J. Pharm. Pharmacol. 1998, 50: 795–801
Received December 15, 1997
Accepted March 3, 1998

In-vitro Characterization of YM872, a Selective, Potent and Highly Water-soluble α-Amino-3-hydroxy-5-methylisoxazole-4-propionate Receptor Antagonist

ATSUYUKI KOHARA, MASAMICHI OKADA, RIE TSUTSUMI, KAZUSHIGE OHNO, MASAYASU TAKAHASHI, MASAO SHIMIZU-SASAMATA, JUN-ICHI SHISHIKURA, HIROSHI INAMI, SHUICHI SAKAMOTO AND TOKIO YAMAGUCHI

Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co. Ltd, 21 Miyukigaoka, Tsukuba City, Ibaraki 305, Japan

Abstract

The in-vitro pharmacological properties of (2,3-dioxo-7-(1*H*-imidazol-1-yl)-6-nitro-1,2,3,4-tetrahydro-1-quinoxalinyl)-acetic acid monohydrate, YM872, a novel and highly water-soluble α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA)-receptor antagonist were investigated.

YM872 is highly water soluble (83 mg mL^{-1} in Britton-Robinson buffer) compared with 2,3-dihydroxy-6-nitro-7-sulphamoyl-benzo(F)quinoxaline (NBQX), 6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione hydrochloride (YM90K) or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). YM872 potently inhibits [^{3}H]AMPA binding with a K_i (apparent equilibrium dissociation constant) value of 0·096 ± 0·0024 μM. However, YM872 had very low affinity for other ionotropic glutamate receptors, as measured by competition with [^{3}H]kainate (high-affinity kainate binding site, concentration resulting in half the maximum inhibition (IC50) = 4·6 ± 0·14 μM), [^{3}H]glutamate (*N*-methyl-D-aspartate (NMDA) receptor glutamate binding site, IC50 > 100 μM) and [^{3}H]glycine (NMDA receptor glycine-binding site, IC50 > 100 μM). YM872 competitively antagonized kainate-induced currents in *Xenopus laevis* oocytes which express rat AMPA receptors, with a pA_2 value of 6·97 ± 0·01. In rat hippocampal primary cultures, YM872 blocked a 20-μM AMPA-induced increase of intracellular Ca^{2+} concentration with an IC50 value of 0·82 ± 0·031 μM, and blocked 300-μM kainate-induced neurotoxicity with an IC50 value of 1·02 μM.

These results show that YM872 is a potent and highly water-soluble AMPA antagonist with great potential for treatment of neurodegenerative disorders such as stroke.

Glutamate antagonists show potential in treating pathophysiological disorders including stroke (McDonald & Johnston 1990; Meldrum & Garthwaite 1990). In cerebral ischaemia the activation of ionotropic glutamate receptors after marked elevation of extracellular glutamate concentration is a key factor in the sequence of cellular events that result in ischaemic damage to neurons.

Ionotropic glutamate receptors, mediating the preponderance of excitatory synapses in the vertebrate CNS, were originally classified through both pharmacological and biophysical characters into *N-methyl-D*-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) and kainate receptors. NMDA-receptor antagonists have been shown to protect neurons in experimental models of ischaemia (Park et al 1988a, b; Bullock et al 1990). However, NMDA antagonists have limited utility as therapeutic agents, because these compounds also induce psychotomimesis (Koek et al 1988), impairment of learning and memory (Morris et al 1986) and neuronal vacuolization (Olney et al 1989; Hargreaves et al 1993).

An important milestone in the development of AMPA-receptor antagonists was the discovery of quinoxalinediones such as 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 6,7-dinitroquinoxaline-2,3-dione (DNQX) (Watkins et al 1990). On the basis of these structures, more potent and selective AMPA-receptor antagonists, 2,3-dihydroxy-6-nitro-7-sulphamoyl-benzo(F)quinoxaline (NBQX) and 6-(1*H*-imidazol-1-yl)-7-nitro-2,3-(1*H*,4*H*)-quinoxalinedione hydrochloride (YM90K), were discovered. These compounds greatly contributed to assessing the role of AMPA receptors in

Correspondence: M. Okada, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co. Ltd, 21 Miyukigaoka, Tsukuba City, Ibaraki 305, Japan.

