Biochimica et Biophysica Acta 1860 (2016) 2345–2354

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Tryptophan 2,3-dioxygenase and indoleamine 2,3-dioxygenase 1 make separate, tissue-specific contributions to basal and inflammation-induced kynurenine pathway metabolism in mice

Paul B. Larkin [a,b,1], Korrapati V. Sathyasaikumar [c,1], Francesca M. Notarangelo [c], Hiroshi Funakoshi [d], Toshikazu Nakamura [e], Robert Schwarcz [c], Paul J. Muchowski [a,b,f,g,h,*]

[a] Gladstone Institute of Neurological Disease, San Francisco, CA, USA
[b] Neuroscience Graduate Program, University of California, San Francisco, CA, USA
[c] Maryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD, USA
[d] Center for Advanced Research and Education (CARE), Asahikawa Medical University, 1-1-1- Higashinijo Midorigaoka, Asahikawa 078-8510, Japan
[e] Neurogen Inc., 1-1-52-201 Nakahozumi, Ibaraki 567-0034, Japan
[f] Department of Biochemistry and Biophysics, University of California, San Francisco, CA, USA
[g] Department of Neurology, University of California, San Francisco, CA, USA
[h] The Taube-Koret Center for Huntington's Disease Research, San Francisco, CA, USA

ARTICLE INFO

Article history:
Received 21 March 2016
Received in revised form 25 June 2016
Accepted 4 July 2016
Available online 5 July 2016

Keywords:
Tryptophan
Inflammation
Neurodegeneration
Cancer
Schizophrenia

ABSTRACT

Background: In mammals, the majority of the essential amino acid tryptophan is degraded via the kynurenine pathway (KP). Several KP metabolites play distinct physiological roles, often linked to immune system functions, and may also be causally involved in human diseases including neurodegenerative disorders, schizophrenia and cancer. Pharmacological manipulation of the KP has therefore become an active area of drug development. To target the pathway effectively, it is important to understand how specific KP enzymes control levels of the bioactive metabolites *in vivo*.
Methods: Here, we conducted a comprehensive biochemical characterization of mice with a targeted deletion of either tryptophan 2,3-dioxygenase (TDO) or indoleamine 2,3-dioxygenase (IDO), the two initial rate-limiting enzymes of the KP. These enzymes catalyze the same reaction, but differ in biochemical characteristics and expression patterns. We measured KP metabolite levels and enzyme activities and expression in several tissues in basal and immune-stimulated conditions.
Results and conclusions: Although our study revealed several unexpected downstream effects on KP metabolism in both knockout mice, the results were essentially consistent with TDO-mediated control of basal KP metabolism and a role of IDO in phenomena involving stimulation of the immune system.

1. Introduction

The essential amino acid tryptophan is incorporated into protein or degraded to a variety of bioactive molecules, including 5-hydroxytryptamine (serotonin), melatonin, tryptamine and metabolites of the kynurenine pathway (KP) [1]. Generation of KP metabolites is initiated by the enzymes tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO), which catalyze the conversion of tryptophan to N-formylkynurenine, which is then degraded further to the pivotal KP metabolite kynurenine. From kynurenine, one branch of the KP leads to the formation of kynurenic acid (KYNA), and another results in the production of NAD^+ via 3-hydroxykynurenine (3-HK) and quinolinic acid (QUIN) (Fig. 1).

TDO and IDO are crucial to understanding KP metabolism, which accounts for the majority of tryptophan degradation in mammals. The two enzymes catalyze the oxidative opening of tryptophan's indole ring at a comparable rate and have only subtle differences in substrate binding [2], but differ greatly in tissue distribution and regulation of expression. Under physiological conditions, TDO activity is highest in the liver [3], but the enzyme and its mRNA are also detectable in a number of other organs, including brain [4–7] and endometrium [8]. Enzyme activity is induced by tryptophan and glucocorticoids [9,10], and allosterically

Abbreviations: 3-HAO, 3-hydroxyanthranilic acid dioxygenase; 3-HK, 3-hydroxykynurenine; 5-HT, serotonin; IDO, indoleamine 2,3-dioxygenase; KATs, kynurenine aminotransferases; KMO, kynurenine 3-monooxygenase; KYNA, kynurenic acid; KYNU, kynureninase; QUIN, quinolinic acid; TDO, tryptophan 2,3-dioxygenase.

