Cellular and Molecular Neurobiology, Vol. 25, No. 6, September 2005 (©2005)
DOI: 10.1007/s10571-005-8469-y

Kynurenic Acid Inhibits the Release of the Neurotrophic Fibroblast Growth Factor (FGF)-1 and Enhances Proliferation of Glia Cells, *in vitro*

Claudia Di Serio,[1] Andrea Cozzi,[2] Ilaria Angeli,[1] Laura Doria,[1] Isabella Micucci,[1] Silvia Pellerito,[1] Patrizia Mirone,[1] Giulio Masotti,[1] Flavio Moroni,[2] and Francesca Tarantini[1,3]

Received January 11, 2005; accepted June 6, 2005

SUMMARY

1. Kynurenic (KYNA) and quinolinic (QUIN) acids are neuroactive tryptophan metabolites formed along the kynurenine pathway: the first is considered a non-competitive antagonist and the second an agonist of glutamate receptors of NMDA type. The affinity of these compounds for glutamate receptors is, however, relatively low and does not explain KYNA neuroprotective actions in models of post-ischemic brain damage.

2. We evaluated KYNA effects on the release of fibroblast growth factor (FGF)-1, a potent neurotrophic cytokine. Because KYNA exhibits a neuroprotective profile *in vitro* and *in vivo*, we anticipated that it could function as an autocrine/paracrine inducer of FGF-1 release. Studies were performed in several models of FGF-1 secretion (FGF-1 transfected NIH 3T3 cells exposed to heat shock, A375 melanoma cells exposed to serum starvation, growth factor deprived human endothelial cells). To our surprise, KYNA, at low concentration, inhibited FGF-1 release in all cellular models. QUIN, a compound having opposite effects on glutamate receptors, also reduced this release, but its potency was significantly lower than that of KYNA.

3. KYNA and QUIN also displayed a major stimulatory effect on the proliferation rate of mouse microglia and human glioblastoma cells, *in vitro*.

[1] Department of Critical Care Medicine and Surgery, Geriatric Medicine Unit, University of Florence, School of Medicine, Viale Pieraccini n. 6, Florence, Italy, 50139.

[2] Department of Pharmacology, University of Florence, Florence, Italy.

[3] To whom correspondence should be addressed at Department of Critical Care Medicine and Surgery, Geriatric Medicine Unit, University of Florence, School of Medicine, Centro Polivalente per la Didattica e la Ricerca (Cubo Alto), Viale Pieraccini n. 6, 50139, Florence, Italy; e-mail: taranf@unifi.it.

Abbreviations used: CL, cell lysate; CM, conditioned medium; DMEM, Dulbecco's modified Eagle's medium; DTT, dithiothreitol; FBS, fetal bovine serum; FGF, fibroblast growth factor; HS, heat shock; HUVEC, human umbilical vein endothelial cell; IDO, indoleamine 2,3-dioxygenase; KP, kynurenine pathway; KYN, kynurenine; KYNA, kynurenic acid; LDH, lactate dehydrogenase; PAGE, polyacrylamide gel electrophoresis; QUIN, quinolinic acid; SDS, sodium dodecyl sulfate; TEB, Tris EDTA buffer.

0272-4340/05/0900-0981/0 © 2005 Springer Science+Business Media, Inc.

4. Our data suggest that minor changes of local KYNA concentration may modulate FGF-1 release, cell proliferation, and ultimately tissue damage in different pathological conditions.

KEY WORDS: kynurenic acid; quinolinic acid; fibroblast growth factor-1; secretion; neuroprotection; proliferation; glia.

INTRODUCTION

Kynurenines are tryptophan metabolites formed along the pathway leading to nicotinamide adenine dinucleotide synthesis (Stone and Connick, 1985; Moroni, 1999). Among them, quinolinic (QUIN) and kynurenic (KYNA) acids have been widely investigated in the last 20 years because of their neuroactive properties: QUIN is an agonist of glutamate receptors of NMDA type and may cause excitotoxic neuronal death (Stone and Perkins, 1981; Schwarcz *et al.*, 1983) while KYNA is considered a non-competitive NMDA receptor antagonist, able to interact with the glycine recognition site present on the NMDA receptor–ion channel complex (Moroni *et al.*, 1989; Kessler *et al.*, 1989; Stone, 1993). Procedures aimed at increasing KYNA concentrations in the brain cause sedation, reduce neuronal excitability, and excitotoxic or post-ischemic brain damage, both *in vivo* and *in vitro* (Russi *et al.*, 1992; Carpenedo *et al.*, 1994; Chiarugi *et al.*, 1995; Cozzi *et al.*, 1999; Wu *et al.*, 2000). KYNA concentrations in the cerebrospinal fluid and in rat brain extracellular spaces are approximately 10–100 nM (Moroni *et al.*, 1988; Turski *et al.*, 1988; Swartz *et al.*, 1990) and an increase of three times these concentrations is sufficient to exert a potent neuroprotective action (Cozzi *et al.*, 1999; Carpenedo *et al.*, 2002). However, KYNA affinity for the glycine site of NMDA receptors is relatively low (approximately 30 μM) (Stone, 2000) suggesting that KYNA neuroprotective action in the brain is likely not mediated by the antagonism of these receptors.

It has been reported that low micromolar concentrations (1–2 μM) of KYNA are sufficient to antagonize, in a non-competitive manner, a subgroup of nicotinic receptors (alpha 7 subtype) permeable to calcium (Hilmas *et al.*, 2001) and recently Rassoulpour *et al.* demonstrated that even low nanomolar concentrations of KYNA were able to block nicotinic receptors (Rassoulpour *et al.*, 2005).

