

# Tryptophan metabolism in the central nervous system: medical implications

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The metabolism of the amino acid L-tryptophan is a highly regulated physiological process leading to the generation of several neuroactive compounds within the central nervous system. These include the aminergic neurotransmitter serotonin (5-hydroxytryptamine, 5-HT), products of the kynurenine pathway of tryptophan metabolism (including 3-hydroxykynurenine, 3-hydroxyanthranilic acid, quinolinic acid and kynurenic acid), the neurohormone melatonin, several neuroactive kynuramine metabolites of melatonin, and the trace amine tryptamine. The integral role of central serotonergic systems in the modulation of physiology and behaviour has been well documented since the first description of serotonergic neurons in the brain some 40 years ago. However, while the significance of the peripheral kynurenine pathway of tryptophan metabolism has also been recognised for several decades, it has only recently been appreciated that the synthesis of kynurenines within the central nervous system has important consequences for physiology and behaviour. Altered kynurenine metabolism has been implicated in the pathophysiology of conditions such as acquired immunodeficiency syndrome (AIDS)-related dementia, Huntington's disease and Alzheimer's disease. In this review we discuss the molecular mechanisms involved in regulating the metabolism of tryptophan and consider the medical implications associated with dysregulation of both serotonergic and kynurenine pathways of tryptophan metabolism.

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The essential amino acid L-tryptophan (tryptophan) is ingested as part of the diet and is required by every living cell in order to synthesise protein. Metabolism of tryptophan yields several biologically active molecules, including many important neuromodulators of central nervous system (CNS) function. The mechanisms involved in regulating tryptophan metabolism within the CNS, as well as the medical implications resulting from dysregulation of these mechanisms, are discussed in this review.

### Regulation of plasma free tryptophan concentrations

Tryptophan is the only amino acid to bind plasma albumin and exists in an equilibrium between albumin-bound and free forms in the peripheral circulation (Ref. 1). At rest, about 90% of total plasma tryptophan is bound to albumin, forming a complex that cannot cross the blood–brain barrier (BBB); the remainder circulates in a free form that is available for transport across the BBB into the brain (Ref. 2).

A major determinant of the ratio of free tryptophan to albumin-bound tryptophan is the plasma concentration of non-esterified fatty acids (NEFAs), which also bind to albumin and by doing so displace tryptophan from its binding site (Ref. 3), resulting in an elevation of free tryptophan in the plasma (Refs 4, 5). Increases in plasma NEFA concentrations can come about under many different circumstances, including upon activation of the sympathetic nervous system during sustained exercise (Ref. 6), when  $\beta$ -adrenoceptor-mediated lipolysis leads to increased release of NEFAs from adipose tissue (Ref. 7). Other exogenous factors may also displace tryptophan from albumin, as is the case for numerous drugs (albeit at pharmacological doses), including the benzodiazepines (Ref. 8) and the lipid-lowering drug clofibrate (Ref. 9).

Until recently, it was widely argued that the increase in the free pool of tryptophan following NEFA elevation was the primary determinant of the increase in brain tryptophan concentrations in response to exercise or other challenges; however, several recent findings argue against this simple interpretation (Ref. 6). While it remains a matter for debate whether plasma concentrations of total tryptophan or free tryptophan are most critical for

determining the rate of tryptophan uptake into the brain (Ref. 6), there is convincing evidence to suggest that enhanced dissociation of tryptophan from albumin within the local environment of the cerebral microvascular system is an important factor (as discussed further below) (Refs 10, 11).

### Tryptophan transport across the BBB

The transport of tryptophan across the BBB and into the extracellular fluid of the CNS plays a critical role in the regulation of brain tryptophan metabolism. For example, as tryptophan hydroxylase (TPH; the rate-limiting enzyme in the metabolism of tryptophan to serotonin) is not saturated at baseline concentrations of tryptophan (Refs 12, 13), the rate of serotonin synthesis in the brain can be either increased or decreased by altering the rate of tryptophan transport across the BBB (Refs 14, 15).

The activation of the sympathetic nervous system appears to play a significant role in the regulation of tryptophan availability to the brain. Indeed, sympathetic nervous system activation has been shown to contribute to the increases in brain tryptophan concentrations seen after stress-related and immune challenges in experimental animals (Ref. 16). Furthermore, animal studies have established that tryptophan availability to the brain, but not other organs, is increased by peripheral administration of  $\beta$ -adrenoceptor agonists (Refs 17, 18, 19, 20, 21) – specifically agonists at the  $\beta_2$ - and  $\beta_3$ -adrenoceptors (Ref. 22) – and decreased by peripheral administration of  $\beta$ -adrenoceptor antagonists (Ref. 16). The mechanisms underlying the effects of  $\beta$ -adrenoceptor agonists on brain tryptophan concentrations are not clear, but the weight of the evidence suggests that they are independent of changes in NEFAs (Ref. 23). Some studies have suggested that there might be a direct stimulatory influence of the sympathetic nervous system on the transporter mediating tryptophan uptake into the CNS (Refs 20, 21), a hypothesis consistent with the observation that capillary endothelial cells isolated from rat and human cerebral microvessels possess  $\beta_2$ -adrenoceptors (Ref. 24).

Tryptophan is transported from the blood across the BBB by a competitive transport carrier that is shared by several large neutral amino acids (LNAAs), including the aromatic

amino acids tyrosine and phenylalanine and the branched-chain amino acids leucine, isoleucine and valine, in rats (Refs 13, 25, 26) and in humans (Ref. 27). The functional properties of this transporter correspond to those of the classically characterised sodium-independent amino acid transport system L (Ref. 28). However, the L-type amino acid transporter at the BBB (LAT1; Fig. 1) has a much higher affinity (lower  $K_m$ ) for amino acids compared with L-type amino acid transport systems in peripheral tissues (Ref. 29) and is expressed at much higher levels at the BBB compared with other tissues (Ref. 30). LAT1, which spans the membrane 12 times, requires an additional single-membrane-spanning protein, the heavy chain of 4F2 cell surface antigen (4F2 heavy chain; 4F2hc; CD98), for its functional expression (Ref. 31). If the subcellular expression of the BBB LAT1 is similar to that of other amino acid transporters that have been characterised at the BBB (Ref. 32), it is likely to be expressed on both the luminal surface of the endothelial cell (transporting tryptophan into the cell) and the abluminal surface of the endothelial cell (transporting tryptophan out of the cell into the extracellular fluid of the brain). This hypothesis is supported by immunohistochemical studies (Ref. 33). Owing to the competitive nature of amino acid transport across the BBB by LAT1 and the low abundance of tryptophan relative to other LNAAs, increases in the ratio of free tryptophan to other LNAAs (at least within the

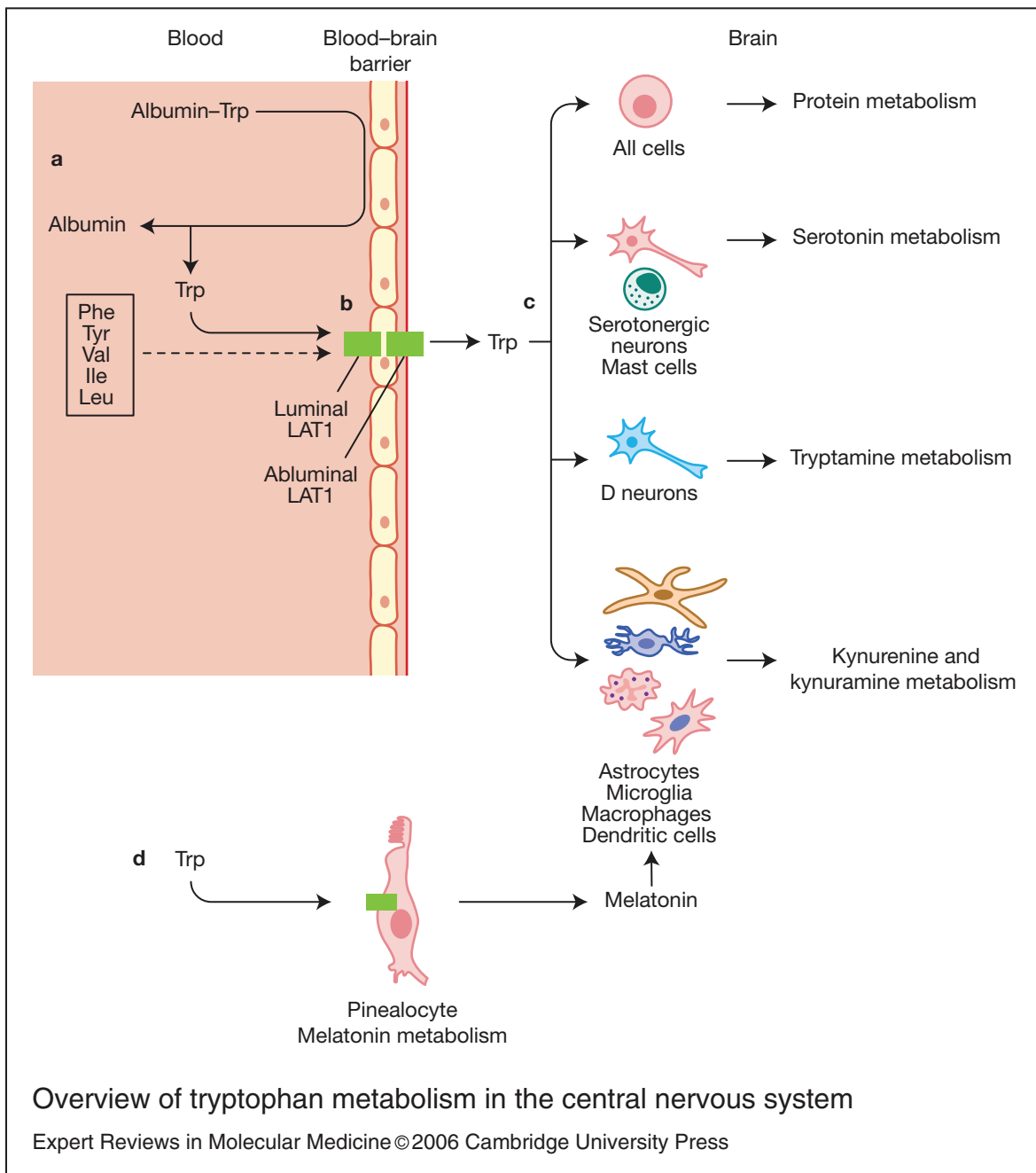
local cerebral microvasculature) increase the rate of tryptophan uptake into the brain. The reverse is also true: increases in the plasma concentrations of other LNAAs decrease the rate of tryptophan uptake into the brain (Ref. 34).

