Stable isotope labeled *L*-kynurenine (KYN) metabolism investigated using *in vivo* microdialysis coupled with a novel high content LC/MS/MS method

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Introduction

Degradation of the amino acid tryptophan along the kynurenine pathway (KP) yields several neuroactive intermediates regulated by enzymes localized in astrocytes and microglial cells. The limited ability of many KYN-derived metabolites to cross the blood brain barrier suggests that CNS concentrations of these metabolites is largely regulated by local enzyme activity. KYN itself is actively transported into the brain by large neutral amino acids transporter. The fate of KYN in the brain is poorly understood and differences between species further complicate the issue. We investigated the metabolism of KYN in mouse and rat brains using a stable labeled analogue of KYN ([$^{13}C_6$]L-KYN), LC/MS/MS and *in vivo* microdialysis to provide a time course for stable isotope labeled metabolite formation.

Methods

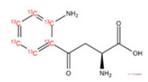
Animals, surgery and *in vivo* microdialysis

Mice and rats were implanted with guide cannula placed into the striatum. One week after surgery a probe (3 mm polyacrylonitrile membrane, BrainLink, NL), was inserted for the microdialysis experiment. The probes were perfused at 1 µL/min. Microdialysis samples were collected every hour for 24 hrs after an i.p. administration of [$^{13}C_6$]*L*-KYN (5 mg/kg) and analyzed for labeled and unlabeled *L*-kynurenine (KYN), kynurenic acid (KYNA), 3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA) and quinolinic acid (QUIN), using LC/MS/MS method.

LC/MS/MS method for detection of metabolites

Standard curves were prepared using pure components including KYN, KYNA, 3-HK, 3-HAA, and QUIN dissolved in 0.2% acetic acid to correct for sample and instrument variability.

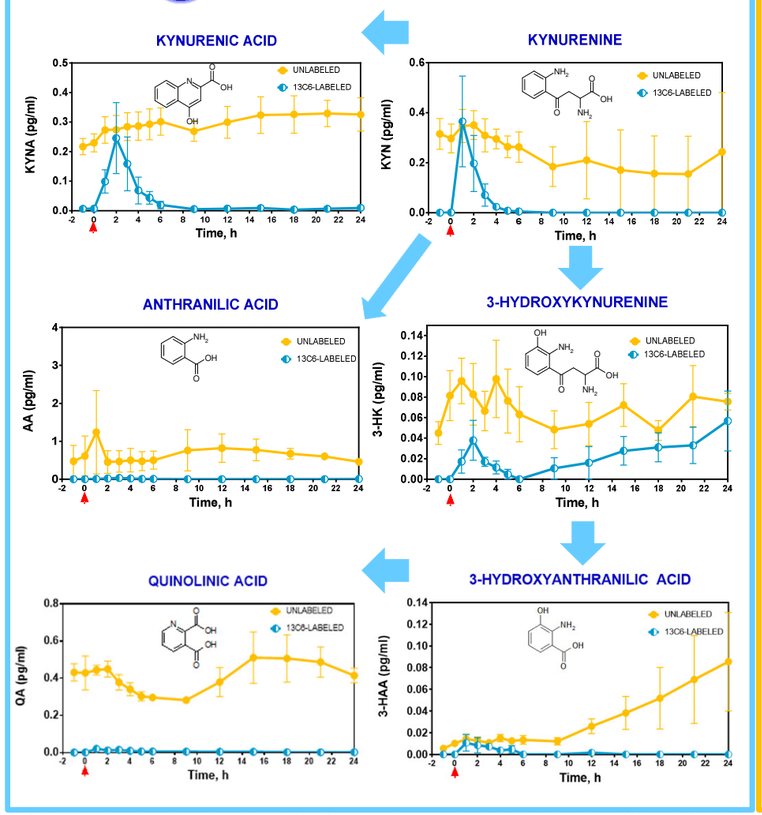
Microdialysis samples were directly injected into a Waters (Milford, MA, USA) Acquity HPLC system equipped with an YMC™ ODS-AQ™ 2 mm × 100 mm, 3 µm particle column. Separated kynurenine analytes (labeled and unlabeled) were detected by a Waters Quattro Premier XE triple quadrupole mass spectrometer (Waters, Milford, MA, USA), operating in the MS/MS mode. Column and pre-column tubing were maintained at 40 ℃ while eluting kynurenine metabolites with a mobile phase consisting of an aqueous component (A: 0.5% formic acid in milliQ water) and an organic component (B: 1% formic acid in acetonitrile). Relative levels of the analytes (labeled and unlabeled) were determined by utilizing analyte response observed in the standard curves prepared for each unlabeled compound as there is no difference in response for carbon 13 analogues vs corresponding unlabeled compounds. Limits of detection were determined based on a signal to noise ratio of >10 and found to be well below required limits for analysis of rodent microdialysates