We have previously demonstrated that low nanomolar concentrations of KYNA, applied to the rat striatum with a microdialysis technique, significantly reduced glutamate output (Carpenedo *et al.*, 2001), although the mechanism of this inhibition and the cell type involved have not been clarified. However, the ability of KYNA to interfere with the release of a neuroactive compound and its overall neuroprotective profile prompted us to investigate the effects of KYNA on the release of a powerful neurotrophic molecule, fibroblast growth factor (FGF)-1. FGF-1 is a prototype member of the FGF family (Friesel and Maciag, 1999). In the central nervous system, FGF-1 is produced by glia, astrocytes, and neuronal cells (Bizon *et al.*, 1996) and is able to support the growth of cortical neurons and to facilitate survival of injured cells (Le and Esquenazi, 2002). FGF-1 and FGF-2, released by astrocytes, have been shown to reduce the effect of experimental injury mediated by neurotoxins (Mocchetti and Wrathall, 1995), including QUIN (Hossain *et al.*, 2002). To our

surprise, we found that KYNA and, less efficiently, QUIN had a major inhibitory effect on the release of FGF-1. Moreover, we also demonstrated that KYNA and QUIN induced proliferation of glia cells.

METHODS

Cell Culture

NIH 3T3 cells stably transfected with the human FGF-1 gene (Jackson *et al.*, 1992) were grown on human fibronectin (10 μg/cm^2)-coated dishes (Falcon) in Dulbecco's modified Eagle's medium (DMEM) (EuroClone), supplemented with 10% (v/v) fetal bovine serum (FBS) (EuroClone), 1% (v/v) antibiotic/antimycotic solution (Gibco), and 400 (μg/ml G418 (EuroClone). The human melanoma cell line A375 (ATCC, Rockville, MD, USA), the murine microglia cell line N11 (Sassano *et al.*, 1994), and the human glioblastoma cell line U-343 MG (a generous gift of Dr. M. Landriscina, University of Foggia, Italy) (Xie *et al.*, 2004) were grown in DMEM supplemented with 10% (v/v) FBS and 1% (v/v) antibiotic/antimycotic solution. Human umbilical vein endothelial (HUVE) cells (ATCC, Rockville, MD, USA) were grown on human fibronectin (10 μg/cm^2)-coated dishes in M199 (EuroClone), supplemented with 10% (v/v) FBS, 1% (v/v) antibiotic/antimycotic solution, 5 U/ml heparin (Heparin Vister, Pfizer) and 1$\times$ endothelial cell growth (ECG) supplement (Sigma).

Heat Shock, Processing of Conditioned Media and Immunoblot Analysis

Heat shock (HS) was performed as previously described (Tarantini *et al.*, 1998). Briefly, the day before the experiment, the same number of FGF-1 transfected NIH 3T3 cells were plated for each experimental sample, in 15-cm fibronectin-coated dishes. The next day (cell density around 70–80%) HS was performed in DMEM, containing 5 U/ml heparin, exposing the cells to a temperature of 42°C for 110 min. Cells maintained at 37°C were used as controls. To analyze the effect of kynurenine (KYN), KYNA, and QUIN (Sigma) on FGF-1 release, HS experiments were performed pre-incubating the cells with increasing concentrations of KYN, KYNA, and QUIN, at 37°C, 1 h before the experiment, and then exposing the cells to high temperature in the presence of tryptophan metabolites. Cells maintained at 37°C in the presence of KYN, KYNA, and QUIN were used as controls. Following HS, cells were counted again and the same number of cells was processed over a heparin column (heparin-Sepharose CL-6B column; Amersham Pharmacia Biotech) to obtain total cell lysates (CL). Cell number was also used to normalize the amount of conditioned medium (CM) processed over heparin. CL were obtained by washing the cells in cold phosphate-buffered saline (PBS) and then collecting the cells by centrifugation (1000$\times g$, 2 min). Cell pellets were resuspended in 2 ml of cold 1% (v/v) Triton X-100 (Sigma) and lyzed by incubation on ice for 10 min. Lysates were then clarified by centrifugation (2000$\times g$, 10 min). CM were collected, filtered through a 0.22 μm filter and treated with 0.1% (w/v) dithiothreitol (DTT) (Amersham Pharmacia Biotech)

for 2 h at 37°C. CM and one-third of each CL were adsorbed to 1 ml heparin-Sepharose column, pre-equilibrated with 50 mM Tris, 10 mM EDTA, pH 7.4 (TEB). The column was washed with TEB and adsorbed proteins were eluted with TEB containing 1.5 M NaCl. Eluted proteins were concentrated through a 10 kDa concentrator (Vivaspin, Vivascience), resolved by 12% (w/v) sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE), transferred to a nitrocellulose membrane (Hybond C, Amersham Pharmacia Biotech) and immunoblotted using anti-FGF-1 polyclonal antibodies (a generous gift of Dr. T. Maciag and Dr. I. Prudovsky, Center for Molecular Medicine MMCRI, Maine, USA), as previously described (Jackson *et al*., 1992). FGF-1 immunoreactive bands were visualized by chemioluminescence (ECL, Amersham Pharmacia Biotech), following manufacturer's instructions. The activity of lactate dehydrogenase (LDH) in CM was utilized as an assessment of cell lysis in all experiments and was measured by a colorimetric assay using pyruvate (Sigma) as a substrate. Experiments were performed at least three times and similar results were obtained. Representative autoradiograms are shown.

