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Excitotoxicity of Quinolinic Acid: Modulation by Endogenous Antagonists

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Quinolinic acid (QUIN), a product of tryptophan metabolism by the kynurenine pathway, produces excitotoxicity by activation of NMDA receptors. Focal injections of QUIN can deplete biochemical markers for dopaminergic, cholinergic, gabaergic, enkephalinergic and NADPH diaphorase neurons, which differ in their sensitivity to its neurotoxic action. This effect of QUIN differs from that of other NMDA receptor agonists in terms of its dependency on the afferent glutamatergic input and its sensitivity to the receptor antagonists. The enzymatic pathway yielding QUIN produces metabolites that inhibit QUIN-induced neurotoxicity. The most active of these metabolites, kynurenic acid (KYNA), blocks NMDA and non-NMDA receptor activity. Treatment with kynurenine hydroxylase and kynureninase inhibitors increases levels of endogenous KYNA in the brain and protects against QUIN-induced neurotoxicity. Other neuroprotective strategies involve reduction in QUIN synthesis from its immediate precursor, or endogenous synthesis of 7-chloro-kynurenic acid, a NMDA antagonist, from its halogenated precursor. Several other tryptophan metabolites – quinaldic acid, hydroxyquinaldic acid and picolinic acid – also inhibit excitotoxic damage but their presence in the brain is uncertain. Picolinic acid is of interest since it inhibits excitotoxic but not neuroexcitatory responses. The mechanism of its anti-excitotoxic action is unclear but might involve zinc chelation.

Neurotoxic actions of QUIN is modulated by nitric oxide (NO). Treatment with inhibitors of NO synthase can augment QUIN toxicity in some models of excitotoxicity suggesting a neuroprotective potential of endogenous NO. In recent studies, certain nitroso compounds which could be NO donors have been reported to reduce the NMDA receptor-mediated neurotoxicity.

The existence of endogenous compounds which inhibit excitotoxicity provides a basis for future development of novel and effective neuroprotectants.

Keywords: Excitotoxicity, Kynurenic acid, Neuroprotection, Nitric oxide, Quinolinic acid

INTRODUCTION

The concept of excitotoxicity – over stimulation of excitatory amino acid (EAA) receptors on neuronal cell bodies leading to cell death – was developed by Olney (1969) on the basis of studies demonstrating that L-glutamate is highly toxic to the developing brain. In 1976, two independent studies by Coyle and Schwarcz and McGeer and McGeer showed that focal injections of kainic acid, now widely recognized as a non-NMDA EAA receptor agonist, produced axon-sparing lesions of rat striatum and yielded a pattern of

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neurotransmitter deficits resembling that seen in Huntington's disease (HD), an inherited neurodegenerative disorder involving marked striatal degeneration and choreiform movements. These and subsequent studies, demonstrating that activation of all ionotropic EAA receptor types – NMDA, AMPA and kainate – produced axon-sparing lesions in the central nervous system (CNS), led to the proposal that excitotoxicity may underlie the neurodegeneration in certain acute conditions such as hypoglycemia, ischemia anoxia, status epilepticus, and chronic neurodegenerative disorders such as HD, Parkinson's disease (PD), Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) (see Olney, 1990). The excitotoxicity in chronic neurodegenerative disease can be conceived to result from abnormal production of *endogenous* excitotoxins or increased sensitivity of specific neurons to normal levels of such substances and/or the EAA transmitters such as L-glutamate and L-aspartate. The excitotoxic hypothesis gained credance by the discovery that certain endogenous agents can indeed mimic the action of exogenous excitotoxins such as ibotenic acid or kainic acid. One such compound, quinolinic acid (QUIN), a pyridine dicarboxylic acid and tryptophan metabolite (Fig. 1), has been designated as an endogenous excitotoxin (Schwarcz *et al.*, 1983) and it has been implicated in the neuropathology of neurodegenerative (Beal *et al.*, 1986) and inflammatory disorders (Heyes, 1993) of the CNS.

The presence of QUIN and enzymes involved in the production or metabolism of QUIN have been demonstrated in different brain areas (Moroni *et al.*, 1984a; Wolfensberger *et al.*, 1983), and recently it has become feasible to alter levels of QUIN and other neuroactive metabolites by influencing the activity of enzymes that yield QUIN (Fig. 1). The distribution of QUIN in the CNS is uneven, and its concentrations increase with age (Moroni *et al.*, 1984b). Immunocytochemical studies on, 3-OH-anthranilate oxygenase (3-HAO), the enzyme catalysing QUIN production, have shown its predominant localization in glial cells (astrocytes). Similar

