10 and 30 mg/kg p.o. (Fig. 2D). In contrast with motor seizures, administration of either of the doses showed a similar reduction in afterdischarge.

Administration of phenobarbital caused a significant reduction of motor seizure and afterdischarge duration at doses of 30 and 60 mg/kg p.o. (Fig. 2B,E). The efficacy of phenobarbital at 60 mg/kg was similar to that of YM928 at 30 mg/kg. Phenobarbital at 60 mg/kg induced sedation in some rats (3/8).

Table 1
Effects of YM928, phenobarbital and diazepam on seizure induced by stimulation at twice the generalized seizure-triggering threshold (GST) intensity

Drug	Treatment	n	Seizure score (range)	Afterdischarge (s)
YM928	Control GST	8	5.0 (5)	66.2 ± 4.9
	30 mg/kg GST	8	0.8 (0–1)	22.9 ± 1.4
	30 mg/kg GST × 2	6	1.3 (1–3)	25.6 ± 2.8
Phenobarbital	Control GST	8	5.0 (5)	82.3 ± 3.6
	60 mg/kg GST	8	1.3 (0–4)	23.2 ± 6.3
	60 mg/kg GST × 2	8	4.4 (3–5)[a]	65.3 ± 4.6[b]
Diazepam	Control GST	8	5.0 (5)	75.5 ± 6.7
	10 mg/kg GST	8	0.9 (0–1)	23.2 ± 4.1
	10 mg/kg GST × 2	8	1.0 (1)	28.0 ± 2.6

Afterdischarges are shown as means ± S.E.M.
[a] $P < 0.01$, Wilcoxon signed-rank test as compared with the GST stimulation group.
[b] $P < 0.01$, Student's t-test as compared with the GST stimulation group.

Administration of diazepam at doses of 1–10 mg/kg p.o. caused a significant reduction of motor seizure and afterdischarge duration ($n = 8$, Fig. 2C,F). Efficacy of diazepam at 10 mg/kg was similar to that of YM928 at 30 mg/kg.

In tests evaluating YM928 and phenobarbital, when motor seizure was suppressed (mean seizure score less than 2), afterdischarge duration was less than 50% of control values. In contrast, diazepam at a dose of 1 mg/kg suppressed motor seizure, but afterdischarge was only moder-

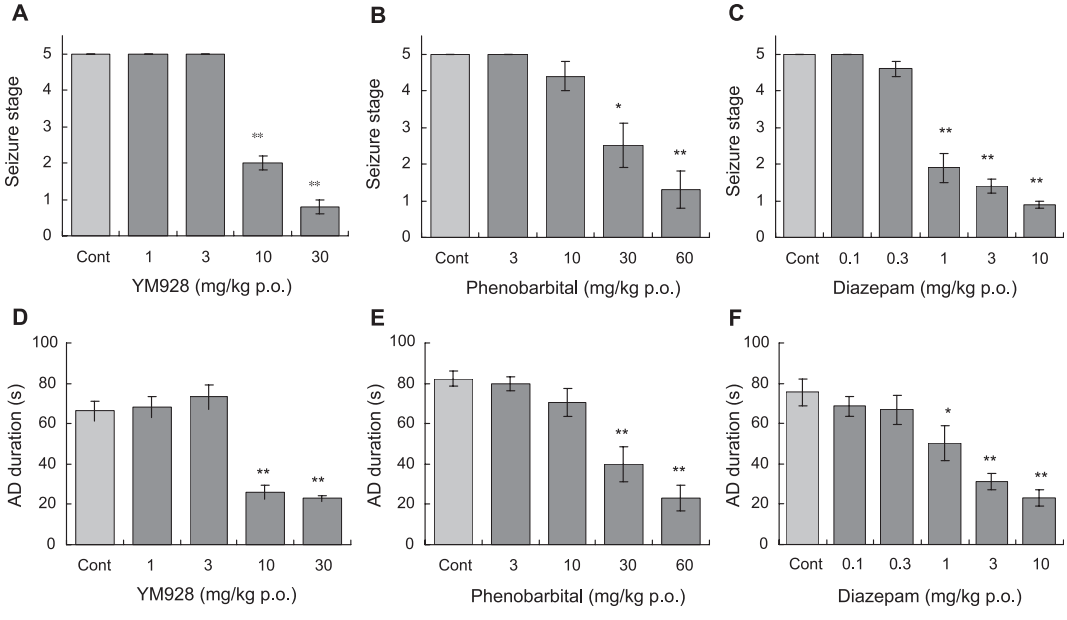

Fig. 2. Anticonvulsant effect of YM928, phenobarbital and diazepam on kindling in rats. Seizure stages (score 0, no seizure; score 1, mouth movement; score 2, head nodding; score 3, forepaw up and/or forelimb clonus; score 4, rearing; score 5, generalized convulsive seizure with falling) of YM928 (A), phenobarbital (B) and diazepam (C) were monitored. Afterdischarge (AD) duration of YM928 (D), phenobarbital (E) and diazepam (F) were used to evaluate anticonvulsant effects. Each drug was administered 60 min prior to electrical stimulation of the amygdala. Each column represents the mean ± S.E.M. Significantly different from control group values: *$P < 0.05$, **$P < 0.01$ (A, B, C: Steel test; D, E, F: one-way analysis of variance followed by post hoc comparisons using Dunnett's test) ($n = 8$/group).

ately inhibited. In the diazepam group, a 50% reduction of afterdischarge duration was achieved at 3 mg/kg.

