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NMDA and AMPA/kainate receptors are involved in the anticonvulsant activity of riluzole in DBA/2 mice

Giovambattista De Sarro [a,*], Antonio Siniscalchi [a], Guido Ferreri [a], Luca Gallelli [a], Angela De Sarro [b]

[a] *Department of Experimental and Clinical Medicine, Faculty of Medicine and Surgery, University of Catanzaro "Magna Graecia", Policlinico Mater Domini, Via T. Campanella, 88100 Catanzaro, Italy*

[b] *Institute of Pharmacology, School of Medicine, University of Messina, Messina, Italy*

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Abstract

The anticonvulsant activity of riluzole against sound-induced seizures was studied in the DBA/2 mouse model. Riluzole (0.1–4 mg kg^{-1}, intraperitoneal (i.p.)) produced dose-dependent effects with ED_{50} values for the suppression of tonic, clonic and wild running phases of 0.72, 1.38 and 2.71 mg kg^{-1}, respectively. Riluzole also protected DBA/2 mice from seizures induced by an intracerebroventricular (i.c.v.) injection of *N*-methyl-D-aspartate (NMDA) with ED_{50} values of 3.03 and 5.0 mg kg^{-1} for tonus and clonus, respectively. Pretreatment with glycine, an agonist to the glycine/NMDA receptors, shifted the dose–response effect of riluzole to the right (ED_{50} = 6.53 against tonus and 9.34 mg kg^{-1} vs. clonus). Similarly, D-serine, an agonist at the glycine site, shifted the ED_{50} of riluzole against the tonic component of audiogenic seizures from 0.72 to 1.97, and that against clonus from 1.38 to 2.77 mg kg^{-1}. Riluzole was also potent to prevent seizures induced by administration of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), an AMPA/kainate receptor agonist (ED_{50} = 1.80 and 3.35 mg kg^{-1}, against tonus and clonus, respectively). Pretreatment with aniracetam, a positive allosteric modulator of AMPA/kainate receptors, shifted the dose–response curve of riluzole to the right (ED_{50} = 1.78 against tonus and 2.58 mg kg^{-1} vs. clonus). The data indicate that riluzole is an effective anticonvulsant drug in the genetic model of seizure-prone DBA/2 mice. Our findings suggest that the anticonvulsant properties of riluzole depend upon its interaction with neurotransmission mediated by both the glycine/NMDA and the AMPA/kainate receptor complex.

Keywords: Epilepsy; Riluzole; Audiogenic seizure; AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid); Glycine/NMDA receptor; DBA/2 mouse

1. Introduction

Riluzole (2-amino-6-trifluoromethoxybenzothiazole) is a neuroprotective agent, which blocks glutamate neurotransmission before it become toxic. In particular, it acts on excitatory amino acid neurotransmission through at least four pharmacological mechanisms: (1) it inhibits the activity of the α-subunit of the Na^+ channel in the hyperpolarizing direction, thus increasing the inactivation state (Hebert et al., 1994); (2) it prevents Ca^{2+} mobilization by activating G-proteins (Kretschmer et al.,1998); (3) it reduces the release of excitatory amino acid by a similar G-protein-dependent mechanism (Chéramy et al., 1992; Martin et al., 1993; Kretschmer et al., 1998); and (4) it block post-synaptic glutamate receptors without a direct receptor interaction (Doble, 1996). Early data showed that riluzole prevents hippocampal neuronal damage in ischemic gerbils and in another rodent model of cerebral ischemia (Malgouris et al., 1989; Pratt et al., 1992), it blocks Na^+ channels in their inactivated state in myelinated fibres (Benoit and Escande, 1991). Recently, it has been demonstrated that the agent reduces the amplitude of the Na^+ currents in cortical neurons in culture, shifts the steady state inactivation curve of the Na^+ currents towards more negative values and reduces the amplitude of the late component of the outward K^+ currents (Zona et al., 1998). Moreover, riluzole depresses excitatory cortico-cortical synaptic transmission, reduces the tonic firing of the neocortical neurons in brain slices (Siniscalchi et al., 1997)

* Corresponding author. Tel.: +39-961-774424; fax: +39-961-774424.
E-mail address: desarro@unicz.it (G. De Sarro).

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and also interact with other excitatory amino acid-mediated responses (Hubert and Doble, 1989; Debono et al., 1993; Mantz, 1996; Keita et al., 1997).

In clinical studies, riluzole has been tested in patients with amyotrophic lateral sclerosis and it appears to improve the survival of patients suffering from this neurodegenerative disease and to slow their muscle-strength deterioration (Benismon et al., 1994; Hugon, 1996; Festoff, 1996; Lacomblez et al., 1996; Meininger et al., 1997; Couratier et al. 1994).

The cellular mechanisms through which riluzole exerts its anticonvulsant activity have yet to be established. Results of a previous study suggested that riluzole may primarily interact with excitatory amino acid-mediated neurotransmission (Mizoule et al., 1985; Siniscalchi et al. 1997, 1999; Stutzmann and Doble, 1994; Jimonet et al. 1999). In an attempt to understand more about its mode of action, we studied its activity in audiogenic seizure-prone DBA/2 mice, an animal model of reflex epilepsy (Chapman et al., 1984; Seyfried and Glaser, 1985; Engstrom and Woodbury, 1988). The aim of the present experiments was to characterize the type of glutamate ionotropic receptor mainly involved in the anticonvulsant activity of riluzole. Specifically, we studied whether excitatory amino acid neurotransmission is involved in the anticonvulsant activity of riluzole. Since it has been demonstrated that riluzole could act as an antagonist of the glycine/*N*-methyl-D-aspartate (NMDA) receptor complex (Doble, 1996; Kretschmer et al., 1998), we tested whether either glycine itself or the agonist at glycine sites, D-serine, interacts with the anticonvulsant effects of riluzole in the DBA/2 seizure models. In addition, the anticonvulsant effects of riluzole were evaluated against seizures induced by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), an agonist at the non-NMDA receptor complex (Honoré et al., 1988). Furthermore, we studied whether aniracetam, a positive allosteric modulator of AMPA/kainate receptor was able to affect the anticonvulsant properties of riluzole.