As mentioned above, one mechanism involved in controlling the availability of tryptophan for transport into the brain appears to involve enhanced dissociation of tryptophan from albumin within the cerebral microvasculature (Ref. 10). This process is dependent upon cerebral haemodynamics, with greater dissociation rates under conditions of low cerebral blood flow, possibly due to increased interactions between the albumin-bound tryptophan complex and the glycocalyx of the endothelial cell membrane (Refs 10, 35). These findings suggest that temporally dynamic or spatial differences in regional cerebral blood flow may have important consequences for rates of tryptophan uptake within specific regions of the CNS. Other mechanisms that might regulate brain tryptophan concentrations include alterations in the kinetics of tryptophan transport across the BBB, changes in brain protein metabolism, or changes in the rate of tryptophan efflux from the brain (Refs 36, 37).

#### Tryptophan uptake into cells of the CNS

Once tryptophan has crossed the BBB into the extracellular fluid or cerebrospinal fluid (CSF) of the CNS, it is freely available for uptake into cells of the CNS for utilisation in multiple

**Figure 1. Overview of tryptophan metabolism in the central nervous system.** (*Legend; see next page for figure.*) (a) The majority of tryptophan (Trp) in the periphery (approximately 90%) is bound to plasma albumin. As the albumin–tryptophan complex travels through the cerebrovasculature, the dissociation of tryptophan from albumin is facilitated, possibly by interactions between the complex and the glycocalyx of the endothelial cell membrane. This dissociation is also facilitated by a decrease in the rate of blood flow, suggesting that changes in regional cerebral blood flow could alter the local availability of tryptophan for uptake into the brain. (b) Free tryptophan is transported across the blood–brain barrier via the L-type amino acid transporter (LAT1)/4f2 heavy chain complex, at the luminal and abluminal surfaces of endothelial cells. The aromatic amino acids tryptophan, tyrosine (Tyr) and phenylalanine (Phe) compete with the branched-chain amino acids leucine (Leu), isoleucine (Ile) and valine (Val) for transport by LAT1. (c) Once in the extracellular fluid or cerebrospinal fluid in the brain, tryptophan is available for uptake into all cells for protein synthesis, and specialised cells that synthesise neuroactive metabolites of tryptophan, including serotonin (in serotonergic neurons and mast cells), tryptamine (possibly in ‘D’ neurons that express aromatic amino acid decarboxylase), and kynurenines (in astrocytes, microglia, macrophages and dendritic cells). The molecular mechanisms of high-affinity transport of tryptophan into cellular compartments within the central nervous system are not known. (d) Melatonin synthesised in the pineal gland (which is not protected by the blood–brain barrier) has ready access to the brain, where it is involved in neural signalling through G-protein-coupled receptors, but also has antioxidant effects and can serve as a precursor for synthesis of other molecules with antioxidant effects, including kynuramines.

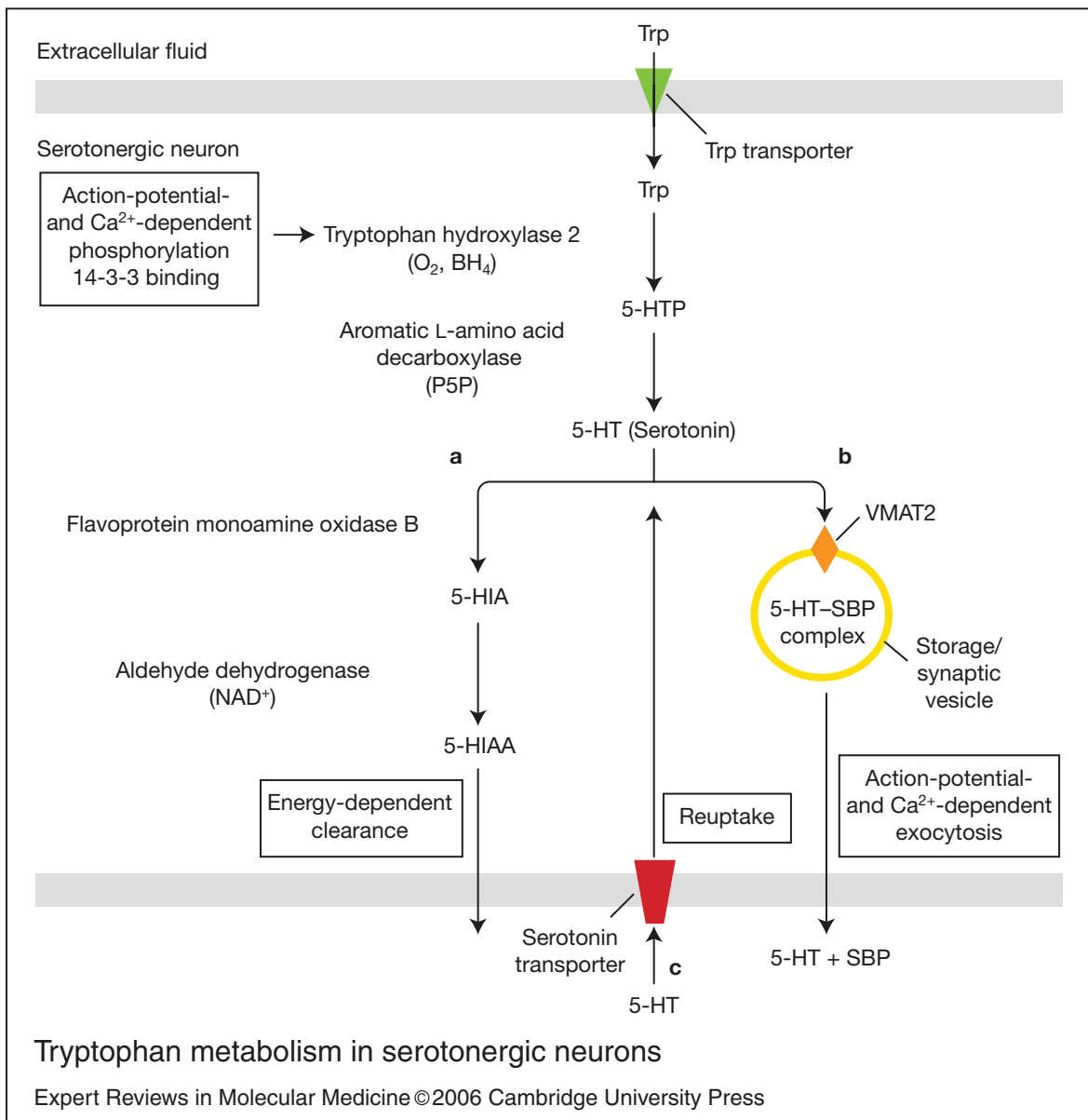


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**Figure 1. Overview of tryptophan metabolism in the central nervous system.** (See previous page for legend.)

metabolic pathways (Fig. 1). The molecular mechanisms underlying the uptake of tryptophan into different cell types within the CNS are virtually unknown as neither the purification of neutral amino acid transport systems nor the cloning of the genes coding for these transport systems has yet been reported (Ref. 38). It is also unknown if different cell

types within the CNS utilise different neutral amino acid transport systems, and whether or not different transport systems are used to deliver tryptophan to specific intracellular metabolic pathways (e.g. pathways for protein synthesis versus pathways for synthesis of serotonin in serotonergic neurons or mast cells, kynurenines in astrocytes, microglia,



**Figure 2. Tryptophan metabolism in serotonergic neurons.** Tryptophan (Trp) in the extracellular fluid is transported into the serotonergic neuron by a high-affinity neuronal tryptophan transporter. In the serotonergic neuronal cell body, the rate-limiting enzyme tryptophan hydroxylase 2 (TPH2) is located in the cytoplasm and, using molecular oxygen ( $O_2$ ) and the cofactor tetrahydrobiopterin ( $BH_4$ ) catalyses the hydroxylation of tryptophan to 5-hydroxytryptophan (5-HTP), which is rapidly converted to 5-hydroxytryptamine (5-HT, serotonin) by aromatic L-amino acid decarboxylase, using pyridoxal-5'-phosphate (P5P) as cofactor. TPH2 activity is increased by action-potential- and  $Ca^{2+}$ -dependent phosphorylation, and the phosphorylated form of TPH2 is stabilised by binding to 14-3-3 protein. Serotonin is then either: (a) metabolised by flavoprotein monoamine oxidase B (located on the mitochondrial membrane) to 5-hydroxyindole acetaldehyde (5-HIA) and then by aldehyde dehydrogenase to the acid metabolite 5-hydroxyindoleacetic acid (5-HIAA), which is excreted from the cell by an energy-dependent clearance mechanism; or (b) packaged in synaptic vesicles, via the action of vesicular monoamine transporter 2 (VMAT2), bound by the carrier protein serotonin-binding protein (SBP), and then released into the extracellular fluid by calcium-dependent exocytosis. (c) Released serotonin can be recycled within the cell following reuptake by the serotonin transporter (SERT) or can be metabolised to 5-HIAA by postsynaptic cells containing monoamine oxidase. For chemical structures of key metabolites, see Ref. 106.

