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Functional Characterization of YM928, a Novel Noncompetitive α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid (AMPA) Receptor Antagonist

KAZUSHIGE OHNO, RIE TSUTSUMI, NAOYUKI MATSUMOTO, HIROSHI YAMASHITA, YOKO AMADA, JUN-ICHI SHISHIKURA, HIROSHI INAMI SHIN-ICHI YATSUGI, MASAMICHI OKADA, SHUICHI SAKAMOTO, and TOKIO YAMAGUCHI

Neuroscience Research, Pharmacology Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., Tsukuba, Japan

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ABSTRACT

The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor is thought to play an important role in the pathogenesis of several neurological disorders as well as normal brain function. The search for AMPA receptor antagonists as potential therapeutics is ongoing. Here, we describe the functional characterization of a novel noncompetitive AMPA receptor antagonist, 2-[*N*-(4-chlorophenyl)-*N*-methylamino]-4*H*-pyrido[3,2-*e*]-1,3-thiazin-4-one (YM928). This compound inhibited AMPA receptor-mediated toxicity in primary rat hippocampal cultures with an IC_{50} of 2 μM. Its manner of inhibition was noncompetitive as the agonist concentration was increased. YM928 blocked AMPA-induced intracellular calcium influx with an IC_{50} of 3 μM and antagonized AMPA-induced inward currents with an IC_{50} of 1 μM in cultured cells. YM928 displaced neither [^{3}H]AMPA binding nor other existing glutamate receptor-related ligand binding in rat brain membranes. In terms of in vivo activity, YM928 had an anticonvulsant effect in sound-induced seizures in DBA/2 mice 45 min after oral administration at 3 mg/kg. Thus, YM928 has potential as an oral therapeutic drug for various types of neurological disorders.

The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor belongs to the ionotrophic glutamate receptor family that is regulated by the neurotransmitter glutamate (for review, see Seeburg, 1993). Other members of this family are the *N*-methyl-D-aspartate (NMDA) and kainate (KA) receptors. The AMPA receptor provides the majority of fast excitatory transmission in the brain. It is composed of four subunits (GluR1–4) that can assemble to form functional ion channels through which Na^+/K^+ or Ca^{2+} is permeable, depending on the subtype composition.

Excessive activation of ionotrophic glutamate receptors is thought to be implicated in the pathogenesis of a diverse group of neurological disorders (for review, see Gill et al., 1999; Lees, 2000). These disorders include epilepsy, focal and global ischemia, central nervous system trauma, and various forms of neurodegeneration such as Parkinson's disease and Huntington's disease. Indeed, glutamate can induce neuronal death in vitro, and several glutamate receptor antagonists have been shown to have neuroprotective effects in animal models of brain ischemia and neurodegenerative disorders. Both the AMPA and the NMDA receptor seem to play an important role in such pathological conditions. The cerebroprotective effects of NMDA receptor antagonists have been well documented in focal ischemia models (Park et al., 1988; Gill et al., 1991). However, NMDA receptor antagonists may have limited utility as therapeutic agents, since these also produce psychotomimetic effects (Koek et al., 1988), impairment of learning and memory (Morris et al., 1986), and ultrastructural changes in cortical neurons (Olney et al., 1989). Therefore, the development of AMPA receptor antagonists has been encouraged to create therapeutics for neurological disorders.

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ABBREVIATIONS: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, *N*-methyl-D-aspartate; KA, kainate; YM928, 2-[*N*-(4-chlorophenyl)-*N*-methylamino]-4*H*-pyrido[3,2-*e*]-1,3-thiazin-4-one; NBQX, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[*f*]quinoxaline-7-sulfonamide; GYKI52466, 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-[5*H*-2,3]-benzodiazepine; LY293558, (3*S*,4*aR*,6*R*,8*aR*)-6-[2-(1(2)*H*-tetrazole-5-yl)ethyl]decahydroisoquinoline-3-carboxylic acid; CP-465,022, (+)-(*aS*)-3-(2-chlorophenyl)-2-[(*E*)-2-[6-(diethylaminomethyl)pyridin-2-yl]vinyl]-6-fluoroquinazolin-4(3*H*)-one; TTX, tetrodotoxin; fura 2-AM, 1-[6-amino-2-(5-carboxy-2-oxazolyl)-5-benzofuranyloxy]-2-(2′-amino-5′-methylphenoxy)-ethane-*N,N,N′,N′*-tetraacetic acid pentaacetoxymethyl ester; LY300164/talampanel, (*R*)-7-acetyl-5-(4-aminophenyl)-8,9-dihydro-8-methyl-7*H*-1,3-dioxolo[4,5-*h*][2,3] benzodiazepine; CGS19755, *cis*-4-(phosphonomethyl)piperidine-2-carboxylic acid; LDH, lactate dehydrogenase; MK-801, dizocilpine; CP-526,427, (*S*)-2-{2-[3-(2-chloro-phenyl)-6-fluoro-4-oxo-3,4-dihydro-quinazolin-2-yl]-vinyl}-nicotinonitrile; B169, 2-(β-pyridyl-(2″)-ethenyl)-3-(2′-methylphenyl)-quinazolinone-(4).