2. Materials and methods

2.1. Animals

Male and female DBA/2 mice weighing 8–12 g (22–26-days old) were used (Charles River, Calco, Como, Italy). The animals were housed in groups of 8–10 under a 12-h light/dark cycle (lights on at 7:00 AM) with food and water available ad libitum.

2.2. Experimental design

Seizures were induced by auditory stimulation or intracerebroventricular (i.c.v.) injections of NMDA or AMPA.

DBA/2 mice were exposed to auditory stimulation 45 min after intraperitoneal (i.p.) administration of vehicle or riluzole. Each mouse was placed under a hemispheric Perspex dome (diameter 58 cm) and 1 min was allowed for habituation and assessment of locomotor activity. Auditory stimulation (12–16 kHz, 109 dB) was applied for 1 min or until tonic extension occurred. Seizure response as previously reported (De Sarro et al., 1984) was assessed using the following scale: 0 = no response, 1 = wild running, 2 = clonus, 3 = tonus, 4 = respiratory arrest. The maximum response was recorded for each animal. Rectal temperature was recorded with an Elektrolaboratoriet thermometer type T.E.3 immediately prior to auditory testing. Behavioral changes were observed during the period between drug administration and auditory testing.

For i.c.v. injections, the mice were anesthetized with ether and injections were made in the left or right lateral ventricle (coordinates 1-mm posterior and 1-mm lateral to the bregma; depth 2.4 mm) using a 10-μl Hamilton microsyringe (type 701N) fitted with a nylon cuff on the needle as previously described (De Sarro et al., 1994a). Injections of drugs by this procedure led to a uniform distribution throughout the ventricular system within 10 min (De Sarro et al., 1988). The animals were placed singly in a 30 × 30 × 30 cm box and the observation time was 20 min after 2,3 dihydroxy-6-nitro-7-sulfamoyl-benzo(*F*)-quinoxaline (NBQX) injection, 30 min after AMPA, NMDA, 5,7-dichlorokynurenic acid or D-serine, 45 min after riluzole and 60 min after CPPene, glycine or aniracetam injections. The occurrence of clonic and tonic seizure signs and their latency were recorded. DBA/2 mice were pretreated with aniracetam (50 nmol i.c.v.), 60 min before testing, in order to evaluate whether it was able to reverse the anticonvulsant effects of riluzole in DBA/2 mice.

2.3. Statistical analysis

Groups of control and drug-treated DBA/2 mice were compared using Fisher's exact probability test (incidence of the seizure phases) or an analysis of variance (ANOVA) with Dunnett's *t*-test (rectal temperatures). The percentage incidence of each phase of the audiogenic seizure was determined for each drug. These values were plotted against the corresponding doses by means of computer construction of the dose–effect curves for calculation of ED_{50} (with 95% confidence limits). The ED_{50} values for each compound were calculated using a computer programme of the method of Litchfield and Wilcoxon (1949). At least 32 animals were used to calculate each ED_{50} value.

2.4. Drugs

Riluzole was kindly supplied by Rhône–Poulenc–Rorer (Paris, France) 3-((±)-2-carboxypiperazin-4-yl)-1-propenyl-1-phosphonic acid (CPPene) by Novartis (Berne, Switzerland) and 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(*F*)-quinoxaline (NBQX) by Novo Nordisk (Malov, Denmark). AMPA and 5,7-dichlorokynurenic acid were

purchased from Tocris (Buckhurst Hill, UK), NMDA, D-serine and glycine from Sigma (St. Louis, MO, USA).

For systemic injections, all compounds were given intraperitoneally (0.1 ml/10 g body weight) as a freshly prepared solution in 10% dimethylsulphoxide and 90% sterile saline (0.9% NaCl). Doses and time of administration are reported in the tables. Previous experiments had shown that this vehicle, when administered i.p., does not affect either behavior or response to auditory stimulation of DBA/2 mice (De Sarro et al., 1994a).

All drugs administered i.c.v. were dissolved in Na^+ phosphate buffer 67 mM, microinjected in a volume of 5 μl per mouse. Doses and time of administration are reported in the tables. NBQX was dissolved in a minimum amount of 1 N NaOH and the final volume was made up with Na^+ phosphate buffer. When necessary, the pH was adjusted to 7.3–7.4 by adding 0.2 N HCl. Because of the light sensitivity of some compounds, weighing and handling were carried out under sodium vapor lamps and the substances were protected from light during the experiments.

3. Results

3.1. Anticonvulsant activity of riluzole

Riluzole (0.1–4 mg kg^{-1} i.p.), administered 45 min before the auditory stimulation, dose-dependently reduced the severity of the audiogenic seizures in DBA/2 mice. In particular, a significant reduction of the incidence ($P < 0.01$) of the tonic phase of the audiogenic seizure was observed after 1 mg kg^{-1}, whereas no significant protection occurred after the low dose of 0.5 mg kg^{-1} (Table 1). Higher doses of riluzole (2–4 mg kg^{-1} i.p.) were necessary to obtain a significant reduction ($P < 0.01$) of the clonic phase of the audiogenic seizures (Table 1). In addition, significant protection ($P < 0.05$) against the wild running phase of the audiogenic seizure was observed following administration of 3 and 4 mg kg^{-1}. The ED_{50} values (±95% confidence limits) were 0.72 (0.47–1.12) against tonus 1.38 (0.96–1.99) against clonus (Table 2) and 2.71 (2.17–3.39)] mg kg^{-1} against wild running induced by auditory stimulation. Riluzole at the doses of 3 and 4 mg kg^{-1} affected body temperature (Table 1) and behavior (not shown). In fact, mild ataxia was observed for 20–30 min following the injection of such doses of riluzole.

