Manipulation of Brain Kynurenines: Glial Targets, Neuronal Effects, and Clinical Opportunities

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ABSTRACT
Degradation of the essential amino acid tryptophan along the kynurenine pathway (KP) yields several neuroactive intermediates, including the free radical generator 3-hydroxykynurenine, the excitotoxic N-methyl-D-aspartate (NMDA) receptor agonist quinolinic acid, and the NMDA and α7 nicotinic acetylcholine receptor antagonist kynurenic acid. The ambient levels of these compounds are determined by several KP enzymes, which in the brain are preferentially localized in astrocytes and microglial cells. Normal fluctuations in the brain levels of neuroactive KP intermediates might modulate several neurotransmitter systems. Impairment of KP metabolism is functionally significant and occurs in a variety of diseases that affect the brain. Pharmacological agents targeting specific KP enzymes are now available to manipulate the concentration of neuroactive KP intermediates in the brain. These compounds can be used to normalize KP defects, show remarkable efficacy in animal models of central nervous system disorders, and offer novel therapeutic opportunities.

Neuroactive Tryptophan Metabolites
In mammals, the vast majority of dietary tryptophan is metabolized via the kynurenine pathway (KP) (Scheme 1), which is initiated by the oxidative opening of the indole ring and eventually produces the ubiquitous enzyme cofactor NAD⁺.

This catabolic cascade is notable for the fact that it contains three neuroactive intermediates, all of which derive directly or indirectly from L-kynurenine (L-KYN), the primary major degradation product of tryptophan. One of the three compounds, kynurenic acid (KYNA), is formed in a "dead end" side-arm of the pathway, whereas the other two, 3-hydroxykynurenine (3-HK) and quinolinic acid (QUIN), are synthesized from L-KYN en route to NAD⁺. Although all kynurenines—the collective term used for KP intermediates shown in Scheme 1—are found in high concentration in urine (hence the name), none of them has so far been assigned an important physiological function in peripheral organs.

The first indication that kynurenines might play a role in the brain was provided by Lapin (1978), who noted convulsions after an intracerebroventricular QUIN injection in mice. Soon thereafter, ionophoretically applied QUIN was found to excite rat cortical neurons (Stone and Perkins, 1981), and intracerebrally injected QUIN was shown to cause excitotoxic lesions in rat brain (Schwarcz et al., 1983). Both QUIN-induced excitation and neurotoxicity are mediated by N-methyl-D-aspartate (NMDA) receptors, leading to the suggestion that endogenous QUIN might participate in physiological and pathological processes that are associated with NMDA receptor activation. Indeed, QUIN occurs naturally in the mammalian brain, although the low QUIN content of cerebral tissue (50–1000 nM) is difficult to reconcile with its low receptor affinity (ED₅₀ >100 μM). It appears that the remarkably high in vivo potency of QUIN, particularly as an excitotoxin, is caused by a combination of factors, including the absence of effective removal mechanisms for extracellular QUIN (Foster et al., 1984), its ability to readily generate damage-promoting free radicals (Rios and Santamaria, 1991), and, possibly, its specific interaction with the NR2A

ABBREVIATIONS:
KP, kynurenine pathway; L-KYN, L-kynurenine; KYNA, kynurenic acid; 3-HK, 3-hydroxykynurenine; QUIN, quinolinic acid; NMDA, N-methyl-D-aspartate; KAT, kynurenine aminotransferase; CNS, central nervous system.
and NR2B NMDA receptor subtypes (de Carvalho et al., 1996). These and other characteristics distinguish QUIN from other excitotoxins, such as NMDA itself, and might account for the compound’s unique neuroexcitatory and neurotoxic profile (Foster and Schwarcz, 1989; Stone, 1993). Notably, the distinct properties of QUIN are of more than academic interest since they likely account for the fact that QUIN-induced pathological changes in animals provide remarkably accurate models for human brain diseases (Schwarcz et al., 1984; Beal et al., 1991).