Serum Starvation, Supplement Withdrawal, Processing of Conditioned Media and Immunoblot Analysis

The day before the experiment, the same number of A375 and HUVE cells were plated for each experimental sample, in 15-cm dishes. A375 were grown until 70–80% confluency in DMEM supplemented with 10% FBS, until starvation was started by exposing the cells to DMEM containing 2% FBS and 5 U/ml heparin (starvation medium) for 10–12 h. HUVE cells were grown until 70–80% confluency in M199 supplemented with 10% FBS and 1× ECG supplement, until supplement withdrawal was started by exposing the cells to supplement-free M199, containing 10% FBS and 5 U/ml heparin for 10–12 h. To evaluate the effect of tryptophan metabolites on FGF-1 release, KYNA and QUIN at the indicated concentrations were added to the A375 cell culture medium. Only 1 μM KYNA was tested on HUVE cells. Normalization among samples was obtained by counting the number of cells at the end of each experiment. CM were then collected, filtered through a 0.22 μm filter, and treated with 0.1% (w/v) DTT as described above. To analyze the level of expression of endogenous FGF-1 in A375 and HUVE cells, cell pellets were collected by centrifugation and total cell lysates were obtained in lysis buffer, as previously described. CM and one-half of each CL were processed over a heparin-Sepharose CL-6B column and analyzed by FGF-1 immunoblotting, as described above. In all experiments, cell viability was assessed by measuring LDH activity in CM, as reported previously. A375 starvation experiment was performed three times; HUVE cell supplement withdrawal experiment was performed twice and similar results were obtained. Representative autoradiograms are shown.

Cell Proliferation Assay

N11 cells were seeded at a density of 4×10^4 cells/well in 6-well cluster plates and grown in DMEM supplemented with 0.5% FBS with or without KYNA or

QUIN. U-343 MG cells were seeded at a density of 4×10^4 cells/well in 6-well cluster plates and grown in DMEM supplemented with 10% or 3% FBS, with or without KYNA. HUVE cells at passage 3 were seeded at a density of 2×10^4 cells/well in 6-well cluster plates and grown in M199 supplemented with 10% FBS, 5 U/ml heparin, with or without KYNA, in the presence or absence of 20 ng/ml FGF-1 recombinant protein (a generous gift of Dr. T. Maciag and Dr. I. Prudovsky, Center for Molecular Medicine MMCRI, Maine, USA). Cells were fed every 2 days with fresh medium. The number of viable cells was counted after trypsinization by hemacytometer at timed intervals, in triplicates, using two separate measurements per well. A statistical comparison between growth curves at each observation point was performed by using the Student's *t*-test. A *p*-value less than 0.05 was considered statistically significant.

Measurement of KYN and KYNA in Cell Culture Medium

Cells (1×10^6) were incubated with 2 ml of cell culture medium for 24 h. The medium was collected, treated with an equal volume of 100% trichloroacetic acid and centrifuged at $3000\times g$ for 15 min. Supernatants (500 μl) were injected into an HPLC apparatus with an UV detector and analyzed as previously described (Holmes, 1988; Moroni *et al.*, 1991). Briefly, a reverse phase column (Spherisorb S5 ODS2, 25 cm) was used with a mobile phase containing 0.1 mmol/l ammonium acetate, 0.1 mol/l acetic acid, and 2% (v/v) acetonitrile, at 1 ml/min flow rate. KYN was detected at 365 nm and KYNA at 338 nm (UV detector: Perkin Elmer model LC 90). Concentration of KYNA in CM derived from NIH 3T3 cells treated with 1, 10, and 100 μM KYN for 3 h was determined by HPLC as described above.

RESULTS

KYNA and QUIN Inhibit the Release of FGF-1 from Fibroblasts and Melanoma Cells

FGF-1 transfected NIH 3T3 cells pre-incubated with increasing concentrations of KYNA, QUIN, or their precursor KYN, were subjected to heat shock to induce secretion of FGF-1 (Tarantini *et al.*, 1998). As shown in Fig. 1, KYNA was a very potent inhibitor of FGF-1 release (Fig. 1B, CM); on the other hand KYN, even at the highest concentrations, was unable to completely block secretion (Fig. 1A, CM). A noticeable concentration-dependent reduction of FGF-1 release was also observed when the cells were treated with QUIN, although at higher concentrations than KYNA (Fig. 1C, CM). Treatment with KYN, KYNA and QUIN had no effect on the level of FGF-1 protein detected in 37°C cell lysates (Fig. 1A–C, CL). Interestingly, under basal conditions the concentration of KYNA in the medium derived from confluent cultures of NIH 3T3 cells was 4.8 nM (Table I). These data indicated that a relatively modest increase of KYNA concentrations above basal level was sufficient to significantly inhibit FGF-1 release in this experimental system. Because KYN can be metabolized to KYNA inside the cells, although quite inefficiently (Moroni, 1999), we analyzed the amount of KYNA that was produced and released in the

