The kynurenine pathway and neurodegenerative disease

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Abstract

Neuroactive metabolites of the kynurenine pathway (KP) of tryptophan degradation have been closely linked to the pathogenesis of several neurodegenerative diseases. Tryptophan is an essential amino acid required for protein synthesis, and in higher eukaryotes is also converted into the key neurotransmitters serotonin and tryptamine. However, in mammals >95% of tryptophan is metabolized through the KP, ultimately leading to the production of nicotinamide adenosine dinucleotide (NAD^+). A number of the pathway metabolites are neuroactive; e.g. can modulate activity of several glutamate receptors and generate/scavenge free radicals. Imbalances in absolute and relative levels of KP metabolites have been strongly associated with neurodegenerative disorders including Huntington's, Alzheimer's, and Parkinson's diseases. The KP has also been implicated in the pathogenesis of other brain disorders (e.g. schizophrenia, bipolar disorder), as well as several cancers and autoimmune disorders such as HIV. Pharmacological and genetic manipulation of the KP has been shown to ameliorate neurodegenerative phenotypes in a number of model organisms, suggesting that it could prove to be a viable target for the treatment of such diseases. Here, we provide an overview of the KP, its role in neurodegeneration and the current strategies for therapeutic targeting of the pathway.

Abbreviations: 3-HANA, 3-hydroxyanthranilic acid; 3-HAO, hydroxyanthranilate 3,4- dioxygenase; 3-HK, 3-hydroxykynurenine; α7nACh, α7-nicotinic acetylcholine; Aß, ß-amyloid peptide; ACMS, 2-amino-3-carboxymuconic 6-semialdehyde; AD, Alzheimer's Disease; aSyn, α –synuclein; BBB, blood-brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; FAD, flavin adenine dinucleotide; GAS, INF-γ activated site; GST, glutathione S-transferase; HD, Huntington's disease; HDAC, histone deacetylase; *HTT*, huntingtin gene; HTT, Huntingtin protein; IDO, indoleamine-2,3-dioxygenase; INF-γ Interferon-γ; IL, interleukin; IL-1ß, Interleukin-1 beta; iNOS, inducible nitric oxide synthase; KAT, kynurenine aminotransferase; KMO, kynurenine monooxygenase; KP, kynurenine pathway; KYNA, kynurenic acid; KYNU, kynureninase; L-KYN, L-kynurenine; LPS, lipopolysaccharide; L-TRP, L-tryptophan; mHTT, mutant HTT; NAD+, nicotinamide adenosine dinucleotide; NMDA, N-methyl-D-aspartate; nNOS, neuronal nitric oxide synthase; OGT, Oren-gedoku-to; PD, Parkinson's disease; QUIN, quinolinic acid; QPRT, quinolinate phosphoribosyltransferase; TDO tryptophan 2,3-dioxygenase; TNFα, tumor necrosis factor α; ROS, reactive oxygen species; SOD, superoxide dismutase.

1. Introduction

In mammals, the kynurenine pathway (KP) is initiated by the oxidative cleavage of the indole-ring of L-tryptophan (L-TRP) by either one of two indoleamine-2,3-dioxygenases (IDO1, IDO2) or tryptophan 2,3-dioxygenase (TDO2) to produce *N*-formylkynurenine (see **Figure 1**). This is followed by the synthesis of the first stable molecule of the pathway, L-kynurenine (L-KYN). The subsequent metabolism of L-KYN occurs via one of three mechanisms: 1) deamination of L-KYN by the kynurenine aminotransferase (KAT) family of enzymes results in the production of kynurenic acid (KYNA); 2) degradation of L-KYN by kynureninase results in the production of anthranilic acid; or 3) hydroxylation of L-KYN by kynurenine monooxygenase (KMO) produces 3-hydroxykynurenine (3-HK). 3-HK is subsequently converted into 3-hydroxyanthranilic acid (3- HANA) by kynureninase (KYNU), and then oxidized by 3-hydroxyanthranilate 3,4-dioxygenase (3-HAO) into 2-amino-3-carboxymuconic 6-semialdehyde (ACMS). Under physiological conditions, this intermediate spontaneously reassembles to form quinolinic acid (QUIN), which is subsequently transaminated by quinolinate phosphoribosyltransferase (QPRT) to generate nicotinic acid, and ultimately NAD^+. QPRT levels in the brain are low [1], thereby limiting the rate of NAD^+ production. The metabolite ACMS can also be metabolized to produce picolinic acid via the activity of 2-amino-3-carboxymuconic-6-semialdehyde decarboxylase [2].