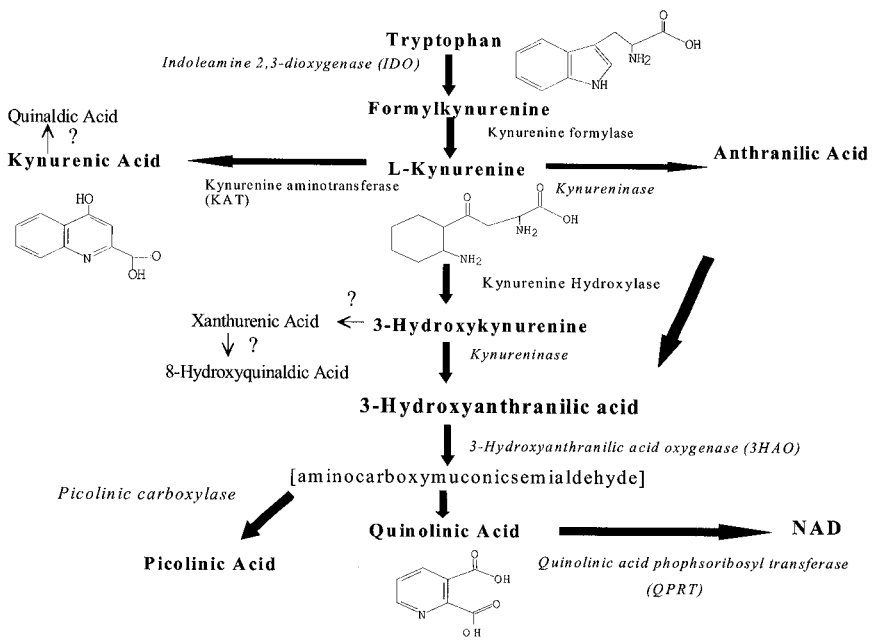


FIGURE 1 Representation of tryptophan metabolism by the kynurenine pathway.

observations have been made with quinolinic acid phosphoribosyltransferase (QPRTase) which degrades QUIN (Köhler *et al.*, 1987). This enzyme, localized to astrocytes and ependymal cells of the ventricles, may serve as safety mechanism that prevents QUIN from reaching toxic levels.

The tryptophan metabolism pathway yielding QUIN also produces several metabolites which act as EAA receptor antagonists and inhibit excitotoxicity. Kynurenic acid (KYNA), acts as a NMDA and non-NMDA receptor antagonist and inhibits the neurotoxic effects of QUIN and other EAA receptor agonists. Thus, endogenous KYNA may function as a modulator of EAA receptor- mediated physiological and pathological responses in the CNS, and augmentation of its levels may limit the excitotoxicity-based damage. Indeed, recent studies have yielded agents that increase the content of brain KYNA and afford protection against this type of damage.

NEUROTOXIC ACTIONS OF QUIN

The neuroactive property of QUIN was first identified by Lapin (1978, 1982) who discovered that its intracerebroventricular injection provoked seizures in rodents. Subsequently, electrophysiological studies of Stone and Perkins (1981) demonstrated that QUIN activates NMDA receptors and produces excitation in different brain areas. In 1983, Schwarcz *et al.* demonstrated the excitotoxic potential of QUIN by showing its ability to produce axon-sparing lesions in the rat brain. They reported depletion of cholineacetyltransferase (ChAT) activity, a marker for cholinergic neurons, following intrastriatal QUIN injections and inhibition of this effect by an NMDA receptor antagonist. A number of subsequent studies, including those from this laboratory, have shown that focal injections of QUIN in the rodent brain produce loss of histochemical and biochemical markers for specific neurotransmitters and induce specific behavioural deficits (see Jhamandas *et al.*, 1994). Thus, QUIN injections into the nucleus basalis deplete cholinergic markers in the cortex and amygdala (Cockhill *et al.*, 1992; El-Defrawy *et al.*, 1985), and similar injections in the substantia nigra produces loss of dopaminergic markers in the striatum (Connop *et al.*, 1995). Animals bearing excitotoxic lesions of the cholinergic and dopaminergic pathways show deficits in memory (Wirsching *et al.*, 1989) and motor behaviour (Beninger *et al.*, 1994), respectively. The nigrostriatal dopaminergic neurons (Connop *et al.*, 1995) show a greater vulnerability than the forebrain cholinergic neurons to the action of QUIN (Jhamandas *et al.*, 1990). The nigral neurons are surrounded by astrocytes containing enzymes of the kynurenine pathway (Roberts *et al.*, 1994). This structural characteristic thus provides an anatomical basis for the modulation of nigral dopamine cells by QUIN or other tryptophan-derived metabolites, and it could explain the high vulnerability of these neurons to its excitotoxic action (Connop *et al.*, 1995). Whether the neuronal populations that differ in their sensitivity to QUIN, also differ with respect to their sensitivity to effects of the antagonist metabolites co-generated with this excitotoxin is not clear. In animals, intrastriatal QUIN has been found to very largely replicate the loss of gabaergic, cholinergic and substance P neurons that occurs in HD (Beal *et al.*, 1986). The striatal NADPH diaphorase-containing interneurons are relatively spared in HD, and according to an early report this also applies to the QUIN model of striatal neurotoxicity, (Beal *et al.*, 1986). However, subsequent studies have revealed a severe depletion of such neurons in this model (Boegman *et al.*, 1987b; Davies and Roberts, 1987; Kalisch *et al.*, 1999). The discrepant findings obtained in the QUIN model have been explained on basis of methodological differences in assessment of the neuron loss (Roberts *et al.*, 1993).

The toxic effects of QUIN differ in certain respects from that of other excitotoxins – ibotenic acid and NMDA – that also target the NMDA receptor. The full expression of QUIN

neurotoxicity depends on the presence of an intact glutamatergic input to the target neuron (see Schwarcz *et al.*, 1984), a characteristic that is shared by kainic acid but not by other toxins (NMDA or ibotenate) acting on the NMDA receptor. Additionally, neurotoxic actions of QUIN and NMDA differ in sensitivity to the inhibitory effect of NMDA receptor antagonists, a finding that suggests activation of different NMDA receptor populations by the two toxins (Pawley *et al.*, 1996; Winn *et al.*, 1991). It has also been suggested that QUIN itself exerts two separate actions: a direct agonist action on the NMDA receptor, and a positive modulatory action on both the NMDA and non-NMDA receptors (Schurr *et al.*, 1991; Schurr and Rigor, 1993). Indeed, certain QUIN analogs, such as 6-methyl-QUIN, are reported to exert only the modulatory action: enhancing the acute toxicity (Schurr *et al.*, 1991). Given the differences between QUIN and other neurotoxins, it may be feasible to selectively influence the QUIN-based pathology without globally affecting the diverse functions associated with brain NMDA receptors.