3-HK, a biological precursor of QUIN present in the brain in nanomolar concentrations, also has neurodestructive properties. In contrast to QUIN, however, 3-HK does not interact directly with specific recognition sites but kills nerve cells by generating toxic free radicals (Eastman and Guilarte, 1989), which initiate a cascade of intracellular events resulting in cellular disintegration (Okuda et al., 1998). These events are also responsible for the substantial potentiation of excitotoxicity that is seen when neurons are exposed to both 3-HK and QUIN (Guidetti and Schwarz, 1999). In addition, 3-HK and its degradation product, the immediate QUIN bioprecursor 3-hydroxyanthranilic acid, generate superoxide and hydrogen peroxide in a copper-dependent manner and thus promote copper-dependent, oxidative protein damage (Goldstein et al., 2000). Although 3-HK-induced cell death conceivably provides benefits to the organism by triggering apoptosis during development or in pathological situations (Okuda et al., 1998), no physiological role of 3-HK in the brain has been established so far.

Among the three neuroactive kynurenines, KYNA has recently received the most attention. First described as a neuroinhibitory compound two decades ago (Perkins and Stone, 1982), KYNA, at high, nonphysiological concentrations, is a broad spectrum antagonist of ionotropic excitatory amino acid receptors. As such, it serves as a valuable experimental tool and is widely used to block excitatory neurotransmission in vitro and in vivo. Accordingly, high concentrations of KYNA are anticonvulsant and provide excellent protection against excitotoxic injury (Foster et al., 1984). At much lower concentrations, KYNA acts as a competitive blocker of the glycine coagonist site of the NMDA receptor (IC50 ~8 μM; Kessler et al., 1989) and as a noncompetitive inhibitor of the α7 nicotinic acetylcholine receptor (IC50 ~7 μM; Hilmas et al., 2001). The fact that the affinity of KYNA to these two Ca2+-permeable receptors is in the range of KYNA levels in the human brain and reasonably close to the (lower) KYNA content of the rodent brain suggests a physiological function in glutamatergic and cholinergic neurotransmission. Direct support for such a role has been provided, for example, by in vivo studies in the rat striatum where a reduction in KYNA levels enhances vulnerability to an excitotoxic insult (Poeggeler et al., 1998) and, conversely, modest elevations of KYNA inhibit glutamate release (Carpendedo et al., 2001).

### Enzymes of the Kynurenine Pathway

The major enzymes of the KP are illustrated in Scheme 1 and Fig. 1. First, indoleamine 2,3-dioxygenase (or, in the liver, the more specific enzyme tryptophan 2,3-dioxygenase) metabolizes tryptophan to N-formylkynurenine, which is further degraded to L-KYN by formamidase. L-KYN in turn serves as a substrate of several distinct enzymes: kynureninase (yielding anthranilic acid), kynurenine 3-hydroxylase (yielding 3-HK), and kynurenine aminotransferases (KATs), which catalyze the irreversible transamination of L-KYN to KYNA. 3-HK is metabolized by the same KATs to yield xanthurenic acid, a metabolically inert side product of the pathway, or by kynureninase to give rise to 3-hydroxyanthranilic acid. 3-Hydroxyanthranilic acid oxygenase then converts 3-hydroxyanthranilic acid to α-amino-ω-carboxymuconic acid semialdehyde, which either rearranges nonenzymatically to form QUIN or serves as a substrate of α-amino-ω-carboxymuconic acid semialdehyde decarboxylase, eventually producing picolinic acid and its downstream metabolites. Finally, QUIN is metabolized by quinolinic acid phosphoribosyltransferase, yielding nicotinic acid mononucleotide and subsequent degradation products including the end product NAD+.