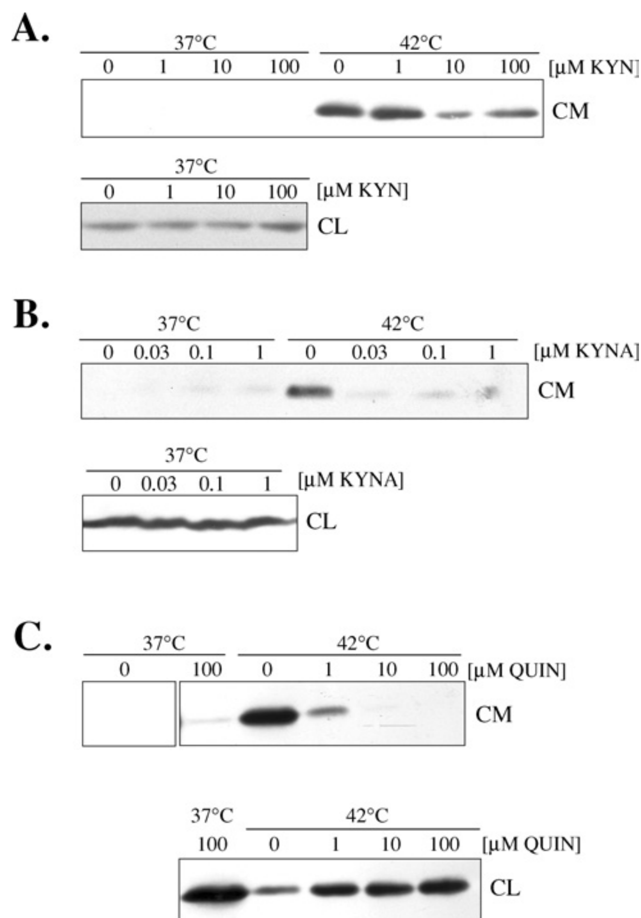

Fig. 1. Effect of kynurenines on FGF-1 release from NIH 3T3 cells. Conditioned media and cell lysates from cells exposed to heat shock in the presence or absence of increasing concentrations of KYN (A), KYNA (B), or QUIN (C). (A) 37 and 42°C CM in the presence of increasing concentrations of KYN; 37°C CL in the presence of increasing concentrations of KYN. (B) 37 and 42°C CM, in the presence of increasing concentrations of KYNA; 37°C CL, in the presence of increasing concentrations of KYNA. (C) 37 and 42°C CM, in the presence of increasing concentrations of QUIN; 37 and 42°C CL, in the presence of increasing concentrations of QUIN. Note that in (C), in CL corresponding to cells exposed to HS (42°C), the level of FGF-1 protein detected in the intracellular compartment is consistent with the amount of protein that had been released in CM.

medium when NIH 3T3 cells were incubated with 1, 10, and 100 μM KYN for 3 h: the levels of KYNA found in the extracellular space remained undetectable in cells incubated with 1 and 10 μM KYN, and reached 15 nM when cells were exposed to 100 μM KYN. These data explained why we did not detect a significant inhibition of FGF-1 secretion in the presence of KYN.

Table I. Concentrations of KYN and KYNA in CM of Confluent Cultured Cells

	A375	NIH 3T3	N11
KYN (μM)	0.22 ± 0.04	0.40 ± 0.02	0.40 ± 0.03
KYNA (nM)	6.8 ± 0.7	4.8 ± 0.3	4.2 ± 0.9

Note. Each value represents the average of four different experiments and is expressed as mean ± SD.

We next analyzed the effect of KYNA and QUIN in another model of FGF-1 secretion: A375 melanoma cells subjected to serum starvation. As shown in Fig. 2, when the cells were grown in DMEM containing 10% serum, only a small amount of endogenous FGF-1 was detected in CM (Fig. 2A, CM). Under these experimental conditions concentration of KYNA in the medium was 6.8 nM (Table I). When the

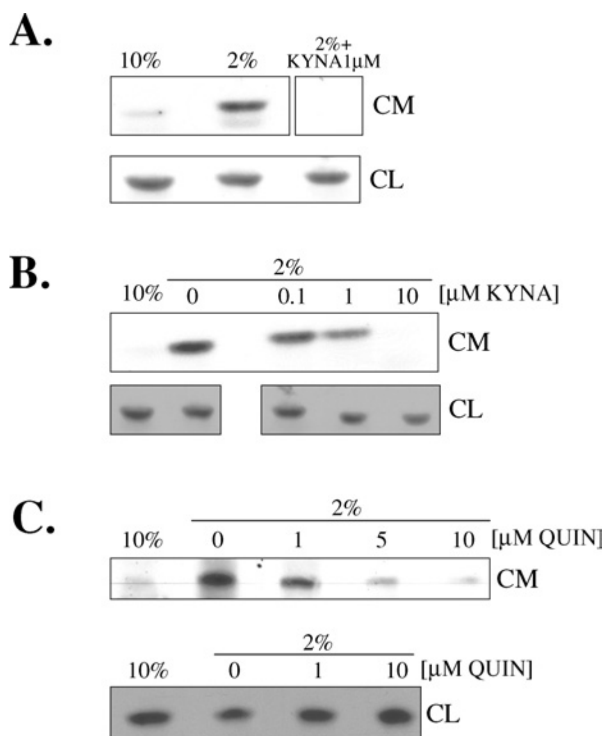

Fig. 2. Effect of kynurenines on FGF-1 release from A375 cells. Conditioned media and cell lysates from cells exposed to serum starvation in the presence or absence of KYNA or QUIN. (A) CM and CL from cells exposed to 10% serum, 2% serum, and 2% serum + 1 μM KYNA. (B) 10 and 2% serum CM and CL, in the presence of increasing concentrations of KYNA; (C) 10 and 2% serum CM and CL, in the presence of increasing concentrations of QUIN.

cells were shifted from 10 to 2% serum, an increased amount of secreted FGF-1 was recovered from CM after 10 h incubation (Fig. 2A, CM). However, FGF-1 release significantly decreased in the presence of KYNA (Fig. 2A, CM). When we looked at the concentration–response of KYNA and QUIN we found that both compounds inhibited FGF-1 secretion in a concentration-dependent manner (Fig. 2B and C, CM). Again, KYNA was able to block the secretory pathway at lower concentrations than QUIN. There was never a correlation with LDH release. Moreover, KYNA (Fig. 2A and B, CL) and QUIN (Fig. 2C, CL) did not change the expression of endogenous FGF-1 in A375 cells, suggesting that tryptophan metabolites did not interfere with the mechanisms of transcription and translation of this growth factor.