QUIN has been implicated as a factor in the pathology associated with inflammatory disorders of the CNS (Heyes, 1993). It is present in immune cells (Moffett *et al.*, 1993) and immune stimulation markedly augments production of QUIN in the CNS (Heyes *et al.*, 1988). High levels of QUIN have been detected in the brain as well as cerebrospinal fluid of subjects with HIV infections (Heyes, 1989) and it has been proposed that this agent plays a role in AIDS-associated damage to the CNS. Levels of QUIN in brains of patients with AD (Moroni *et al.*, 1986) and HD (Heyes *et al.*, 1991) are not changed, however, such changes may be highly localized or may have preceded the onset of pathology. The role of endogenous QUIN in the production of neurotoxic damage in inflammatory or degenerative diseases of the CNS is not proven, however, factors such as its presence in the CNS, the ability to produce NMDA receptor-mediated excitotoxic responses in animal models and to augment the deleterious effects of hypoxia (Schurr and Rigor, 1993), suggest that it likely contributes to the process of neuronal damage.

ENDOGENOUS ANTI-EXCITOTOXIC AGENTS

Remarkably, the kynurenine pathway-producing QUIN, the NMDA receptor agonist, also yields KYNA, the NMDA and non-NMDA receptor antagonist. At the NMDA receptor, KYNA blocks the glycine site that facilitates receptor function (Kessler *et al.*, 1989) and its synthetic analogue, 7-chloro-kynurenic acid, exhibits both a greater potency and selectivity at this site (Kemp *et al.*, 1988). In neurotoxicity experiments, KYNA exerts a differential action against the NMDA receptor-based excitotoxins, being more potent against QUIN than against ibotenic acid or NMDA (Foster *et al.*, 1984; Winn *et al.*, 1991). This observation has been interpreted as evidence for the existence of NMDA receptor populations, at which KYNA shows preference for the QUIN-sensitive receptor subtype. We have observed that KYNA attenuates the neurotoxic action of QUIN (Boegman *et al.*, 1985) as well as that of ibotenic and kainic acid but not quisqualic acid (Cockhill *et al.*, 1992). Thus, although widely regarded as a non-specific EAA antagonist, in the neurotoxicity experiments KYNA exhibits a measure of selectivity against ligands targeting NMDA and non-NMDA receptors. While KYNA, like QUIN, does not readily cross the blood–brain barrier (Schwarcz, 1993), large systemic doses of the antagonist have been reported to impair the physiological and neurotoxic responses produced by EAA receptor activation (Salvati *et al.*, 1999; Scharfman and Goodman, 1998). There appears to be no apparent reuptake or enzymatic metabolism of KYNA, and its efflux from the brain occurs through a mechanism that is sensitive to probenecid. The latter therefore has been employed in experimental studies on endogenous neuroprotection to promote retention of KYNA in the brain (see below).

Brain KYNA is synthesized from L-kynurenine (which crosses the blood–brain barrier) by the enzyme kynurenine aminotransferase (KAT). The latter is evenly distributed in different brain areas (Okuno *et al.*, 1991), and, like other enzymes of the kynurenine pathway, is predominantly localized in astrocytes (Du *et al.*, 1992). The KYNA produced in astrocytes is detectable in the extracellular fluid, as revealed by brain slice experiments and by *in vivo* brain microdialysis studies (Speciale *et al.*, 1990; Turski *et al.*, 1989; Wu *et al.*, 1992). However, unlike classical neurotransmitters, the endogenous KYNA is not released in response to depolarizing stimuli. In the striatal microdialysis experiments, focal application of EAA agonists (NMDA, QUIN or kainate) also does not release KYNA to the extracellular space (Swartz *et al.*, 1990). Administration of the precursor, L-kynurenine, can increase extracellular KYNA, a response that is due to the enzymatic synthesis of the antagonist since it is blocked by non-selective aminotransferase inhibitors (Swartz *et al.*, 1990). The extracellular levels of KYNA increase following excitotoxic lesions, possibly due to astrocytosis accompanying such lesions (Turski *et al.*, 1989; Wu *et al.*, 1992). Recent studies by Schwarcz and colleagues have revealed an important link between energy metabolism and production of KYNA in the brain. Energy depletion significantly lowers production of brain KYNA (Hodgkins and Schwarcz, 1998a,b; Hodkins *et al.*, 1999). This finding is of considerable interest since defects in energy metabolism are considered to play a key role in neurodegenerative phenomena (Beal, 1998). The neuron loss associated with energy deficits has been linked to NMDA receptor activation. Thus, a lowered production of the endogenous antagonist and an increase in sensitivity to QUIN under conditions of hypoxia (Schurr and Rigor, 1993) may contribute to overactivity of NMDA receptors and produce excitotoxic cell death.