Despite the fact that all enzymes involved in the peripheral degradation of tryptophan to QUIN and KYNA were known and had been well characterized by 1970, their presence, properties, and cellular localization in the brain were only investigated once the neurobiological significance of KP in-
termediates was recognized. In essence, these studies confirmed that all enzymes of the catabolic cascade were present in the brain, albeit with much lower activity than in peripheral organs. Although some enzymes show severalfold differences between brain regions, and activities vary between species and with age, no consistent patterns have emerged so far to identify specific functions of the cerebral KP. Qualitatively, the characteristics of enzymes along the QUIN branch of the pathway do not appear to differ between the brain and the periphery, showing the same high substrate specificity and high substrate affinity (i.e., low \( K_m \) values). Moreover, cerebral and peripheral kynurenine 3-hydroxylase, kynureninase, 3-hydroxyanthranilic acid oxygenase, and quinolinic acid phosphoribosyltransferase have been confirmed to be identical using specific antibodies and molecular cloning.

Comparison of peripheral and central KATs reveals a more complex picture. In contrast to peripheral organs, which contain several aminotransferases capable of forming KYNA and xanthurenic acid from L-KYN and 3-HK, respectively, only two such enzymes appear to exist in the brain. Arbitrarily termed KAT I and KAT II, these enzymes have \( K_m \) values for L-KYN in the low millimolar range and differ substantially with regard to their pH optimum and substrate specificity. Thus, KAT I has a pH optimum of 9.5 to 10.0 and shows relatively little substrate specificity, whereas KAT II operates best at physiological pH and preferentially recog-
nizes l-KYN as a substrate. In the brain, KAT II is therefore primarily responsible for the de novo formation of KYNA (Guidetti et al., 1997).

Immunocytochemical and lesion studies, as well as experiments with primary cell cultures, have provided additional information regarding the cellular localization of KP enzymes (Guidetti et al., 1995; Heyes et al., 1996; Schwarcz et al., 1999; Guillemin et al., 2001). Most of this work was designed to identify the cellular and subcellular source of QUIN and KYNA in cerebral tissue and revealed unequivocally that glial cells, rather than neurons, harbor the enzymatic machinery for the biosynthesis of brain kynurenines. Although there is some evidence for the sporadic presence of KATs, kynurenine 3-hydroxylase, and quinolinic acid phosphoribosyltransferase in neurons, all enzymes of the pathway are primarily contained in astrocytes and microglial cells. Of functional significance, astrocytes do not appear to contain kynurenine 3-hydroxylase and therefore favor KYNA synthesis, whereas microglial cells harbor very little KAT activity and preferentially form intermediates of the QUIN branch of the pathway (Guillemin et al., 2001).

**Dynamics of Kynurenine Pathway Metabolism in the Periphery and in the Brain**

The existence of catabolic enzymes with high capacity accounts for the efficient degradation of tryptophan in the periphery, contrasting starkly with the relatively poor efficacy of the corresponding brain KP. Although l-KYN can be produced in the brain to a moderate degree, the cerebral pathway is driven mainly by blood-borne l-KYN, which enters from the circulation using the large neutral amino acid transporter (Fukui et al., 1991). In the brain, l-KYN is then rapidly taken up by astrocytes and, presumably, microglial cells. Some l-KYN is also actively transported into neurons, but this process is much slower and, unlike glial l-KYN uptake, critically dependent on the supply of Na$^+$ (Speciale and Schwartz, 1990). Of possible functional significance, 3-HK also penetrates into the brain and is then accumulated by brain cells, using the same uptake mechanisms as l-KYN (Eastman et al., 1992; Reinhardt et al., 1994; cf. Fig. 1). The subsequent intracellular degradation of these substrates appears to be dictated primarily by the differential distribution of individual enzymes in astrocytes and microglial cells.

In vivo microdialysis experiments and studies with tissue slices in vitro have demonstrated that newly formed QUIN readily enters the extracellular compartment. However, attempts to identify an ionic dependence or other control mechanisms of these release processes either in peripheral organs or in the brain have not been successful to date. Because of the efficient synthesis of QUIN by 3-hydroxyanthranilic acid oxygenase and the very low activity of QUIN’s degradative enzyme, quinolinic acid phosphoribosyltransferase, the levels of QUIN in the extracellular milieu are essentially governed by the bioavailability of 3-hydroxyanthranilic acid. Thus, both in the brain and in the periphery, sudden or prolonged increases in QUIN formation lead to corresponding surges in extracellular QUIN. Eventually, QUIN is removed from the brain by a probenecid-dependent transport process and eliminated by urinary excretion.

