5 Kynurenines in the Brain: Preclinical and Clinical Studies, Therapeutic Considerations

C. Kiss · L. Vécsei

Abstract: In mammalian cells, the kynurenine pathway (KP) is a major biochemical route for the conversion of tryptophan, yielding L-kynurenine (L-KYN) and ultimately several other metabolites, called kynurenines, which derive directly or indirectly from L-KYN. Kynurenines have been shown to be involved in various physiological and pathological processes. Alteration of KP metabolism is functionally significant and occurs in a variety of diseases of the central nervous system. The discovery of the importance of kynurenines in brain function under physiological and pathologic conditions has led to the identification of potential new drug targets exploiting the therapeutic potential of the pathway. Some of these compounds proved to provide neuroprotection in animal models of various human diseases which holds promise that their effectiveness will translate to the clinic in the future.

List of Abbreviations: EAA, excitatory amino acid; HD, Huntington's disease; IDO, indoleamine 2,3-dioxygenase ; KAT II KO, kynurenine-aminotransferase knockout; KP, The kynurenine pathway; KYNA, kynurenic acid; L-KYN, L-kynurenine; mNBA, meta-nitrobenzoylalanine; MS, Multiple sclerosis; NAD, nicotinamide adenine dinucleotide; NMDA, N-methyl-D-aspartate; oMBA, Ortho-methoxybenzoylalanine; PD, Parkinson's disease; QUIN, quinolinic acid; TDO, tryptophan dioxygenase; 3-HAO, 3-hydroxyanthranilate-oxygenase; 3-HK, 3-hydroxykynurenine

1 Introduction

The kynurenine pathway (KP) is a major route for the conversion of tryptophan, yielding L-kynurenine (L-KYN) and ultimately several other metabolites, called kynurenines, which are derived directly or indirectly from L-KYN (*Figure 5-1*). The metabolic cascade was originally known to be a source of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), two coenzymes of basic cellular processes, and only later it was discovered that some of its metabolites could exhibit neuromodulatory actions. Interest in the research of KP emerged as it turned out that two metabolites of the pathway, quinolinic acid (QUIN) and kynurenic acid (KYNA) exhibit activity at glutamate receptors (Stone and Perkins, 1981; Perkins and Stone, 1982). QUIN was shown to inhibit *N*-methyl-D-aspartate (NMDA) receptors, whereas KYNA proved to be a broad-spectrum antagonist of excitatory amino acid (EAA) receptors with particular high affinity to the glycine-coagonist site of the NMDA receptor (Kessler et al., 1989). Subsequently, studies from several laboratories have clarified the role of kynurenines in the brain function under physiological and pathological conditions. This has led to the identification of potential new drug targets for various neurological disorders.

2 L-Kynurenine and Neuroactive Metabolites of the Kynurenine Pathway

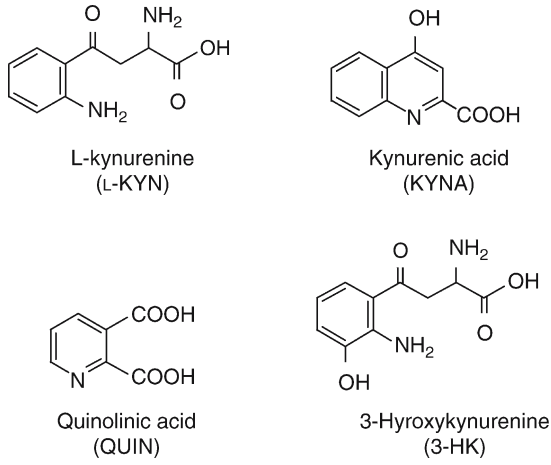
2.1 L-Kynurenine

L-KYN (*Figure 5-1*) is a major compound of the KP, serving as a source for the synthesis of all the other metabolites of the pathway. L-KYN is present in the blood, brain, and peripheral organs in low micromolar concentrations, and gets transported through the blood–brain barrier by the neutral amino acid carrier (Fukui et al., 1991). Although L-KYN does not directly influence neuronal function, systemic or intracerebral administration of it decreases blood pressure (Lapin, 1976) and evokes convulsions (Lapin, 1978, 1981, 1982; Pinelli et al., 1984), probably by getting converted to its neuroactive metabolites.

2.2 Kynurenic Acid

KYNA (*Figure 5-1*) was long known to be a side product of tryptophan metabolism but no particular biological function was assigned to it until more recently when in neurophysiological experiments it was shown to inhibit neurons (Perkins and Stone, 1982). Subsequently, KYNA was recognized as a broad-spectrum antagonist of ionotropic EAA receptors. Since EAA receptor activation takes place in a variety of

Figure 5-1
Chemical structure of L-kynurenine and neuroactive kynurenines



pathological states, KYNA has been tested as a neuroprotective agent. Indeed, at high concentrations, KYNA was shown to block excitotoxic damage and seizures induced by QUIN in rats (Foster et al., 1984) and to protect against various conditions like ischemia, traumatic brain injury (Germano et al., 1987; Andiné et al., 1988; Hicks et al., 1994; Salvati et al., 1999). Intracerebroventricular administration of KYNA was shown to dose-dependently evoke characteristic behavior in the rat: increased stereotypy and ataxia (Vécsei and Beal, 1990a, 1991). At lower concentrations, KYNA was recognized to act as a competitive antagonist at the glycine site of the NMDA receptor ($IC_{50} \cong 8$ μM) (Kessler et al., 1989) and as a noncompetive blocker at the α7-nicotonic receptor ($IC_{50} \cong 7$ μM) (Hilmas et al., 2001). These receptors could be the major sites of action of KYNA in the brain and thus endogenous KYNA could modify glutamatergic and cholinergic neurotransmission. Indeed, the level of KYNA seems to determine the vulnerability of the brain against excitotoxins, because intraperitoneal (i.p.) injection of amphetamine – which leads to reduction in the brain concentration of KYNA-potentiated quinolinate but not kainate excitotoxicity, and readjusting the level of KYNA to control levels by pharmacological manipulation of the KP, restored the vulnerability (Poeggeler et al., 1998; Rassoulpour et al., 2002). Furthermore, endogenous KYNA might influence glutamatergic neurotransmission presynaptically because modest elevations of KYNA in the rat striatum in vivo and in synaptosomes in vitro are able to inhibit glutamate release (Carpenedo et al., 2001).

Generation of kynurenine aminotransferase knockout (KAT II KO) mice – which have reduced brain KYNA levels early in development (<28 days) – has provided a useful tool for studying the role of KYNA in brain function and underlined the importance of endogenous KYNA in modulating glutamatergic and cholinergic neurotransmission. Intrastriatal injection of EAA receptor agonist QUIN induced significantly larger lesion in KAT II KO mice compared with wild-type mice (Sapko et al., 2003). Furthermore, the observation that KAT II KO mice have increased activity of α7-nicotonic receptor has proved the previous hypothesis that endogenous KYNA is an important regulator of α7-nicotonic receptor activity (Alkondon et al., 2003). The changes observed in the KAT II KO mice are evident early in development (<28 days), when cerebral KYNA levels are significanly decreased but tend to be reverted as the KYNA levels return to normal levels. These observations suggest that changes in endogenous KYNA levels have profound effects on the function of glutamatergic and cholineric receptors.

2.3 Quinolinic Acid

QUIN (*Figure 5-1*) is present in the brain in concentrations similar to that of KYNA (50–100 nmol). It has pronounced effects on neuronal activity being an agonist at the NMDA receptor (Stone and Perkins,

1981), preferentially activating the NR2A and NR2B NMDA receptor subtypes (de Carvalho et al., 1996). Besides acting at the NMDA receptor, QUIN produces toxic free radicals (Rios and Santamaria, 1991). Due to the compound's excitotoxic and free-radical-generating property, injection of QUIN into the rat striatum leads to excitotoxic damage (Schwarcz et al., 1983) duplicating the neurochemical features of Huntington's disease (HD) (Beal et al., 1986). In addition, long-term exposure to submicromolar concentrations of QUIN results in neuronal death in vitro (Whetsell and Schwarcz, 1989). In pathological states, like neuroinflammatory diseases, dramatic increases of its level in the brain could occur which could result in neuronal damage.