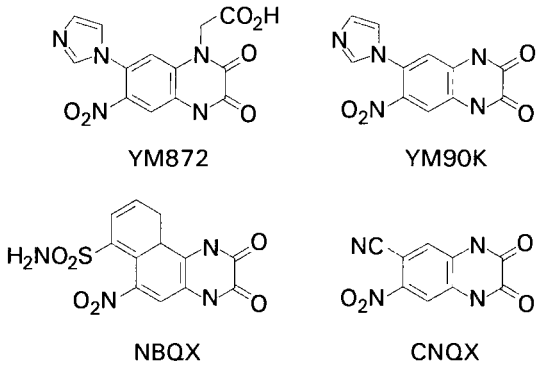

Figure 1. The chemical structures of YM872, YM90K, NBQX and CNQX.

many different animal models of glutamate-mediated neuropathology (Sheardown et al 1990; Bullock et al 1994; Shimizu-Sasamata et al 1996; Yatsugi et al 1996). Although these compounds do not share the unfavourable properties of NMDA-receptor antagonists (Burchuladze & Rose 1992; Parada et al 1992), the use of these compounds in animal models and clinical trials is limited because of their low water-solubility (Xue et al 1994).

Recently, a competitive AMPA-receptor antagonist, (2,3-dioxo-7-(1*H*-imidazol-1-yl)-6-nitro-1,2,3,4-tetrahydro-1-quinoxalinyl)-acetic acid monohydrate (YM872), which is highly water-soluble and has high affinity and selectivity for AMPA receptors (Figure 1) was discovered. The pharmacological properties of YM872 have been characterized in-vitro.

Materials and Methods

Drugs

YM872, YM90K and NBQX were synthesized by the Institute for Drug Discovery Research, Yamanouchi Pharmaceutical. CNQX and AMPA (*S*-AMPA) were purchased from Tocris Neuramin (Essex, UK). Kainate was purchased from Sigma (St Louis, MO) and MK-801 from Research Biochemicals (Natick, MA). [^{3}H]AMPA (45 Ci mmol^{-1}), [^{3}H]kainate (58 Ci mmol^{-1}) and [^{3}H]glycine (45-51 Ci mmol^{-1}) were purchased from New England Nuclear (Boston, MA) and [^{3}H]glutamate (49 Ci mmol^{-1}) from Amersham (Bucks, UK). All other reagents were obtained from commercial sources.

Determination of solubility

Each compound was weighed into a micro-tube and Britton-Robinson buffer (pH 5–12) was added. The mixtures were shaken vigorously for 30 min at 25°C, centrifuged, and filtered to remove insoluble material. After separation the pH of each solution was determined with a pH meter. The concentration of each compound in the solution was determined by photometry by use of a standard curve.

Radioligand binding

The potency of YM872, YM90K, NBQX and CNQX in inhibiting specific binding of [^{3}H]AMPA (the AMPA receptor), [^{3}H]kainate (the high-affinity kainate receptor), [^{3}H]glutamate (glutamate binding site of the NMDA receptor) and strychnine-insensitive [^{3}H]glycine (glycine-binding site of the NMDA receptor) to brain membranes in-vitro was determined as previously published.

The binding of [^{3}H]AMPA was conducted in the presence of 100 mM potassium thiocyanate as described by Honoré et al (1982). Before assay crude membranes of rat whole-brain were washed twice with 5 mM Tris-HCl buffer (pH 7·2) containing 0·25 mM $CaCl_2$ and 10 mM potassium thiocyanate. [^{3}H]Kainate binding was determined according to the method described by Braitman and Coyle (1987) using crude membranes from rat cortex; before assay the membranes were washed twice with 5 mM Tris-HCl buffer containing 0·5 mM ethylene glycol bis(β-aminoethyl ether) *N,N'*-tetra-acetic acid (pH 7·4). [^{3}H]Glutamate binding was determined according to the method described by Monahan & Michel (1987) using crude post-synaptic densities (Foster & Fagg 1987) of whole brain (except brain stem and cerebellum) washed twice with 5 mM Tris-HCl buffer (pH 7·4). [^{3}H]Glycine binding was determined according to the method described by Monahan et al (1989) using crude postsynaptic densities (Foster & Fagg 1987) of whole brain (except brain stem and cerebellum) washed twice with 5 mM Tris-acetate buffer (pH 7·4).

Each radioligand was used at a concentration near the K_d (equilibrium dissociation constant of the ligand) value for each binding assay (Table 1). Non-specific binding was defined as the residual in the presence of 30 μM quisqualate ([^{3}H]AMPA), 600 μM L-glutamate ([^{3}H]kainate), 100 μM NMDA ([^{3}H]glutamate) and 1 mM glycine ([^{3}H]glycine). Non-specific binding usually amounted to 5–30% of the total binding.

