Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects

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International Journal of Tryptophan Research
Volume 10: 1–20

DOI: 10.1177/1178646917691938

ABSTRACT: Regulatory and functional aspects of the kynurenine (K) pathway (KP) of tryptophan (Trp) degradation are reviewed. The KP accounts for ~95% of dietary Trp degradation, of which 90% is attributed to the hepatic KP. During immune activation, the minor extrahepatic KP plays a more active role. The KP is rate-limited by its first enzyme, Trp 2,3-dioxygenase (TDO), in liver and indoleamine 2,3-dioxygenase (IDO) elsewhere. TDO is regulated by glucocorticoid induction, substrate activation and stabilization by Trp, cofactor activation by heme, and end-product inhibition by reduced nicotinamide adenine dinucleotide (phosphate). IDO is regulated by IFN-γ and other cytokines and by nitric oxide. The KP disposes of excess Trp, controls hepatic heme synthesis and Trp availability for cerebral serotonin synthesis, and produces immunoregulatory and neuroactive metabolites, the B_3 "vitamin" nicotinic acid, and oxidized nicotinamide adenine dinucleotide. Various KP enzymes are undermined in disease and are targeted for therapy of conditions ranging from immunological, neurological, and neurodegenerative conditions to cancer.

KEYWORDS: kynureninase, kynurenine aminotransferase, indoleamine 2,3-dioxygenase, tryptophan 2,3-dioxygenase, tryptophan disposition, nicotinamide

RECEIVED: November 21, 2016. **ACCEPTED:** January 11, 2017.

PEER REVIEW: Five peer reviewers contributed to the peer review report. Reviewers' reports totaled 611 words, excluding any confidential comments to the academic editor.

TYPE: Review

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Introduction

In addition to its indispensable role in protein synthesis, the essential amino acid L-tryptophan (Trp) is the precursor of many physiologically important metabolites produced during the course of its degradation along 4 pathways, 3 of which are of quantitatively minor significance, with the fourth, the kynurenine (K) pathway (KP), accounting for ~95% of overall Trp degradation.[1,2] The 3 minor pathways (and their important products) are (1) hydroxylation (serotonin, 5-hydroxytryptamine or 5-HT in brain, and melatonin in the pineal); (2) decarboxylation (tryptamine); and (3) transamination (indolepyruvic acid [IPA]). The KP produces many biologically active metabolites, including the important redox cofactors oxidized nicotinamide adenine dinucleotide (phosphate) [$NAD^+(P^+)$] and their reduced forms NAD(P)H [reduced nicotinamide adenine dinucleotide (phosphate)], pellagra-preventing factor, niacin (collectively referring the to nicotinamide and nicotinic acid) or vitamin B_3, Zn-binding compound picolinic acid (PA), *N*-methyl-D-aspartate (NMDA) receptor agonist quinolinic acid (QA) and antagonist kynurenic acid (KA), neuroactive K, and immunosuppressive K metabolites 3-hydroxykynurenine (3-HK) and 3-hydroxyanthranilic acid (3-HAA), with QA and PA also similarly active. The KP has received greater attention in recent years with the discovery of new and important roles its products play in health and disease and, in particular, conditions associated with immune dysfunction and central nervous system disorders. This article will be concerned mainly with the enzymatic, regulatory, and functional features of the KP with brief reference to clinical consequences of disturbances in activity of the pathway enzymes. This review is not intended to be exhaustive, but will cover the main features of the KP and, where appropriate, will refer to original sources.

General Description of the KP

The KP exists mainly in the liver, which contains all the enzymes necessary for NAD^+ synthesis from Trp and is responsible for ~90% of overall Trp degradation under normal physiologic conditions. The KP also exists extrahepatically, but its contribution to Trp degradation is normally minimal (5%-10%) but becomes quantitatively more significant under conditions of immune activation.[3] The extrahepatic KP does not include all enzymes of the pathway, and this determines which intermediates are produced and hence the type of functional modulation that results. An outline of the KP is given in Figure 1[4] and of the NAD^+ biosynthetic pathway from QA and via the salvage pathway in Figure 2.[4]

Tryptophan is converted to *N*′-formylkynurenine (NFK) by the action of either Trp 2,3-dioxygenase (TDO, formerly Trp pyrrolase; EC 1.13.11.11) mainly in liver or indoleamine 2,3-dioxygenase (IDO; EC 1.13.11.17) extrahepatically. *N*′-formylkynurenine is then hydrolyzed to kynurenine (K) by NFK formamidase (FAM). Kynurenine is metabolized mainly by hydroxylation to 3-HK by K hydroxylase (K monooxygenase [KMO]) followed by hydrolysis of 3-HK to 3-HAA by kynureninase. This latter enzyme can also hydrolyze K to anthranilic acid (AA). Both K and 3-HK can also be transaminated to KA and xanthurenic acid (XA) by K aminotransferase (KAT). For the sake of simplicity, KAT will be described as KAT A (K → KA) and KAT B (3-HK → XA), although 4

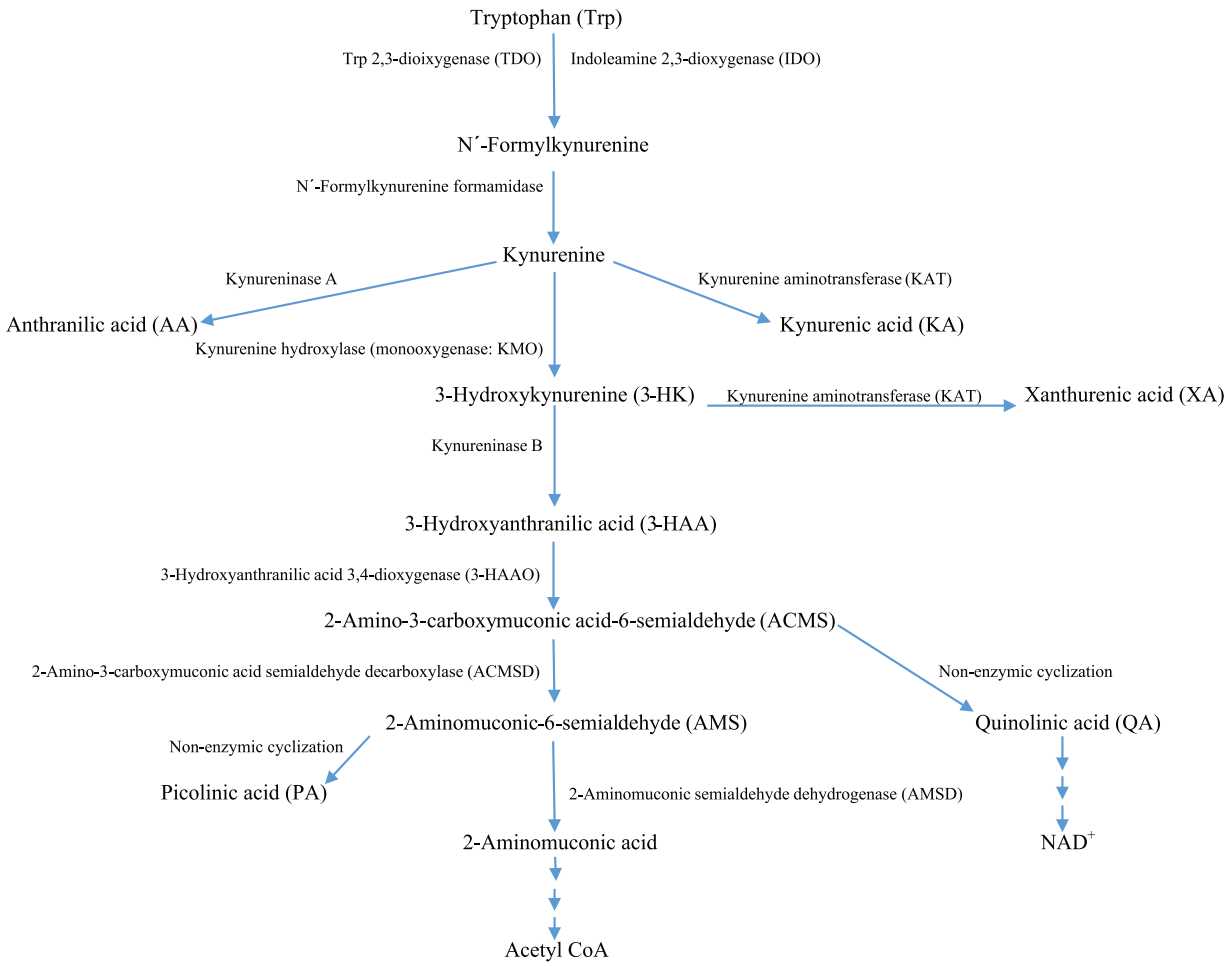

Figure 1. The kynurenine pathway of tryptophan degradation.

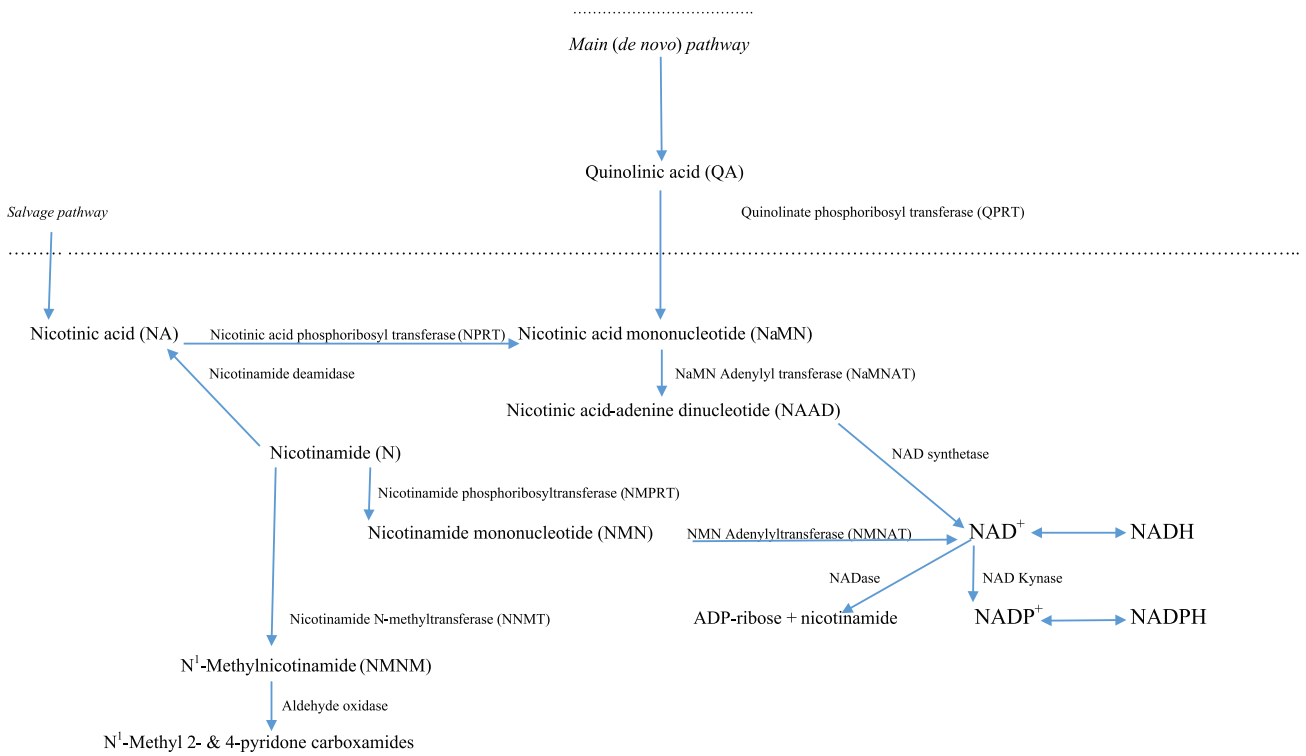

Figure 2. $NAD^+(P^+)$ synthesis from quinolinic acid (main pathway) and nicotinic acid (salvage pathway).