The exact significance of endogenous KYNA in the brain remains uncertain, but considering its ability to block several EAA receptor types, its strategic location in cells surrounding neurons and co-production with an excitotoxin, it is highly likely that this antagonist metabolite acts as a shield against devastating consequences of excessive EAA receptor activity. The excitotoxic lesions of neostriatum in the mature (Ceresoli-Borroni *et al.*, 1999) or developing brain (Ceresoli *et al.*, 1994) are associated with an increased brain KYNA, a response that may be produced to limit the manifestations of neuronal injury. Decreases in the content endogenous brain KYNA have been noted under conditions of hypoxia (Gramsbergen *et al.*, 1997), and in HD (Beal *et al.*, 1990; Jauch *et al.*, 1995), and these possibly predispose neurons to the EAA receptor-based damage. However, a causal link between the decline in brain KYNA levels and neuronal pathology has not been established, although experimental evidence obtained with agents that lower the antagonist levels points to such a link (see below). In recent years, there has thus been interest in the idea that procedures which augment the production of endogenous brain KYNA or reduce the production of QUIN may protect neurons against excitotoxic insults.

ENDOGENOUS NEUROPROTECTION

The existence of a metabolic pathway co-generating an excitotoxin (QUIN) and its antagonist (KYNA) from a common precursor, L-kynurenine, suggests that a critical balance between these opposing factors may be an important determinant of the neuronal viability. Recently, attempts have been made to promote brain KYNA synthesis from its precursor by blocking its conversion to QUIN (Fig. 2). An increase in KYNA production thus would increase its extracellular levels, prevent activation of the neuronal NMDA and non-NMDA receptors, and inhibit the excitotoxic response. A novel variant of this strategy involves endogenous synthesis of 7-chloro-kynurenic acid, the KYNA analogue which is not a brain constituent and lacks affinity for non-NMDA

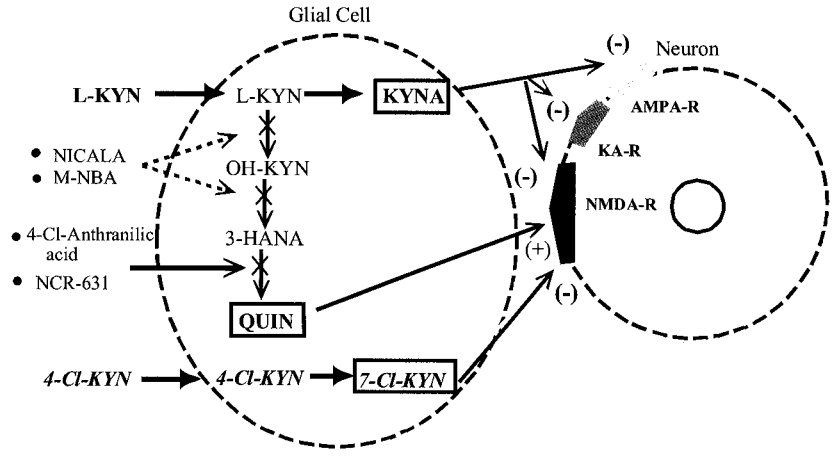


FIGURE 2 Neuroprotection strategies based on pharmacological manipulation of the kynurenine pathway of tryptophan metabolism. *Production of endogenous kynurenic acid (KYNA):* The enzymatic conversion of the tryptophan metabolite, L-kynurenine (L-KYN) to 3-hydroxykynurenine (OH-KYN), 3-hydroxyanthranilinic acid (3-HANA) and quinolinic acid (QUIN), and to the excitatory amino receptor antagonist, KYNA, occurs primarily in glial cells. QUIN activates NMDA receptor on neurons and produces toxicity. KYNA inhibits the neurotoxic response mediated by activity of the NMDA, kainate (KA-R) and AMPA receptor (AMPA-R). Nicotinylalanine (NICALA) or m-nitrobenzoylalanine (M-NBA) inhibit activity of enzymes producing QUIN and this diverts L-kynurenine to KYNA synthesis. The increase in endogenous KYNA production blocks receptor activity and inhibits neurotoxicity. *Inhibition of QUIN production:* Blockade of the enzyme activity converting 3-hydroxyanthranilinic acid (3-HANA) to QUIN by 4-chloro-anthranilinic acid or NCR-631, reduces the production of the excitotoxin and inhibits toxicity. *Endogenous synthesis of 7-chloro-kynurenic acid (7-Cl-KYN), in the brain:* Administration of the halogenated precursor, 4-chloro-kynurenine (4-Cl-KYN), a structural analogue of L-kynurenine, produces 7-chloro-kynurenic acid, *the NMDA receptor-selective antagonist*, via activity of enzymes that normally catalyse KYNA production. The antagonist synthesized in the brain blocks activation of NMDA receptors and inhibits neurotoxicity.

receptors. The approach taken involves administration of the halogenated precursor, 4-chloro-kynurenine, to generate 7-chloro-kynurenic acid in the brain via the pathway that normally convert L-kynurenine to KYNA (Fig. 2). Several recent studies have been undertaken to determine whether production of these antagonists in the brain indeed translates into neuronal protection against the damage produced by EAA receptor activation.