Table 2
Effects of riluzole on tonic and clonic seizures in the DBA/2 mouse model

Convulsant stimulus	Anticonvulsant dose of riluzole (mg kg^{-1}, i.p.) ED_{50} (±95% confidence limits)	
	Tonic extension	Clonic seizures
Audiogenic stimulation	0.72 (0.47–1.12)	1.38 (0.96–1.99)
NMDA injection	3.03 (1.85–4.96)	5.0 (3.67–6.81)
AMPA injection	1.80 (1.12–2.90)	3.35 (2.22–5.07)

DBA/2 mice were exposed to auditory stimulation 45 min following i.p. administration of riluzole. NMDA and AMPA were administered intracerebroventricularly at the CD_{97} for either clonus or forelimb tonic extension, 1 h after riluzole. All data were calculated according to the method of Litchfield and Wilcoxon (1949).

3.2. Involvement of NMDA receptors

NMDA by itself (0.2–10 nmol/mouse, i.c.v.) produced generalized seizures. In particular, tremor and head-bobbing, hypermotility, jumping and circling preceded the first clonic episode, which consisted of wild running, jumping and loss of righting. The tonic component of the seizures occurred at the highest doses tested and was occasionally

Table 1
Effects of riluzole on audiogenic seizures in DBA/2 mice

Drug	Dose (mg kg^{-1}, i.p.)	Sound-induced response (% of mice)				SR (mean)	Temperature (°C) Mean ± S.E.M.
		WR	Clonus	Tonus	RA		
Vehicle		100	100	100	50	3.5	37.7 ± 0.10
Riluzole	0.1	100	100	90	50	3.4	37.7 ± 0.16
	0.5	100	80	70	10	2.6	37.5 ± 0.14
	1	100	70	40[a]	0[a]	2.1	37.4 ± 0.22
	1.5	80	60	20[a]	0[a]	1.6	37.2 ± 0.18
	2	70	40[a]	0[b]	0[a]	1.1	37.1 ± 0.24
	2.5	60	30[a]	0[b]	0[a]	0.9	36.9 ± 0.32
	3	50[c]	20[a]	0[b]	0[a]	0.7	36.2 ± 0.21[c]
	4	20[a]	0[b]	0[b]	0[a]	0.2	36.0 ± 0.21[a]

Groups of DBA/2 mice ($n = 10$/dose) were injected intraperitoneally with riluzole or vehicle (dimethylsulphoxide and saline) and auditory stimulation was delivered 45 min later. WR = wild running; RA = respiratory arrest; SR = mean maximal seizure response (see Materials and methods for grading). Riluzole 3 and 4 mg kg^{-1} significantly affected body temperature.

[a] $P < 0.01$.

[b] $P < 0.01$ compared with vehicle-treated group, Fisher's exact probability test (incidence of seizures phases) or ANOVA and Dunnett's *t*-test (body temperature).

[c] $P < 0.05$.

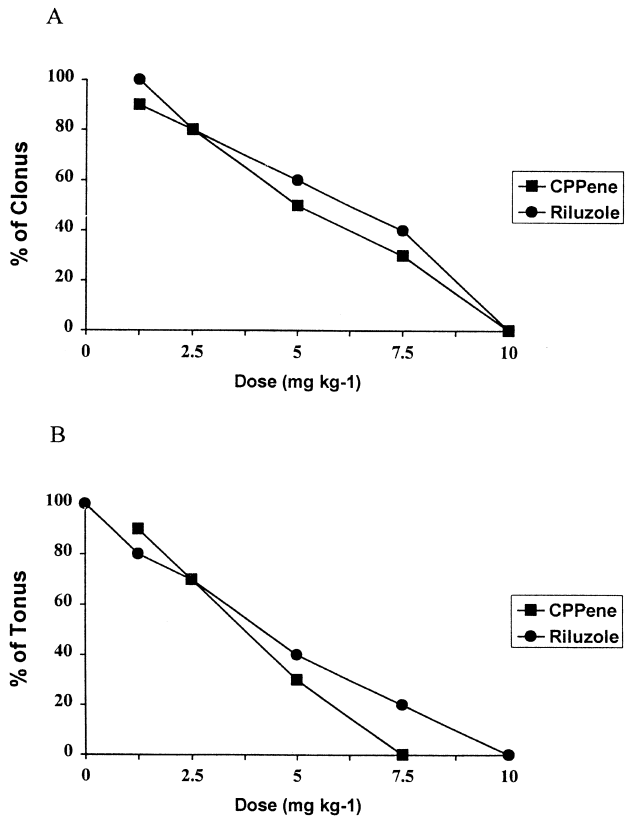

Fig. 1. Influence of riluzole or CPPene on clonic (A) and tonic (B) phases of NMDA-induced seizures in DBA/2 mice. Groups of 10 mice were pretreated with increasing doses of riluzole or CPPene (0.1–10 mg kg^{-1}, i.p.). NMDA (CD_{97} for tonus: 18.7 nmol/mouse, i.c.v.) was administered 1 h later. In another series of experiments, mice were pretreated with riluzole or CPPene, 1 h before NMDA (CD_{97} for clonus: 1.9 nmol/mouse, i.c.v.) injection. The ED_{50} values (±95% confidence limits) for riluzole against tonus and clonus were 3.03 (1.85–4.96) and 5.0 (3.67–6.81) mg kg^{-1}, respectively. The ED_{50} values (±95% confidence limits) for CPPene against tonus and clonus were 2.95 (1.88–4.61) and 4.61 (3.00–7.09) mg kg^{-1}, respectively. All data were calculated according to the method of Litchfield and Wilcoxon (1949). (●–●) Riluzole + NMDA; (■–■) CPPene + NMDA.