isoforms exist (KAT I, II, III, and IV). The KAT reactions are normally of minor significance because of the high K_m for its 2 substrates, compared with those of KMO and kynureninase, but can be enhanced after loading with Trp and/or K.[1] KAT A can also be enhanced after inhibition of KMO.[5] The most active among KP enzymes is 3-HAA 3,4-dioxygenase (oxidase: 3-HAAO).[6,7] The unstable product of this reaction, acroleyl aminofumarate (2-amino-3-carboxymuconate-6-semialdehyde [ACMS]), occupies a central position at 2 junctions in the KP. The pathway favors its nonenzymic cyclization to QA and hence production of NMN and then NAD^+. Picolinic acid is also formed by nonenzymic cyclization of aminomuconic acid semialdehyde. However, PA formation depends on extent of the substrate saturation of the enzyme (2-aminomuconic acid semialdehyde dehydrogenase) competing with this cyclization. Only when such saturation is maximal, PA production can proceed at a faster rate.

Substrates, Cofactors, and Tissue Distribution of Enzymes of the KP

These are outlined in Table 1, the information of which is derived from literature data referred to in the text and also the BRENDA (BRaunschweig ENzyme DAtabase) database for enzymes.[8] The L-Trp-specific TDO and mainly liver-specific TDO use O_2 as cosubstrate and heme as cofactor. The extrahepatic IDO, by contrast, can oxidize a broad range of substrates, including D-Trp, indoles, and indoleamines. It also uses molecular O_2 as cosubstrate and not superoxide as was previously assumed[9] and, although it is also a hemoprotein, it exists in the active holoenzyme form that cannot be further activated by heme. Some of the properties of TDO and IDO have been compared.[10] Affinity for Trp is one important difference. Although TDO has a much lower affinity but higher capacity for Trp, with a K_m of 100 μM in rat hepatocytes, 400 μM in human liver, and 190 μM in the purified human enzyme, IDO has a much greater affinity and lower capacity, with K_m values of 3 to 50 μM in various sources.[10] Of important relevance to functional differences is that IDO activity, unlike that of TDO, can be substrate inhibited by high [Trp], with a K_i of ⩾200 μM[10] (see below).

Although TDO is mainly liver specific, it also exists in brain, with 2 variants having been identified at relatively different expression levels in various mouse brain structures and suggested to play important roles during postnatal developent.[11] Trp 2,3-dioxygenase is also actively expressed in brain tumors[12] (see further below). Indoleamine 2,3-dioxygenase has a much wider tissue distribution, including peripheral blood and other immune cells. Indoleamine 2,3-dioxygenase exists in 2 forms: the traditional IDO 1 and the more recently discovered IDO 2. The latter is expressed in murine kidney, liver, and the male and female reproductive systems.[13,14] The human IDO 2 is expressed in kidney, liver, and brain and has a much higher K_m for L-Trp than IDO 1 and a much reduced catalytic activity.[15] However, as will be described below, IDO 2 exerts important immunoregulatory activity under certain conditions.

N-formylkynurenine formamidase exists in liver, kidney, and brain and is very active in hydrolyzing NFK to K and formate, with the recombinant mouse liver enzyme exhibiting a catalytic rate of 42 μmol/min/mg of protein and a high capacity for NFK (K_m: 180-190 μM).[16]

Kynurenine monooxygenase (or kynurenine hydroxylase) is a mitochondrial flavoprotein that uses O_2 as cosubstrate and NADPH as cofactor.[17] As a flavin adenine dinucleotide–dependent enzyme, its activity can be expected to be decreased in riboflavin (vitamin B_2) deficiency, as suggested by a 10-fold decrease in 3-HK, a 3-fold decrease in both 3-HAA and *N*-methyl nicotinamide, and a 2-fold increase in KA urinary excretion in riboflavin-deficient baboons.[18] Decreased KMO in a 9-year-old girl with pellagra with colitis is accompanied with increased KA and K and decreased XA excretion.[19] The decreased KMO activity in B_2 deficiency has been suggested to involve decreased cofactor availability because of inhibition of $NAD^+(P^+)$ synthesis.[20]

As kynureninase (kynase) is a pyridoxal 5′-phosphate (PLP)–dependent enzyme, its activity is impaired by vitamin B_6 deficiency,[21–23] whether induced by malnutrition or functionally. Malnutrition-induced inhibition of kynase activity can occur in B_6 deficiency or with Leu-rich diets, such as maize or sorghum.[24] With Leu, it has been suggested that PLP stores may also become depleted by increased consumption during transamination of Leu by branched-chain amino acid aminotransferase.[24] The pellagragenic effect of Leu additionally involves activation of liver TDO and enhancement of the flux of Trp through TDO and possibly also 2-amino-3-carboxymuconate-6-semialdehyde decarboxylase or picolinate carboxylase (ACMSD) of renal origin[24] (see further below). Drugs can also inhibit kynase activity by inducing a functional B_6 deficiency through binding and thereby inactivating PLP. Examples include estrogens[25,26] and hydrazine drugs, such as isoniazid, benserazide, and carbidopa.[7] It is noteworthy that incidence of pellagra during the first half of the 20th century was twice as high in women than men, suggesting that estrogens may be a precipitating factor in the absence of adequate Trp or niacin intake.[7] The purified recombinant human Kynase appears to selectively use 3-HK as substrate, with a K_m value of 3 μM and is inhibited by L-K and D-K with K_i values of 20 and 12 μM, respectively.[27] Earlier, it has been reported[28] that the human liver enzyme shows a much greater preference to 3-HK (K_m = 77 μM) than to K (K_m = 1 mM), with a 15:1 ratio of activity toward these 2 substrates. In our recent study[29] in normal US subjects of various ethnic backgrounds, we observed ratios of Kynase B:Kynase A (estimated from the fasting plasma [3-HAA]/[3-HK] and [AA]/[K] ratios, respectively) consistent with a much greater Kynase B activity, with ratios of 28:1, 20:1, and 40:1 for the whole group (n = 114), men (n = 54), and women (n = 60), respectively.

Table 1. Enzymes of the kynurenine pathway.

ENZYME	EC NUMBER	SUBSTRATE(S)	PRODUCT(S)	COFACTOR	$K_{M,}$ μM	MAIN SOURCES
TDO	1.13.11.11	L-Trp, O_2	NFK	Heme	190	Liver, CNS
IDO	1.13.11.17	L-Trp, various, O_2	NFK	Heme	20–50	Placenta, lung, intestine, others
FAM	3.5.1.9	NFK	K + formate	None	50–180	Liver, kidney, brain
KMO	1.14.13.9	K + O_2	3-HK	NADPH	14–25	Liver, kidney, CNS, placenta
Kynase A	3.7.1.3	K	AA	PLP	1000	Liver, kidney, wide tissue distribution
Kynase B	3.7.1.3	3-HK	3-HAA	PLP	77	Same as above
KAT A	2.6.1.7	K	KA	PLP	960–4700	Liver, kidney, brain, heart
KAT B	2.6.1.7	3-HK	XA	PLP	3800–5700	Liver, kidney, brain
3-HAAO	1.13.11.6	3-HAA + O_2	ACMS	Fe^{2+}	2-3.6	Liver, kidney, brain
ACMSD	4.1.1.45	ACMS	AMS + CO_2	Nil	6.5-14	Kidney, liver, various organs
AMSD	1.2.1.32	2-AMSD + H_2O	2-AMA + 2 H^+	NAD^+	11–26	Liver
QPRT	2.4.2.19	QA + PRPP	NaMN + PPi + CO_2	Nil	21.6	Liver, kidney
NPRT	2.4.2.11	NA + PRPP	NaMN + PPi	Nil	1	Liver. kidney, pancreas, heart
NMPRT	2.4.2.12	NM + PRPP	NMN + PPi	Nil	0.855–42	Liver, kidney, spleen, heart, brain
NMD	3.5.1.19	NM + H_2O	NA + NH_3	Nil	0.2–50	Liver, small intestine
NMNAT	2.7.7.18	NMN (NaMN) + ATP	NAD^+ ($NaAD^+$) + PPi	Nil	21–209	Liver, kidney, spleen, pancreas, brain
NADS2	6.3.1.5	$NaAD^+$ + ATP + NH_3	NAD^+ + AMP + PPi	Nil	130–490	Brain, human cells, kidney
NADS1	6.3.1.5	$NaAD^+$ + ATP + Gln/NH_3	Same as above + Glu	Nil	130–490	Small intestine, kidney, liver, testis
NAD kynase	2.7.1.23	NAD^+ + ATP	$NADP^+$ + ADP	Nil	110–6500	Various (K_m: 0.22 mM in brain)
NADase	3.2.2.5	NAD^+ + H_2O	ADP-ribose + nicotinamide	Nil	28–260	Lung, serum, blood, and other cells
NNMT	2.1.1.1	Nicotinamide + SAM	NMNM + SAC	Nil	105–400	Liver, lung, muscle, kidney, heart, adipose
NMNO	1.2.3.1	NMN + H_2O + O_2	4-Py + 2-Py + H_2O_2	FAD^+	355–1895	Liver, kidney, brain, adrenals, etc