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macrophages or dendritic cells, or melatonin in pinealocytes; Fig. 1).

### Serotonergic neurons

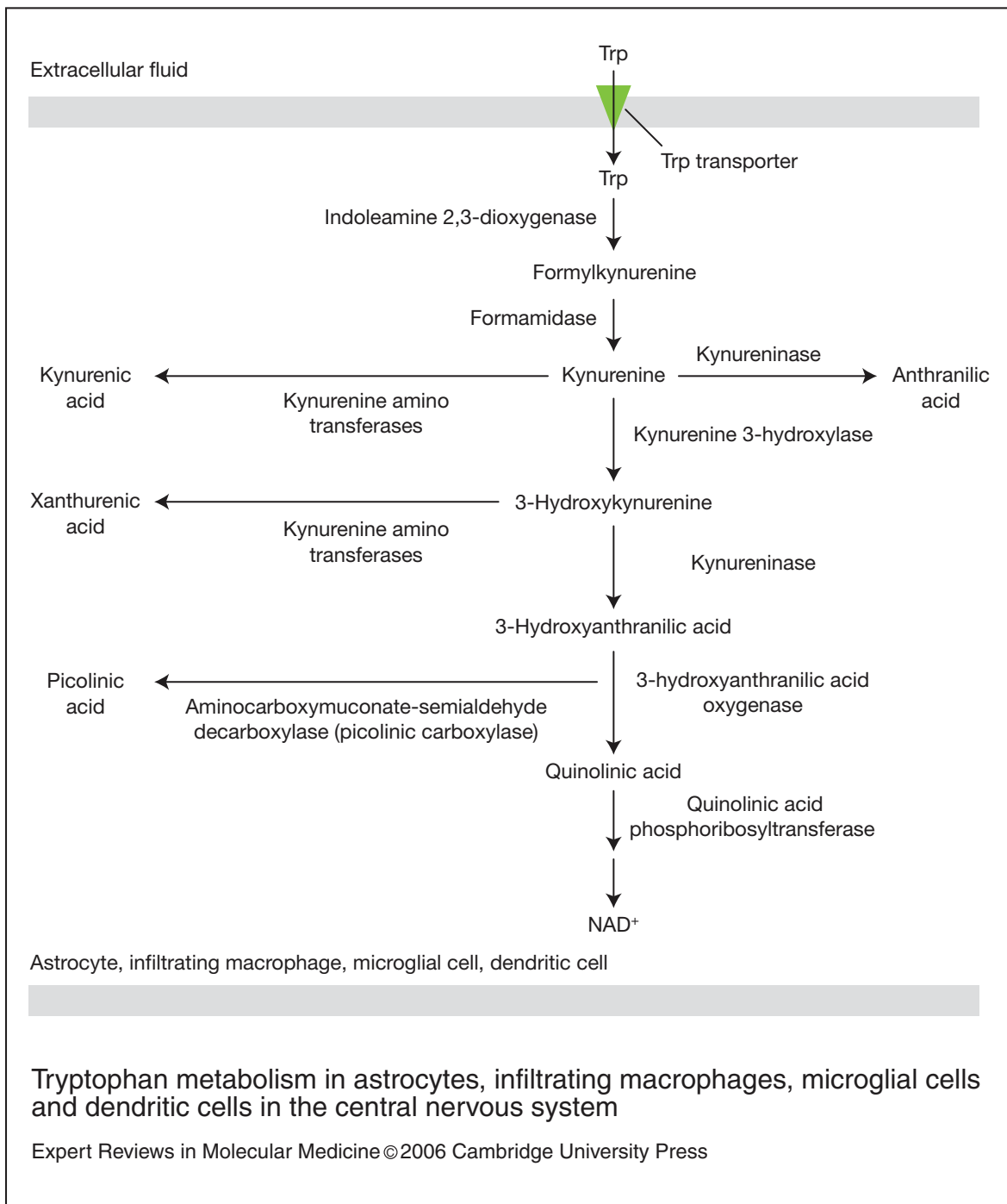
Very little is known about the mechanisms underlying tryptophan uptake into serotonergic neurons in the brain. Although there is good evidence supporting the hypothesis that a dynamic, regulatory barrier exists between extracellular brain levels of tryptophan and intraneuronal enzymatic pathways (Fig. 2), the molecular identity of the neuronal tryptophan transporter(s) in serotonergic neurons is not known. Nevertheless, two uptake processes with low ( $K_m = 1 \times 10^{-3}$  M) and high ( $K_m = 1 \times 10^{-5}$  M) affinities for tryptophan have been elucidated using synaptosomal preparations prepared from the septum, a region of the brain with high concentrations of serotonin nerve endings (Ref. 39). The high-affinity uptake system is sensitive to ion species and concentration, pH, temperature and uncouplers of energy metabolism, whereas the low-affinity system does not appear to be temperature-dependent, drug-sensitive or even stereospecific (Refs 40, 41). The high-affinity system has some specificity, as competition of radiolabelled tryptophan against equimolar amounts of unlabelled tyrosine has no effect on tryptophan conversion to serotonin (Ref. 42). Although tryptophan is accumulated by both high-affinity and low-affinity systems, it has been proposed that the high-affinity, low-capacity uptake system may maintain appropriate neuronal levels of tryptophan for serotonin synthesis whereas the low-affinity, high-capacity uptake system may subserve general metabolic functions (Ref. 43). It should also be emphasised that tryptophan uptake assays do not differentiate between uptake into serotonergic neuronal cell bodies or fibres, where tryptophan can be converted to serotonin, and uptake in other cellular compartments, where it is not. Nevertheless, tryptophan transport into brain synaptosomes is a dynamic and regulated process that varies over the circadian cycle (Ref. 44) and can be regulated by a range of endogenous and pharmacological compounds including corticosterone (Ref. 45), hydrocortisone acetate (Ref. 45), cocaine (Ref. 39) and parachlorophenylalanine (PCPA) (Ref. 40), a drug that selectively lesions serotonergic neurons.

Although we do not know which specific tryptophan transport systems are present in serotonergic neurons within the CNS, lesion studies in developing rats suggest that the uptake of tryptophan during postnatal development is not more active in serotonergic terminals than it is in other types of nerve terminals (Ref. 46). However, we cannot assume that serotonergic neurons are homogeneous in their cellular and molecular properties (Ref. 47) and therefore it remains possible that there are subpopulations of serotonergic neurons with unique tryptophan transport mechanisms. Consistent with a meaningful heterogeneity of tryptophan transport mechanisms within the CNS, both the  $V_{max}$  and  $K_m$  of high-affinity tryptophan transport vary across different brain regions (Ref. 48).

### Non-neuronal cells

In addition to serotonergic neurons, four different non-neuronal cell types within the CNS (or with access to the CNS) – including astrocytes, infiltrating macrophages/microglia, dendritic cells and mast cells – are able to synthesise neuroactive metabolites using tryptophan as a precursor. Astrocytes, infiltrating macrophages/microglia and dendritic cells synthesise multiple neuroactive metabolites via the actions of the rate-limiting synthetic enzyme indoleamine 2,3-dioxygenase (IDO) and the kynurenine pathway of tryptophan metabolism (Ref. 49) (Fig. 3). Mast cells utilise tryptophan to synthesise serotonin (Ref. 50), which may contribute to neuroimmune interactions within the brain, including modulation of the permeability of the BBB (Ref. 51). The neuroactive metabolites generated by the kynurenine pathway include 3-hydroxykynurenine and 3-hydroxyanthranilic acid, the excitotoxic *N*-methyl-D-aspartate (NMDA) receptor agonist quinolinic acid, and the NMDA and  $\alpha 7$  nicotinic acetylcholine receptor antagonist kynurenic acid.

As is the case with serotonergic neurons, we do not currently know which specific tryptophan transport systems are present in any of these cell types, but convincing evidence suggests that specific transport systems exist and that their regulation may be critical for cell function. For example, while we do not know the identity of tryptophan transport systems in mast cells, it is clear that tryptophan availability



**Figure 3. Tryptophan metabolism in astrocytes, infiltrating macrophages, microglial cells and dendritic cells in the central nervous system.** Tryptophan (Trp) in the extracellular fluid is transported into glial or immune cells by a high-affinity tryptophan transporter. The rate-limiting enzyme indoleamine 2,3-dioxygenase (IDO) catalyses the initial enzymatic step in the kynurenine pathway leading to the synthesis of a number of neuroactive compounds, including the free-radical generators 3-hydroxykynurenine and 3-hydroxyanthranilic acid, the excitotoxic *N*-methyl-*D*-aspartate (NMDA) receptor agonist and free-radical generator quinolinic acid, and the neuroprotective NMDA receptor antagonist kynurenic acid. IDO is induced by interferon  $\gamma$ , and therefore kynurenine metabolism is increased during many inflammatory conditions involving the central nervous system. For chemical structures of key metabolites, see Ref. 106.

