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Comparative study on the effects of kynurenic acid and glucosamine–kynurenic acid

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Abstract

Kynurenic acid (KYNA) is the only known endogenous *N*-methyl-D-aspartate (NMDA) receptor inhibitor and might therefore come into consideration as a therapeutic agent in certain neurobiological disorders. However, its use as a neuroprotective compound is practically excluded because KYNA does not readily cross the blood-brain barrier (BBB). We recently synthetized a new compound, glucosamine–kynurenic acid (KYNA-NH-GLUC), which is presumed to cross the BBB more easily. In this study, the effects of KYNA and KYNA-NH-GLUC on behavior and cortical activity were investigated in adult rats. The results show that (1) on intracerebroventricular application, the behavioral changes induced by KYNA and by KYNA-NH-GLUC are quite similar; (2) on intravenous administration, KYNA (25 mg/kg) has no effect on the somatosensory-evoked cortical potentials, whereas KYNA-NH-GLUC (25 mg/kg) causes transient but appreciable reductions in the amplitudes of the evoked responses within 5 min after application; and (3) the results of in vitro studies demonstrated that both KYNA and KYNA-NH-GLUC reduced the amplitudes of the field excitatory postsynaptic potentials (fEPSPs). These observations suggest that the two compounds have similar effects, but that KYNA-NH-GLUC passes the BBB much more readily than does KYNA. These results imply that the conjugated NH-GLUC is of importance in the passage across the BBB.
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1. Introduction

It is widely accepted that activation of the excitatory amino acid (EAA) receptors plays a role in neuronal death in stroke, hypoglycemia, degenerative diseases (Choi, 1988), and the genesis of epileptic seizures (Dingledine et al., 1990). By definition, excitotoxicity is caused by the overexcitation of EAA receptors by exogenous or endogenous receptor agonists. In turn, neuroprotective strategies have utilized antagonists of EAA receptors to prevent excitotoxic neuronal loss (Hodgkins and Schwarcz, 1998).

The kynurenine pathway, which accounts for around 80% of the nonprotein tryptophan metabolism (Stone, 2001), involves both an EAA receptor antagonist, kynurenic acid (KYNA), and an EAA receptor agonist, quinolinic acid.

KYNA is one of the few known endogenous *N*-methyl-D-aspartate (NMDA) receptor inhibitors (Swartz et al., 1990); it may influence important physiological and pathological processes. At physiological concentrations, the main effect of KYNA is at the glycine allosteric site on the NMDA receptor complex (Birch et al., 1988; Danysz et al., 1989), though presynaptic kynurenate-sensitive receptors have recently been described, which inhibit glutamate release (Carpenedo et al., 2001, Hilmas et al., 2001). Experimental data and theoretical considerations suggest that KYNA, its derivatives, or its metabolic precursor kynurenine could have therapeutic effects in neurological disorders (Vécsei and

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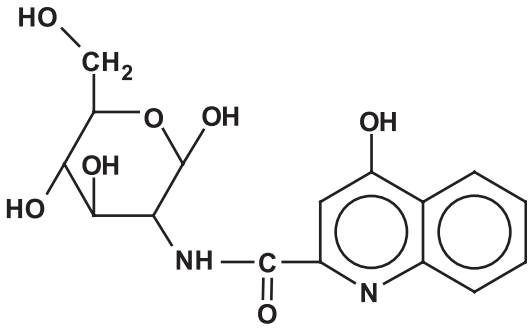

Fig. 1. The structure of KYNA-NH-GLUC.

Beal, 1991). However, its use as a neuroprotective agent is rather limited because KYNA has only a very limited ability to cross the blood-brain barrier (BBB) (Fukui et al., 1991).