The enzymatic formation of KYNA, too, is determined by the intracellular concentration of its immediate biological precursor, l-KYN. In contrast to QUIN synthesis, however, the generation of KYNA is critically influenced by additional modulatory factors. For example, KYNA production both in peripheral organs and in the brain is stimulated by cosubstrates of KAT, such as pyruvate or 2-oxoglutarate (Hodgkins et al., 1999), and inhibited by amino acids that compete with l-KYN as substrates of KAT (Guidetti et al., 1997). In the brain but not in periphery, depolarizing agents like high K+ or veratridine, or compromised cellular energy metabolism, substantially reduce KYNA formation and hence extracellular KYNA levels. Interestingly, these effects are not seen in lesioned, i.e., neuron-depleted, brain tissue, suggesting that neuronal activity normally controls glial KYNA synthesis in the brain (Gramsbergen et al., 1997). The recently discovered fluctuations in extracellular KYNA levels following the systemic administration of dopaminergic (Poeppel et al., 1998) or cholinergic (Hilmas et al., 2001) agents are also brain-specific, but the mechanisms underlying these remarkable interactions have not yet been fully elaborated. Taken together, it is clear that an intricate machinery has evolved to regulate the extracellular concentration of KYNA in the brain. This seems fitting for a neuroactive metabolite without an efficacious extracellular removal mechanism (Moroni et al., 1988; Turski and Schwarz, 1988).

**Endogenous Kynurenines and Brain Dysfunction**

The distinct convulsant and excitotoxic properties of QUIN, the pro-excitotoxic properties of 3-HK, and the anti-convulsant and neuroprotective properties of KYNA in experimental animals soon led to the idea that endogenous kynurenines might be involved in human brain diseases that are caused by excitotoxic mechanisms (Schwarcz et al., 1984). Notably, the term “excitotoxicity” is frequently applied loosely and speculatively in this regard, sometimes merely referring to localized hyperfunction of excitatory neurotransmission. Kynurenines may therefore participate not only in the pathophysiology of neurodegenerative and seizure disorders, as originally assumed, but could play a role in a large number of etiologically diverse CNS diseases, including neuroimmunological disorders, drug abuse, or chronic pain (Table 1).

A link between endogenous kynurenines and excitotoxic phenomena is supported by experimental studies demonstrating, for example, neurotoxic effects after chronic application of nanomolar concentrations of QUIN (Whetsell and Schwarz, 1989; Kerr et al., 1998), acute neurotoxic consequences of 1 μM 3-HK (Okuda et al., 1998), and anticonvulsant efficacy of nanomolar concentrations of KYNA (Scharfman et al., 1999). Regardless of the precise nature of the cellular and molecular events that underlie these effects, it is therefore reasonable to assume that an impairment of KP metabolism may have untoward effects on brain function. It follows that QUIN, 3-HK, and KYNA, alone or in concert, might in certain cases be the *primary* cause of CNS pathology.

A large number of studies in experimental animals have also explored a possible role of kynurenines as *secondary* mediators of dysfunctional states. Thus, the levels of brain kynurenines are often abnormal as a result of pathogenic events (see Stone, 2001, for a recent review). In essence,
Putative links between neuroactive KP intermediates and CNS pathology

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Molecular Event</th>
<th>Pathology (Cellular/Clinical)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-HK</td>
<td>Free radical generation</td>
<td>Apoptosis, potentiation of excitotoxicity, cataract formation, neurodegenerative diseases, stroke, traumatic injury, neuroinflammation</td>
</tr>
<tr>
<td>QUIN</td>
<td>NMDA receptor activation, free radical generation</td>
<td>Excitotoxicity, neurodegenerative diseases, stroke, traumatic injury, epilepsy, cerebral malaria, perinatal hypoxia, neuroinflammatory</td>
</tr>
<tr>
<td>KYNA</td>
<td>NMDA receptor blockade, α7 nicotinic acetylcholine receptor blockade</td>
<td>Cognitive impairment, schizophrenia, Neurodegenerative diseases, pain, drug abuse</td>
</tr>
</tbody>
</table>