Endogenous KYNA-based Neuroprotection

Increases in the brain KYNA content can be achieved with inhibitors of the two enzymes, L-kynurenine 3-hydroxylase and kynureninase, whose activity contributes to the production of QUIN (Fig. 1). The first such inhibitor, nicotinylalanine (NICALA), a kynurenine analogue introduced by Decker *et al.* (1963), was successfully demonstrated to increase brain KYNA content and to produce sedative and anticonvulsant effects in rats (Connick *et al.*, 1992; Russi *et al.*, 1992). These findings suggested that NICALA may have a neuroprotectant action and we evaluated this possibility, in two *in vivo* models of excitotoxin-induced damage – the nigrostratial dopamine model (Miranda *et al.*, 1997) and the striatal NADPH-diaphorase and GABA neuron model (Harris *et al.*, 1998) – using the strategy represented in Fig. 3. In the nigrostriatal model, intraventricular NICALA prevented loss of the markers of dopaminergic neurons – striatal tyrosine hydroxylase (TH) activity and nigral TH immunostaining – produced by intranigral injections of QUIN or NMDA. The NICALA treatment also attenuated deficits of motor function associated with the dopamineregic cell loss (Miranda *et al.*, 1999). The protective effect of NICALA coincided with peak brain levels of

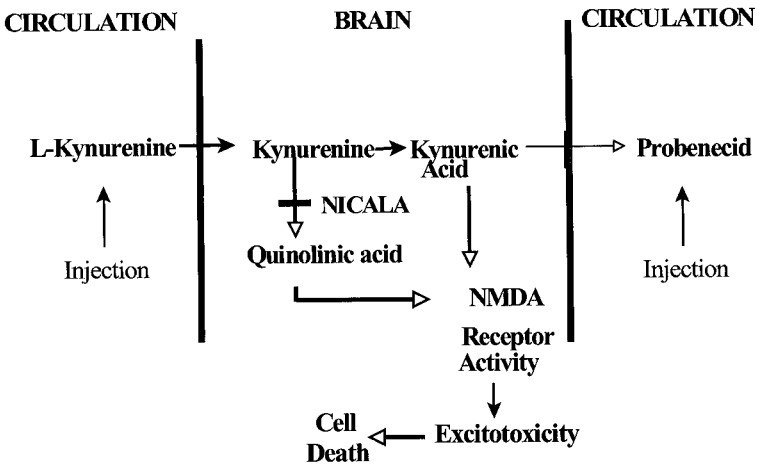


FIGURE 3 Experimental strategy for inhibition of excitotoxic damage by the endogenous kynurenic acid (KYNA). The blockade of enzymes converting L-kynurenine to quinolinic acid by nicotinylalanine promotes KYNA synthesis in the brain. The systemic administration of L-kynurenine augments production of KYNA, and probenecid inhibits its egress from the brain. Endogenous KYNA blocks the NMDA receptor and thus prevents excitotoxic action of quinolinic acid.

KYNA and was not observable after a decline of the antagonist levels. Similarly, the QUIN-induced loss of the NADPH diaphorase or GABA neurons in the striatal model was significantly reduced by pretreatment with intraventricular NICALA (Harris *et al.*, 1998). We have observed that systemic administration of NICALA also inhibits QUIN-induced toxicity (unpublished results). Although studies demonstrating the NICALA effects entailed use of L-kynurenine to increase precursor availability for the KYNA synthesis, and probenecid to prevent its egress from the brain, the co-treatments alone were not neuroprotective, indicating the importance of the enzymatic inhibition by NICALA. To further validate this approach we have recently examined the effects of NICALA analogues (Fig. 4) in the dopaminergic model of neurotoxicity. When given intraventricularly, these agents increased the content of KYNA in the rat brain to varying degrees (Fig. 5). The QUIN-induced dopaminergic neuron loss, as reflected by depletion of the striatal tyrosine hydroxylase activity, was attenuated by NICALA and the analogue, 2-amino-4-hydroxy-4-(3′pyridyl) butyric acid, which produced a high peak level of the KYNA (Fig. 6).

Recent experimental studies have also revealed the potential of the endogenous KYNA-based strategy to influence neuronal loss produced by cerebral ischemia. Two systemically injected L-kynurenine hydroxylase inhibitors – m-nitrobenzoylalanine (mNBA) and 3,4-dimethoxy-[nitrophenyl]-benzenesulfonamide (Ro 61-8048) – were reported to increase extracellular KYNA levels and inhibit hippocampal neuron loss in the gerbil ischemia model (Cozzi *et al.*, 1999). The neuroprotective effect of these agents, as well as their ability to reduce locomotor activity and audiogenic seizures, has been attributed to EAA receptor blockade by endogenous KYNA (Carpenedo *et al.*, 1994; Chiarugi *et al.*, 1995). As the systemic use of these enzyme inhibitors would block both the peripheral and central metabolism of L-kynurenine, both pools of this precursor likely contribute to elevation of the brain KYNA levels.

If KYNA present in the brain indeed represents a physiological restraint against the EAA receptor-based neurotoxicity, then a decrease in its synthesis predictably should precipitate toxicity. Currently, pharmacological agents that specifically inhibit activity of KAT, the aminotransferase

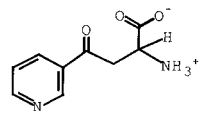
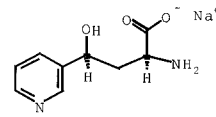
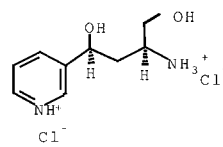
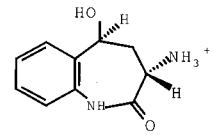
Compound	Chemical Name	Structure
Nicotinylalanine (NICALA)	4-(3'-pyridyl)-4-oxo-2-aminobutyrate	
Analog #1 (AN#1)	2-amino-4-hydroxy-4-(3'-pyridyl) butyric acid	
Analog #2 (AN#2)	2-amino-1,4 dihydroxy-4-(3'-pyridyl) butane dihydrochloride	
Analog #3 (AN#3)	(3S,5RS)-3-amino-5-hydroxy-2-oxo-[1H]-1-benzazepine	

FIGURE 4 Structural representation of nicotinylalanine and related agents that influence brain kynurenic acid.