During the past 15 years, a great amount of evidence has accumulated on the effects of kynurenergic manipulations and especially on the behavioral and pharmacological effects of KYNA (Speciale et al., 1987, 1988; Vécsei and Beal, 1990a,b, 1991; Vécsei et al., 1992; Stone, 1993; Cini et al., 1996; Speciale et al., 1996a,b; Wu et al., 2000). Its electrophysiological effects have also been studied. Experiments were recently performed mainly on the hippocampus (Scharfman and Goodman, 1998; Wu et al., 2000). The aims of the present study were to characterize a derivative of KYNA, which better crosses the BBB, and to compare the behavioral and electrophysiological effects of this derivative with those of KYNA itself. Battaglia et al. (2000) have shown that a glucose conjugate is effective in transporting the KYNA analogue 7-chloro-KYNA into the brain. We recently synthetized a new compound, glucosamine–kynurenic acid (KYNA-NH-GLUC; Fig. 1), which was presumed to cross the BBB better than does KYNA. In particular, we set out (i) to learn whether intracerebroventricularly applied KYNA-NH-GLUC has the same behavioral effects in rats as those well known for pure KYNA (see the cited papers above), (ii) to compare the electrophysiological effects of intravenously applied KYNA and KYNA-NH-GLUC on the evoked cortical activity in rats, and (iii) to compare the effects of the two compounds in in vitro electrophysiological experiments. In these experiments, the compounds were applied in perfusate (“bath application”).

As the first step in this complex study, we repeated some of the behavioral experiments of Vécsei and Beal (1991); however, this is the first report on the effects of this new synthetized KYNA derivative and the first comparison of the effects of these two compounds.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 200–250 g were housed individually and had free access to food and water. All efforts were 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 Hungarian Health Committee (1998) and the European Communities Council Directive of 24 November 1986 (86/609/EEC) were followed.

2.2. Drugs

KYNA was obtained from Sigma (Steinheim, Germany). The NH-GLUC conjugate of KYNA, which is a new compound, was synthetized in the Institute of Medical Chemistry, University of Szeged (Fig. 1). The synthesis steps were as follows: 1,3-dicyclohexylcarbodiimide (2.27 g, 11 mmol) was added to a solution of 4-hydroxyquinoline-2-carboxylic acid (1.89 g, 10 mmol), (+)-D-glucosamine hydrochloride (2.15 g, 10 mmol), 1-hydroxybenzotriazole (1.35 g, 10 mmol), and *N*-ethylmorpholine (1.3 ml, 10 mmol) in dimethylformamide (100 ml) at 0 °C. The mixture was stirred for 1 h at 0 °C and for another 3 h at room temperature. The precipitate was filtered off and the filtrate was concentrated to dryness in vacuo. The residue was dissolved in methanol and the crude product was obtained as a yellowish powder by precipitation with ethyl acetate [yield: 2.5 g (71%)]. Five hundred milligrams of the crude product was purified by column chromatography on silica gel (particle size 0.02–0.063 mm) with an 8:2 dichloromethane/methanol solvent mixture. The appropriate fractions were combined and the residue was crystallized from methanol/diethyl ether [yield: 350 mg (60%), melting point: >260 °C (decomposition)]. TLC was performed on 60 F_{254} silica gel-precoated glass plates; R_f: 0.42 (dichloromethane/methanol 7:3). The chemical purity of the product was checked by HPLC (LKB Bromma instrument, column: Nucleosil 5C-8, 4 × 250 mm; eluents: A: 0.1% trifluoroacetic acid/H_2O; B: 80% acetonitrile in A; gradient: 0–70% B for 20 min; flow rate: 0.8 ml/min; detection: UV at 254 nm); R_f: 9.60. The MS measurements were made with a Finnigan Mat TSQ 7000 mass spectrometer; the result (Mw: 350.2) agreed with the calculated value.

2.3. Surgery and intracerebroventricular injection

Before surgery, the rats (48) were handled every day for 2 weeks. For surgery, they were anaesthetized intraperitoneally with a mixture of ketamine (10 mg/100 g) and xylazine (0.8 mg/100 g). The animals were then placed in a Kopf stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA), and in a diagonal direction two stainless-steel anchor screws were secured in the skull. A stainless-steel 20-gauge guide cannula was placed into the left ventricle. The coordinates were 1.5 mm caudal and 1.5 mm lateral to the bregma, with the cannula extending 3.5 mm ventrally to the skull surface (coordinates given according to the rat stereotaxic atlas of Paxinos and Watson, 1998). The cannula was fixed to the skull with duracryl and made

fast with the aid of the two stainless-steel anchor screws. The rats were used after a recovery period of 6–7 days; during this period, they were handled daily. The correct positioning of the cannula was checked by dissection of the brain at the end of the experiments.