The IC_{50} for the inhibitory effect of KYNA and QUIN on FGF-1 release was calculated from three separate experiments and was about 200 nM for KYNA and 3.5 μM for QUIN.

KYNA Inhibits the Release of FGF-1 from Human Endothelial Cells and Affects Cell Proliferation

Human endothelial cells are known to be FGF-dependent for growth (Basilico and Moscatelli, 1992). In the absence of exogenously added FGF-1 or FGF-2, HUVE cells undergo only a limited number of cell divisions in culture, likely mediated by the production and release of endogenous growth factors. Indeed, HUVE cells grown in the absence of ECG supplement released endogenous FGF-1 in the culture medium (Fig. 3A, CM lane 1). However, the addition of KYNA significantly inhibited the release of FGF-1 (Fig. 3A, CM lane 2) which remained intracellular (Fig. 3A, CL). To confirm these data, we evaluated the effects of KYNA on the proliferation rate of HUVE cells in culture. As expected, when HUVE cells were grown in the absence of exogenous FGF-1 (Fig. 3B, light gray bar), their proliferation rate was significantly hampered compared to cells grown in the presence of recombinant growth factor (white bar) and was sustained by the production and secretion of endogenous FGF. The addition of KYNA to this culture totally prevented cell proliferation (dark gray bar), confirming that KYNA was able to interfere with the release of FGF. Indeed, when we added recombinant FGF-1 to the medium, KYNA effect on HUVE cell growth was abolished (black bar).

KYNA Increases the Proliferation Rate of Mouse Microglia and Human Glioblastoma Cells

The inhibitory effect of KYNA on the release of a potent neurotrophic factor like FGF-1 was totally unanticipated and could not explain the neuroprotective action of KYNA. In the effort to identify which biological function of this tryptophan metabolite could support its neuroprotective outcome, we investigated the ability of KYNA to influence the proliferation rate of cells of the immune system. Using a mouse microglia cell line, N11, we demonstrated that the proliferation rate of glia cells significantly increased in the presence of KYNA (Fig. 4A). A higher proliferation rate was also observed when QUIN was added to the culture but, in agreement with the results obtained on FGF-1 release, QUIN was active at higher

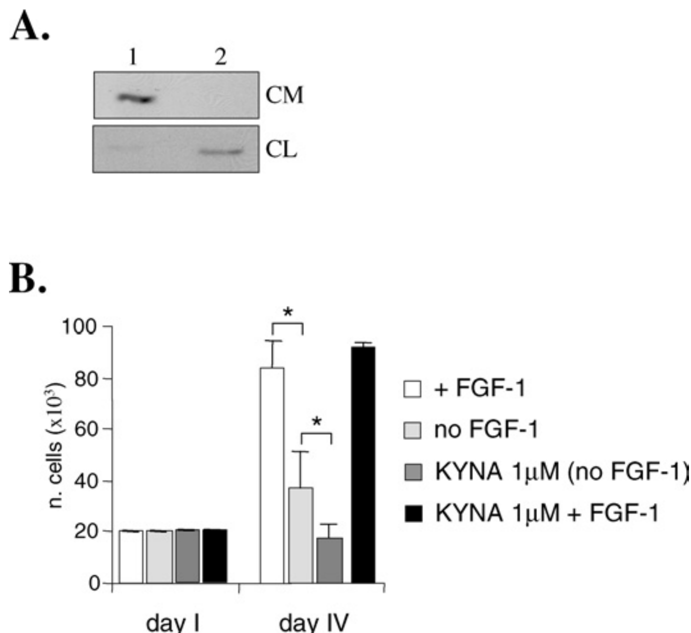

Fig. 3. Effect of KYNA on FGF-1 release from HUVE cells. (A) Cell lysates and conditioned media from cells exposed to growth factor supplement withdrawal in the absence (lane 1) or presence (lane 2) of 1 μM KYNA. (B) HUVE cells at passage 2 were plated at low density (2×10^4 cells/well, day I) and grown in 10% serum without KYNA or with 1 μM KYNA, in the absence or presence of recombinant FGF-1 (20 ng/ml). Cells were detached by trypsinization and counted by hemacytometer at day IV. Each value represents the mean ± SD of three different wells, with two separate measurements per well. Student's paired *t*-test was used for statistical analysis. $^*p < 0.05$.

concentration (Fig. 4A). Interestingly, when we looked at the human and mouse macrophage cell lines J774 and U937, neither KYNA nor QUIN were able to modify cell growth (data not shown). To examine whether the proliferative effect of KYNA was selective for glia cells, we studied the proliferation rate of a human glioblastoma cell line, U-343 MG, in the presence or absence of KYNA. As shown in Fig. 4B, KYNA significantly increased the growth rate of these cells in culture. The ED_{50} for the proliferative effect of KYNA and QUIN on N11 cells was calculated from three separate experiments and was about 25 nM for KYNA and 530 nM for QUIN.