Abbreviations: 3-HAA, 3-hydroxyanthranilic acid; 3-HAAO, 3-hydroxyanthranilic acid oxygenase; 3-HK, 3-hydroxykynurenine; AA, anthranilic acid; ACMS, 2-amino-3-carboxymuconate-6-semialdehyde; ACMSD, 2-amino-3-carboxymuconate-6-semialdehyde decarboxylase or picolinate carboxylase; ADP, adenosine diphosphate; AMS, alpha-aminomuconate semialdehyde; AMSD, 2-aminomuconic-6-semialdehyde dehydrogenase; CNS, central nervous system; EC, Enzyme Commission; FAD^+, oxidized flavin adenine dinucleotide; FAM, formylkynurenine formamidase; IDO, indoleamine 2,3-dioxygenase; KA, kynurenic acid; KAT, kynurenine aminotransferase; KMO, kynurenine monooxygenase or kynurenine hydroxylase; Kynase, kynureninase; NADase, NAD^+ glycohydrolase; $NADP^+$, oxidized nicotinamide adenine dinucleotide phosphate; NADS, NAD^+ synthetase; NaMN, nicotinic acid mononucleotide; NFK, *N*′-formylkynurenine; NM, nicotinamide; NMD, nicotinamide deamidase; NMN, nicotinamide mononucleotide; NMNAT, nicotinamide/nicotinic acid mononucleotide adenylyltransferase; NMNO, *N*-methylnicotinamide oxidase or aldehyde oxidases; NMPRT, nicotinamide phosphoribosyltransferase; NNMT, nicotinamide *N*-methyltransferase; NPRT, nicotinate phosphoribosyltransferase; PLP, pyridoxal 5′-phosphate; QPRT, quinolinate phosphoribosyltransferase; SAC, *S*-adenosyl cysteine; SAM, *S*-adenosyl methionine; TDO, Trp 2,3-dioxygenase;XA, xanthurenic acid.
Data were obtained from the general literature and www.brenda-enzymes.org.[8]

The other PLP-dependent enzyme of the KP, KAT, is also subject to inhibition in B_6 deficiency[21,22] and by benserazide and carbidopa.[7] As stated above, the KAT reaction is of minor quantitative significance under normal conditions because of the much higher K_m for its 2 substrates, K and 3-HK, compared with those for Kynase. Various aminotransferases capable of catalyzing the conversion of K to KA (and also of 3-HK to XA) exist in different organs and across species and can use a wide range of substrates. There appears to be 4 KATs (KAT I, II, III, and IV) in humans, mice, and rats. KAT I, II, III, and IV are identical to (1) glutamine aminotransferase and cysteine conjugate β lyase (CCBL), (2) α-ketoadipate aminotransferase, (3) CCBL, and (4) aspartate aminotransferase and glutamate oxaloacetate aminotransferase. In the mouse, these KATs show

different thermal stabilities, pH dependence, and responses to inhibitors.[30] The biochemical behaviors of KAT I and KAT III appear very similar and could reflect the same enzyme.[31] In the brain, KAT II appears particularly responsible for transamination of K to KA and can almost equally act on 3-HK as on K.[32] Although its K_m for K and 3-HK is very high (4.7 and 3.8 mM, respectively), the irreversible nature of the transamination reaction allows for the accumulation of KA. As will be discussed below, KAT II inhibition is a strategy for lowering KA under certain neurological conditions.

3-HAA 3,4-dioxygenase (oxidase: 3-HAAO) is by far the most active among KP enzymes,[6,7] which explains the rapid conversion of 3-HAA into the unstable ACMS and nonenzymic cyclization of the latter to QA. The enzyme is a nonheme iron-dependent dioxygenase that uses molecular O_2 as cosubstrate and Fe^{2+} as cofactor. Its activity in human, mouse, and rat brain is stimulated by the addition of Fe^{2+} and inhibited by iron chelators.[33] The important role of QA in neurodegenerative diseases emphasizes the critical role of 3-HAAO activity and its control by fluctuations in endogenous iron and proteins that regulate iron bioavailability.[33] The enzyme exhibits a high affinity for its 3-HAA substrate, with a K_m of 3 μM for the purified rat liver and brain enzyme[34] and 2 μM for the cloned human enzyme.[35]

2-Amino-3-carboxymuconate-6-semialdehyde decarboxylase or picolinate carboxylase is expressed in the rat only in liver and kidney, but not in 10 other tissues and organs, with the kidney enzyme content being 2.52-fold that in liver.[36] The K_m values of the 2 purified enzymes toward the ACMS substrate are, however, close (14 and 13 μM, respectively).[36] In humans, 2 alternatively spliced ACMSD transcripts (ACMSD I and II) have been examined in brain, liver, and kidney.[37] Although both transcripts are present in kidney and liver, no ACMSD II expression is detected in brain. However, the enzymatically active ACMSD I is present at very low levels in brain.[37] The enzyme exhibits a K_m of 6.5 μM and is inhibited by QA, PA, and KA.[37] As is the case in rats, the highest activity of ACMSD is present in the human kidney. As stated earlier, enhancement of ACMSD by Leu explains in part the pellagragenic effect of high Leu diets.[38]

2-Aminomuconate semialdehyde dehydrogenase catalyzes the conversion of AMS to 2-aminomuconic acid, which is further degraded to acetyl CoA. 2-Aminomuconate semialdehyde dehydrogenase has been widely studied in bacteria. In *Pseudomonas pseudoalcaligenes*, the enzyme can act on several analogues and shows a great identity to 2-hydroxymuconic semialdehyde,[39] which is often used in kinetic studies. Its K_m for the latter substrate is 11 to 26 μM. In cat (*Felis catus*), the K_m of the enzyme for the 2-hydroxy substrate and for NAD^+ is 16 and 19 μM, respectively.[8]

Quinolinate phosphoribosyltransferase (QPRT) is a key enzyme in the synthesis of NAD^+. Quinolinate phosphoribosyltransferase uses QA and 5-phosphoribosyl-1-pyrophosphate (PRPP) as cosubstrates and produces nicotinic acid mononucleotide (NaMN), inorganic pyrophosphate (PPi), and CO_2. The human enzyme exhibits a K_m value of 21.6 μM for QA and of 23 μM for PRPP.[40] The PRPP exerts substrate inhibition at concentrations greater than 300 μM.[41] In the Wistar rat, the enzyme is localized in liver and kidney, with an activity ratio of 6:1.[41]

Nicotinic acid mononucleotide can also be formed by the action of nicotinate phosphoribosyltransferase (NPRT). Like QPRT, NPRT also uses PRPP as cosubstrate along with nicotinic acid. The enzyme from beef liver cytosols has a K_m for nicotinic acid of 1 μM and for PRPP of 50 μM and undergoes product inhibition by NaMN.[42] Nicotinate phosphoribosyltransferase is concentrated in liver, kidney, and small intestine of rats and mice but is also expressed to a lesser extent in 7 other organs, including the brain[41,42] and in cultured human hepatoma HepG2, kidney epithelia HEK293, and cervical carcinoma HeLa cells.[43] Nicotinamide is a poor substrate for NPRT, with a K_m of 100 mM.[44] Also, the recombinant human NPRT does not catalyze the formation of the mononucleotides of NMN or nicotinic acid from NMN or QA, respectively.[45]

In the study by Ikeda et al,[41] the activity of NMN phosphoribosyltransferase (NMPRT), assayed under the same experimental conditions as those for NPRT but using NMN as substrate, was found to be lower than that of NPRT, with the activity in rat liver, kidney, brain, and heart being only 23%, 48%, 49%, and 45%, respectively,[41] of that of NPRT. The NMPRT from rat liver has a K_m for NMN of 42 μM,[44] whereas the affinity of the human enzyme to NMN (K_m = 0.855 μM) is enhanced 10-fold by adenosine triphosphate (ATP) (K_m = 0.057 μM).[45] Similarly, the K_m of the enzyme for PRPP of 7 to 12 μM is decreased to 0.63 μby ATP.[45]

Nicotinamide deamidase (NMD) converts NMN to nicotinic acid, releasing ammonia in the process. The K_m of the enzyme has been widely measured in bacteria and varies between 0.2 and 0.65 mM.[8] In mouse liver, the K_m is 1 μM,[46] whereas that in rat liver is considerably higher, possibly because of the presence of an endogenous inhibitor.[47]

Nicotinamide/nicotinic acid mononucleotide adenylyltransferases are a group of isoenzymes, collectively referred to here as NMNATs, that transfer the adenylate moiety of ATP to the mononucleotides of nicotinamide (NMN) and nicotinic acid (NaMN) to produce NAD^+ and $NaAD^+$, respectively, plus PPi. These NMNATs are critical for NAD^+ synthesis. Although the transfer reaction is reversible, net NAD^+ formation is likely to be due to substrate availability. In humans, 3 nicotinate/nicotinamide mononucleotide adenylyltransferase (NMNAT) isoforms have been characterized—NMNAT 1, 2, and 3—and an excellent review of their structure, properties, and biology has been published.[48] NMNAT 1 in humans has a wide tissue distribution and a dominant activity. It is most highly expressed in skeletal muscle and heart and strongly expressed in liver and kidney. NMNAT 2 is strongly expressed in brain but is also

expressed in heart, skeletal muscle, and pancreas. NMNAT 3 is strongly expressed in lung and spleen and generally in tissues in which NMNAT 2 expression is weak. Subcellular localization of NMNATs also differs. Thus, NMNAT 1 activity is present in the nucleus, whereas that of NMNAT 2 and 3 is located in Golgi apparatus and mitochondrion, respectively. With NMN as substrate, the K_m of NMNAT 1 and 2 is similar (21-34 μM), whereas that of NMNAT 3 is higher (66-209 μM).[48] With NaMN, the K_m values of the 3 isoforms are 68 to 116, 15, and 111 μM, respectively.[48]

NAD synthetase converts nicotinic acid adenine dinucleotide (NaAD$^+$) to NAD$^+$ using as cosubstrates either ATP and NH_3 or ATP and glutamine (Gln) and producing, along with NAD$^+$, either AMP and PPi or AMP + PPi + glutamate (Glu). Most studies of NAD synthetase have been performed with enzymes from various bacteria. However, the human enzyme has been studied in detail,[49] and 2 species have been identified with different tissue distributions and substrate activities. Nicotinamide adenine dinucleotide synthetase 1 can use as an amide donor either NH_3 or Gln, whereas NAD synthetase 2 uses only NH_3. The former is strongly expressed in small intestine, kidney, and liver, whereas the latter is predominant in brain and human cells such as HepG2 and Huh7.[49] The tissue distribution of 6 enzymes of NAD$^+$ synthesis (from QPRT to NAD synthetase) in rats has been reported.[50]

NAD kinase catalyzes the phosphorylation of NAD$^+$ to oxidized nicotinamide adenine dinucleotide phosphate (NADP$^+$) using ATP and releasing adenosine diphosphate (ADP). The enzyme is widely distributed throughout organs and tissues of rats and humans.[8]

NAD glycohydrolase (NADase) hydrolyzes NAD$^+$ to NMN and ADP-ribose. It occurs in many tissues, including erythrocytes and other blood cells,[8] and, as will be described below, its activity can be enhanced in infections and cancers.

Nicotinamide *N*-methyltransferase catalyzes the methylation of nicotinamide to *N*^{1}-methylnicotinamide by *S*-adenosyl-L-methionine (SAM), producing, along with NMN, *S*-adenosyl-L-cysteine. It is strongly expressed in liver, but also occurs in lung, muscle, kidney, heart, and adipose tissue. The K_m of the human enzyme varies between 105 and 200 μM (mutant protein), 380 μM (recombinant protein), 400 μM (wild type), and 1360 μM (liver homogenates).[8] The enzyme activity can lead to depletion of both NAD$^+$ and SAM and thus plays very important roles in development and disease (see below).

