

Characterisation of kynurenine pathway metabolism in human astrocytes and implications in neuropathogenesis

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The role of astrocytes in the production of the neurotoxin quinolinic acid (QUIN) and other products of the kynurenine pathway (KP) is controversial. Using cytokine-stimulated human astrocytes, we assayed key enzymes and products of the KP. We found that astrocytes lack kynurenine-hydroxylase so that large amounts of kynurenine (KYN) and kynurenic acid (KYNA) were produced, while minor amounts of QUIN were synthesised that were completely degraded. We then showed that kynurenine added to macrophages led to significant production of QUIN. These results suggest that astrocytes alone are neuroprotective by minimising QUIN production and maximising synthesis of KYNA. However, it is likely that, in the presence of macrophages and/or microglia, astrocytes are neurotoxic by producing large concentrations of KYN that can be metabolised by neighbouring monocytic cells to QUIN.

INTRODUCTION

The KP is potentially important in the pathogenesis of a number of neurological diseases.¹ Moreover, it may play a role in certain physiological functions such as behavior, sleep, thermo-regulation and pregnancy.¹⁻³ The KP is a major route of L-tryptophan catabolism, resulting in production of NAD and various neuro-active intermediates (Fig. 1).¹⁻³ Of these metabolites, the N-methyl-D-aspartate receptor agonist and neurotoxin QUIN is the most important.

The cellular localisation of the KP has been shown primarily to be in macrophages and potentially in astrocytes.^{4,5} It is not clear whether enzymes in the KP are present and functional in man.^{1,5,6}

To define the KP in astrocytes, human fetal astrocytes were cultured and stimulated by cytokines. Developing original primer sets, we assessed mRNA expression of seven of the major KP enzymes using RT-PCR. In addition, we also measured the concentration of important KP products. KYN, KYNA and 3-HK synthesis was quantitated using HPLC and QUIN and picolinic acid (PIC) production was quantitated using gas chromatography/mass spectrometry (GC/MS).

MATERIALS AND METHODS

Cultured human fetal astrocytes⁷ were stimulated by 100 U/ml of the cytokines IL-1 β , IL-6, GM-CSF, IFN- γ and TNF- α , or by different concentrations of QUIN in the presence or absence of 100 μ M 3HAA. Macrophage cultures⁸ were treated with 12.5 μ M QUIN (equivalent to the concentration of KYN produced by cultured astrocytes after IFN- γ stimulation) or 50 μ M KYN.

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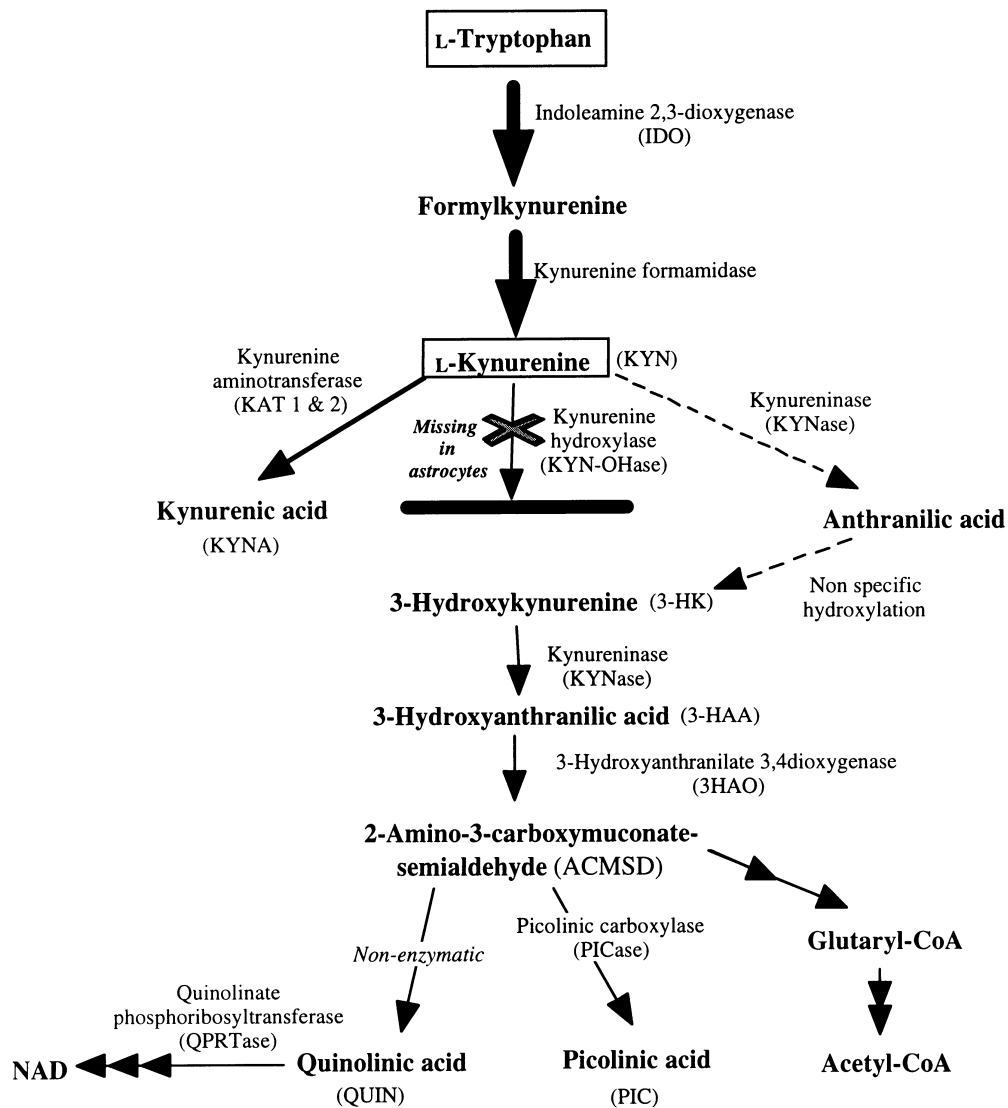


Fig. 1. Simplified kynurenine pathway in human fetal astrocytes.