2.4 3-Hydroxykynurenine

No important physiological function in the brain has so far been assigned to 3-hydroxykynurenine (3-HK) (*Figure 5-1*), but in the primate lenses it could act as major UV filter together with its glucoside derivative, L-KYN and 4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid *O*-β-D-glucoside and may be useful in protecting the retina from UV radiation (Vazquez et al., 2002). However, the autooxidation of these metabolites has been proposed in the processes leading to opacification of the lens and cataract formation (Chiarugi et al., 1999). In the brain, 3-HK can cause neuronal death in cell cultures by generating toxic free radicals. 3-HK is present in nanomolar concentrations in the mammalian brain, but under pathological conditions similarly to QUIN its level could increase dramatically reaching the micromolar range (0.8–1.2 μM) (Eastman and Guilarte, 1989). Chronic exposure of neuronal cultures to these levels of 3-HK could cause neuronal death (Okuda et al., 1996). 3-HK should get into neurons to induce toxicity, because the inhibition of its uptake by large neutral amino acid prevents from neuronal death (Okuda et al., 1998). 3-HK potentiates QUIN toxicity in the rat striatum, which suggests that these metabolites may act in concert to induce neuronal damage (Guidetti and Schwarcz, 1999).

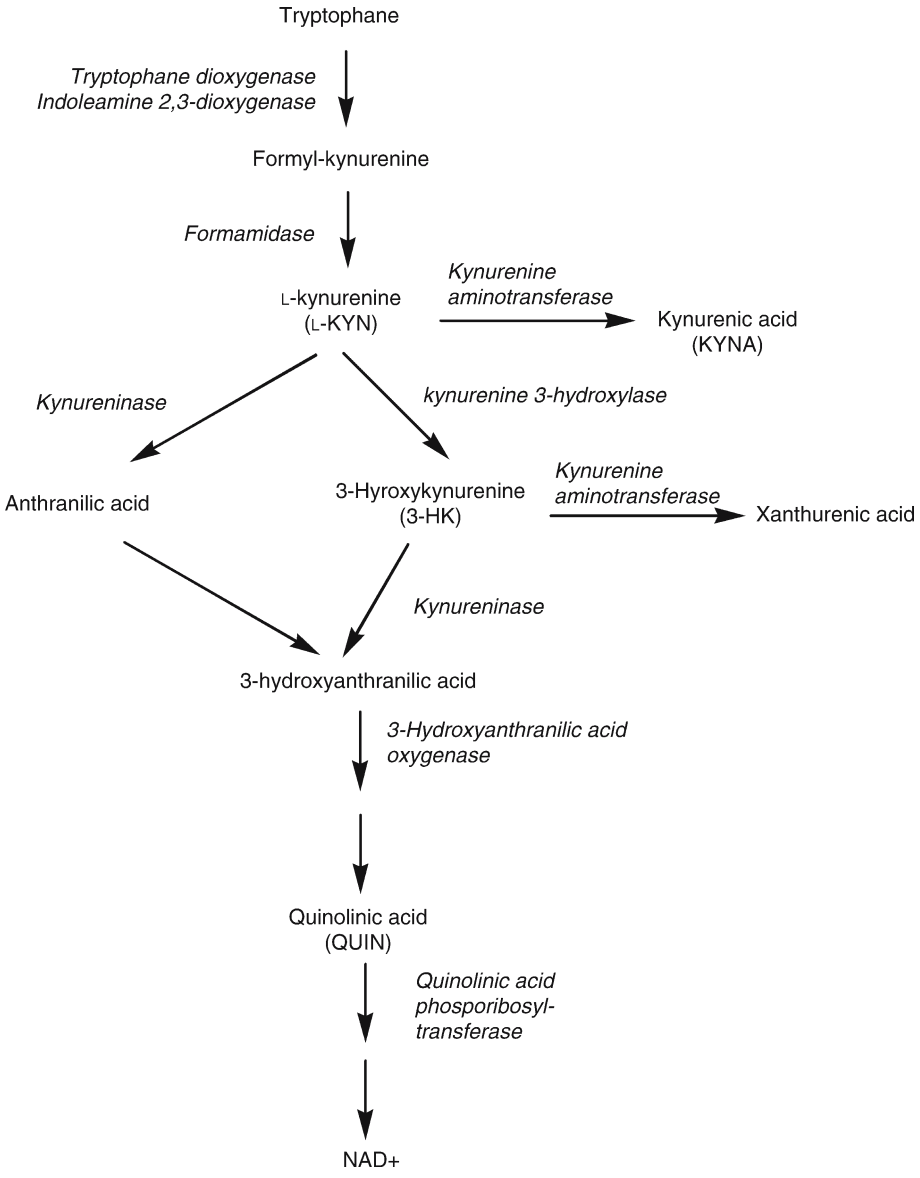
3 Enzymes of the Kynurenine Pathway

Figure 5-2 summarizes the enzymes and metabolites of the KP. The first and rate-limiting step in the formation of L-KYN is the conversion of L-tryptophan to formyl kynurenine, which is catalyzed by two distinct heme-containing enzymes. In peripheral tissues and particularly in the liver, *tryptophan dioxygenase* (TDO; tryptophan pyrrolase; EC 1.13.1.2.) is mainly responsible for yielding L-KYN but in most mammalian organs including intestine, lung, epididymis, placenta, central nervous system, reticuloendothelial system, *indoleamine 2,3-dioxygenase* (IDO; EC 1.13.11.42) is able to convert tryptophan to formyl kynurenine. In the second step, formyl kynurenine is rapidly metabolized into L-KYN by *formamidase*, an enzyme abundant in most mammalian organs (Mehler and Knox, 1950).

TDO is able to metabolize L- but not D-tryptophan (Schutz et al., 1972), whereas IDO not only acts on L-tryptophan, but it can also cleave the indole ring of the D-tryptophan, L- or D-5-hydroxytryptophan, indoleamins like 5-HT, tryptamine, and melatonine (Hirata and Hayaishi, 1975; Yoshida and Hayaishi, 1987). The gene encoding TDO contains glucocorticoid-responsive elements (Danesh et al., 1983, 1987) which explains that glucocorticoid administration increases TDO formation in the liver, which causes a decrease in blood tryptophan levels with L-KYN accumulation. The transcription of the gene of IDO is under tight immunological control, and it contains two interferon-stimulated response elements (ISRE) and at least one gamma-interferon-activated sequence (GAS) (Dai and Gupta, 1990; Tone et al., 1990). Interferons and proinflammatory cytokines stimulate (Carlin et al., 1989; Taylor and Feng, 1991), whereas other cytokines and growth factors, like interleukin-4 or TGF-beta, inhibit IDO expression and activity (Musso et al., 1994; Yuan et al., 1998).

L-KYN is the key player of the pathway serving as a substrate of several enzymes: *kynurenine 3-hydroxylase* (EC 1.14.13.9; yielding 3-HK), *kynureninase* (EC 3.7.1.3; yielding anthranilic acid), and *kynurenine aminotransferases* (KATs; yielding KYNA). *Kynurenine 3-hydroxylase* is present in the liver, placenta, spleen, kidney, and brain (Erickson et al., 1992) and requires NADPH and molecular oxygen

Figure 5-2
The kynurenine pathway



for the conversion of L-KYN to 3-HK. The enzyme has a high affinity for its substrate ($K_m \cong 1$ μM), implicating that it converts most of the available L-KYN under physiological conditions.

Kynureninase has been characterized from rodent liver, kidney, and spleen (Kawai et al., 1988). It is a pyridoxal phosphate-dependent enzyme that converts L-KYN and 3-HK into L-alanine and anthranilic or 3-hydroxyanthranilic acid, respectively. It has been shown that the affinity of the enzyme is tenfold higher for 3-HK than for L-KYN (K_m for L-KYN: 250 μM, K_m for 3-HK: 25 μM; Alberati-Giani et al., 1996; Toma et al., 1997).

3-Hydroxyanthranilate oxygenase (3-HAO, EC 1.13.11.6) has been purified from mammalian liver or kidney and it requires ferrous ions (Fe^{2+}) and sulfhydryl groups for its activity (Long et al., 1954; Decker et al., 1961; Koontz and Shiman, 1976). 3-HAO cleaves the benzene ring of 3-hydroxyanthranilate into α-amino-β-carboxymuconate-ε-semialdehyde, an unstable compound, which nonenzymatically gets transformed into QUIN. A portion of α-amino-β-carboxymuconate-ε-semialdehyde is metabolized by α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (EC 4.1.1.45) to produce picolinic acid or aminomuconic acids.