KYNA (0.3 or 0.6 μmol) or KYNA-NH-GLUC (0.3 or 0.6 μmol), dissolved in saline and neutralized to pH 7.4 or saline alone, was injected intracerebroventricularly over a period of 60 s. The injection was performed through a 26-gauge internal cannula, which was attached to a Hamilton 60 μl syringe via approximately 50 cm of polyethylene tubing, allowing the animals to move freely during the injection period. Between injections, the guide cannula was closed with copper wire to prevent contamination.

2.4. Treatments

Rats were tested with KYNA (0.3 and then 0.6 μmol icv, with a 3-day interval) or with KYNA-NH-GLUC (0.3 and then 0.6 μmol icv, with a 3-day interval). The control rats received the same volume (10 μl) of saline.

2.5. Behavioral tests

Ten minutes after the intracerebroventricular treatment, the animals were placed in an open-field box (a 40 × 40-cm rectangular wooden box, 40 cm high). The activity of each animal was videorecorded during a 5-min session. The videorecorded behavior was examined by an investigator who was unaware of the experimental protocol.

2.6. Stereotyped behavior and ataxia

The stereotyped behavior was characterized by the total time spent in grooming, sniffing, and rearing according to the literature (Vécsei and Beal, 1991). For ataxia, the ratings were as "1": awkward and jerky movements; "2": stumbling or awkward posture; "3": falling; "4": cannot move beyond a small area or supports weight on stomach or haunches; and "5": unable to move except for twitching movements (modified after Sturgeon et al., 1979, and Contreras, 1990).

2.6.1. Electrophysiology

2.6.1.1. In vivo study.

Surgical procedure. Thirty-five animals were used in this study. The rats were anaesthetized with urethane (1.25 g/kg ip) and were then placed in the Kopf stereotaxic frame, and the skin above the skull was opened. On the left side, above the primary somatosensory cortex (SI), the skull was opened with a dental drill, but the dura was left intact.

Stimulation and recording. Electrical stimulation of the right vibrissa pad was employed to induce evoked potentials (EPs) in the left SI. The details of the stimulation and cortical recordings have been published elsewhere (Toldi