There are two prototype AMPA receptor antagonists, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide (NBQX) and, 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-[5H-2,3]-benzodiazepine (GYKI52466) (Fig. 1) (for review, see Nikam and Kornberg, 2001). NBQX belongs to the quinoxalinedione class and is a competitive AMPA receptor antagonist. On the other hand, GYKI52466 belongs to the 2,3-benzodiazepine class and is a noncompetitive AMPA receptor antagonist. They have been excellent tools for investigating the function of the AMPA receptor. They were shown to be neuroprotective in global (Sheardown et al., 1990, 1993; Buchan et al., 1991; Judge et al., 1991; Le Peillet et al., 1992; Li and Buchan, 1993; Lodge et al., 1996) and focal (Gill et al., 1992; Smith and Meldrum, 1992; Xue et al., 1994; Graham et al., 1996) models of ischemia. Their anticonvulsant activities were also described in several animal models (Chapman et al., 1991; Smith et al., 1991; Yamaguchi et al., 1993; Durmuller et al., 1994). NBQX, however, is poorly soluble and precipitates in the kidney at projected therapeutic plasma levels. Although some efforts have provided an improvement in the water solubility of this class of compounds, for example YM872, the problem of brain penetrability still remains (Kohara et al., 1998). Recently decahydroisoquinolines typified by LY293558 (Bullock et al., 1994; O'Neill et al., 1998) and quinazolinones typified by CP-465,022 (Lazzaro et al., 2002; Menniti et al., 2000) have become known as new classes of competitive and noncompetitive AMPA receptor antagonists, respectively.

To create new orally active AMPA receptor antagonists, a hundred thousand compounds have been screened against KA-induced toxicity in rat primary cortical cultures, which is mediated by the AMPA receptor (Ohno et al., 1997). Several active compounds with potentially useful chemical structures were found, and from these, a pyridothiazine derivative was selected as a lead compound. 2-[*N*-(4-Chlorophenyl)-*N*-methylamino]-4*H*-pyrido[3,2-*e*]-1,3-thiazin-4-one (YM928) arose from medicinal chemistry based on this compound (Fig. 1). In the present study, we describe the functional characterization of YM928.

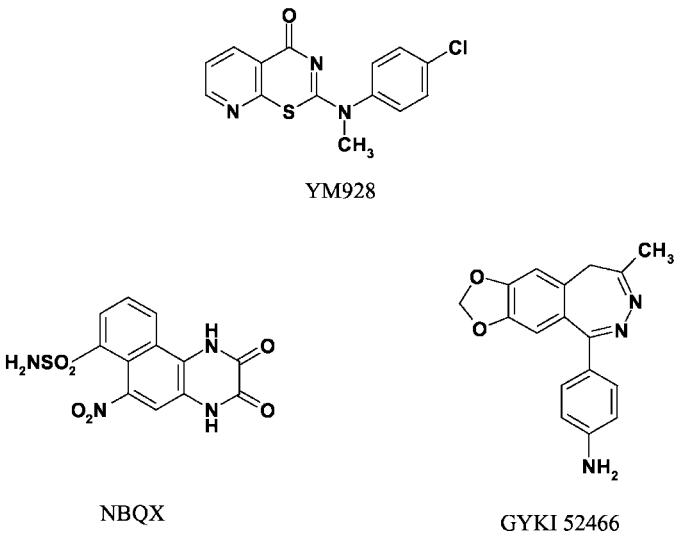

Fig. 1. Chemical structures of YM928, NBQX, and GYKI52466.

Materials and Methods

Materials. Drugs used in toxicity, calcium measurement, and electrophysiological experiments were purchased from the following sources: KA, Sigma/RBI (Natick, MA); NMDA, Sigma/RBI; NBQX, Tocris Cookson Inc. (Bristol, UK); AMPA [(*S*)-AMPA], Tocris Cookson Inc.; veratridine, Sigma/RBI; tetrodotoxin (TTX), Sankyo (Tokyo, Japan); and fura 2-AM, Dojin Chemical (Tokyo, Japan). YM928, GYKI52466, LY300164/talampanel and CGS19755 were synthesized in the Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd.

Rat Hippocampal Cell Cultures. Hippocampi were isolated from embryonic day 18 to 20 Wistar rats, and dissociated by incubation with papain and DNase I, followed by pipetting. These cells were suspended in SUMILON Medium/Neuron (containing glial conditioned medium, No. MB-X9501; Sumitomo Bakelite Co. Ltd., Tokyo, Japan) and were plated on poly-L-lysine-coated 48-well test plates at a density of 1.3×10^5 cells/cm^2 for the KA-induced toxicity assay, and on poly-L-lysine-coated coverslips at a density of 1×10^5 cells/cm^2 for the intracellular Ca^{2+} influx assay and patch-clamp analysis. Cultures were maintained without exchange of culture medium for 7 to 17 days. All experiments were performed in compliance with the regulations of the Animal Ethical Committee of Yamanouchi Pharmaceutical Co., Ltd.