3-HAO has been shown to be present in the brain (Foster et al., 1986), and increased activity of the enzyme has been detected in various pathological states, like in the striatum of HD patients (Schwarcz et al., 1988a), in gerbil hippocampus after global ischemia (Saito et al., 1993), and in epileptic rats (Du et al., 1993). QUIN is metabolized by *quinolinic acid phosphoribosyltransferase* (QPRT; EC 2.4.2.19) yielding nicotinic acid mononucleotide and subsequent degradation products, including NAD^+. Since QPRT has a very low activity, the levels of QUIN in the extracellular space are essentially determined by its rate of synthesis.

KYNA is converted from L-KYN in the brain by two distinct KATs (Okuno et al., 1991; Guidetti et al., 1997). Arbitrarily termed KAT I and KAT II, these enzymes have substantially different pH optimum and substrate specificity. KAT I (EC 2.6.1.14; also called glutamine transaminase K) has an optimal pH of 9.5–10, whereas KAT II has a maximal activity in the neutral pH. KAT I prefers pyruvate as a cosubstrate and it is potently inhibited by glutamine. KAT II shows no preference for pyruvate, and it is not sensitive to inhibition by glutamine. These differences suggest that KAT II might be responsible for most part of the KYNA synthesis in the brain. Lesion and pharmacological studies have also confirmed that in most brain regions KYNA derives primarily from KAT II activity (Guidetti et al., 1997). Under pathological conditions, however, such as after exposure to mitochondrial toxins, a massive increase in KAT I immunostaining has been observed in the neurons in the affected brain areas (Csillik et al., 2002; Knyihár-Csillik et al., 1999). Both KATs have high affinity for their substrate (K_m in the millimolar range), suggesting that L-KYN bioavailability determines the rate for KYNA biosynthesis. Indeed, in experiments, when rat brain slices or human astrocyte cultures are exposed to L-KYN, the levels of KYNA increase linearly with L-KYN availability (Turski et al., 1989; Kiss et al., 2003).

No catabolic enzyme or cellular re-uptake system of KYNA exists in mammals (Turski et al., 1989); so KYNA is extruded from the brain by a probenecid-sensitive carrier system (Moroni et al., 1988). More recently, QUIN has been also shown to exit the brain by the same carrier system (Morrison et al., 1999).

All of the enzymes of the KP are detectable in the brain, but their activities are much lower than in the peripheral organs (Stone, 1993). KP enzymes are predominantly localized in glial cells according to immunocytochemical, lesion, and molecular biological studies (Guidetti et al., 1995; Schwarcz et al., 1996; Guillamin et al., 2001).

Astrocytes seem to produce most part of KYNA in the brain (Guillamin et al., 2001), whereas microglial cells are the primary source of metabolites of the QUIN branch of the pathway (Lehrmann et al., 2001).

4 Alterations of the KP under Pathological Conditions

Elucidation of the importance of the KP in brain function has facilitated extensive research, investigating the alterations of the pathway in various neurological disorders. In the literature, a recent extensive review is available on this topic (Stone, 2001). In a great number of diseases – especially in neuroimmunological disorders – dramatic increases in the level of QUIN have been detected, suggesting its role in the pathological processes. Whereas, in diseases with cognitive alterations, elevated levels of cerebral KYNA have been shown which could contribute to cognitive defects by inhibiting NMDA receptor function.

4.1 Diseases with Increased Cerebral QUIN Levels

Infection of the CNS with viruses, bacteria, parazites: Significant increases in QUIN levels in the CNS have been described in patients with AIDS–dementia complex (Heyes et al., 1991, 1998), Lyme borelliosis (Halperin and Heyes, 1992), in mice infected with Herpes simlex virus type 1, cerebral malaria, and

Toxoplasma gondii (Reinhard, 1998; Sanni et al., 1998; Fujigaki et al., 2002), in macaques with poliovirus infection and septicaemia (Heyes and Lackner, 1990; Heyes et al., 1992). Reinhard et al. (1994) listed many other infectious diseases in which alterations of the KP have been described.

Multiple sclerosis (*MS*): Experimental allergic encephalomyelitis is an autoimmune inflammatory disorder and serves as an animal model of human MS. The levels of neurotoxic kynurenine metabolites, 3-HK and QUIN, are elevated in the spinal cord of affected animals (Flanagan et al., 1995; Chiarugi et al., 2001). Since QUIN has been shown to be toxic to oligodendrocytes (Cammer, 2001), elevated levels of QUIN might contribute to the pathology of the disease.

Huntington's disease: QUIN has long been hypothesized to play an important role in HD, because intrastriatal injection of QUIN duplicates many of the distinct neuropathological features of the striatum of patients with HD (Schwarcz et al., 1983; Beal et al., 1996). However, no changes in QUIN levels in tissue samples have been detected in HD patients dying after a prolonged illness (Reynolds et al., 1998; Schwarcz et al., 1988b). Of potential interest is the finding that elevated cortical and striatal content of 3-HK and QUIN has been shown in early grade HD, suggesting a causal role of QUIN in nerve cell loss in HD (Guidetti et al., 2000, 2003).

Parkinson's disease (*PD*): 3-HK has been shown to be significantly increased in the putamen and substantia nigra in patients with PD, which could play a causal role in the neuronal loss in PD (Ogawa et al., 1992).

Ischaemia: Dramatic increases in cerebral QUIN levels have been observed several days after global ischemia in a gerbil model of the disease (Heyes and Nowak, 1990; Saito et al., 1993). The changes have been observed in brain areas exposed to ishemia, but not in areas with an uninterrupted blood supply (Saito et al., 1992).

Traumatic brain/spinal cord injury: Both in humans and in the animal model of the disease, massive increases (50-fold) in the level of QUIN in the CSF (Sinz et al., 1998) and in affected areas have been shown (Blight et al., 1995, 1997).

4.2 Diseases with Increased Cerebral KYNA Levels

Alzheimer's disease (*AD*): Increased level of KYNA has been measured in the putamen and caudate nucleus of AD patients, which could be responsible for the cognitive deficits seen in AD patients (Baran et al., 1999).

Down's syndrome: The cortical levels of KYNA have been shown to be elevated in the postmortem specimens of Down's syndrome patients that could play a causal role in the impaired memory and learning defects seen in these patients (Baran et al., 1996).

Schizophrenia: Elevated levels of KYNA have been detected in the prefrontal cortex (Brodmann area 9), but not in other cortical areas of schizophrenic patients (Schwarcz and Pellicciari, 2002). Increased KYNA levels may contribute to the hypofunction of glutamatergic neurotransmission that plays a critical role in schizophrenia (Tamminga, 1998).

5 Therapeutic Approaches based on Kynurenines

After KYNA was recognized as a broad-spectrum antagonist of EAA receptors, its neuroprotective potential has been investigated in a variety of disorders. KYNA itself poorly enters the brain due to its polar structure and lack of efficient transport across the blood–brain barrier, but if systematically given in high doses, it protects against anoxia (Simon et al., 1986), ischemia (Germano et al., 1987; Andiné et al., 1988; Salvati et al., 1999), traumatic brain injury (Hicks et al., 1994), and antagonizes the toxic effects of QUIN (Foster et al., 1984). There are several strategies to generate neuroprotective drugs that have superior therapeutic potency to KYNA.

One strategy to exploit the therapeutic potential of KYNA is to develop chemically related drugs with better bioavailability and higher potency on the glycine site of the NMDA receptor.

The second approach uses prodrugs of KYNA or its analogs, which readily penetrate the blood–brain barrier, and are hydrolyzed in the CNS to form active compounds.

Third way is manipulating the KP by administering compounds that block the activity of the KP enzymes. With this strategy, one can shift L-KYN metabolism to the KYNA or QUIN branch with the aim to inhibit or excite EAA receptors, respectively.

Recently, several reviews have been published on the therapeutic alternatives based on kynureninergic manipulations (Stone, 2000, 2001; Schwarcz and Pellicciari, 2002; Stone and Darlington, 2002).