N^1-methylnicotinamide oxidases (aldehyde oxidases) oxidize this nicotinamide metabolite to *N*^{1}-methyl-4-pyridone-3-carboxamide (4-Py) and N^1-methyl-2-pyridone-5-carboxamide (2-Py). Little information is available on kinetics of the human enzymes toward *N*^{1}-methylnicotinamide, but the K_m for this substrate is much higher for the rat (0.355-1.895 mM) than the mouse (0.0255-0.1285 mM) enzyme.[8] Those of the rabbit and hog liver enzymes are 0.66 and 0.36 mM, respectively.[51] The enzyme has a wide human tissue distribution, including liver, kidney, brain, adrenals, lung, spleen, stomach, and testis. Urinary excretion of these 2 metabolites is used as a measure of dietary niacin intake.[52] This aldehyde oxidase acts on a wide range of substrates, and it appears that the unusual characteristics of the enzyme enable it to produce 2 oxidation products (2-Py and 4-Py) from 1 substrate.[51] The ratio of 2-Py to 4-Py is 100 for the rabbit, and only 3.8 for the hog, enzyme.[51] The ratio in humans, determined from urinary excretion data, is ~91.[52]

Species Differences in KP Enzymes and Choice of Experimental Models of Trp-Related Human Diseases

There are wide species differences in the activity of the KP and its enzymes, necessitating the importance of choice of the most suitable animal model to study human diseases related to Trp metabolism. In human liver and that of rat, mouse and some, but not all, other animal species, TDO exists in both the heme-free apoenzyme and heme-containing holoenzyme in roughly equal proportions.[53] Some species (including cat, gerbil, golden hamster, and guinea pig) lack the free apoenzyme and are therefore TDO deficient. They also lack the glucocorticoid induction mechanism (see below) and suffer from toxicity of an excess of Trp.[53] For example, a comparative study of cat and rat showed marked differences in several enzymes of the KP other than TDO.[54] Thus, cat to rat ratios of 0.4, 0.8, 0.1, 0.03, and 1.7 for liver TDO, liver kynureninase, liver KAT, kidney KAT, and liver KMO, respectively, were observed. The most remarkable difference between cat and rat is the much greater activity of ACMSD, the cat/rat ratio of which is 32.17 in liver and 4.06 in kidney.[41] This high ACMSD activity in the cat renders it vulnerable to niacin and NAD$^+$ deficiency, with the conversion of 3-HK to niacin ribonucleotides being only 11% of that in the rat.[41]

Significant differences in up to 6 enzymes of the KP in liver, kidney, lung, and brain have also been reported in more recent comparative studies in rats, mice, rabbits, gerbils, and guinea pigs.[55,56] The distinction between these 2 groups of species based on TDO differences does not apply to IDO.[56] The above TDO-deficient species are therefore unsuitable as animal models of human diseases involving Trp metabolism, although they would be valid models in studies addressing issues related to their specific KP characteristics. Rats and mice are the most common animal models in Trp-related studies. The author recommends the Wistar rat as the most suitable animal model in this regard. Significant mouse strain differences in Trp metabolism exist,[3] which necessitates careful choice of the mouse strain model.

Regulation of the KP

Most previous studies on KP regulation have focused on the first and most rate-limiting enzyme, hepatic TDO. Because of its short half-life (~2 hours for the apoenzyme) and hence rapid inducibility, TDO served as a model for enzyme regulation

Table 2. Regulatory mechanisms of TDO and IDO.

MECHANISM AND MAIN FEATURE	TDO	IDO1
Glucocorticoid induction (de novo enzyme synthesis at transcription level)	+	–
Substrate (Trp) activation (enhanced heme conjugation + stabilization + normal synthesis)	+	(Inhibition by excess Trp through reverse binding sequence)
Cofactor (heme) activation (enhanced heme conjugation)	+	–
Feedback inhibition by NAD(P)H (allosteric modulation)	+	(Unknown)
Cytokine induction (IFN-γ) (enhanced transcription)	–	+
Inhibition by nitric oxide (by binding to heme)	(Unknown)	+

Abbreviations: IDO, indoleamine 2,3-dioxygenase; NAD(P)H, reduced nicotinamide adenine dinucleotide (phosphate); TDO, Trp 2,3-dioxygenase; Trp, tryptophan.

during the 1950s and 1960s established by the pioneering work, in the United States, of W. Eugene Knox, Olga Greengard, and Philip Feigelson.[57–59] Similarly, the pioneering work in Japan of Osamu Hayaishi,[60] discoverer of IDO and its induction by IFN-γ, deserves recognition. The half-lives of other KP enzymes are unknown, but, even if they exhibit slower turnover rates, their important roles in regulating the pathway activity is nevertheless equally important and are best described below from a functional perspective. In this section, the main emphasis will therefore be on regulation of TDO and IDO and the flux of Trp down the pathway.

TDO regulation

Four mechanisms exist for broad regulation of TDO activity (Table 2). These are hormonal induction by glucocorticoid and some other hormones, substrate activation by Trp, cofactor activation by heme, and feedback inhibition by NAD(P)H. The main features of the first 3 mechanisms are induction of new apoenzyme synthesis by glucocorticoids, activation of preexisting apoenzyme and its stabilization by Trp, and activation of preexisting apoenzyme by heme. Glucocorticoid induction involves de novo synthesis of new apoenzyme, whereas substrate activation by Trp involves enhanced conjugation of the apoenzyme with its heme cofactor and stabilization of the holoenzyme.[57–59] The increase in TDO activity after Trp administration therefore results from a combination of activation, stabilization, and normal rate of synthesis. Only Trp stabilizes TDO. Estimates of the half-life of the basal enzyme in rat liver following inhibition of further enzyme synthesis by cycloheximide showed[61] that the basal holoenzyme and apoenzyme exhibit half-lives of 7.7 and 2.3 hours, respectively, and administration of a 500 mg/kg dose of Trp increases these values to 11.4 and 6.7 hours, respectively. Neither cortisol nor sources of heme prolong the TDO $T_{1/2}$. Administration of a 50 mg/kg Trp dose either alone or with cortisol doubles the half-life of the apoenzyme.[61] Thus, increased TDO activity can be expected in situations involving long-term provision of Trp either via nutritional or therapeutic interventions. Increased Trp breakdown along the hepatic KP leading to decreased availability of Trp for cerebral serotonin synthesis may explain in part the moderate and variable antidepressant activity of this serotonin precursor.[62] The increased saturation of TDO with heme is thought to involve enhancement of heme biosynthesis by Trp at the 5-aminolevulinate (5-ALA) dehydratase and possibly also subsequent steps in the heme biosynthetic pathway.[63] Although heme itself only activates TDO by virtue of being its cofactor, evidence suggests that it mediates glucocorticoid induction of TDO messenger RNA (mRNA) transcription and translation.[64] Furthermore, it appears that heme regulates the TDO gene posttranslationally through enhanced phosphorylation of the α subunit of the eukaryotic initiation factor, eIF2α.[65]

Hormonal induction of TDO by glucocorticoids involves de novo enzyme synthesis effected at the transcriptional level.[66,67] The TDO gene from rat liver has earlier been isolated and characterized.[68] Induction of the TDO gene is mediated by glucocorticoid-responsive elements, and glucocorticoid receptors play an important role in TDO induction. In response to experimental stress in rats, a new glucocorticoid receptor, termed receptor C, appears and is associated specifically with induction of TDO, but not with that of another glucocorticoid-inducible enzyme, tyrosine aminotransferase.[69,70] A potential appearance of this new receptor in humans in response to stress can be assumed to enhance TDO induction by the stress hormone cortisol and may thus undermine cerebral serotonin synthesis.[62]

Glucocorticoid induction of TDO in primary cultures of adult rat hepatocytes is potentiated by glucagon but inhibited by insulin and adrenaline, all of which act at the transcriptional level.[67] In cultured hepatocytes, however, glucagon enhances TDO activity at the translational step provided Trp is present to restore the decreased activity to levels observed in freshly isolated cells, and this enhancement appears to be synergistic to that by glucocorticoids, thus suggesting different mechanisms of action.[71] Thus, glucagon seems to exert a permissive effect

on glucocorticoid induction of TDO and to act additionally in the presence of Trp. Glucagon can be replaced by cyclic adenosine monophosphate (c-AMP).[71]

The effects of insulin on TDO activity are controversial. Thus, although insulin inhibits TDO induction by glucocorticoids in cultured rat hepatocytes,[67,71] its administration to rats does not inhibit TDO, whether administered alone[72,73] or jointly with glucagon or additionally with cortisol.[73] Others[74] have, however, reported that insulin, as well as glucagon and dibutyryl c-AMP, increases the heme saturation of TDO by enhancing heme synthesis. Dibutyryl c-AMP and glucagon can do so via Trp following stimulation of lipolysis[75,76] (see below), whereas insulin may act on TDO by enhancing heme synthesis.[77] Studies on experimental (streptozotocin-induced) diabetes have resulted in greater agreement. Thus, TDO activity is enhanced[78,79] and the flux of Trp through TDO is increased[80] in this experimental model. It is possible that the TDO enhancement in this model is mediated by the decreases in [NAD(P)H],[78] resulting in removal of the negative feedback inhibition by these end products of the KP (see below).

Although adrenaline inhibits glucocorticoid induction of TDO in hepatocytes,[67] it activates TDO after administration.[81,82] Noradrenaline administration also activates TDO.[82] Both act via Trp by enhancing lipolysis, leading to increased release of serum albumin–bound Trp. As catecholamine release is increased in stress, a TDO enhancement can be expected in stressed individuals. The effects on TDO activity of catecholamines and cortisol in stress will be additive,[61] thereby potentiating the serotonin deficiency under these conditions.

The effect of thyroxine on TDO activity is also controversial, with reports suggesting enhancement[81] or no change.[73,83]

TDO is also regulated by a feedback mechanism involving allosteric inhibition by NAD(P)H, as has been demonstrated in vitro[84,85] and after administration of agents causing increases in the hepatic levels of these reduced dinucleotides, eg, glucose and nicotinamide[86] and chronic ethanol intake.[2] It has been suggested[1,84] that 3-HK and 3-HAA may also exert feedback inhibition of TDO activity at (in vitro) concentrations near the physiologic levels or moderately higher. However, administration of these K metabolites to rats resulting in hepatic concentrations within the in vitro inhibitory range does not cause TDO inhibition.[87] In fact, 3-HK exerts no effect on TDO, whereas 3-HAA causes an enhancement. A similar effect mediated by 3-HAA is also observed after KA administration.[87] These and other changes induced by the above metabolites may be important in situations where levels of immunosuppressive kynurenines are raised in certain clinical conditions.