2. Neuroactive kynurenine metabolites

KP metabolites show diverse properties that can cause contrasting effects in neurons. The arm of L-KYN metabolism catalyzed by KMO produces the metabolites 3-HK, 3-HANA and QUIN, all of which are neurotoxic. QUIN selectively activates N-methyl-D-aspartate (NMDA) receptors [3] and was first identified as a potential neurotoxin when intracerebroventricular injection in mice caused strong convulsions [4]. Subsequent work found that striatal injection of QUIN into rodent brains was excitotoxic, causing axon-sparing lesions of dose-dependent size, proximal to the site of injection [5], which could be rescued by co-administration of a selective NMDA receptor antagonist [6]. QUIN levels marginally above physiological levels are also sufficient to cause rapid neurodegeneration in rat corticostriatal cell culture [7]. The highly efficacious nature of QUIN is likely due to the number of mechanisms through which this neurotoxin can cause neuronal insult. Not only does QUIN stimulate neuronal release of glutamate, it also inhibits the astroglial reuptake of this neurotransmitter [8] and reduces the activity of glutamine synthetase [9] - which facilitates glutamine production from glutamate and ammonia. High concentrations of extracellular glutamate and persistent activation of excitatory neurons causes excitotoxicity due to augmented Ca^{2+} influx through the ion-channel complex, leading to mitochondrial dysfunction, cytochrome C release, the activation of proteases and caspases, as well as NOS activation[10].

QUIN promotes lipid peroxidation [11] in an NMDA receptor [12] and iron (II) [13] dependent manner. Furthermore, QUIN-iron complexes produce reactive

oxygen species (ROS) upon auto-oxidation [14]. In cultured human neurons and astrocytes, treatment with QUIN results in a dose-dependent increase in the activity of inducible and neuronal nitric oxide synthase (iNOS and nNOS respectively), leading to increased cellular toxicity, depletion of NAD^+ and activation of the NAD^+ dependent nuclear DNA repair enzyme PARP-1 [15]. Inhibition of iNOS and nNOS is sufficient to rescue all of these effects, indicating that nitric oxide production likely plays a causative role in QUIN excitotoxicity. QUIN-induced increases in both iNOS and nNOS are accompanied by lipid peroxidation and neuronal excitotoxicity in rat brains, both of which are prevented by inhibiting both forms of NOS [16] Furthermore the antioxidants melatonin [17], alpha-phenyl-t-butyl nitrone and U-83826E [18] are able to reduce QUIN-induced cell death in rat striatal neurons *in vivo* and *in vitro.*

The neuroactive KP metabolite 3-HK is synthesized by KMO and generates free-radicals by oxidizing interacting molecules [19] [20]. Indeed, 3-HK treatment of cultured striatal and cortical neurons results in reduced viability, shrunken, irregular somata and reduced neuritic outgrowths, which is prevented by the antioxidant catalase, but not superoxide dismutase (SOD) [21] [22] [23]. Notably, intrastriatal co-injection of 3-HK with QUIN potentiates excitotoxic neuronal lesions [24]. The formation of these lesions can be blocked via either NMDA receptor inhibition or scavenging of free radicals using N-tert-butyl-a-(2-sulphophenyl)-nitrone. Synthesized further downstream in the pathway, the metabolite 3-HANA readily auto-oxidizes and consequently generates highly reactive species such as hydrogen peroxide and hydroxyl radicals [25] [26], which is enhanced by SOD but abolished by catalase [26] [23]. These data suggest that 3-HK and 3-HANA mediated neurotoxicity is caused by ROS, which can be specifically counteracted by catalase activity. It has also been reported, however, that both 3-HK and 3-HANA are also capable of antioxidant activity [27] [28]. Notably, 3-HK dependent toxicity is observed in neuronal cell cultures [22] [23], but not in cultured glioma cells [28]. As the KP takes place predominantly in microglia and astrocytes in the central nervous system (CNS), these data may indicate that glial cells are more likely to tolerate the presence of 3-HK than neurons, where 3-HK is not endogenously produced [29]. Interestingly, it has been observed via both *in vitro* and *in vivo* approaches that at high concentrations (50-100 μM), 3-HK was in fact protective against known neurotoxins in the rat striatum, likely due stimulation of the antioxidant enzymes glutathione S-transferase (GST) and SOD [29].

KYNA, on the other hand, possesses antioxidant properties due to its ability to scavenge free radicals such as hydroxyls and superoxide anions [30]. At physiological levels, it also acts as a non-competitive antagonist of α7-nicotinic acetylcholine (α7nACh) receptors, subsequently reducing acetylcholine, dopamine and glutamate signaling [31]. At high micromolar concentrations, KYNA is a non-selective antagonist of NMDA receptors [32]. Through this modulation of glutamate signaling and antioxidant activity, KYNA is likely able to counteract the neurotoxicity conveyed by QUIN, 3-HK, and 3-HANA. During normal physiological conditions, the relative flux through the two arms of L-KYN metabolism must therefore be tightly regulated to ensure the ratio of these metabolites is maintained at a level that prevents cellular toxicity.