5.1 KYNA Analogs

Several attempts have been made to use the KYNA structure to develop NMDA glycine site antagonists with better bioavailability and higher potency on the glycine site of the NMDA receptor. Substitution of KYNA with halogen atoms (7-chlorokynurenic acid and 5,7-dichlorokynurenic acid; Baron et al., 1990), replacement of the 4-hydroxy group of KYNA with amido substituents (MDL 100,748, L-689,560; Baron et al., 1992; Leeson et al., 1992), substitution with a phenyl or more complex lipophil groups at position 3 of the KYNA nucleus (MDL 104,653, L-701,324; Kulagowski et al., 1994; Bristow et al., 1996), or replacment of the six-membered nitrogen containing ring by a five-carbon ring (GV150526A or gavestinel; Glaxo, 1993) provided compounds that have superior neuroprotective abilities compared with KYNA. Gavestinel has already entered clinical trials but the Glycine Antagonist in Neuroprotection (GAIN) Americas trial, a randomized, double-blind placebo-controlled phase III trial has failed to show any benefit of gavestinel treatment in acute ischemic stroke patients (Sacco et al., 2001).

5.2 Prodrugs of KYNA and of KYNA Analogs

L-KYN readily penetrates the blood–brain barrier by the neutral amino acid transporter (Fukui et al., 1991) and serves as a precursor for the neuroprotectant KYNA in the brain. This explains that systemic administration of L-KYN has been shown to protect against cerebral ischemia or local injection of NMDA in neonatal rats (Nozaki and Beal, 1992) and to counteract pentylenetetrazol- and NMDA-induced seizures in mice (Vécsei et al., 1992), but only modest effects of L-KYN treatment havebeen observed on kainate-induced seizures in rats (Vécsei et al., 1990b). However, it should be noted that L-KYN gets converted not only to KYNA but also to 3-HK and QUIN which poses considerable limitations to L-KYN therapy and explains the poor efficacy of L-KYN treatment since systemical administration of L-KYN is not capable of selectively increasing the levels of the neuroprotectant KYNA. For this reason, more potent KYNA precursors have been searched that are not getting metabolized in the QUIN branch of the pathway.

L-4-Chlorokynurenine or 4,6-dichlorokynurenines are potent precursors that meet the previously mentioned criteria of not getting metabolized to QUIN. These compounds are converted to two NMDA glycine site antagonists, namely 7-chlorokynurenic acid and 5,7-dichlorokynurenic acid, respectively (Hokari et al., 1996). 4-Chlorokynurenine is also metabolized to 4-chloro-3-hydroxyanthranilic acid, which is a potent and selective inhibitor of 3-hydroxyanthranilic acid oxygenase, and thus the administration of 4-chlorokynurenine causes a reduction of QUIN synthesis besides inhibiting NMDA receptors. It penetrates into the brain easier than its metabolite7-chlorokynurenic acid, and provides several additional benefits compared with other glycine site NMDA receptor antagonists: it preferentially gets metabolized in brain areas where neurodegeneration takes place, allowing lower dosage of the drug (Lee and Schwarcz, 2001). 4-Chlorokynurenine diminishes brain damage induced by focal application of QUIN and malonate into the rat striatum and hippocampus (Guidetti et al., 2000; Wu et al., 1997) and inhibits convulsions and neurotoxicity after systemic application of kainate (Wu et al., 2002). The conversion of 4-chlorokynurenine to 7-chlorokynurenic acid has also been observed in human astrocytes, implicating that 4-chlorokynurenine therapy might be used in humans in diseases of excitotoxic origin (Kiss et al., 2003).

Another prodrug strategy uses D-glucose and D-galactose conjugates of KYNA and its analogs for allowing better penetration of these drugs into the brain, assuming that the conjugates are recognized by the glucose transporter facilitating their entry and get hydrolyzed in the brain to release the active

compound (Battaglia et al., 2000a; Bonina et al., 2000). Systemic administration of 7-chlorokynurenic acid-D-glucopyranos-6′-yl ester or 7-chlorokynurenic acid-D-glucopyranos-3′-yl ester has provided protection against seizures induced by NMDA in mice.

A glucosamine–kynurenic acid conjugate has also been synthesized which induced stereotypy and ataxia in freely moving rats and reduced the evoked excitatory postsynaptic potentials in rat motor cortical slices after intracerebroventricular administration (Füvesi et al., 2004). Further studies are under way to characterize the bioavailability and therapeutic efficacy of the glucosamine–kynurenic acid conjugate after systemic administration.

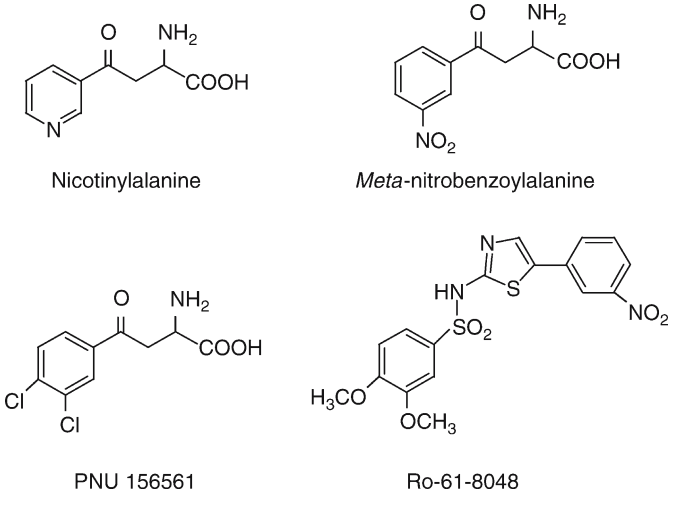
5.3 KP Enzyme Inhibitors

5.3.1 Manipulation of the KP with the Aim to Elevate the Cerebral Level of KYNA

Modulation of the KP by inhibiting enzymes of QUIN synthesis is a rational approach to divert the kynurenine metabolism toward the neuroprotective KYNA. This therapy would be particularly useful in clinical situations when excessive EAA receptor activation takes place. The administration of *kynurenine 3-hydroxylase* inhibitors is the most rational approach to evoke marked elevation in cerebral KYNA levels with concomitant attenuation of 3-HK and QUIN formation. While application of compounds inhibiting *kynureninase* and 3-hydroxyanthranilic acid oxygenase – enzymes that act downstream from the 3-HK – has limited therapeutic potency, these drugs cause an increase in 3-HK levels with modest KYNA elevations.

Kynurenine 3-hydroxylase inhibitors: The first drug reported to exhibit *kynurenine 3-hydroxylase* inhibiting activity was nicotinylalanine (Figure 5-3, Moroni et al., 1991), which is an analog of L-KYN. Subsequently, analogs with more potency and higher selectivity have been synthesized: *meta*-nitrobenzoylalanine (mNBA) is 1,000 times more active and by far more selective than nicotinylalanine (Figure 5-3, Pellicciari et al., 1994). Modification of the aromatic ring region and the side chain yielded (*R,S*)-3,4-dichlorobenzoylalanine (PNU 156561, formerly known as FCE 288833A), which showed further improvements in its potency and selectivity, compared with mNBA (Figure 5-3, Speciale et al., 1996). The

Figure 5-3
Chemical structure of selected kynurenine 3-hydroxylase inhibitors



screening of a library of sulfonamides led to the identification of 3,4-dimethoxy-*N*-[4-(3-nitrophenyl) thiazol-2-yl]benzenesulfonamide (IC_{50} = 37 nM, Ro-61-8048), which is currently the most potent non-competitive inhibitor of the *kynurenine 3-hydroxylase* (*Figure 5-3*, Röver et al., 1997).

Systemic administration of *kynurenine 3-hydroxylase* inhibitors results in inhibition of maximal electroshock-induced seizures in rats and audiogenic seizures in DBA/2 mice (Russi et al., 1992; Carpenedo et al., 1994) and provides protection in a rat focal ischemia and a gerbil global ischemia model (Cozzi et al., 1999). Furthermore, these drugs significantly reduce blood and brain accumulation of QUIN in immunostimulated mice, suggesting that the application of *kynurenine 3-hydroxylase* inhibitors may be of benefit to the patient with neuroimmunological disorders (Chiarugi and Moroni, 1999b).

Kynureninase inhibitors: These compounds inhibit the QUIN branch of the pathway downstream of 3-HK, which explains the limited therapeutic potency of these drugs compared with the *kynurenine 3-hydroxylase* inhibitors. Application of these drugs also results in accumulation of 3-HK in the brain besides elevating cerebral KYNA levels. *ortho*-Methoxybenzoylalanine (oMBA) is a prototype inhibitor of kynurenase, which is chemically related to mNBA but preferentially inhibits *kynureninase* (Pellicciari et al., 1994). Administration of oMBA results in an increase in the amount of KYNA in the brain in vivo and antagonizes audiogenic convulsion in DBA2 mice (Carpenedo et al., 1994; Chiarugi et al., 1995). Although oMBA is a selective inhibitor of *kynureninase* in vitro, it also inhibits 3-hydroxyanthranilic acid oxygenase in vivo, making it difficult to study the metabolic effects of *kynureninase* inhibitors in vivo (Chiarugi and Moroni, 1999a).

3-Hydroxyanthranilic acid oxygenase inhibitors: Halogenated substrate analogs, such as 4-chloro-3-hydroxyanthranilic acid, were the first potent, selective, and competitive blockers reported to inhibit 3-hydroxyanthranilic acid oxygenase (Todd et al., 1989; Walsh et al., 1991); but more recently, dihalogenated analogs of 3-hydroxyanthranilic acid have also been described with even higher potency and selectivity (Linderberg et al., 1999). These drugs reduce functional deficits in animals exposed to experimental spinal cord injury and reduce QUIN accumulation in the brain of traumatized animals (Blight et al., 1995) and in the blood and brain of immunoactivated mice (Saito et al., 1994).

5.3.2 KP Enzyme Inhibition with the Aim to Decrease Cerebral KYNA Levels

Since certain cognition enhancers have been shown to decrease KYNA levels in the brain (Poeggeler et al., 1998; Rassoulpour et al., 1998) and others to prevent the KYNA antagonism of the NMDA receptor (Pittaluga et al., 1995), one might hypothesize that KYNA plays an important role in cognitive processes and pharmacological inhibition of cerebral KYNA levels might be a useful strategy to produce cognition enhancer drugs. Furthermore, the cognitive deficits might be attenuated with the use of these drugs in diseases with elevated cerebral KYNA levels such as schizophrenia, Alzheimer's disease, or Down's syndrome.

KAT inhibitors: KAT II is responsible for producing large part of KYNA present in the brain that makes it a prime target for influencing cerebral KYNA levels. α-Aminoadipate, quisqualate, DL-5-bromocriptine, and certain metabotropic glutamate receptor agonists, such as L-(+)-2-amino-4-phosphonobutyric acid (L-AP4), 4-carboxy-3-hydroxyphenylglycine (4C3HPG), and L-serine-*o*-phosphate (L-SOP) selectively block KAT II in vitro (Battaglia et al., 2000b). However, all KAT II inhibitors known so far influence other cellular processes besides acting on the KP, rendering it difficult to characterize the behavioral consequences of decreased KYNA content in the brain. Synthesis of more selective KAT II inhibitors is essential to study the importance of KYNA in the brain function.

6 Conclusion

The discovery of the importance of kynurenines in brain function under physiological and pathologic conditions has led to the identification of potential new drug targets exploiting the therapeutic potential of the pathway. Some of these compounds proved to provide neuroprotection in animal models of various human diseases, which holds promise that their effectiveness will translate to the clinic in the future.

References

Alberati-Giani D, Buchli R, Malherbe P, Broger C, Lang G, et al. 1996. Isolation and expression of a cDNA clone encoding human kynureninase. Eur J Biochem 239: 460-468.

Alkondon M, Pereira, EFR, Fawcett WP, Randall WR, Guidetti P, et al. 2003. Endogenous kynurenic acid regulates alpha7 nicotinic receptor activity in the hippocampus. Program No. 248.9. Abstract Viewer/Itinerary Planner. Washington, D.C.: Society for Neuroscience.

Andiné P, Lehmann A, Ellren K, Wennberg E, Kjellmer I, et al. 1988. The excitatory amino acid antagonist kynurenic acid administered after hypoxic-ischemia in neonatal rats offers neuroprotection. Neurosci Lett 90: 208-212.

Baran H, Cairns N, Lubec B, Lubec G. 1996. Increased kynurenic acid levels and decreased brain kynurenine aminotransferase I in patients with Down syndrome. Life Sci 58: 1891-1899.

Baran H, Jellinger K, Derecke L. 1999. Kynurenine metabolism in Alzheimer's disease. J Neural Transm 106: 165-181.

Baron BM, Harrison BL, Miller FP, McDonald IA, Salituro FG, et al. 1990. Activity of 5,7-dichlorokynurenic acid, a potent antagonist at the NMDA receptor-associated glycine binding site. Mol Pharmacol 38: 554-561.

Baron BM, Harrison BL, McDonald IA, Meldrum BS, Palfreyman MG, et al. 1992. Potent indole- and quinoline-containing NMDA antagonists at the strychnine-insensitive glycine binding site. J Pharmacol Exp Ther 262: 947-956.

Battaglia G, La Russa M, Bruno V, Arenare L, Ippolito R, et al. 2000a. Systemically administered D-glucose conjugates of 7-chlorokynurenic acid are centrally available and exert anticonvulsant activity in rodents. Brain Res 860: 149-156.

Battaglia G, Rassoulpour A, Wu HQ, Hodgkins PS, Kiss C, et al. 2000b. Some metabotropic glutamate receptor ligands reduce kynurenate synthesis in rats by intracellular inhibition of kynurenine aminotransferase II. J Neurochem 75: 2051-2060.

Beal MF, Kowall NW, Ellison DW, Mazurek MF, Swartz KJ, Martin JB, et al. 1986. Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. Nature 321: 168-171.

Blight AR, Cohen TI, Saito K, Heyes MP. 1995. Quinolinic acid accumulation and functional deficits following experimental spinal cord injury. Brain 118: 735-752.

Blight AR, Leroy Jr EC, Heyes MP. 1997. Quinolinic acid accumulation in injured spinal cord: Time course, distribution, and species differences between rat and guinea pig. J Neurotrauma 14: 89-98.

Bonina FP, Arenare L, Ippolito R, Boatto G, Battaglia G, et al. 2000. Synthesis, pharmacokinetics and anticonvulsant activity of 7-chlorokynurenic acid prodrugs. Int J Pharm 202: 79-88.

Bristow LJ, Flatman KL, Hutson PH, Kulagowski JJ, Leeson PD, et al. 1996. The atypical neuroleptic profile of the glycine/*N*-methyl-D-aspartate receptor antagonist, L-701324, in rodents. J Pharmacol Exp Ther 277: 578-585.

Cammer W. 2001. Oligodendrocyte killing by quinolinic acid in vitro. Brain Res 896: 157-160.

Carlin JM, Ozaki Y, Byrne GI, Brown RR, Borden EC. 1989. Interferons and indoleamine 2,3-dioxygenase: Role in antimicrobial and antitumor effects. Experientia 45: 535-541.

Carpenedo R, Chiarugi A, Russi P, Lombardi G, Carla V, et al. 1994. Inhibitors of kynurenine hydroxylase and kynureninase increase cerebral formation of kynurenate and have sedative and anticonvulsant activities. Neuroscience 61: 237-244.

Carpenedo R, Pittaluga A, Cozzi A, Attucci S, Galli A, et al. 2001. Presynaptic kynurenate-sensitive receptors inhibit glutamate release. Eur J Neurosci 13: 2141-2147.

Chiarugi A, Moroni F. 1999a. Effects of mitochondria and *o*-methoxybenzoylalanine on 3-hydroxyanthranilic acid dioxygenase activity and quinolinic acid synthesis. J Neurochem 72: 1125-1132.

Chiarugi A, Moroni F. 1999b. Quinolinic acid formation in immune-activated mice: Studies with (*m*-nitrobenzoyl)-Alanine (mNBA) and 3,4-dimethoxy-[*N*-4-(3-nitrophenyl) thiazol-2-yl]benzenesulfonamide (Ro 61-8048), two potent and selective inhibitors of kynurenine hydroxylase. Neuropharmacology 38: 1225-1233.

Chiarugi A, Carpenedo R, Molina MT, Mattoli L, Pellicciari R, et al. 1995. Comparison of the neurochemical and behavioural effects resulting from the inhibition of kynurenine hydroxylase and/or kynureninase. J Neurochem 65: 1176-1183.