IDO regulation

Unlike TDO, IDO in rats or mice does not require heme for its activation nor is it inducible by glucocorticoids[88,89] (Table 2). Consequently, neither adrenalectomy nor starvation influence IDO activity,[88] unlike the case with TDO where the former inhibits, whereas the latter enhances, TDO synthesis.[88,90] Also, unlike the several-fold activation of rat liver TDO,[86] administration of a large dose of Trp (750 mg/kg) enhances IDO activity in rat intestine (the largest source) by only 50%.[89] This may be explained in part by a possible substrate inhibition, reported for the mouse epididymal enzyme to occur at [Trp] above 50 μM.[91] The mechanism of this inhibition involves a reversed sequence of binding to the enzyme of Trp and O_2.[92] At low [Trp], Trp is bound first followed by O_2, whereas at high [Trp], this order is reversed, with the heme reduction potential playing an important role.

The principal effector of IDO is IFN-γ,[93] and a less efficient inducer is IFN-α.[94] Other cytokines and mediators (both proinflammatory and anti-inflammatory) exert various effects on IDO.[62] Thus, anti-inflammatory cytokines (interleukin [IL]-4, IL-10, and transforming growth factor β) inhibit IDO induction by IFN-γ. The proinflammatory IL-1β and tumor necrosis factor α potentiate this induction and IL-2 acts via IFN-γ. Thus, the IDO status can be assumed to be determined by the balance between proinflammatory and anti-inflammatory cytokines. Interestingly, IFN-γ induction of IDO is potentiated by the synthetic glucocorticoid dexamethasone, which exerts no effect by itself.[94]

Nitric oxide (NO) may play an important role in the control of IDO activity. It inhibits the human enzyme.[95] Activity of the recombinant human IDO is reversibly inhibited by NO by binding to heme, with the inactivated enzyme complex being the Fe^{2+}-NO-Trp adduct.[96] This inhibition may represent an important mechanism of regulating the immune function of IDO.[96]

Regulation of the flux of tryptophan down the KP

The flux of Trp down the KP is determined primarily by plasma free Trp and secondarily by activities of TDO in liver and IDO elsewhere in the body. Generally, 5% to 10% of plasma Trp exists in the unbound (free) state, with the remaining 90% to 95% being albumin bound. Equilibration between the free and bound fractions is rapid. Free Trp is a labile parameter that can be influenced by many physiological and pharmacological factors, and it is important that Trp binding, usually expressed as the percentage free Trp (100 × [free Trp]/[total Trp]), is assessed at baseline and following experimental treatments or interventions, as it can establish the Trp metabolic status of an individual and its biological determinants and also facilitate accurate interpretation of clinically or experimentally induced changes.[97] Failure to consider the free Trp status in pregnancy led to the widely accepted concept of "Trp depletion as a defence mechanism against fetal rejection" being invalid.[98] It is also important that free Trp is determined in freshly isolated serum or plasma to avoid the increased albumin binding caused by frozen storage leading to erroneously lower [free Trp] values.[97]

Table 3. Correlations between plasma-free or total tryptophan and parameters of tryptophan oxidation.

TREATMENT GROUPS	PARAMETER					
	K	TDO	TDOF	KS	TTOX	TTOXF
	CORRELATION COEFFICIENT *R* AND SIGNIFICANCE (*P*)					
Baseline (n = 111)						
Free	0.22 (.019)					
Total						
F3 (n = 96)						
Free	0.27 (.008)	−0.35 (.000)	−0.62 (.000)		−0.32 (.002)	0.50 (.000)
Total	0.56 (.000)	−0.46 (.000)		0.35 (.000)	−0.45 (.000)	
F0 (n = 96)						
Free	0.20 (.05)	−0.43 (.000)	−0.53 (.000)		−0.39 (.000)	−0.43 (.000)
Total	0.24 (.02)	−0.58 (.000)	−0.46 (.000)		−0.54 (.000)	−0.42 (.000)
ATL 5.15 (n = 199)						
Free	0.36 (.000)		−0.32 (.000)	0.44 (.000)		−0.49 (.000)
Total	0.35 (.000)	−0.27 (.000)	−0.17 (.015)	0.37 (.000)	−0.31 (.000)	−0.47 (.000)
ATL 10.30 (n = 160)						
Free		−0.18 (.021)	−0.38 (.000)	0.26 (.001)	−0.17 (.029)	−0.42 (.000)
Total	0.27 (.000)		−0.27 (.000)	0.45 (.000)		−0.29 (.000)

Abbreviations: K, kynurenine; Ks, sum of total kynurenines; TDO, Trp dioxygenase: 100 × [K]/[total Trp]; TDOF, Trp dioxygenase relative to free Trp: 100 × [K]/[free Trp]; TTOX, total Trp oxidation: 100 × [Ks]/[total Trp]; TTOXF, total Trp oxidation relative to free Trp: 100 × [Ks]/[free Trp].
Definition of treatment groups: baseline, fasting plasma; F3, a small Trp load of 1.15 g with minimal contribution to the Trp flux from a small dose of leucine; F0, same as F3, but with a larger dose of leucine; ATL 5.15, acute Trp loading with a 5.15 g Trp dose and the same dose of leucine as in F0; ATL 10.30, a larger Trp dose with double the leucine content as that in ATL 5.15. Only the significant Pearson product moment correlations are given.

The flux of Trp down the KP was studied in isolated rat hepatocytes under a variety of conditions, including adrenalectomy, experimental diabetes, fasting, glucocorticoid treatment, and manipulation of Trp binding by albumin and nonesterified fatty acids (NEFA).[80,99–102] The results of these hepatocyte studies have generally confirmed the observations described above for these treatments and established that Trp availability to the liver and, in particular, free Trp, is the major determinant of TDO activity in vivo. Tryptophan oxidation by TDO in hepatocytes is enhanced 5-fold by increasing the [Trp] from the physiological level of 0.1 to 0.5 mM, with a further increase to 2.5 mM exerting no additional effect. From a recent study in intact rats,[87] the author has now calculated that liver Trp was significantly correlated (Pearson product moment) with liver K (r = 0.616; P = .0381) and liver total kynurenines (r = −0.711; P = .0211; n = 10). After acute Trp loading (50 mg/kg intraperitoneally), liver Trp correlated only with total Trp oxidation (r= −0.472; P = .0171; n = 25). Thus, as in hepatocytes, liver Trp (derived from plasma free Trp) appears to play an important role in flux down the hepatic KP.

In parallel agreement with the above findings in hepatocytes with various Trp concentrations,[80,98–101] our recent human study[29] showed that TDO activity (expressed as the [K]/[Trp] ratio %) was increased maximally by a 5.15 g Trp dose (~74 mg/kg in a 70 kg human). However, flux of Trp through the KP continued to increase dose dependently beyond maximum TDO activation, further suggesting that flux is primarily determined by Trp availability. In this human study,[29] the combined TDO/IDO activity in fasting subjects (n = 114) was estimated to account for a maximum of ~70% of total Trp oxidation, and a similar value was obtained for the [kynurenine]/[total kynurenines] ratio. By taking into account quinolinate formation, which was not measured, this value can be revised to 63%. A similar value (of 60%) can be calculated for the contribution of TDO to Trp oxidation to CO_2 in rat hepatocytes.[100]

Further analysis of the data from our previous 2 studies in humans[24,29] was performed to establish the potential role of plasma free and total Trp in the flux down the KP under the conditions listed in Table 3. Here, Pearson product moment correlations were examined for free and total plasma Trp and a number of parameters indicative of the Trp flux, namely, K, TDO, TDOF (TDO relative to free Trp), total Trp oxidation (TTOX), and TTOXF (TTOX relative to free Trp). Under basal fasting conditions, the only significant correlation was between free Trp and K. Significance was then extended to most of the other parameters, as shown, when Trp was administered at

3 dose levels. In the 2 groups (F3 and FO) receiving a small Trp dose (1.15 g) with a, respectively, minimal and greater contribution to the Trp flux from a small and a larger Leu (branched-chain amino acids) dose, there were significant correlations between both free and total Trp and most of the above parameters. Only total kynurenines did not correlate, which may reflect the contribution of extrahepatic tissues to further metabolism of K. After loading with larger doses of Trp, both free and total Trp correlated with total kynurenines in addition to other parameters. From the data in Table 3, it appears that total Trp is as important as free Trp in the flux down the KP. This is not surprising given the rapid equilibration between the free and bound fractions. However, this does not minimize the importance of free Trp as it can only enter tissues after being released from albumin.

Functions of the KP

The KP performs a number of functions (outlined in Table 4) both within the liver and other organs/tissues and at the general body level. These functions will be discussed with special reference to KP enzymes at the physiological level, their disturbances in disease conditions, and their targeting for therapeutic purposes. It is, however, important to emphasize that all enzymes of the pathway are important, irrespective of their catalytic activities or turnover rates, and that the term "regulatory" should be used with caution when the expression or function of a particular enzyme is undermined with resultant negative health consequences.

Detoxification of excess tryptophan

As stated above, species lacking the TDO free apoenzyme and its glucocorticoid induction mechanism are sensitive to toxicity of an excess of Trp.[53] When adrenalectomized, the rat becomes vulnerable to this toxicity as it no longer possesses the glucocorticoid induction mechanism necessary to process the excess of Trp down the KP.[103] Administration of cortisol to adrenalectomized rats confers protection,[103] and it is thought that toxicity arises from diversion of Trp degradation toward the indole(amine) pathways.[104] This function is exclusive to liver TDO and so is unlikely to involve IDO because although species lacking the TDO apoenzyme, eg, gerbil and rabbit, possess a higher IDO activity than rats, they are still vulnerable to Trp toxicity. To (partially) mitigate this vulnerability, these species metabolize Trp at a faster rate and a lower dosage than does the rat (see Badawy[105] for reference). Also, as Trp inhibits IDO activity at concentrations greater than 50 μM,[91] it is unlikely that IDO will be able to process any excess Trp. Another mechanism through which Trp-sensitive species process Trp is its oxidation to acetyl CoA along the second arm of the KP. As stated above, the cat possesses a greatly elevated ACMSD activity,[41] and a study in isolated hepatocytes[106] showed that although the rate of Trp oxidation and QA formation is lower than in rats, guinea pigs, gerbils, and sheep metabolize a much larger part of Trp through the citric acid cycle.

Table 4. Functions of the kynurenine pathway.

FUNCTION	ENZYME(S) RESPONSIBLE
1. Detoxification of excess Trp	TDO
2. Control of hepatic heme biosynthesis	TDO
3. Control of plasma Trp availability	TDO (physiological conditions)
	IDO (immune activation)
4. Production of kynurenines	TDO/IDO → 3-HAAO
5. Production of picolinic acid	ACMSD
6. Production of NAD^+	QPRT → NAD synthetase
7. Production of niacin	QPRT → NADase and PARP

Abbreviations: ACMSD, 2-amino-3-carboxymuconate-6-semialdehyde decarboxylase or picolinate carboxylase; HAAO, hydroxyanthranilic acid oxygenase; IDO, indoleamine 2,3-dioxygenase; NAD^+, oxidized nicotinamide adenine dinucleotide; NADase, NAD^+ glycohydrolase; PARP, poly-(ADP-ribose) polymerase; QPRT, quinolinate phosphoribosyl transferase; Trp, tryptophan; TDO, Trp 2,3-dioxygenase.