We developed original primer sets for 7 of the major KP enzymes and then assessed their expression using RT-PCR. They were IDO (EC 1.13.11.17), kynurenine-pyruvate aminotransferase (KAT-I; EC 2.6.1.64), kynurenine-2-oxoglutarate aminotransferase (KAT-II; EC 2.6.1.7), kynurenine hydroxylase (KYN-OHase; EC 1.14.13.9), kynureninase (KYNase; EC 3.7.1.3), 3-hydroxyanthranilate dioxygenase (3-HAO; EC 1.13.11.6) and quinolate phosphoribosyltransferase (QPRTase; EC 2.4.2.19).

We also measured the concentration of KP intermediates and end-products: KYN and KYNA synthesis was quantitated using HPLC,⁹ and QUIN and picolinic acid production was quantitated using GC/MS.⁸

Experiments were performed in triplicate on cultures derived from 3 different fetal brains.

RESULTS

Production of KP metabolites by human fetal astrocytes

Human fetal astrocytes can produce high amounts of KYN (μM) and to a lesser extent KYNA (nM) only after IFN- γ stimulation (Table 1). Synthesis of 3-(OH)KYN was never detected in astrocyte culture supernatants. Cultured astrocytes significantly degrade QUIN. PIC was produced in low amounts (nM).

Expression of KP enzymes in human fetal astrocytes

Human fetal astrocytes express IDO only after IFN- γ stimulation (Table 2). They express both subtypes of

Table 1. Quantification of the *in vitro de novo* synthesis of KP metabolites by human fetal astrocytes after 48 h following various cytokine treatments

	No cytokine	IFN- γ	TNF- α	TNF- α + IFN- γ	IL1- β	IL6	GMCSF	QUIN (350 nM)
[KYN] (μ M)	0	11.7 \pm 1.5	ND	10.3 \pm 0.9	0	0	0	0
[KYNA] (nM)	13.2 \pm 5.3	60.5 \pm 14.7	ND	25.0 \pm 8.1	13.0 \pm 4.6	8.2 \pm 7.6	6.7 \pm 7.6	3.7 \pm 5.5
[3-(OH)KYN] (nM)	0	0	0	0	0	0	0	0
[QUIN] (nM)	-150.24 \pm 7.4	-166.28 \pm 19	-159.86 \pm 10	ND	-191.94 \pm 4	-202.63 \pm 15	-175.90 \pm 9	ND
[PIC] (nM)	13.4 \pm 8.1	1.6 \pm 0.7	7.2 \pm 4.8	ND	17.6 \pm 2.8	24.3 \pm 8	7.7 \pm 7.2	ND

Table 2. Semi-quantification of KP enzyme expression by RT-PCR in human fetal astrocytes and human macrophages

	Unstimulated human fetal astrocytes	IFN- γ stimulated human fetal astrocytes	IFN- γ stimulated macrophages
IDO	-	+++	+++
KAT-I	+	++	++
KAT-II	+/-	+	+
KYN(OH)ase	-	-	++
KYNase	+/-	+/-	++
3-HAO	++	+++	++
QPRTase	+/-	+	+/-

No expression, -; low expression, +/-; medium expression, +; strong expression, ++; very high expression, +++.

KAT, KYNase, 3-HAO, and QPRTase. Expression of KYN(OH)ase was not detected in human fetal astrocytes.

DISCUSSION

The present study provides the first comprehensive analysis of KP metabolism in human astrocytes and has implications for the pathogenesis of inflammatory neurological diseases.

Essentially, we showed that the KP is 'split' in half in astrocytes compared to macrophages where it is fully present. Indeed, we have demonstrated that KYN-OHase, a critical enzyme in the KP, is missing in astrocytes. This explains our findings that astrocytes produce large amounts of the early KP metabolites KYN, and to a lesser extent KYNA, and only minute amounts of the late metabolites QUIN (only when 3-HAA is added) and PIC. Moreover, we have shown that cultured astrocytes preferentially degrade QUIN in most situations.

By studying, for the first time, expression of 7 of the major KP enzymes, we have demonstrated that the IDO can only be induced by IFN- γ and leads to further excess production of the early metabolites, KYN and KYNA.

While there is over-production of those early metabolites, the amount of KYNA, an antagonist of QUIN, is 200-fold less than KYN, probably due to a 30-fold decrease in expression of KAT-II, compared to KAT-I. Astrocytes express 3-HAO and an IFN- γ inducible QPRTase constitutively thereby explaining our findings that astrocytes degrade QUIN. Nevertheless, the accumulation of QUIN within the CNS during inflammatory diseases, suggests the activity of QPRTase is probably saturated by QUIN concentrations, above those which are physiological.

We also proved that the amount of KYN synthesised by stimulated astrocytes is large enough to potentiate a significant increase of QUIN production by macrophages.

The function of astroglial KP is certain to provide KYN within the brain. If KYN is used firstly for synthesis of KYNA, it can possibly be used as a substrate by infiltrated macrophages and/or activated resident microglia for the synthesis of high amounts of QUIN. Through this potential way, astrocytes can probably play an indirect role in KP regulation of other brain cells and be thus involved in the global amplification of inflammatory responses within the brain.¹⁰ Therefore, further experiments are required to clearly elucidate the way in which astrocytes act in concert with other neural cells to regulate KP metabolism within the CNS.

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