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Anticonvulsant activity of a novel NMDA/glycine site antagonist, MDL 104,653, against kindled and sound-induced seizures

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Abstract

MDL 104,653 (3-phenyl-4-hydroxy-7-chloro-quinolin-2(1*H*)-one), acts as an antagonist at the glycine site of the NMDA receptor. MDL 104,653 protects against sound-induced clonic seizures in DBA/2 mice following intracerebroventricular (ED_{50} = 19.1 nmol, 30 min), intraperitoneal (i.p.; ED_{50} = 6.1 μmol/kg, 45 min), or oral (ED_{50} = 23.0 μmol/kg, 2 h) administration. Optimal protection by MDL 104,653 was observed 15–60 min after i.p. administration, and the therapeutic index, as assessed by rotarod performance, was 4.0 at 45 min after i.p. administration. Fully amygdala-kindled motor seizures in rats were significantly reduced at 15, 30 and 60 min, and the duration of the after-discharge was significantly shortened at 30 min after the i.p. administration of 74 μmol/kg MDL 104,653. A lower dose of MDL 104,653 (37 μmol/kg) had no significant effect on either motor seizures or after-discharge duration. The rate of amygdala kindling was also significantly retarded following the daily administration of 56 μmol/kg MDL 104,653 (1 times daily for 6 days; i.p. 30 min before kindling stimulus).

Keywords: Amygdala kindling; (DBA/2 mouse); Strychnine-insensitive glycine site; NMDA receptor antagonist; Anticonvulsant

1. Introduction

Antagonists acting at the glycine site of the NMDA receptor reverse the glycine potentiation of NMDA responses in in vivo or in vitro systems (Palfreyman and Baron, 1991; Baron et al., 1992; Kemp and Leeson, 1993) and have anticonvulsant activity in many experimental seizure models (Skolnick et al., 1989; Chiamulera et al., 1990; Croucher and Bradford, 1990, 1991; Koek and Colpaert, 1990; Saywell et al., 1991; Peterson, 1992; Smith and Meldrum, 1992). The anticonvulsant activity of the glycine site antagonists is analogous to the better characterized anticonvulsant properties of antagonists acting at other sites of the NMDA receptor (competitive antagonists; channel site antagonists) and at the non-NMDA AMPA/kainate receptor (see Chapman, 1991; Chapman and Meldrum, 1993). Although antagonists acting at different NMDA and non-NMDA sites share many anticonvulsant properties, there are some important differences between the different groups of antagonists with respect to their therapeutic indices, uptake into the brain, or selectivity for different seizure types. For instance, competitive NMDA receptor antagonists are not very effective in blocking fully kindled seizures (Löscher and Hönack, 1991; Morimoto et al., 1991; Cotterell et al., 1992; Dürmüller et al., 1994), even though they potently inhibit the development of kindling (Morimoto et al., 1991; see Chapman, 1991). The non-NMDA receptor antagonists, NBQX (2,3-dihydroxy-6-nitro-7-sulphamoyl-benzo(*F*)quinoxaline) or GYKI 52466 (1-(α-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine), on the other hand, inhibit fully amygdala-kindled seizures, while having less effect on the development of kindling (Dürmüller et al., 1994; Namba et al., 1994). It is therefore of interest to compare the anticonvulsant action of a glycine site antagonist against the kindling process and against fully kindled seizures.

In addition, most of the currently available glycine site antagonists (e.g. the kynurenic acid derivatives)

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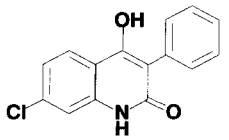

Fig. 1. Chemical structure of MDL 104,653 ((3-phenyl-4-hydroxy-7-chloro-quinolin-2(1*H*)-one).

have a short time course of action and penetrate the blood-brain barrier poorly. We are therefore interested in identifying potent antagonists for this site with a favourable brain uptake and improved time course of action. MDL 104,653 (3-phenyl-4-hydroxy-7-chloro-quinolin-2(1*H*)-one; Fig. 1) belongs to a novel class of 3′-substituted 3-phenyl-4-hydroxy-2-quinolones with selective glycine/NMDA receptor antagonist activity (McQuaid et al., 1992; Kulagowski et al., 1994). Anticonvulsant activity against sound-induced seizures in DBA/2 mice has been reported in preliminary form for MDL 104,653 and closely related structural analogues (Chapman et al., 1993; Rowley et al., 1993; Kulagowski et al., 1994).