Control of plasma tryptophan availability

Under normal conditions, this control is exerted mainly, if not exclusively, by hepatic TDO. Under conditions of immune activation, IDO assumes the major role, although TDO may also play a part. This major contribution of TDO, which has a much greater capacity for Trp than IDO, is best illustrated by the observation[107,108] that deletion of the mouse TDO gene increases plasma [Trp] by 9.3- to 12.7-fold. Plasma [Trp] was not measured in a study involving deletion of the mouse IDO1 gene,[109] but brain [Trp] was not altered after mouse IDO1 and IDO2 gene deletion, in contrast to a ~10.6-fold elevation of brain [Trp] by TDO2 gene deletion.[110] Thus, IDO under normal physiological conditions does not influence plasma or brain Trp. Earlier work has clearly demonstrated the impact of the TDO control of plasma Trp availability on tissue Trp levels. In relation to CNS function, an inverse relationship exists between TDO activity and brain [Trp] and 5-HT synthesis.[111] A potential enhancement of TDO activity by the elevated cortisol levels may explain the serotonin deficiency in major depressive disorder.[62] The TDO inhibition by chronic administration to rats of drugs of dependence increases brain [Trp] and enhances 5-HT synthesis, whereas TDO induction by corticosterone during subsequent drug withdrawal exerts the opposite effects.[111] The dramatic increase in plasma [Trp] induced by deletion of the mouse TDO gene results in an equally dramatic increase in circulating Trp availability to the brain (expressed as the [Trp]/[competing amino acids] ratio) and consequently in brain Trp, 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA).[107] The control of Trp availability to the brain by TDO has also been suggested[107] as a modulator of anxiety through changes in brain 5-HT. The deletion of TDO gene also increases Trp availability for the decarboxylation and

transamination of Trp,[107] and it is of interest that the Trp transamination product IPA possesses anxiolytic properties.[112]

Although the extrahepatic IDO plays a minimal role in the control of plasma Trp availability, it can influence KP activity even in the absence of TDO, as suggested by the finding[107] that plasma [K] and [KA] are maintained at wild-type levels in TDO knockout mice. In the absence of preformed niacin, synthesis of K and its metabolites KA, XA, and 3-HAA is increased, whereas that of QA is decreased, in these mice,[108] and it was suggested that K formed by IDO extrahepatically can be used by the liver to form adequate amounts of nicotinamide and NAD^+ nucleotides to maintain growth. Also, the hepatic KP can contribute to extrahepatic kynurenine metabolite formation through the availability of the kynurenine precursor. In some situations, this is likely to be quantitatively more important than the modest contribution of IDO itself. The role of IDO in control of plasma Trp availability takes center stage when the immune system is activated. The induction of IDO by IFN-γ and agents acting through it leads to depletion of [Trp] and increased K formation in cultures of monocytes[94] and serum.[113,114]

Production of kynurenine metabolites

Kynurenine metabolites have been the subject of intense research activity over the past 2.5 decades with the discovery of their important roles in neuronal and immune function. In this section, a brief discussion of the functions of KP metabolites from K to QA and PA and the roles of the relevant enzymes will be presented with reference to disease states. Functions of enzymes leading to NAD^+ synthesis from QA or nicotinamide will be discussed in the next section and subsequently in relation to their targeting for therapeutic purposes.

A landmark discovery was that of identification of QA and KA, respectively, as agonist and antagonist of the NMDA receptors of the excitatory amino acid glutamate,[115] thus heralding a new era of research in neuroscience and neuropsychopharmacology. Another equally important landmark discovery is that of identification of a number of K metabolites as immunomodulators, in particular, 3-HK, 3-HAA, QA, and PA.

In the minor transamination step in the KP, KA formation is controlled by availability of K through either K or Trp loading or KMO inhibition. KAT inhibition is a new strategy for the treatment of schizophrenia,[116–118] which is now considered a disease of glutamatergic hypoactivity induced by elevation of the NMDA receptor antagonist and the KAT A reaction product KA.[119] Preliminary preclinical results show that inhibition of the brain KAT II isoenzyme by BFF-816 improves performance in spatial and contextual memory tests[116] and that by PF-04859989 improves other cognitive functions.[117] The NMDA receptors are activated by glycine, but clinical trials of chemicals that inhibit the glycine transporter 1 have given disappointing results.[120] A more promising approach is to address the status of KA in schizophrenia with KAT inhibitors. Benserazide inhibits KAT A[87] and therefore has the potential to influence cognition in schizophrenia. Two previous studies, however, did not demonstrate an antipsychotic action of benserazide administered either alone or with Trp.[121,122] The rationale here was to correct a potential serotonin deficiency by Trp and benserazide based on the ability of (large doses of) the latter to inhibit liver TDO activity. A more likely rationale is KAT A inhibition, and perhaps, efficacy could be demonstrated with doses larger than those used in the above clinical studies.

Another important aspect of KA and KAT A that requires further investigation is that of the greater incidence of schizophrenia in female, compared with male, whites. A potential explanation is that of estrogens inhibiting the KAT A reaction leading to a lower [KA] in women.[29] The limitation of this gender difference to white women, as opposed to African American or American Hispanic women, may be explained by the greater use of estrogen contraception by white women.[29]

KA may also modulate immune function. It has been shown to exert anti-inflammatory effects in mice and to lower levels of proinflammatory cytokines.[123] This is likely to influence IDO activity. KA also protects against lipopolysaccharide toxicity in mice.[124] A novel feature of relevance to these properties is that KA may modulate levels of AA and thereby lower the [3-HAA]/[AA] ratio. A decrease in this ratio has been reported[125] in a wide range of neurological conditions and was suggested as a protective mechanism limiting further damage, particularly in brain. In nonsurviving patients with acute stroke, this decrease is associated with elevated plasma [KA].[126] In agreement with these findings in humans, a recent study in rats[87] reported that KA administration raises [AA] in liver by enhancing kynase A activity (K → AA) and thus lowers the [3-HAA]/[AA] ratio. These effects of KA have also been established from fasting baseline data in humans.[29] In patients with schizophrenia, raised [AA] has recently been reported.[127] Thus, apart from its NMDA antagonistic function, KA possesses anti-inflammatory properties, and the potential role of AA in these latter effects is worthy of investigation. The role of AA analogues and derivatives as anti-inflammatory agents is currently the subject of active investigation.

Modulation of immune function has been established for 3-HK, 3-HAA, QA, and PA. Thus, during the 1990s, many observations have demonstrated the immunomodulatory effects of the above K metabolites, namely, (1) PA acting as a second signal in the activation of IFN-γ–primed macrophages and a co-stimulus for induction of reactive nitrogen intermediate, (2) QA as an immune system signaling agent, and (3) 3-HAA as inhibitor of NO synthase expression and activity (for reference, see Badawy et al[98]). Subsequently, the immunomodulatory effects of kynurenines were extended to include (1) suppression of allogeneic T-cell proliferation by 3-HK and 3-HAA additively by an apoptotic mechanism, (2) apoptosis

undermining T-helper type 1 (Th1) cells by 3-HAA and QA shifting the Th1/Th2 balance toward Th2, and (3) prolonged survival of cerebral malaria in mice by KMO inhibition.[98]

Plasma Trp depletion caused by IFN-γ induction of IDO was originally proposed to underpin the antibacterial, antiparasitic, and antiviral effects of this cytokine[128–130] and protection against fetal rejection.[131] However, with the demonstration of the immunomodulatory effects of K metabolites, it became necessary to modify the depletion hypothesis in infection to a combination of Trp depletion and K metabolite elevation. Even so, because bacteria, fungi, and parasites can synthesize Trp and do not suffer from host Trp depletion, an alternative Trp utilization concept in infection was proposed based on immunomodulation by K metabolites and the importance of NAD^+ synthesis.[132] Regarding pregnancy, it would be a physiological disadvantage if Trp were to be depleted, as the Trp requirements in pregnancy are dramatically increased, with the need for increased protein synthesis by mother and fetus, serotonin for signaling pathways, KA for neuronal protection, QA for NAD^+ synthesis, and immunosuppressive kynurenines for fetal tolerance. A Trp utilization concept in pregnancy has been proposed satisfying these requirements.[3,133] The Trp depletion concept in pregnancy[131] did not take into consideration the elevation of plasma free [Trp] during pregnancy but relied on the decrease in total (free + albumin-bound) [Trp], which occurs as a result of increased uptake and utilization of the free fraction. As stated above, accurate interpretation of changes in Trp disposition requires measurement of both free and total plasma [Trp].[97] It seems that changes in Trp metabolism and disposition during pregnancy also occur in infections of various types, including cancer, and a hypothesis proposing future Trp-related studies in these areas of public health has been published.[98]

As the NMDA agonist, QA exerts excitotoxic effects and has been implicated in many neurological conditions, including epilepsy, Huntington disease, trauma, and hypoxia, and in inflammatory conditions, such as HIV infection (for reference, see Malherbe et al[35]). QA levels are increased in plasma, cerebrospinal fluid (CSF), and/or brain in some of these conditions,[134] with activity of 3-HAAO being vital for QA synthesis. QA levels in schizophrenia are not decreased,[135] and so, the glutamatergic hypoactivity can be attributed solely to the raised [KA]. Strategies for lowering QA synthesis involve inhibition of IDO, kynureninase, or 3-HAAO. However, this should be accompanied with provision of nicotinic acid to ensure adequate NAD^+ synthesis. Although excitotoxic, QA is a very important precursor of NAD^+, as will be described below.