KA-Induced Toxicity. Cultures were used after 8 or 9 days in vitro. KA and other drugs were dissolved in 20 mM HEPES-buffered Eagle's minimal essential medium (Earle's salts) containing 6 mg/ml glucose, 1 mM sodium pyruvate, 1 mg/ml BSA, 2 mM L-glutamine for exposure to neurons. In all experiments, neurons were exposed to KA and one of the study drugs at the same time. Overall neuronal cell injury was quantitatively assessed by measurement of lactate dehydrogenase (LDH) released from damaged or destroyed cells into the extracellular fluid 24 h after KA exposure. LDH activity was measured in 300-μl aliquots of media using an LDH assay kit ([BMY] LDH SFBC; Roche Diagnostics, Indianapolis, IN) in a 7250 Automatic Analyzer (Hitachi, Tokyo, Japan). The IC_{50} (concentration of drug producing half-maximal inhibition) and the n (the Hill coefficient) was determined from the logistic equation: $R = 100/(1 + ([\text{Drug}]/IC_{50})^n)$, in which R is the normalized response of KA-induced LDH efflux in the presence of each drug. Values are expressed with their 95% confidence intervals. In competition studies, LDH activities were scaled to the total LDH activity induced by 0.05% Triton X-100 (= 100) in sister cultures.

Measurements of Intracellular Calcium Concentration. The intracellular calcium concentration ($[Ca^{2+}]_i$) in hippocampal neurons was determined using fura 2-AM. The cells were loaded for 60 to 90 min with fura 2-AM (6 μM). After incubation, the cells were placed on an inverted-stage microscope and continuously perfused with artificial cerebrospinal fluid (ACSF; 135 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.9 mM $MgCl_2$, 10 mM glucose, and 10 mM HEPES buffer, pH 7.4) at 32°C. In the experiment on NMDA-induced $[Ca^{2+}]_i$, ACSF without $MgCl_2$ and with 0.1 μM TTX was used as the perfusion medium. $[Ca^{2+}]_i$ measurements were made using an ARGUS-50/CA (Hamamatsu Photonics, Hamamatsu, Japan) as described previously (Ogura et al., 1987). The ratio of fluorescence obtained at 340 nm to that obtained at 380 nm was converted to an absolute value of $[Ca^{2+}]_i$ according to a Molecular Probes assay kit (Molecular Probes, Eugene, OR). Drugs were dissolved in the perfusing solution. YM928 and other inhibitors were perfused from 1 min before the stimulators. $[Ca^{2+}]_i$ was induced by 10-s application of 20 μM AMPA, 10 s of 100 μM NMDA, or 30 s of 5 μM veratridine, depending on experiments. IC_{50} values were determined as before. Values are expressed with their 95% confidence intervals. In the examination of the inhibitors, only data obtained from neurons whose peak $[Ca^{2+}]_i$ following agonist stimulation before and after the administration of the inhibitors remained stable (within 80–120%) were used.

Patch-clamp Analysis. Whole-cell recording at a holding potential of −60 mV was performed with an on-line patch clamp system

(Axopatch 1D patch-clamp amplifier, Digidata 1200 digitizer, pCLAMP6 acquisition and analysis computer program; Axon Instruments Inc., Union City, CA) and a thermal pen recorder (recti-horiz 8K20; NEC, Tokyo, Japan). The pipette solution contained 140 mM CsF and 5 mM CsCl in 10 mM HEPES, adjusted to pH 7.2 with CsOH. The perfusion solution contained 140 mM NaCl, 5 mM KCl, 2.4 mM $CaCl_2$, and 10 mM glucose in 10 mM HEPES, adjusted to pH 7.4 with NaOH. Cells were perfused at 5 to 8 ml/min of perfusion solution at room temperature. Inward currents were induced by application of 20 μM AMPA for 10 s. YM928 was perfused from 1 min before AMPA application at the indicated concentrations. IC_{50} values were determined as previously. Values are expressed with their 95% confidence intervals.

Radioligand Binding Competition Assays. The studies were performed at NovaScreen (Hanover, MD), using published protocols. Values are expressed as percentage of inhibition of specific binding and represent the average of two tubes at each concentration tested.

Sound-Induced Seizure in DBA/2 Mice. Male DBA/2 mice, weighing 9.5 to 12.5 g (Charles River Japan, Inc., Yokohama, Japan) were exposed to auditory stimulation (12 kHz, 120 dB for 60 s or until tonic extension occurred) in a soundproof box at 45 min after oral administration of vehicle or YM928. The drug was suspended in 0.5% aqueous methylcellulose as vehicle. The dosing volume was 0.3 ml/10 g, which was calculated on the basis of the body weight on the day of the experiment. Anticonvulsant effects were evaluated according to the following scores: 0, no response; 1, wild running; 2, clonic seizure; 3, tonic seizure; 4, death (De Sarro et al., 1988). Maximum response was measured for each mouse.