**Table 1. Tryptophan uptake systems in pineal and central nervous system tissues**

Uptake system	Tissue	$K_m$	Species	Refs
LAT1 (light chain) and 4F2 heavy chain	Bovine blood–brain barrier	10–100 $\mu\text{M}$	Cow	30, 199
High-affinity synaptosomal Trp transport system	Brain synaptosomes (septum)	10 $\mu\text{M}$	Rat	39
ND	Neurons (cortical synaptosomes)	67 $\mu\text{M}$ (light phase) 42 $\mu\text{M}$ (dark phase)	Rat	44
System-L-related Trp transport system	Monocyte-derived macrophages	19 $\mu\text{M}$	Human	53
High-affinity Trp transport system	Monocyte-derived macrophages	230 nM	Human	53
High-affinity Trp transport system; aromatic amino acid transport system T	Dissociated pinealocytes	4 nM	Rat	55

Abbreviations: LAT1, L-type amino acid transporter 1; ND, not determined; Trp, tryptophan.

is a determinant of the proportion of serotonin, compared with histamine, released from this cell type (Ref. 50). It has been proposed that tryptophan uptake, coupled to metabolism of tryptophan, is an important factor in the immunoregulatory functions (e.g. immune tolerance; see below) of antigen-presenting cells such as macrophages or dendritic cells (Ref. 52), and a preliminary report has described a very high-affinity ( $K_m = 230$  nM) tryptophan-selective transport system in human monocyte-derived macrophages (Ref. 53).

### Pinealocytes

Some progress has been made in identifying transport systems mediating tryptophan uptake in pinealocytes within the pineal gland. Pinealocytes synthesise serotonin from tryptophan as a precursor for synthesis of melatonin. Melatonin is then released from the cell and acts as a neurohormone to affect CNS function. Unlike cells in the CNS, the pineal is not protected by the BBB; therefore, as expected, reductions in plasma tryptophan concentrations can reduce melatonin synthesis and secretion from the pineal gland (Ref. 54). Tryptophan is transported into pinealocytes by both low- and high-affinity transport mechanisms. Studies

using rat pineal gland have demonstrated that high-affinity tryptophan uptake is mediated by the aromatic amino acid transport system T (Ref. 55) (Table 1).

### ATP-binding cassette transporter G (ABCG) family members

One candidate protein for facilitation of tryptophan transport across the plasma membrane in cells of the CNS is the product of the human homologue of the *Drosophila white* gene, so named because mutations in this gene cause flies to have white eyes rather than wild-type red eyes. In *Drosophila*, the white protein is thought to facilitate the transmembrane transport of tryptophan or a tryptophan metabolite in the eye (Ref. 56). Human (*ABCG1*) and murine (*ABC8*) homologues of the *Drosophila white* gene have been cloned (Ref. 57) and, together with the closely related *ABCG4* gene, have been shown to be expressed in the brain and eye (Refs 58, 59, 60, 61). Indeed, *ABCG1* is expressed in areas of the brain associated with serotonergic function (e.g. the dorsal raphe nucleus) and in the pineal gland (Ref. 58). While there is presently no direct evidence for a role for *ABCG1* and *ABCG4* in facilitation of tryptophan transport in



mammalian systems, based on arguments of functional homology, the well-established function of the white protein in *Drosophila*, and the high expression levels of ABCG1 and ABCG4 in macrophages (Refs 62, 63), ABCG1 and related proteins are candidate proteins for a role in modulation of tryptophan transport or metabolism in mammals. Intriguingly, polymorphisms in the *ABCG1* gene (Refs 57, 64, 65) have been associated with bipolar depression and mood disorders, conditions associated with serotonergic dysfunction. Further studies are warranted to study the role of this family of proteins in tryptophan transport and metabolism in the CNS.

### Summary of tryptophan uptake

In summary, preliminary reports looking at homogeneous populations of cells (pinealocytes and monocyte-derived macrophages) with unique metabolic demands for tryptophan have demonstrated the presence of high-affinity tryptophan uptake systems with  $K_m$  values in the nanomolar range. It remains to be seen if serotonergic neurons or subpopulations of serotonergic neurons also have high-affinity uptake systems associated with their unique metabolic demands for tryptophan. Given the diversity of tryptophan uptake mechanisms in different cellular compartments (Table 1), it is possible that pharmacological approaches could be used to selectively regulate tryptophan uptake into different cellular compartments in order to regulate the metabolism of tryptophan via specific metabolic pathways. This is an exciting direction for future research, with broad medical implications.

### Serotonin biosynthesis and metabolism

Once tryptophan gains access to the cytoplasm of mast cells or serotonergic dendrites, somata, fibres or presynaptic nerve terminals, a number of highly regulated enzymes and co-factors convert tryptophan to serotonin (Fig. 2). Evidence suggests that access of tryptophan to serotonin-producing neurons in the brain is a critical step in regulation of serotonin biosynthesis (Ref. 42). Elevations in brain tryptophan concentrations lead to increased serotonin biosynthesis, and thus mechanisms controlling brain tryptophan levels (see above) contribute to the tonic regulation of serotonin synthesis in serotonergic neurons (Refs 14, 15,

66, 67). Indeed, brain tryptophan concentrations are positively and significantly correlated with brain serotonin and 5-hydroxyindoleacetic acid (5-HIAA; the major serotonin catabolite) concentrations (Ref. 66).

### Tryptophan hydroxylase

Although availability of tryptophan in the brain is rate-limiting for the synthesis of serotonin in serotonergic neurons, the rate-limiting enzyme in the synthesis of serotonin is TPH (tryptophan hydroxylase; tryptophan 5-monoxygenase; EC 1.14.16.4) (Fig. 2). Until recently, it was assumed that TPH found in both the periphery and in the CNS was derived from a single gene. However, in 2003, Walther and colleagues discovered a new, brain-specific TPH gene (*tph2*) located on the long arm of chromosome 12 (Ref. 68), a region reported to contain susceptibility genes for bipolar disorder and major depressive disorder (Refs 69, 70, 71, 72). Levels of *tph2* mRNA are elevated in the dorsal and median raphe nuclei of depressed suicide patients (Ref. 73), and, indeed, subsequent analysis of polymorphisms in the *tph2* gene has supported an association between *tph2* genotype and affective disorders (Refs 74, 75).

TPH expression is limited to a few specialised tissues: raphe neurons (Ref. 76), pinealocytes (Ref. 76), mast cells (Ref. 77), mononuclear leukocytes (Ref. 78), intestinal enterochromaffin cells (Ref. 79) and bronchopulmonary neuroendocrine epithelial cells (Refs 80, 81). Tryptophan hydroxylase 1 (TPH1) is highly expressed in the pineal and peripheral tissues, while TPH2 is highly expressed in the brain (Refs 68, 82). Transcripts of *tph1* and *tph2* have been colocalised in multiple brain regions in post mortem human brain using quantitative real-time PCR, with a ratio favouring *tph2* mRNA expression in the primary site of serotonin synthesis, the raphe nuclei (Ref. 83).

TPH1 and TPH2 are tetrameric holoenzymes that catalyse the hydroxylation of tryptophan to form 5-hydroxytryptophan (5-HTP), which is then readily converted to serotonin (5-HT) by aromatic amino acid decarboxylase (AADC; EC 4.1.1.28). Both TPH1 and TPH2 utilise molecular oxygen and the cofactor tetrahydrobiopterin to convert tryptophan to 5-HTP. The  $K_m$  value for tryptophan is reported to be 10–20  $\mu\text{M}$  for TPH1 and 40–50  $\mu\text{M}$  for TPH2 (Refs 84, 85). As tryptophan concentrations in the brain range

from 10 to 30  $\mu\text{M}$  (Ref. 86) and are reportedly as low as 2  $\mu\text{M}$  in CSF (Ref. 87), these  $K_m$  values are consistent with findings that brain TPH is unsaturated under baseline conditions (Refs 88, 89, 90). It follows, therefore, that tryptophan loading results in increases in serotonin and 5-HIAA levels in the brain (Refs 91, 92).

The activity of TPH can be modified by several factors. Stress-induced increases in TPH activity have been correlated with increased serotonin release in forebrain serotonergic projection regions as well as locally within the midbrain raphe nuclei (Ref. 93). TPH2 is regulated by action-potential- and  $\text{Ca}^{2+}$ -dependent phosphorylation (Refs 85, 94) and by an activating protein, 14-3-3, which binds to the phosphorylated enzyme, preventing dephosphorylation and deactivation (Ref. 95).