3.2. Effects on generalized seizure-triggering threshold

Since YM928 at 30 mg/kg, phenobarbital at 60 mg/kg and diazepam at 10 mg/kg showed similar efficacy on motor seizure and afterdischarge, these doses were used to examine the effects on generalized seizure-triggering threshold. When stimulation was increased to twice the generalized seizure-triggering threshold intensity, YM928 showed a similar anticonvulsant effect to that at the threshold intensity (Table 1). In contrast, motor seizure and afterdischarge values for phenobarbital were markedly increased when the stimulation was doubled. The effect of diazepam on kindled seizures induced by stimulation at twice the threshold intensity was not significantly changed (Table 1).

3.3. Effect on seizure acquisition

Assessment of the effect on fully kindled seizure revealed that YM928 at a dose of 10 mg/kg reduced motor seizure and afterdischarge without inducing obvious behavioral changes. Doses of 3 and 10 mg/kg were therefore chosen to examine the effect on seizure acquisition. On day 1, the values of motor seizure and afterdischarge duration were not significantly different among groups (Fig. 3A,B). In the vehicle control group, motor seizure was progressively intensified and on day 15, all rats showed stage 5 motor seizures (Fig. 3A; Table 2). The increase in afterdischarge duration and seizure score in the control group appeared parallel (Fig. 3B). Daily treatment of YM928 at a dose of 3 mg/kg showed little effect on motor seizures. On day 8, afterdischarge duration in rats receiving 3 mg/kg was significantly ($P<0.05$) shorter than that of the control, but