Although emphasis on immunosuppression by K metabolites has focused on their ability to suppress T-cell reactivity, less emphasis is given to their effects on innate immunity and in particular cerebral microglial reactivity. It has been known for some time that 3-HK and 3-HAA possess the highest antioxidant properties among all Trp and serotonin metabolites.[136] Despite the widely reported pro-oxidant property of 3-HK and 3-HAA in peripheral blood cells and rodent neurons, an anti-inflammatory and neuroprotective role for 3-HAA in brain during inflammation has been suggested.[137] A possible explanation of these opposing views is that of species differences (rodents vs humans) and composition of cultures (pure vs mixed neuronal-glial[137]). Microglia are the major sites of innate immunity, and the above effects of 3-HAA have been shown to be mediated by induction in astrocytes and microglia of heme oxygenase 1, an enzyme of heme degradation that possesses anti-inflammatory and cytoprotective properties.[137]

Other effects of XA, QA, and PA

Some KP metabolites can influence carbohydrate metabolism in various ways. Activity of the key gluconeogenic enzyme, phosphoenol pyruvate carboxykinase, is inhibited by QA, and Trp administration to rat, but not gerbil, guinea pig, or sheep, inhibits gluconeogenesis,[100,106,138] presumably via QA in rats, as QA production from Trp in the 3 other species is poor. As the human KP resembles that of rats in its preference for QA formation, it is likely that under conditions of excessive QA production, gluconeogenesis could be inhibited in humans. It is of interest that animal models of diabetes are mainly those of rodents and pigs, but not species in which QA production is limited. Carbohydrate metabolism could also be influenced via insulin by XA and PA. Thus, XA has been known for some time to be diabetogenic through binding and thus inactivating insulin. A high plasma [XA] is associated with high insulin resistance and higher odds of having diabetes.[139] Urinary and plasma [XA] is elevated in patients with diabetes and in experimental diabetes.[140,141] In the urinary study,[140] the increased XA excretion is accompanied by that of Zn in the form of an XA-Zn complex. Zn is essential for insulin at many levels,[142] and its absorption and bioavailability are controlled by PA. As far as the author could ascertain, no information on urinary or plasma [PA] in diabetes is available. The ACMSD activity and mRNA expression are increased in experimental diabetes.[143–145] However, hepatocyte PA production is not impaired.[145] ACMSD activity is, however, greater in kidney than in liver. In hepatitis C viral infection, plasma [PA] is elevated and more so if diabetes is present.[146] Thus, whether the increased urinary excretion of Zn in diabetes is due in part to a potential defect in binding to PA in addition to complex formation with XA remains to be established.

Both PA and XA appear to be the least studied metabolites of the KP. As stated above, PA exerts immunomodulatory effects,[98] and its plasma levels are increased in hepatitis C viral infection and hepatic cirrhosis.[146] Other than the increase in CSF [PA] in cerebral malaria,[147] little is known about the status of this KP metabolite in other CNS conditions.[148] Similarly, XA is poorly studied. Other than its insulin and Zn binding, described above, and activation of the malaria gametocyte,[149]

evidence exists for its role in the brain in synaptic signaling and hence neurotransmission.[150] Clearly, more work on these 2 KP metabolites is required.

An important feature of K and its metabolites is their acting as ligands of the aryl hydrocarbon receptor (AhR), the significance of which will be described below.

Regulation of hepatic heme biosynthesis

Induction of heme oxygenase causes an initial decline in hepatic [heme], thereby removing the negative feedback repression of the rate-limiting enzyme of heme biosynthesis, 5-aminolevulinate synthase (5-ALAS). This results in enhancement of heme biosynthesis and consequently heme availability for hemoproteins under normal physiological conditions. This feedback mechanism is achieved by a small pool of heme, so-called free, unassigned, or readily exchangeable, which exists in the hepatic cytosol at a 10^{-7} M concentration.[151] TDO utilizes this pool and exhibits an exquisite response to changes in its level under various conditions involving altered heme synthesis and degradation.[151] TDO (in rat and presumably also human liver) thus serves as a sensitive marker of changes in the regulatory heme pool, and this property forms the basis of a screening test for exacerbation of hepatic porphyrias by drugs and other chemicals.[152] Thus, by using this regulatory heme pool, TDO can control heme biosynthesis at the 5-ALAS step. Although unaltered in hepatic porphyrias, 5-ALAS is induced during acute porphyric attacks with a resultant accumulation of 5-ALA and intermediates of the pathway proximal to 1 of 6 points of the genetic defect and occurrence of neurological and bodily symptoms. Disturbed Trp metabolism could also contribute to these symptoms. Thus, in experimental hepatic porphyria, the relative heme deficiency decreases the heme saturation of TDO and hence its activity, thereby causing elevation of plasma [Trp] and brain [5-HT].[153] Evidence for similar changes during acute attacks in porphyric patients includes elevation of plasma [Trp] and urinary excretion of the major serotonin metabolite 5-HIAA (for reference, see Badawy[10]). The increased serotonin synthesis and turnover may explain the gastrointestinal disturbances of the acute attacks. In porphyria, urinary excretion of K, 3-HK, KA, and XA is increased after an acute Trp load.[154] Although this resembles the excretion pattern in functional vitamin B_6 deficiency (defined as decreased availability of the PLP cofactor), it is not corrected by B_6 supplementation, and it is thought that the large excretion of K may involve KMO inhibition.[154] The 5-ALAS induction during acute attacks could deplete PLP.

Therapy of acute attacks involves intravenous administration of heme arginate or oral glucose, the mechanisms of which involve downregulating 5-ALAS transcription and its mitochondrial import posttranslationally by heme, and downregulation by glucose of the peroxisome proliferator–activated receptor gamma coactivator 1α (PGC-1α).[10] A third mechanism could involve TDO participation, based on its utilization of heme during starvation (as fasting precipitates acute attacks) and the ability of glucocorticoids to reverse the 5-ALAS induction through increased heme conjugation with newly synthesized apo-TDO.[10] Inhibition of TDO could therefore represent a novel strategy for the treatment of acute hepatic porphyrias.

Niacin synthesis and pellagra prevention

In the absence of adequate intake of niacin, its synthesis is achieved by Trp through the QA arm of the pathway. Niacin deficiency is the central feature of pellagra, usually referred to as the disease of the 3 Ds (dermatitis, diarrhea, and dementia, though more appropriately delirium). The relevant tissues (skin, gastrointestinal tract, and the nervous system) suffer most from the resultant NAD^+ deficiency because of the greater demand for this dinucleotide to satisfy their rapid cellular turnover.

Nutritional pellagra therefore occurs only if diets are deficient in both niacin and Trp. With marginal niacin, but adequate Trp, intake, clinical or subclinical pellagra can be induced by drugs interfering with 1 or more steps during the conversion of Trp to nicotinamide dinucleotides, eg, by TDO inhibition by some antibiotics and antiviral drug or kynureninase inhibition by hydrazine compounds or estrogens.[1,4] The widespread incidence of pellagra in Southern Europe during the 18th century and in the United States following the American Civil War was due to subsistence of a largely maize staple.[1,4] Although maize (and sorghum, widely used in India) are Trp-deficient, they contain sufficient amounts of niacin, but in a polysaccharide-bound form (niacytin), they cannot be hydrolyzed by mammalian digestive enzymes. It was unfortunate in Southern Europe that those who introduced it ignored the need to follow the liming process, a procedure used for millennia by the peasants of Central America in the preparation of tortillas that causes the release of niacin from niacytin.[4] The presence of high levels of leucine in maize and sorghum aggravates the pellagra by actions at a number of steps in the KP, namely, activation of TDO and ACMSD and inhibition of kynureninase and QPRT.[1,4,24] Although pellagra continues to be caused by malnutrition in certain parts of the world, it appears occasionally in developed countries in association with alcoholism, and it is noteworthy that among other effects, chronic ethanol consumption inhibits TDO activity.[2,4]

NAD^+ synthesis

The final main function of the hepatic KP is production of the all-important redox cofactor NAD^+, from which $NADP^+$ is formed by the action of NAD^+ kinase. Both oxidized dinucleotides and their reduced forms play vital roles in metabolism at multiple levels and in other cell functions and are therefore essential to life. On this basis, it could be argued that NAD^+ synthesis is the most important function of the KP. As the KP

favors the arm leading to QA formation (Figures 1 and 2), it could be concluded that NAD^+ synthesis from QA and hence Trp is quantitatively more important than that from nicotinamide or nicotinic acid. This is illustrated by the findings[44,155,156] that dietary Trp is more effective than dietary nicotinamide or nicotinic acid in elevating liver nicotinamide dinucleotides and urinary levels of N^1-methylnicotinamide. The group of Bender[1,44,156] investigated activities of the relevant enzymes and concluded that (1) incorporation of nicotinamide into the dinucleotides is limited by the activities of NMD and NMPRT, both of which are substrate saturated at normal (steady-state) levels of liver nicotinamide. (2) Although activities of the above 2 enzymes show a significant correlation with hepatic nicotinamide dinucleotide levels, this is not case with NPRT, which functions normally just below its V_{max}. (3) Quinolinate phosphoribosyltransferase, whose activity also does not correlate with liver dinucleotides, by contrast, operates at [QA] well below its K_m, thus suggesting that increased availability of QA from Trp should result in greater formation of NaMN and hence NAD^+. (4) Although liver dinucleotide levels are increased after a single large dose of nicotinamide, this is more likely to result from decreased NAD^+ catabolism, rather than increased synthesis, because both nicotinamide and its N^1-methyl metabolite inhibit the NAD^+-degrading (depleting) enzyme poly-(ADP-ribose) polymerase (PARP; EC 2.4.2.30). The significance of PARP will be discussed below.

Although QA is the quantitatively most important NAD^+ precursor, it does not accumulate in liver after Trp loading, presumably because of its rapid metabolism to NAD^+. By contrast, activated cells of the immune system accumulate relatively large amounts of QA, and it has been suggested that this is to provide the substrate for NAD^+ synthesis and the PARP reaction in response to immune-related oxidative damage.[132]

It is generally accepted that 1 mg of niacin arises from intake of 60 mg of Trp,[157,158] although this ratio can vary among individuals and by factors, including nutrients, hormones, pregnancy, drugs, and diseases, with some nutrients and hormones enhancing and others suppressing the conversion of Trp to nicotinamide.[159] The niacin status is generally determined by measuring urinary excretion of N^1-methylnicotinamide and its 2 oxidation products 2-PY and 4-PY.[52,160] The correlations between daily niacin intake and urinary excretion of 4-PY and 2-PY are comparable and more superior to those between niacin intake and N^1-methylnicotinamide excretion.[52] The deletion of TDO gene in mice, however, still allows sufficient synthesis of nicotinamide and NAD^+.[108]

Disturbances in KP Enzymes in Disease and Their Inhibition in Therapeutic Strategies

Disturbed KP functions could have clinical and therapeutic implications, and targeting various KP enzymes is now a strategy for addressing a variety of immune, cognitive, and neurodegenerative diseases[116,118,161–163] (Table 5).

The disturbance of TDO in the hepatic porphyrias suggests that its inhibition could form a therapeutic strategy to increase the availability of free heme to block 5-ALAS synthesis during acute attacks. As discussed above, current therapy with glucose may involve TDO inhibition. Similarly, accelerated Trp degradation through TDO induction by cortisol or activation by catecholamines in major depressive disorder[62] suggests that TDO inhibition may be a useful therapeutic strategy to correct the serotonin deficiency in this illness and, in fact, a large number of antidepressant drugs of various chemical structures and pharmacologic profiles also inhibit TDO.[62] TDO is constitutively expressed in some types of cancers and is capable of suppressing antitumor immune responses,[164] hence the need to develop TDO inhibitors as antitumor agents. Pantouris and Mowat[165] identified 7 TDO inhibitors among 2800 compounds from the library of the US National Cancer Institute, all of which possess antitumor activity, one of which, dihydroquercetin or taxifolin, is a specific TDO inhibitor, with the other 6 also being IDO inhibitors. A specific TDO inhibitor, LM10 (a 6-fluoroindole substituted in the 3-position by a tetrazolyl-vinyl side chain), was developed and shown[166] to reverse tumoral immune resistance in mice.

