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Synthesis and biological effects of some kynurenic acid analogs

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ABSTRACT

The overactivation of excitatory amino acid receptors plays a key role in the pathomechanism of several neurodegenerative disorders and in ischemic and post-ischemic events. Kynurenic acid (KYNA) is an endogenous product of the tryptophan metabolism and, as a broad-spectrum antagonist of excitatory amino acid receptors, may serve as a protective agent in neurological disorders. The use of KYNA is excluded, however, because it hardly crosses the blood–brain barrier. Accordingly, new KYNA analogs which can readily cross this barrier and exert their complex anti-excitatory activity are generally needed. During the past 6 years, we have developed several KYNA derivatives, among others KYNA amides. These new analogs included one, *N*-(2-*N*,*N*-dimethylaminoethyl)-4-oxo-1*H*-quinoline-2-carboxamide hydrochloride (KYNA-1), that has proved to be neuroprotective in several models.

This paper reports on the synthesis of 10 new KYNA amides (KYNA-1–KYNA-10) and on the effectiveness of these molecules as inhibitors of excitatory synaptic transmission in the CA1 region of the hippocampus. The molecular structure and functional effects of KYNA-1 are compared with those of other KYNA amides. Behavioral studies with these KYNA amides demonstrated that they do not exert significant nonspecific general side-effects. KYNA-1 may therefore be considered a promising candidate for clinical studies.

1. Introduction

It is well established that excitotoxicity is the hallmark of several neurodegenerative processes that result from acute events, such as stroke, traumatic brain injury or bacterial meningitis.[1–4] The background of these processes includes an abnormally high glutamate (Glu) level in the brain. In the course of the pathological events, neurons are damaged or killed by the overactivation of receptors of excitatory amino acids (EAAs) such as *N*-methyl-5-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and kainate.[5] Glu may also play important roles in the pathogenesis of other brain diseases, for example, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, multiple sclerosis, epilepsy and migraine.[6,7]

A reduction of the excitotoxicity, which may be achieved by the blockade of NMDA or other Glu receptors, attenuates some of the pathological symptoms in experimental models of acute and chronic neurodegenerative diseases.[8–11]

Although most of the NMDA receptor antagonists prevent excitotoxicity in cellular and animal models, these drugs have only limited clinical use. A complete NMDA receptor blockade is frequently accompanied by side-effects such as psychosis, nausea, vomiting and a memory impairment, which underlines the key role of the NMDA receptors in normal neuronal processes.[12] However, glycine (Gly) and polyamine site agents, NR2B subunit-specific antagonists and ion channel blockers with lower affinity may come into consideration, as they exert attenuated side-effects.[13] Kynurenic acid (KYNA), an intermediate of the tryptophan metabolism,[14–16] would be a good candidate, because it is a broad-spectrum endogenous antagonist at ionotropic EAA receptors.[17,18] KYNA inhibits NMDA receptors at the Gly-binding site[19] and it can non-competitively inhibit α7-nicotinic acetylcholine receptors,[20] whereby it can also mediate the inhibition of Glu release in the striatum.[21]

However, the use of KYNA as a neuroprotective agent must be excluded because it is barely able to cross the blood–brain barrier (BBB),[22] which rules out its systemic use. This has stimulated the design of several new KYNA analogs, prodrugs or derivatives.[14] A few years ago, we began to create new KYNA amides.[23] These compounds are of promise because they are potentially capable of selective inhibition of the NR2B subunit containing NMDA receptors.[24] In the present work we have designed and synthesized new KYNA amides which the following structural properties: (1) containing a water-soluble side-chain; (2) containing a new

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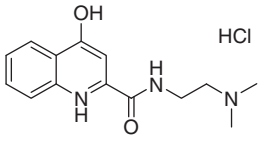
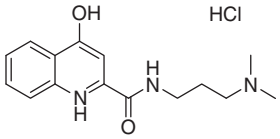
A: KYNA-1



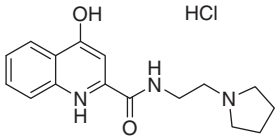
$C_{14}H_{18}ClN_3O_2$ MW: 295.76

B: KYNA-2



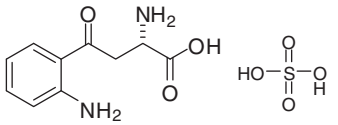
$C_{15}H_{20}ClN_3O_2$ MW: 309.79

C: KYNA-6



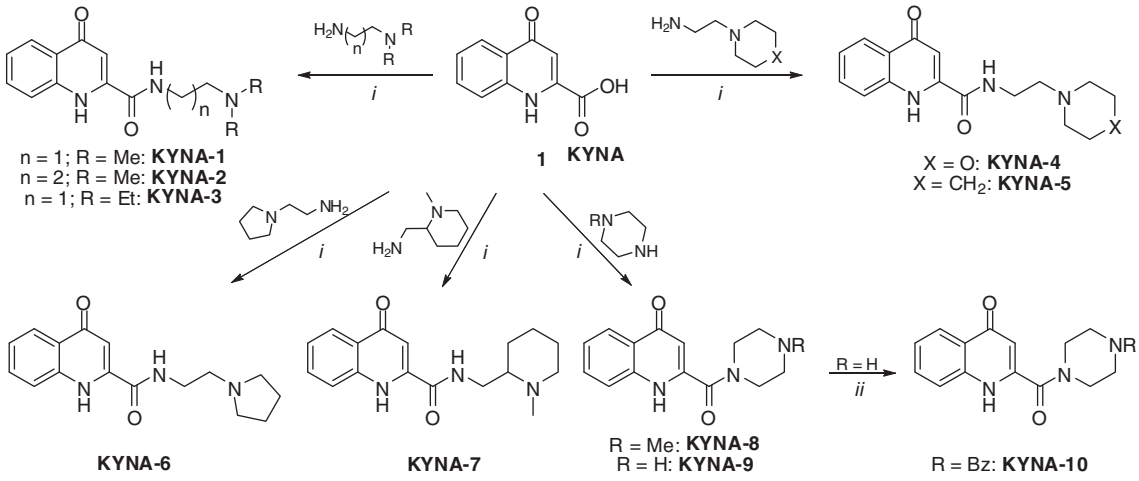
$C_{16}H_{20}ClN_3O_2$ MW: 321.80

D: KYNA-11



$C_{10}H_{14}N_2O_3\ H_2SO_4$ MW: 306.30

Figure 1. Kynurenic acid (KYNA) analogs: (A) KYNA-1, (B) KYNA-2, (C) KYNA-6. (D) KYNA-11 is L-kynurenine sulfate.