Kynurenergic Drugs

The apparent involvement of kynurenines in inflammatory diseases and in neurodegenerative and psychiatric disorders suggested that KP enzymes might be useful targets for rational therapeutic intervention. This, and the general need to define the biological implications of modulating individual steps of the KP, has led to the design and synthesis of potent and selective enzyme inhibitors. Since very limited structural information on these proteins exists, the design strategy has so far been mainly based on iterative processes of intuitive medicinal chemistry and biological evaluation. Combinatorial chemistry and high throughput screening have appeared on the scene only recently. Notably, these new compounds complement a large series of KYNA analogs, which have been extensively investigated for their ability to antagonize glutamate receptor function (Stone, 2001). We focus here on the major compounds that have been synthesized to specifically interfere with the disposition and function of neuroactive KP intermediates (Schemes 2–7).

Enzymes Responsible for Tryptophan Degradation

As mentioned above, two heme-dependent enzymes, tryptophan 2,3-dioxygenase and indoleamine 2,3-dioxygenase, catalyze the oxidative cleavage of the 2,3-bond of the indole ring of L-tryptophan to yield N-formylkynurenine. Tryptophan 2,3-dioxygenase is present specifically in the liver, and its activity is rate-limiting for the entry of tryptophan into the KP. Indoleamine 2,3-dioxygenase, on the other hand, occurs in many extrahepatic tissues and in macrophages and recognizes a wide variety of indoleamine derivatives, including d-tryptophan, serotonin, and melatonin, as a substrate. These differences in tissue distribution and substrate specificity indicate a functional and structural distinction between the two enzymes and explain the chemical diversity of their inhibitors.

Tryptophan 2,3-Dioxygenase Inhibitors. Although a vast array of substances inhibit this enzyme in vitro, many of these compounds have very little effect on tryptophan levels in vivo, probably due to inadequate bioavailability or rapid metabolism. The first potent, selective, and bioactively active inhibitors, belonging to the class of 3-(2-pyridylethenyl)indoles, were described in the mid-1990s (Madge et al., 1996). Among these, (E)-3-[2-(4′-pyridyl)-vinyl]-1H-indole (1) and the corresponding 6-fluoro derivative (2) were shown to be effective and specific, whereas (E)-6-fluoro-3-[2-(3′-pyridyl)vinyl]-1H-indole (3), differing from 1 only by the position of the pyridyl moiety, also potently blocked serotonin reuptake (Salter et al., 1995, 1996; Madge et al., 1996). Using
these compounds, it was possible to demonstrate that inhibition of tryptophan 2,3-dioxygenase decreases the catabolism of peripheral tryptophan, raising its concentration in both blood and brain. Notably, since tryptophan 2,3-dioxygenase is the rate-limiting enzyme for the conversion of tryptophan through the KP, inhibition of this enzyme makes more tryptophan available for conversion to 5-hydroxytryptophan and, eventually, serotonin. Inhibitors of both tryptophan 2,3-dioxygenase and serotonin uptake such as 3 therefore produce an elevation of serotonin in the cerebrospinal fluid. This dual action could constitute an interesting, new approach for the development of antidepressant drugs.

**Indoleamine 2,3-Dioxygenase Inhibitors.** Indoleamine 2,3-dioxygenase is activated in a variety of inflammatory diseases affecting the brain, such as acquired immunodeficiency syndrome, meningitis, hepatic encephalopathy, septicemia, and neurovirological disorders (Heyes et al., 1993). In particular, macrophages express the enzyme in response to interferon-γ and other signals derived from activated T cells. Unfortunately, only a small number of potent and selective indoleamine 2,3-dioxygenase inhibitors are currently available as tools to provide further functional characterization of this important enzyme. A class of noncompetitive inhibitors belonging to the β-carboline family, including norharman (4), was introduced by Hayashi and his collaborators (Eguchi et al., 1984). A more potent noncompetitive inhibitor of the same class, 3-butyl-β-carboline (5), was recently described (Peterson et al., 1993). Brassilexin (6), a phytoalexin isolated from plants of the Cruciferae family, was reported by the same authors to be a potent, noncompetitive enzyme inhibitor (Peterson et al., 1993) and may represent a useful lead for further structural elaboration.