* Corresponding author at: KynurRex Inc., 634 Los Palmos Drive, San Francisco, CA 94127, USA.
E-mail address: paulmuchowski@kynurex.com (P.J. Muchowski).
[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.bbagen.2016.07.002
0304-4165/

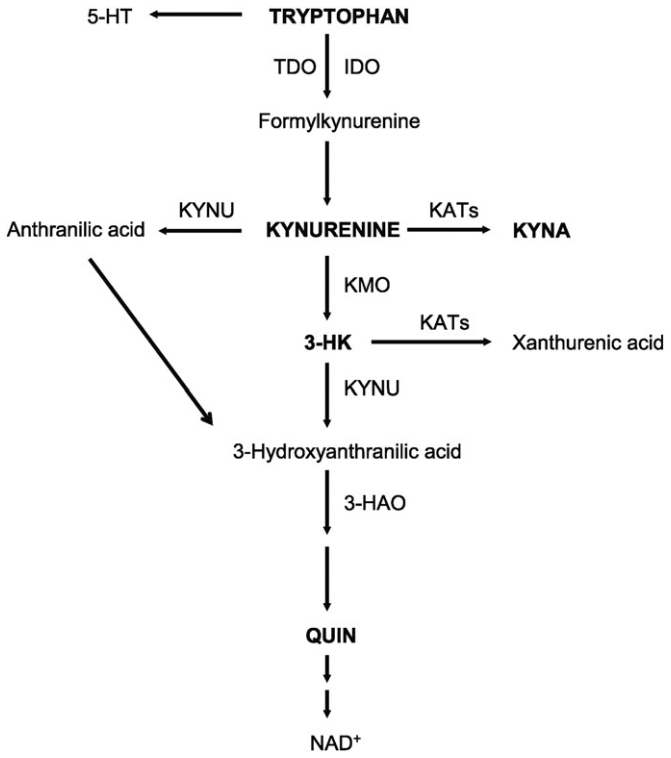

Fig. 1. Kynurenine pathway of tryptophan degradation in mammals. Indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) catalyze the first step of the kynurenine pathway. 5-HT: serotonin; KATs: kynurenine aminotransferases; KYNU: kynureninase; KMO: kynurenine 3-monooxygenase; KYNA: kynurenic acid; 3-HK: 3-hydroxykynurenine; 3-HAO: 3-hydroxyanthranilic acid dioxygenase; QUIN: quinolinic acid.

modulated by the downstream KP metabolite 3-hydroxyanthranilic acid [11]. In contrast, the less substrate-specific IDO, which was discovered much later than TDO [12,13], is expressed in a wide variety of tissues including epididymis, gut, lung, spleen, kidney, vascular endothelium and brain [14–16]. Notably, IDO is only expressed at low levels under basal conditions, but is strongly induced by interferon-γ, lipopolysaccharide (LPS), or several other pro-inflammatory stimuli [17,18].

A second isoform of IDO (IDO2) also catalyzes the conversion of tryptophan to kynurenine [19,20]. This enzyme, which likely arose via gene duplication of IDO, has distinctive kinetic characteristics and substrate specificity [21,22], is expressed in different tissues, and responds to immune stimulation differently than IDO [19,23]. The physiological relevance of IDO2 is still being elaborated, but the enzyme, like IDO (now re-named IDO1) and TDO, may have a role in cancer [20].