2. Materials and methods

2.1. Animals and anticonvulsant testing

DBA/2 mice, male and female, aged 3–4 weeks (weight 6–13 g, Institute of Psychiatry stock) were used in this study. Sound-induced seizure response was assessed as previously described (Patel et al., 1990). Briefly, at a fixed time following drug administration the rectal temperatures of the animals were determined, and the mice were placed individually under a Plexiglas dome fitted with a bell at the apex, which generated a mixed frequency sound of 109 dB. The sound stimulus triggered a characteristic sequence of responses in the mice in the control group, consisting of (1) a wild running phase (onset 1–4 s), (2) clonic seizures (onset 4–10 s), (3) tonic extension (onset 10–25 s), frequently followed by (4) respiratory arrest (onset 15–45 s). In this study we only report ED_{50} values for suppression of the clonic phase of the seizure response.

The kindling procedure in male Wistar rats (386 g ± 38 S.D., $n = 35$) was carried out as previously described (Dürmüller et al., 1994). Bipolar depth electrodes were implanted into the left amygdala under pentobarbital anaesthesia 13 ± 4 S.D. ($n = 35$) days before the rats received the first kindling stimulus. Amygdala stimulation was given for 2 s (parameters: bipolar, 50 pps, 1 ms pulse-width, 8 V amplitude) on a daily basis until the rats exhibited at least three consecutive stage 5 seizures, according to a slightly modified Racine score – stage 1: mouth and facial movements; stage 2: head nodding and/or eyelid or facial clonus; stage 3: unilateral/bilateral forelimb clonus; stage 4: same as stage 3 plus rearing; stage 5: same as stage 4 plus loss of balance (Racine, 1972; Dürmüller et al., 1994). A short pre-stimulus EEG period and the afterdischarges (AD) were recorded from the sensorimotor cortex and the amygdala.

2.2. Drugs and drug administration

MDL 104,653 (3-phenyl-4-hydroxy-7-chloro-quinolin-2(1*H*)-one; molecular weight = 271.7) was a gift from Marion Merrell Dow Research Institute, Cincinnati, OH, USA).

Intraperitoneal (i.p.) administration, DBA/2 mice: MDL 104,653 was dissolved in saline adjusted to pH 7 with 1 N NaOH. The drug was administered i.p. (3.5–18.5 μmol/kg; 0.1 ml of drug solution per 10 g body weight) to groups of DBA/2 mice ($n = 9$–10) 45 min before a sound stimulus was applied to evoke a seizure response.

Oral (p.o.) administration, DBA/2 mice: MDL 104,653 was dissolved in saline adjusted to pH 7 with 1 N NaOH and administered orally (18.5–74 μmol/kg; 0.2 ml of drug solution per 10 g body weight) to groups of DBA/2 mice ($n = 8$–9). The DBA/2 mice were tested for sound-induced seizure response 2 h following the p.o. administration of the drug.

Intracerebroventricular (i.c.v.) administration, DBA/2 mice: MDL 104,653 was dissolved in 67 mM sodium phosphate buffer, pH 7.3 and administered i.c.v. to lightly ether-anaesthetized mice (1.8–37 nmol; 10 μl per mouse; $n = 10$–11) 30 min prior to sound exposure.

I.p. administration, fully kindled rats: MDL 104,653 was dissolved in saline adjusted to pH 7 with 1 N NaOH and administered i.p. (37 or 74 μmol/kg; 1 ml of drug solution per 100 g body weight) to groups of fully kindled rats ($n = 6$ per dose and per time point). The rats received control amygdala stimulations the day before the drug test and were assessed for afterdischarge duration and Racine seizure score. The following day the rats received another amygdala stimulation at 15, 30 or 60 min following drug administration (only a single time-point was assessed on a given day) and finally another control stimulation 24 h after drug administration.

