Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 513 (2005) 75–80
www.elsevier.com/locate/ejphar

Systemically administered glucosamine-kynurenic acid, but not pure kynurenic acid, is effective in decreasing the evoked activity in area CA1 of the rat hippocampus

Hermina Robotka[a], Hajnalka Németh[a,b,c], Csaba Somlai[d], László Vécsei[b,c], József Toldi[a,*]

[a]Department of Comparative Physiology, University of Szeged, POB 533, H-6701 Szeged, Hungary
[b]Department of Neurology, University of Szeged, POB 427, H-6701 Szeged, Hungary
[c]Neurology Research Group of the Hungarian Academy of Sciences and University of Szeged, Semmelweis u. 6, H-6725 Szeged, Hungary
[d]Department of Medical Chemistry, University of Szeged, Dóm tér 6, H-6725 Szeged, Hungary

Received 28 October 2004; received in revised form 23 February 2005; accepted 24 February 2005
Available online 7 April 2005

Abstract

The metabolism of tryptophan along the kynurenine pathway yields several neuroactive intermediates, including kynurenic acid, which is one of the few known endogenous *N*-methyl-D-aspartate receptor inhibitors; in parallel with this, it is an α7 nicotinic acetylcholinergic receptor antagonist. On the basis of these properties, kynurenic acid might therefore come into consideration as a therapeutic agent in certain neurobiological disorders. However, the use of kynurenic acid as a neuroprotective agent is practically excluded because kynurenic acid hardly crosses the blood–brain barrier. We recently synthetized a new compound, glucosamine-kynurenic acid, which is presumed to cross the blood–brain barrier more easily. In this study, the effects of systemically administered kynurenic acid and glucosamine-kynurenic acid on CA3 stimulation-evoked population spike activity in region CA1 of the rat hippocampus were compared. The effect of kynurenic acid or glucosamine-kynurenic acid was augmented by probenecid (200 mg/kg), which inhibits kynurenic acid excretion from the cerebrospinal fluid. The results showed that, while kynurenic acid administered i.p. or i.v. in doses of 17, 34, 68 or 136 μmol/kg did not cause any observable change in the animals, 136 μmol/kg glucosamine-kynurenic acid (either i.p. or i.v.) resulted in the sudden death of all the animals. The dose of 68 μmol/kg i.v., but not i.p., resulted in a sudden stoppage of breath, but the animals could be reanimated. As small a dose of glucosamine-kynurenic acid as 17 μmol/kg i.p. resulted in a reduction in population spike amplitudes; this effect was further augmented by probenecid, whereas neither 17 μmol/kg nor higher doses of pure kynurenic acid had a similar effect. The results presented here suggest that glucosamine-kynurenic acid passes the blood–brain barrier much more readily than does kynurenic acid.

Keywords: Kynurenic acid; Probenecid; Glucosamine-kynurenic acid; Neuroprotection; Hippocampus; (Rat)

1. Introduction

Overactivation of the excitatory amino acid receptors plays a definitive role in neuronal death in stroke, hypoglycaemia and degenerative disorders (Choi, 1988). The kynurenine pathway converts tryptophan into various compounds, including L-kynurenine, which in turn can be converted to the excitatory amino acid receptor antagonist kynurenic acid (Stone, 2001), which may therefore serve as a protective agent in neurological disorders (Kiss et al., 2004; Klivényi et al., 2004; Stone et al., 2003). However, its use as a neuroprotective agent is rather limited because kynurenic acid has only a very poor ability to cross the blood–brain barrier (Fukui et al., 1991). Battaglia et al. (2000) have shown that a glucose conjugate is effective in transporting the kynurenic acid analogue 7-chloro-kynurenic acid into the brain.

We recently synthetized a new compound, glucosamine-kynurenic acid, which was presumed to degrade more slowly than an ester, and to cross the blood–brain barrier

* Corresponding author. Tel.: +36 62 544153; fax: +36 62 544291.
E-mail address: toldi@bio.u-szeged.hu (J. Toldi).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.02.043

better than does kynurenic acid, while it has the same effects on the central nervous system when it is applied intraventricularly (Füvesi et al., 2004).