3. Regulation of the kynurenine pathway in the CNS and periphery

The initial rate-determining step of the KP is controlled by TDO/IDO activity, enzymes which show low sequence similarity, different affinities for specific tryptophan isoforms and are regulated by separate mechanisms [33] [34]. TDO is a heme-containing dioxygenase that can be induced by the binding of corticosteroids to their receptors [35]. Reduction of the TDO heme group from heme-Fe^{3+} to heme-Fe^{2+} allows it to oxidise L-TRP, therefore its activity is modulated by reducing agents and ROS [36]. TDO activation can also be induced by proinflammatory cytokines, although this is thought to occur indirectly through the activation of glucocorticoid receptors [37]. IDO1 is induced directly by proinflammatory cytokines which are released during immune response. Interferon-γ (INF-γ) is one of the key mediators of *IDO1* transcription, binding to one of two INF-γ activated sites (GAS) in the 5' flanking region of the *IDO1* gene [38]. INF-γ-dependant activation of IDO1 can in turn be potentiated by other proinflammatory cytokines such as interleukin-1 beta (IL-1ß) and toll-like receptor agonists [e.g. lipopolysaccharide (LPS)], synergistically promoting IDO1 activity in response to proinflammatory stimuli [reviewed 39]. Notably, activation of IDO1 increases the L-KYN/TRP ratio [40] [41]. In addition, KMO activity can also be influenced by proinflammatory stimuli; systemic administration of LPS in rats increases levels of KMO in the hippocampus [42] [43] and whole brain extracts [44], while in human hippocampal progenitor cells IL-1ß treatment induces transcription of the genes encoding both KMO and KYNU [41]. Immune response can also cause activation of microglia, as well as influx of proinflammatory cytokines and macrophages into the brain. As macrophages have a ~20-fold higher capacity for the production of QUIN than microglia [45], macrophage/ cytokine influx can cause significant changes to the levels and ratio of KP metabolites in the CNS.

Although TRP readily crosses the blood-brain barrier (BBB), levels of IDO1 and TDO2 are much lower in the brain than in the periphery [46]) and thus ~60% of KP metabolism in the brain is initiated by brain penetrant L-KYN synthesized in the periphery [47], which is taken up by glial cells [48]. The two predominant arms of L-KYN metabolism are physically separated in the brain: KMO is expressed in microglia but not in astrocytes, thus the 3-HK arm of the pathway leading to QUIN production, takes place in microglia [45], while KATs are expressed in astrocytes - but not microglia – and therefore production of KYNA within the CNS occurs in astrocytes [49](**Figure 2**). KYNA synthesis and release from astrocytes is influenced by a wide range of factors, such as K^+, glutamate receptor agonist levels and glucose concentrations [50]. Dopamine may also play a regulatory role, as the dopamine reuptake-inhibitor D-amphetamine reduces KYNA levels in the brain but not the periphery, whereas L-KYN levels are unaffected [51]. The aforementioned influences are lost in the absence of neurons, indicating that neuronal signaling is crucial for the regulation of KYNA levels. QUIN levels are dependent on the abundance of its precursors 3-HK and 3-HANA [52], as well as the activity of enzymes KMO, KYNU and 3-HAO [53] [54]. Both KYNA and QUIN lack efficient active transport mechanisms and are unable to cross the BBB, and therefore must be produced locally for use in the CNS.

However 3-HK is able to penetrate the brain, and therefore alterations in blood concentrations of 3-HK produced in the periphery can influence the relative concentrations of QUIN to KYNA in the CNS [55] (See **Figure 2**).

4. Kynurenines in neurodegenerative disease

The KP has also become a key area of research in neurodegenerative disorders such as Huntington's disease (HD), Alzheimer's disease (AD) and Parkinson's disease (PD), due to the association of aberrant KP metabolite levels with all of these diseases. Of these, HD is the best documented with regards to a contribution of the KP to pathogenesis.

4.1. Huntington's disease

HD is an autosomal-dominantly inherited disease, caused by the expansion of a CAG tract within exon 1 of the huntingtin gene (*HTT)*, which encodes a polyglutamine stretch in the HTT protein. Beyond a threshold number of CAG repeats (~36), the translated HTT protein is prone to misfolding and forms toxic aggregates, which leads to the progressive loss of neurons in the brain, specifically in the striatum and cortex [56]. The age of onset of HD shows an inverse correlation with the length of the CAG tract, however there is still a great deal of variability, particularly in the 40-50 repeat range, which is likely due to environmental and genetic modifiers [57]. The modulation of HD progression by a number of genetic factors thus suggests that there may be a number of potential targets for the treatment of the disease.