Chiarugi A, Rapizzi E, Moroni F, Moroni F. 1999. The kynurenine metabolic pathway in the eye: Studies on 3-hydroxykynurenine, a putative cataractogenic compound. FEBS Lett 453: 197-200.

Chiarugi A, Cozzi A, Ballerini C, Massacesi L, Moroni F. 2001. Kynurenine 3-mono-oxygenase activity and neurotoxic kynurenine metabolites increase in the spinal cord of rats with experimental allergic encephalomyelitis. Neuroscience 102: 687-695.

Cozzi A, Carpenedo R, Moroni F. 1999. Kynurenine hydroxylase inhibitors reduce ischaemic brain damage. Studies with (*m*-nitrobenzoyl)-alanine (mNBA) and 3,4-dimethoxy-[*N*-4-(nitrophenyl)thiazol-2-yl]benzenesulfonamide (Ro61-8048) in models of focal or global brain ischaemia. J Cereb Blood Flow Metab 19: 771-777.

Csillik A, Knyihár E, Okuno E, Krisztin-Peva B, Csillik B, et al. 2002. Effect of 3-nitropropionic acid on kynurenine aminotransferase in the rat brain. Exp Neurol 177: 233-241.

Dai W, Gupta SL. 1990. Molecular cloning, sequencing and expression of human interferon-gamma-inducible indoleamine 2,3-dioxygenase cDNA. Biochem Biophys Res Commun 169: 1-8.

Danesh U, Hashimoto S, Renkawitz R, Schutz G. 1983. Transcriptional regulation of the tryptophan oxygenase gene in rat liver by glucocorticoids. J Biol Chem 258: 4750-4753.

Danesh U, Gloss B, Schmid W, Schutz G, Schule R, et al. 1987. Glucocorticoid induction of the rat tryptophan oxygenase gene is mediated by two widely separated glucocorticoid-responsive elements. EMBO J 6: 625-630.

de Carvalho LP, Bochet P, Rossier J. 1996. The endogenous agonist quinolinic acid and the non endogenous homoquinolinic acid discriminate between NMDAR2 receptor subunits. Neurochem Int 28: 445-452.

Decker RH, Kang HH, Leach FR, Henderson LM, 1961. Purification and properties of 3-hydroxyanthranilic acid oxidase. J Biol Chem 236: 3076-3082.

Du F, Williamson J, Bertram E, Lothman EW, Okuno E, et al. 1993. Kynurenine pathway enzymes in a rat model of chronic epilepsy: Immunohistochemical study of activated glial cells. Neuroscience 55: 975-989.

Eastman CL, Guilarte TR. 1989. Cytotoxicity of 3-hydroxykynurenine in a neuronal hybrid cell line. Brain Res 495: 225-231.

Erickson JB, Reinhard JFJ, Flanagan EM, Russo S. 1992. A radiometric assay for kynurenine 3-hydroxylase based on the release of $^{3}H_2O$ during hydroxylation of L-[3.5-^{3}H]-kynurenine. Anal Biochem 205: 257-262.

Flanagan EM, Erickson JB, Viveros OH, Chang SY, Reinhard Jr JF, 1995. Neurotoxin quinolinic acid is selectively elevated in spinal cords of rats with experimental allergic encephalomyelitis. J Neurochem 64: 1192-1196.

Foster AC, Vezzani A, French ED, Schwarcz R. 1984. Kynurenic acid blocks neurotoxicity and seizures induced in rats by the related brain metabolite quinolinic acid. Neurosci Lett 48: 273-278.

Foster AC, White RJ, Schwarcz R. 1986. Synthesis of quinolinic acid by 3-hydroxyanthranilic acid oxygenase in rat brain tissue in vitro. J Neurochem 47: 23-30.

Fujigaki S, Saito K, Takemura M, Maekawa N, Yamada Y, et al. 2002. L-tryptophan-L-kynurenine pathway metabolism accelerated by Toxoplasma gondii infection is abolished in gamma interferon-gene-deficient mice: Cross-regulation between inducible nitric oxide synthase and indoleamine-2,3-dioxygenase. Infect Immun 70: 779-786.

Fukui S, Schwarcz R, Rapoport SI, Takada Y, Smith QR. 1991. Blood-barrier transport of kynurenines: Implications for brain synthesis and metabolism. J Neurochem 56: 2007-2017.

Füvesi J, Somlai C, Németh H, Varga H, Kis Z, et al. 2004. Comparative study on the effects of kynurenic acid and glucosamine–kynurenic acid. Pharmacol Biochem Behav 77: 95-102.

Germano IM, Pitts LH, Meldrum BS, Bartkowski HM, Simon RP. 1987. Kynurenate inhibition of cell excitation decreases stroke size and deficits. Ann Neurol 22: 730-734.

Glaxo SPA. 1993. Patent 2266091.

Guidetti P, Eastman CL, Schwarcz R. 1995. Metabolism of [5-3H]kynurenine in the rat brain in vivo: Evidence for the existence of a functional kynurenine pathway. J Neurochem 65: 2621-2632.

Guidetti P, Okuno E, Schwarcz R. 1997. Characterization of rat brain kynurenine aminotransferases I and II. J Neurosci Res 50: 457-465.

Guidetti P, Schwarcz R. 1999. 3-Hydroxykynurenine potentiates quinolinate but not NMDA toxicity in the rat striatum. Eur J Neurosci 11: 3857-3863.

Guidetti P, Wu HQ, Schwarcz R. 2000. In situ produced 7-chlorokynurenate provides protection against quinolinate- and malonate-induced neurotoxicity in the rat striatum. Exp Neurol 163: 123-130.

Guidetti P, Luthi-Carter RE, Augood SJ, Schwarcz R. 2003. Quinolinic acid levels are increased in early grade Huntington's disease. Program No. 208.7. Abstract Viewer/Itinerary Planner. Washington, D.C.: Society for Neuroscience.

Guillemin GJ, Kerr SJ, Smythe GA, Smith DG, Kapoor V, et al. 2001. Kynurenine pathway metabolism in human astrocytes: A paradox for neuronal protection. J Neurochem 78: 842-853.

Halperin JJ, Heyes MP. 1992. Neuroactive kynurenines in Lyme borreliosis. Neurology 42: 43-50.

Heyes MP, Lackner A. 1990. Increased cerebrospinal fluid quinolinic acid, kynurenic acid and L-kynurenine in acute septicemia. J Neurochem 55: 338-341.

Heyes MP, Nowak Jr TS. 1990. Delayed increases in regional brain quinolinic acid follow transient ischemia in the gerbil. J Cereb Blood Flow Metab 10: 660-667.

Heyes MP, Brew BJ, Martin A, Price RW, Salazar AM, et al. 1991. Quinolinic acid in cerebrospinal fluid and serum in HIV-1 infection: Relationhip to clinical and neurologic status. Ann Neurol 29: 202-209.

Heyes MP, Saito K, Jocobowitz D, Markey SP, Takikawa O, et al. 1992. Poliovirus induces indoleamine-2,3-dioxygenase and quinolinic acid synthesis in macaque brain. FASEB J 6: 2977-2989.

Heyes MP, Saito K, Lackner A, Wiley CA, Achim CL, et al. 1998. Sources of the neurotoxin quinolinic acid in the brain

of HIV-1 infected patients and retrovirus-infected macaques. FASEB J 12: 881-896.

Hicks RR, Smith DH, Gennarelli TA, McIntosh T. 1994. Kynurenate is neuroprotective following experimental brain injury in the rat. Brain Res 655: 91-96.

Hilmas C, Pereira EFR, Alkondon M, Rassoulpour A, Schwarcz R, et al. 2001. The brain metabolite kynurenic acid inhibits α7 nicotinic receptor activity and increases non-7 nicotinic receptor expression: Physiopathological implications. J Neurosci 21: 7463-7473.

Hirata H, Hayaishi O. 1975. Studies on Indoleamine 2,3-dioxygenase. Superoxide anion as substrate. J Biol Chem 250: 5960-5966.

Hokari M, Wu H-Q, Schwarcz R, Smith QR. 1996. Facilitated brain uptake of 4-chlorokynurenine and conversion to 7-chlorokynurenic acid. Neuroreport 8: 15-18.

Kawai J, Okuno E, Kido R. 1988. Organ distribution of rat kynureninase and changes of its activity during development. Enzyme 39: 181-189.