DISCUSSION

Astrocytes and other glia cells synthesize KYNA under physiological conditions and upregulate its production after neurotoxic insults or inflammatory stimulation (Wu *et al.*, 1992; Guillemin *et al.*, 2001). Because astrocytes and glia cells are also

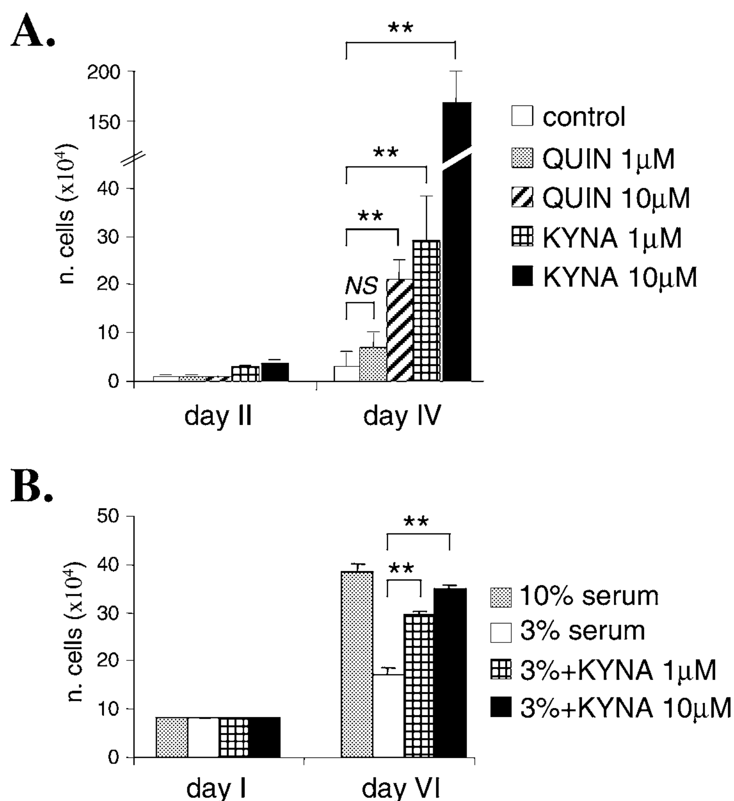

Fig. 4. Effect of KYNA on the proliferation rate of glia cells. (A) N11 cells were plated at low density (4×10^4 cells/well) and grown in 0.5% serum with or without KYNA and QUIN. Cells were detached by trypsinization and counted by hemacytometer at day II and IV. (B) U-343 MG cells were plated at low density (8×10^4 cells/well, day I) and grown in 3% serum with or without KYNA. Cells grown in 10% serum were used as control of optimal growth. Cells were detached by trypsinization and counted by hemacytometer at day VI. Each value represents the mean ± SD of three different wells, with two separate measurements per well. Student's paired *t*-test was used for statistical analysis. NS, not significant; $^{**}p < 0.001$.

the major producers of FGF-1, a potent neurotrophic polypeptide, and because KYNA exhibits a neuroprotective profile *in vitro* and *in vivo*, we anticipated that KYNA could function as an autocrine/paracrine inducer of FGF-1 secretion in the brain. To our surprise, KYNA displayed a potent inhibitory effect on the release of FGF-1 in all three models of secretion: FGF-1 transfected NIH 3T3 cells subjected to HS, A375 melanoma cells subjected to serum starvation and human endothelial cells. Unfortunately, all brain-derived cell lines that we tested, showed low level of expression of endogenous FGF-1, making it difficult for us to study the effect of KYNA on the growth factor release in such experimental systems. Therefore, the assumption that KYNA would have the same biological effect on neuronal cells or brain tissues *in vivo* should be made with care.

The inhibition of FGF-1 secretion was certainly not in line with KYNA neuroprotective actions. We also noticed that KYNA was able to selectively induce proliferation of microglia cells, another unexpected biological effect not in agreement with the potent neuroprotective action of KYNA in post-ischemic brain damage.

Do our data challenge the biological definition of KYNA as a neuroprotective metabolite? We previously reported that nanomolar concentrations of KYNA extensively reduce the depolarization-induced glutamate release in isolated synaptosomal preparations and glutamate extracellular concentrations in different brain regions (Carpenedo *et al.*, 2001). Although the molecular mechanism of such inhibition and the possible receptors involved are still not identified, it is likely that the same effect may apply to the inhibition of FGF-1 release as well as the release of other neuroactive molecules. Indeed, we previously demonstrated that the mechanism of interleukin (IL)-1α secretion is very similar, if not identical, to that of FGF-1 (Tarantini *et al.*, 2001). Therefore, even though blocking the release of FGF-1 does not explain neuroprotection, inhibiting glutamate output or the release of inflammatory cytokines, such as IL-1, would have a potent neuroprotective effect on brain tissues. The ability of KYNA to block IL-1 release from activated immune cells is the subject of an ongoing investigation.

During the preparation of this manuscript, Rassoulpour and colleagues have published that nanomolar concentrations of KYNA reduce the extracellular levels of dopamine in the striatum, *in vivo*. They also demonstrated that the effect is mediated by KYNA-stimulated blockade of alpha 7 nicotinic acetylcholine receptors (Rassoulpour *et al.*, 2005). Therefore, a modest increase in brain levels of endogenous KYNA was capable of interfering with dopaminergic neurotransmission. Because the effect of nicotinic receptor activation on FGF-1 release was not investigated, we cannot exclude the possibility that the inhibition of FGF-1 secretion could be mediated by the interaction of KYNA with acetylcholine receptors of the alpha 7 subtype.