The few known competitive indoleamine 2,3-dioxygenase inhibitors include 1-methyl-tryptophan (7) (Sono and Cady, 1989). Interestingly, probing of the steric requirements of the enzyme's catalytic site revealed that l-(-)-1-methyl-tryptophan (7) is significantly more active than its enantiomer (Peterson et al., 1994). Moreover, a nonindolic compound, 3-amino-2-naphthoic acid (8), was introduced as a novel, potent, and selective inhibitor of potential clinical relevance (Peterson et al., 1994).

![Scheme 2. Selected inhibitors of tryptophan 2,3-dioxygenase.](image)

$K_i = 0.030$

Madge et al. 1996

$K_i = 0.040$

Saiter et al. 1995

$K_i = 0.042$

Saiter et al. 1995

**Scheme 2.** Selected inhibitors of tryptophan 2,3-dioxygenase. $K_i$ values (micromolar) for the inhibition of the formation of l-KYN from l-tryptophan in rat liver are indicated.

**Scheme 3.** Selected inhibitors of indoleamine 2,3-dioxygenase. $K_i$ values (micromolar) for the inhibition of the formation of l-KYN from l-tryptophan in rat liver are indicated.

**Enzymes Regulating the KYNA/QUIN Balance**

The first enzymatic product of tryptophan degradation, N-formylkynurenine, is readily transformed to l-KYN by N-formylkynureninase. No specific inhibitors of this enzyme have been described so far. In contrast, the degradative enzymes of l-KYN, especially kynureninase and kynurenine 3-hydroxylase (cf. Scheme 1), are increasingly recognized as interesting targets for kynurenenergic drug development. This is based on the assumption that interference with these enzymes, acting at a branching point of the KP, might favorably alter the QUIN/KYNA ratio and thus correct pathophysiologically relevant chemical impairments in the brain.

**Kynurenine 3-Hydroxylase Inhibitors.** Kynurenine 3-hydroxylase is a FAD-dependent monooxygenase, which catalyzes the hydroxylation of l-KYN to 3-HK. Inhibition of this enzyme has provided critical information regarding the relationship between the QUIN and KYNA branches of the KP.

A simple isoster of l-KYN, nicotinoylalanine (9), was the first competitive but non selective inhibitor of kynurenine 3-hydroxylase reported (Moroni et al., 1991). Pellicciari et al. (1994) subsequently described m-nitrobenzoylalanine (10) as a potent inhibitor, and this compound was used as a prototype to examine the effect of kynurenine 3-hydroxylase inhibition on brain injury in animal models of stroke. In these studies, 10 showed remarkable, dose-dependent tissue protection, with 85% reduction in damage at the highest concentration used (Cozzi et al., 1999). Moreover, 10 had a significant protective effect even when drug administration was delayed by 60 min after the ischemic insult. These effects were obtained both after occlusion of the middle cerebral artery in rats and in the gerbil global ischemia model (Cozzi et al., 1999).

Systematic evaluation of structure-activity relationships, varying either the aromatic ring region (Giordani et al., 1996) or the side chain (Giordani et al., 1998), revealed that the
2-amino group of the benzoylalanine moiety was not necessary for enzyme inhibition, with 11 exhibiting high activity (Speciale et al., 1996). Conformational restriction of the side chain turned out to be the key to obtaining the first competitive inhibitor of kynurenine 3-hydroxylase that was active in the nanomolar range. Thus, the (JS,2S) isomer of 2-(3,4-dichlorobenzoyl)cyclopropane-1-carboxylic acid (12, UPF 648) selectively inhibits kynurenine 3-hydroxylase with an IC\textsubscript{50} of 20 nM (R. Pellicciani, L. Amori, G. Costantino, P. Pevarello, C. Speciale, H. Q. Wu, R. Schwarz, and M. Varasi, manuscript in preparation).