In the present experiments, we studied the effects of peripherally administered glucosamine-kynurenic acid on the hippocampal evoked activity, in comparison with peripherally administered pure kynurenic acid. These drugs were administered alone, or in combination with probenecid (which is known to inhibit kynurenic acid excretion from the extracellular fluid).

The evoked responses of the hippocampal neurones were chosen as an end-point for these electrophysiological experiments because of the high concentrations of glutamate receptors on the dendrites of these neurones (Martin et al., 1993; Monyer et al., 1994; Watanabe et al., 1993), and because they receive glutamatergic afferents that can be stimulated preferentially in vivo. In these experiments, when kynurenic acid and glucosamine-kynurenic acid were administered i.p., the CA3 pyramidal cells were stimulated during recordings from the neurones that they innervate, the area CA1 pyramidal cells. As shown in our recent work (Németh et al., 2004), this paradigm is very suitable for study of the effects of manipulations of the kynurenergic pathway.

2. Materials and methods

2.1. Animals

Male Wistar rats ($n=51$) weighing 200–250 g were housed individually and had free access to food and water. Two times 15 animals were used to study the effects of kynurenic acid and glucosamine-kynurenic acid. The effects of probenecid were studied on 3 animals, while two times 6 animals were used in the experiments with kynurenic acid+probenecid and glucosamine-kynurenic acid+probenecid. Studies on the changes in blood pressure were carried out on 6 animals. Every effort was made to minimize animal suffering. The principles of laboratory animal care (NIH publication No. 85-23), and the protocol for animal care approved by the European Communities Council Directive of 24 November 1986 (86/609/EEC) were followed.

2.2. Drugs

Kynurenic acid and probenecid were obtained from Sigma (Steinheim, Germany), while the glucosamine conjugate of kynurenic acid, which is a new compound, was synthetized in the Institute of Medical Chemistry, University of Szeged. Probenecid was dissolved in 1 M sodium hydroxide diluted with physiological saline and adjusted with 0.1 M hydrochloric acid to pH 7.4. For the structure of glucosamine-kynurenic acid, and for the detailed steps of the synthesis, see Füvesi et al. (2004).

2.3. Electrophysiology

Animals were anaesthetized with urethane (1.25 g/kg, i.p.). In some cases, the tail vein of the animals was catheterized. In most of the experiments, the drugs were administered i.p., through a syringe implanted at the beginning of the experiments. For recordings in area CA1,