The effect of KYNA on endothelial cells deserves special attention. Because FGF-1 is also a potent pro-angiogenic polypeptide (Friesel and Maciag, 1999), the ability of KYNA to block FGF-1 release and, as a consequence, proliferation of endothelial cells, strongly suggest that by changing local KYNA concentrations it may be possible to regulate angiogenesis.

It is interesting to note that in our experimental system QUIN had effects qualitatively similar to those of KYNA but it was less potent. Since it is largely accepted that KYNA and QUIN have opposite actions on glutamate receptors of NMDA type, it is reasonable to assume that these receptors are not involved in the biological effects here described.

All cell lines we tested were able to synthesize KYNA that reached nanomolar levels in their growth media. Synthesis of KYNA from tryptophan is a three-step process. In most cell types, the first step is mediated by indoleamine 2,3-dioxygenase (IDO) that metabolizes the amino acid in *N*-formyl-kynurenine. Inflammatory mediators such as bacterial lipopolysaccharides (LPS), interferon (IFN)-γ, or tumor necrosis factor (TNF)-α strongly induce this enzyme (Chiarugi *et al.*, 2000; Chiarugi *et al.*, 2001). The second step leads to the formation of KYN (mostly spontaneously) and finally, the third step, is catalyzed by different transaminases and leads to KYNA

production (Moroni, 1999). We propose that minor changes in KYNA concentration above basal levels may have important biological consequences deriving from the inhibition of growth factor, inflammatory cytokine, or transmitter release. This inhibition may affect cell growth and differentiation, immune response and synaptic function.

REFERENCES

Basilico, C., and Moscatelli, D. (1992). The FGF family of growth factors and oncogenes. *Adv. Cancer Res*. **9:**15–165.

Bizon, J. L., Lauterborn, J. C., Isackson, P. J., and Gall, C. M. (1996). Acidic fibroblast growth factor mRNA is expressed by basal forebrain and striatal cholinergic neurons. *J. Comp. Neurol*. **366:**379–389.

Carpenedo, R., Chiarugi, A., Russi, P., Lombardi, G., Carlà, V., Pellicciari, R., Mattoli, L., and Moroni, F. (1994). Inhibitors of kynurenine hydroxylase and kynureninase increase cerebral formation of kynurenate and have sedative and anticonvulsant activities. *Neuroscience* **61:**237–244.

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

Carpenedo, R., Meli, E., Peruginelli, F., Pellegrini-Giampietro, D. E., and Moroni, F. (2002). Kynurenine 3-mono-oxygenase inhibitors attenuate post-ischemic neuronal death in organotypic hippocampal slice cultures. *J. Neurochem*. **82:**1465–1471.

Chiarugi, A., Carpenedo, R., Molina, M. T., Mattoli, L., Pellicciari, R., and Moroni, F. (1995). Comparison of the neurochemical and behavioral effects resulting from the inhibition of kynurenine hydroxylase and/or kynureninase. *J. Neurochem*. **65:**1176–1183.

Chiarugi, A., Dello Sbarba P., Paccagnini, A., Donnini, S., Filippi, S., and Moroni, F. (2000). Combined inhibition of indoleamine 2,3-dioxygenase and nitric oxide synthase modulates neurotoxin release by interferon-gamma-activated macrophages. *J. Leukoc. Biol.* **68:**260–266.

Chiarugi, A., Cozzi, A., Ballerini, C., Massacesi, L., and Moroni, F. (2001). Kynurenine 3-mono-oxygenase activity and neurotoxic kynurenine metabolites increase in the spinal cord of rats with experimental allergic encephalomyelitis. *Neuroscience* **102:**687–695.

Cozzi, A., Carpenedo, R., and Moroni, F. (1999). Kynurenine hydroxylase inhibitors reduce ischemic brain damage: Studies with (*m*-nitrobenzoyl)-alanine (mNBA) and 3,4-dimethoxy-[-*N*-4-(nitrophenyl)thiazol-2yl]-benzenesulfonamide (Ro 61-8048) in models of focal or global brain ischemia. *J. Cereb. Blood Flow Metab*. **19:**771–777.

Friesel, R., and Maciag, T. (1999). Fibroblast growth factor prototype release and fibroblast growth factor receptor signaling. *Thromb. Haemost*. **82:**748–754.

Guillemin, G. J., Kerr, S. J., Smythe, G. A., Smith, D. G., Kapoor, V., Armati, P. J., Croitoru, J., and Brew, B. J. (2001). Kynurenine pathway metabolism in human astrocytes: A paradox for neuronal protection. *J. Neurochem.* **78:**842–853.

Hilmas, C., Pereira, E. F. R., Alkondon, M., Rassoulpour, A., Schwarcz, R., and 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.

Holmes, E. W. (1988). Determination of serum kynurenine and hepatic tryptophan dioxygenase activity by high-performance liquid chromatography. *Anal. Biochem.* **172:**518–525.

Hossain, M. A., Russell, J. C., Gomez, R., Laterra, J., and Gomes, R. (2002). Neuroprotection by scatter factor/hepatocyte growth factor and FGF-1 in cerebellar granule neurons is phosphatidylinositol 3-kinase/akt-dependent and MAPK/CREB-independent. *J. Neurochem.* **81:**365–378.