Several strands of evidence indicate that targeting the KP may be relevant to HD. 3-HK and QUIN levels are raised in the neostriatum and cortex of HD patients at early stages of disease progression [58], whereas striatal levels of KYNA are significantly reduced in the brain [59] and cerebrospinal fluid (CSF) [60]. In HD mouse models, levels of 3-HK and QUIN are also increased in the brain, and correlate with the onset of HD phenotypes [61]. The upregulation of *IDO1* transcription has been observed in the YAC128 HD mouse model [62] and suggests a cause for the increased ratio of L-KYN/TRP in the blood of HD patients [63] [64]. Increased KMO activity has also been implicated as the source of elevated brain 3-HK levels in mice [65]. Furthermore, KAT activity is reduced in the striatum of HD patients [59]. This combination of factors suggests that in HD an abundance of L-KYN leads to high flux through the 3-HK/QUIN producing arm of the pathway and low flux through the KYNA producing arm. It has therefore been hypothesized that the imbalance in neurotoxic and neuroprotective kynurenine metabolites plays a causative role in HD pathogenesis.

The mechanism(s) by which mutant HTT (mHTT) influences the KP are not fully understood. It is clear that immune activation occurs during HD progression, with an abundance of inflammatory cytokines such as tumor necrosis factor α (TNFα) and interleukins (IL) found to be higher in the blood plasma [66] and post-mortem brain tissue [67] of HD patients compared to controls. Plasma IL-6

levels are elevated ~16 years before predicted age of onset [66] and IL-2 levels correlate with both disease severity and increases in the L-KYN/TRP ratio [64]. Microglia are the primary mediators of CNS immune response and their activation correlates with HD progression, detectable up to 15 years before predicted age of onset [68]. The activation of microglia and neuroinflammatory response in HD could therefore lead to enhanced IDO1 activity, increasing levels of downstream KP metabolites.

In parallel, transcriptional dysregulation caused by mHTT expression has been shown to activate the KP in microglia, both *in vitro* and *in vivo* [44]. Indeed, elevated levels of 3-HK and increased KMO activity in primary microglia and brains from R6/2 HD model mice can be normalized via treatment with histone deacetylase (HDAC) inhibitors which modulate gene expression [44]. In addition, HDAC inhibition in WT mice was found to attenuate increases in levels of L-KYN and 3-HK levels, as well as KMO activity induced by LPS stimulation, but had no effect on KP metabolites in untreated WT mice. This provides a strong link between neuroinflammation, activation of microglia and transcriptional dysregulation in HD with regards to the KP.

4.2. Alzheimer's disease

Currently affecting ~30 million people worldwide - a number that is expected to triple by 2050 - AD is the most common neurodegenerative disease. It is characterized by the accumulation of misfolded ß-amyloid peptide (Aß) plaques in the brain and neurofibrillary tangles caused by phosphorylated tau protein. Unlike HD, there is not a single cause of AD, however the diseases are similar in the sense that the pathology of both is modulated by a number of genetic and environmental factors. As in HD, perturbation of the KP is strongly implicated in AD, with an increased L-KYN/TRP ratio found in the blood and CSF of AD patients compared to healthy individuals [69]. This altered ratio coincides with increased levels of IDO in the brain [70] and 3-HK in blood serum [71]. Immunoreactivity for both IDO and QUIN have been observed in microglia, astrocytes and neurons of hippocampal tissue from AD patients, with the highest signal being observed at the perimeter of senile plaques in the brain [72]. Both are also found in neurofibrillary tangles and QUIN is present in intracellular granular deposits in cortical neurons [70] [72].

The amyloid peptide $Aß_{1-42}$ induces expression of IDO1 and increases the production of QUIN in human macrophages and microglia [73]. Studies in both human neurons and mouse models indicate that elevated $Aß_{1-42}$ coincides with a profound induction of the KP by proinflammatory cytokines, which induce IDO, TDO and KMO activity [74] [75] [76]. Furthermore, treatment of human neurons with QUIN results in upregulation of genes involved in tau phosphorylation, potentially providing a mechanism by which neurofibrillary tangles are formed in AD [77]. Several studies also indicate that 3-HK and QUIN levels are increased, while KYNA levels are decreased, in blood and CSF from AD patients. This shift towards production of neurotoxic metabolites over neuroprotective species is considered to contribute towards AD pathology. However, the relationship between peripheral and CNS KP metabolism - and how this contributes to neuronal death - is not yet fully understood.

4.3. Parkinson's disease

KP alterations in PD follow a similar pattern to those described in HD and AD. Indeed, the L-KYN/TRP ratio in serum and CSF has been reported to be higher in PD patients than controls, indicating an upregulation in IDO/TDO activity [78]. 3-HK levels have also been found to be increased in the putamen, prefrontal cortex and pas compacta of the substantia nigra from PD patients [79]. Thus, KP dysfunction may be a general hallmark of neurodegenerative disorders, and therefore alterations in kynurenine metabolite levels could contribute to pathogenesis in a wide variety of these diseases.

5. Achieving neuroprotection through manipulation of the KP

5.1. Targeting KMO in neurodegenerative disease

The branch point in the KP at which L-KYN is converted into either 3-HK or KYNA is a key determinant in the resultant ratio of neurotoxic to neuroprotective metabolites. The activity of KMO - which catalyzes 3-HK synthesis - has significant influence over the direction of flux at this branching point, and therefore KMO inhibition is a leading strategy for normalizing flux in the KP in neurodegenerative diseases. We first discovered the therapeutic potential of KMO inhibition in HD using a *Saccharomyces cerevisiae* model of the disease, which recapitulates several disease-relevant phenotypes, including formation of intracellular aggregates and cellular toxicity [80]. Notably, we found that deletion of the yeast gene encoding KMO reduced toxicity and ROS generation due to mutant HTT expression, and that this amelioration of disease-related phenotypes correlated to decreased levels of 3-HK and QUIN. In subsequent work, we found that either pharmacological or genetic inhibition of KMO in a fruit fly model of HD dramatically reduced neurodegeneration of rhabdomeres – photoreceptor neurons present in the fly's compound eye [81]. Critically, this improvement is correlated to a neuroprotective shift in KP metabolites. We found that by direct manipulation of 3-HK and KYNA levels in the fly brain (by feeding of the metabolites), we were able to modulate neurodegeneration – indicating that changes in KP metabolites are causative. Notably, 3-HK feeding independent of mutant HTT expression did not lead to neurodegeneration, suggesting that modulation of KP metabolite levels is only one of the factors contributing to toxicity in neurodegenerative disease.

The neuroprotective potential of KMO inhibition has also been validated *in vivo* using mammalian models of neurodegenerative disease [82]. Inhibition of KMO in the blood of HD and AD model mice via oral administration of the peripherally-acting pro-drug JM6 has been found to increase KYNA levels and reduce extracellular glutamate in the brain, leading to amelioration of disease phenotypes. Indeed, JM6 treatment reduced microglial activation, decreased synaptic loss, and extended lifespan in HD mice, and improved synaptic loss, spatial memory deficits and anxiety-related behavior in AD mice [82]. Similarly to JM6, the KMO inhibitor Ro-61-8048 does not cross the BBB [83]. Nonetheless, oral administration of Ro-61-8048 has been found to increase brain

concentrations of KYNA in extracellular hippocampal fluid in rats [84] and convey neuroprotection in a primate model of PD [85]. Therefore a reduction in 3-HK synthesis in the periphery due to peripherally-acting KMO inhibitors may lead to increased blood concentrations of L-KYN, which is transported across the BBB into the CNS, and preferentially converted into neuroprotective KYNA [82](**Figure 2**). However, subsequent analyses of JM6 have led to debate on whether this compound acts as a KMO inhibitor [86]. Nonetheless, additional promising compounds are being developed; for example, a peripheral KMO inhibitor (Compound 75; CHDI-340246) has been generated which increases KYNA levels in mice and the CSF of primates [87] [88].

We recently developed KMO knockout mice in order to better understand the biological ramifications of KMO inhibition *in vivo*, as well as to use as a tool for genetic validation of KMO inhibition in mouse models of neurodegenerative disorders [89]. As expected, in homozygous KMO knockout mice levels of 3-HK and QUIN were significantly decreased in the brain and periphery, and levels of KYNA were significantly increased. Interestingly, while KMO activity was eliminated in the brain and liver of these animals, the presence of residual 3-HK was detected – particularly in the liver – indicating that other enzymes may be able to synthesize this metabolite in the absence of KMO. In addition, while levels of L-KYN and KYNA were dramatically increased in both the CNS and periphery in these animals compared with controls, this upregulation was more pronounced in the periphery. We also observed that QUIN levels were only reduced by ~20% in the CNS, whereas in the periphery this metabolite was essentially eliminated – suggesting a re-routing of the KP in the CNS via synthesis of anthranilic acid by kynureninase, and the subsequent generation of 3-HANA (**Figure 1**). Although QUIN was reduced by a much smaller proportion in the brain as compared to the periphery, the increase in anthranilic acid was quantitatively similar, indicating that anthranilic acid is much more readily converted into 3-HANA in the brain than in the periphery. It also suggests that pharmacological KMO inhibition is unlikely to cause large-scale reduction of QUIN in the brain, so potential neuroprotection conveyed by KMO inhibition would need to be conferred solely by the combinatorial action of reducing 3-HK levels and increasing KYNA levels.

Difficulties in KMO purification have meant that until recently, little was known about the structure of this key KP enzyme. The mechanisms by which KMO inhibitors acted were therefore unclear, making it difficult to design novel inhibitory compounds – which is critical as brain penetrant inhibitors are currently lacking. The first crystal structures of KMO were obtained in *S. cerevisiae* revealing its similarity to members of the flavin-dependent hydroxylase family [90]. The structure features a Rossman fold with five ß-sheets and four α-helices as part of the domain that interacts with flavin adenine dinucleotide (FAD), the cofactor upon which KMO is dependent. UPF 648 was found to bind close to this domain, altering the active site at which L-KYN binds, thus hindering KMO activity. Furthermore, the chemical similarity of UPF 648 to L-KYN has permitted predictive modelling of the KMO active site. Critically, mutagenesis and functional assays found that the residues involved in substrate binding are well conserved across different organisms, facilitating translation of

this work to human KMO. The obtained *S. cerevisiae* structure is therefore a valid template for use in high-throughput KMO inhibitor screens, using virtual compound libraries to identify novel scaffolds capable of inhibition, which will likely aid in the development of novel compounds able to cross the BBB.

5.1. Targeting TDO/IDO in neurodegenerative disease

As well as KMO inhibition, intervention at other stages in the KP has shown neuroprotective potential. We first identified TDO as a potential therapeutic target when we found that HD flies with reduced TDO activity - either through RNAi or via hypomorphic mutations - exhibit neuroprotection, as demonstrated by rescue of rhabdomere degeneration [81]. Critically, this amelioration of neuron loss correlated to a strong shift in L-KYN metabolism favouring KYNA production and decreased synthesis of 3-HK – suggesting that alterations in KP metabolites may play a role in the protection conferred by TDO inhibition.

Interestingly, complementary - though partially conflicting - results have been obtained in the nematode *C. elegans* [91]. Genetic inhibition of TDO-2 in worms was found to suppress toxicity of α –synuclein (aSyn) – a key protein involved in PD pathology, as well as $A\beta_{1\text{-}42,}$ and a polyglutamine (40Q) protein. Interestingly, these findings were likely independent of downstream KP metabolites, as knockdown of *tdo-2* in worms lacking key downstream KP metabolites had a similar effect to knockdown in wild-type animals, indicating that the observed effects were likely a consequence of increased concentrations of L-TRP via an unknown mechanism, and not due to alterations in levels of KP metabolites. Indeed, supplementation of L-TRP resulted in a dose-dependent suppression of aSyn toxicity in the worms. Unpublished work from our group has also shown that supplementation of L-TRP in food fed to *Drosophila* rescues neurodegeneration in HD flies, and leads to an increase in KYNA relative to 3-HK (Breda et al, unpublished). Notably, reintroduction of 3-HK into the fly via feeding abolishes the protective effect of TDO inhibition, indicating that a reduction in 3-HK levels is likely a key facet of this neuroprotective approach in fruit flies. Thus, it is possible that TDO inhibition may be neuroprotective via a combination of increased L-TRP levels and altered flux in KP metabolites.

Further evidence supporting the neuroprotective potential of TDO/IDO inhibition has been observed with the compound coptisine - the main active component of the Chinese prescription medication Oren-gedoku-to (OGT) - which is a potent inhibitor of IDO1 [92]. Indeed, treatment of an AD mammalian cell model with coptisine A increased viability while reversing the raised activity of IDO1. Coptisine treatment also effectively reduced microglial and astrocyte activation, amyloid plaque formation and neuronal loss in an AD mouse model while inhibiting IDO1 activity in the blood [93]. Notably, striatal levels of 3-HK were found to be reduced in *Ido1* deficient mice [94], which upon intrastriatal QUIN injection also exhibit smaller neuronal lesions and greater number of surviving neurons in comparison to WT. In total, these data support the therapeutic potential for IDO1 inhibition in neurodegenerative disease.

6. Concluding remarks

An ever-growing body of evidence implicates kynurenine pathway metabolites in the pathology of many neurocognitive and neurodegenerative disorders. Recent genetic and pharmacological approaches in model organisms have complemented past metabolic studies in patients to suggest that normalizing flux through the KP may be a valid therapeutic approach in these disorders. It is clear that a shift towards QUIN and 3-HK synthesis – and away from KYNA production - is found in the majority of KP-related neurodegenerative diseases, a situation that can be addressed by interventions which reverse this flux. KMO, in particular among the KP enzymes, has shown great potential as a therapeutic target for the treatment of neurodegeneration, as inhibition of this enzyme causes an increase in production of neuroprotective KYNA, while decreasing levels of its product 3-HK, as well as the downstream metabolite QUIN. The elucidation of the crystal structure of KMO will allow the design of novel KMO inhibitors with increased efficacy and brain penetrance, permitting modulation of the KP both in the periphery and CNS, which will ultimately improve the arsenal of KP-modulating therapeutic strategies. Recent findings also indicate that targeting the initial rate-limiting step of the pathway through either IDO1 or TDO2 inhibition could also prove to be an effective neuroprotective strategy in these disorders. Planned clinical trials with KMO inhibitors – particularly in the context of HD – will serve as an important first step in the understanding of the therapeutic value of KP manipulation in patients with HD, as well as in the context of neurodegenerative diseases in general.

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Figure Legends

Figure 1. Schematic overview of the kynurenine pathway (KP), which is responsible for >95% of tryptophan metabolism in higher eukaryotes. In the context of neurodegeneration, the pathway features a key branching point at L-KYN metabolism; KMO converts L-KYN into the neurotoxic, free-radical generator 3-HK, which leads to the synthesis of the neurotoxic metabolites 3-HANA and QUIN (3-HK, 3-HANA, and QUIN are highlighted in red). L-KYN can also be metabolised by the KAT family of enzymes to produce KYNA (highlighted in green), which conveys neuroprotection.

Figure 2. The location of KP metabolites in the brain. Only TRP, L-KYN and 3-HK readily cross the BBB - the synthesis of 3-HK by KMO in the periphery is therefore capable of influencing the ratio of KYNA to QUIN in the CNS. The synthesis of KYNA and QUIN is physically separate in the brain - KYNA is produced and released from astrocytes whereas QUIN synthesis and release takes place in microglia. QUIN causes excitotoxicity by selectively binding to postsynaptic NMDARs, causing augmented glutamate signalling and neuronal Ca^{2+} influx. KYNA is able to modulate QUIN-induced excitotoxicity by acting as a non-competitive antagonist at α7nAChRs, thus reducing glutamate signalling. At micromolar concentrations, KYNA also acts antagonistically at NMDA receptors, inhibiting excitatory glutamate signalling.

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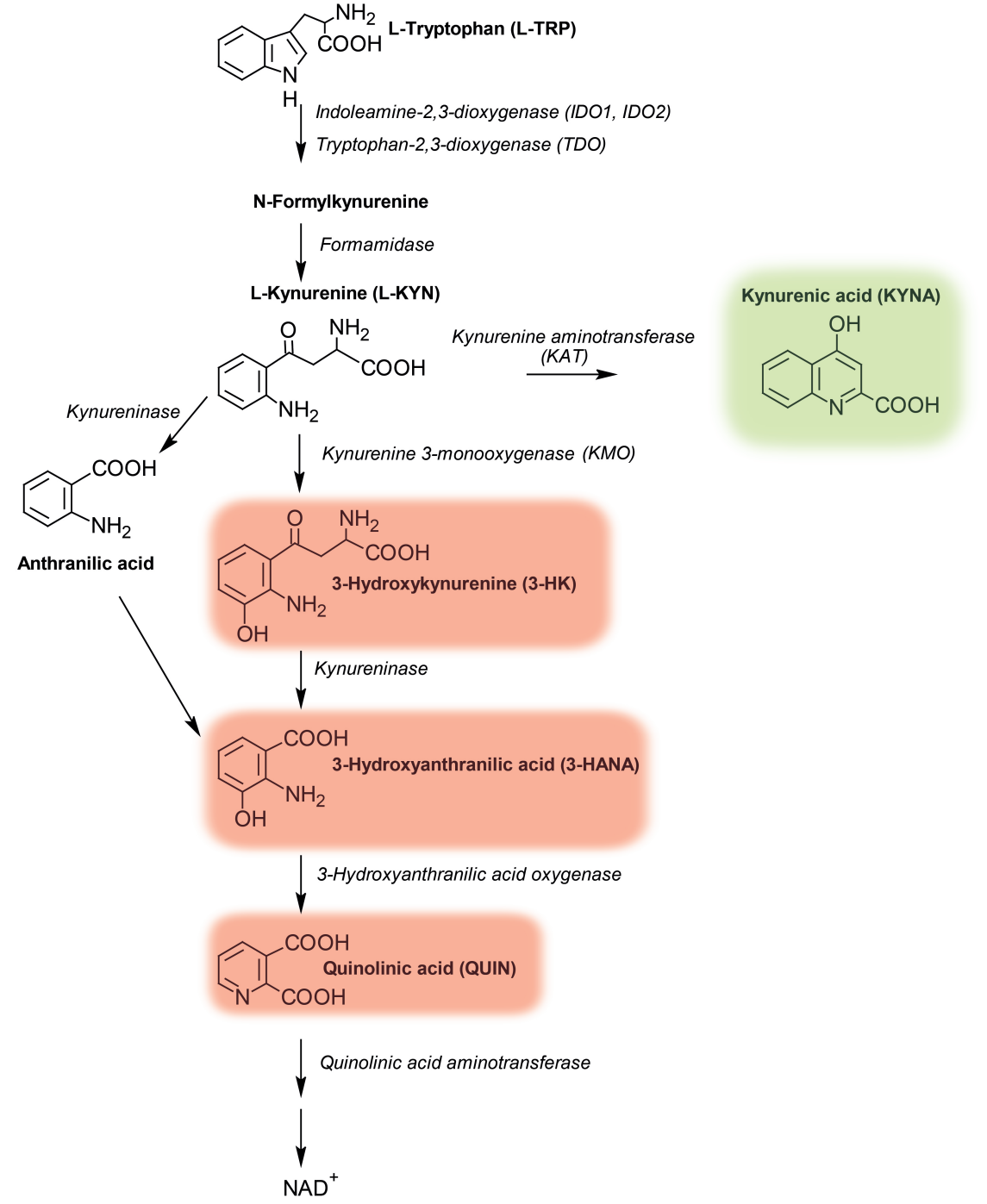
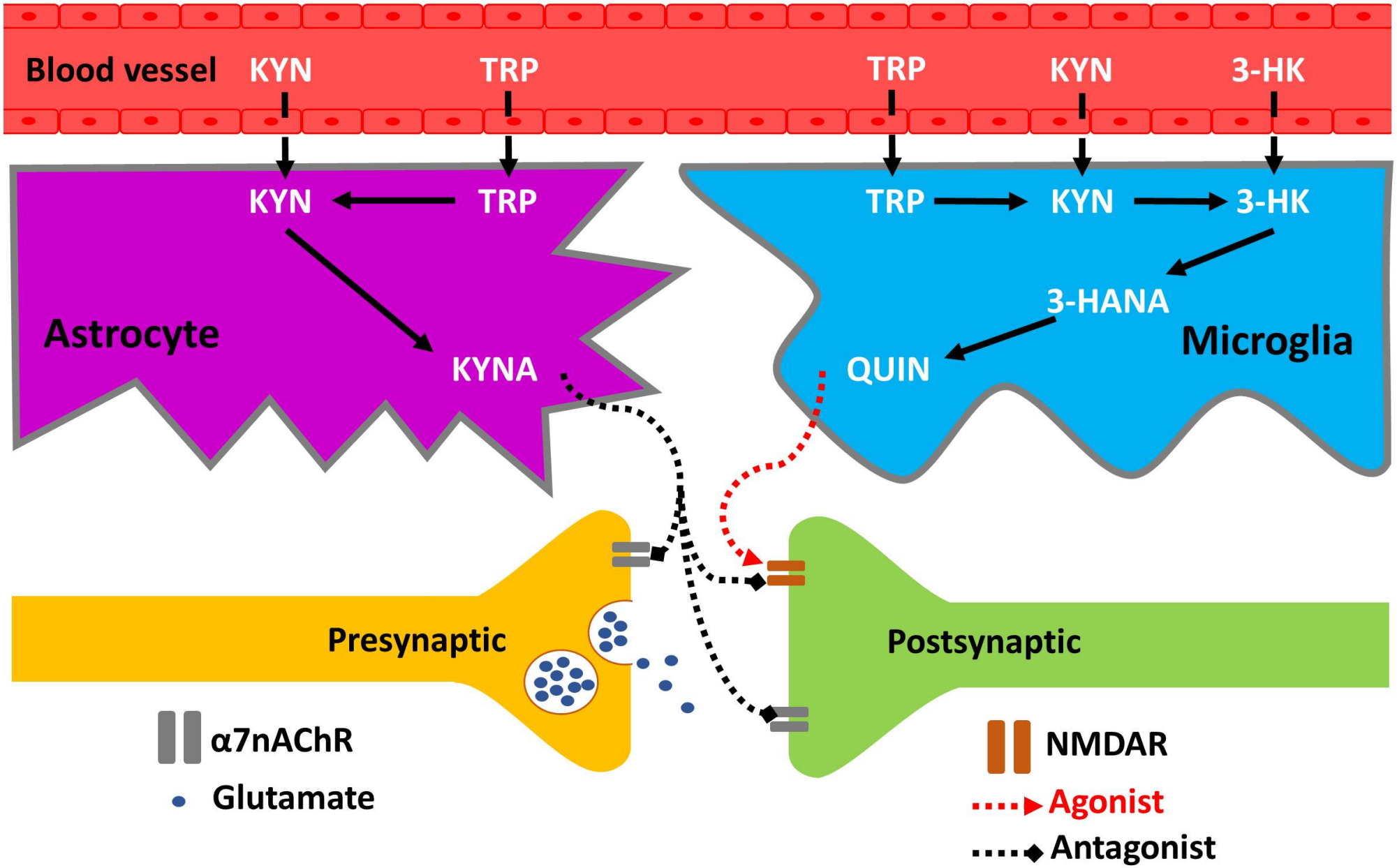

L-Tryptophan (L-TRP)
Indoleamine-2,3-dioxygenase (IDO1, IDO2)
Tryptophan-2,3-dioxygenase (TDO)
N-Formylkynurenine
Formamidase
L-Kynurenine (L-KYN)
Kynurenine aminotransferase (KAT)
Kynurenic acid (KYNA)
Kynureninase
Kynurenine 3-monooxygenase (KMO)
Anthranilic acid
3-Hydroxykynurenine (3-HK)
Kynureninase
3-Hydroxyanthranilic acid (3-HANA)
3-Hydroxyanthranilic acid oxygenase
Quinolinic acid (QUIN)
Quinolinic acid aminotransferase
NAD+


Blood vessel
KYN
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TRP
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Astrocyte
KYNA
3-HANA
Microglia
QUIN
Presynaptic
Postsynaptic
α7nAChR
Glutamate
NMDAR
Agonist
Antagonist