followed by death. The calculated CD_{97} of NMDA for tonic extension seizures (18.7 nmol/mouse) induced characteristic limbic seizures and 8 out of 10 mice died. Riluzole pretreatment (0.1–10 mg kg^{-1}, i.p., 1 h) was able to reduce, in a dose-dependent manner, the incidence of forelimb tonic extension seizures induced by the i.c.v. administration of the CD_{97} of NMDA (Fig. 1). At the higher doses, riluzole provided complete protection against limbic seizures and only 2 out of 10 animals treated with 7.5 mg kg^{-1} died. Riluzole pretreatment (0.1–10 mg kg^{-1}, i.p., 1 h) was able to reduce, in a dose-dependent manner, the incidence of clonus induced by the calculated NMDA CD_{97} for clonic seizures (1.9 nmol/mouse) (Fig. 1). The ED_{50} value (±95% confidence limits) of riluzole against tonus was 3.03 (1.85–4.96) and that against clonic seizures was 5.0 (3.67–6.81) mg kg^{-1} (Table 2). The mice that received i.p.i. 5.75 or 10 mg kg^{-1} showed ataxia, piloerection and a reduction of body temperature and of locomotor activity. CPPene pretreatment (0.1–10 mg kg^{-1}, i.p.i. 1 h) was also able to reduce in a dose-dependent manner the incidence of clonus and tonus induced by NMDA (Fig. 1). The ED_{50} value (±95% confidence limits) of CPPene against tonus was 2.95 (1.88–4.61) and that against clonus was 4.61 (3.0–7.09) mg kg^{-1}.

3.3. Interaction with the glycine recognition site at the NMDA receptor complex

The i.p. administration of glycine on its own (800 mg kg^{-1}, i.p.) 60 min before testing did not significantly modify the seizures induced by i.c.v. administration of NMDA (De Sarro et al., 1994a). When glycine (800 mg kg^{-1}, i.p.) was injected 60 min before riluzole, it shifted the dose–response curve of riluzole against NMDA-induced seizures to the right (Fig. 2). The ED_{50} value for

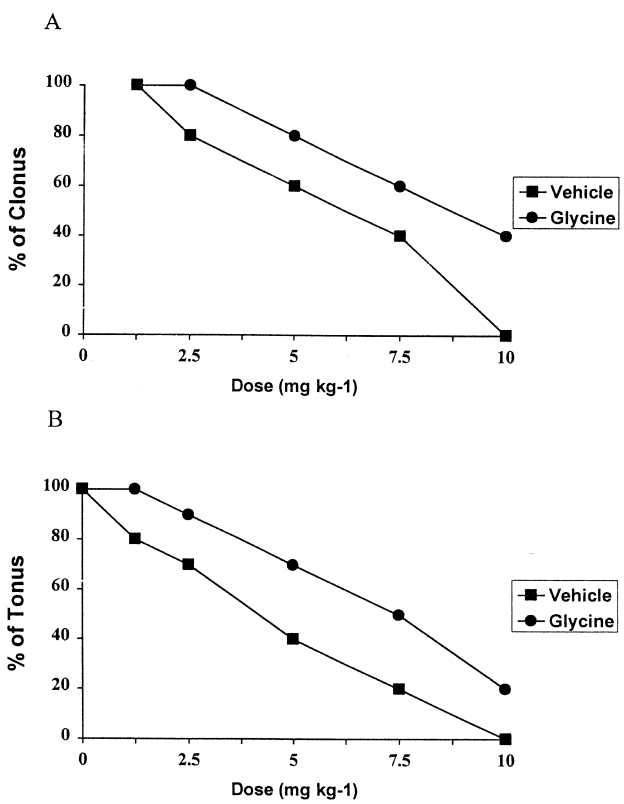

Fig. 2. Influence of glycine, a glycine receptor site agonist of the NMDA receptor complex on riluzole anticonvulsant activity against clonic (A) and tonic (B) phases induced by NMDA in DBA/2 mice. Groups of 10 mice were pretreated with increasing doses of riluzole (0.1–10 mg kg^{-1}, i.p.) and then received either vehicle (i.p.) + NMDA (i.c.v.) or glycine (800 mg kg^{-1}, i.p.) + NMDA (i.c.v.). 30 min after the latter treatment mice were placed singly under a hemispheric Perspex dome (diameter 58 cm) and observed for the presence or absence of seizure activity for 30 min. The doses of NMDA administered were 1.9 nmol/mouse for clonic seizure and 18.7 nmol/ mouse for tonic seizures. All data were calculated according to the method of Litchfield and Wilcoxon (1949). (●–●) Vehicle + Riluzole; (■–■) Glycine + Riluzole.

suppression of tonic extension seizures (NMDA CD_{97}: 18.7 nmol/mouse, i.c.v.) increased 2.2-fold from 3.03 (1.85–4.96) to 6.53 (4.63–9.22) mg kg^{-1}. For clonic seizures (NMDA CD_{97}: 1.9 nmol/mouse, i.c.v.), glycine was able to increase the ED_{50} value for suppression of clonic seizures 1.9-fold from 5.0 (3.67–6.81) to 9.34 (6.46–13.49) mg kg^{-1} i.p. Both increases of the ED_{50} values for glycine plus riluzole were significantly different from those of vehicle plus riluzole. The adverse effect of riluzole were reduced by pretreatment (60 min before) with glycine (800 mg kg^{-1}, i.p.). The animals pretreated with glycine did not show either ataxia or a reduction in body temperature.