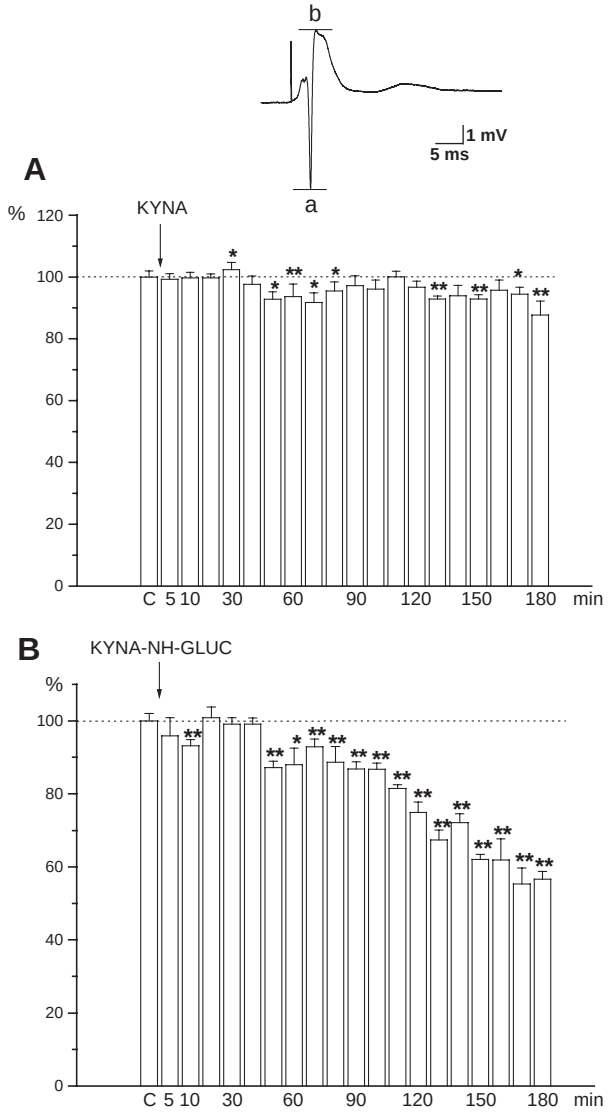

Fig. 1. Examples of the effects of the administered compounds on the population spike amplitudes recorded in CA1. Control population spikes (C in panels A and B and top right) were evoked in the pyramidal layer of the CA1 region with contralateral CA3 stimulation during 30 min. (A) After kynurenic acid (KYNA) injection (17 μmol/kg) there were some decreases in amplitude, but these changes were not marked. The observations were similar after the i.p. injection of kynurenic acid in higher doses up to 136 μmol/kg. Top right: an example of the evoked spikes, the amplitudes of which were measured between peaks *a* and *b*. Calibration: 1 mV, 5 ms. (B) Glucosamine-kynurenic acid (KYNA-NH-GLUC) injection (17 μmol/kg, i.p.) resulted in marked and significant decreases in the CA1 population spike amplitudes with a 50–60 min delay. Ordinate: spike amplitudes as percentages of the controls (C). Abscissa: time in min after drug injection. Each column represents the mean ± S.D. for 5 potentials. $*P<0.05$, $**P<0.01$.

a 2–3 mm diameter hole was drilled over the dorsal hippocampus (3.0–3.8 mm posterior and 1.8–2.3 mm lateral to the sagittal suture) and the recording electrode was lowered 3.3 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: final electrode depth 3.8 mm below the dura). Electrodes were lowered and final positions were adjusted so that the maximum CA1 population spike was obtained in response to contralateral CA3 stimulation (Fig. 1A, insert). The sites in areas CA1 and CA3 were confirmed histologically in creosyl violet-stained sections. Responses to a range of stimulus intensities were recorded under control conditions to produce an input–output curve by changing the duration (10–100 μs), using current (up to 200 μA) square pulses. Stimuli were triggered at low frequency (0.05 Hz). Response stability was monitored for 30 min prior to drug administration. The electrophysiological recording continued during the following 3 h recording period after drug administration.

2.4. Drug administration

The doses of kynurenic acid and glucosamine-kynurenic acid that were chosen were based on earlier pilot experiments, in which both kynurenic acid and glucosamine-kynurenic acids were administered i.v. or i.p., in doses of 17, 34, 68 or 136 μmol/kg. Since i.p. and i.v. administration resulted in similar effects, i.p. administration was chosen in further studies, because this seemed to be more appropriate in the scheduled behavioural experiments. We endeavoured to find the minimum doses that were still effective; accordingly, on the basis of these pilot experiments, both kynurenic acid and glucosamine-kynurenic acids were administered in a dose of 17 μmol/kg throughout the main study. The dose of probenecid (200 mg/kg, i.p.) was chosen on the basis of earlier work (Németh et al., 2004).

2.5. Data analysis

Population spikes evoked by CA3 stimulation were measured from peak to peak (Scharfman and Goodman, 1998). Differences between the amplitudes were determined statistically (paired t test, P value set at 0.05 for significance).

3. Results

3.1. Effects of kynurenic acid

The responses of the area CA1 pyramidal cells to contralateral CA3 stimulation were tested before and after the injection of kynurenic acid. Kynurenic acid was administered in doses of 17, 34, 68 or 136 μmol/kg. Three animals were tested at each dose, with the exception of the 17 μmol/kg dose, at which 6 animals were examined. Marked effects of the intraperitoneally injected drug were not observed in any of the treated animals, apart from some transient, inconsistent deviations from the control level. In the main series of experiments, to facilitate comparability with the results of administration of glucosamine-kynurenic acid (see below), 17 μmol/kg (equimolar) kynurenic acid was administered (i.p.); this resulted in only a slight, if any decrease in the amplitude of the CA1 responses (Fig. 1A and Table 1).