Scheme 1. Reagents and conditions: (i) 1-Hydroxybenztriazole-hydrate, *N*,*N*′-di-isopropylcarbodiimide, DMF, rt, 48 h; (ii) Bz–Br, Na_2CO_3, H_2O/toluene, rt, 10 h.

cationic center; (3) with side-chain substitution enhancing penetration into the brain.

A further aim was to compare the effects of some of these analogs in electrophysiological and behavioral experiments.

2. Materials and Methods

2.1. Animals

Experiments were carried out on both mice and rats: in vitro electrophysiological experiments on rats, behavioral studies on mice, and ex vivo electrophysiological experiments on both mice and rats.

First of all, in vitro testing of the 10 new KYNA amides was carried out on hippocampal slices of rats ($n = 10$). Six-week-old C57Bl/6 male mice ($n = 60$) were used in the behavioral experiments, while the ex vivo electrophysiological study involved the use of adult Wistar rats ($n = 47$, 280–300 g) and mice ($n = 12$, 28–36 g). The animals were kept under controlled environmental conditions at 22 ± 2 °C under a 12-h light/dark cycle. Food and water were available *ad libitum*. The local Animal Ethics Committee had approved all experiments. The care and use of the

experimental animals were in full accordance with the 86/609/EEC directive.

2.2. Drugs

L-Kynurenine sulfate (KYNA-11; Fig. 1D) and probenecid (PROB) were from Sigma–Aldrich (Steinheim, Germany), and were administered by the intraperitoneal (ip) route in dosages of 200 mg/kg.

New KYNA amide derivatives (KYNA-1–KYNA-10) were prepared from KYNA and the appropriate amine, by using *N*,*N*′-diisopropylcarbodiimide as coupling reagent according to Scheme 1 (for details, see Section 2.3).

Among the new KYNA amides,[23] *N*-(2-*N*,*N*-dimethylaminoethyl)-4-oxo-1*H*-quinoline-2-carboxamide hydrochloride (KYNA-1) proved to be outstandingly effective as a neuroprotective agent.

KYNA and the 10 new KYNA amides were subjected to electrophysiological testing (in vitro studies). These studies revealed that KYNA and only one of the new molecule (KYNA-1) displayed inhibitory effects, KYNA-1 and two other relatively non-effective KYNA amides (KYNA-2 and KYNA-6) were chosen to carry out behavioral experiments. The electrophysiological and neuroprotective effects of L-kynurenine sulfate (KYNA-11) were studied earlier,[9–11,25] and for purposes of comparison, KYNA-11 was therefore also included in the present studies.

The mice received ip injections of KYNA-1 (Fig. 1A; 200 mg/kg/day, in a volume of 5 ml/kg, dissolved in distilled water, the pH of which was adjusted to 6.5 with 1 N NaOH). For comparison, KYNA-2, KYNA-6 (Fig. 1B,C) in equimolar dosages (likewise in a volume of 5 mL/kg, dissolved in distilled water, the pH of which was adjusted to 6.5 with 1 N NaOH) or the vehicle (0.1 M PBS, in a volume of 5 ml/kg) was administered at the same time each day from 6 weeks of age.

In behavioral experiments, the mice were treated once (in acute experiments) or for 9 days (in chronic experiments) according to the regime detailed above. Acute electrophysiological experiments were carried out on both rats and mice.

2.3. Experimental procedures

2.3.1. Synthesis

Melting points were determined on a Kofler micro melting apparatus and are uncorrected. Elemental analyses were performed with a Perkin-Elmer 2400 CHNS elemental analyzer. Merck Kieselgel 60F_{254} plates were used for TLC. The ^{1}H NMR spectra were recorded in D_2O solution in 5 mm tubes, at room temperature, on a Bruker *Avance* DRX400 spectrometer at 400.13 (^{1}H) and 100.61 (^{13}C) MHz, with the deuterium signal of the solvent as the lock and TMS as internal standard.

2.4. General method for the synthesis of KYNA amides (KYNA-1–KYNA-10)

To a stirred solution of KYNA (0.40 g; 2.13 mmol) in 30 mL of DMF, 1-hydroxy-benztriazole hydrate (0.28 g; 2.13 mmol) and the appropriate amine (2.13 mmol) were added. The mixture was stirred at 0 °C for 30 min and 0.4 mL (2.34 mmol) of *N*,*N*′-diisopropylcarbodiimide was then added. The reaction mixture was stirred for an additional 48 h at room temperature. After evaporation of the solvent, the crude product was purified by column chromatography, with MeOH as eluent. The free base was suspended in EtOH (10 mL) and 1 mL of ethanolic HCl solution (22%) was next added. The mixture was stirred for 0.5 h, after which Et_2O (30 mL) was added. The precipitate was filtered off and washed with Et_2O (2 × 20 mL).

2.4.1. *N*-(2-*N*,*N*-Dimethylaminoethyl)-4-oxo-1*H*-quinoline-2-carboxamide hydrochloride (KYNA-1)

0.54 g (86%); mp 178–179 °C. ^{1}H NMR (D_2O); 3.00 (6H, s, N–CH_3); 3.47 (2H, t, *J* = 5.8 Hz, CH_2); 3.87 (2H, t, *J* = 5.7 Hz, CH_2); 6.90 (1H, s, C3-*H*); 7.58 (1H, t, *J* = 7.2 Hz, Ar-*H*); 7.81–7.86 (2H, m, Ar-*H*); 8.18 (1H, d, *J* = 8.1 Hz, Ar-*H*)

2.4.2. *N*-(3-*N*,*N*-Dimethylaminopropyl)-4-oxo-1*H*-quinoline-2-carboxamide hydrochloride (KYNA-2)

