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European Journal of Pharmacology 319 (1997) 13–20

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Enzyme-catalyzed production of the neuroprotective NMDA receptor antagonist 7-chlorokynurenic acid in the rat brain in vivo

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Received 25 July 1996; revised 8 October 1996; accepted 15 October 1996

Abstract

NMDA receptors play a critical role in neurotransmission and are also involved in the occurrence of excitotoxic nerve cell death. Synthetic halogenated analogs of the endogenous broad spectrum excitatory amino acid receptor blocker kynurenic acid are among the most potent and selective antagonists of the glycine co-agonist site of the NMDA receptor complex. Pharmacological blockade of this site provides neuroprotection in animal models of cerebral ischemia, epilepsy and neurodegenerative disorders, and does not appear to be associated with some of the undesirable side effects linked to classic competitive and non-competitive NMDA receptor antagonists. Here we demonstrate that neuroprotective quantities of 7-chloro-kynurenic acid (7-Cl-KYNA), one of the most selective and well-studied glycine site antagonists, can be synthesized in the brain from its bioprecursor L-4-chlorokynurenine (4-Cl-KYN). Intracerebral infusion of 4-Cl-KYN dose-dependently reduced quinolinate neurotoxicity in the rat hippocampus after enzymatic conversion to 7-Cl-KYNA by kynurenine aminotransferase. In accordance with previous studies demonstrating that kynurenine aminotransferase is preferentially localized in astrocytes, both the enzymatic formation of 7-Cl-KYNA and the neuroprotective potency of 4-Cl-KYN were substantially reduced following an intrahippocampal injection of the gliotoxin fluorocitrate. In situ produced 7-Cl-KYNA offers a novel neuroprotective strategy for targeting the glycine/NMDA site while avoiding excessive receptor blockade and reducing the clinical risks associated with conventional NMDA receptor antagonism.

Keywords: Astrocyte; Excitotoxicity; Glycine site; NMDA receptor; Kynurenic acid; Neurodegenerative disorder; Quinolinic acid

1. Introduction

During the past decade, the NMDA receptor has emerged as one of the most prominent targets for drug development in the treatment of neurological and psychiatric diseases (Watkins and Collingridge, 1994). Based on a large body of preclinical evidence, pharmacological manipulation of NMDA receptor function offers opportunities for the treatment of neurodegenerative and convulsive disorders (Schwarcz et al., 1982; Rogawski, 1992; Lipton and Rosenberg, 1994), pain (Näsström et al., 1992; Kristensen et al., 1994), anxiety (Trullas et al., 1989), depression (Paul et al., 1994), opiate tolerance and withdrawal symptoms (Trujillo and Akil, 1991; Herman et al., 1995) and schizophrenia (Olney and Farber, 1995). In many cases, NMDA receptor blockade is required to prevent, attenuate or arrest imminent or progressing neurodegeneration. In preparation for clinical assessment, a large number of NMDA receptor antagonists have been demonstrated to possess neuroprotective potency in vitro and in vivo, suggesting that neuroprotection can be achieved by individually blocking several of the sites of the NMDA receptor complex (Watkins and Collingridge, 1994).

The glycine co-agonist site of the NMDA receptor has been identified by many as the preferred target for pharmacological intervention (cf., Leeson and Iversen, 1994 for review). This is based primarily on data suggesting that glycine/NMDA receptor blockers are not, or far less, associated with some of the side effects seen with the use

Abbreviations: 4-Cl-KYN, L-4-chlorokynurenine; 7-Cl-KYNA, 7-chlorokynurenic acid; GAD, glutamate decarboxylase; KYNA, kynurenic acid; NMDA, *N*-methyl-D-aspartate; QUIN, quinolinic acid.