3.2. Effects of glucosamine-kynurenic acid

In pilot experiments, glucosamine-kynurenic acid was administered in doses of 17 ($n=6$), 34 ($n=3$), 68 ($n=3$) or 136 ($n=3$) μmol/kg. Glucosamine-kynurenic acid, administered either i.v. or i.p., in a dose of 136 μmol/kg resulted in 100% mortality within 5 min. Similarly, glucosamine-kynurenic acid administered in a dose of 68 μmol/kg i.v. resulted in a stoppage of breath, but the animals could be resuscitated. I.p. injection of this dose (68 μmol/kg) of drug did not stop the breathing of the animals. These findings led us to reduce the dose of the drug to a level as low as possible which still resulted in a marked and clear-cut decrease in amplitude of the CA1 pyramidal cell responses. We found that, in a dose of ≥17 μmol/kg, glucosamine-kynurenic acid induced consistent and appreciable decreases in the population spike amplitudes in all cases. This change started 50–60 min after drug administration, regardless of the mode of application. In some cases, a small and transient increase

Table 1
Changes in population spike amplitudes of CA1 pyramidal cells

	C	0–30 min	30–60 min	60–90 min	90–120 min	120–150 min	150–180 min
KYNA	100	95.88 ± 3.9	90.55 ± 2.1*	88.61 ± 1.5**	90.08 ± 2.8*	87.20 ± 2.6*	87.39 ± 2.4*
KYNA-NH-GLUC	100	100.64 ± 0.5	91.03 ± 4.2	83.96 ± 3.8*	78.55 ± 4.6*	63.93 ± 8.2*	61.65 ± 3.0**
KYNA+PROB	100	97.79 ± 1.5	105.94 ± 11.9	98.43 ± 4.2	85.75 ± 8.1	84.35 ± 5.7*	77.76 ± 1.4**
KYNA-NH-GLUC+PROB	100	93.11 ± 2.5*	88.43 ± 0.8**	81.47 ± 2.9**	74.51 ± 4.2**	73.50 ± 2.9**	65.13 ± 6.6**

Data summarising the effects of kynurenic acid (KYNA), glucosamine-kynurenic acid (KYNA-NH-GLUC), kynurenic acid+probenecid (KYNA+PROB) and glucosamine-kynurenic acid+probenecid (KYNA-NH-GLUC+PROB) on the amplitudes of the population spikes recorded during 30 min blocks in the pyramidal layer of region CA1 of the hippocampus. C: Normalized amplitudes of responses before drug injections (100%). Amplitudes (means ± S.D.). KYNA-NH-GLUC+PROB treatment was the most effective in inducing a persistent and long-lasting decrease in the amplitudes.

* $P<0.05$ ($n=6$ animals in each group).

** $P<0.01$ ($n=6$ animals in each group).

in amplitude was observed shortly after the application of the drug, but this was followed by a significant lasting decrease in amplitude in all cases (Fig. 1B and Table 1).