I.p. administration during the kindling process: 56 μmol/kg MDL 104,653 was administered daily for 6 days (days 3–8 of the kindling protocol) 30 min before the rats ($n = 6$) received the kindling stimulation. There were two different control groups for the kindling procedure: one kindled control group ($n = 9$) received daily saline i.p. administrations, while the other control group ($n = 11$) received no treatment. There were no statistically significant differences between these two

control groups with respect to their rates of development of after-discharge or motor seizures.

2.3. Rotarod performance

Prior to drug administration the mice were trained to remain for 2 min on a rod 25 mm in diameter and rotating at 18–22 rotations per minute. MDL 104,653 was administered i.p. (11–74 μmol/kg) to groups of DBA/2 mice ($n = 9$–10). The rotarod performance of the mice was assessed 45 min and 2 h after the i.p. administration of MDL 104,653.

2.4. Statistics

ED_{50} values for inhibition against sound-induced clonic seizures in DBA/2 mice were calculated with 95% confidence limits according to Litchfield and Wilcoxon (1949).

The Wilcoxon two-sample test was used for statistical analysis of drug-induced effects on cortical after-discharge duration and seizure score in kindled rats. The responses to control amygdala stimulation 24 h before drug administration were used for comparison. Asterisks denote responses significantly different from control responses: $^{*}P < 0.05$, $^{**}P < 0.01$. The Wilcoxon two-sample test was also used to compare the delay to the first stage 5 seizure between control and drug-treated animals.

An analysis of variance test (ANOVA) for repeated measures was used to compare the after-discharge duration (cortex and amygdala) between control and drug-treated rats.

3. Results

3.1. Sound-induced seizures in DBA / 2 mice

MDL 104,653 administration (i.p., p.o. or i.c.v.) had no significant effect on the body temperature of DBA/2 mice at any of the doses tested (data not shown).

A fully anticonvulsant dose (18.5 μmol/kg or 5 mg/kg) of MDL 104,653 gave complete protection against sound-induced clonic and tonic seizures in DBA/2 mice, and partial protection against the wild running phase, for 15–60 min after its i.p. administration, with a gradual return to control seizure response during the following 2 h (Fig. 2).

Dose-dependent suppression of clonic seizures in DBA/2 mice following i.p., p.o. or i.c.v. administration of MDL 104,653 is shown in Fig. 3a–c. The corresponding ED_{50} values (μmol/kg) for the protection against sound-induced clonic seizures by MDL 104,653 are listed in Table 1.

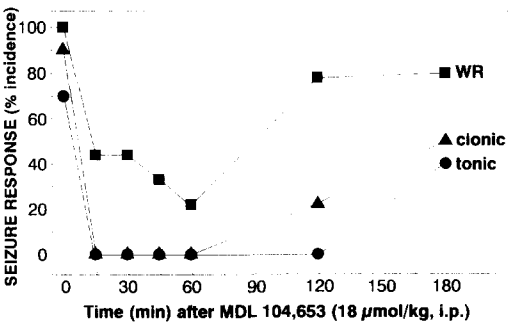

Fig. 2. The duration of the MDL 104,653-induced suppression of sound-induced wild running (WR), clonic, and tonic seizures in DBA/2 mice following the i.p. administration of a fully anticonvulsant dose (18 μmol/kg) of MDL 104,653 ($n = 9$–10 per dose).

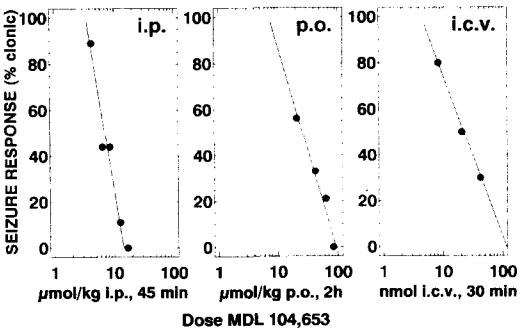

Fig. 3. Dose-dependent suppression of sound-induced clonic seizures in DBA/2 mice by MDL 104,653 (a) 45 min after i.p. administration, (b) 2 h after p.o. administration, and (c) 30 min after i.c.v. administration. $n = 8$–11 per group. See Materials and methods for further experimental details.