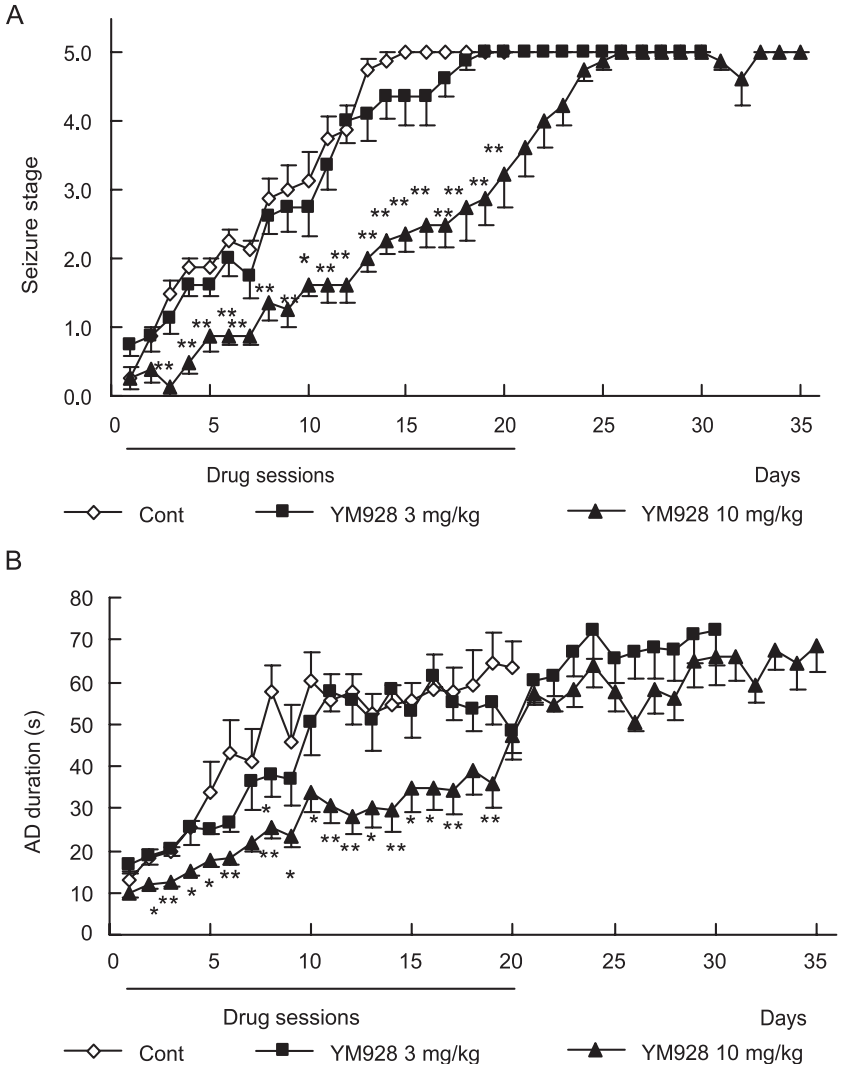

Fig. 3. Effect of YM928 on the development of kindling in rats. From day 1 to day 20, rats received oral administration of YM928 (3–10 mg/kg) or vehicle once daily. Kindled seizure (A) and afterdischarge (AD) duration (B) induced by electrical stimulation were monitored 60 min after administration. Significantly different from control group values: *$P<0.05$, **$P<0.01$ (A: Steel test; B: one-way analysis of variance followed by post hoc comparisons using Dunnett's test) ($n=7$ or 8/group).

Table 2
Effect of YM928 on the number of stimulations required for the development of the first seizure of scores 2 and 5

Drug	Dose (mg/kg p.o.)	First score 2, no. of stimulations (range)	First score 5, no. of stimulations (range)
Control		3.8 (3–6)	12.5 (9–15)
YM928	3	4.8 (2–8)	14.0 (9–19)
	10	9.5 (5–13)[a]	22.5 (18–26)[a]

[a] $P<0.01$ (one-way ANOVA followed by Dunnett's test compared with the control group).

the values of other days were similar to those of the control group. Pretreatment with YM928 at a dose of 10 mg/kg markedly retarded the increase in motor seizure and afterdischarge (Fig. 3A,B). From day 3 to the last drug session day (day 20), seizure scores of rats administered 10 mg/kg were significantly reduced compared to those of control rats. Afterdischarge duration in rats receiving 10 mg/kg was significantly reduced from day 2 to day 19, except on days 7 and 17.

The number of stimulations required for the first inductions of stages 2 and 5 was significantly increased in the group that received 10 mg/kg YM928 (Table 2). In this group, six of eight animals did not develop full seizure (seizure stage 5) in the drug session period. The effect of YM928 at a dose of 3 mg/kg was not significantly different from that of the control. All rats examined displayed stage 5 seizure eventually.

4. Discussion

In fully kindled rats, YM928 exerted potent anticonvulsant effects against generalized seizure induced by the afterdischarge threshold current at a dose which had no apparent side effects (10 mg/kg). Since amygdala kindling is thought to be a model of complex partial seizures with secondary generalization (Sato et al., 1990; Albertson et al., 1980), YM928 is expected to be effective against complex partial seizures in humans. Administration of 30 mg/kg YM928 further suppressed the motor seizure with little change in afterdischarge at the amygdala. Since afterdischarge the amygdala was not completely suppressed at these doses, it is likely that YM928 exerted an anticonvulsant action by reducing the propagation of seizure activity. Anticonvulsant effects of phenobarbital and diazepam were also detected. The doses of diazepam effective in decreasing afterdischarge were found to be higher than those effective in suppressing generalized seizure. These profiles of phenobarbital and diazepam were consistent with those reported by Voits and Frey (1994), and the maximum efficacy of YM928 obtained here was closely similar to their results. Local epileptic activity (seizure score 1–2) in amygdala kindling was reported to be resistant to treatment with antiepileptics (Albright and Burnham, 1980). The present results therefore suggest that the efficacy of YM928 against fully kindled seizures is comparable to that of conventional antiepileptics.

In vitro pharmacologic studies revealed that YM928 blocked kainate-induced toxicity, AMPA-induced intracellular Ca^{2+} increase, and AMPA-induced inward currents in rat hippocampal cultures. YM928 showed little effect on NMDA- and veratridine-induced intracellular Ca^{2+} increase in rat hippocampal cultures. These results and those of binding studies suggest that YM928 is a selective AMPA antagonist (Ohno et al., 2003). The beneficial effects of YM928 in the present study are therefore considered to be due to antagonistic action at AMPA receptors.

Further, YM928 has shown anticonvulsant effects in the maximal electroshock seizure test, pentylenetetrazol-induced seizure test in mice and rats and AMPA- and strychnine-induced seizure test in mice (Yamashita et al., 2004). These and the present results suggest that YM928 inhibits both partial and generalized seizure, indicating that it may represent a novel antiepileptic drug with a broad spectrum of action. In theory, it is preferable to use noncompetitive antagonists to suppress seizures, because an equivalent degree of inhibition is expected whatever the glutamate level is. On the other hand, the effect of competitive antagonists could be overcome by excessive release of glutamate (Rogawski, 1993). YM928 suppressed kainate-induced toxicity noncompetitively and did not inhibit the binding of [^{3}H]AMPA (Ohno et al., 2003). YM928 is therefore considered to be a noncompetitive AMPA antagonist, and is expected to show a better safety profile than competitive AMPA antagonists.