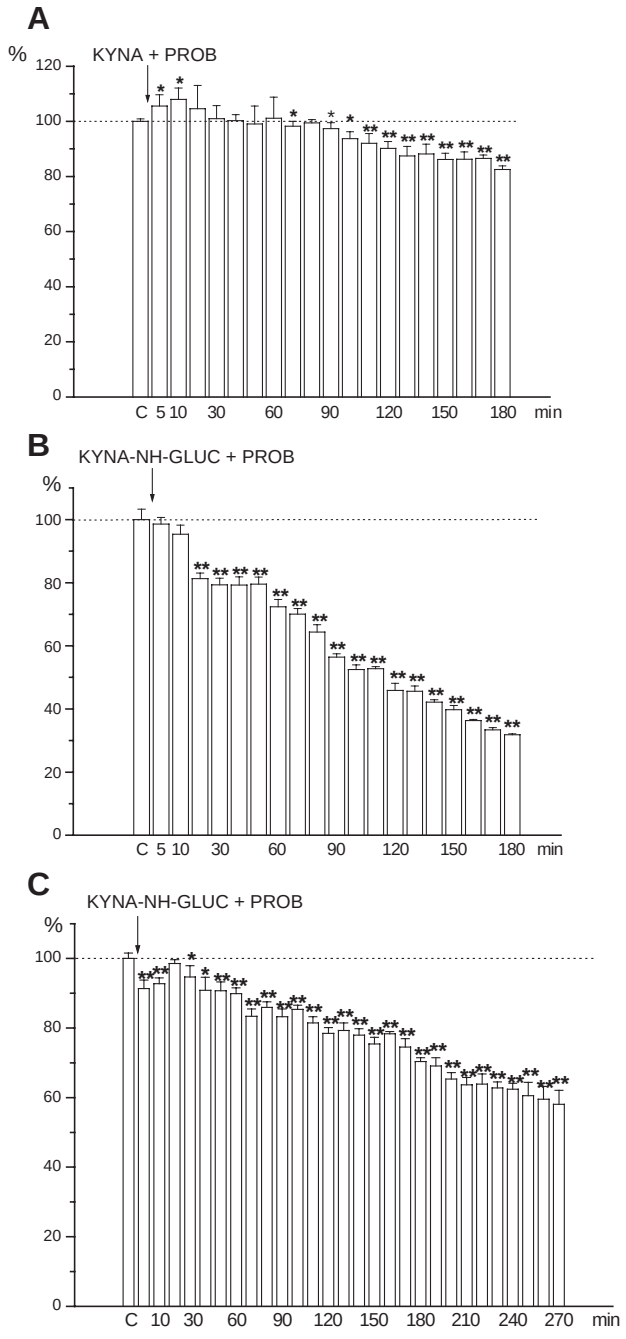

Fig. 2. Examples of the effects of kynurenic acid or glucosamine-kynurenic acid administered together with probenecid (PROB). (A) After a transient increase, kynurenic acid+probenecid (17 μmol/kg and 200 mg/kg, respectively, injected i.p.) resulted in decreased CA1 population spikes with a 1.5–2 h delay. (B) Glucosamine-kynurenic acid+probenecid i.p. injection (17 μmol/kg and 200 mg/kg, respectively) resulted in considerable decreases in amplitude, shortly (10–20 min) after the administration. In some cases (as presented here), the amplitudes fell to 35–40% of the controls, while in most cases, as in panel C below, the amplitudes fell only to 60–70% of the controls, in spite of the relatively long recording period. The ordinate and abscissa are the same as in Fig. 1.

3.3. Effects of probenecid

Probenecid administered in a dose of 200 mg/kg i.p. did not cause an immediate significant change in the amplitude of the evoked population spike activity. In most cases, it resulted in a decrease in amplitude, but this needed a longer period (60–90 min) to develop. This observation is fully consistent with our recently published results (Németh et al., 2004), and is therefore not shown here.

3.4. Effects of kynurenic acid+probenecid

The results were similar when kynurenic acid was administered together with probenecid. In most cases when kynurenic acid (17 μmol/kg) and probenecid (200 mg/kg) were administered together, there was no change in the amplitudes of the responses during 1 h following the drug application, except for a transient, slight facilitation in a few cases, but with a 1.5–2 h delay, a slight decrease in amplitude was registered in all cases (Fig. 2A and Table 1).

3.5. Effects of glucosamine-kynurenic acid+probenecid

The results were quite different when glucosamine-kynurenic acid was administered together with probenecid. Glucosamine-kynurenic acid (17 μmol/kg) administered together with probenecid (200 mg/kg) resulted in a progressive and, by the end of the recording period, a considerable decrease in amplitude of the CA1 population spikes. The decrease in amplitude began and became significant within 10–20 min, and continued over the 3 h registration time (Fig. 2B and Table 1). In some of the experiments, the registration continued over 4.5 h. In these animals, the reduction in amplitude was progressive throughout the whole experiment (Fig. 2C).

3.6. Blood pressure

In 6 animals, the basal blood pressure and the effects of kynurenic acid and of glucosamine-kynurenic acid on the blood pressure were examined. In all animals, the intracarotid blood pressure was found to be 100–119 mm Hg. Neither kynurenic acid nor glucosamine-kynurenic acid influenced the intracarotid blood pressure markedly.

4. Discussion

The primary finding of this study was that the peripheral administration of glucosamine-kynurenic acid in a dose as low as 17 μmol/kg, especially together with probenecid, effectively reduced the responses to glutamatergic input in region CA1 of the hippocampus, while pure kynurenic acid, injected either equimolarly or in higher doses, did not do so.