The screening of a small library of sulfonamides synthesized in a conventional manner allowed the identification of compound 13 as the most potent noncompetitive inhibitor of kynurenine 3-hydroxylase reported so far. This compound, too, has impressive anti-ischemic neuroprotective properties and can be effectively used as an experimental tool to raise brain KYNA levels (Röver et al., 1997; Chiarugi and Moroni, 1999; Cozzi et al., 1999).

**Kynureninase Inhibitors.** Kynureninase is a pyridoxal phosphate-dependent enzyme that catalyzes the hydrolysis of both L-KYN and 3-HK into L-alanine and anthranilic acid, respectively. Structural elaboration around the kynurenine nucleus has provided the first competitive enzyme inhibitors. Based on the proposed mechanism of action of kynureninase, which includes as a key step the base-catalyzed nucleophilic attack of a water molecule on the γ-carbonyl carbon atom of the kynurenine-ketamine-pyridoxal phosphate complex, Phillips and Dua (1991) reported the (2S,4S) isomer of dihydroxykynurenine (14) as a transition state analog inhibitor of *Pseudomonas fluorescens* kynureninase. The activity of 14 was ascribed to its similarity with the postulated gem-diolate intermediate formed upon the nucleophilic attack of water. Subsequently, similarity with the gem-diolate intermediate prompted the evaluation of a series of S-aryl-L-cysteine S,S-dioxides as enzyme inhibitors (Dua et al., 1993). S-(2-Aminophenyl)-L-cysteine-S,S-dioxide (15) turned out to be a particularly potent inhibitor. When tested on human recombinant enzyme, 15 showed a dramatic reduction in apparent potency when compared with the bacterial enzyme. The 5-methyl derivative (16) was 3 times more potent against human kynureninase (Drysdale and Reinhard, 1998).

Substitution of the ortho amino group of L-KYN by a methoxy moiety yields α-methoxykynurenine (17), which was identified as a potent and selective competitive inhibitor of rat liver kynureninase (Carpenedo et al., 1994; Chiarugi et al., 1995). Experiments with this compound alone and in combination with a kynurenine 3-hydroxylase inhibitor demonstrated that 3-hydroxylation is the preferred route of L-KYN metabolism in the rodent brain. Not unexpectedly, selective inhibition of kynureninase causes a marked increase in the levels of the neurotoxic KP intermediate 3-HK (Chiarugi et al., 1995).

**3-Hydroxyanthranilic Acid Oxygenase Inhibitors.** 3-Hydroxyanthranilic acid oxygenase is a monomeric cytosolic protein belonging to the family of intramolecular dioxygenases containing nonheme ferrous iron. Halogenated substrate analogs such as 4-chloro-3-hydroxyanthranilic acid (18) were the first KP enzyme inhibitors described (Todd et al., 1989) and are highly potent, reversible, and competitive inhibitors (Walsh et al., 1991). These compounds are active in the CNS after systemic administration (Saito et al., 1993, 1994), and reduce QUIN accumulation and functional deficits following spinal cord injury (Blight et al., 1995). Because of the location of 3-hydroxyanthranilic acid oxygenase distant from the branching point of the KP (Scheme 1), enzyme inhibition provides a method to selectively attenuate QUIN formation without a concomitant increase in KYNA levels. More recently (Linderberg et al., 1999), the new 4,5-dihalogenated compounds 19 to 22 have also been reported to be highly potent and selective enzyme inhibitors.

**Kynurenine Aminotransferase Inhibitors.** KAT II, which accounts for the majority of KYNA formation in the normal rat brain, is relatively substrate-specific, whereas the less essential KAT I is more promiscuous with regard to its substrate preference. No preferential KAT I inhibitor has been identified to date, but α-amino adipic acid (23), quis-
Qualic acid (24) and DL-5-bromokynurenine (25) are selective KAT II (over KAT I) inhibitors in vitro, with IC\textsubscript{50} values in the micromolar range. These compounds may provide structural clues for the development of novel, specific KAT II inhibitors, which could prove useful in situations that require a down-regulation of brain KYNA (Schwarcz et al., 2001).

**A Special Case: 4-Chlorokynurenine.** Like its parent compound L-KYN, 4-chlorokynurenine (26) (Scheme 8) is metabolized by both branches of the KP, yielding both the 3-hydroxyanthranilic acid oxygenase inhibitor 18 and 7-chlorokynurenic acid, a potent and selective antagonist of the glycine coagonist site of the NMDA receptor (Guidetti et al., 2000). Using the large neutral amino acid transporter, 26 readily enters the brain after peripheral administration and can thus provide anti-excitotoxic neuroprotection (Wu et al., 2000). Its dual action may offer unique advantages in clinical situations where both a reduction of brain QUIN and NMDA receptor blockade are desirable.

**Outlook: The Road to Kynurenergic Therapies**

The availability of a host of new compounds that selectively target individual KP enzymes has led to new neuropharmacological concepts and provided novel opportunities for therapeutic intervention. These enzyme inhibitors allow the judicious up- or down-regulation of the brain concentration of 3-HK, QUIN, and KYNA. Although the levels of brain kynurenes can be effectively manipulated by influencing the activity of peripheral KP enzymes (Reinhard et al., 1994), new agents with better brain access and pharmacodynamic properties should soon make it possible to target cerebral KP enzymes directly. Such compounds are currently under development and hold promise as investigative tools and therapeutically useful drugs. In common to all these agents is the use of glial cells for the delivery of kynurenes into the extracellular compartment, providing a fundamentally new method of influencing glutamatergic and cholinergic neurotransmission. Compared with other intervention strategies, the advantages of this approach include: 1) increased drug efficacy due to highly localized delivery of neuroactive kynurenes at tight glia-neuron junctions (Scharfman et al., 1999); 2) the use of activated glial cells to preferentially target certain neuronal populations or brain areas after systemic drug application (Lee and Schwarcz, 2001); and 3) the opportunity to differentially affect KYNA formation (in astrocytes) or 3-HK and QUIN synthesis (in microglial cells) based on the different cellular localization and function of KP enzymes (cf. Fig. 1). In practical terms, this heightened pharmacological efficacy and the option to affect the two pathway branches separately provide significant benefits for experimental studies and might substantially reduce side effect hazards in clinical situations.

Four related scenarios may warrant the use of kynurenergic drugs. First, enzyme inhibitors could be used to purposefully interfere with the physiological effects of neuroactive kynurenes (Wu et al., 2000; Carpenedo et al., 2001; Erhardt et al., 2001). Thus, a relatively modest reduction in brain KYNA levels may be a desirable means to enhance cognitive function (Pittaluga et al., 1997; Poeggeler et al., 1998). Second, appropriate kynurenergic manipulations should prevent or correct impairments that result from a primary defect in KP metabolism, e.g., those caused by an enzyme deficiency. Third, kynurenergic drugs should be useful in situations where abnormal 3-HK, QUIN, and/or KYNA levels occur secondarily in response to an insult and subsequently participate in pathological events. For example, inhibition of 3-hydroxyanthranilic acid oxygenase attenuates QUIN accumulation following traumatic spinal cord injury and reduces the severity of injury-related functional deficits (Blight et al., 1995). Finally and more generally, kynurenergic drugs could be used with the sole purpose of limiting pathological excitatory overactivity, for instance in attempts to provide anti-excitotoxic neuroprotection (Wu et al., 2000).
To date, none of these concepts and predictions has been tested in humans. In addition to safety and tolerability, important questions for kyureninergic drug development include the selection of appropriate enzyme targets, the possibility of harmful drug-induced reductions in NAD+ formation, and the feasibility of success in chronic treatment regimens. These issues are not likely to pose insurmountable obstacles, so that first evaluations of kyureninergic agents in humans should be forthcoming before too long.

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References


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