Jackson, A., Friedman, S., Zhan, X., Engleka, K. A., Forough, R., and Maciag, T. (1992). Heat shock induces the release of fibroblast growth factor 1 from NIH 3T3 cells. *Proc. Natl. Acad. Sci. U.S.A.* **89:**10691–10695.

Kessler, M., Terramani, T., Lynch, G., and 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.

Le, R., and Esquenazi, S. (2002). Astrocytes mediate cerebral cortical neuronal axon and dendrite growth, in part, by release of fibroblast growth factor. *Neurol. Res.* **24:**81–92.

Mocchetti, I., and Wrathall, J. R. (1995). Neurotrophic factors in central nervous system trauma. *J. Neurotrauma* **12:**853–870.

Moroni, F., Russi, P., Lombardi, G., Beni, M., and Carlà, V. (1988). Presence of kynurenic acid in the mammalian brain. *J. Neurochem*. **51:**177–181.

Moroni, F., Pellegrini-Giampietro, D. E., Alesiani, M., Cherici, G., Mori, F., and Galli, A. (1989). Glycine and kynurenate modulate the glutamate receptors in the myenteric plexus and in cortical membranes. *Eur. J. Pharmacol.* **163:**123–126.

Moroni, F., Russi, P., Gallo-Mezo, M. A., Moneti, G., and Pellicciari, R. (1991). Modulation of quinolinic and kynurenic acid content in the rat brain: Effects of endotoxins and nicotinylalanine. *J. Neurochem*. **57:**1630–1635.

Moroni, F. (1999). Tryptophan metabolism and brain function: Focus on kynurenine and other indole metabolites. *Eur. J. Pharmacol*. **375:**87–100.

Rassoulpour, A., Wu, H. Q., Ferre, S., and Schwarcz, R. (2005). Nanomolar concentrations of kynurenic acid reduce extracellular dopamine levels in the striatum. *J. Neurochem.* **93:**762–765.

Russi, P., Alesiani, M., Lombardi, G., Davolio, P., Pellicciari, R., and Moroni, F. (1992). Nicotinylalanine increases the formation of kynurenic acid in the brain and antagonizes convulsions. *J. Neurochem*. **59:**2076–2080.

Sassano, M., Granucci, F., Seveso, M., Marconi, G., Foti, M., and Ricciardi-Castagnoli, P. (1994). Molecular cloning of a recombinant retrovirus carrying a mutated envAKR-mycMH2 fusion gene immortalizing cells of the monocytic-macrophage lineage. *Oncogene* **9:**1473–1477.

Schwarcz, R., Whetsell, W. O., and Mangano, R. M. (1983). Quinolinic acid: An endogenous metabolite that produces axon-sparing lesions in rat brain. *Science* **219:**316–318.

Stone, T. W., and Perkins, M. N. (1981). Quinolinic acid: A potent endogenous excitant at amino acid receptors in CNS. *Eur. J. Pharmacol.* **72:**411–412.

Stone, T. W., and Connick, J. (1985). Quinolinic acid and other kynurenines in the central nervous system. *Neuroscience* **15:**507–617.

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

Stone, T. W. (2000). Development and therapeutic potential of kynurenic acid and kynurenine derivatives for neuroprotection. *Trends Pharmacol. Sci*. **21:**149–154.

Swartz, K. J., Matson, W. R., MacGarvey, U., Ryan, E. A., and Beal, M. F. (1990). Measurement of kynurenic acid in mammalian brain extracts and cerebrospinal fluid by high-performance liquid chromatography with fluorometric and coulometric electrode array detection. *Anal. Biochem.* **185:**363–376.

Tarantini, F., LaVallee, T., Jackson, A., Gamble, S., Mouta Carreira, C., Garfinkel, S., Burgess, W. H., and Maciag, T. (1998). The extravesicular domain of synaptotagmin-1 is released with the latent fibroblast growth factor-1 homodimer in response to heat shock. *J. Biol. Chem*. **273:**22209–22216.

Tarantini, F., Micucci, I., Bellum, S., Landriscina, M., Garfinkel, S., Prudovsky, I., and Maciag, T. (2001). The precursor but not the mature form of IL1α blocks the release of FGF1 in response to heat shock. *J. Biol. Chem*. **276:**5147–5151.

Turski, W. A., Nakamura, M., Todd, W. P., Carpenter, B. K., Whetsell, W. O., and Schwarcz, R. (1988). Identification and quantification of kynurenic acid in human brain tissue. *Brain Res.* **454:**164–169.

Wu, H. Q., Ungerstedt, U., and Schwarcz, R. (1992). Regulation of kynurenic acid synthesis studied by microdialysis in the dorsal hippocampus of unanesthetized rats. *Eur. J. Pharmacol.* **213:**375–380.

Wu, H. Q., Guidetti, P., Goodman, J. H., Varasi, M., Ceresoli-Borroni, G., Speciale, C., Scharfman, H. E., and Schwarcz, R. (2000). Kynurenergic manipulations influence excitatory synaptic function and excitotoxic vulnerability in the rat hippocampus in vivo. *Neuroscience* **97:**243–251.

Xie, D., Yin, D., Tong, X., O'Kelly, J., Mori, A., Miller, C., Black, K., Gui, D., Said, J. W., and Koeffler, H. P. (2004). Cyr61 is overexpressed in gliomas and involved in integrin-linked kinase-mediated Akt and beta-catenin-TCF/Lef signaling pathways. *Cancer Res.* **64:**1987–1996.