The DBA/2 mice exhibited normal behaviour at the therapeutic dose ranges of MDL 104,653 (3.5–18.5 μmol/kg i.p.; 18.5–74 μmol/kg p.o.; or 1.8–37 nmol i.c.v.). Rotarod performance was assessed following i.p. administration of MDL 104,653 (11–74 μmol/kg). At high drug doses (37–74 μmol/kg i.p.), the mice exhibited severe ataxia during the first hour after drug administration. This is reflected in an impairment of rotarod performance with an ED_{50} of 24.3 μmol/kg. The ratio of the ED_{50} for rotarod performance divided

Table 1
The inhibition of sound-induced clonic seizures and the impairment of rotarod performance in DBA/2 mice following systemic and central administration of MDL 104,653

	ED_{50}		T.I. value
	μmol/kg	nmol	
Anticonvulsant activity (clonic seizures)			
i.p. 45 min	6.1 (4.6–8.0)	–	
p.o. 2 h	23.0 (13.2–39.8)	–	
i.c.v. 30 min	–	19.1 (11.8–31.1)	
Rotarod impairment			
i.p., 45 min	24.3 (19.2–30.7)	–	4.0

T.I.: therapeutic index.

by the ED_{50} for the anticonvulsant effect gives a therapeutic index of 4.0 (Table 1). By 2 h following i.p. administration of MDL 104,653 the rotarod performance had returned to normal at all doses tested (data not shown).

3.2. Effect of MDL 104,653 on fully kindled seizures

The i.p. administration of 37 μmol/kg MDL 104,653 to amygdala-kindled rats had no significant effect on either the behavioural seizure score or the duration of the cortical (Fig. 4a) or amygdala (data not shown) after-discharge when tested 15, 30 and 60 min after drug administration. A higher dose of MDL 104,653 (74 μmol/kg i.p.) significantly reduced the seizure score from 5.0 in the pre-drug controls to 3.8 at 15 min, 1.3 at 30 min, and 2.7 at 60 min after drug administration (Fig. 4b). The cortical after-discharge duration was significantly reduced from 95.5 s in pre-drug controls to 37.8 s at 30 min after the i.p. administration of 74 μmol/kg MDL 104,653. The amygdala after-discharge duration was also significantly reduced from 98.0 s in pre-drug controls to 37.8 s in the same post-drug group (data not shown).

3.3. Effect of MDL 104,653 on the rate of kindling

Rats were injected i.p. with 56 μmol/kg MDL 104,653 30 min before receiving kindling stimuli, starting on the third day of the kindling procedure with daily repeats for 6 days. The rate of expression of stage 1 and stage 2 seizures in the experimental group was the same as in the two control groups, but stage 3–5 seizures (containing a clonic component) were not expressed as long as MDL 104,653 was administered (Fig. 5). The overall average numbers of stimulations needed for reaching stage 5 seizures in the two control groups were 7.6 (no treatment) or 8.7 (saline treatment), while 11.3 stimulations were required in the MDL 104,653-treated group. The difference between the experimental group and the control groups in the rate of achieving stage 5 seizures was statistically significant ($P < 0.05$). When the entire kindling procedure (pre-, during-, and post-drug periods) was considered, there were no overall significant differences between the rates of

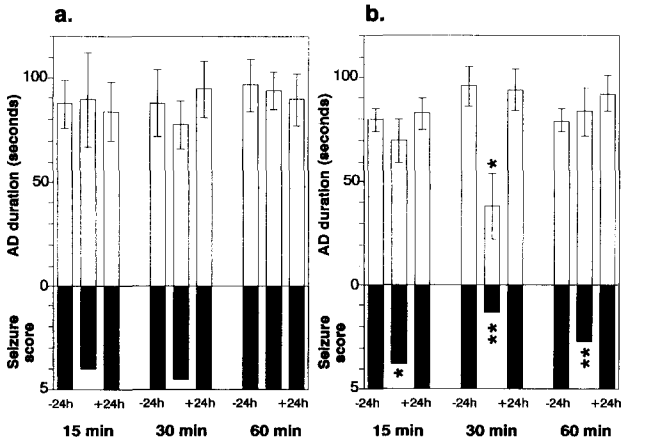

Fig. 4. The effect of (a) 37 μmol/kg, and (b) 74 μmol/kg of MDL 104,653 (15–60 min pretreatment) on cortical after-discharge (AD) duration (top, open bars) and seizure score (bottom, filled bars) in fully amygdala-kindled rats ($n = 6$ per dose and time point). Pre-drug control responses (−24 h), and post-drug recovery responses (+24 h) are also included in the figure. Asterisks denote responses significantly different from pre-drug responses (see Materials and methods for further details).