0.51 g (77%); mp 149–150 °C. ^{1}H NMR (D_2O); 2.10 (2H, t, *J* = 8.2 Hz, CH_2); 2.92 (6H, s, N–CH_3); 3.25 (2H, t, *J* = 7.9 Hz, CH_2); 3.55 (2H, t, *J* = 6.7 Hz, CH_2); 6.87 (1H, s, C3-*H*); 7.57 (1H, t, *J* = 7.4 Hz, Ar-*H*); 7.83–7.86 (2H, m, Ar-*H*); 8.18 (1H, d, *J* = 8.2 Hz, Ar-*H*)

2.4.3. *N*-(2-*N*,*N*-Diethylaminoethyl)-4-oxo-1*H*-quinoline-2-carboxamide hydrochloride (KYNA-3)

0.55 g (80%); mp 192–194 °C. ^{1}H NMR (D_2O); 1.34 (6H, t, *J* = 7.3 Hz, N–CH_2–CH_3); 3.30–3.38 (4H, m, N–CH_2–CH_3); 3.47 (2H, t, *J* = 6.4 Hz, CH_2); 3.86 (2H, t, *J* = 6.4 Hz, CH_2); 6.89 (1H, s, C3-*H*); 7.57 (1H, t, *J* = 7.6 Hz, Ar-*H*); 7.59–7.87 (2H, m, Ar-*H*); 8.16 (1H, d, *J* = 8.3 Hz, Ar-*H*)

2.4.4. *N*-(2-*N*-Morpholinoethyl)-4-oxo-1*H*-quinoline-2-carboxamide hydrochloride (KYNA-4)

0.54 g (75%); mp 230–232 °C. ^{1}H NMR (D_2O); 3.30 (2H, br s, CH_2), 3.51 (2H, t, *J* = 6.12 Hz, CH_2); 3.67 (2H, br s, CH_2); 3.90 (2H, t, *J* = 5.9 Hz, CH_2); 3.91 (2H, br s, CH_2); 4.13 (2H, br s, CH_2); 6.89 (1H, s, C3-*H*); 7.57 (1H, t, *J* = 7.4 Hz, Ar-*H*); 7.86 (1H, d, *J* = 8.4 Hz, Ar-*H*); 8.19 (1H, t, *J* = 7.1 Hz, Ar-*H*)

2.4.5. *N*-(2-*N*-Piperidinylethyl)-4-oxo-1*H*-quinoline-2-carboxamide hydrochloride (KYNA-5)

0.58 g (81%); mp 206–208 °C. ^{1}H NMR (D_2O); 1.48–1.99 (6H, m), 3.02 (2H, t, *J* = 12.5 Hz, CH_2); 3.41 (2H, t, *J* = 6.2 Hz, CH_2); 3.63–3.67 (2H, m, CH_2); 3.86 (2H, t, *J* = 6.2 Hz, CH_2); 6.88 (1H, s, C3-*H*); 7.57 (1H, t, *J* = 7.4 Hz, Ar-*H*); 7.81–7.89 (2H, m, Ar-*H*); 8.19 (1H, d, *J* = 8.2 Hz, Ar-*H*)

2.4.6. *N*-(2-*N*-Pyrrolidinylethyl)-4-oxo-1*H*-quinoline-2-carboxamide hydrochloride (KYNA-6)

0.56 g (82%); mp 185–187 °C. ^{1}H NMR (D_2O); 2.02–2.19 (4H, m), 3.15–3.20 (2H, m CH_2); 3.52 (2H, t, *J* = 6.0 Hz, CH_2); 3.74–3.77 (2H, m, CH_2); 3.85 (2H, t, *J* = 6.0 Hz, CH_2); 6.89 (1H, s, C3-*H*); 7.56 (1H, t, *J* = 7.4 Hz, Ar-*H*); 7.79–7.86 (2H, m, Ar-*H*); 8.17 (1H, d, *J* = 8.2 Hz, Ar-*H*)

2.4.7. *N*-[2-(2-*N*-Methylpiperidinyl)methyl]-4-oxo-1*H*-quinoline-2-carboxamide hydrochloride (KYNA-7)

0.55 g (77%); mp 218–220 °C. ^{1}H NMR (D_2O); 1.55–2.32 (6H, m), 3.02 (3H, s); 3.11–3.95 (5H, m); 6.85 (1H, s, C3-*H*); 7.53 (1H, t, *J* = 7.4 Hz, Ar-*H*); 7.77–7.82 (2H, m); 8.17 (1H, d, *J* = 8.2 Hz, Ar-*H*)

2.4.8. *N*-(4-Methylpiperazinyl)-4-oxo-1*H*-quinoline-2-carboxamide hydrochloride (KYNA-8)

0.60 g (82%); mp 192–194 °C. ^{1}H NMR (D_2O); 2.92 (3H, s), 3.12–3.97 (8H, m); 6.65 (1H, s, C3-*H*); 7.51 (1H, t, *J* = 7.4 Hz, Ar-*H*); 7.67 (1H, d, *J* = 8.4 Hz, Ar-*H*); 7.80 (1H, t, *J* = 7.1 Hz, Ar-*H*); 8.12 (1H, d, *J* = 8.2 Hz, Ar-*H*)

2.4.9. *N*-(Piperazinyl)-4-oxo-1*H*-quinoline-2-carboxamide hydrochloride (KYNA-9)

0.47 g (67%); mp >350 °C. ^{1}H NMR (D_2O); 3.21–3.98 (8H, m), 6.51 (1H, s, C3-*H*); 7.51 (1H, t, *J* = 7.4 Hz, Ar-*H*); 7.67 (1H, d,

J = 8.4 Hz, Ar-*H*); 7.80 (1H, t, J = 7.1 Hz, Ar-*H*); 8.15 (1H, d, J = 8.2 Hz, Ar-*H*)

2.4.10. *N*-(4-Benzylpiperazinyl)-4-oxo-1*H*-quinoline-2-carboxamide hydrochloride (KYNA-10)

To a stirred solution of KYNA-9 (0.165 g (0.5 mmol) and Et_3N (0.126 g, 1.25 mmol) in 10 mL of EtOH, benzyl bromide (0.073 g, 0.55 mmol) dissolved in 5 mL of EtOH was added. The reaction mixture was stirred for 10 h at room temperature. After the evaporation of the solvent, the crude product was purified by column chromatography, with MeOH as eluent. The process to obtain the hydrochloride salt of KYNA-10 was as described above.