Kessler M, Terramani T, Lynch G, Baudry M. 1989. A glycine site associated with *N*-methyl-D-aspartic acid receptors: Characterization and identification of a new class of antagonists. J Neurochem 52: 1319-1328.

Kiss C, Ceresoli-Borroni G, Guidetti P, Zielke CL, Schwarcz R. 2003. Kynurenic acid production by human astrocytes. J Neural Transm 110: 1-14.

Knyihár-Csillik E, Okuno E, Vécsei L. 1999. Effects of in vivo sodium azide administration on the immunohistochemical localization of kynurenine aminotransferase in the rat brain. Neuroscience 94: 269-277.

Koontz WA, Shiman R. 1976. Beef kidney 3-hydroxyanthranilic acid oxygenase. J Biol Chem 251: 368-377.

Kulagowski JJ, Baker R, Curtis NR, Leeson PD, Mawer IM, et al. 1994. 3′-(Arylmethyl)- and 3′-(aryloxy)-3-phenyl-4-hydroxyquinolin-2(1H)-ones: Orally active antagonists of the glycine site on the NMDA receptor. J Med Chem 37: 1402-1405.

Lapin IP. 1976. Depressor effect of kynurenine and its metabolites in rats. Life Sci 19: 1479-1484.

Lapin IP. 1978. Stimulant and convulsive effects of kynurenines injected into brain ventricles in mice. J Neural Transm 42: 37-43.

Lapin IP. 1981. Kynurenines and seizures. Epilepsia 22: 257-265.

Lapin IP. 1982. Convulsant action of intracerebroventricularly administered kynurenine sulphate, quinolinic acid and other derivatives of succinic acid and effects of amino acids: Structure–activity relationships. Neuropharmacology 21: 1227-1233.

Lee SC, Schwarcz R. 2001. Excitotoxic injury stimulates prodrug-induced 7-chlorokynurenate formation in the rat striatum in vivo. Neurosci Lett 304: 185-188.

Leeson PD, Carling RW, Moore KW, Moseley AM, Smith JD, et al. 1992. 4-Amido-2-carboxytetrahydroquinolines. Structure–activity relationships for antagonism at the glycine site of the NMDA receptor. J Med Chem 35: 1954-1968.

Lehrmann E, Molinari A, Speciale C, Schwarcz R. 2001. Immunohistochemical visualization of newly formed quinolinate in the normal and excitotoxically lesioned rat striatum. Exp Brain Res 141: 389-397.

Linderberg M, Hellberg S, Björk S, Gotthammar B, Högberg T, et al. 1999. Synthesis and QSAR of substituted 3-hydroxyanthranilic acid derivatives as inhibitors of 3-hydroxyanthranilic acid dioxygenase (3-HAO). Eur J Med Chem 34: 729-744.

Long CL, Hill HN, Weinstock IM, Henderson LM. 1954. Studies on the enzymatic transformation of 3-hydroxyanthranilic acid to quinolinic acid. J Biol Chem 205: 411-413.

Mehler AH, Knox WE. 1950. Conversion of tryptophan to kynurenine in liver. II. The enzymatic hydrolysis of formylkynurenine. J Biol Chem 187: 431-433.

Moroni F, Russi P, Carla V, Lombardi G. 1988. Kynurenic acid is present in the rat brain and its content increases during development and aging processes. Neurosci Lett 94: 145-150.

Moroni F, Alesiani N, Galli A, Mori F, Pecorari R, et al. 1991. Thiokynurenates – a new group of antagonists of the glycine modulatory site of the NMDA receptor. Eur J Pharmacol 199: 227-232.

Morrison PF, Morishige GM, Beagles KE, Heyes MP. 1999. Quinolinic acid is extruded from the brain by a probenecid-sensitive carrier system: A quantitative analysis. J Neurochem 72: 2135-2144.

Musso T, Gusella GL, Brooks A, Varesio L. 1994. Interleukin-4 inhibits indoleamine 2,3-dioxygenase expression in human monocytes. Blood 83: 1408-1411.

Nozaki K, Beal MF. 1992. Neuroprotective effects of L-kynurenine on hypoxia-ischemia and NMDA lesions in neonatal rats. J Cereb Blood Flow Metab 12: 400-407.

Ogawa T, Matson WR, Beal MF, Myers RH, Bird ED, et al. 1992. Kynurenine pathway abnormalities in Parkinson's disease. Neurology 42: 1702-1706.

Okuda S, Nishiyama N, Saito H, Katsuki H. 1996. Hydrogen peroxide-mediated neuronal cell death induced by an endogenous neurotoxin, 3-hydroxykynurenine. Proc Natl Acad Sci USA 93: 12553-12558.

Okuda S, Nishiyama N, Saito H, Katsuki H. 1998. 3-Hydroxykynurenine, an endogenous oxidative stress generator, causes neuronal cell death with apoptotic features and region selectivity. J Neurochem 70: 299-307.

Okuno E, Nakamura M, Schwarcz R. 1991. Two kynurenine aminotransferases in human brain. Brain Res 542: 307-312.

Pellicciari R, Natalini B, Costantino G, Mahmoud MR, Mattoli L, et al. 1994. Modulation of the kynurenine pathway in search for new neuroprotective agents. Synthesis and preliminary evaluation of (*m*-nitrobenzoyl)alanine, a potent inhibitor of kynurenine 3-hydroxylase. J Med Chem 37: 647-655.

Perkins MN, Stone TW. 1982. An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. Brain Res 247: 184-187.

Pinelli A, Ossi C, Colombo R, Tofanetti O, Spazzi L. 1984. Experimental convulsions in rats induced by intraventricular administration of kynurenine and structurally related compounds. Neuropharmacology 23: 333-337.

Pittaluga A, Pattarini R, Raiteri M. 1995. Putative cognition enhancers reverse kynurenic acid antagonism at hippocampal NMDA receptors. Eur J Pharmacol 272: 203-209.

Poeggeler B, Rassoulpour A, Guidetti P, Wu HQ, Schwarcz R. 1998. Dopaminergic control of kynurenate levels and NMDA toxicity in the developing rat striatum. Dev Neurosci 20: 146-153.

Rassoulpour A, Wu HQ, Poeggeler B, Schwarcz R. 1998. Systemic D-amphetamine administration causes a reduction of kynurenic acid levels in rat brain. Brain Res 802: 111-118.

Rassoulpour A., Guidetti P., Kiss C., Schwarcz R. 2002. Amphetamine potentiates quinolinate but not kainate excitotoxicity in the rat striatum. Society for Neuroscience Abstract Viewer/Itinerary Planner. Program No. 92.6.

Reinhard Jr JF, Erickson JB, Flanagan EM. 1994. Quinolinic acid in neurological disease: Opportunities for novel drug discovery. Adv Pharmacol 30: 85-126.

Reinhard Jr JF. 1998. Altered tryptophan metabolism in mice with herpes simplex virus encephalitis: Increases in spinal cord quinolinic acid. Neurochem Res 23: 661-665.

Reynolds GP, Pearson SJ, Halket J, Sandler M. 1988. Brain quinolinic acid in Huntington's disease. J Neurochem 50: 1959-1960.

Rios C, Santamaria A. 1991. Quinolinic acid is a potent lipid peroxidant in rat brain homogenates. Neurochem Res 16: 1139-1143.

Röver S, Cesura AM, Hugenin P, Kettler R, Szente A. 1997. Synthesis and biochemical evaluation of *N*-(4-phenylthiazol-2-yl)benzenesulfonamides as high-affinity inhibitors of kynurenine 3-hydroxylase. J Med Chem 40: 4378-4385.

Russi P, Alesiani M, Lombardi G, Davolio P, Pellicciari R, et al. 1992. Nicotinylalanine increases the formation of kynurenic acid in the brain and antagonizes convulsions. J Neurochem 59: 2076-2080.

Sacco RL, De Rosa JT, Haley Jr EC, Levin B, Ordronneau P, et al. 2001. The glycine antagonist in neuroprotection Americas investigators. Glycine antagonist in neuroprotection for patients with acute stroke: GAIN Americas: A randomized controlled trial. JAMA 285: 1719-1728.

Saito K, Nowak Jr TS, Markey SP, Heyes MP. 1992. Delayed increases in kynurenine pathway metabolism in damaged brain regions following transient cerebral ischemia. J Neurochem 60: 180-192.