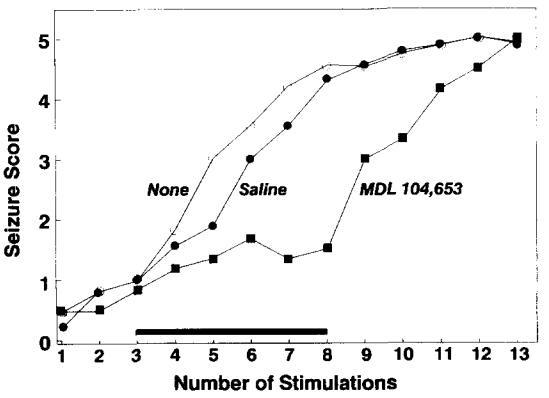

Fig. 5. The effect of daily (56 μmol/kg i.p., 30 min pre-stimulation; $n = 6$; filled squares) MDL 104,653 administration on the rate of appearance of motor seizures during amygdala kindling in Wistar rats, compared with untreated (None; $n = 11$; open circles) and saline-treated (Saline; 30 min pre-stimulation; $n = 9$; filled circles) control groups. The horizontal bar indicates the 6-day period when drug or saline injections preceded the kindling stimulus (see Materials and methods for further details).

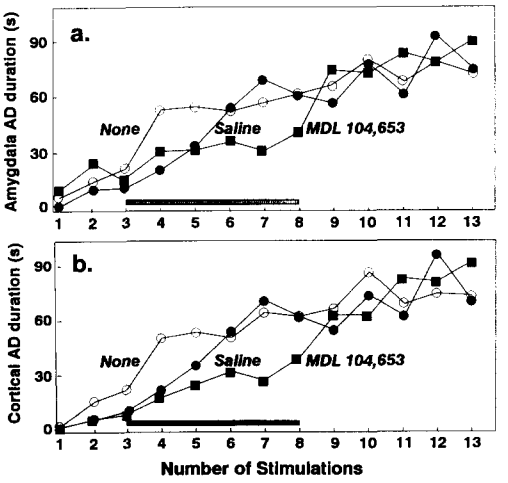

Fig. 6. The effect of daily (56 μmol/kg i.p., 30 min pre-stimulation; $n = 6$; filled squares) MDL 104,653 administration on the rate of increase in amygdala (a) and cortical (b) after-discharge (AD) duration during amygdala kindling in Wistar rats, compared with untreated (None; $n = 11$; open circles) and saline-treated (Saline; 30 min pre-stimulation; $n = 9$; filled circles) control groups. The horizontal bar indicates the 6-day period when drug or saline injections preceded the kindling stimulus (see Materials and methods for further details).

increases in cortical and amygdala after-discharge duration between the experimental and control groups (Fig. 6a and b). However, when tested 30 min post-drug during the six consecutive days of drug administration, 56 μmol/kg MDL 104,653 caused a significant reduction in cortical after-discharge duration ($P < 0.05$; Fig. 6b) and a non-significant reduction in amygdala after-discharge duration during the drug treatment period (Fig. 6a).

4. Discussion

MDL 104,653 belongs to a large class of 3′-substituted 3-phenyl-4-hydroxy-2-quinolones that have recently been shown to be selective antagonists at the glycine site of the NMDA receptor complex (McQuaid et al., 1992; Rowley et al., 1993; Kulagowski et al., 1994). Their glycine/NMDA receptor antagonist activity is demonstrated by displacement of tritiated glycine or L-689,560 binding with potencies in the low to mid nanamolar range, and with K_b values against NMDA depolarization of cortical slices in the low micromolar range. A number of this class of glycine/NMDA receptor antagonists have been shown to be active against sound-induced seizures in DBA/2 mice, with a broad correlation between their anticonvulsant potencies and their affinities for the glycine/NMDA site (Carling et al., 1993; Rowley et al., 1993; Kulagowski et al., 1994). MDL 104,653 is not the most potent compound in this series. Certain 3′-substitutions in the 3-phenyl ring of the compounds improve both receptor binding by up to 50–100-fold and anticonvulsant efficacy by 5–10-fold (Kulagowski et al., 1994). The affinity and selectivity of MDL 104,653 for the glycine/NMDA site is demonstrated by IC_{50} values of 273 ± 46 nM versus [^{3}H]glycine and 1.1 ± 0.1 μM versus NMDA-stimulated cGMP accumulation in cerebellar slices, and IC_{50} values > 100 μM for tritiated kainate and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionate) binding (Chapman et al., 1993; B. Siegel, A. Slone and B. Baron, Marion Merrell Dow, Cincinnati, OH, USA, unpublished results).