IC50 values (concentrations resulting in half the maximum inhibition) were calculated from the logistic equation:

$$B = B_N + (B_T - B_N) \times IC50^n / ((\text{inhibitor})^n + IC50^n)$$

Table 1. Binding affinities of YM872, YM90K, NBQX and CNQX for various glutamate receptor subtypes.

Compound	Ki (μM)	IC50 (μM)		
	AMPA site	High-affinity kainate site	NMDA receptor-complex	
	[^{3}H]AMPA (31 nM)	[^{3}H]Kainate (5·4 nM)	NMDA site [^{3}H]Glutamate (9·8 nM)	Glycine site [^{3}H]Glycine (35 nM)
YM872	0·096 ± 0·0024	4·6 ± 0·14	> 100	> 100
YM90K	0·071 ± 0·0020	4·4 ± 0·15	> 100	55 ± 7·4
NBQX	0·046 ± 0·00079	6·8 ± 0·20	> 100	> 100
CNQX	0·250 ± 0·012	2·7 ± 0·074	45 ± 4·0	9·3 ± 1·2

Values are means ± s.e.m., determined for triplicate samples in two or three separate experiments.

where B is the amount of bound radiolabelled ligand in the presence of inhibitor, B_T is the total binding and B_N is the non-specific binding. The parameters n (the Hill coefficient) and IC50 (concentration of inhibitor producing 50% of B) were calculated with SAS ver. 6.11 resident in an IBM RS-6000 computer.

Values of apparent equilibrium dissociation constant of drugs (K_i) were calculated by the method of Cheng & Prussoff (1973).

RNA preparation, oocyte injection and electrophysiological recording

These were performed as described by Okada et al (1996). Briefly, poly(A)$^+$ RNA was prepared from the cerebral cortex of male Wistar rats, age 6 weeks, by the combined use of a total RNA Separator Kit and an mRNA Separator Kit (Clontech Laboratories). mRNA was dissolved in diethyl pyrocarbonate-treated water at a final concentration of 1 mg mL^{-1} and stored as single-use samples at −80°C until injection into the oocytes. Oocytes, stage V–VI, were obtained surgically from adult oocyte-positive female *Xenopus* frogs anaesthetized by immersion in 0·3% Tricaine for 20 min and then placed on an ice bed. One or more lobes of the ovary were removed into the culture solution (modified Barth's saline, MBS) containing (mM): 88·0 NaCl, 1·0 KCl, 15·0 Hepes, 0·5 $Ca(NO_3)_2$, 0·41 $CaCl_2$, 0·82 $MgSO_4$ and supplemented with 10 mg L^{-1} each of penicillin and streptomycin and 40 mg L^{-1} gentamycin. After incubation for 6–8 h at 19°C, poly(A)$^+$ RNA from rat cortex (50 ng) was pressure-injected directly into the oocytes by means of a Drummond Nanojet. The oocytes were then incubated at 19°C. Four days after incubation the oocytes were treated with collagenase I (Sigma, 2 mg mL^{-1} in MBS) for 10 min at room temperature, and the follicle-cell layer was then removed manually with fine forceps. The oocytes were then incubated for one day at 19°C. For recording, oocytes were transferred to a recording chamber perfused at 5 mL min^{-1} with modified Barth's solution (96·0 mM NaCl, 5·0 mM KCl, 1·0 mM $MgCl_2$, 1·8 mM $CaCl_2$, 5·0 mM HEPES, pH 7·6). Drugs were dissolved in the perfusing solution. Oocytes were voltage-clamped to −60 mV via two 3 M KCl-filled electrodes connected to an amplifier. Currents and voltage signals were recorded on a chart recorder. Values are expressed as the means ± standard errors of the number of oocytes tested.

Rat hippocampal cell cultures

Rat hippocampal cell cultures were established as described by Ohno et al (1997). Briefly, the hippocampus was isolated from embryonic day-18–20 Wistar rats and dissociated by incubation with papain and DNase I, followed by pipetting. These cells were suspended in modified Eagle's medium (MEM) supplemented with 10% foetal bovine serum and were plated on either poly-L-lysine-coated 48-well test plates at a density of 4×10^5 cells cm^{-2} for neurotoxicity tests, or on poly-L-lysine-coated cover slips at a density of 1×10^5 cells cm^{-2} for measurement of $[Ca^{2+}]_i$ (intracellular calcium). After 24 h in humidified 5% CO_2–95% air at 37°C, cultures plated in 48-well test plates were switched from serum-containing to serum-free, chemically defined 20 mM HEPES-buffered MEM (CDM: 6 mg mL^{-1} glucose, 1 mM sodium pyruvate, 1 mg mL^{-1} bovine serum albumin, 2 mM L-glutamine, 25 μg mL^{-1} transferrin, 25 μg mL^{-1} insulin, 25 ng mL^{-1} selenium, 100 μM putrescine, 1 nM triiodothyronine, 20 nM progesterone, 20 ng mL^{-1} corticosterone, 1 μg mL^{-1}