It is not possible to review here the medical implications of tryptophan metabolism in the CNS related to serotonergic neurotransmission, but serotonin has been implicated in many physiological and behavioural responses, as well as stress-related neuropsychiatric disorders including anxiety and major depression (Ref. 96). Stress, stress-related hormones and stress-related neuropeptides have effects on tryptophan availability for uptake into the brain, on the rate of tryptophan uptake in brain synaptosomal preparations, on serotonin synthesis, release and metabolism, and on postsynaptic signalling mechanisms. These complex relationships are beyond the scope of this article but are detailed in several excellent reviews (Refs 97, 98, 99). Evidence supporting a role for serotonin in depression includes: (1) lowered CSF and brain concentrations of the serotonin catabolite 5-HIAA in depressed patients and particularly in depressive suicides (Ref. 100); (2) increased susceptibility to depression associated with polymorphisms in the serotonin transporter (Ref. 101); (3) elevation of chronic serotonergic function with clinically effective antidepressants (Ref. 102); and (4) precipitation of decreased mood or depressive relapse caused by depletion of tryptophan (Ref. 103).

### **Kynurenine biosynthesis and metabolism**

In addition to the ubiquitous use of tryptophan for protein synthesis and its role in serotonin metabolism in the CNS, tryptophan is also a

precursor for the kynurenine pathway of metabolism in astrocytes, infiltrating macrophages, microglia and dendritic cells (Fig. 3). The first step in the synthesis of kynurenines is the oxidative opening of the indole ring of tryptophan, and two different haem-dependent enzymes can catalyse this reaction. The first, tryptophan 2,3-dioxygenase (TDO; tryptophan pyrrolase; EC 1.13.11.11) is almost entirely localised to hepatic cells and regulates homeostatic plasma tryptophan concentrations. The second, IDO (EC 1.13.11.17), is found largely in macrophages in the periphery but is also expressed in the CNS by astrocytes, infiltrating macrophages, microglia and dendritic cells. The expression of IDO is upregulated in response to infection and tissue inflammation (Ref. 104). Neuroactive kynurenine metabolites include the free-radical generators 3-hydroxykynurenine and 3-hydroxyanthranilic acid (Refs 105, 106), the excitotoxic NMDA receptor agonist and free-radical generator quinolinic acid (Ref. 107), and the neuroprotective NMDA and  $\alpha 7$  nicotinic acetylcholine receptor antagonist kynurenic acid (Refs 108, 109). Other products of the kynurenine pathway of tryptophan metabolism also may have important physiological functions; for example, picolinic acid appears to play an important role in modulation of immune function (Ref. 110). The expression of specific enzymes varies across different brain regions, and species and age differences have been noted in the activity of specific enzymes within the kynurenine pathway of tryptophan metabolism (and therefore may result in differences in the amounts of specific metabolites produced) (Refs 111, 112).

Given that the kynurenine pathway produces both neurotoxic (e.g. quinolinic acid) and neuroprotective (e.g. kynurenic acid) metabolites, it is necessary to consider the ratio of these products when attempting to correlate metabolite concentrations with neural actions and the pathology of neurodegenerative disease (Ref. 106).

### **Tryptophan 2,3-dioxygenase**

TDO is the rate-limiting enzyme in the kynurenine pathway of tryptophan metabolism in the periphery (Ref. 104), catalysing the oxidative cleavage of tryptophan and regulating homeostatic plasma tryptophan concentrations.

The tetrameric holoenzyme contains two haem units per tetramer, is iron- and copper-dependent, and utilises molecular oxygen in the catalytic cycle (Ref. 104). TDO expression can be induced or its activity can be increased by tryptophan and some of its analogues (including  $\alpha$ -methyltryptophan) via actions at a distinct allosteric activation site (separate from the active site for substrate binding) (Ref. 113); consequently, tryptophan loading can increase activity four- to tenfold within a period of several hours (Ref. 114). Although multiple tryptophan analogues can activate TDO, the only substrate is tryptophan. TDO is competitively inhibited by indoleamines [including tryptamine, 5-HTP and melatonin (Ref. 104)] and is subject to end-product inhibition by the reduced form of nicotinamide-adenine dinucleotide phosphate and other nicotinamide analogues (Ref. 114).

It has been estimated that in peripheral tissue, only 1% of dietary tryptophan not utilised for protein synthesis is converted to serotonin, while greater than 95% is metabolised via the kynurenine pathway (Ref. 104). Not surprisingly, plasma free tryptophan concentrations can be influenced by the activity of hepatic TDO (Ref. 115). Although the major site of expression of TDO is the liver, studies using reverse transcriptase PCR (RT-PCR) and immunohistochemical techniques have described TDO mRNA and protein expression in astrocytes within the human frontal cortex (Ref. 116), and recent studies suggest that the enzyme and its activity are upregulated in the anterior cingulate cortex of patients with schizophrenia and bipolar disorder (Ref. 117).

Although it now seems clear that peripheral TDO activity is inversely related to brain tryptophan concentrations (Ref. 118) and that brain tryptophan concentrations can be dramatically increased by inhibiting TDO activity (Ref. 119), it remains unclear how important moderate changes in peripheral tryptophan concentrations are in the aetiology and pathophysiology of major depression or other stress-related neuropsychiatric disorders. It is clear, however, that TDO expression and activity can be increased by stress-related glucocorticoid hormones via actions at both transcriptional and translational levels (Refs 120, 121). In addition, several antidepressant drugs including imipramine,

tranylcypamine (Ref. 122), lofepramine (Ref. 123), desmethylimipramine (Ref. 123), paroxetine (Ref. 124), fluoxetine (Refs 125, 126) and others (Ref. 127) have been shown to inhibit TDO activity – effects that are likely to increase plasma and brain tryptophan concentrations and to increase the rate of serotonin biosynthesis in the brain (Ref. 128). A full understanding of tryptophan metabolism in the CNS during health and disease states will require an integrative approach that also considers the expression and activity of peripheral, and potentially brain, TDO.

### Indoleamine 2,3-dioxygenase

IDO, like TDO, catalyses the oxidative cleavage of tryptophan. While TDO expression is largely restricted to hepatocytes, IDO is widely distributed in mammalian peripheral tissues as well as within glial and immune cells in the CNS (Ref. 104). Like TDO, IDO is a haem-containing glycoprotein, but it functions as a monomer (Ref. 104). IDO utilises superoxide as well as molecular oxygen in the catalytic process of the enzyme (Ref. 129) and is thought to use dihydroflavin mononucleotide (FMN<sub>H2</sub>) and tetrahydrobiopterin as cofactors (Ref. 130), but binding sites have not been identified (Ref. 104). By contrast with TDO, IDO has a broad substrate specificity and will accept several different indoleamines including D- and L-tryptophan, tryptamine, 5-HTP, serotonin and melatonin (see section on kynuramine biosynthesis and metabolism, below; Ref. 131). Furthermore, unlike TDO, IDO is not induced by glucocorticoid hormones and contains no allosteric activating site for tryptophan or tryptophan analogues (Ref. 104). IDO is, however, activated by interferon  $\gamma$  (IFN- $\gamma$ ), which is released during immunological stress (Refs 132, 133).

Because IDO is widely distributed in different cell types in brain and peripheral tissues, tryptophan metabolism via IDO activity is likely to have many different functions, dependent on the tissue type, cell type and physiological context. In the periphery, IDO is expressed in macrophages and dendritic cells (Ref. 49) but can also be induced in other cell types, such as alveolar interstitial cells of the lung following treatment of mice with lipopolysaccharide (Ref. 134), or the vascular endothelium of brain, heart, lung, spleen and

uterus following malaria infection (Ref. 135). Expression of IDO in the vascular endothelium of the brain and within the brain itself under normal or pathological conditions may have important consequences for tryptophan availability and might influence rates of serotonin synthesis. IDO is distributed evenly throughout the brain but high IDO activity has been reported in the pineal gland and choroid plexus (Ref. 136). There is also evidence that IDO expression in astrocytes and infiltrating macrophages or microglia is differentially controlled (Ref. 137). Thus, IDO expression is dynamically regulated in different cell types in the periphery and in the brain.

### Kynurenine metabolites

Kynurenine metabolites have been implicated in diverse aspects of physiology and pathophysiology (Ref. 106). For example, induction of IDO has been found to suppress T-cell proliferation, leading to immune tolerance. There are currently two main working hypotheses to explain how activation of IDO can suppress T-cell proliferation. First, it has been proposed that IDO-mediated depletion of tryptophan from the local environment deprives T cells of tryptophan needed for proliferation (Refs 138, 139). However there is also evidence that metabolites such as 3-hydroxykynurenine, 3-hydroxyanthranilic acid and quinolinic acid actively suppress the proliferation of activated T cells (Refs 140, 141, 142, 143). Indeed IDO can mediate suppression of T-cell proliferation even when tryptophan levels remain high or are deliberately supplemented (Ref. 143). These two hypotheses for the mechanisms of action of IDO-induced immunosuppression are not mutually exclusive and suggest IDO facilitates tolerance to antigens, with implications for protection of the fetus during pregnancy (Ref. 144), tumour immune escape in cancer therapy (Ref. 145), and allograft survival (Ref. 141). The current understanding of the respective contributions of local tryptophan depletion and tryptophan utilisation in the functions of IDO in the immune response has been addressed in comprehensive reviews (Refs 142, 146, 147). In addition, recent interest has focused on the possibility that IDO, through its effects on tryptophan metabolism via the kynurenine pathway, might play a role in the

aetiology and pathophysiology of depression (Ref. 148).

The main neuroactive kynurenine metabolites are quinolinic acid, 3-hydroxykynurenine, 3-hydroxyanthranilic acid and kynurenic acid (Ref. 106).