The potent oral availability and sustained anticonvulsant action of MDL 104,653 against reflex epilepsy (sound-induced seizures in DBA/2 mice), epileptogenesis (kindling process) and 'complex partial epilepsy' (fully amygdala-kindled seizures) represent a major advance towards finding therapeutically useful anticonvulsants among the glycine/NMDA receptor antagonists. MDL 104,653 has a much more favourable time course and systemic anticonvulsant efficacy than most of the previously available glycine site antagonists, such as kynurenic acid derivatives. Interestingly, following i.c.v. administration MDL 104,653 has an ED_{50} value of 19.1 nmol (Table 1), which is very similar to the anticonvulsant ED_{50} values (nmol, i.c.v., 15 min pretreatment, clonic seizures; 95% lower and upper confidence limits given in parentheses) listed below for the more potent of the kynurenic acid derivatives, using the same DBA/2 seizure model: 7-chloro-kynurenic acid, 18.9 (15.1–23.6) nmol; 5,7-dichloro-kynurenic acid, 11.2 (9.3–16.7) nmol; 5,7-dichloro-thiokynurenic acid, 17.2 (13.8–24.1) nmol (Chapman, unpublished results). The quite potent anticonvulsant action of MDL 104,653 following systemic (i.p. or p.o.) administration, compared to the kynurenic acid derivatives that in our hands have no anticonvulsant activity against sound-induced seizures in DBA/2 mice at doses of up to 50 μmol/kg following systemic administration, must therefore be due to a favourable uptake system for this compound, rather than a higher intrinsic anticonvulsant activity. The therapeutic index of 4.0 observed in DBA/2 mice following i.p. drug administration is similar to values previously observed after the administration of competitive NMDA receptor antagonists and compares favourably to therapeutic index values previously observed after the administration of other glycine/NMDA site antagonists (see Chapman, 1991). We observed no overt difference between naive and kindled rats with respect to the degree of ataxia and other locomotor impairment induced by 56 μmol/kg MDL 104,653 (the dose used for chronic administration). This is in contrast to the behavioural side-effects of competitive NMDA receptor antagonists that appear more pronounced in kindled than in naive rats (Löscher and Hönack, 1991).

The anticonvulsant protection achieved with MDL 104,653 both against fully kindled seizures (suppression of both after-discharge duration and motor seizures) and against the kindling process, is in interesting contrast to what has previously been observed using competitive NMDA receptor antagonists, that suppress the kindling process (after-discharge duration and motor seizures) more potently than they suppress fully kindled seizures (Morimoto et al., 1991; Löscher and Hönack, 1991; Dürmüller et al., 1994). AMPA/kainate receptor antagonists, conversely, have no significant effect on the motor seizure manifestation during the kindling process, while potently suppressing the seizure score in fully amygdala-kindled rats (Dürmüller et al., 1994; Namba et al., 1994). The involvement of glycine/NMDA receptor antagonists in regulating both the kindling development and the expression of fully kindled seizures has been suggested earlier by using intra-amygdaloid or i.c.v. injections of the centrally (but not systemically) active glycine/NMDA receptor antagonist, 7-chloro-kynurenic acid (Croucher and Bradford, 1990, 1991; Namba et al., 1993). These studies demonstrated a significant retardation of the development of both the after-discharge and motor seizure components of kindled seizures associated with chronic

7-chloro-kynurenic acid administration (Croucher and Bradford, 1990; Namba et al., 1993). The effect of 7-chloro-kynurenic acid (10–50 nmol) on fully kindled seizures was less pronounced: only the generalized seizure threshold was significantly elevated by focal injections of 7-chloro-kynurenic acid, while after-discharge duration and expression of motor seizures were not affected by 7-chloro-kynurenic acid (Croucher and Bradford, 1991; Namba et al., 1993).