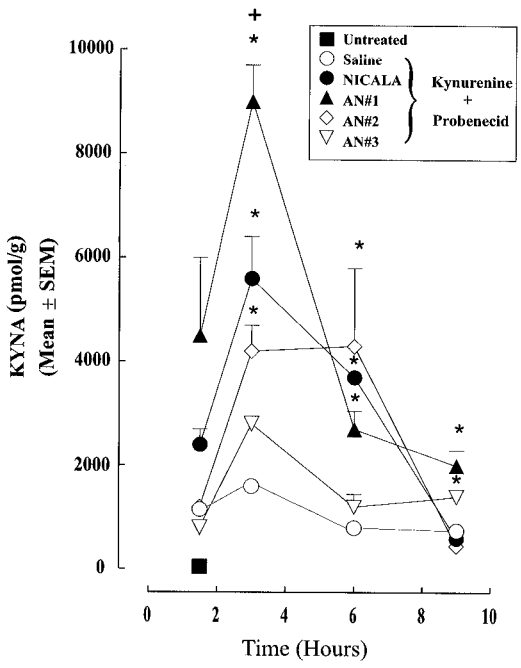


FIGURE 5 Elevation of brain kynurenic acid (KYNA) content by intraventricular injection (5.6 nmol/5 μl) of nicotinylalanine (NICALA) and related agents (see Fig. 4). All agents were administered in combination with systemic L-kynurenine and probenecid. *Value is significantly higher than that obtained after saline treatment ($p < 0.01$) (see Miranda *et al.*, 1997 for experimental details).

that catalyses KYNA synthesis, are unavailable, but administration of non-specific aminotransferase inhibitors indeed produces axon-sparing lesions in the brain (McMaster *et al.*, 1993). This toxic response likely results from a diversion of L-kynurenine from KYNA to QUIN synthesis and consequent activation NMDA receptors since it was attenuated by NMDA receptor antagonism. However, the toxicity could also result from unrestrained release of EAA transmitters due to a decrease in KYNA production. This possibility is suggested by the fact that the NMDA (Bustos *et al.*, 1992) and kainate activity stimulates brain glutamate release (Vrooman *et al.*, 1993; Young *et al.*, 1988) and endogenous KYNA may function as an inhibitory modulator of this release. Reductions in endogenous KYNA would compromise its inhibitory function and provoke unrestrained brain glutamate release and excitotoxicity. The extent to which these different mechanisms contribute to the toxicity of non-selective aminotransferase inhibitors is not clear, and potential involvement of some other unrelated action in this toxicity is arguable. However, an association between reductions in the brain KYNA levels

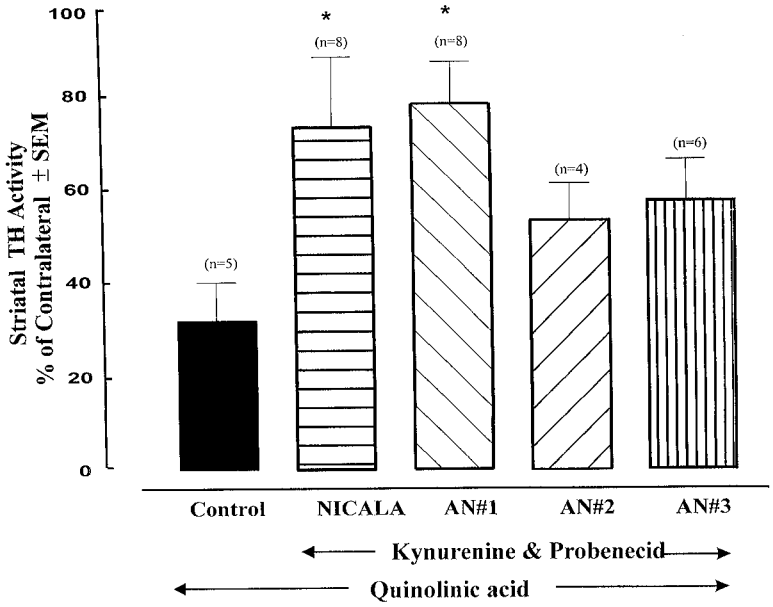


FIGURE 6 The effects of intraventricular nicotinylalanine and related agents (see Figs. 4 and 5) on the depletion of the rat striatal tyrosine hydroxylase (TH) activity produced by an intranigral infusion of quinolinic acid (60 nmol/0.5 µl). L-kynurenine and probenecid were administered by systemic route. The TH activity is expressed as per cent of activity in the contralateral (uninjected) striatum in the same animal. *Value is significantly greater than that in the saline group ($p < 0.05$) (see Miranda *et al.*, 1997 for experimental details).

and the production of an excitotoxic response favours the notion that this antagonist metabolite functions as a physiological neuroprotectant in the brain.