### Quinolinic acid

Quinolinic acid can selectively activate the NMDA-sensitive subpopulation of glutamate receptors (Ref. 107), allowing it not only to excite neurons but also to cause neuronal damage resulting from overstimulation of the receptor (Ref. 149) – a process that is exacerbated by activation of metabotropic glutamate receptors by endogenous glutamate (Ref. 150). A large volume of evidence has accumulated suggesting that quinolinic acid might play an important pathological role in several CNS disorders, such as acquired immunodeficiency syndrome (AIDS) dementia complex (Ref. 151), Huntington's disease (Ref. 152) and Alzheimer's disease (Refs 153, 154, 155), while IDO activity is increased in a variety of other neuroinflammatory diseases including hepatic encephalopathy, septicaemia and neurovirological disorders (Ref. 156; for reviews, see Refs 157, 158). However, quinolinic acid is selectively stored in macrophages and may be a substrate for extrahepatic nicotinamide adenosine dinucleotide (NAD<sup>+</sup>) synthesis for alleviation of neuronal damage caused during an immune response. NAD<sup>+</sup> is required in a poly(ADP-ribose) polymerisation reaction used to repair DNA damage incurred by reactive oxygen and nitrogen species during an immune response (Ref. 137). Indeed, IFN- $\gamma$ , released during immune stress, induces NAD<sup>+</sup> synthesis in macrophages with quinolinic acid as a substrate (Ref. 159).

### 3-Hydroxykynurenine

3-Hydroxykynurenine (and its metabolite 3-hydroxyanthranilic acid) are less potent toxins than quinolinic acid and the neuronal damage produced appears to be mediated by free radicals and not through glutamate receptors (Ref. 160). Elevated levels of 3-hydroxykynurenine have been reported in patients with Huntington's disease (Ref. 161), and in cell culture 3-hydroxykynurenine can induce neuronal cell death, particularly in cortical and striatal neurons (Ref. 162).

### Kynurenic acid

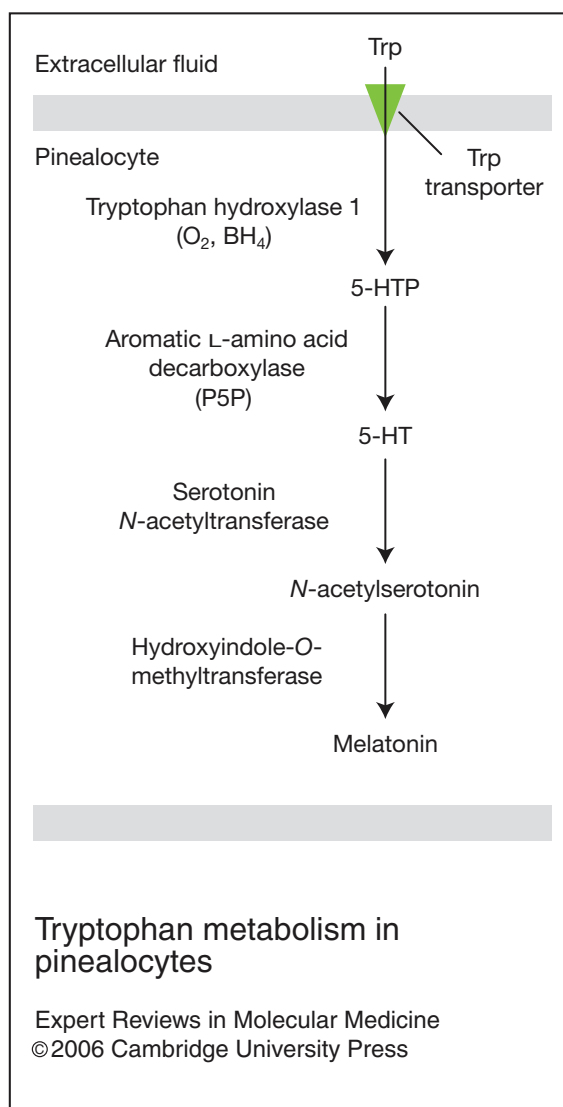
Kynurenic acid acts as an antagonist at NMDA receptors and has been found to be particularly active as an antagonist at an allosteric site on the NMDA receptor for which glycine or serine appears to be the endogenous, essential coagonist (Ref. 163). The NMDA antagonist activity of kynurenic acid is probably responsible for its ability to prevent brain damage following ischaemia in rats (Ref. 164). These properties of kynurenic acid have led to the development of several compounds with potential therapeutic value in the treatment of stroke, epilepsy and neurodegenerative disorders (Refs 49, 106, 165).

### Melatonin biosynthesis and metabolism

Melatonin is synthesised within the pineal gland and to a lesser extent in the retina (Ref. 166). Melatonin synthesis in the pineal gland varies dramatically in a circadian fashion (with high levels of synthesis and secretion during the dark phase of the circadian cycle) and is thought to be an important neuroendocrine signal conveying circadian and seasonal information to multiple organ systems, including the brain. Figure 4 illustrates the biosynthetic pathway for the synthesis of melatonin from tryptophan. The rate-limiting step in the synthesis of melatonin from serotonin is catalysed by the cytoplasmic enzyme serotonin *N*-acetyltransferase (arylalkylamine *N*-acetyltransferase, AANAT; EC 2.3.1.5), an enzyme that displays a circadian rhythm in activity, with higher activity during the dark phase of the circadian cycle (Refs 167, 168). The final step in the biosynthesis of melatonin is catalysed by hydroxyindole-*O*-methyltransferase (HIOMT), which does not show circadian variations in activity (Ref. 168). Thus, the rhythm in serotonin *N*-acetyltransferase activity regulates the circadian rhythm of melatonin synthesis. Melatonin regulates many physiological and behavioural responses including sexual maturation and reproductive behaviour, thermoregulation, sleep patterns, metabolism, haematopoiesis, immune responses, and reduction of oxidative stress (Refs 169, 170).

### Serotonin *N*-acetyltransferase

Serotonin *N*-acetyltransferase, the penultimate enzyme in melatonin synthesis, belongs to the



**Figure 4. Tryptophan metabolism in pinealocytes.** Tryptophan (Trp) in the extracellular fluid is transported into the pinealocyte by a high-affinity tryptophan transporter. In the pinealocyte, tryptophan hydroxylase 1, using molecular oxygen ( $O_2$ ) and the cofactor tetrahydrobiopterin ( $BH_4$ ), catalyses the hydroxylation of tryptophan to 5-hydroxytryptophan (5-HTP), which is rapidly converted to 5-hydroxytryptamine (5-HT, serotonin) by aromatic L-amino acid decarboxylase, using pyridoxal-5'-phosphate (P5P) as cofactor. Serotonin *N*-acetyltransferase catalyses the conversion of serotonin to *N*-acetylserotonin, which is rapidly converted to melatonin by hydroxyindole-*O*-methyltransferase. The activity of serotonin *N*-acetyltransferase is responsible for the circadian fluctuations of melatonin synthesis in the pineal gland. For chemical structures of key metabolites, see Ref. 106.

GCN5-related *N*-acetyltransferase superfamily of acetyltransferases (Ref. 171). The mechanisms underlying the rhythmic expression of serotonin *N*-acetyltransferase have been extensively studied (Refs 172, 173). These studies highlight an important role for the adrenergic induction of the cyclic AMP pathway in regulation of enzyme activity, as well as post-translational control of enzyme activity as proteolysis is responsible for the rapid decrease in serotonin *N*-acetyltransferase activity following sudden exposure to light during the dark phase (Ref. 174). As described above for TPH, serotonin *N*-acetyltransferase binds to 14-3-3 protein in a phosphorylation-dependent manner, which decreases the  $K_m$  for substrate binding (Ref. 175) and is believed to protect the enzyme from proteolysis (Refs 174, 176).

The cDNA for serotonin *N*-acetyltransferase has been cloned (Ref. 177). Unexpectedly, in addition to high expression in the pineal gland and retina, serotonin *N*-acetyltransferase mRNA was found in the pituitary gland and also in several brain regions (Ref. 177). Although the function of serotonin *N*-acetyltransferase in extra-pineal tissues including the brain is not certain, recent studies have shown that the expression of serotonin *N*-acetyltransferase mRNA in brain can be upregulated by chronic cocaine or antidepressant treatment (Ref. 178).