tocopherol acetate, 1 $\mu g\,mL^{-1}$ arachidonic acid and 500 $ng\,mL^{-1}$ docosahexaenoic acid), and maintained for 6 to 10 days by changing the medium every three days. Cultures plated on cover slips were switched from serum-containing medium to Sumilon Medium/Neuron (containing glial-conditioned medium, Sumitomo Bakelite No. MB-X9501) 24 h after preparation and maintained without exchange of culture medium for 7 to 11 days.

Measurements of increases of intracellular calcium concentration ($[Ca^{2+}]_i$)

The $[Ca^{2+}]_i$ of the neurons in the hippocampal cultures was determined by use of fura-2-acetoxymethyl ester as previously published (Kawabata et al 1996). Drugs were dissolved in the perfusing solution.

Kainate-induced neurotoxicity in-vitro

Kainate-induced neurotoxicity in-vitro was performed as described by Ohno et al (1997). In brief, kainate and other drugs were dissolved in CDM for delivery to neurons. 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 overnight exposure of sister cultures to 300 μM kainate and 10 μM MK-801. LDH was measured using an LDH assay kit in a 736-10 automatic analyser (Hitachi) by a standard rate assay. In potency estimation studies values of LDH activity were normalized to kainate-induced LDH activities in sister-culture experiments and the IC50 was calculated by use of a four-parameter logistic equation ($n = 12$ from four independent cultures) with SAS ver. 6.11 resident in an IBM RS-6000 computer.

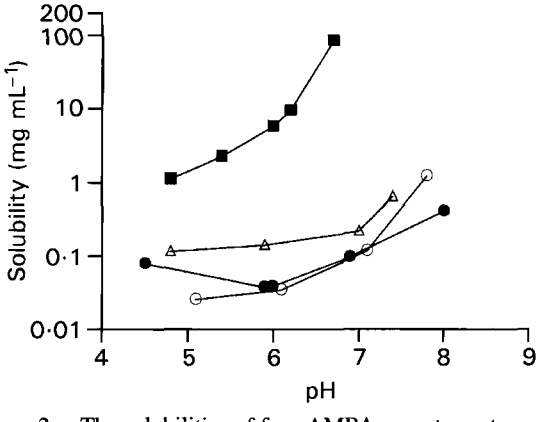

Figure 2. The solubilities of four AMPA-receptor antagonists in Britton-Robinson buffer: ■ YM872; ● YM90K; ○ NBQX; △ CNQX.

Results and Discussion

YM872 was the most soluble of the four compounds tested (Figure 2). At neutral pH (pH 6·7), the solubility of YM872 was $83\,mg\,mL^{-1}$ in Britton-Robinson buffer. This is approximately 500–1000 times the solubility of YM90K ($0{\cdot}096\,mg\,mL^{-1}$ at pH 6·9), NBQX ($0{\cdot}12\,mg\,mL^{-1}$ at pH 7·1) or CNQX ($0{\cdot}22\,mg\,mL^{-1}$ at pH 7·0). By introducing an acetate group at position 4 of YM90K (Figure 1), the solubility of YM872 in Britton-Robinson buffer was dramatically improved.

Among the glutamate-receptor subtypes tested, YM872 had the highest affinity for the AMPA receptor ($0{\cdot}096 \pm 0{\cdot}0024$ μM, Table 1). The affinity of YM872 for AMPA receptors was similar to that of YM90K or NBQX. YM872 bound only weakly to high-affinity kainate receptors. It failed to affect the glutamate binding site of NMDA receptors and the strychnine-insensitive glycine-binding site of NMDA receptors at doses of 100 μM or more. The acetate group at position 4 of YM90K does not dramatically change the affinity for ionotropic glutamate receptors.

YM872 had weak affinity for the benzodiazepine binding site of the γ-aminobutyric acid ($GABA_A$) receptor-complex ($IC50 = 27{\cdot}9$ μM, using [^{3}H]flunitrazepam as ligand), and was devoid of activity ($IC50 > 100$ μM) at the inhibitory neurotransmitter binding sites [^{3}H]muscimol (GABA binding site of $GABA_A$ receptor complex), [^{3}H]2-chloro-N^6-cyclopenthyladenosine (adenosine A_1 receptor) and [^{3}H]CGS-21680 (adenosine A_2 receptor) (data not shown).