0.118 g (62%); mp 213–215 °C. ^{1}H NMR (D_2O); 3.15–4.17 (8H, m); 4.38 (2H, s); 6.55 (1H, s, C3-*H*); 7.42–7.61 (6H, m, Ar-*H*); 7.70 (1H, d, J = 8.4 Hz, Ar-*H*); 7.82 (1H, t, J = 7.1 Hz, Ar-*H*); 8.17 (1H, d, J = 8.2 Hz, Ar-*H*)

2.5. Behavioral studies

2.5.1. Open-field test

The spontaneous locomotor and exploration activities of the mice were examined 2 h after the first treatment (acute), and then on the last day (day 9) of treatment (chronic). The tests were performed at the same time of day so as to avoid possible changes due to the diurnal rhythm. Each mouse was placed at the center of a black box (48 × 48 × 40 cm) and its behavior during a 5-min period was recorded with the aid of Conducta 1.0 software (Experimetria Ltd., Budapest, Hungary). The ambulation time, the mean velocity, the local time and the number of rearings were evaluated. For a few rats, the corresponding parameters were also tested under acute conditions at the same dosages, but the complete experiments were performed on mice, as described above.

2.6. Electrophysiology

2.6.1. In vitro electrophysiology

Coronal slices (400 μm; 4 slices/animal), prepared from the middle part of the rats' hippocampi with a vibratome (Campden Instruments, UK), were kept in an ice-cold artificial cerebrospinal solution (ACSF) composed of (in mM): 130 NaCl, 3.5 KCl, 1 NaH_2PO_4, 24 $NaHCO_3$, 1 $CaCl_2$, 3 $MgSO_4$ and 10 D-glucose (all from Sigma, Germany), saturated with 95% O_2 + 5% CO_2. The slices then were transferred to a Haas recording chamber and incubated at room temperature for 1 h to allow the slices to recover in the solution used for recording (differing only in that it contained 3 mM $CaCl_2$ and 1.5 mM $MgSO_4$). The flow rate of the recording solution was 1.5–2 mL/min and the experiments were performed at 34 °C. In order to achieve orthodromic stimulation of the Schaffer collateral/commissural pathway, the stimulating electrode (either a micropipette pulled from a theta glass or a bipolar concentric stainless steel electrode developed by Neuronelektrod Ltd, Hungary) was placed in the stratum radiatum between areas CA1 and CA2. The stimulus intensity was adjusted to between 30 and 60 μA (constant current, 0.2-ms pulses delivered at 0.05 Hz) to evoke the half-maximal response.

Field excitatory postsynaptic potentials (fEPSPs) were recorded with glass micropipettes with a resistance of 1.5 MΩ filled with ACSF. The recordings were amplified with a neutralized, high-input-impedance preamplifier and filtered (1 Hz–3 kHz). The fEPSPs were digitized (AIF-03, Experimetria Ltd. Hungary), acquired at a sampling rate of 10 kHz, saved to a PC and analyzed off-line with Origin 6.0 software (OriginLab Corporation, USA).

KYNA and the new KYNA amides were dissolved in ACSF and presented at 16 μM to the hippocampal slices via perfusion for 30 min.

2.6.2. Ex vivo electrophysiology

The rats were anesthetized with urethane (1.25 g/kg, ip). In some cases, the tail vein was catheterized. In most of the experiments, the drugs were administered ip, through a syringe implanted at the beginning of the experiments. For recordings in area CA1, a 2–3-mm diameter hole was drilled over the dorsal hippocampus (3.0–3.8 mm posterior to the bregma and 1.8–2.3 mm lateral to the sagittal suture) and the recording electrode was lowered 2.2–2.8 mm from the cortical surface. Contralaterally, a 1–2-mm hole was drilled for the CA3 stimulating electrode (3.7 mm posterior to the bregma, and 3.3 mm lateral to the sagittal suture: the final electrode depth was 3.8 mm below the dura). The electrodes were lowered and the final position was adjusted so that the maximal CA1 population spike was obtained in response to contralateral CA3 stimulation (Fig. 3, inset). The sites in areas CA1 and CA3 were confirmed histologically. Responses to a range of stimulus intensities were recorded under control conditions so as to produce an input-output curve by changing the duration (10–100 μs), using current (up to 50 μA) square pulses. Stimuli were triggered at low frequency (0.05 Hz). Response stability was monitored for 30 min prior to drug administration. KYNA derivatives were administered in the same dosage as in the behavioral experiments. The mice were anesthetized with urethane (2.4 g/kg, ip).

We earlier carried out a number of electrophysiologial experiments on rats.[11,25,26] Accordingly, for the present results to be fully comparable with the previous ones, we detail here only those obtained in rats; otherwise, the results obtained in mice were quite similar to those in rats.

2.7. Statistical analysis

Population spikes evoked by CA3 stimulation were measured from peak to peak (see Scharfman and Goodman, 1998, and Fig. 3, inset). To test the statistical significance of the differences in amplitudes of the responses recorded during the control and drug-treatment periods, one-way ANOVA following the *post hoc* Bonferroni test was applied. Values of $p < 0.05$ were considered significant. For statistical evaluation of the data in the behavioral tests, one-way ANOVA was used, followed by Fisher's LSD *post hoc* test with StatView 4.53 for Windows software (Abacus Concept Inc., Berkeley, CA, USA). These data were expressed as means ± standard error of the mean (S.E.M.). Statistical significances in the behavioral tests were taken as $^{*}p \leqslant 0.5$ and $^{**}p \leqslant 0.01$.

3. Results

Following the in vitro electrophysiological studies on KYNA and the 10 new KYNA amides, 4 molecules were further studied in ex vivo and behavioral experiments. They were chosen for the following reasons: KYNA-1 was the only new amide effective as an inhibitor in the in vitro study, and it had proved to be outstandingly effective in earlier studies of neuroprotection;[27–30] KYNA-2 is structurally very similar to KYNA-1, differing only in containing an additional –CH_2- group in the side-chain; KYNA-6 and KYNA-11 (KYNA-11 is identical with L-kynurenine sulfate, which was already tested before[9,25]) differ appreciably in structure not only from each other, but also from KYNA-1 and KYNA-2 (see Fig. 1).