### **Kynuramine (kynurenamine) biosynthesis and metabolism**

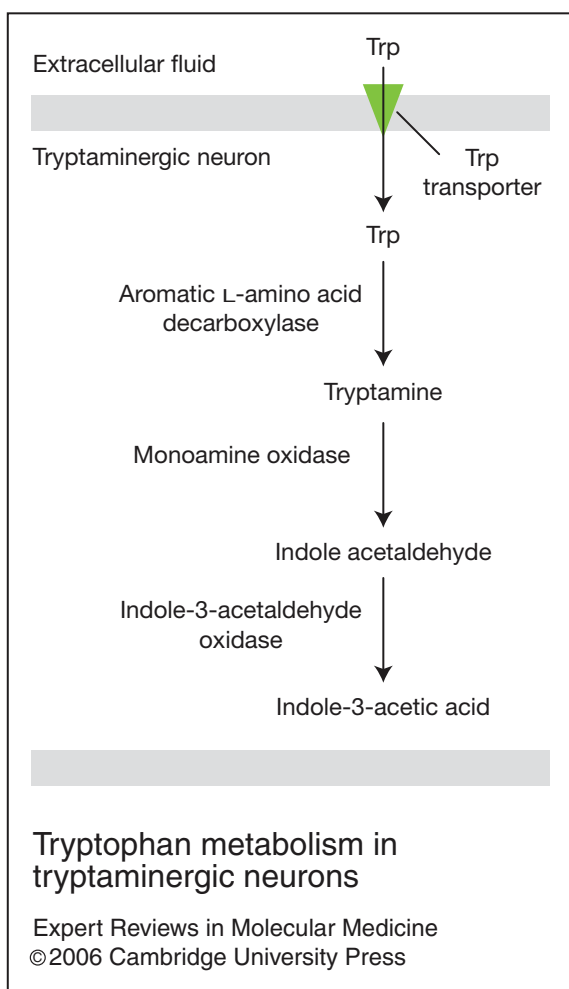
Although melatonin itself is known to signal within the CNS via actions on high-affinity melatonin 1 (MT1) and melatonin 2 (MT2) G-protein-coupled receptors (Ref. 179), it has also been proposed that kynuramine metabolites of melatonin [also called kynurenamines – not to be confused with kynurenines (Ref. 180)], may also have important physiological functions. Kynuramine metabolites of melatonin include *N*-formyl-*N*-acetyl-5-methoxy kynuramine (AFMK; *N*-formyl-*N*-acetyl-5-methoxy kynurenamine) formed in the brain by the action of IDO, the same enzyme responsible for the conversion of tryptophan to formylkynurenine in the kynurenine pathway of tryptophan metabolism (Ref. 104), and *N*-acetyl-5-methoxy kynuramine (AMK; *N*-acetyl-5-methoxy kynurenamine) formed in the brain by the action of

formamidase (EC 3.5.1.49), the same enzyme responsible for conversion of formylkynurenine to kynurenine in the kynurenine pathway of tryptophan metabolism (Ref. 104). Peripheral administration of melatonin leads to increases in AFMK concentrations in retina, plasma and CSF in rats (Ref. 181). Proposed functions of AMK and AFMK include inhibition of prostaglandin synthesis (Ref. 182), regulation of cytokine release from neutrophils (Ref. 183), and scavenging of free radicals (Ref. 184). Physiological and behavioural effects of other kynuramines [potentially derived from kynurenine, tryptamine (see below) or serotonin (Refs 106, 180)], including kynuramine and 5-hydroxykynuramine, have been studied (Refs 180, 185) but little is known about the physiological or behavioural significance of these endogenous kynuramines. The finding that concentrations of the melatonin metabolite AFMK are elevated in the CSF of viral meningitis patients and are correlated with pro-inflammatory cytokines has led to the hypothesis that inflammation favours the formation of AFMK and that this compound has immunomodulatory activity in vivo (Ref. 186).

### **Trace amine biosynthesis and metabolism**

Trace amines are endogenous amine compounds structurally related to the classical monoamine neurotransmitters. Trace amines include  $\beta$ -phenylethylamine, *p*-tyramine, *m*-tyramine, *m*-octopamine, *p*-octopamine and tryptamine (Refs 187, 188, 189). Although the presence of trace amines in the vertebrate CNS was well documented during the 1970s and 1980s (for references, see Ref. 188) they were often considered metabolic by-products. The recent identification of a novel family of G-protein-coupled receptors that includes members with high affinity for specific trace amines (Ref. 189) has led to a resurgence of interest in these compounds and the possibility that they might play important physiological or pathophysiological roles (Ref. 188).

For the purposes of this review, we focus on the decarboxylation of tryptophan by AADC to form tryptamine (Ref. 189). Tryptamine in turn is converted to the secondary amine *N*-methyltryptamine, or inactivated by monoamine oxidase, which converts tryptamine



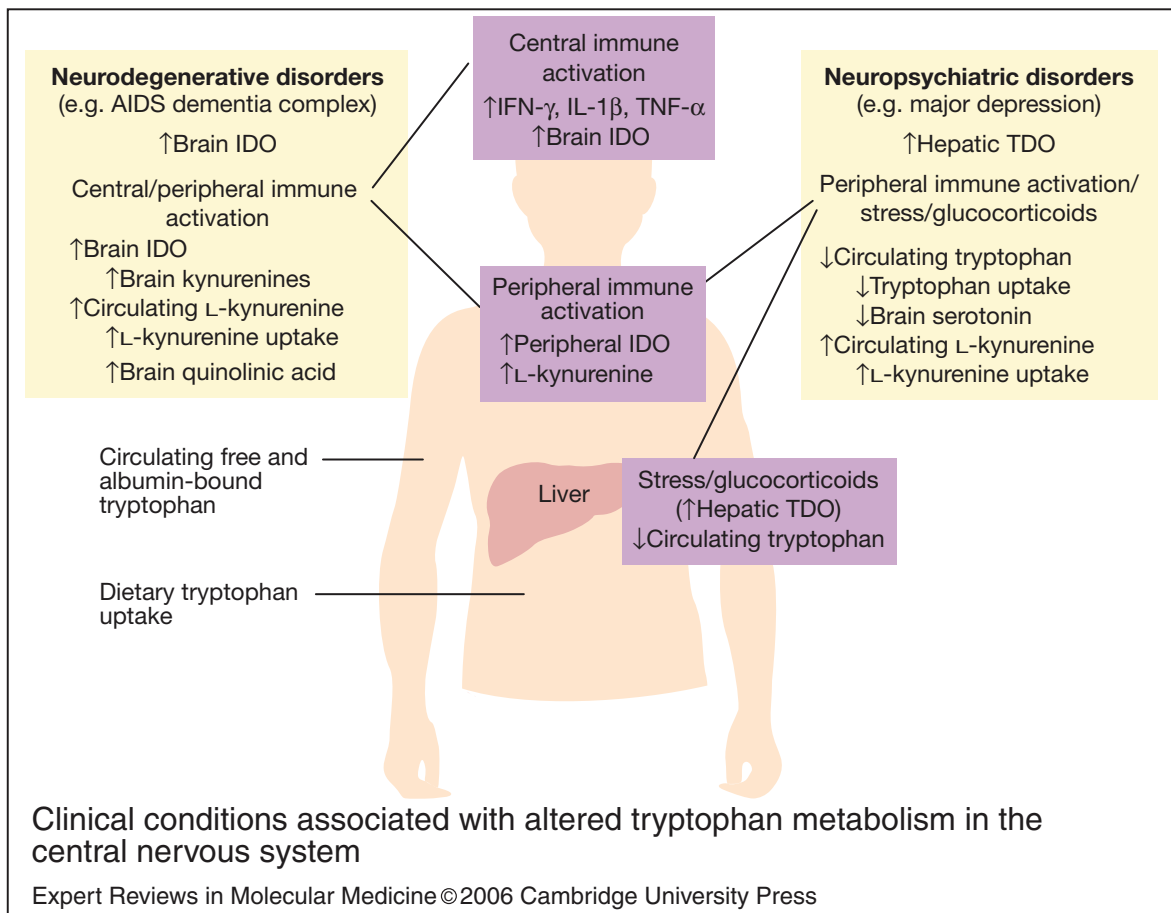
**Figure 5. Hypothetical model of tryptophan metabolism in tryptaminergic neurons.** Tryptophan (Trp) in the extracellular fluid is transported into the tryptaminergic neuron by a high-affinity neuronal tryptophan transporter. In tryptaminergic cells, aromatic L-amino acid decarboxylase (AADC) catalyses the decarboxylation of tryptophan to form tryptamine, in the absence of either tryptophan hydroxylase or tyrosine hydroxylase. This reaction may occur in 'AADC-only' neurons, also called 'D' neurons, that have been described in specific cell clusters in brain. Tryptamine is metabolised by monoamine oxidase and indole-3-acetaldehyde oxidase to the acid metabolite indole-3-acetic acid, which is excreted from the cell. For chemical structures of key metabolites, see Ref. 106.

to the acid metabolite indoleacetic acid (Fig. 5). Immunohistochemical studies suggest that tryptamine-containing cell bodies are present around the aminergic cell groups within the interpeduncular nucleus, substantia nigra, and

rostral dorsal raphe nucleus (Ref. 190). This distribution overlaps with the distribution of the 'D4' group of AADC-only neurons in the rat brain (Ref. 191) – neurons containing AADC that are neither catecholaminergic nor serotonergic and therefore are ideal candidates for neurons that synthesise trace amines. Tryptamine has a unique distribution of binding sites in brain (Ref. 192) and activates the recently cloned and characterised trace-amine-associated receptors 1 (TAAR1) and 4 (TAAR4), albeit at micromolar concentrations. Electrophysiological studies have demonstrated that tryptamine modulates cortical serotonergic neurotransmission (Ref. 193), and a general role of trace amines in modulation of monoaminergic neurotransmission has been proposed (Ref. 188). Although it has been hypothesised that trace amines may play an important role in major depression, schizophrenia or other neuropsychiatric disorders (Refs 189, 194), full characterisation of the physiological or pathophysiological functions of tryptamine and other trace amines awaits identification of high-affinity receptors and development of selective antagonists.