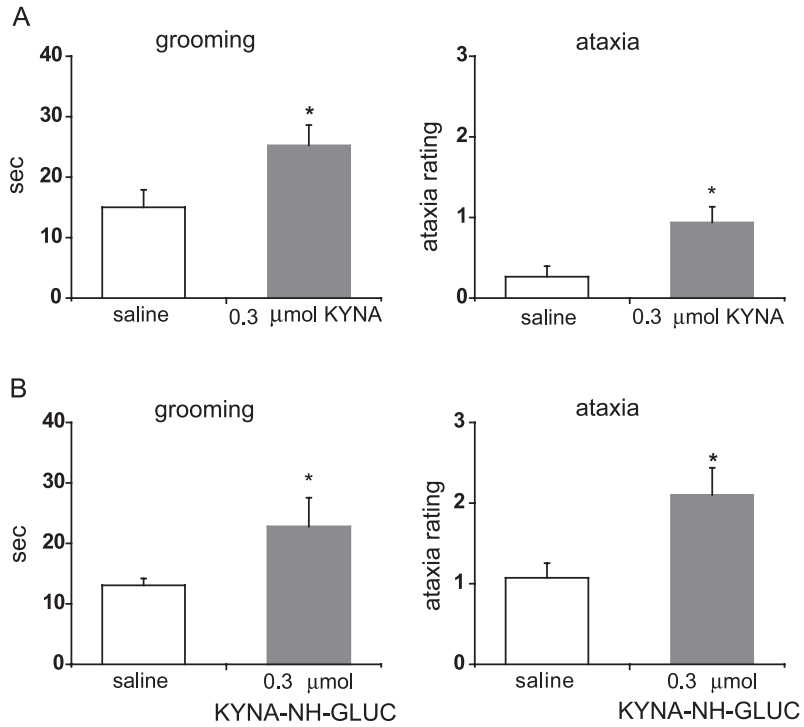

Fig. 2. Effects of intracerebroventricularly administered 0.3 μmol KYNA (A, number of animals: $n = 12$) and KYNA-NH-GLUC (B, $n = 14$) on grooming and ataxia in rats. Controls ($n = 2 \times 11$) received the same volume (10 μl) of saline. The behavior was examined between 10 and 15 min after drug administration. Vertical lines represent the S.E.M. * $P < .05$ (ANOVA). Both KYNA and KYNA-NH-GLUC resulted in the development of grooming and ataxia.

et al., 1994). In brief, the whisker pad was stimulated with a bipolar needle electrode pair (0.5 Hz; 0.3 ms; 100–150 μA). The EPs in the punctum maximum above the barrel field contralateral to the stimulation were fed into a differential amplifier (Tektronix AM 502) and visualized on a Tektronix storage oscilloscope. The amplified responses were fed into a computer via an interface (Digidata 1200, pClamp 6.0.4. software, Axon Instruments) and stored for further processing. Averaged potentials were produced from the registrations, each containing 20 trials.

KYNA or KYNA-NH-GLUC (10, 20, 25, or 50 mg/kg body weight, in 0.5 ml saline) was applied through the tail vein. Each dose of drugs was given to three animals, with the exception of the 25 mg/kg dose, which was tested on six animals receiving KYNA and on six receiving KYNA-NH-GLUC. Controls (5 animals) received the same volume (0.5 ml) of saline. In all these experiments, the animals were left at rest for 1 h after surgery. The core temperature was maintained at 37 ± 0.3 °C. After a 1-h resting period, the stimulation and recording were started. A 30-min stimulation and recording control period was followed by the intravenous administration of KYNA or KYNA-NH-GLUC, during and after which the recording of evoked activity continued.

2.6.1.2. In vitro study. Nine young rats (20–30 days old) were decapitated and coronal slices (400 μm) were prepared from their primary motor cortex with a vibratome (Campden Instruments) in a solution 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 saturated with 95% O_2 and 5% CO_2. The slices were then transferred into a Haas-type recording chamber and incubated at room temperature for 1 h in the solution used for the recordings, differing only in that it contained 3 mM $CaCl_2$ and 1.5 mM $MgSO_4$. To stimulate the layer II/III horizontal pathways (constant current, 0.2 ms pulses delivered at 0.033 Hz), a bipolar stainless-steel microelectrode was positioned approximately 350 μm below the surface and 2 mm from the midline. The stimulus intensity (20–70 μA) was adjusted to evoke a half-maximum response. Recording micropipettes were filled with artificial cerebrospinal fluid (ACSF) and broken off (1.0–1.5 MΩ). The recordings were carried out at the same depth, 300–500 μm away from the stimulation, at ~ 34 °C. In the course of these experiments, KYNA was added to the perfusate (“bath application”). There were two reservoirs, one containing ACSF and one containing ACSF + KYNA (10^{-5} M) or ACSF + KYNA-NH-GLUC (10^{-5} M). After the control field excitatory postsynaptic potentials (fEPSPs) had been recorded, the flow was switched from pure ACSF to ACSF + KYNA or ACSF + KYNA-NH-GLUC. The flow rate was approximately 2.5 ml/min and the dead space (the tubing leading to the chamber plus the chamber volume) was approximately 12.5 ml. To ensure enough time for the exchange of the drug solution, ACSF + KYNA (or ACSF + KYNA-NH-GLUC) was applied for at least 30–40 min. The time for the drug solution to enter the recording chamber was estimated as 5 min. This was done by introducing into the tubing a bubble that could be followed from the exterior to the point of entry into the recording chamber. The recorded data were amplified (SEC-LX05, npi, Germany), filtered (1 Hz–3 kHz), acquired at a