Results

KA-Induced Toxicity. The effect of YM928 was examined on KA-induced toxicity in primary rat hippocampal cultures, which is mediated by the AMPA receptor (Ohno et al., 1997). Neurons were exposed to KA with or without a test drug, and 24 h later, cell death was assessed by the amount of LDH activity in the culture media. YM928 inhibited KA-induced toxicity completely and concentration dependently (Fig. 2A). YM928 reduced the maximum response of KA-dose response curves (Fig. 2B). Higher KA concentrations than those shown here were not used because of its poor solubility and nonspecific effects. The IC_{50} value for YM928 was 2.0 (1.5–2.6) μM. Other AMPA antagonists were also evaluated (Table 1). As has already been shown, NBQX showed quite strong activity. However, two noncompetitive antagonists, GYKI52466 and LY300164/talampanel, had weaker activity than that of YM928.

Intracellular Calcium Influx. The effect of YM928 on AMPA-induced $[Ca^{2+}]_i$ in primary rat hippocampal cultures was investigated. YM928 inhibited AMPA-induced $[Ca^{2+}]_i$ completely and concentration dependently (Fig. 3). The IC_{50} value of YM928 was 3.0 (2.3–3.7) μM.

To examine the effect of YM928 on NMDA receptors, voltage-dependent Na^+ channels, and voltage-dependent Ca^{2+} channels in hippocampal neurons, the compound was tested against NMDA- and veratridine-induced $[Ca^{2+}]_i$ (Fig. 4). Against NMDA-induced $[Ca^{2+}]_i$, YM928 showed no effect at 30 μM. At 100 μM, YM928 slightly inhibited NMDA-induced $[Ca^{2+}]_i$ (Fig. 4A). On the other hand, the competitive NMDA receptor antagonist CGS19755 markedly inhibited NMDA-induced $[Ca^{2+}]_i$ at 10 μM. Against veratridine-induced $[Ca^{2+}]_i$, YM928 had a slight effect at 30 and 100 μM (Fig. 4B). NBQX, a relatively selective inhibitor of the AMPA receptor, inhibited it by approximately 15% at 10 μM as well.

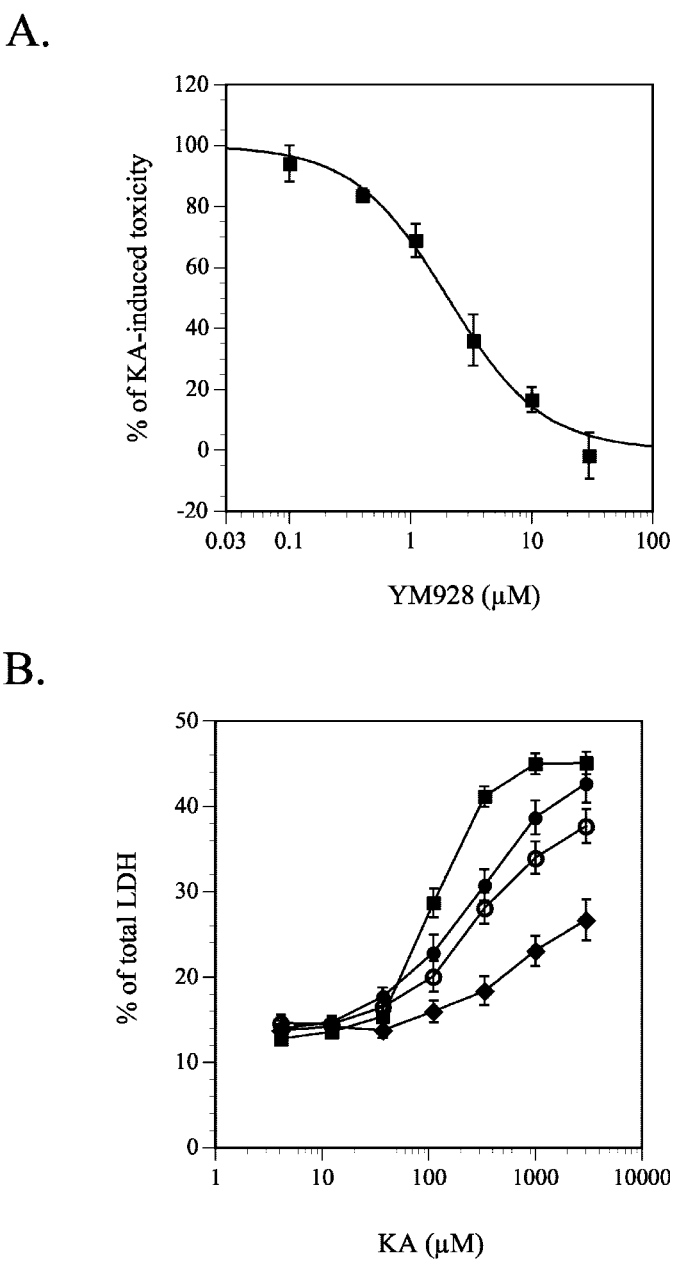

Fig. 2. Effects of YM928 on kainate (KA)-induced toxicity in primary rat hippocampal cultures. A, cultures were exposed to 300 μM KA for 24 h in the presence of YM928 at the indicated concentrations. Values represent the percentage of KA-induced toxicity (mean ± S.E.) scaled to the mean LDH release induced by 300 μM KA after subtraction of basal LDH values (= 100). The IC_{50} value is shown in Table 1. B, effect of YM928 on KA dose-response curves. Cultures were exposed to the indicated concentrations of KA either alone (■) or in the presence of 1 μM (●), 3 μM (○), or 10 μM (♦) YM928 for 24 h. Values represent percentage of total LDH (mean ± S.E.) scaled to LDH release induced by 0.05% Triton X-100 (= 100). For all data, n = 9.

TTX, at 1 μM, completely inhibited veratridine-induced $[Ca^{2+}]_i$.

AMPA-Induced Inward Currents. To confirm the effect of YM928 on the AMPA receptor electrophysiologically, AMPA-induced inward currents were examined by whole-cell patch-clamp analysis in rat hippocampal cultures (Fig. 5). YM928 inhibited 20 μM AMPA-induced inward currents completely and concentration dependently. The IC_{50} value was 1.03 (0.91–1.12) μM.

TABLE 1
Potency of AMPA antagonists in inhibiting kainate-induced toxicity

AMPA Antagonist	IC_{50}	95% Confidence Intervals	
		Lower	Upper
	μM		
YM928	2.0	1.5	2.6
NBQX	0.46	0.39	0.52
GYKI52466	17.0	13.7	20.3
LY300164	6.0	4.9	7.1

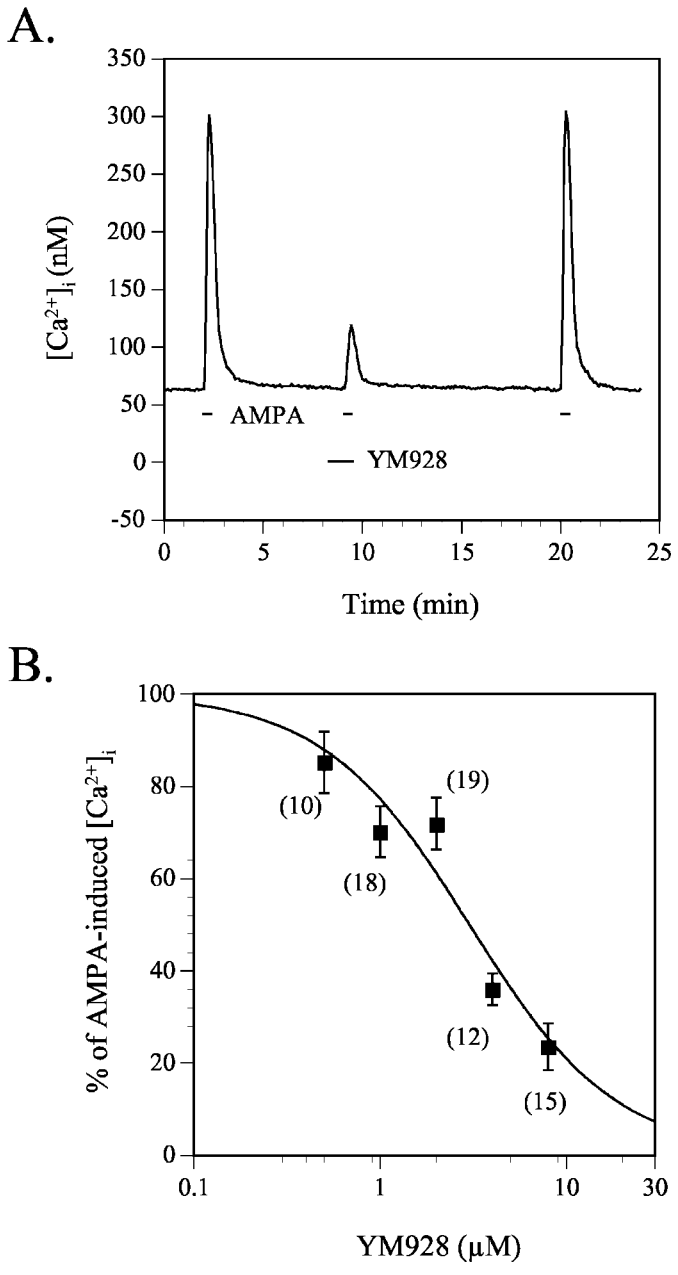

Fig. 3. Effect of YM928 on AMPA-induced intracellular calcium concentration ($[Ca^{2+}]_i$) in rat hippocampal cultures. A, fluorometric measurements of $[Ca^{2+}]_i$ induced by AMPA and inhibition by 8 μM YM928. Data are the mean from 15 neurons. B, concentration-dependent inhibition of AMPA-induced $[Ca^{2+}]_i$ by YM928. Values in parentheses are the number of cells tested.

Radioligand Binding Competition Assays. The interaction of YM928 with known glutamate-related ligand binding sites was investigated. YM928 showed pIC_{50} values <4 at rat

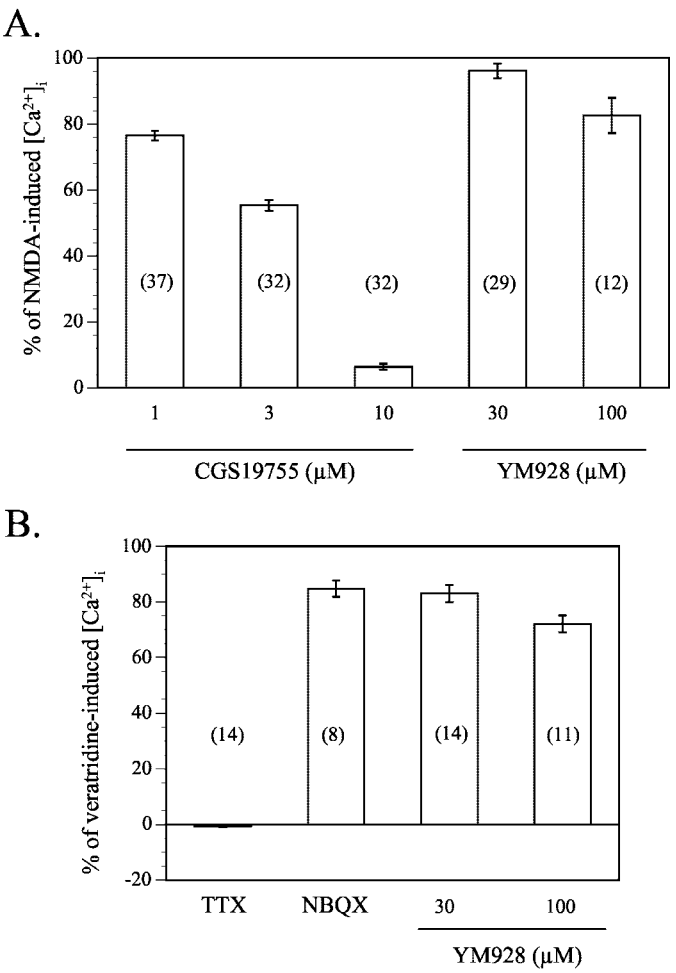

Fig. 4. Effect of YM928 on NMDA- and veratridine-induced $[Ca^{2+}]_i$ in rat hippocampal cultures. A, effects of YM928 and CGS19755 on NMDA-induced $[Ca^{2+}]_i$. NMDA (100 μM)-induced $[Ca^{2+}]_i$ is scaled to the basal level of $[Ca^{2+}]_i$. Data represent mean ± S.E.M. and values in parentheses are the number of cells tested. B, effects of YM928, TTX (1 μM) and NBQX (10 μM) on veratridine-induced $[Ca^{2+}]_i$. Veratridine-induced $[Ca^{2+}]_i$ is scaled to the basal level of $[Ca^{2+}]_i$. Data represent mean ± S.E. and values in parentheses are the number of cells tested.

brain sites labeled by [^{3}H]AMPA, [^{3}H]KA, [^{3}H]CGP39653, [^{3}H]glycine, [^{3}H]MK-801, or [^{3}H]glutamate, indicating no significant affinity for ionotropic glutamate channels, or the glycine or MK-801 site of the NMDA receptor complex, chloride channels, or glutamate uptake sites (Table 2). In an additional competition assay screen using 36 ligands for the main types of autonomic and ion-channel receptors, YM928 exerted less than 50% inhibition at 10 μM (data not shown), indicating no relevant affinity for any of the investigated receptor types.

Sound-Induced Seizure. To determine the in vivo activity of YM928, it was tested on sound-induced seizures in DBA/2 mice, which are often used for the evaluation of glutamate receptor antagonists (Shimizu-Sasamata et al., 1996) (Fig. 6). YM928 induced a dose-dependent reduction of sound-induced seizures 45 min after oral administration. The effect was statistically significant at doses of 3 mg/kg and 10 mg/kg.

Discussion

The present studies show that YM928 is an orally active and noncompetitive AMPA receptor antagonist. Thus, the

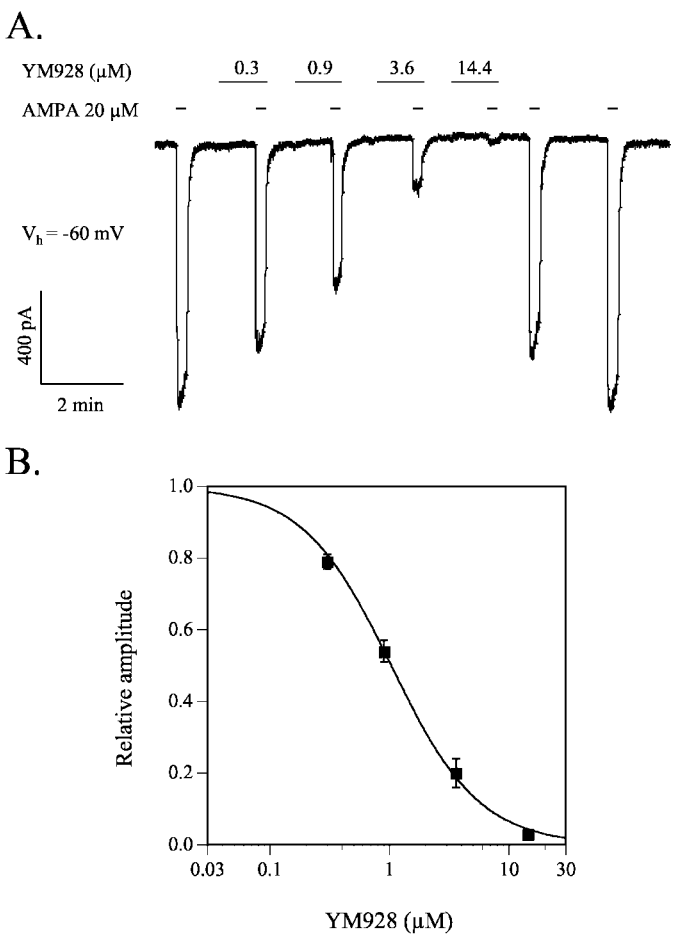

Fig. 5. Concentration-dependent inhibitory effect of YM928 on AMPA-induced inward currents. A, a representative trace of the inhibition of an AMPA-induced inward current by YM928. B, relative amplitudes of steady-state currents are plotted as a function of drug concentration. The symbols represent mean ± S.E. (n = 6).

compound blocked KA-induced toxicity, AMPA-induced $[Ca^{2+}]_i$, and AMPA-induced inward currents in rat primary hippocampal cultures in a concentration-dependent manner. In the KA-induced toxicity assay, potency was observed similar to that of existing AMPA receptor antagonists. YM928 inhibited the maximum response of KA and it did not displace [^{3}H]AMPA binding to rat forebrain membranes at concentrations up to 100 μM, suggesting that the manner of its inhibition is noncompetitive. In terms of in vivo activity, YM928 significantly reduced sound-induced seizures in DBA/2 mice after oral administration at 3 mg/kg.

YM928 was able to inhibit AMPA-induced current completely in whole-cell patch-clamp experiments, suggesting that YM928 directly acts on the AMPA receptor. YM928 also blocked the maximum response of the KA dose-response curve in KA-induced toxicity experiments. However, [^{3}H]AMPA binding experiments indicated that YM928 does not act on the glutamate binding site on the AMPA receptor. Therefore, YM928 seems to be a noncompetitive AMPA receptor antagonist and to act at a distinct site on the AMPA receptor. Recently, another class of noncompetitive AMPA receptor antagonists typified by CP-465,022 and CP-526,427 was identified (Menniti et al., 2000; Lazzaro et al., 2002). Interestingly, Menniti and colleagues did identify [^{3}H]CP-526,427 binding in rat forebrain membranes; however, the [^{3}H]CP-526,427 binding site did not interact directly with the glutamate binding site. The binding affinity of a series of compounds for the [^{3}H]CP-526,427 binding site was well related to potency for inhibition of a functional AMPA receptor-mediated response. Among noncompetitive AMPA receptor antagonists, 2,3-benzodiazepines can displace [^{3}H]CP-526,427 binding, but Evans blue cannot. Therefore, on the AMPA receptor, there seem to be at least two allosteric modulatory sites that noncompetitive AMPA receptor antagonists can interact with. YM928 might bind to these sites. Further investigation is needed on this matter.

In terms of the selectivity of YM928 for other receptors, YM928 did not have any affinity for glutamate-related ligand binding sites. Although YM928 had a slight inhibitory effect on veratridine- and NMDA-induced $[Ca^{2+}]_i$, it was much less effective than known antagonists at these sites. Since veratridine activates sodium influx, causing depolarization and increasing $[Ca^{2+}]_i$ in cells, these experiments suggest that YM928 does not interact with voltage-dependent sodium channels or calcium channels on rat hippocampal neurons, and that the inhibitory effect of YM928 on $[Ca^{2+}]_i$ is specific for the AMPA-induced response. Moreover, in dozens of typical neurotransmitter-ligand binding assays, YM928 at 10 μM showed no inhibitory activity. Taken together, these results suggest that YM928 is specific for the AMPA receptor.

YM928 significantly inhibited sound-induced seizures in DBA/2 mice 45 min after oral administration at 3 mg/kg, suggesting that its brain penetrability might be excellent. Quinoxalinediones typified by NBQX have poor brain penetrability, and this restricts their potential use in the treatment of chronic diseases. The oral activity of YM928 may extend its application to several kinds of disease conditions. Moreover, its noncompetitive action may be preferable to competitiveness for protection against neurological disorders with high synaptic glutamate levels, such as stroke and epilepsy, because high synaptic concentrations of glutamate

TABLE 2
Effects of YM928 in glutamate-related radioligand binding competition assays

Binding Site	Radioligand	Membrane Source	Percent Inhibition (Average; n = 2)		
			1.0E-6 M	1.0E-5 M	1.0E-4 M
AMPA site	[^{3}H]AMPA	Rat forebrain	0.08	−5.36	11.70
Kainate site	[^{3}H]Kainate	Rat forebrain	9.87	−6.30	11.18
NMDA agonist site	[^{3}H]CGP 39653	Rat forebrain	−14.94	2.34	18.90
NMDA glycine site	[^{3}H]Glycine	Rat cortex	3.34	3.97	5.39
NMDA MK801 site	[^{3}H]MK801	Rat forebrain	−10.29	0.03	−3.64
Chloride-dependent site	[^{3}H]Glutamate	Rat cerebellum	−1.34	5.22	16.54
Glutamate uptake site	[^{3}H]Glutamate	Rat cortex	3.98	6.09	9.87

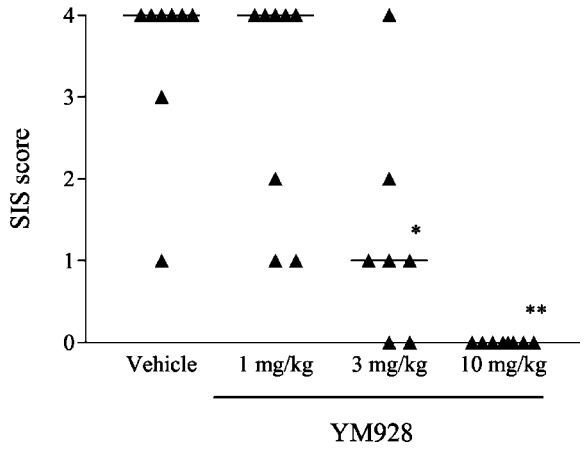

Fig. 6. Anticonvulsant effect of YM928 against sound-induced seizure (SIS) in DBA/2 mice. Anticonvulsant effects were evaluated according to the following score: 0, no response; 1, wild running; 2, clonic seizure; 3, tonic seizure; 4, death. Maximum response was measured for each mouse. YM928 was orally administered 45 min before SIS. Horizontal bar represents median score. *, $p < 0.05$, **, $p < 0.01$ significant difference relative to control (Steel test).

could surmount the blocking action of a competitive antagonist.

We screened a large chemical library for inhibitors of KA-induced toxicity in rat cortical primary neurons, an assay that is well characterized (Ohno et al., 1997, 1998). The toxicity is blocked completely by AMPA receptor antagonists, attenuated by AMPA, and enhanced by cyclothiazide, but not concanavalin A, which enhances KA responses at the KA receptor. KA is a low-affinity agonist for the AMPA receptor. In electrophysiological experiments, KA induces a nondesensitizing and long-lasting response at the AMPA receptor, whereas AMPA produces a rapid response; that is, the response desensitizes very quickly within the order of milliseconds. AMPA can desensitize the KA-induced long-lasting response due to its higher affinity for the receptor. The KA-induced long-lasting response at the AMPA receptor is thought to be involved in the mechanism of KA-induced toxicity in neurons. The pharmacology of KA-induced toxicity seems to reflect the electrophysiological properties of a nondesensitizing response at the AMPA receptor. The KA-induced toxicity assay thus allows us to identify AMPA receptor antagonists. It might also detect novel neuroprotectants that act by inhibiting the signaling cascade that causes the toxicity.

Using this screening process, we have identified the inhibitory effect of piriqualone/B169 on the AMPA receptor independently of Menniti's group (Menniti et al., 2000). Several open label clinical studies on piriqualone/B169 for the treatment of epilepsy were conducted in the early 1970s in Japan (Mukawa and Jinnai, 1970; Seki and Fukuyama, 1971). The reports of these clinical studies indicated that piriqualone/B169 was effective for refractory epilepsy and was well tolerated. Recently, LY300164/talampanel also showed efficacy in reducing seizures in a clinical setting (Chappell et al., 2002). Therefore, AMPA receptor antagonists seem to be promising antiepileptics.

In conclusion, YM928 is an orally active noncompetitive AMPA receptor antagonist. The compound is chemically distinct compared with existing AMPA receptor antagonists, and belongs to a novel class of AMPA receptor antagonists. YM928 has potential as an oral therapeutic drug for various types of neurological disorders.

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Address correspondence to: Dr. Kazushige Ohno, Neuroscience Research, Pharmacology Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba 305-8585, Japan. E-mail: ohno@yamanouchi.co.jp