Schild analysis was performed to determine the potency of YM872 at AMPA receptors in oocytes to evaluate its antagonistic effect. In this study, kainate, not AMPA, was used as the agonist. The response of kainate is mediated through AMPA receptors (Okada et al 1996). A reduction of kainate-evoked currents was seen upon co-application of AMPA. These currents have also been shown to be potentiated by co-application with cyclothiazide, but not con-canavalin A (Okada et al 1996). Figure 3A shows that increasing doses of YM872 elicited a parallel right-ward shift of the kainate-concentration–response curves. Schild regression of YM872 was linear (Figure 3B), with an average slope of $1{\cdot}03 \pm 0{\cdot}02$ and a mean pA_2 value of $6{\cdot}97 \pm 0{\cdot}01$ (Table 2). This parallel shift in the log-concentration–response curve to kainate and near unity value ($1{\cdot}03 \pm 0{\cdot}02$) of the slope of the Schild plot are consistent with the idea that YM872 acts as a competitive antagonist against AMPA receptors.

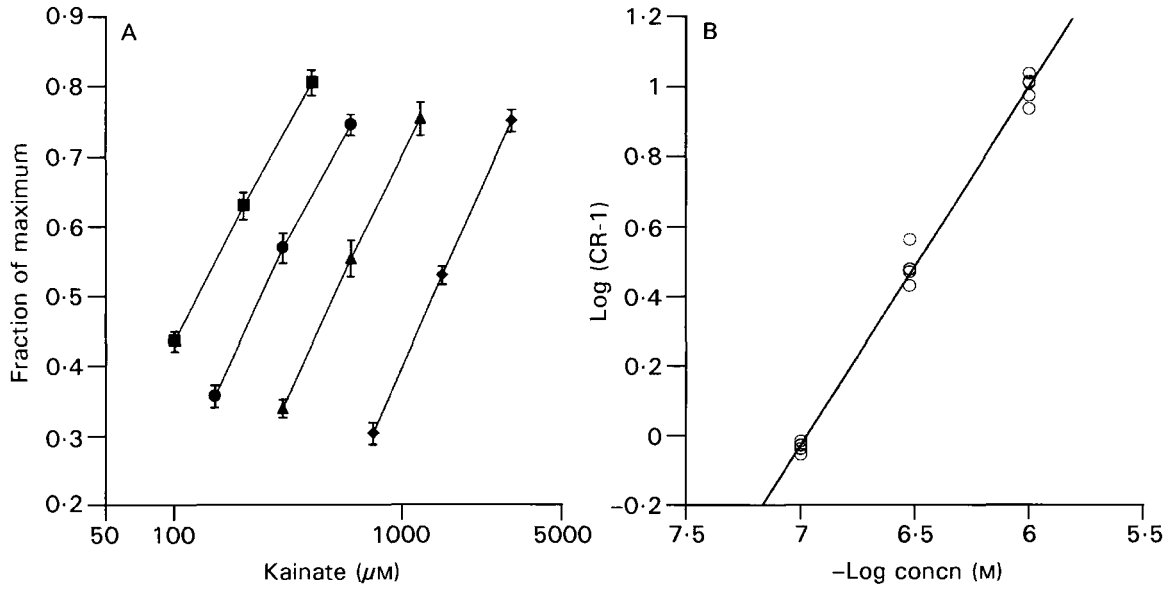

Figure 3. Schild analysis of the antagonism by YM872 of currents activated by kainate. Kainate currents were measured from *Xenopus* oocytes injected with rat cortex poly(A)$^+$ RNA. A. Partial kainate concentration–response curves in the presence of increasing concentrations of YM872: ■ 0 μM; ● 0·1 μM; ▲ 0·3 μM; ● 1·0 μM. Each point represents the mean ± s.e.m. of results from five determinations. The maximum response amplitude elicited by 3 mM kainate was tested before and after each curve was plotted. B. Schild regression of YM872 antagonism of currents activated by kainate.

Table 2. Potencies of YM872, YM90K and NBQX at AMPA receptors.