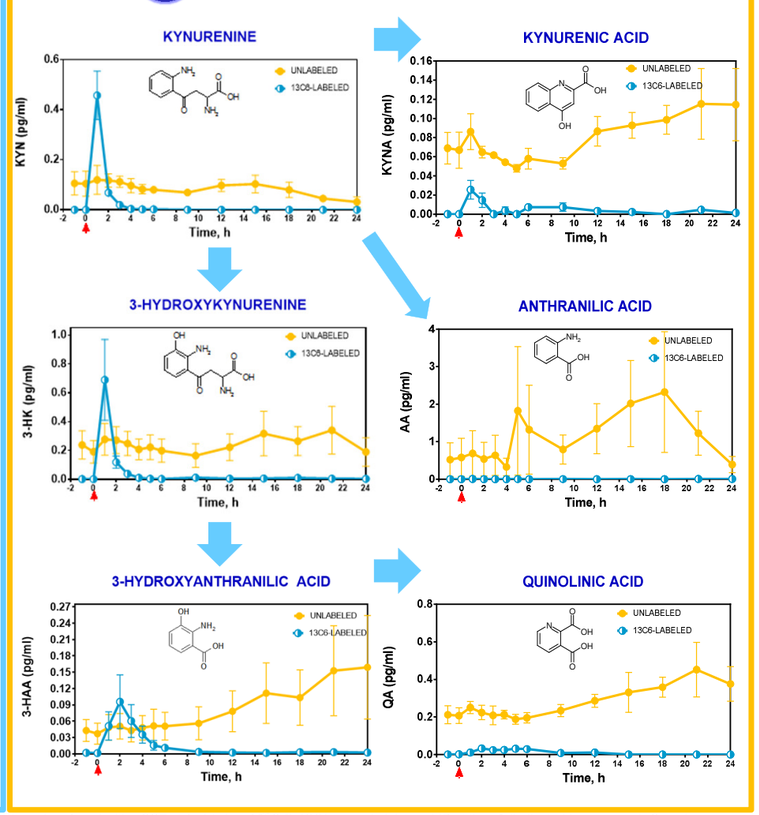
Compound

[$^{13}C_6$]*L*-KYNURENINE

1 Kynurenine metabolism in rats

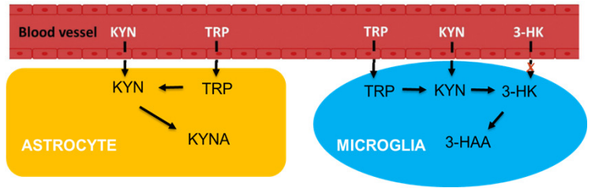


2 Kynurenine metabolism in mice



BrainLink (NL) probes with polyacrylonitrile (PAN) 3 mm membrane were implanted into the striatum 16 hours before the study and perfused with aCSF at 1 µL/min. of [$^{13}C_6$]L-KYN was administered IP at time 0 (indicated by an arrow). Samples were collected every 60 min and analyzed for KP metabolites by LC/MS/MS method . Data are presented as mean ± SEM. N=3 in each group

3 The location of KP metabolites in the brain



TRP and *L*-KYN readily cross the BBB. The extent to which 3-HK crosses the BBB is believed to be dependant on the species involved. Neither KYNA or QUIN are believed to cross the BBB. The synthesis of KYNA and QUIN is physically separated in the brain – KYNA is produced and released from astrocytes whereas QUIN synthesis and release takes place in microglia.

4 Conclusions

- In mice, [$^{13}C_6$]*L*-KYN metabolism shows a stronger kynurenine 3-monooxygenase (KMO) branch response whereas in rats the kynurenine aminotransferease (KAT) pathway is more prevalent.
- LC/MS/MS analysis of stable isotope labeled KP metabolite from in vivo microdialysis samples provides a powerful tool for examining the mechanism of L-KYN metabolism across species.
- Improved success rates of studies involving brain-penetrable modulators of KP may be achieved with a better understanding of the complex L-KYN metabolism process.

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