Reduction of Endogenous QUIN

An alternate neuroprotection strategy involves reduction of the brain QUIN synthesis from 3-hydroxyanthranilinic acid (3-HANA) that is catalysed by 3-HANA 2,4-dioxygenase (3-HAO). The activity this enzyme is blocked by 4-chloro-3-hydroxyanthranilinic acid and related analogues (Heyes, 1993; Todd *et al.*, 1989), and it is increased in inflammatory neurological disorders (Heyes, 1993), experimental epilepsy (Nakagawa *et al.*, 1995), and brain ischemia (Saito *et al.*, 1993a). This suggests that neuronal pathology in these conditions could arise from increased brain QUIN production and overactivity of NMDA receptors. If this premise were valid, then the 3-HAO inhibitors would reduce QUIN synthesis and prevent neuronal damage. The halogenated 3-HANA analogue, 4-chloro-3-hydroxy-anthranilate indeed prevents QUIN accumulation produced by inflammatory stimuli (Saito *et al.*, 1993b), and another 3-HAO inhibitor, NCR-631, in hippocampal cultures reduces the loss of pyramidal cells due to tissue anoxia or neuroinflammatory factors (Luthman *et al.*, 1998). The applicability of this strategy to other models of acute and chronic neuron loss, especially the *in vivo* models, needs to be determined. However, chronic inhibition of 3-HAO aimed at reducing QUIN synthesis might lead to accumulation of 3-HANA in the brain, and therefore its biological activity, especially in relation to the neuronal viability, becomes an important consideration. In this respect, our previous experiments on the cholinergic model (Jhamandas *et al.*, 1990) and a more recent study on the dopaminergic model (Fig. 7) have revealed the neurotoxic potential of high doses of 3-HANA. The toxic

effect of 3-HANA, unlike that of QUIN, does not depend on activation of the NMDA receptor, either directly or indirectly (through its conversion of QUIN, Luthman *et al.*, 1996), since it is not antagonized by acute or chronic MK-801 (Fig. 8). While the exact mechanism of 3-HANA toxicity remains unclear, the generation of reactive oxygen species may be a contributing factor (Dykens *et al.*, 1987), as suggested by the ability of ascorbic acid to attenuate this effect. Interestingly, exposure of cell cultures to physiological concentrations of 3-hydroxy kynurenine, the immediate precursor of 3-HANA (Fig. 1), has been reported to produce hydrogen peroxide and neuronal cell loss (Okuda *et al.*, 1996). Thus, pharmacological approaches based on reductions in QUIN synthesis might involve accumulation of precursors with toxic potential. In the same vein, the neuronal damage resulting from overactivity of the L-kynurenine pathway might involve not only QUIN-induced stimulation of NMDA receptor activity, but also free radical-induced damage by the QUIN precursors. In view of this, agents producing blockade of NMDA receptor activity as well as free radical generation could potentially be better neuroprotectants than those producing either effect alone (Moroni *et al.*, 1992).

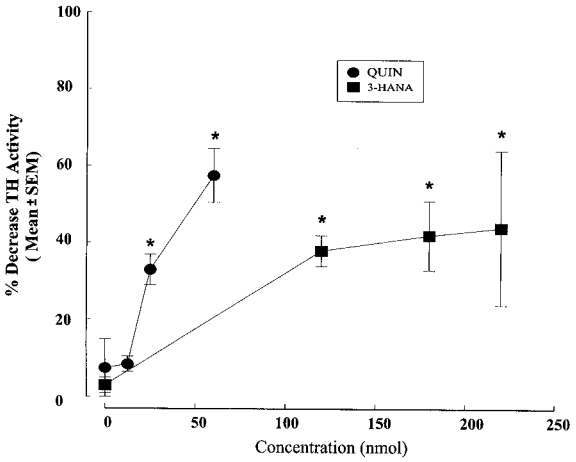


FIGURE 7 The depletion of rat striatal tyrosine hydroxylase (TH) activity by increasing intranigral infusions (0.5 µl) of quinolinic acid (QUIN) or its precursor, 3-hydroxyanthranilinic acid (3-HANA). The TH activity is expressed as a percentage of activity in the uninjected side in the same animal. *Values are significantly different ($p < 0.05$) from those in saline-infused animals.

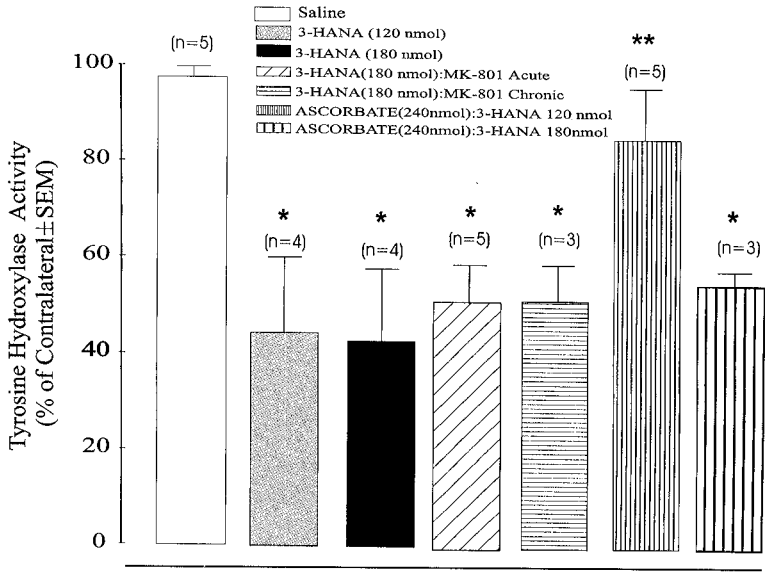


FIGURE 8 The effects of MK-801 or ascorbic acid on the depletion of tyrosine hydroxylase activity produced by intranigral infusion of 3-hydroxyanthranilinic acid (3-HANA). MK-801 was administered acutely (5 mg/kg ip) at 30 min, or chronically (every four hours) for 24 h prior to the infusion of 3-HANA. Ascorbic acid was co-infused with 3-HANA. *Values significantly different from those in saline-injected animals ($p < 0.01$).