Targeting IDO has preceded that of TDO, as the former has been well established as a modulator of immune responses, and clinical trials of IDO1 inhibitors are currently in progress.[167] Controversy surrounded one such inhibitor, 1-methyltryptophan. Thus, although the D isomer is superior to the L isomer as an antitumor inhibitor in preclinical studies, it possesses little IDO inhibitory activity compared with the L isomer.[168,169] The controversy has been resolved in part by the finding that the isoenzyme IDO2 is the preferred target for inhibition by the D isomer.[170] IDO2 appears to play important roles in cellular immunity. For example, its gene deletion causes defects in immune responses and the ability of IDO1 to influence the generation of T regulatory cells, and it has been suggested that IDO2 is uniquely regulated by the AhR and involved in shaping immune tolerance in humans.[171] In contrast, the absence of IDO1 induces upregulation of IDO2, as shown in epididymis.[14] Many other IDO1 inhibitors have been developed, some of which are currently under investigation in phases 1 and 2 trials (for reference, see Frèdèrick[162]).

The discovery that K and some of its metabolites are endogenous ligands of the AhR is an important landmark in KP research. The AhR is a ligand-activated transcription factor mediating the toxicity of environmental chemicals, such as the dioxins. On binding of these chemicals to the cytosolic AhR, the AhR-ligand complex translocates to the nucleus, where it dimerizes with the AhR nuclear translocator and binds to the ligand response elements in the promoter of target genes. Activation of the AhR then elicits toxic responses, including cell damage and carcinogenesis.[172] In addition to modulating xenobiotic metabolism by activating the cytochrome P450 family, the AhR also plays an important role in the control of immune responses.[173] Here, the AhR, however, plays both a

protective and a destructive role. Thus, although endogenous ligands of the AhR, such as kynurenine, facilitate a dampening of the immune response to prevent excessive inflammation and autoimmunity, exogenous ligands act as signals to enhance inflammatory responses to infection and resistance of cancer to its own destruction.[174] In this latter scenario, the overwhelming immune response may represent a state of "pathological immunosuppression," the mechanism(s) of which is currently unclear. A potential explanation has been proposed[98] based on a possible effect of an excess of Trp. The flux of this excess Trp can lead to increased production of immunosuppressive K metabolites that can be further augmented by an upregulated IDO or TDO. Conditions favoring this possibility are met in the case of 2,3,7,8-tetrachlorodibenzo *p*-dioxin (TCDD), which has been shown to upregulate mouse IDO1 and IDO2[175] and to induce a strong elevation of rat plasma free [Trp] by a combination of NEFA elevation and liver TDO inhibition.[176] It is important to emphasize here the existence of rat and mouse strain differences in sensitivity to TCDD and the free Trp elevation.[176,177]

The Trp metabolites with the highest ligand activity for the AhR are KA, XA, and K. In HepG2 reporter cell lines, 10 μM KA activates AhR to the same extent as TCDD, and the relative activation by a similar concentration of XA is 20%.[178] By contrast, in mouse Hepa1 cells, K activates by 55% at 2 μM and by 72% at 50 μM.[179] It would appear that KA is the strongest KP metabolite ligand of the AhR. Two observations with KA further illustrate the dual (harmful and protective) role of AhR activation. Activation of the AhR by KA may allow certain tumor cells to escape immune surveillance mechanisms by secreting large amounts of IL-6,[178] whereas deletion of the mouse AhR gene increases production of KA and expression of KAT II in mouse cortex and striatum, thereby protecting the brain against excitotoxicity and oxidative stress.[180]

It should be emphasized that targeting TDO, IDO, or subsequent enzymes of the KP will limit the formation of various metabolites, which could result in niacin and NAD^+ deficiencies, unless adequate niacin intake is assured. Targeting KMO, kynureninase, or 3-HAAO has been proposed to limit the formation of QA, an excitotoxic K metabolite implicated in a variety of neurological conditions.[33,35] Various KMO inhibitors have been developed, the most potent of which is Ro61-8048.[162] This compound prolongs survival of a murine model of cerebral malaria.[181] Theoretically, KMO inhibition should result in elevation of K (and consequently KA and possibly also AA: see below) and a decrease in 3-HK and subsequent metabolites. Indeed, deletion of the KMO gene in mice increases the concentrations of K, KA, and AA and decreases those of 3-HK and QA in liver, plasma, and brain.[182] The deletion of KMO gene, however, does not influence activities of other KP enzymes or tissue [NAD^+] and causes a modest decrease in brain [QA], and Giorgini et al[182] suggest that brain QA levels may be preserved through increased formation of its precursor 3-HAA by the action of AA oxidase. Similarly, in the study of the murine model of cerebral malaria,[181] normal levels of brain QA are maintained in control mice treated with the KMO inhibitor Ro61-8048, possibly because of the strong elevation of [AA]. However, when brain [AA] is further increased by Ro61-8048 in mice infected with *Plasmodium berghei*, no additional increase in [QA] is caused by this KMO inhibitor.

Recently, KMO inhibition by Ro61-8048 has been shown to inhibit alcohol- and cocaine-seeking behavior and relapse in rat models: an effect attributed to increased cerebral KA.[183] Possible mechanisms of these KA-dependent novel effects of KMO inhibition include decreased release of glutamate and/or dopamine by antagonism at the glycine site of NMDA receptors, negative modulation of the α-7-nicotinic acetylcholine receptors, and aversion to alcohol by aldehyde dehydrogenase inhibition.[183,184] Although an increase in cerebral KA by KMO inhibition may be desirable for combating drug dependence, the opposite is true in schizophrenia, wherein decreasing the elevated KA levels requires KAT inhibition. As discussed in detail above, KAT inhibition is actively pursued as a strategy for treatment of schizophrenia.[116–120]

Kynureninase inhibition can also be a strategy to lower QA levels in neurodegenerative conditions, such as the Huntingdon and Alzheimer diseases and AIDS dementia. Various compounds with moderately potent inhibitory potencies have been developed.[162] The strongest inhibition is observed with 3-hydroxydesaminokynurenine, with K_i value of 5 nM with recombinant human kynureninase.[185] As discussed above, the kynureninase inhibitor benserazide has shown little efficacy in the treatment of schizophrenia.[121,122] Inhibitors of 3-HAAO have been developed, the most potent of which are derivatives of AA, with K_i of 0.3 to 6 nM.[162] 4-Chloro-3-hydroxyanthranilate (K_i = 6 nM)[186] has been shown to improve functional recovery and to preserve white matter after spinal cord injury in guinea pigs.[187]

The KP enzymes closer to NAD^+ synthesis have also been targeted for therapeutic purposes on the basis of a variety of observations related to the wide range of NAD^+ functions. Thus, QPRT inhibits spontaneous cell death by decreasing the overproduction of active caspase 3.[188] Another key enzyme of NAD^+ synthesis, NMPRT, possesses multiple functions in health and disease.[189] For example, the overexpression of NMPRT in a number of cancers suggests that its inhibition can form a therapeutic strategy, the basis of which involves inhibition of glycolysis at the glyceraldehyde 3-dehydrogenase step leading to ATP depletion.[190] In colorectal cancer, NMPRT overexpression is inversely related to microRNA-26b, which targets the enzyme.[191] The NMNAT protects against acute neurodegeneration in developing CNS by inhibiting excitotoxic-necrotic cell death.[192]

The KP enzymes involved in NAD^+ degradation perform equally important functions in health and disease and are therefore also targeted for therapeutic intervention. Thus, NADase secreted by Group A streptococci enhances their

Table 5. Targeting enzymes of the kynurenine pathway for therapeutic purposes.

ENZYME	IDENTIFIED CLINICAL CONDITION(S) FOR TARGETING
TDO	Cancer, major depressive disorder, porphyria
IDO	Cancer, immune diseases, neurological disorders, neurodegenerative diseases
KAT	Schizophrenia,
KMO	Schizophrenia, drug dependence, infectious diseases
Kynase	Neurodegenerative conditions
3-HAAO	Neurological disorders
QPRT	Inflammatory diseases
NMPRT	Cancer
NNMT	Cancer, diabetes, schizophrenia
NADase	Infectious diseases
PARP	Cancer, inflammatory, metabolic, and neurological disorders

Abbreviations: 3-HAAO, 3-hydroxyanthranilic acid oxygenase; IDO, indoleamine 2,3-dioxygenase; KAT, kynurenine aminotransferase; KMO, kynurenine monooxygenase or kynurenine hydroxylase; Kynase, kynureninase; NADase, NAD^+ glycohydrolase; NMPRT, nicotinamide phosphoribosyltransferase; NNMT, nicotinamide *N*-methyltransferase; QPRT, quinolinate phosphoribosyltransferase; PARP, poly-(ADP-ribose) polymerase; TDO, Trp 2,3-dioxygenase.

virulence[193] and its targeting can reduce infection.[194] Various inhibitors of intrinsic NADase exist, but their efficacy is species dependent.[195] Poly-(ADP-ribose) polymerase is another important enzyme that plays important roles in various cellular processes, including DNA repair, and targeting it offers an opportunity for therapeutic intervention in cancer and other conditions, including inflammatory, metabolic, and neurological disorders.[196–199] Evidence from preclinical studies and current clinical trials of PARP inhibitors promises a fruitful outcome of these interventions.[199]

Nicotinamide *N*-methyltransferase also plays important roles in development and disease. These include participation in regulating the epigenetic landscape during early embryonic development[200] and potential female-specific association (in Han Chinese) of its gene with schizophrenia,[201] of its mRNA with insulin resistance,[202] and of its protein expression with renal cell cancer,[203] thus rendering it a target for therapeutic intervention strategies.

General Conclusions and Comments

From the account given in this review, it is clear that the KP of Trp degradation is an important vehicle and fertile ground for basic and clinical research. The KP performs many functions, the most important of which is provision of NAD^+, and has been the source of much valuable information over the past 76 years starting with the concept of enzyme physiology and metabolic regulation in the 1950s to 1960s and reaching the current status as a major player in infectious, neurological, and neurodegenerative diseases. Through its wide range of enzymes and intermediates, it uniquely controls many important physiological processes throughout the body and provides multiple opportunities to address various disease states. The KP is currently at the forefront of biomedical research, and experience has proven that it never disappoints those who study it.

Acknowledgements

The author held an honorary professorial appointment at Cardiff Metropolitan University during the period September 2006 to September 2016.

Author Contributions

The author is the sole contributor to this article.

Disclosures and Ethics

The author has provided to the publisher signed confirmation of compliance with legal and ethical obligations including, but not limited to, the following: authorship and contributorship, conflicts of interest, privacy and confidentiality, and (where applicable) protection of human and animal research subjects. The author has read and confirmed his agreement with the ICMJE authorship and conflict of interest criteria. The author has also confirmed that this article is unique and not under consideration or published in any other publication, and that he has permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

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