3.1. Electrophysiology

3.1.1. In vitro study

When the Schaffer collaterals were stimulated, and the fEPSPs and their amplitudes were recorded in the pyramidal layer of region CA1 of the hippocampus before, during and after the administration of KYNA or KYNA-1, micromolar concentrations of these

compounds resulted in decreases in the amplitudes of the fEPSPs. KYNA-1 proved to be an even more effective inhibitor than KYNA itself (Fig. 2). However, non of the other 9 new KYNA amides displayed a similar effect. None of them reduced the amplitudes of the fEPSPs. Indeed, KYNA-6 slightly increased the amplitudes of the fEPSPs, while KYN-2 had no effect at all (Fig. 2). The same was true for the other new KYNA amides (data not presented).

3.1.2. Ex vivo study

The blocking effects of KYNA analogs on NMDA receptors can be tested adequately also via the amplitudes of population spikes recorded in area CA1 of the hippocampus.

The responses of the pyramidal cells in area CA1 to contralateral CA3 stimulation were tested before and after the injection of the vehicle or the KYNA analog. For comparison, KYNA-11 (L-kynurenine sulfate) (200 mg/kg) was administered ip together with PROB (200 mg/kg) to one group of animals; this combination is well known to result in a gradual decrease in amplitude of the population spikes[25] (Fig. 3A).

The electrophysiological effects of these new KYNA analogs on the responses of the area CA1 pyramidal cells to contralateral CA3 stimulation have not been studied previously. In comparison with KYNA-11 + PROB, KYNA-1 proved similarly or even more effective in reducing the amplitude of the population spikes. KYNA-1 started to reduce the amplitude immediately after its administration, without a latency period, The amplitude decreased to 75% of the control level within 60 min, and to 70% within 90 min (Fig. 3B), whereas in the case of KYNA-11 + PROB it had not reached this level even after 180 min (Fig. 3A).

In contrast with KYNA-1, the other analogs did not reduce the population spike amplitudes significantly, as illustrated by the data on KYNA-2 (Fig. 3C), despite its similar structure to that of KYNA-1. Similarly, negative results were observed after KYNA-6 administration (not shown).

3.2. Behavioral studies

Open-field observations were carried out on five groups of animals: a control group (injected with saline), and KYNA-1, KYNA-2,

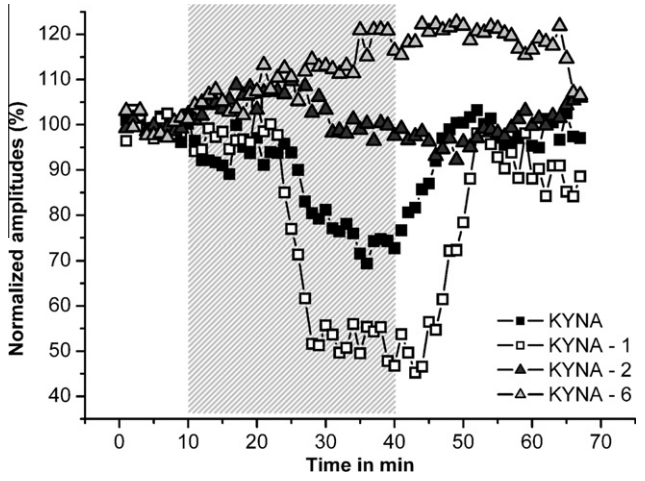


Figure 2. Comparison of suppressor effects of KYNA and some of its new analogs. KYNA (16 μM) induced a definitive decrease (to 70–75%) in the amplitudes of the fEPSPs. However, KYNA-1 (16 μM) proved to be even more effective in decreasing the amplitudes, to 50–55% of the control level. None of the other new KYNA amides showed a similar effect at this concentration. As examples, the lack of an inhibitory effect of KYNA-2 and KYNA-6 is presented. In this Figure, the amplitudes are normalized to the amplitudes in the control period (10 min). The illustrated results are representative examples; similar findings were observed for all four slices tested for each compound. The gray-striped area indicates the period of administration of KYNA or KYNA amide.

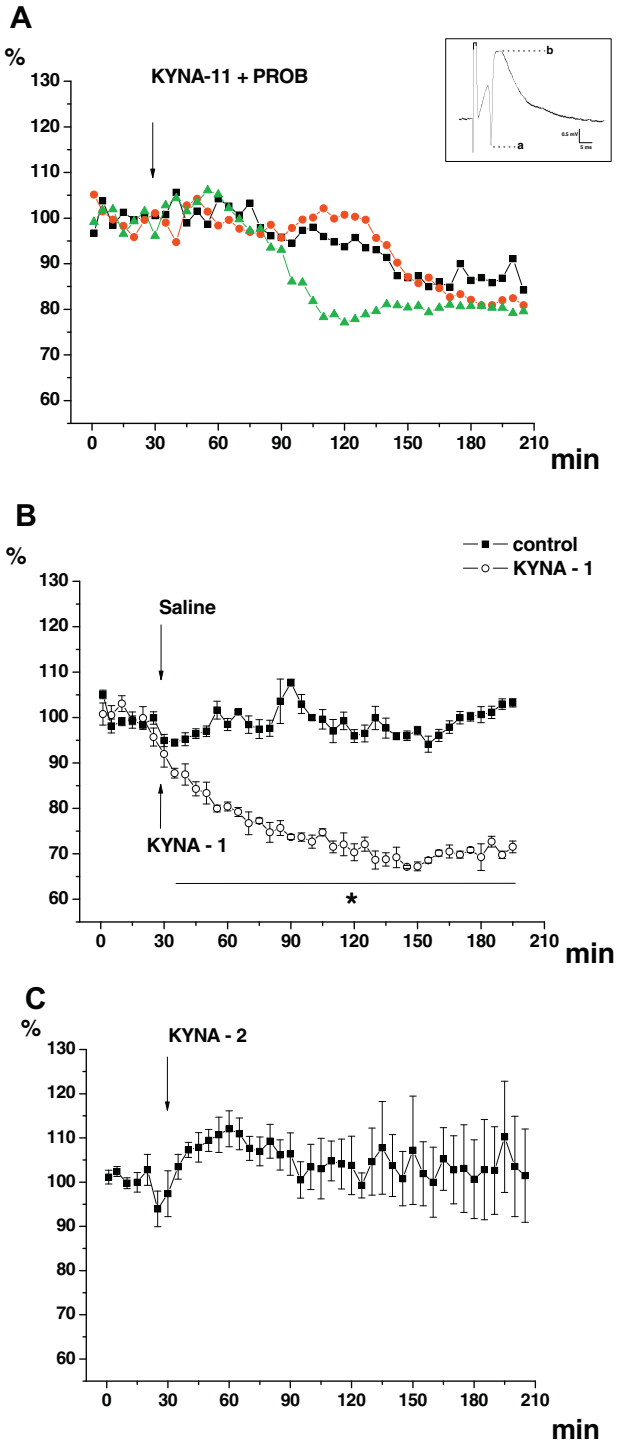


Figure 3. Examples of the effects of the administered compounds on the population spike amplitudes recorded in area CA1. (A) Examples of the inhibitory effect of KYNA-11 + PROB, which resulted in decreases in amplitude with a long delay. Lines with circles, squares or triangles relate to individual experiments carried out on 3 rats. Each circle, triangle and square represents an average of 10 potentials. Inset: an example of evoked spikes whose amplitude was measured between peaks *a* and *b*. Calibration: 0.5 mV; 5 ms. (B) KYNA-1 injection resulted in an immediate decrease in amplitude of the CA1 population spikes (open circles), while the saline injection did not induce any significant change in amplitude (filled squares). Each square and circle denotes the mean ± S.D. for 5 × 10 potentials observed in 5 animals. After the 30-min recordings, the animals received a saline or KYNA-1 injection ip (arrows). *The horizontal line indicates the time range in which the differences between the saline and KYNA-1 effects were significant (p <0.005). (C) Following KYNA-2 injection, hardly any significant change in amplitude was observed. Each square denotes the mean ± S.D. for 3 × 10 potentials observed in 3 animals.

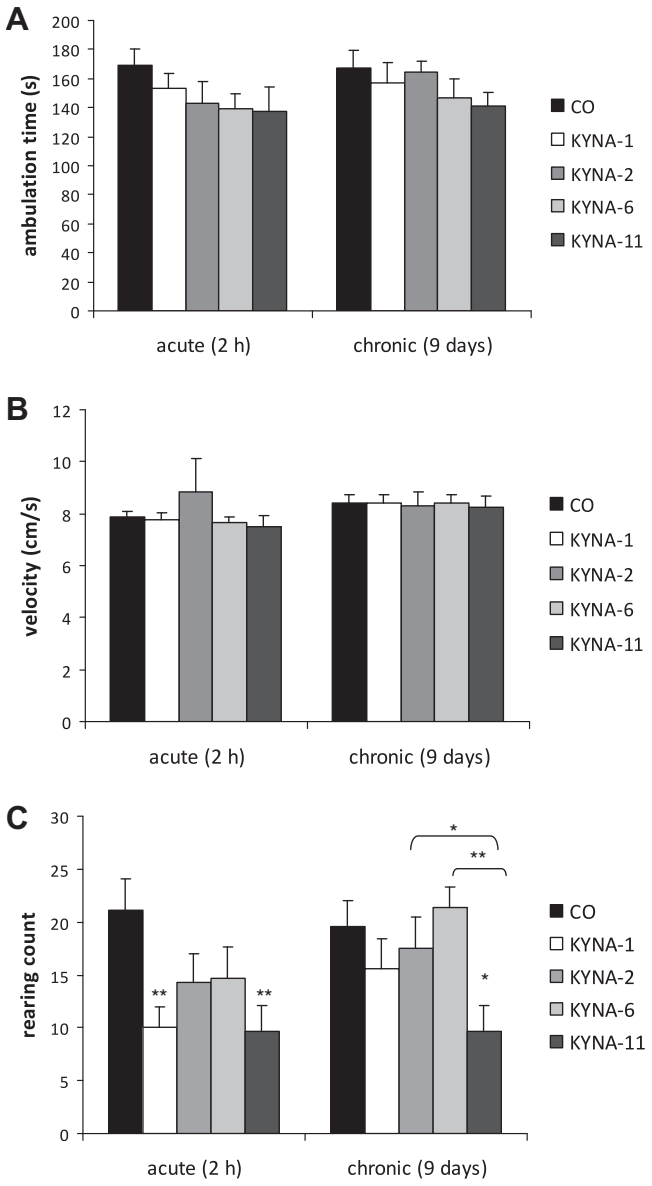


Figure 4. Observations in the open-field arena. (A) Time spent in ambulation. No difference was found between the control and treated groups in either the acute or the chronic experiments. (B) Velocity of the movements of the mice. No difference was found between the control and treated groups in either the acute or the chronic experiments. (C) Number of rearings during 5-min observation periods. KYNA-1 and KYNA-11 significantly reduced the number of rearings in the acute experiments, as did KYNA-11 in the chronic experiments. If not labeled, the comparisons were made with the controls. CO: control.

KYNA-6 or KYNA-11-treated groups. In acute behavioral experiments, the animals were treated with saline (CO) or KYNA amide analog 2 h prior to the behavioral observations, while chronic treatment was administered on nine successive days, with observations on day 9, 2 h after the final injection.

The animals treated with one or other KYNA amide analog did not differ greatly in behavior from those that received the saline vehicle. The posture and activity were quite similar in each group. The ambulation time, the mean velocity and the number of rearings did not exhibit highly significant differences in most cases (Fig. 4). Although the ambulation time was somewhat decreased after KYNA-11 administration, the changes were not significant (Fig. 4A). The mean velocities values were nearly the same in each group (Fig. 4B). Significant changes in performance were observed only in the numbers of rearings. Both KYNA-1 and KYNA-11 decreased the number of rearings in the acute experiments, and KYNA-11 did so in the chronic experiments too (Fig. 4C).

Similar behavioral observations were made on rats, but those results are not detailed here.

4. Discussion

KYNA is one of the few known endogenous NMDA receptor inhibitors.[31] It can influence important physiological and pathological processes. The available experimental data and theoretical considerations suggest that KYNA, as an EAA receptor antagonist, could exert therapeutic effects in neurological disorders,[32–37] but its use as a neuroprotective agent is rather restricted because it has only limited ability to cross the BBB.