Indeed, 7-chloro-thiokynurenic acid, a synthetic KYNA analogue, which possesses these two properties, is reported to exert a greater *in vitro* neuroprotective action than 7-chloro-kynurenic, which only has anti-NMDA activity (Priestley *et al.*, 1990). Whether such a difference between these agents also prevails in the *in vivo* models of neuron damage is not known, however, the thio derivative of KYNA exerts a neuroprotective action in an *in vivo* model of cerebral ischemia (Pellegerini-Giampietro *et al.*, 1994).

Endogenous Production of 7-Chloro-kynurenic Acid

The halogenated KYNA analogue, 7-chloro-kynurenic acid, which has a high affinity at the NMDA receptor sites, can produce complete protection against the toxic effect of NMDA or QUIN on the forebrain cholinergic neurons (Pawley *et al.*, 1996). Hokari *et al.* (1996) have demonstrated that L-4-chloro-kynurenine, an analogue of L-kynurenine, gains access from circulation to the brain where it can be converted to 7-chloro-kynurenic acid by activity of the aminotransferases that catalyse KYNA synthesis. Focal infusions of 4-chloro-kynurenine have been demonstrated to prevent the QUIN- induced loss of hippocampal GABA neurons, an effect that has been attributed to formation of the antagonist, 7-chloro-kynurenic acid, at the delivery site. This synthesis occurs primarily in the astrocytes since fluorocitrate, a gliotoxin, reduces both the production of the antagonist and abolishes neuroprotective effect against QUIN (Wu *et al.*, 1997). Thus, the enzymatic mechanisms normally involved in the synthesis of endogenous KYNA could be harnessed to produce an antagonist that selectively targets brain NMDA receptors. This type of strategy could be useful in reducing the NMDA receptor-based pathology while sparing functional activity of the non-NMDA receptors since 7-chloro-kynurenic has poor affinity for the latter.

Other Endogenous Antagonists

The extent to which several other L-kynurenine-derived metabolites are produced centrally and participate in endogenous neuroprotection is not known, although an original study by Gal *et al.* (1978) identified some of these in the brain. However, several metabolites (quinaldic acid, hydroxyquinaldic acid, and picolinic acid), when co-infused locally with QUIN, were found to attenuate the brain cholinergic neuron loss, albeit less potently and efficaciously than KYNA (Jhamandas *et al.*, 1990). Additionally, picolinic acid has been observed to partially reduce the QUIN-induced loss of dopaminergic and NADPH diaphorase neurons (Beninger *et al.*, 1994; Kalisch *et al.*, 1994). However, unlike QUIN, picolinic acid is devoid of neurotoxicity (Cockhill *et al.*, 1992), even after prolonged focal infusions (Kalisch *et al.*, 1994). Its lower potency and efficacy notwithstanding, picolinic acid is of interest since it influences neurotoxicity but, unlike KYNA, does not affect the neuroexcitatory responses (Beninger *et al.*, 1994; Robinson *et al.*, 1985). On the basis of experiments with several QUIN analogues, it was reported that the neuroexcitatory actions of certain analogues were dissociable from excitotoxic effects (Lehman *et al.*, 1985). In view of such dissociations, it may be possible to modulate excitotoxic damage without interference with phyisological functions of neurons.

The mechanism of picolinic acid action is not known. Since it selectively inhibits the action of the EAA agonists (QUIN and kainic acid) that require an intact glutamatergic afferent input to produce the excitotoxic effect, a reduction in presynaptic release of glutamate may explain its action. Experiments utilizing different picolinic acid analogues to test this possibility, however, did not provide support for such a mechanism (Vrooman *et al.*, 1993). Picolinic acid is a known chelator of zinc (Suzuki, 1957) and we have recently observed that its ability to antagonize QUIN is compromised on combination with zinc,

suggesting a role for this cation in its effect (Jhamandas *et al.*, 1998). Indeed, several zinc chelators – dipicolinic acid, diethyldithiocarbamate and TPNN (N,N,N′,N′-tetrakis(2-pyridylmethyl)-ethylenediamine) – also exhibit anti-excitotoxic properties (Boegman *et al.*, 1987a; Lee *et al.*, 1996). Thus, zinc chelation might underlie the protective action of picolinate, but additional studies are needed to determine the exact mechanism of this action. Recent reports implicate zinc in the damage due to cerebral ischemia, and Ca EDTA, a zinc chelator, has been found to ameliorate this damage (Koh *et al.*, 1996). In view of the emerging role of this cation in the pathophysiology of brain ischemia, the anti-excitotoxic potential of picolinic acid and related zinc chelators should of interest in developing newer neuroprotectants.

NITRIC OXIDE-BASED NEUROPROTECTION

The role of nitric acid (NO) in QUIN-induced neuron loss has been of interest to us as considerable evidence favours this gaseous molecule as an intermediary in the expression of NMDA-induced physiological and neurotoxic responses (see Strijbos *et al.*, 1996). The latter response has been attributed to formation of the toxic peroxynitrate (Lipton *et al.*, 1993). