Analytical Biochemistry 421 (2012) 573–581

Contents lists available at SciVerse ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

# Gas chromatography/tandem mass spectrometry detection of extracellular kynurenine and related metabolites in normal and lesioned rat brain

Francesca M. Notarangelo [a], Hui-Qiu Wu [a], Anthony Macherone [b], David R. Graham [c], Robert Schwarcz [a,*]

[a] Maryland Psychiatric Research Center, University of Maryland School of Medicine, Baltimore, MD 21228, USA
[b] Agilent Technologies, Santa Clara, CA 95051, USA
[c] Department of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

ARTICLE INFO

*Article history:*
Received 9 September 2011
Received in revised form 19 December 2011
Accepted 20 December 2011
Available online 28 December 2011

*Keywords:*
3-Hydroxykynurenine
Microdialysis
Quinolinic acid
Selective reaction monitoring
Tandem mass spectrometry
Tryptophan

ABSTRACT

We describe here a gas chromatography–tandem mass spectrometry (GC/MS/MS) method for the sensitive and concurrent determination of extracellular tryptophan and the kynurenine pathway metabolites kynurenine, 3-hydroxykynurenine (3-HK), and quinolinic acid (QUIN) in rat brain. This metabolic cascade is increasingly linked to the pathophysiology of several neurological and psychiatric diseases. Methodological refinements, including optimization of MS conditions and the addition of deuterated standards, resulted in assay linearity to the low nanomolar range. Measured in samples obtained by striatal microdialysis in vivo, basal levels of tryptophan, kynurenine, and QUIN were 415, 89, and 8 nM, respectively, but 3-HK levels were below the limit of detection (<2 nM). Systemic injection of kynurenine (100 mg/kg, i.p.) did not affect extracellular tryptophan but produced detectable levels of extracellular 3-HK (peak after 2–3 h: ~50 nM) and raised extracellular QUIN levels (peak after 2 h: ~105 nM). The effect of this treatment on QUIN, but not on 3-HK, was potentiated in the *N*-methyl-D-aspartate (NMDA)-lesioned striatum. Our results indicate that the novel methodology, which allowed the measurement of extracellular kynurenine and 3-HK in the brain in vivo, will facilitate studies of brain kynurenines and of the interplay between peripheral and central kynurenine pathway functions under physiological and pathological conditions.



Mounting evidence indicates that metabolites of the kynurenine pathway (KP),[1] the major catabolic route of dietary tryptophan in mammals (Fig. 1), play a significant role in biology. Although several of these metabolites (collectively termed kynurenines) have been credibly linked to peripheral immune function [1,2], age-associated endocrine disorders [3], regulation of vascular tone [4], and cancer [5], most major advances have come from studies of the central nervous system (CNS). Interest in the role of the KP in the CNS is mostly related to the neuroactive properties of kynurenic acid (a neuroprotective glutamate and α7 nicotinic receptor antagonist [6–8]) and the excitotoxic *N*-methyl-D-aspartate (NMDA) receptor agonist quinolinic acid (QUIN) [9,10]. Also of interest to biologists is the ability of two other KP metabolites, 3-hydroxykynurenine (3-HK) and 3-hydroxyanthranilic acid, to both generate and scavenge reactive free radicals [11,12]. Alone or jointly, these metabolites are involved in a number of important neurophysiological processes and participate causally in neurological and psychiatric diseases (see Refs. [13–15] for reviews). Pharmacological manipulation of the KP, therefore, offers novel attractive approaches to influence brain physiology and pathology [16–19].

The dynamics of the KP within the normal and dysfunctional brain and the effect of peripheral KP manipulations on the brain have been explored in a series of studies conducted mainly in rodents. Briefly, tryptophan, kynurenine, and 3-HK readily enter the brain from the circulation, whereas the acidic metabolites kynurenic acid and QUIN appear to be largely precluded from crossing the blood–brain barrier [20,21]. In the brain, KP metabolism, initiated by either local synthesis or systemic influx of tryptophan, kynurenine, or 3-HK, occurs mainly in glial cells (cf. Ref. [16]), although neurons have been implicated as well [22]. Interestingly, the two branches of the KP (Fig. 1) are segregated, with the formation of kynurenic acid occurring in astrocytes, and the competing cascade, leading to 3-HK and its downstream metabolites, taking place in microglial cells. However, the interplay

* Corresponding author. Fax: +1 410 747 2434.
*E-mail address:* rschwarc@mprc.umaryland.edu (R. Schwarcz).

[1] *Abbreviations used:* KP, kynurenine pathway; CNS, central nervous system; NMDA, *N*-methyl-D-aspartate; QUIN, quinolinic acid; 3-HK, 3-hydroxykynurenine; GC, gas chromatography; MS, mass spectrometry; MS/MS, tandem MS; CI, chemical ionization; EI, electron impact; HD, Huntington's disease; PFAA, pentafluoropropionic anhydride; PFP, 2,2,3,3,3-pentafluoro-1-propanol; PFDTD, perfluoro-5,8-dimethyl-3,6,9-trioxidodecane; SRM, selected reaction monitoring; ECNI, electron capture negative ionization; PBS, phosphate-buffered saline; GAD, glutamate decarboxylase; ANOVA, analysis of variance.

0003-2697/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ab.2011.12.032

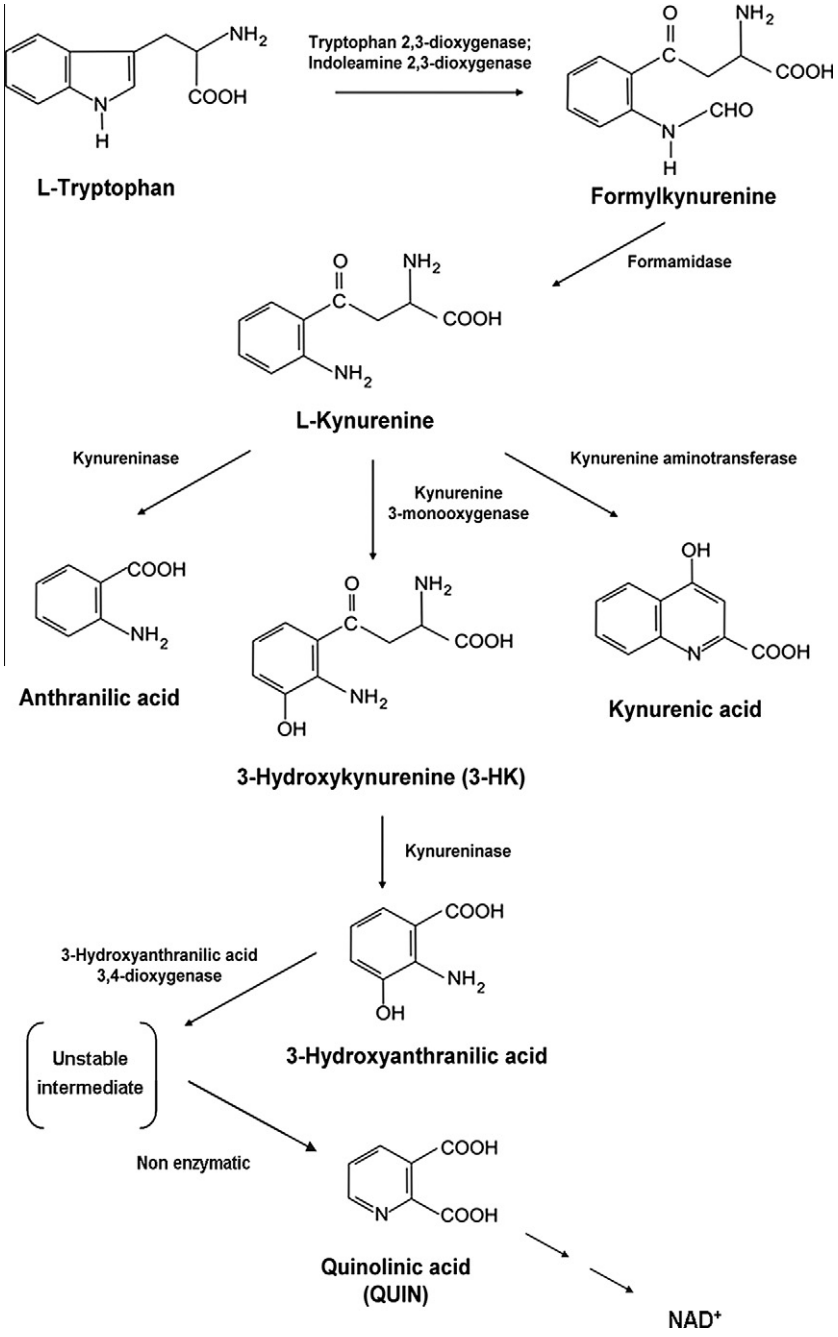


**Fig.1.** Kynurenine pathway of tryptophan degradation.

between peripherally derived and locally produced kynurenines is complex, depending on the integrity of the brain tissue and, more generally, on the health status of the entire organism [23–25].

Because several cell types are involved in cerebral KP metabolism, and because the blood-borne metabolites must enter the extracellular space before being accumulated by cells, the dynamics of neuroactive KP metabolites in the brain are best studied by in vivo microdialysis. This method has been successfully used to elaborate features of kynurenic acid and QUIN neurochemistry in experimental animals, providing valuable information on the role of these compounds in health and disease [26–30]. Other KP metabolites, on the other hand, have so far barely been studied by microdialysis, and there is only sporadic mention of extracellular brain tryptophan in the literature [31,32].

Because the concentration of neuroactive kynurenines in the mammalian brain is in the nanomolar range, sensitive detection methods (e.g., fluorescence or gas chromatography/mass spectrometry [GC/MS]) are required for analysis. Here we used GC/tandem MS (GC/MS/MS) detection with negative chemical ionization (CI), rather than the traditional electron impact (EI) ionization or positive CI, focusing on the simultaneous measurement of tryptophan, kynurenine, and the two microglial KP metabolites 3-HK and QUIN in brain microdialysate samples. This methodology, adapted from Eckstein and coworkers [33], provides improved selectivity and sensitivity compared with other ionization techniques [34] and was selected because our molecules of interest, after derivatization, can stabilize a negative charge and produce a stable negative ion. We report the successful application of this methodology to a paradigmatic microdialysis study that was performed in normal and excitotoxin-lesioned rat striata. Our goal was to simultaneously identify tryptophan and kynurenines in the brain's extracellular compartment, to determine the effects of