Kynurenic acid is an excitatory amino acid receptor antagonist that can partially act at both the α-amino-3-

hydroxy-5-methylisoxazole-4-propionic acid and *N*-methyl-D-aspartate subunits of the glutamate receptors. Its action at the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors requires relatively high concentrations (ED_{50} > 100 μM) as compared with the concentrations which are known to affect the *N*-methyl-D-aspartate receptors (Stone, 1993). Besides these effects of kynurenic acid, it has recently been demonstrated in an electrophysiological study that α7 nicotinic acetylcholinergic receptors are also targets for kynurenic acid. The experimental data indicate that the potency at α7 is around 30-fold higher than for *N*-methyl-D-aspartate in the presence of 10 μM glycine. These results suggest functionally significant cross-talk between the nicotinic cholinergic system and the kynurenine pathway of the brain (Hilmas et al., 2001).

The affinity of kynurenic acid for the glycine coagonist site of the *N*-methyl-D-aspartate receptor ($IC_{50} \approx 8$ μM; Kessler et al., 1989) and in non-competitive inhibition of the α7 nicotinic acetylcholinergic receptors ($IC_{50} \approx 7$ μM; Hilmas et al., 2001) is in the range of the kynurenic acid levels in the human brain, and reasonably close to the (lower) kynurenic acid content of the rodent brain, which suggests a physiological function of kynurenic acid in the glutamatergic and cholinergic neurotransmission. Moreover, it may be supposed that kynurenic acid inhibits the glutamate release through the presynaptic α7 nicotinic acetylcholinergic receptors. This was directly supported by experiments in which a modest elevation of kynurenic acid inhibited glutamate release (Carpenedo et al., 2001).

These results suggest that the manipulation of brain kynurenines, e.g. increase of the kynurenic acid level in the brain, may reduce the overactivation of excitatory amino acid receptors in more than one way, and offers novel therapeutic opportunities (Schwarcz and Pellicciari, 2002).

However, its use as a neuroprotective agent is rather restricted because kynurenic acid has only a very limited ability to cross the blood–brain barrier (Fukui et al., 1991). This is supported by the results we have presented here: systemically administered kynurenic acid in doses of 17, 34, 68 or 136 μmol/kg did not cause any observable change in the anaesthetized animals, either in their breathing, or in the electrophysiological activity of their CA1 region. Against this, glucosamine-kynurenic acid in a dose of 136 μmol/kg resulted in the death of the animals in all cases, while 68 μmol/kg (i.v.) glucosamine-kynurenic acid induced the stoppage of breathing of the animals, though they could be resuscitated.

A dose of glucosamine-kynurenic acid as small as 17 μmol/kg was effective in reducing the CA3 stimulation-evoked activity of the CA1 pyramidal cells in the hippocampus. This effect was augmented when glucosamine-kynurenic acid was administered together with probenecid, which is known to be an inhibitor of the transport of organic acids from the cerebrospinal fluid, and has been demonstrated to increase the brain concentration of kynurenic acid (Vécsei et al., 1992). Probenecid administered with kynurenic acid was not so effective, probably because i.p. or i.v. injected kynurenic acid does not cross the blood–brain barrier. A slight decrease in evoked activity was observed with a long (1–1.5 h) delay in these experiments too. This suggests that probenecid itself induced an increase in the brain kynurenic acid content, which is probably based only on the endogenous kynurenic acid. This observation is in accordance with our own recent finding (Németh et al., 2004).

Determination of the kynurenic acid content in the cerebrospinal fluid by high-performance liquid chromatography or mass spectrography is needed to acquire definite proof that glucosamine-kynurenic acid, in contrast with pure kynurenic acid, crosses the blood–brain barrier more easily. We are currently working on this field.

Acknowledgements

This work was supported by the following grants: OTKA T046687, ETT 10/2003, NKFP 1/027, RET-08/2004 and BIO-00100/2002OM.

References

Battaglia, G., La Russa, M., Bruno, V., Arenare, L., Ippolito, R., Copani, A., Bonina, F., Nicoletti, F., 2000. Systemically administered D-glucose conjugates of 7-chlorokynurenic acid are centrally available and exert anticonvulsant activity in rodents. Brain Res. 860, 149–156.