For comparison, we studied the effects of 5,7-dichlorokynurenic acid, (0.6–10 nmol/mouse, i.c.v.), a glycine antagonist. A significant reduction of the incidence ($P < 0.01$) of the tonic and clonic phases of the audiogenic seizure was evident 30 min after 5,7-dichlorokynurenic acid starting at 3 nmol/mouse. The ED_{50} values (±95% confidence limits) for 5,7-dichlorokynurenic acid against the tonus were 2.2 (1.2–3.9), for the clonus, 2.4 (1.2–5), and for the wild running 5.3 (3.7–7.5) nmol/mouse. Neither hypothermia nor behavioral changes were noted following administration of 5,7-dichlorokynurenic acid at the doses, which induced significant protection against the audiogenic seizures.

The administration of 5,7-dichlorokynurenic acid (5–60 nmol/mouse, i.c.v., 30 min before) was able to reduce in a dose-dependent manner, the incidence of both clonic and tonic extension seizures induced by the i.c.v. administration of the CD_{97} of NMDA for clonus (1.9 nmol/mouse) or forelimb tonic extension (18.7 nmol/mouse). The ED_{50} values of 5,7-dichlorokynurenic acid against clonus and tonus were 25.5 (15.4–42.2) and 21.7 (13.3–35.5) nmol/mouse, respectively. Therefore, 5,7-dichlorokynurenic acid was equipotent against forelimb tonic extension and all limb clonus (ED_{50}: 21.7 vs. 25.5 nmol/mouse).

Pretreatment with glycine (800 mg kg^{-1} i.p., 1 h) shifted to the right the dose–response curve of 5,7-dichlorokynurenic acid against seizures induced by NMDA. The ED_{50} value for suppression of tonic extension seizures increased twofold from 21.7 (13.2–35.5) to 42.7 (30.2–60.3) nmol/mouse. In addition, glycine was able to increase the ED_{50} value for suppression of clonic seizures induced by NMDA by 2.3-fold from 25.5 (15.5–42.2) to 59.8 (32.9–108.5) nmol/mouse.

Similarly, D-serine (300 nmol/mouse, i.c.v.), an agonist at the glycine receptor site, interfered with the effects of riluzole against audiogenic seizures. The treatment with D-serine shifted the dose–response curve for riluzole to the right (Fig. 3). The ED_{50} values (±95% confidence limits) for the combined treatment, riluzole + D-serine, was 1.97 (1.59–2.44) for tonus, 2.77 (1.59–2.44) for clonus, and 6.7 (5.6–8.02) mg kg^{-1}, for wild running. Thus, the ED_{50} values for suppression of tonic and clonic seizures increased 2.7- and 2-fold, respectively. Our previous studies had shown that D-serine, administered directly into the lateral cerebral ventricle at 300 nmol/mouse is not itself convulsant (De Sarro et al., 1993; 1994a). The coadministration of D-serine had no effect on ataxia, piloerection, reduction in body temperature or locomotor activity observed following i.p. injection of riluzole (5, 7.5 and 10 mg kg^{-1}). The coadministration of D-serine (300 nmol/mouse, i.c.v.) with 5,7-dichlorokynurenic acid (1–20 nmol/mouse, i.c.v.), 30 min before the auditory stimulation, significantly reduced the anticonvulsant effects of 5,7-dichlorokynurenic acid. D-serine shifted the dose–response curve for 5,7-dichlorokynurenic acid to the right. The ED_{50} value (±95% confidence limits) for the combined treatment, 5,7-dichlorokynurenic acid + D-serine, was 8.5 (6–12.2), for tonus, 11.7 (7.5–18.4), for clonus,

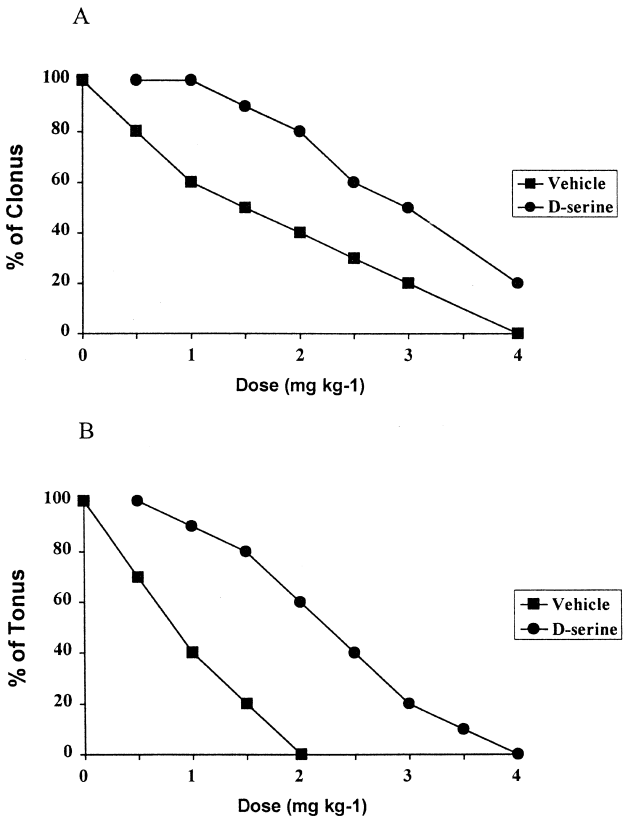

Fig. 3. Influence of D-serine, a glycine receptor site agonist of the NMDA receptor complex on anticonvulsant effects of riluzole against clonic (A) and tonic (B) phases of the audiogenic seizures in DBA/2 mice. Groups of 10 mice were pretreated concomitantly with increasing doses of riluzole (0.1–4 mg kg^{-1}, i.p.), and either vehicle (i.c.v.) or D-serine (300 nmol/mouse i.c.v.). Mice were placed singly under a hemispheric Perspex dome (diameter 58 cm) 30 min after the treatment and after 1 min were exposed to auditory stimulation (12–16 kHz, 109 dB). Mice were observed for the presence or absence of seizure activity for 1 min or until tonic extension occurred. All data were calculated according to the method of Litchfield and Wilcoxon (1949). The coadministration with D-serine increased the ED_{50} values for suppression of clonic and tonic seizures of 2- and 2.7-fold, respectively. (●–●) Vehicle + riluzole; (■–■) D-serine + riluzole.

and 19.1 (11.8–31.1) nmol/mouse, for wild running. Therefore, the concomitant treatment with 5,7-dichlorokynurenic acid + D-serine increased the ED_{50} values for suppression of audiogenic seizure phases from 3.6 to 4.9 times in comparison to the groups receiving 5,7-dichlorokynurenic acid only.