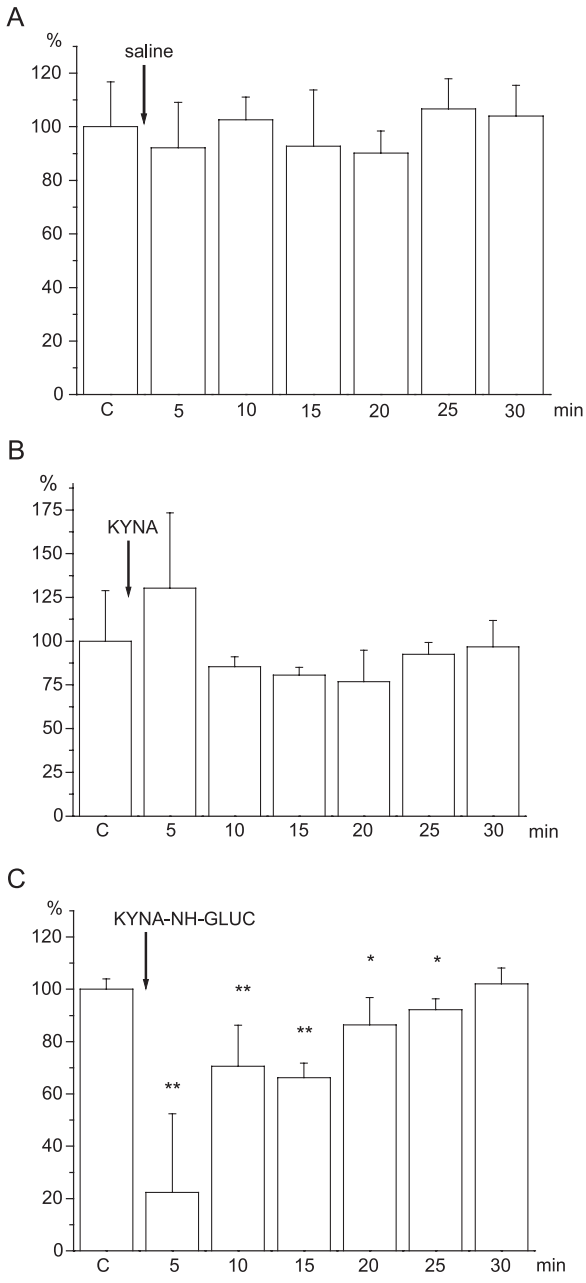

Fig. 3. Representative examples of the effects of intravenously injected saline (A, $n=5$), KYNA (B, $n=6$), and KYNA-NH-GLUC (C) on the amplitudes of barrel field potentials evoked by contralateral whisker pad stimulation. Each column represents the average of 5×30 potentials recorded before (C, $n=6$) and 5, 10, 15, 20, 25, and 30 min after intravenous injection. Amplitudes are normalized and the changes are expressed as percentages of the controls. Arrows show saline, KYNA and KYNA-NH-GLUC application. Control responses (C: 100% = 400–600 μV) were recorded before saline, KYNA, or KYNA-NH-GLUC injection. $**P<.01$; $*P<.05$.

sampling rate of 10 kHz on a pClamp8 system (Axon Instruments, USA, Digidata 1320 A/D board), and analyzed off line with Origin software (Microcal Software, USA). The averaged amplitude ($n=4$) of the initial negative component of the fEPSPs evoked by stimuli applied through horizontally displaced electrodes in layers II/III was used as a measure of the population excitatory synaptic potential.

2.6.2. Statistics

The behavioral data were evaluated by repeated measures ANOVA and the paired sample *t* test, followed by Bonferroni's correction (SPSS 9.0). For electrophysiological studies, statistical significance was determined by means of Student's *t* test. In all cases, *P* was set at .05 prior to the experiments.