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References

Baron, B.M., B.L. Harrison, I.A. McDonald, B.S. Meldrum, M.G. Palfreyman, F.G. Salituro, B.W. Siegel, A.L. Slone, J.P. Turner and H.S. White, 1992, Potent indole- and quinoline-containing *N*-methyl-D-aspartate antagonists acting at the strychnine-insensitive glycine binding site, J. Pharmacol. Exp. Ther. 262, 947.

Carling, R.W., P.D. Leeson, K.W. Moore, J.D. Smith, C.R. Moyes, I.M. Mawer, S. Thomas, T. Chan, R. Baker, A.C. Foster, S. Grimwood, J.A. Kemp, G.R. Marshall, M.D. Tricklebank and K.L. Saywell, 1993, 3-Nitro-3,4-dihydro-2(1*H*)-quinolones. Excitatory amino acid antagonists acting at glycine-site NMDA and (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors, J. Med. Chem. 36, 3397.

Chapman, A.G., 1991, Excitatory amino acid antagonists and therapy of epilepsy, in: Excitatory Amino Acid Antagonists, ed. B.S. Meldrum (Blackwell Scientific Publications, Oxford) p. 265.

Chapman, A.G. and B.S. Meldrum, 1993, Excitatory amino acid antagonists and epilepsy, Biochem. Soc. Trans. 21, 106.

Chapman, A.G., N. Dürmüller, N. Parvez, B.L. Harrison, B.M. Baron and B.S. Meldrum, 1993, The anticonvulsant action of a novel NMDA/glycine site antagonist, MDL 104,653, following systemic administration to DBA/2 mice or kindled rats, Soc. Neurosci. Abstr. 19, 197.13 (Abstract).

Chiamulera, C., S. Costa and A. Reggiani, 1990, Effect of NMDA- and strychnine-insensitive glycine site antagonists on NMDA-mediated convulsions and learning, Psychopharmacology 102, 551.

Cotterell, K.L., M.J. Croucher and H.F. Bradford, 1992, Weak anticonvulsant activity of CGP 37849 and CGP 39551 against kindled seizures following systemic administration, Eur. J. Pharmacol. 214, 285.

Croucher, M.J. and H.F. Bradford, 1990, 7-Chlorokynurenic acid, a strychnine-insensitive glycine receptor antagonist, inhibits limbic seizure kindling, Neurosci. Lett. 118, 29.

Croucher, M.J. and H.F. Bradford, 1991, The influence of strychnine-insensitive glycine receptor agonists and antagonists on generalized seizure thresholds, Brain Res. 543, 91.

Dürmüller, N., M. Craggs and B. Meldrum, 1994, The effect of the non-NMDA antagonists GYKI 52466 and NBQX and the competitive NMDA receptor antagonist D-CPPene on the development of amygdala kindling and on amygdala-kindled seizures, Epilepsy Res. 17, 167.

Kemp, J.A. and P.D. Leeson, 1993, The glycine site of the NMDA receptor – Five years on, Trends Pharmacol. Sci. 14, 20.

Koek, W. and F.C. Colpaert, 1990, Selective blockade of *N*-methyl-D-aspartate (NMDA)-induced convulsions by NMDA antagonists and putative glycine antagonists: relationship with phencyclidine-like behavioral effects, J. Pharmacol. Exp. Ther. 252, 349.

Kulagowski, J.J., R. Baker, N.R. Curtis, P.D. Leeson, I.M. Mawer, A.M. Moseley, M.P. Ridgill, M. Rowley, I. Stansfield, A.C. Foster, S. Grimwood, R.G. Hill, J.A. Kemp, G.R. Marshall, K.L. Saywell and M.D. Tricklebank, 1994, 3′-(arylmethyl)- and 3′-(aryloxy)-3-phenyl-4-hydroxyquinolin-2(1*H*)-ones: orally active antagonists of the glycine site on the NMDA receptor, J. Med. Chem. 37, 1402.