Coadministration of glycine (800 mg kg^{-1} i.p.) or of D-serine (300 nmol/mouse, i.c.v.) with CPPene did not significantly increase the ED_{50} values (±95% confidence limits) of CPPene against audiogenic seizures or seizures induced by NMDA (data not shown).

3.4. Involvement of AMPA receptors

Injections of AMPA (1–15 nmol/mouse, i.c.v.), an agonist at non-NMDA receptors, induced generalized seizures, similar to those caused by NMDA administration. Its latency was 1–3 min after the treatment with 5, 8, 10, 12 and 15 nmol and it was longer for AMPA at 1 and 3 nmol (up to 5 min). The CD_{97} for clonus was 9.7 nmol/mouse, while that for forelimb tonic extension was 11.7 nmol/mouse. Mice treated with the latter doses (10, 12 and 15 nmol/mouse) showed the characteristic limbic seizures and all animals died following tonic extension.

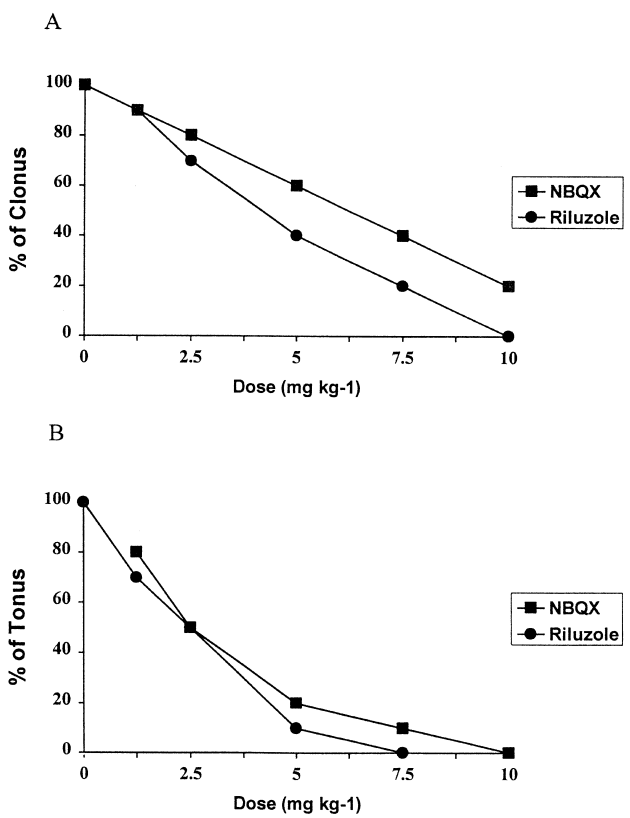

Fig. 4. Effects of riluzole on clonic (A) and tonic (B) phases of the seizures induced by i.c.v. injection of AMPA in DBA/2 mice. The selective AMPA receptor antagonist, NBQX was used for comparison. Groups of 10 mice were pretreated with increasing doses of riluzole (0.1–10 mg kg^{-1}, i.p.; 60 min before) or NBQX (1–10 mg kg^{-1}, i.p.; 20 min before) and then individual mice were challenged with either the CD_{97} of AMPA for clonus (9.7 nmol/mouse, i.c.v.) or tonus (11.8 nmol/mouse, i.c.v.). Mice were observed for the presence or absence of seizure activity for 30 min. All data were calculated according to the method of Litchfield and Wilcoxon (1949). (■–■) NBQX + AMPA; (●–●) Riluzole + AMPA.

As shown in Fig. 4, riluzole pretreatment (1 h) reduced the incidence of both the limb clonus and the forelimb tonic extension seizures induced by the i.c.v. administration of the CD_{97} of AMPA for either clonus or forelimb tonic extension. The ED_{50} values of riluzole against clonus and tonus were 1.80 (1.12–2.90) mg kg^{-1} and 3.35 (2.22–5.07) mg kg^{-1}, respectively (Table 2). Therefore, riluzole was approximately twice as potent against forelimb tonic extension as against all limb clonus. At the doses of 2.5, 5, 7.5 and 10 mg kg^{-1} riluzole provided complete protection against limbic seizures and there were no deaths at 120 min. Even at the lower dose of 1 mg kg^{-1}, riluzole protected 8 out 10 mice against limbic seizures and mortality.

As shown in Fig. 4, pretreatment (20 min) with NBQX (1–10 mg kg^{-1}, i.p.), a non-NMDA receptor antagonist, prevented the incidence for all limb clonus induced by the i.c.v. injection of the CD_{97} of AMPA for either clonus (9.7 nmol/mouse) or tonus (11.8 nmol/mouse). NBQX, at 7.5 and 10 mg kg^{-1}, significantly reduced the incidence of forelimb clonus and tonus. Animals pretreated with 7.5 and 10 mg kg^{-1} of NBQX did not show limbic seizures and at 120 min there were no death. The ED_{50} values of NBQX against clonus and tonus were 4.9 (3.2–7.5) and 2.2 (1.4–3.5) mg kg^{-1}, respectively. Therefore, NBQX was also approximately twice as potent against forelimb tonic extension as against all limb clonus.