### Summary

Tryptophan is an essential amino acid that is ingested as part of the diet. Diverse mechanisms contribute to the regulation of tryptophan availability within the CNS. An important factor is the rate of tryptophan transport across the BBB, determined by plasma concentrations of free tryptophan and the ratio of tryptophan to other LNAAs, which in turn are determined by (1) peripheral TDO activity, (2) regulation of the affinity of tryptophan for circulating albumin (for example by NEFAs or by interactions between the tryptophan–albumin complex and the glycocalyx of the endothelial cell membrane), and (3) sympathetic nervous system activity. Tryptophan in the extracellular fluid or CSF in the CNS is available for cellular uptake by multiple cell types with unique metabolic demands for tryptophan, including serotonergic neurons, mast cells, astrocytes, microglia, macrophages and dendritic cells, emphasising the need to understand the molecular mechanisms underlying cellular uptake of tryptophan by different cell types. The diversity of metabolites of tryptophan in the CNS, including serotonin, products of the kynurenine



**Figure 6. Clinical conditions associated with altered tryptophan metabolism in the central nervous system.** Diverse neurological and neuropsychiatric conditions have been associated with altered tryptophan metabolism in the central nervous system (CNS) (for reviews see Refs 49, 105, 106, 157, 158, 200, 201). Neurodegenerative disorders involving central immune activation, including infections of the CNS (e.g. AIDS dementia complex, meningitis), autoimmune disorders (e.g. multiple sclerosis, systemic lupus erythematosus), and other inflammatory conditions (e.g. Lyme disease, malaria), are associated with activation of brain indoleamine 2,3-dioxygenase (IDO) and increases in brain concentrations of kynurenines as secondary mediators of dysfunctional states. Increased brain IDO activity is due to actions of interferon  $\gamma$  (IFN- $\gamma$ ), acting in concert with other immune signalling molecules including interleukin 1 $\beta$  (IL-1 $\beta$ ) and tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ). By contrast, in stress-related neuropsychiatric disorders, glucocorticoid hormones increase transcription of tryptophan 2,3-dioxygenase (TDO) and peripheral degradation of tryptophan via the kynurenine pathway, limiting tryptophan availability for serotonin synthesis. Complex interactions between kynurenine and serotonin pathways of tryptophan metabolism are likely in many conditions. For example, L-kynurenine competes with tryptophan and other large neutral amino acids for transport across the blood–brain barrier (Ref. 202). Increased L-kynurenine from peripheral sources may contribute to the formation of brain quinolinic acid in the presence of central immune activation (left). In contrast, increased L-kynurenine from peripheral sources, by competing with tryptophan for uptake into the brain, may further limit tryptophan availability for serotonin synthesis in neuropsychiatric disorders (right). Consequently, concurrent activation of IDO and TDO by peripheral immune activation and stress, respectively, may have additive effects, decreasing tryptophan availability, serotonin synthesis, and serotonergic neurotransmission, as has been described for example in patients with major depression (see text). This effect may be reversed by antidepressants that inhibit TDO activity and serotonin reuptake, increasing tryptophan availability and serotonergic neurotransmission (see text), while subsequent acute tryptophan depletion results in a transient recurrence of depressive symptoms in patients who had responded to treatment with selective serotonin reuptake inhibitors (Ref. 103). It remains uncertain if the effects of acute tryptophan depletion in neuropsychiatric disorders are a result of changes in serotonergic neurotransmission, changes in signalling by other tryptophan metabolites, or both.



pathway of tryptophan metabolism (including 3-hydroxykynurenine, 3-hydroxyanthranilic acid, quinolinic acid and kynurenic acid), the neurohormone melatonin, several neuroactive kynuramine metabolites of melatonin, and the trace amine tryptamine, points towards a need for integrative approaches to understand the functional consequences of tryptophan metabolism in the CNS.

### Clinical implications and future directions

Tryptophan depletion has been shown to result in a rapid induction of depressive symptoms in patients that have been treated with selective serotonin-reuptake inhibitors (Refs 195, 196). Although these results and others (Ref. 103) have been interpreted as evidence for a role for serotonergic systems in the regulation of mood, tryptophan depletion has the potential to simultaneously alter tryptophan metabolism within the serotonin, kynurenine, melatonin and trace amine pathways of metabolism. The clinical significance of selectively altering tryptophan metabolism within each of these metabolic pathways or shuttling of tryptophan into the kynurenic acid versus the quinolinic acid pathway of metabolism (e.g. by inhibiting kynureninase and kynurenine 3-hydroxylase), effectively altering the ratio between neuroprotective and neurotoxic kynurenine metabolites, has immense and obvious clinical significance (Ref. 106) (Fig. 6). Given the diversity of bioactive metabolites of tryptophan derived from different metabolic pathways in the CNS, caution should be exercised in the use of tryptophan loading, particularly during inflammatory disease when IDO expression is often upregulated, or in subjects in whom the induction of oxidative stress would be contra-indicated (Ref. 197).

Evidence is accumulating that there is anatomical, physiological and functional diversity among subpopulations of serotonergic neurons (Refs 47, 198). It will be important in future studies to determine the cellular mechanisms regulating tryptophan uptake into specific cellular compartments, including not only subpopulations of serotonergic neurons but also astrocytes, infiltrating macrophages, microglia, dendritic cells and mast cells. Identification and characterisation of the molecular mechanisms underlying high-affinity tryptophan uptake into different cell types

within the CNS might lead to novel therapeutic strategies based on specific regulation of different metabolic pathways for tryptophan. It will also be important to determine how stress interacts with tryptophan metabolism at the level of cellular uptake as well as intraneuronal trafficking and compartmentalisation of tryptophan and enzyme activity.

Further studies of associations between genetic polymorphisms in genes regulating different aspects of tryptophan metabolism – for example, *TPH2*, *TDO*, and *ABCG* transport genes – and specific neuropsychiatric disorders should lead to important advances in our understanding of the medical implications of altered tryptophan metabolism in the CNS. Advances in imaging technology and the application of this technology to preclinical models should aid in determining the distribution of specific receptors for neuroactive tryptophan metabolites, while development of specific receptor antagonists will be critical in defining their function. Given the diversity of neuroactive signalling molecules derived from tryptophan, and the broad substrate specificity of IDO, integrative approaches will be required to understand interactions among different pathways of tryptophan metabolism in health and disease states.

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### Note added in proof

Wang and colleagues have reported that kynurenic acid is a ligand for the orphan G-protein-coupled receptor GPR35, adding another dimension to the complexity of signalling mechanisms for metabolic intermediates of the kynurenine pathway (Ref. 203).

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## Further reading, resources and contacts

### Resources

Information about tryptophan and tryptophan metabolites:

<http://en.wikipedia.org/wiki/5-HTP>  
<http://en.wikipedia.org/wiki/Melatonin>  
<http://en.wikipedia.org/wiki/Niacin>  
<http://en.wikipedia.org/wiki/Serotonin>  
<http://en.wikipedia.org/wiki/Tryptamines>  
<http://en.wikipedia.org/wiki/Tryptophan>

Health information on tryptophan from the United States National Library of Medicine and the National Institutes of Health:

<http://www.nlm.nih.gov/medlineplus/druginfo/uspdi/500179.html>

OMIM (Online Mendelian Inheritance in Man) database of human genes and genetic disorders:

<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=omim>

Reference pathway for tryptophan metabolism from Kyoto Encyclopedia of Genes and Genomes:

<http://www.genome.jp/kegg/pathway/map/map00380.html>

Reference pathway for nicotinic acid and nicotinamide metabolism from Kyoto Encyclopedia of Genes and Genomes:

<http://www.genome.jp/kegg/pathway/map/map00760.html>

United States National Library of Medicine and the National Institutes of Health including access to PubMed (biomedical journal literature):

<http://www.nlm.nih.gov/>

ExpASY Proteomics Server. The ExpASY (Expert Protein Analysis System) proteomics server of the Swiss Institute of Bioinformatics (SIB) is dedicated to the analysis of protein sequences and structures, and includes an enzyme nomenclature database with a search engine for EC (Enzyme Commission) numbers:

<http://ca.expasy.org/>

Related tools and databases:

Biochemical Pathways – interactive access to Roche Applied Science ‘Biochemical Pathways’:

<http://www.expasy.org/tools/pathways/>

BRENDA – comprehensive enzyme information system:

<http://www.brenda.uni-koeln.de/>

KEGG – Kyoto Encyclopedia of Genes and Genomes:

<http://www.genome.ad.jp/kegg/>

MetaCyc – Metabolic Encyclopedia of enzymes and metabolic pathways:

<http://www.metacyc.org/>

IUBMB enzyme nomenclature:

<http://www.chem.qmul.ac.uk/iubmb/enzyme/>

Enzymes and Metabolic Pathways (EMP) database:

<http://www.empproject.com/>

Authors’ institute URLs: <http://www.bris.ac.uk/Depts/URCN/labs/lowry.html>;  
<http://www.bristol.ac.uk/neuroscience>

### Features associated with this article

#### Figures

Figure 1. Overview of tryptophan metabolism in the central nervous system.

Figure 2. Tryptophan metabolism in serotonergic neurons.

Figure 3. Tryptophan metabolism in astrocytes, infiltrating macrophages, microglial cells and dendritic cells in the central nervous system.

Figure 4. Tryptophan metabolism in pinealocytes.

Figure 5. Hypothetical model of tryptophan metabolism in tryptaminergic neurons.

Figure 6. Clinical conditions associated with altered tryptophan metabolism in the central nervous system.

#### Table

Table 1. Tryptophan uptake systems in pineal and central nervous system tissues.

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