Litchfield, J.T. and F. Wilcoxon, 1949, A simplified method of evaluating dose-effect experiments, J. Pharmacol. Exp. Ther. 96, 99.

Löscher, W. and D. Hönack, 1991, Anticonvulsant and behavioral effects of two novel competitive *N*-methyl-D-aspartate acid receptor antagonists, CGP 37849 and CGP 39551, in the kindling model of epilepsy. Comparison with MK-801 and carbamazepine, J. Pharmacol. Exp. Ther. 256, 432.

McQuaid, L.A., E.C.R. Smith, D. Lodge, E. Pralong, J.H. Wikel, D.O. Calligaro and P.J. O'Malley, 1992, 3-Phenyl-4-hydroxyquinolin-2(1*H*)-ones: potent and selective antagonists at the strychnine-insensitive glycine site on the *N*-methyl-D-aspartate receptor complex, J. Med. Chem. 35, 3423.

Morimoto, K., K. Katayama, K. Inoue and K. Sato, 1991, Effects of competitive and noncompetitive NMDA receptor antagonists on kindling and LTP, Pharmacol. Biochem. Behav. 40, 893.

Namba, T., K. Morimoto, N. Yamada and S. Otsuki, 1993, Antiepileptogenic action of 7-chlorokynurenic acid on amygdala kindling of rats, Pharmacol. Biochem. Behav. 46, 275.

Namba, T., K. Morimoto, K. Sato, N. Yamada and S. Kuroda, 1994, Antiepileptogenic and anticonvulsant effects of NBQX, a selective AMPA receptor antagonist, in the rat kindling model of epilepsy, Brain Res. 638, 36.

Palfreyman, M.G. and B.M. Baron, 1991, Non-competitive NMDA antagonists acting on the glycine site, in: Excitatory Amino Acid Antagonists, ed. B.S. Meldrum (Blackwell Scientific Publications, Oxford) p. 101.

Patel, S., A.G. Chapman, J.L. Graham, B.S. Meldrum and P. Frey, 1990, Anticonvulsant activity of the NMDA antagonists, D(−)4-(3-phosphonopropyl)piperazine-2-carboxylic acid (D-CPP) and D(−)(E)-4-(3-phosphonoprop-2-enyl)piperazine-2-carboxylic acid (D-CPPene) in a rodent and a primate model of reflex epilepsy, Epilepsy Res. 7, 3.

Peterson, S.L., 1992, 7-Chlorokynurenic acid antagonizes the anticonvulsant activity of D-cycloserine in maximal electroshock seizures, Epilepsy Res. 13, 73.

Racine, R.J., 1972, Modification of seizure activity by electrical stimulation. II. Motor seizure, Electroencephalogr. Clin. Neurophysiol. 32, 281.

Rowley, M., P.D. Leeson, G.I. Stevenson, A.M. Moseley, I. Stansfield, I. Sanderson, L. Robinson, R. Baker, J.A. Kemp, G.R. Marshall, A.C. Foster, S. Grimwood, M.D. Tricklebank and K.L. Saywell, 1993, 3-Acyl-4-hydroxyquinolin-2(1*H*)-ones. Systemically active anticonvulsants acting by antagonism at the glycine site of the *N*-methyl-D-aspartate receptor complex, J. Med. Chem. 36, 3386.

Saywell, K., L. Singh, R.J. Oles, C. Vass, P.D. Leeson, B.J. Williams and M.D. Tricklebank, 1991, The anticonvulsant properties in the mouse of the glycine/NMDA receptor antagonist, L-687,414, Br. J. Pharmacol. 102, 66P.

Skolnick, P., J.C.G. Marvizón, B.W. Jackson, J.A. Monn, K.C. Rice and A.H. Lewin, 1989, Blockade of *N*-methyl-D-aspartate induced convulsions by 1-aminocyclopropanecarboxylates, Life Sci. 45, 1647.

Smith, S.E. and B.S. Meldrum, 1992, The glycine-site NMDA receptor antagonist, *R*-(+)-*cis*-β-methyl-3-amino-1-hydroxypyrrolid-2-one, L-687,414 is anticonvulsant in baboons, Eur. J. Pharmacol. 211, 109.