3.5. Pretreatment with aniracetam

The i.c.v. injection of aniracetam on its own (12.5, 50 and 100 nmol), 60 min before testing, did not significantly modify the audiogenic seizure response in DBA/2 mice (De Sarro et al., 1994a), but aniracetam (50 nmol i.c.v.), 60 min before testing, reversed the anticonvulsant effects of riluzole in DBA/2 mice. Aniracetam (50 nmol i.c.v., 30 min before AMPA administration) reduced the anticonvulsant efficacy of riluzole (Table 2), resulting in increases of anticonvulsant ED_{50} values ranging from 1.9- to 2.5-fold. In particular, the ED_{50} values (±95% confidence limits) for the combined treatment, riluzole + aniracetam, were 1.78 (1.43–2.21 mg kg^{-1}) for tonus and 2.58 (2.15–3.10 mg kg^{-1}) for clonus. The corresponding aniracetam-induced shifts to the right of the dose–response curves for protection by riluzole against AMPA-induced clonic and tonic seizures are shown in Fig. 5.

The highest doses of riluzole induced ataxia, splayed hind limbs and tremor in some animals during the period of maximal anticonvulsant activity.

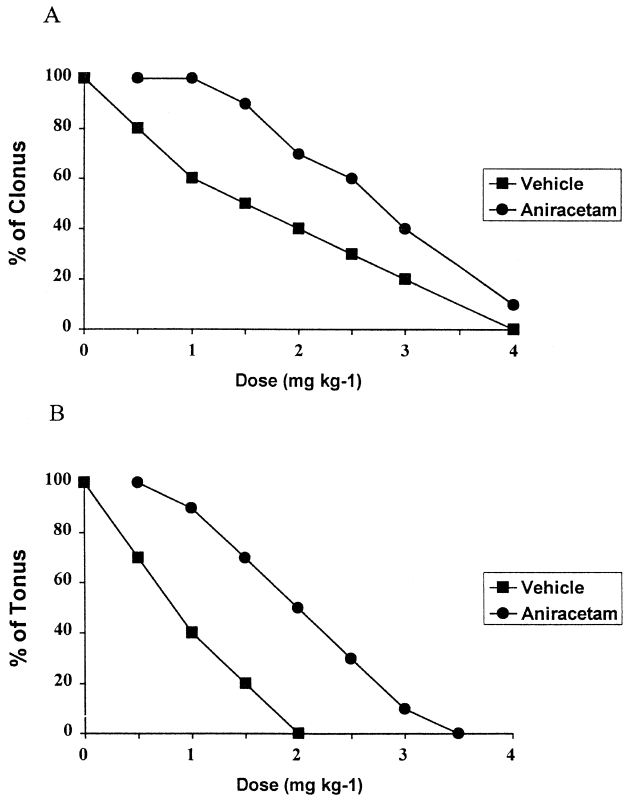

Fig. 5. Influence of aniracetam, a positive allosteric modulator of AMPA/kainate receptors on anticonvulsant effects of riluzole against clonic (A) and (B) tonic phases of the audiogenic seizures in DBA/2 mice. Groups of 10 mice were pretreated with increasing doses of riluzole (0.1–4 mg kg^{-1}, i.p.) and then received either vehicle (i.c.v.) or aniracetam (50 nmol/mouse i.c.v.). Mice were placed singly under a hemispheric Perspex dome (diameter 58 cm) 40 min after the latter treatment and after 1 min were exposed to auditory stimulation (12–16 kHz, 109 dB). Mice were observed for the presence or absence of seizure activity for 1 min or until tonic extension occurred. All data were calculated according to the method of Litchfield and Wilcoxon (1949). Coadministration with aniracetam increased the ED_{50} values for suppression of clonic and tonic seizures of 1.9 and 2.5 times, respectively. (■–■) Vehicle + Riluzole; (●–●) Aniracetam + Riluzole.

The coadministration of aniracetam was able to partially reduce the ataxia, piloerection, reduction in body temperature and locomotor activity induced by i.p. injection of riluzole.

4. Discussion

The present results indicate that riluzole has an interesting profile in an established mouse model of reflex epilepsy. The drug fully protects DBA/2 mice from sound-induced tonic extension and has an ED_{50} of 0.72 mg kg^{-1}, i.p. This value is quite similar to that (0.66 mg kg^{-1}, i.p.) previously reported by Mizoule et al. (1985). This is the most pronounced anticonvulsant activity of riluzole that has been observed thus far in animal models (see Romettino et al., 1991). For example, in the maximal electroshock seizure model, riluzole has anticonvulsant activity with an ED_{50} value of 8.5 mg kg^{-1}, i.p. and is not active against pentylenetetrazole, bicuculline or picrotoxin (Mizoule et al., 1985). Over a low-dose range, riluzole also antagonizes seizures induced by various chemoconvulsants 3-mercaptopropionic acid, thiosemicarbazide, isoniazide, harmaline and ouabain (Mizoule et al., 1985). Riluzole was able to prevent the neuronal epileptic depolarization induced by L-glutamate and kainate (Hubert et al., 1998; Debono et al., 1993; Keita et al., 1997). Thus, NMDA-induced tonic extension seizure was prevented at low doses, giving ED_{50} values of 3.03 mg kg^{-1} i.p. Our data showing that riluzole protects markedly against convulsions induced by both NMDA and AMPA provides strong support for the hypothesis that the drug interferes primarily with neurotransmission occurring at both NMDA and non-NMDA excitatory amino acid receptor complexes. These data therefore suggest that riluzole exerts its anticonvulsant effects through specific actions on neurotransmission mediated by excitatory amino acids. In addition, the present data and earlier in vivo and in vitro data (Mizoule et al., 1985; Romettino et al., 1991; Hubert et al., 1998; De Sarro et al., 1993; Keita et al., 1997) clearly show that the involvement of alternative mechanisms (sodium channel blockade, prevention of calcium ion mobilization, reduction of excitatory amino acid release) in the antiseizures activity of riluzole is as important as that of its interaction with NMDA and AMPA/kainate receptors.

The NMDA receptor is activated by L-glutamate only in the presence of glycine which binds at a discrete modulatory site located on the NMDA receptor-ionophore complex (Johnson and Ascher, 1987; Monahan et al., 1989). In radioligand binding studies, riluzole has no affinity for the NMDA, AMPA/kainate or metabotropic receptors (Doble, 1996; Kretschmer et al., 1998). The latter results have stimulated further research on the role of glycine in the mode of action of riluzole. In mice, D-cycloserine was found to markedly affect the pharmacological properties of riluzole (Kretschmer et al., 1998). In keeping with these data, we have demonstrated that glycine reduces the protective effects of riluzole against seizures induced by NMDA, and that D-serine, an agonist at the glycine recognition site, markedly attenuates the anticonvulsant properties of riluzole in the genetic model of the audiogenic seizure-prone DBA/2 mouse.

Together, these findings clearly suggest that the activity at the glycine/NMDA receptor complex might be an important mechanism underlying the anticonvulsant properties of riluzole.

In contrast to the blocking action observed on riluzole, glycine potentiates some anticonvulsant drugs, e.g. phenobarbital, carbamazepine, phenytoin and diazepam (Peterson, 1986, 1990, 1991; Peterson et al., 1990) and γ-vinyl-GABA (Seiler and Sarhan, 1984). However, glycine reduces the anticonvulsant properties of felbamate (De Sarro et al., 1994a). It is not certain that this action of

riluzole depends on an interaction with the strychnine-insensitive glycine sites of the NMDA receptors (Kretschmer et al., 1998). It is, however, difficult to interpret the different response of the various anticonvulsant drugs to glycine. This is particularly true in view of the fact that glycine has a rather complex mechanism of action. This is also evident when one considers that, under our experimental conditions, glycine by itself is devoid of anticonvulsant activity, whereas Toth and Lajtha (1984) have found that it partially protects against 3-mercaptopropionic acid-induced seizures in mice. Conversely, when administered intratecally, glycine potentiates the convulsant effects of NMDA and strychnine (Larson and Beitz, 1988; Singh et al., 1990). It may well be that the contrasting effects of glycine on the anticonvulsant activity of drugs depend upon their different mode of interaction with the glycine sites distributed either in the brain or in the spinal cord (Peterson, 1991).

Other data, however, support the hypothesis that riluzole also interacts with AMPA/kainate receptors (Debono et al., 1993; Keita et al., 1997; Siniscalchi et al., 1997; Hubert et al., 1998). Specifically, the drug has been found to provide effective protection against seizures induced by kainic acid (Mizoule et al., 1985), a chemical agent that stimulates the AMPA/kainate receptor complex. The present studies demonstrated that convulsions induced directly by AMPA itself are potently prevented by riluzole. Interestingly, the drug was able to prevent tonic extension seizures ($ED_{50} = 1.80$ mg kg^{-1}) and clonus ($ED_{50} = 3.35$ mg kg^{-1}) with a pattern of activity similar to that shown by NBQX, a specific antagonist of the AMPA/kainate receptor. However, the mechanism by which riluzole interacts functionally with the AMPA/kainate receptor remains unclarified.

Comparing riluzole's effective doses in various convulsion models, it emerges that the drug is more potent against seizures induced by the stimulation of AMPA/kainate receptors than against seizures induced by NMDA receptor activation. Conversely, the drug is much less effective against a variety of other chemical agents that induce seizures through the blockade of different mechanisms, and is not active against those drugs that act by blockade of γ-aminobutyric acid (GABA) neurotransmission. Consistent with these data, there are results of in vitro studies, with rat cortical neurones showing that riluzole interacts with excitatory neurotransmission mediated by both NMDA and AMPA/kainate receptors (Debono et al., 1993; Estevez et al., 1995; Hubert et al., 1998; Keita et al., 1997; Mary et al., 1995). Under these experimental conditions, the drug at 100 μM was able to fully prevent the action of kainate and to partially antagonize the action of glutamate.

Antagonists of the excitatory amino acid receptors, especially non-competitive NMDA receptor antagonists which block ion channels, e.g. MK 801, may induce cognitive deficits and a variety of other neurological and behavioral side-effects (e.g. reduction of locomotor activity, stereotypies and impaired motor coordination) (Kretschmer et al., 1992; McEntee and Crook, 1993; Rogawski, 1993). There are, however, studies showing that potent antagonists at either the glycine site, NMDA receptor or AMPA/kainate receptor have anticonvulsant effects at doses below those impairing behavior (Chiamulera et al., 1990; Löscher et al., 1993; Kretschmer, 1998; Kretschmer and Schmidt, 1996; Kretschmer et al., 1994, 1998; Danysz et al., 1994; De Sarro et al., 1994b, 1996). Regarding riluzole, there is evidence from a variety of studies with experimental animals and with humans that the drug is devoid of the psychotomimetic or other behavioral side-effects commonly associated with excitatory amino acid antagonists (Doble, 1996; Kretschmer et al., 1998). This profile of riluzole makes it of interest in the search for new drugs interacting with excitatory amino acid receptors and with a therapeutic potential (Meldrum and Garthwaite, 1990; Rogawski, 1992, 1993). Some other antiepileptic drugs such as lamotrigine, oxcarbazepine, phenytoin show neuroprotective activity by reducing the synaptic excitatory transmission mediated by excitatory amino acid receptors (Calabresi et al., 1996, 2000; Centonze et al., 1998; Siniscalchi et al., 1997). In agreement with a recent study of Kretschmer et al. (1998), we suggest that riluzole possesses the behavioral and anticonvulsant profile of a drug acting upon glutamate receptors. The interaction with AMPA/kainate receptors merits further investigation.

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