Antagonist	pA_2	Slope	Reference
YM872	6·97 ± 0·01	1·03 ± 0·02	–
YM90K	6·83 ± 0·01	0·94 ± 0·01	Okada et al (1996)
NBQX	7·24 ± 0·01	0·91 ± 0·01	Okada et al (1996)

This table summarizes the results of Shild analyses of YM872, YM90K and NBQX in *Xenopus* oocytes injected with rat cortex poly(A)$^+$ RNA. Values are means ± s.e.m. (n = 5 or 6) derived from mean EC50 values for individual cells.

The potency of YM872 was very similar to those of YM90K and NBQX. The pA_2 value was in close agreement with the results obtained from the [^{3}H]AMPA binding.

To evaluate the antagonism of YM872 on native AMPA receptors in rat hippocampal neurons, the effect of YM872 on the increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was examined. YM872 dose-dependently inhibited the increase in $[Ca^{2+}]_i$ induced by 20 μM AMPA (Figure 4). The IC50 value of YM872 was 0·82 ± 0·031 μM.

Kainate-induced neuronal death in rat primary hippocampal cultures was examined to evaluate the protective effects of YM872. In these experiments kainate was used as an agonist for AMPA receptors. Although kainate has high affinity for kainate receptors, the response of kainate is mediated through AMPA receptors as shown by the reduction of kainate-induced cell death by co-application of AMPA (Ohno et al 1997). Kainate toxicity has also been shown to be potentiated by co-application with cyclothiazide, but not concanavalin A. YM872 protects against kainate (300 μM)-induced cell death with an IC50 value of 1·02 μM (Table 3). The value was closely similar to that given by YM90K or NBQX, and in close agreement with the results obtained in the $[Ca^{2+}]_i$ increase assay.

The discovery of NBQX and YM90K, potent and selective AMPA-receptor antagonists, has greatly facilitated the study of the AMPA receptor and the application of AMPA antagonists to various animal models of neurodegenerative disorders including stroke. However, at the doses of NBQX required for neuroprotection, intravenous administration results in increased drug precipitation in the medullary tubules of the kidney because of its low water-solubility (Xue et al 1994). Because YM872 is a highly water-soluble agent with the same potency and selectivity as NBQX and YM90K, YM872 might not cause the renal crystallization problems encountered with the aforementioned compounds. YM872 has been tested in some in-vivo ischaemia models to evaluate its neuroprotective properties (Kawasaki-Yatsugi et al 1997; Takahashi et al 1998). In these experiments YM872 markedly reduced neuronal damage in focal cerebral ischaemia in cats and rats at a dose which is free from significant side effects, including renal crystallization. These observations strongly suggest

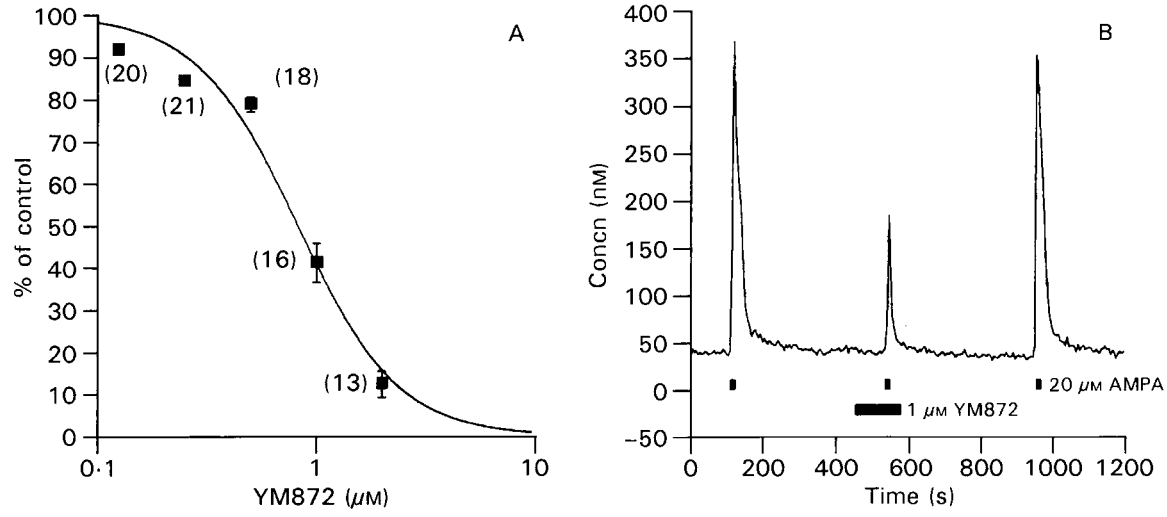

Figure 4. Effect of YM872 on the AMPA-induced $[Ca^{2+}]_i$ increase in rat hippocampal neurons. A. Concentration-dependent inhibition by YM872 of a 20 μM AMPA-induced increase in $[Ca^{2+}]_i$. Values in parentheses are the number of the cells tested. The results were normalized relative to a 20-μM AMPA-induced $[Ca^{2+}]_i$ increase which was taken as a 100% response. The IC50 was determined from the logistic equation $R = 100/(1 + ([YM872]/IC50^n)$ where R is the normalized response of the AMPA-induced $[Ca^{2+}]_i$ increase in the presence of YM872. The parameters n (the Hill coefficient) and IC50 (concentration of YM872 resulting in half the maximum inhibition) were determined by means of a non-linear least squares curve-fitting program. B. Fluorimetric measurement of increase in $[Ca^{2+}]_i$ induced by AMPA (20 μM) and inhibition of 20 μM AMPA-induced $[Ca^{2+}]_i$ increase by 1 μM YM872.

Table 3. Effects of YM872, YM90K and NBQX on kainate-induced neurotoxicity in-vitro.

Compound	Mean IC50 (μM)	95% Confidence intervals		Reference
		Lower	Upper	
YM872	1·02	0·48	1·55	—
YM90K	0·71	0·46	0·97	Ohno et al (1998)
NBQX	0·45	0·34	0·56	Ohno et al (1998)

that YM872 is a useful tool for investigation of the pathophysiologic role of AMPA receptors in the development of neural disorders. Of course, it also shows great promise as a clinical agent in treating disorders such as stroke.

In conclusion, this report shows that YM872 is a potent and highly water-soluble competitive AMPA-receptor antagonist which has great potential as a therapeutic agent in treating neurodegenerative disorders such as stroke.

References

Braitman, D. J., Coyle, J. T. (1987) Inhibition of [^{3}H]kainic acid receptor binding by divalent cations correlates with ion affinity for the calcium channel. Neuropharmacology 26: 1247–1251

Bullock, R., Graham, D. I., Chen, M. H., Lowe, D., McCulloch, J. (1990) Focal cerebral ischaemia in the cat: pretreatment with a competitive NMDA antagonist, D-CPPene. J. Cereb. Blood Flow Metab. 10: 668–674

Bullock, R., Graham, D. I., Swanson, S., McCulloch, J. (1994) Neuroprotective effect of the AMPA-receptor antagonist LY-293558 in focal cerebral ischemia in the cat. J. Cereb. Blood Flow Metab. 14: 466–471

Burchuladze, R., Rose, S. P. R. (1992) Memory formation in day-old chicks requires NMDA but not non-NMDA glutamate receptors. Eur. J. Neurosci. 4: 533–538

Cheng, Y. C., Prussoff, W. H. (1973) Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition of an enzymatic reaction. Biochem. Pharmacol. 22: 3099–3108

Foster, A. C., Fagg, G. E., (1987) Comparison of L-[^{3}H]glutamate, D-[^{3}H]aspartate, DL-[^{3}H]AP5 and [^{3}H]NMDA as ligands for NMDA receptors in crude postsynaptic densities from rat brain. Eur. J. Pharmacol. 133: 291–300

Hargreaves, R. J., Rigby, M., Smith, D., Hill, R. G., Iversen, L. L. (1993) Competitive as well as uncompetitive *N*-methyl-D-aspartate receptor antagonists affect cortical neuronal morphology and cerebral glucose metabolism. Neurochem. Res. 18: 1263–1269

Honoré, T., Lauridsen, J., Krogsgaad-Larsen, P. (1982) The binding of [^{3}H]-AMPA, a structural analogue of glutamic acid, to rat brain membranes. J. Neurochem. 38: 173–178

Kawabata, S., Tsutsumi, R., Kohara, A., Yamaguchi, T., Nakanishi, S., Okada, M. (1996) Control of calcium oscillations by phosphorylation of metabotropic glutamate receptors. Nature 383: 89–92

Kawasaki-Yatsugi, S., Yatsugi, S., Takahashi, M., Toya, T., Ichiki, C., Koshiya, K., Yamagichi, T., Shimizu-Sasa-mata, M. (1997) A novel AMPA-receptor antagonist YM872 reduces infarct size after MCA occlusion in rats. J. Cereb. Blood Flow Metab. 17: S139

Koek, W., Woods, J. H., Winger, G. D. (1988) MK-801, a proposed noncompetitive antagonist of excitatory amino acid neurotransmission, produce phencyclidine-like behavioral effects in pigeons, rats and rhesus monkeys. J. Pharmacol. Exp. Ther. 245: 969–974

McDonald, J. W., Johnston, M. V. (1990) Physiological and pathological roles of excitatory amino acids during central nervous system development, Brain Res. Rev. 15: 41–70

Meldrum, B., Garthwaite, J. (1990) Excitatory amino acid neurotoxicity and neurodegenerative disease. Trends Pharmacol. Sci. 11: 379–387

Monahan, J. B., Michel, J. (1987) Identification and characterization of an *N*-methyl-D-aspartate-specific L-[^{3}H]glutamate recognition site in synaptic plasma membranes. J. Neurochem. 48: 1699–1708

Monahan, J. B., Corpus, V. M., Hood, W. F., Thomas, J. W., Compton, R. P. (1989) Characterization of a [^{3}H]-glycine recognition site as a modulatory site of the *N*-methyl-D-aspartate receptor complex. J. Neurochem. 53: 370–375

Morris, R. G. M., Anderson, E., Lynch, G. S., Baudry, M. (1986) Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-D-aspartate receptor antagonist, AP5. Nature 319: 774–776

Ohno, K., Okada, M., Tsutsumi, R., Kohara, A., Yamaguchi, T. (1997) Kainate excitotoxicity is mediated by AMPA—but not kainate—preferring receptors in embryonic rat hippocampal cultures. Neurochem. Int. 31: 715–722

Ohno, K., Okada, M., Tsutsumi, R., Shuichi, S., Yamaguchi, T. (1998) The AMPA-receptor antagonist YM90K reduces AMPA receptor-mediated excitotoxicity in rat hippocampal cultures. Jpn J. Pharmacol. 76: 105–108

Okada, M., Kohara, A., Yamaguchi, T. (1996) Characterization of YM90K, a selective and potent antagonist of AMPA receptors, in rat cortical mRNA-injected *Xenopus* oocytes. Eur. J. Pharmacol. 309: 299–306

Olney, J. W., Labruyene, J., Price, M. T. (1989) Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. Science 244: 1360–1362

Parada, J., Czuczwer, S. J., Turski, W. A. (1992) NBQX does not affect learning and memory tasks in mice: a comparison with D-CPPene and ifenprodil. Cognitive Brain Res. 1: 67–71

Park, C. K., Nehls, D. G., Graham, D. I., Teasdale, G. M., McCulloch, J. (1988a) Focal cerebral ischaemia in the cat: treatment with the glutamate antagonist MK-801 after induction of ischaemia. J. Cereb. Blood Flow Metab. 8: 757–762

Park, C. K., Nehls, D. G., Graham, D. I., Teasdale, G. M., McCulloch, J. (1988b) The glutamate antagonist MK-801 reduces focal cerebral ischemic brain damage in the rat. Ann. Neurol. 24: 543–554

Sheardown, M. J., Nielsen, E. O., Hansen, A. J., Jacobsen, P., Honoré, T. (1990) 2,3-Dihydroxy-6-nitro-7-sulfamoil-benzo(F)quinoxaline: a neuroprotectant 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., Murase, K. (1996) YM90K: pharmacological characterization as a selective and potent α-amino-3-hydroxy-5-methylisoxasole-4-propionate/kainate receptor antagonist. J. Pharmacol. Exp. Ther. 276: 84–92

Takahashi, M., Ni, J. W., Kawasaki-Yatsugi, S., Toya, T., Yatsugi, S., Shimizu-Sasamata, M., Koshiya, K., Shishikura, J., Sakamoto, S., Yamagichi, T. (1998) YM872, a novel selective α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid-receptor antagonist, reduces brain damage after permanent focal cerebral ischemia in cats. J. Pharmacol. Exp. Ther. 284: 467–473

Watkins, J. C., Krogsgaard-Larsen, P., Honoré, T. (1990) Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists. Trends Pharmacol. Sci. 11: 25–33

Xue, D., Huang, Z. G., Barnes, K., Lesiuk, H. J., Smith, K. E., Buchan, A. M. (1994) Delayed treatment with AMPA, but not NMDA, antagonists reduces neocortical infarction. J. Cereb. Blood Flow Metab. 14: 251–261

Yatsugi, S., Takahashi, M., Kawasaki-Yatsugi, S., Koshiya, K., Sakamoto, S., Uematsu, D., Shimizu-Sasamata, M. (1996) Neuroprotective effect of YM90K, a novel AMPA/kainate receptor antagonist, in focal cerebral ischemia in cats. J. Cereb. Blood Flow Metab. 16: 959–966