During the past decade, we have made great efforts to develop new neuroprotective agents and strategies based on kynurenines. We initially tested L-kynurenine sulfate (KYNA-11) as a neuroprotective compound which crosses the BBB and which can be transformed to the EAA receptor antagonist KYNA by the enzyme kynurenine aminotransferase, located primarily in the glia,[38] which has the ability to take up KYNA-11 and to release KYNA.[39,40] We achieved good results as concerns neuroprotection with KYNA-11,[9–11,25] but its use (even in preclinical studies) is limited, primarily because it is converted to the EAA receptor agonist quinolic acid (QUIN) in high proportion after systemic administration. QUIN may cross the BBB, especially in a diseased organism, and it may induce an opposite effect. We further found that the administration of KYNA-11 in a dosage of 300 mg/kg ip, together with PROB, induces a transient facilitation of the population spike amplitudes, and its inhibitory effect commences only 60–80 min after administration.[25] Similarly, the inhibitory effect of KYNA-11 + PROB was observed only after a long delay when it was administered in a dosage of 200 mg/kg ip. The long delay of its inhibitory effect and its relatively moderate effectiveness are disadvantageous if the aim is an immediate reduction of the excitotoxicity induced by excess Glu, for example, after a stroke or a traumatic brain injury.

This situation turned our interest to KYNA derivatives,[23] one of which (KYNA-1) turned out to be effective as a neuroprotective agent. Its effects have already been tested in several models,[27–30,35,41] but its influence on the NMDA-mediated synaptical function has not been studied previously.

As the first step, KYNA-1 was studied in an in vitro electrophysiological experiment together with the other 9 newly synthesized KYNA amides and with KYNA itself. The molecular structure of KYNA-2 is similar to that of KYNA-1 (differing in a $-CH_2-$ group), while the others are structurally quite different. KYNA-1 was the only KYNA amide which reduced the amplitude of the fEPSPs, which suggests that KYNA-1 exerts an inhibitory effect on the synaptic transmission mediated by NMDA receptors.

In the ex vivo experiments, KYNA-1 (200 mg/kg ip) displayed inhibition of the population spike amplitude without any delay, without any transient facilitation. Its inhibitory effect attained its maximum within 1 h and surpassed that of KYNA-11. It was interesting that, despite its structure being similar to that of KYNA-1, KYNA-2 did not exhibit any significant effect on the population spike amplitudes.

It was also important that KYNA-1, which had proved neuroprotective in several models (cited above), and partially inhibited NMDA-mediated synaptical transmission in the hippocampus, did not induce significant changes in the behavior of the tested animals. This was true for both rats and mice, but we present here only the data observed on mice. The results confirmed that none of the studied KYNA derivatives induced major changes in the

behavior of these animals. Only the number of rearings was reduced somewhat after KYNA-1 and KYNA-11 administration.

There is accumulating evidence that Glu-induced excitotoxicity is mainly mediated by NMDA receptor channels.[42,43] From clinical aspects, the massive blockade of NMDA receptors would result in unacceptable symptoms.[12] Accordingly, Gly and polyamine site agents, NR2B subunit specific antagonists and ion channel blockers with lower affinity may come into consideration, as they exert acceptable side-effects.[13] KYNA would theoretically be a good candidate, because it is a broad-spectrum endogenous antagonist on ionotropic EAA receptors.[17,18] KYNA can inhibit NMDA receptors at the Gly-binding sites[19] and it can non-competitively inhibit α7-nicotinic acetylcholine receptors.[20] Blockade of these nicotinic receptors can also mediate the inhibition of Glu release in the striatum.[21] In addition, in the past few years it has been proposed that KYNA is the endogenous ligand of the G-protein coupled receptor 35 (GPR35) too, which is highly expressed in the gastrointestinal tract, but is also present in the central nervous system.[44] It has recently been suggested that KYNA effects in the brain are mediated, at least partially, by GRP35 activation and reduced Glu release.[45]

However, from pharmacological considerations, KYNA itself has several disadvantages, which rule out its systemic use. Accordingly, several new KYNA analogs or prodrugs have been designed.[14] An important group of these compounds comprises the KYNA amides, which are excellent candidates,[23] because they are known to be capable of the selective inhibition of the NR2B subunit containing NMDA receptors.[24] The most prominent of these KYNA amides is KYNA-1, the beneficial effects of which were reported earlier,[27–30,40] and whose partial inhibitory effect on the hippocampal pyramidal neurons in area CA1 was demonstrated in the present study. Moreover, as KYNA-1 did not significantly influence the behavioral performance in the open-field arena, KYNA-1 treatment does not appear to have any appreciable side-effects.

In summary, we believe that KYNA-1 is an effective neuroprotectant and may therefore be a promising candidate for clinical trials.