The anticonvulsant effects of competitive AMPA receptor antagonists in amygdala kindling have been extensively studied (Löscher, 1998). NBQX was reported to show an anticonvulsant effect in generalized seizure in kindled rats (Meldrum et al., 1992; Löscher and Hönack, 1994; Namba et al., 1994; Dürmüller et al., 1994), but ataxia was also observed at effective doses. Recently, evaluation of a pyrrolyl-quinoxalinedione series of non-NMDA receptor antagonists revealed that compounds acting at both AMPA and kainate receptors are more potent than AMPA receptor-selective drugs (Löscher et al., 1999). Similarly, an anticonvulsant effect was reported for GYKI52466, a noncompetitive antagonist, but severe motor side effects and an irregular electroencephalogram (EEG) were concomitantly observed (Meldrum et al., 1992; Dürmüller et al., 1994). To our knowledge, none of these AMPA receptor antagonists is under active development for the treatment of epilepsy. YM928, which possess an anticonvulsant action without severe motor side effects, may be a candidate for clinical investigation.

It is well recognized that stimulus intensity has a marked effect on the outcome of drug evaluation in amygdala kindling in rats. The effects of phenobarbital, carbamazepine and valproic acid were reduced when stimulus intensity was increased, while those of phenytoin, clonazepam and

diazepam were independent of stimulus intensity (Voits and Frey, 1994). It is thought that compounds showing anticonvulsant effects irrespective of stimulation intensity inhibit seizure spread (Piredda et al., 1985). Several studies have used the generalized seizure-triggering threshold intensity and its doubled intensity in rats to characterize the profile of compounds. Results showed that the effects of tiagabine and valproate were reversed by stimulation at twice the threshold (Morimoto et al., 1997), whereas with NBQX, seizure stage and afterdischarge duration were not significantly changed. This latter result was interpreted to mean that the anticonvulsant effect of NBQX does not depend on stimulation intensity (Namba et al., 1994). In contrast, YM90K, another quinoxalinedione derivative AMPA antagonist, failed to suppress seizures and afterdischarge duration when intensity was doubled (Kodama et al., 1999). In the present study, the profile of YM928 at a dose of 30 mg/kg was similar to that of NBQX. Further study is necessary to elucidate the conditions under which AMPA antagonists elevate seizure threshold. The effect of diazepam at 10 mg/kg was not altered when stimulation was doubled, whereas that of phenobarbital at 60 mg/kg was reversed. These results are consistent with those of a previous report (Voits and Frey, 1994) and indicate that the elevation of seizure threshold is at least partly responsible for the anticonvulsant effect of phenobarbital. Further investigation of the relationship between clinical outcomes in antiepileptics and the effects of drugs on generalized seizure-triggering threshold in rat kindling may clarify those conditions under which the clinical evaluation of potential antiepileptics may be commenced.

We also examined the effect of YM928 on the development of kindling. It is important to note here that some drugs inhibit kindled seizures, but not the development of kindling (Albertson et al., 1984). In the present study, it was clearly shown that YM928 at a dose of 10 mg/kg significantly inhibited the development of kindling seizure, but did not completely block it. One possible explanation is that YM928 does not completely inhibit seizures at a dose of 10 mg/kg and the residual seizure activity is sufficient for the development of kindling. Another possibility is that progression resulted from the development of tolerance to YM928 or a decrease in concentration in the brain. The latter is unlikely; however, in a previous study examining changes in the anticonvulsant effect of YM928 after subchronic administration in rats, the threshold of currents required to elicit electroshock-induced acute tonic seizures was not significantly altered after 16 days of daily administration (Yamashita et al., 2004).

As for the antiepileptogenic effects of known AMPA antagonists, initial reports using NBQX and GYKI52466 showed no effect on the development of kindling (Dürmüller et al., 1994), while a second group demonstrated the antiepileptogenic effect of NBQX (Namba et al., 1994) and YM90K (Kodama et al., 1999). These latter and the present results suggest that AMPA receptors are involved in the development of seizure in amygdala kindling. After the drug sessions, animals of all groups eventually developed stage 5 seizures, indicating that the retardation of kindling acquisition of YM928 did not result from any permanent change in susceptibility to kindling.

In conclusion, this study shows that YM928, a novel AMPA receptor antagonist, has an anticonvulsant effect when administered orally at a dose which does not induce severe central nervous system depression in a rat kindling model of epilepsy. This anticonvulsant effect was preserved when the stimulation was increased to twice the generalized seizure-triggering threshold. YM928 also showed the retardation of kindling acquisition. These results indicate that YM928 may be a suitable tool for evaluating the effects of AMPA antagonists on human complex partial seizure, a condition that is often resistant to conventional drug therapy.

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