3. Results

3.1. Behavioral experiments

The behavioral effects of intracerebroventricularly injected KYNA are already well documented (Vécsei and Beal, 1990a,b, 1991). Since a repeated detailed study and discussion of the behavioral changes induced by KYNA was not the purpose of this article, we focus here only on whether intracerebroventricularly applied KYNA induces similar changes in behavior under the present circumstances as those described more than 10 years ago (Vécsei and Beal, 1990a,b, 1991). In order to confirm that KYNA induces changes in behavior, we injected 0.3 μmol icv KYNA. This amount of the compound-induced characteristic changes in the behavior of the animals after each of the consecutive injections. The animals developed grooming, sniffing, and gnawing behavior 3–5 min after the microinjections. Consequently, their exploration and rearing activities were markedly decreased, as described by Vécsei and Beal (1990a,b) and Vécsei and Beal (1991). As an example, the changes in grooming activity and ataxia after the injection of 0.3 μmol icv KYNA are illustrated in Fig. 2A.

The effects of equimolar KYNA-NH-GLUC were also tested. The results of these experiments were very similar to those found after 0.3 μmol pure KYNA (Fig. 2B).

Similar but more dramatic effects were observed after the injection of 0.6 μmol icv KYNA or KYNA-NH-GLUC (not shown).

3.2. In vivo electrophysiology

Five controls, 15 KYNA, and 15 KYNA-NH-GLUC intravenously injected animals participated in these experiments. The cortical electrophysiological effects of intravenously administered 10, 20, 25, or 50 mg/kg KYNA or KYNA-NH-GLUC were tested on three animals at each dosage. As marked effects of the intravenously injected drugs were not seen at doses of 10 and 20 mg/kg and KYNA-NH-GLUC resulted in 100% mortality within 5 min at 50 mg/kg iv, we tested the effects of both drugs in a dose of 25 mg/kg on three additional animals each. The anaesthetized animals were placed into a stereotaxic frame. The right side vibrissa pad-stimulation EPs were recorded from the contralateral SI transdurally. After observation of the EPs in the punctum maximum of the SI, the effects of KYNA and KYNA-NH-GLUC on the cortical-evoked responses were tested by intravenous injection of the drugs.

The control animals, which received 0.5 ml iv saline, did not exhibit any significant change in the amplitude of the EPs (Fig. 3A). KYNA in a dose of 25 mg/kg also resulted in