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References and notes

1. Foster, A. C.; White, R. J.; Schwarcz, R. *J. Neurochem.* **1986**, *47*, 23.
2. Castillo, J.; Davalos, A.; Naveiro, J.; Noya, M. *Stroke* **1996**, *27*, 1060.
3. Zauner, A.; Bullock, R.; Kuta, A. J.; Woodward, J.; Young, H. F. *Acta Neurochir. Suppl.* **1996**, *67*, 40.
4. Johnston, M. V.; Trescher, W. H.; Ishida, A.; Nakajima, W. *Pediatr. Res.* **2001**, *49*, 735.
5. Olney, J. W. *Science* **1969**, *164*, 719.
6. Choi, D. W.; Rothman, S. M. *Annu. Rev. Neurosci.* **1990**, *13*, 171.
7. Koutsilieri, E.; Riederer, P. *Parkinsonism Relat. Disord.* **2007**, *13*(Suppl 3), S329.
8. Gardoni, F.; Di Luca, M. *Eur. J. Pharmacol.* **2006**, *545*, 2.
9. Gigler, G.; Szénási, G.; Simó, A.; Lévay, G.; Hársing, L. G., Jr.; Sas, K.; Vécsei, L.; Toldi, J. *Eur. J. Pharmacol.* **2007**, *564*, 116.
10. Robotka, H.; Sas, K.; Ágoston, M.; Rózsa, E.; Szénási, G.; Gigler, G.; Vécsei, L.; Toldi, J. *Life Sci.* **2008**, *82*, 915.
11. Sas, K.; Robotka, H.; Rózsa, E.; Ágoston, M.; Szénási, G.; Gigler, G.; Marosi, M.; Kis, Z.; Farkas, T.; Vécsei, L.; Toldi, J. *Neurobiol. Dis.* **2008**, *32*, 302.
12. Muir, K. W.; Lees, K. R. *Stroke* **1995**, *26*, 503.
13. Muir, K. W. *Curr. Opin. Pharmacol.* **2006**, *6*, 53.
14. Schwarcz, R. *Curr. Opin. Pharmacol.* **2004**, *4*, 12.
15. Vécsei, L., Ed.; Nova Biomedical Books: New York, 2005.
16. Robotka, H.; Toldi, J.; Vécsei, L. *Future Med.: Future Neurol.* **2008**, *3*, 169.
17. Perkins, M. N.; Stone, T. W. *Brain Res.* **1982**, *247*, 184.
18. Birch, P. J.; Grossman, C. J.; Hayes, A. G. *Eur. J. Pharmacol.* **1988**, *151*, 313.
19. Kessler, M.; Terramani, T.; Lynch, G.; Baudry, M. *J. Neurochem.* **1989**, *52*, 1319.
20. Hilmas, C.; Pereira, E. F.; Alkondon, M.; Rassoulpour, A.; Schwarcz, R.; Albuquerque, E. X. *J. Neurosci.* **2001**, *21*, 7463.
21. Marchi, M.; Risso, F.; Viola, C.; Cavazzani, P.; Raiteri, M. *J. Neurochem.* **2002**, *80*, 1071.
22. Fukui, S.; Schwarcz, R.; Rapoport, S. I.; Takada, Y.; Smith, Q. R. *J. Neurochem.* **1991**, *56*, 2007.
23. Fülöp, F.; Szatmári, I.; Vámos, E.; Zádori, D.; Toldi, J.; Vécsei, L. *Curr. Med. Chem.* **2009**, *16*, 4828.
24. Borza, I.; Kolok, S.; Galgóczy, K.; Gere, A.; Horváth, C.; Farkas, S.; Greiner, I.; Domany, G. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 406.
25. Németh, H.; Robotka, H.; Kis, Z.; Rózsa, E.; Janáky, T.; Somlai, C.; Marosi, M.; Farkas, T.; Toldi, J.; Vécsei, L. *Neuropharmacology* **2004**, *47*, 916.
26. Robotka, H.; Németh, H.; Somlai, C.; Vécsei, L.; Toldi, J. *Eur. J. Pharmacol.* **2005**, *513*, 75.
27. Vámos, E.; Fejes, A.; Koch, J.; Tajti, J.; Fülöp, F.; Toldi, J.; Párdutz, A.; Vécsei, L. *Headache* **2010**, *50*, 834.
28. Vámos, E.; Párdutz, A.; Varga, H.; Bohár, Z.; Tajti, J.; Fülöp, F.; Toldi, J.; Vécsei, L. *Neuropharmacology* **2009**, *57*, 425.
29. Marosi, M.; Nagy, D.; Farkas, T.; Kis, Z.; Rózsa, É.; Robotka, H.; Fülöp, F.; Vécsei, L.; Toldi, J. *J. Neural. Transm.* **2010**, *117*, 183.
30. Zádori, D.; Nyiri, G.; Szőnyi, A.; Szatmári, I.; Fülöp, F.; Toldi, J.; Freund, T. F.; Vécsei, L.; Klivényi, P. *J. Neural. Transm.* **2010**. PMID: 21194001.
31. Swartz, K. J.; During, M. J.; Freese, A.; Beal, M. F. *J. Neurosci.* **1990**, *10*, 2965.
32. Stone, T. W. *Expert Opin. Investig. Drugs* **2001**, *10*, 633.
33. Stone, T. W.; Mackay, G. M.; Forrest, C. M.; Clark, C. J.; Darlington, L. G. *Clin. Chem. Lab. Med.* **2003**, *41*, 852.
34. Németh, H.; Toldi, J.; Vécsei, L. *Curr. Neurovasc. Res.* **2005**, *2*, 249.
35. Németh, H.; Toldi, J.; Vécsei, L. *J. Neural Transm. Suppl.* **2006**, 285.
36. Zádori, D.; Klivényi, P.; Vámos, E.; Fülöp, F.; Toldi, J.; Vécsei, L. *J. Neural. Transm.* **2009**, *116*, 1403.
37. Kincses, Z. T.; Toldi, J.; Vécsei, L. *J. Cell. Mol. Med.* **2010**, *14*, 2045.
38. Du, F.; Schmidt, W.; Okuno, E.; Kido, R.; Kohler, C.; Schwarcz, R. *J. Comp. Neurol.* **1992**, *321*, 477.
39. Turski, W. A.; Gramsbergen, J. B.; Traitler, H.; Schwarcz, R. *J. Neurochem.* **1989**, *52*, 1629.
40. Speciale, C.; Schwarcz, R. *J. Neurochem.* **1990**, *54*, 156.
41. Knyihar-Csillik, E.; Mihály, A.; Krisztin-Peva, B.; Robotka, H.; Szatmári, I.; Fülöp, F.; Toldi, J.; Csillik, B.; Vécsei, L. *Neurosci. Res.* **2008**, *61*, 429.
42. Heng, M. Y.; Detloff, P. J.; Wang, P. L.; Tsien, J. Z.; Albin, R. L. *J. Neurosci.* **2009**, *29*, 3200.
43. Wee, X. K.; Ng, K. S.; Leung, H. W.; Cheong, Y. P.; Kong, K. H.; Ng, F. M.; Soh, W.; Lam, Y.; Low, C. M. *Br. J. Pharmacol.* **2010**, *159*, 449.
44. Wang, J.; Simonavicius, N.; Wu, X.; Swaminath, G.; Reagan, J.; Tian, H.; Ling, L. *J. Biol. Chem.* **2006**, *281*, 22021.
45. Cosi, C.; Mannaioni, G.; Cozzi, A.; Carlà, V.; Sili, M.; Cavone, L.; Maratea, D.; Moroni, F. *Neuropharmacology* **2010**. PMID:21110987.