Carpenedo, R., Pittaluga, A., Cozzi, A., Attucci, S., Galli, A., Raiteri, M., Moroni, F., 2001. Presynaptic kynurenate-sensitive receptors inhibit glutamate release. Eur. J. Neurosci. 13, 2141–2147.

Choi, D.W., 1988. Glutamate neurotoxicity and diseases of the nervous system. Neuron 1, 623–634.

Fukui, S., Schwarcz, R., Rapoport, S.I., Takada, Y., Smith, Q.R., 1991. Blood–brain barrier transport of kynurenines: implications for brain synthesis and metabolism. J. Neurochem. 56, 2007–2017.

Füvesi, J., Somlai, C., Németh, H., Varga, H., Kis, Z., Farkas, T., Károly, N., Dobszay, M., Penke, Z., Penke, B., Vécsei, L., Toldi, J., 2004. Comparative study on the effects of kynurenic acid and glucosamine-kynurenic acid. Pharmacol. Biochem. Behav. 77, 95–102.

Hilmas, C., Pereira, E.F., Alkondon, M., Rassoulpour, A., Schwarcz, R., Albuquerque, E.X., 2001. The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: physiopathological implications. J. Neurosci. 21, 7463–7473.

Kessler, M., Terramani, T., Lynch, G., Baudry, M., 1989. A glycine site associated with *N*-methyl-D-aspartic acid receptors: characterization and identification of a new class of antagonists. J. Neurochem. 52, 1319–1328.

Kiss, C., Bari, F., Toldi, J., Vécsei, L., 2004. Kynurenine in the Brain: Therapeutic Perspectives. Medicina, Budapest, pp. 77–112.

Klivényi, P., Toldi, J., Vécsei, L., 2004. Kynurenines in neurodegenerative disorders: therapeutic consideration. Adv. Exp. Med. Biol. 541, 169–183.

Martin, L.J., Blackstone, C.D., Levey, A.I., Huganir, R.L., Price, D.L., 1993. AMPA glutamate receptor subunits are differentially distributed in rat brain. Neuroscience 53, 327–358.

Monyer, H., Burnashev, N., Laurie, D.J., Sakmann, B., Seeburg, P.H., 1994. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron 12, 529–540.

Németh, H., Robotka, H., Kis, Z., Rózsa, É., Janáky, T., Somlai, C., Marosi, M., Farkas, T., Toldi, J., Vécsei, L., 2004. Kynurenine administered together with probenecid markedly inhibits pentylenetetrazol-induced seizures. An electrophysiological and behavioural study. Neuropharmacology 47, 916–925.

Scharfman, H.E., Goodman, J.H., 1998. Effects of central and peripheral administration of kynurenic acid on hippocampal evoked responses in vivo and in vitro. Neuroscience 86, 751–764.

Schwarcz, R., Pellicciari, R., 2002. Manipulation of brain kynurenines: glial targets, neuronal effects, and clinical opportunities. J. Pharmacol. Exp. Ther. 303, 1–10.

Stone, T.W., 1993. Neuropharmacology of quinolinic and kynurenic acids. Pharmacol. Rev. 45, 309–379.

Stone, T.W., 2001. Kynurenic acid antagonists and kynurenine pathway inhibitors. Expert Opin. Investig. Drugs 10, 633–645.

Stone, T.W., Mackay, G.M., Forrest, C.M., Clark, C.J., Darlington, L.G., 2003. Tryptophan metabolites and brain disorders. Clin. Chem. Lab. Med. 41, 852–859.

Vécsei, L., Miller, J., MacGarvey, U., Beal, M.F., 1992. Kynurenine and probenecid inhibit pentylenetetrazol- and NMDLA-induced seizures and increase kynurenic acid concentrations in the brain. Brain Res. Bull. 28, 233–238.

Watanabe, M., Inoue, Y., Sakimura, K., Mishina, M., 1993. Distinct distributions of five *N*-methyl-D-aspartate receptor channel subunit mRNAs in the forebrain. J. Comp. Neurol. 338, 377–390.