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PII S0014-2999(96)00829-1

of the more conventional competitive and non-competitive NMDA receptor antagonists (Grotta et al., 1995; Muir and Lees, 1995). For example, at neuroprotective doses, glycine/NMDA receptor antagonists appear to cause few if any neuropathological changes in the posterior cingulate cortex and other brain areas (Hargreaves et al., 1993), and do not present phencyclidine-like discriminative stimulus effects (Koek and Colpaert, 1990). Characterization of the advantageous properties of glycine-NMDA receptor antagonists was accomplished to a large part through the use of 7-chlorokynurenic acid (7-Cl-KYNA), a synthetic derivative of the naturally occurring broad spectrum excitatory amino acid receptor antagonist kynurenic acid (KYNA) (Perkins and Stone, 1982). Introduced shortly after the discovery of glycine's stimulatory effect on NMDA receptor function (Johnson and Ascher, 1987; Kemp et al., 1988), 7-Cl-KYNA has become the prototypical experimental probe for the glycine/NMDA site, although it is less potent at the receptor ($ED_{50} = 0.56$ μM) than some di-halogenated KYNA derivatives and other more recently synthesized compounds. Its major drawback for both experimental work and clinical development is its poor lipophilicity and, hence, its inadequate penetration through the blood-brain barrier (Leeson and Iversen, 1994).

The present study was designed to explore the possibility that biologically significant amounts of 7-Cl-KYNA can be produced by enzymatic transamination of L-4-chlorokynurenine (4-Cl-KYN), a synthetic compound which has negligible affinity to the glycine/NMDA receptor ($ED_{50} = 150$ μM; B. Baron, personal communication) and readily enters the brain from the periphery (Hokari et al., 1996). Preliminary experiments using cell-free enzyme preparations, brain tissue slices and intracerebral injections in vivo had demonstrated that L-4-Cl-KYN can serve as a substrate of the two kynurenine aminotransferases (KATs) which are responsible for the conversion of L-kynurenine to KYNA in the brain (Okuno et al., 1991; Salituro et al., 1994; Buchli et al., 1995). De novo produced 7-Cl-KYNA can be recovered by in vivo microdialysis, indicating that mechanisms are in place to release the compound into the extracellular compartment for possible interaction with NMDA receptors (Salituro et al., 1994). In preparation of future studies using the systemically administered compound, the neuroprotective potency of intrahippocampally injected 4-Cl-KYN was now tested against the excitotoxic effects of quinolinic acid (QUIN), which causes a well-established pattern of neuronal damage (Schwarcz et al., 1983). Moreover, since cerebral KYNA formation appears to take place preferentially in astrocytes (Roberts et al., 1992), an astrocyte poison, fluorocitrate (Paulsen et al., 1987), was used to examine the role of glial cells in the synthesis and delivery of newly formed 7-Cl-KYNA. Our results indicate that the in situ formation of 7-Cl-KYNA constitutes a promising strategy to target astrocytes as cellular vehicles for the pharmacological modulation of NMDA receptor function.

2. Materials and methods

2.1. Animals and surgical procedures

Male Sprague-Dawley rats (220–250 g) were used in all experiments. The animals were housed under standard laboratory conditions with a 12 h:12 h light:dark cycle and free access to food and water. Experimental protocols were in accordance with the guidelines of the Institutional Animal Care and Use Committee of the University of Maryland.

In preparation for intracerebral drug infusions, animals were anesthetized with chloral hydrate (360 mg/kg, i.p.) and placed in a small animal stereotaxic apparatus (David Kopf, Tujunga, CA, USA). Through a burr hole in the calvarium, a 28 gauge stainless steel cannula was then lowered into the dorsal hippocampus, and intracerebral drug infusions were made at a rate of 0.033 μl/min using Teflon tubing attached to the injection cannula and a CMA/100 microinfusion pump (Carnegie Medicin, Stockholm, Sweden). The coordinates for intrahippocampal infusion were (in mm): A, 3.4 posterior to bregma; L, 2.3 from the midline; V, 3.0 below the dura.

Hippocampal lesions were made using the excitotoxin QUIN (Sigma, St. Louis, MO, USA). To this end, 8 nmol QUIN dissolved in 4 μl phosphate-buffered saline (PBS), pH 7.4, were infused over a period of 2 h. Control animals received an intrahippocampal infusion of 4 μl PBS, pH 7.4, over 2 h.

4-Cl-KYN (prepared as described by Salituro et al., 1994) or 7-Cl-KYNA (Research Biochemicals International, Natick, MA, USA) were dissolved in PBS, pH 7.8, and co-infused with 8 nmol QUIN as described above (i.e., over 2 h). In separate experiments, 4-Cl-KYN, 7-Cl-KYNA or PBS, pH 7.8 (controls) were infused alone for 2 h, followed by a 2 h co-infusion of the respective compound with 8 nmol QUIN.

The gliotoxin fluorocitrate (Ba-salt; Sigma), prepared by precipitating Ba^{2+} as the sulfate and buffering the supernatant with PBS, pH 7.4, was injected intrahippocampally over 4 min at a rate of 0.25 μl/min immediately prior to a 4 h infusion of the test compounds. Control animals received an injection of 1 μl PBS, pH 7.4, over 4 min.

2.2. Assessment of neurodegeneration and neuroprotection

Neuronal loss was examined by histological and biochemical methods in animals killed four days after the QUIN infusion. For histological analysis, animals were deeply anesthetized with chloral hydrate (400 mg/kg) and perfused transcardially with buffered 4% formaldehyde. The brains were excised, postfixed, cut coronally at 30 μm in a cryostat, and stained with thionin. Biochemically, the extent of the lesion was quantitated by measuring the activity of glutamate decarboxylase (GAD), a marker for

excitotoxin-sensitive hippocampal neurons (Schwarcz et al., 1979). To this end, animals were decapitated, and the dorsal hippocampus (tissue block cut 1.5 mm anterior and posterior of the injection track) was rapidly dissected on ice. The tissue (weighing approximately 20 mg) was sonicated (1:20, w/v) in 50 mM Tris-HCl, pH 7.4, containing 0.2% (v/v) Triton X-100, and enzyme activity was determined as described, using a $^{14}CO_2$ trapping method and [1-^{14}C]glutamic acid (NEN-Dupont, Wilmington, DE) as a substrate (Schwarcz et al., 1979). Protein was measured according to the method of Bradford (1976) using bovine serum albumin as a standard.

2.3. Determination of 7-Cl-KYNA

Immediately after the termination of a 4 h intrahippocampal infusion of 4-Cl-KYN, animals were decapitated, and the dorsal hippocampus (tissue block cut 1.5 mm anterior and posterior of the injection track) was rapidly dissected on ice. The tissue was homogenized by ultrasonication in 800 μl distilled water, immersed in a boiling water bath for 10 min and centrifuged (12 000 × *g*, 10 min). The resulting supernatant was acidified with 1 N HCl and applied to a Dowex 50 W cation-exchange column prewashed with 0.1 N HCl. Subsequently, the column

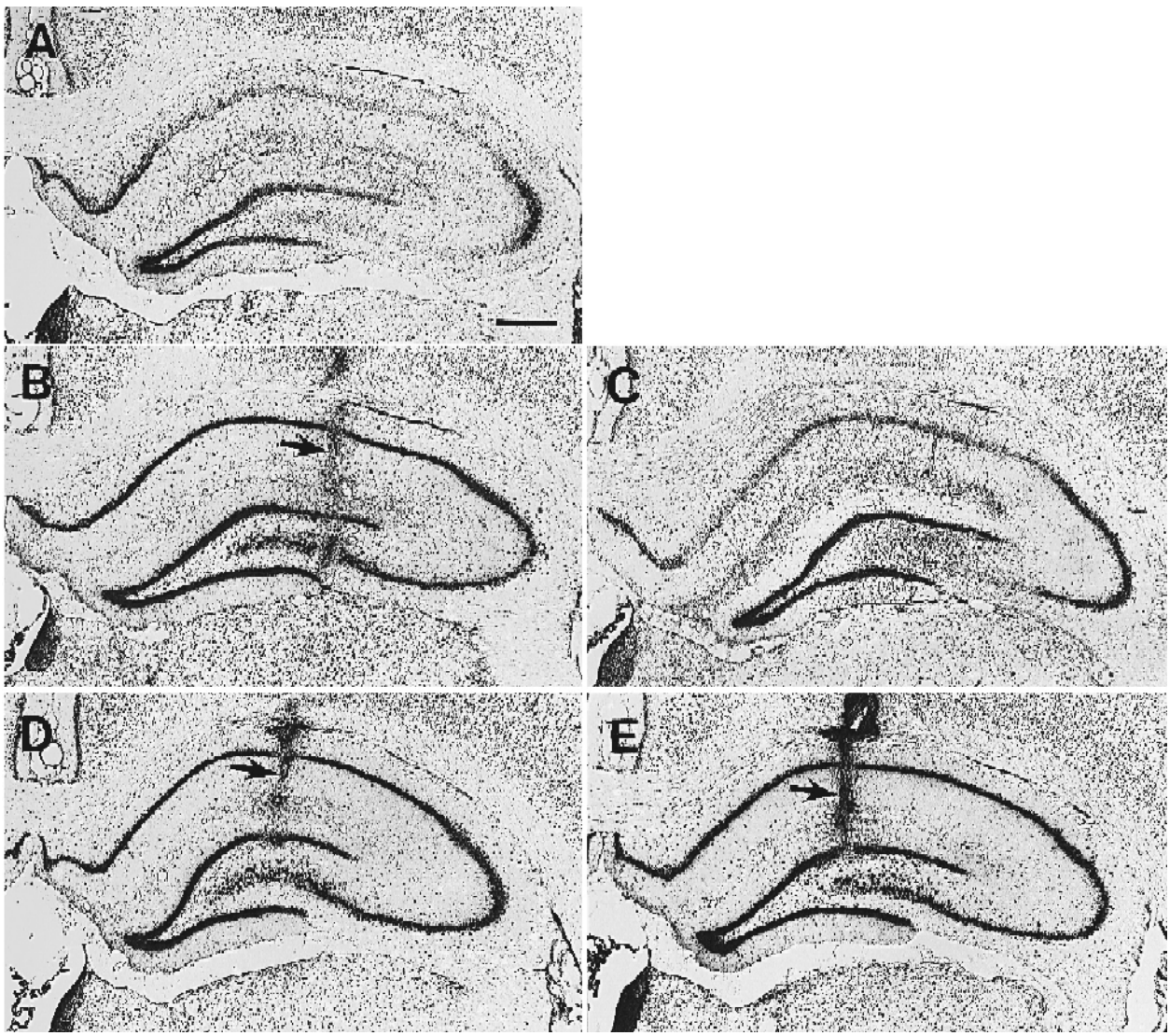

Fig. 1. Photomicrographs of 30 μm coronal sections of the rat hippocampus, depicting the neuroprotective effects of 4-Cl-KYN and 7-Cl-KYNA against QUIN-induced excitotoxic neurodegeneration. The animals were treated, and the tissue was prepared for histological analysis as described in Section 2. (A) pattern of neuronal loss observed 4 days after the infusion of PBS, pH 7.8, for 2 h, followed by 8 nmol of QUIN over 2 h (control); (B) treatment with 24 nmol of 4-Cl-KYN (over 4 h) prevents the neurotoxicity of QUIN; (C) pre-treatment with the astrocyte poison fluorocitrate (1 nmol) partially attenuates the protective action of 4-Cl-KYN (cf., B); (D) treatment with 4 nmol of 7-Cl-KYNA (over 4 h) abolishes QUIN-induced neurotoxicity; (E) pretreatment with fluorocitrate does not interfere with the neuroprotective power of 7-Cl-KYNA (cf., D). Micrographs are representative of 5–7 animals per group. Arrows point to the track of the injection needle. Bar: 50 μm.

was washed with 1 ml of distilled water, and 7-Cl-KYNA was recovered with 2 ml of water. The eluate was subjected to HPLC, using a 3 μm C_{18} column (100 × 3.2 mm i.d., Bioanalytical Systems, West Lafayette, IN, USA), a mobile phase containing 50 mM ammonium acetate and 5% methanol, and a flow rate of 0.5 ml/min. 7-Cl-KYNA was detected at 340 nm using a Beckman 160 UV detector. Under these conditions, the retention time of 7-Cl-KYNA was approximately 15 min.

2.4. Statistics

GAD activities in QUIN-treated and contralateral hippocampi, and the effect of fluorocitrate, were compared using Student's *t* test. The effects of various doses of 4-Cl-KYN or 7-Cl-KYNA on QUIN-induced decreases in GAD activity were evaluated by one-way ANOVA followed by Dunnett's test for multiple group comparisons with the same control.

3. Results

3.1. Effect of co-infusion of QUIN with 4-Cl-KYN or 7-Cl-KYNA

In control rats, intrahippocampal infusion of 8 nmol of QUIN over 2 h resulted in the preferential degeneration of neurons in the CA1 and CA3 sectors (Schwarcz et al., 1983) (Fig. 1A). The lesion was assessed quantitatively as described previously (Schwarcz et al., 1979) by measuring the reduction in the activity of GAD, a marker of hippocampal inhibitory neurons. Lesioned hippocampi showed an approximately 40% decrease in GAD activity (open bars in Fig. 2). Co-treatment with either 4-Cl-KYN (Fig. 2A) or 7-Cl-KYNA (Fig. 2B) dose-dependently attenuated QUIN toxicity, with 48 nmol of 4-Cl-KYN and 4 nmol of 7-Cl-KYNA providing total neuroprotection.

3.2. Effect of pre-infusion of 4-Cl-KYN or 7-Cl-KYNA

Since we had shown previously that 2–3 h of continuous intrahippocampal infusion of 4-Cl-KYN are required to attain steady-state levels of in situ produced 7-Cl-KYNA (Salituro et al., 1994), the next set of experiments was designed to optimize the yield of 7-Cl-KYNA by pre-infusing 4-Cl-KYN for 2 h prior to the co-application of the compound with QUIN. Using this paradigm, we were able to essentially double the neuroprotective potency of 4-Cl-KYN. Thus, 24 nmol of 4-Cl-KYN, which only moderately attenuated QUIN-induced neurodegeneration in the 2 h co-infusion experiment (Fig. 2A), now totally prevented nerve cell loss (Fig. 1B, open bars in Fig. 3). No such increase in neuroprotective power was observed in parallel experiments using 2 nmol of 7-Cl-KYNA (data not shown), supporting the thesis that 4-Cl-KYN, but not 7-Cl-KYNA, must undergo biotransformation in order to become a neuroprotective agent.

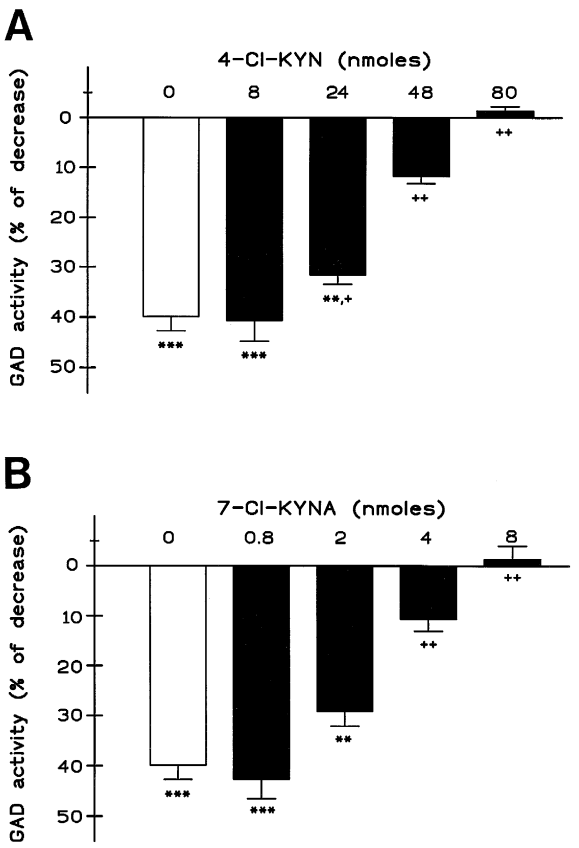

Fig. 2. Biochemical assessment of the neuroprotective potency of 4-Cl-KYN and 7-Cl-KYNA against QUIN-induced neurodegeneration in the rat hippocampus. The test compounds were co-infused with 8 nmol QUIN over 2 h as described in Section 2. Animals were killed 4 days after surgery. Data are expressed in relation to the GAD activity in the contralateral hippocampus (76.7 ± 1.2 nmol/h per mg protein; $n = 60$) and are the mean ± SEM of six animals per group. $^{***}P < 0.001$, $^{**}P < 0.01$, as compared to the contralateral hippocampus (Student's *t* test); $^{++}P < 0.01$, $^{+}P < 0.05$, as compared to QUIN alone (Dunnett's test).

3.3. Effect of fluorocitrate pretreatment

The idea that the enzymatic conversion of 4-Cl-KYN to 7-Cl-KYNA is needed for the former compound to become neuroprotective was tested more directly in another series of experiments. Presumably, a decrease in the activity of kynurenine aminotransferase should reduce the de novo production of 7-Cl-KYNA and thus diminish the neuroprotective potency of 4-Cl-KYN (but not of 7-Cl-KYNA). Since KYNA is preferentially produced in astrocytes (Roberts et al., 1992), fluorocitrate, an established astrocyte poison (Paulsen et al., 1987), was injected into the hippocampus prior to the infusion of 24 nmol of 4-Cl-KYN. As compared to controls, which yielded 18.5 ± 1.6 pmol 7-Cl-KYNA/mg tissue at the end of the 4 h infusion period ($n = 10$), substantially less of the enzymatic prod-

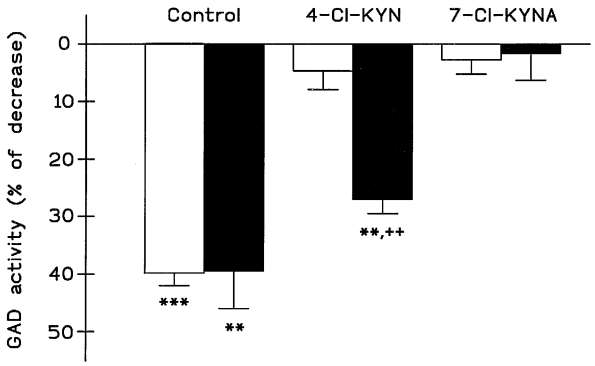

Fig. 3. Biochemical assessment of the effect of fluorocitrate (1 nmol) on the neuroprotective potency of 4-Cl-KYN and 7-Cl-KYNA. As detailed in Section 2, fluorocitrate (solid bars) or vehicle (open bars) was injected intrahippocampally immediately prior to a 4 h infusion of 24 nmol of 4-Cl-KYN or 4 nmol of 7-Cl-KYNA, both co-infused with 8 nmol of QUIN for the last 2 h. Animals were killed 4 days after surgery. Data are expressed as a percentage of GAD activity in the contralateral hippocampus (75.7 ± 2.1 nmol/h per mg protein; $n = 36$) and are the mean ± SEM of 5–6 animals per group. Note that fluorocitrate influenced neither the QUIN-induced lesion nor the neuroprotective effect of 7-Cl-KYNA, but attenuated the neuroprotection caused by 4-Cl-KYN. $^{***}P < 0.001$, $^{**}P < 0.01$ as compared to the contralateral hippocampus; $^{++}P < 0.01$ as compared to the vehicle-treated group (Student's t test).

uct (9.9 ± 1.8 pmol 7-Cl-KYNA/mg tissue, $n = 6$) was recovered from fluorocitrate-treated animals ($P < 0.01$, Student's t test).

In separate animals, fluorocitrate treatment significantly reduced the neuroprotective effect of 4-Cl-KYN, assessed both histologically (Fig. 1B and C) and biochemically (Fig. 3). In contrast, the fluorocitrate injection did not affect the ability of 7-Cl-KYNA to block QUIN-induced neurotoxicity (Fig. 1D and E, Fig. 3).

4. Discussion

The present findings indicate a novel way to target the glycine co-agonist site of the NMDA receptor pharmacologically by producing the receptor antagonist 7-Cl-KYNA in situ from its bioprecursor 4-Cl-KYN. In particular, the study describes the use of the brain's own machinery for the formation of the endogenous excitatory amino acid receptor antagonist KYNA to deliver neuroprotective quantities of 7-Cl-KYNA to glutamatergic synapses. Since limited bioavailability was the major impediment for developing 7-Cl-KYNA for clinical use, the present results may focus renewed attention on the compound's therapeutic potential.

In spite of the development of novel glycine/NMDA receptor antagonists with higher receptor affinity and/or improved bioavailability, such as MDL 104,653 (3-phenyl-4-hydroxy-7-chloro-quinolin-2-(1H)-one), L-701,324 and L-705,022 (McQuaid et al., 1992; Kulagowski et al., 1994; Leeson and Iversen, 1994; Chapman et al., 1995), 7-Cl-KYNA remains the most widely used experimental probe for this receptor. This is due to its commercial availability and, more importantly, to the fact that its pharmacological profile has been very well elaborated in a wide variety of test systems. Those studies established 7-Cl-KYNA as a highly selective, pure antagonist whose actions are readily overcome with increasing concentrations of glycine both in vivo and in vitro. Thus, in spite of its poor penetration into the brain, 7-Cl-KYNA is frequently preferred to the partial receptor agonists (+)-HA-966 ((+)-3-amino-1-hydroxy-pyrrolid-2-one) and ACPC (1-aminocyclopropanecarboxylic acid) which are clearly distinguished from 7-Cl-KYNA in drug discrimination paradigms (Leeson and Iversen, 1994; Witkin et al., 1995), or the trisubstituted

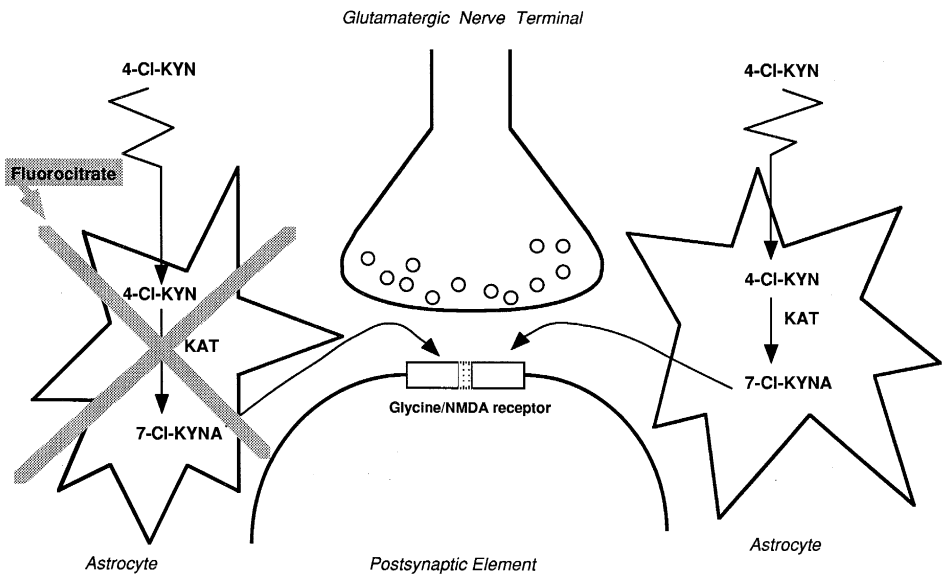

Fig. 4. Schematic representation of the conversion of 4-Cl-KYN to 7-Cl-KYNA in kynurenine aminotransferase (KAT)-containing astrocytes and the attenuation of 7-Cl-KYNA formation by fluorocitrate.

quinoxaline-2,3-dione ACEA-1021, whose actions in vivo require further characterization (Balster et al., 1995). Studies using 7-Cl-KYNA as a prototypical research tool established the role of the glycine/NMDA receptor in excitotoxicity (Foster et al., 1990), in the development and expression of kindling (Croucher and Bradford, 1990; Namba et al., 1993), in anxiolysis (Faiman et al., 1994) and in pain sensitivity (Näsström et al., 1992; Coderre and Van Empel, 1994). In the process, work with 7-Cl-KYNA became instrumental for establishing the glycine/NMDA receptor as a worthwhile target for the development of novel neuroprotective, anticonvulsant, anxiolytic and analgetic agents.

The idea underlying the present set of experiments originated from our studies of the neurobiology of KYNA, an endogenous broad spectrum antagonist of excitatory amino acid receptors (Perkins and Stone, 1982) with a relatively high affinity for the glycine/NMDA receptor (Kessler et al., 1989). In the brain as in the periphery, KYNA is formed through transamination from L-kynurenine, a major metabolite along the kynurenine pathway of tryptophan degradation. Two enzymes, arbitrarily termed KAT I and II, are present in the brain (Okuno et al., 1991; Schmidt et al., 1993; Buchli et al., 1995), but their relative importance for the biosynthesis of KYNA is unknown and may vary under different physiological and pathophysiological conditions. Lesion studies in the rat brain demonstrated an increase in extracellular KYNA levels and a concomitant increase in KAT activity following neuronal ablation, suggesting that non-neuronal elements are the major source of KYNA in pathological (gliotic) tissue (Wu et al., 1992). This was corroborated in immunohistochemical studies using antibodies against KAT I, the better characterized of the two enzymes, which showed a preponderance of immunodeposit in astrocytes (Roberts et al., 1992). Moreover, at the electron microscopic level, KAT I-containing astrocytic processes are frequently seen in close apposition of glutamatergic nerve terminals (labelled with anti-glutamate antibodies), suggesting ready access of astrocyte-derived, newly released KYNA to neuronal excitatory amino acid receptors (Schwarcz et al., 1996).

The conversion of 4-Cl-KYN to 7-Cl-KYNA probably takes place in close analogy to the biosynthesis of KYNA (Fig. 4). In vitro, 4-Cl-KYN serves as a substrate of both KAT I (Salituro et al., 1994) and KAT II (P. Guidetti and R. Schwarcz, unpublished data), and increasing amounts of extracellular 7-Cl-KYNA are recovered in vivo from brain tissue continuously perfused with 4-Cl-KYN (Salituro et al., 1994). The higher neuroprotective efficacy of 4-Cl-KYN after prolonged infusion shown here is in excellent agreement with the notion that a relatively slow, enzyme-catalyzed production of 7-Cl-KYNA occurs in the brain. The results from the experiments using fluorocitrate further substantiated that the in vivo conversion of 4-Cl-KYN employs the same enzymatic machinery which accounts for the transamination of L-kynurenine. Thus, the toxin, which was used at a dose known to incapacitate astrocytes selectively (Paulsen et al., 1987), significantly reduced the accumulation of 7-Cl-KYNA. It follows that de novo produced 7-Cl-KYNA can take advantage of the anatomical arrangement of KYNA-synthesizing astrocytes described above, and that 7-Cl-KYNA, upon release into the extracellular compartment, is in an excellent position to block glycine/NMDA receptors (Fig. 4). This efficient delivery of the newly formed antagonist to the glutamatergic synapse may explain the merely 10-fold difference in neuroprotective potency between 4-Cl-KYN and 7-Cl-KYNA observed in the 2 h co-infusion paradigm (Fig. 2A and B). Our data also raise the possibility that even better neuroprotective efficacy may be obtained by applying the bioprecursor concept to 4, 6-dihalogenated kynurenine analogs, which are transaminated to the corresponding 5, 7-di-substituted kynurenic acids (Salituro et al., 1994), i.e., superior glycine/NMDA antagonists (Baron et al., 1990).

There are several reasons to assume that 4-Cl-KYN holds promise as a novel neuroprotective agent. Because of its structural analogy with L-kynurenine, the compound is an excellent substrate for the large neutral amino acid carrier which is responsible for the facilitated transport of L-kynurenine into the brain (Fukui et al., 1991) and, subsequently, into astrocytes (Speciale et al., 1989). Indeed, recent data indicate that 4-Cl-KYN penetrates the blood-brain barrier better than L-kynurenine (K_m for the cerebrovascular large neutral amino-acid transporter: 4-Cl-KYN: 105 ± 14 μM, L-kynurenine: 255 ± 9 μM; Hokari et al., 1996). In preliminary experiments in mice, systemic administration of 4-Cl-KYN was shown to produce close to micromolar concentrations of 7-Cl-KYNA in the brain (Wu and Schwarcz, 1995), though the neuroprotective potency of peripherally applied 4-Cl-KYN has not been evaluated so far. Notably, 4-Cl-KYN may also be enzymatically converted to the potent QUIN synthesis inhibitor 4-chloro-3-hydroxyanthranilate (Walsh et al., 1991) and may therefore be particularly useful in clinical situations which require a down-regulation of the endogenous excitotoxin QUIN (Schwarcz, 1992; Blight et al., 1995).

In summary, 4-Cl-KYN is a novel pharmacological tool and possible advantageous therapeutic agent designed to attenuate glycine/NMDA receptor function. Through action at the glycine site, 4-Cl-KYN-derived 7-Cl-KYNA may provide neuroprotection in a large number of acute or chronic neurodegenerative diseases affecting various cell populations and CNS regions. In addition, in vivo transamination of 4-Cl-KYN may result in anticonvulsant, antiaddictive, analgesic and anxiolytic actions (cf., Section 1). Importantly, however, and in line with the advantageous side effect profile of other glycine/NMDA receptor antagonists, the use of 4-Cl-KYN is unlikely to cause the neuropathological, cardiovascular and psychotomimetic effects observed with classic competitive or non-competitive NMDA receptor antagonists.

Acknowledgements

This work was supported in part by USPHS grants NS 16102 and NS 28236 (to R.S.). We thank Mrs. J. Burgess for excellent secretarial assistance.

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