Saito K, Nowak TS Jr, Markey SP, Heyes MP. 1993. Mechanism of delayed increases in kynurenine pathway metabolism in damaged brain regions following transient cerebral ischemia. J Neurochem 60: 180-192.

Saito K, Markey SP, Heyes MP. 1994. 6-Chloro-D,L-tryptophan, 4-chloro-3-hydroxyanthranilate and dexamethasone attentuate quinolinic acid accumulation in brain and blood following systemic immune activation. Neurosci Lett 178: 211-215.

Salvati P, Ukmar G, Dho L, Rosa B, Cini M, et al. 1999. Brain concentrations of kynurenic acid after a systemic neuroprotective dose in the gerbil model of global ischaemia. Prog Neuropsychopharmacol Biol Psychiatry 23: 741-752.

Sanni LA, Thomas SR, Tattam BN, Moore DE, Chaudhri G, et al. 1998. Dramatic changes in oxidative tryptophan metabolism along the kynurenine pathway in experimental cerebral and noncerebral malari. Am J Pathol 152: 611-619.

Sapko MT, Yu P, Guidetti P, Pellicciari R, Tagle DA, et al. 2003. Endogenous brain kynurenic acid modulates susceptibility to striatal quinolinic acid excitotoxicity. Program No. 805.20. 2003. Abstract Viewer/Itinerary Planner. Washington, D.C.: Society for Neuroscience.

Schutz G, Chow E, Feigelson P. 1972. Regulatory properties of hepatic tryptophan oxygenase. J Biol Chem 247: 5333-5337.

Schwarcz R, Whetsell Jr WO, Mangano RM. 1983. Quinolinic acid: An endogenous metabolite that produces axon-sparing lesions in rat brain. Science 219: 316-318.

Schwarcz R, Okuno E, White RJ, Bird ED, Whetsell WO. 1988a. 3OH-anthranilic acid oxygenase activity is increased in the brains of Huntington disease victims. Proc Natl Acad Sci USA 85: 4079-4081.

Schwarcz R, Tamminga CA, Kurlan R, Shoulson I. 1988b. CSF levels of quinolinic acid in Huntington's disease and schizophrenia. Ann Neurol 24: 580-582.

Schwarcz R, Pellicciari R. 2002. Manipulation of brain kynurenines: Glial targets, neuronal effects, and clinical opportunities. J Pharmacol Exp Ther 303: 1-10.

Simon RP, Young RS, Stout S, Cheng J. 1986. Inhibition of excitatory neurotransmission with kynurenate reduces brain edema in neonatal anoxia. Neurosci Lett 71: 361-364.

Sinz EH, Kochanek PM, Heyes MP, Wisniewski SR, Bell MJ, et al. 1998. Quinolinic acid is increased in CSF and associated with mortality after traumatic brain injury in humans. J Cereb Blood Flow Metab 18: 610-615.

Speciale C, Wu HQ, Cini M, Marconi M, Varasi M, et al. 1996. (*R,S*)-3,4-dichlorobenzoylalanine (FCE 28833A) causes a large and persistent increase in brain kynurenic acid levels in rats. Eur J Pharmacol 315: 263-267.

Stone TW. 1993. Neuropharmacology of quinolinic and kynurenic acids. Pharmacol Rev 45: 309-379.

Stone TW. 2000. Development and therapeutic potential of kynurenic acid and kynurenine derivatives for neuroprotection. Trends Pharmacol Sci 21: 149-154.

Stone TW. 2001. Kynurenines in the CNS: From endogenous obscurity to therapeutic importance. Prog Neurobiol 64: 185-218.

Stone TW, Perkins MN. 1981. Quinolinic acid: A potent endogenous excitant at amino acid receptors in CNS. Eur J Pharmacol 72: 411-412.

Stone TW, Darlington LG. 2002. Endogenous kynurenines as targets for drug discovery and development. Nat Rev Drug Discov 1: 609-620.

Schwarcz R, Ceresoli G, Guidetti P. 1996. Kynurenine metabolism in the rat brain in vivo. Effect of acute excitotoxic insults. Adv Exp Med Biol 398: 211-219.

Tamminga CA. 1998. Schizophrenia and glutamatergic transmission. Crit Rev Neurobiol 12: 21-36.

Taylor MW, Feng G. 1991. Relationship between interferon gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. FASEB J 5: 2516-2522.

Todd WP, Carpenter BK, Schwarcz R. 1989. Preparation of 4-halo-3-hydroxyanthranilates and demonstration of their inhibition of 3-hydroxanthranilate oxygenase in rat and human brain tissue. Prep Biochem 19: 155-165.

Toma S, Nakamura M, Tone S, Okuno E, Kido R, et al. 1997. Cloning and recombinant expression of rat and human kynureninase. FEBS Lett 408: 5-10.

Tone S, Takikawa O, Habara-Ohkubo A, Kadoya A, Yoshida R, et al. 1990. Primary structure of human indoleamine 2,3-dioxygenase deduced from the nucleotide sequence of its cDNA. Nucleic Acid Res 18: 367.

Turski WA, Gramsbergen JB, Traitler H, Schwarcz R. 1989. Rat brain slices produce and liberate kynurenic acid upon exposure to L-kynurenine. J Neurochem 52: 1629-1636.

Urenjak J, Obrenovitch TP. 2000. Kynurenine 3-hydroxylase inhibition in rats: Effects on extracellular kynurenic acid concentration and *N*-methyl-D-aspartate-induced depolarisation in the striatum. J Neurochem 75: 2427-2433.

Vazquez S, Aquilina JA, Jamie JF, Sheil MM, Truscott RJ. 2002. Novel protein modification by kynurenine in human lenses. J Biol Chem 277: 4867-4873.

Vécsei L, Beal MF. 1990a. Intracerebroventricular injection of kynurenic acid, but not kynurenine, induces ataxia and stereotyped behavior in rats. Brain Res Bull 25: 623-627.

Vécsei L, Beal MF. 1990b. Influence of kynurenine treatment on open-field activity, elevated plus-maze, avoidance behaviors and seizures in rats. Pharmacol Biochem Behav 37: 71-76.

Vécsei L, Beal MF. 1991. Comparative behavioral and pharmacological studies with centrally administered kynurenine and kynurenic acid in rats. Eur J Pharmacol 196: 239-246.

Vécsei L, Miller J, MacGarvey U, Beal MF. 1992. Kynurenine and probenecid inhibit pentylenetetrazol- and NMDLA-induced seizures and increase kynurenic acid concentrations in the brain. Brain Res Bull 28: 233-238.

Walsh JL, Todd WP, Carpenter BK, Schwarcz R. 1991. 4-Halo-3-hydroxyanthanilic acids: Potent competitive inhibitors of 3-hydroxyanthranilic acid oxygenase in vitro. Biochem Pharmacol 42: 985-990.

Watanabe Y, Yoshida R, Sono M, Hayaishi O. 1981. Immunohistochemical localization of indoleamine 2,3-dioxygenase in the argyrophilic cells of rabbit duodenum and thyroid gland. J Histochem Cytochem 29: 623-632.

Whetsell Jr WO, Schwarcz R. 1989. Prolonged exposure to submicromolar concentrations of quinolinic acid causes excitotoxic damage in organotypic cultures of rat corticostriatal system. Neurosci Lett 97: 271-275.

Wu HQ, Salituro FG, Schwarcz R. 1997. Enzyme-catalyzed production of the neuroprotective NMDA receptor antagonist 7-chlorokynurenic acid in the rat brain in vivo. Eur J Pharmacol 319: 13-20.

Wu HQ, Lee SC, Scharfman HE, Schwarcz R. 2002. L-4-Chlorokynurenine attenuates kainate-induced seizures and lesions in the rat. Exp Neurol 177: 222-232.

Yoshida R, Hayaishi O. 1987. Indoleamine 2,3-dioxygenase. Methods Enzymol 142: 188-195.

Yuan W, Collado-Hidalgo A, Yufit T, Taylor MW, Varga J. 1998. Modulation of cellular tryptophan metabolism in human fibroblasts by transforming growth factor beta: Selective inhibition of indoleamine 2,3-dioxygenase and tryptophanyl-tRNA synthetase gene expression. J Cell Physiol 177: 174-186.