The diverse physiological functions of the various KP metabolites, as well as the realization that impaired KP metabolism is *causally* involved in a number of grave disorders in humans [24–34], have prompted efforts to unravel the intricacies of KP regulation in health and disease. As entry points to the enzymatic cascade, TDO and IDO naturally attract special attention in this regard. In addition to ongoing attempts to specifically target these enzymes pharmacologically [35,36], genetic tools have been generated for experimental use. For example, mice genetically deficient in TDO ($Tdo^{-/-}$ mice) [37] or IDO1 ($Ido^{-/-}$ mice) [38] have been successfully used for specific hypothesis testing in the neurosciences [6,37,39–41]. However, these mutant animals have so far not been used for a comprehensive investigation of KP metabolism. The present study was designed to fill this void by directly comparing adult $Tdo^{-/-}$ and $Ido^{-/-}$ mice, as well as respective wild-type animals, under basal conditions and in response to a stimulation of the immune system (by LPS). Our results, which revealed several tissue- and KP metabolite-specific phenomena in these mutants, jointly emphasize the separate biological roles of TDO and IDO1, with the latter being of special relevance in events involving activation of the immune system.

2. Experimental procedures

2.1. Mice

All animals (C57BL/6 background) were bred, housed and handled in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals", and all experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco. Animals were housed in a pathogen-free facility on a 12 h/12 h light/dark cycle. $Tdo^{-/-}$ mice were originally generated in our laboratory in Japan [37], and $Ido^{-/-}$ mice were obtained from the Jackson Laboratory (Bar Harbor, ME). For each strain, heterozygote-to-heterozygote breedings provided gene deficient ($^{-/-}$) mice and wild-type ($^{+/+}$) littermates. All mice received an intraperitoneal (i.p.) injection 24 h before tissues were collected (see below), and all studies were performed using 6–10 mice per group.

2.2. LPS injection and tissue collection

LPS from *Escherichia coli* K-235 was purchased from Sigma-Aldrich (St. Louis, MO; lot #020M4060). Mice received an i.p. injection of 1 mg/kg LPS, prepared freshly using sterile saline, or an equal volume of saline and were euthanized with an overdose of avertin 24 h later. After blood was collected into EDTA-containing tubes via cardiac puncture, animals were transcardially perfused with saline. Forebrain (i.e. brain minus cerebellum) and liver were rapidly removed and frozen on dry ice. Blood was promptly centrifuged to obtain plasma, which was subsequently also frozen on dry ice. All samples were then stored at −80 °C.

2.3. Genotyping

Mouse tail DNA was analyzed by PCR to determine genotype. The $Tdo^{+/+}$ and $Tdo^{-/-}$ alleles were amplified in separate reactions. The forward [AGC AAA CCT GTG TGG TCC TG] and reverse [GCC ATA GAT AAG TCC TCC T] primers were used to amplify the $Tdo^{+/+}$ allele, while the forward [CTT GGG TGG AGA GGC TAT TC] and reverse [AGG TGA GAT GAC AGG AGA TC] primers were used to amplify the Neo cassette in the $Tdo^{-/-}$ allele.

The $Ido^{+/+}$ and $Ido^{-/-}$ alleles were amplified in the same reaction with the forward [TGG AGC TGC CCG ACG C] and reverse [TAC CTT CCG AGC CCA GAC AC] primers for the $Ido^{+/+}$ allele and the forward [CTT GGG TGG AGA GGC TAT TC] and reverse [AGG TGA GAT GAC AGG AGA TC] primers for the $Ido^{-/-}$ allele.

2.4. Chemicals

[$^2H_6$]-L-kynurenine, pentafluoropropionic anhydride (PFPA) and 2,2,3,3,3-pentafluoro-1-propanol (PFP) were obtained from Sigma-Aldrich. Ro 61–8048 was a generous gift from Dr. W. Fröstl (Novartis, Basel, Switzerland). 4-Chloro-3-hydroxyanthranilic acid (4-Cl-3-HANA) was kindly provided by Drs. W.P. Todd and B.K. Carpenter (Department of Chemistry, Cornell University, Ithaca, NY). [$^2H_3$]-QUIN was purchased from Synfine Research (Richmond Hill, Ontario, Canada), and [$^2H_5$]-L-tryptophan was obtained from CDN Isotopes (Pointe-Claire, Quebec, Canada). [1-^{14}C]-3-Hydroxyanthranilic acid (6 mCi/mmol) was obtained from Dupont/New England Nuclear (Boston, MA). All other fine biochemicals and chemicals were purchased from various commercial suppliers and were of the highest available purity.