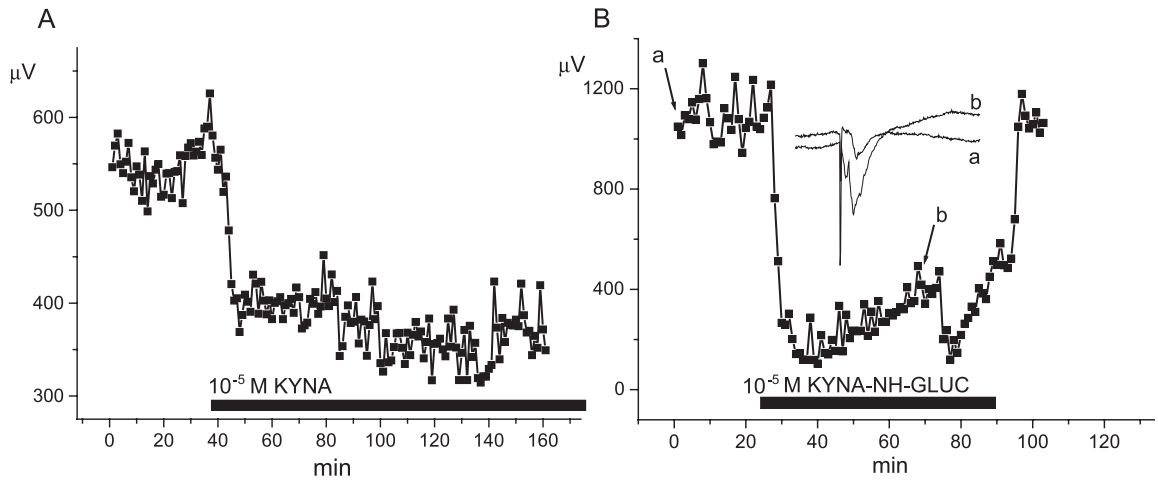

Fig. 4. fEPSP amplitudes in slices from primary motor cortices are plotted as a function of time before and during the application of KYNA (A) and before, during, and after KYNA-NH-GLUC application (B). Both KYNA and KYNA-NH-GLUC were applied in a flow of ACSF. The thick line in (A) shows the time of 10^{-5} M KYNA application. (B) A representative example of the experiments in which ACSF containing KYNA-NH-GLUC (10^{-5} M) was applied for 70 min (thick line). Inset in B, (a) an averaged response to stimuli in the control period (sampled at 24 min before KYNA-NH-GLUC application); (b) an averaged response to stimuli observed during KYNA-NH-GLUC application. Ordinate: fEPSP amplitudes in μV. Abscissa: time in min.

hardly any change in the amplitude of the evoked responses. In some cases, a very slight decrease in amplitude was seen, with a minimum at about 10–15 min after intravenous injection; however, this change was never significant (Fig. 3B). In contrast with the slight, if any effect of KYNA, KYNA-NH-GLUC (25 mg/kg) gave rise to a marked decrease in amplitude of the EPs, with a minimum at 5 min after intravenous injection (Fig. 3C). KYNA-NH-GLUC applied in a dose of 25 mg/kg resulted in the sudden death of 1 animal out of the six (16%), while the mortality within 5 min was 100% when this compound was injected intravenously in a dose of 50 mg/kg.

3.3. In vitro electrophysiology

The effects of KYNA and of KYNA-NH-GLUC on the fEPSPs were studied in vitro on slices from the primary motor cortex of young animals. The amplitude of the initial negative component of the fEPSPs evoked by stimuli applied through horizontally displaced electrodes in layers II/III was recorded. The effects of KYNA and KYNA-NH-GLUC on the amplitude of the fEPSPs were observed at a concentration of 10^{-5} M, when the amplitude of the fEPSPs decreased after drug application. The reduction in amplitude reached its maximum with a 10- to 15-min delay and persisted during the drug application (Fig. 4A and B). The effects of KYNA and of KYNA-NH-GLUC were quite similar and could not be distinguished. In some cases, the reduction in fEPSP amplitude was moderate: it decreased to only 65–70% of the control response (Fig. 4A); while in other cases, the amplitudes of the fEPSPs fell to 20% of the control responses during drug application (Fig. 4B). The degree of reduction did not depend on the drug applied (KYNA or KYNA-NH-GLUC) but rather on the slice, the position of the stimulating, recording electrodes, etc.

4. Discussion

This is the first description of a new KYNA derivative, which displays effects similar to those of KYNA, but which crosses the BBB much better than does KYNA itself. The ability of KYNA to function as an EAA receptor antagonist was originally reported by Perkins and Stone (1982), and it soon became apparent that KYNA preferentially inhibits NMDA receptors (Ganong et al., 1983). KYNA was found to exhibit a particularly high affinity for the glycine/NMDA binding site (Kessler et al., 1989). However, a new interpretation of the KYNA mechanism should also be taken into account, especially at low KYNA concentrations. Hilmas et al. (2001) reported that alpha7 nicotinic receptors are targets for KYNA. On the basis of a microdialysis method, Carpenedo et al. (2001) also suggest that presynaptic kynurenate-sensitive receptors inhibit the release of glutamate. They found that, at a low concentration of KYNA, the kynurenate-induced inhibition of glutamate release is not mediated by glutamate receptors; they suggest that nicotinic acetylcholine receptors may contribute to the inhibitory effects of kynurenate on glutamate release.

Whatever the mechanism of action of KYNA is, KYNA and its analogues came into the center of research because of their possible role as neuroprotectants (Stone, 2001). During the past decade, research into the biological effects of KYNA has accelerated in many fields (Speciale et al., 1996a,b; Wu et al., 2000). The behavioral effects of intracerebroventricularly applied KYNA have been well known since the early 1990s; among others, KYNA induces dose-dependent increases in stereotyped behavior and ataxia (Vécsei and Beal, 1990b, 1991). Our knowledge of its electrophysiological effects has also increased. It has been found, for example, that KYNA inhibits excitatory synaptic transmission in rat hippocampus slices and in the isolated immature rat spinal cord (Ganong et al., 1983). Later, it was observed that KYNA decreases the evoked responses in area CA1 and the dentate gyrus both in vivo and in vitro (Perkins and Stone, 1985; Scharfman and Goodman, 1998). However, not much is known about its cortical effects.

Since the behavioral effects of KYNA have been well documented, we did not set out to perform another detailed behavioral study, but rather to test whether KYNA-NH-GLUC has the same behavioral and electrophysiological effects as those observed with pure KYNA and especially whether KYNA-NH-GLUC is more effective than KYNA on intravenous administration.

In agreement with the literature findings (Vécsei and Beal, 1990b, 1991), the behavioral experiments revealed that intracerebroventricularly microinjected KYNA induces stereotyped behavior and ataxia. Similar changes in behavior were found after intracerebroventricularly microinjected equimolar KYNA-NH-GLUC.

The results of our in vitro studies are in agreement with the findings of behavioral experiments: the two compounds had similar effects, i.e., KYNA and KYNA-NH-GLUC induced a reduction in the fEPSP amplitudes. In these in vitro experiments, we stimulated the layer II/III horizontal pathways; glutamate is known to be the major transmitter of these pathways in the motor cortex (Hess et al., 1994). While most glutamatergic transmissions are relayed by other glutamate receptor subtypes, NMDA receptor activation contributes a consistent proportion of the ordinary transmission in each of these pathways in vitro (Hess et al., 1994). Besides the possible blockade of postsynaptic NMDA receptors, presynaptic nicotinic acetylcholine receptors may also contribute to the inhibitory effects of kynurenate on glutamate release, especially at low concentration, as described by Carpenedo et al. (2001).

Although, similarly to KYNA-NH-GLUC, KYNA was effective in in vitro electrophysiological studies and in behavioral experiments (on intracerebroventricular administration), it cannot be administered intravenous as an NMDA-receptor antagonist because it is practically unable to cross the BBB (Swartz et al., 1990; Fukui et al., 1991). It

can enter the brain after peripheral administration when a 300 mg/kg bolus procedure is used, followed by an infusion at 300 mg/kg/h for 3 h (Scharfman and Goodman, 1998). However, the possible use of KYNA as a putative neuroprotectant requires facilitation of its passage across the BBB. The results of our in vivo experiments suggest that this problem could be solved; the NH-GLUC conjugated to KYNA probably promotes the passage across the BBB. This is supported by the mortality data: KYNA-NH-GLUC administered intravenously in a dose of 50 mg/kg resulted in 100% mortality, whereas KYNA at 50 mg/kg did not induce any detectable effect. This finding is in good accordance with the earlier result of Vécsei and Beal (1991), who observed 100% mortality after the intracerebroventricular administration of 1.6 μmol KYNA. The reason for the deaths was not examined in detail, but brainstem respiratory problems appear probable. The lower KYNA-NH-GLUC dose of 25 mg/kg iv resulted in marked decreases in amplitude of the cortical responses relative to those of KYNA (given intravenously in the same dose).

It should be noted that the 25 mg/kg dose of KYNA-NH-GLUC is probably in excess of that required to decrease the cortical activity effectively. Our ongoing studies are aimed at finding the minimum but still sufficient dose of KYNA-NH-GLUC and the most appropriate means and timing of its application.

Additionally to the passage of KYNA-NH-GLUC across the BBB, these results suggest that the conjugated NH-GLUC does not influence the binding of KYNA to its receptor binding site, either pre- or postsynaptically.

Recent publications indicate that KYNA is becoming more important both in basic studies on the CNS (Tancredi et al., 2000; Ito and Hicks, 2001; Goldstein-Daruech et al., 2002) and in pharmaceutical research (Stone, 2001).

On the basis of the results presented above, we conclude that KYNA and KYNA-NH-GLUC have similar effects, but that KYNA-NH-GLUC passes the BBB much more readily than does KYNA. It raises our hopes that the glucoseamine conjugate of KYNA or its derivatives might be of use in the future as a neuroprotective agent.

Acknowledgements

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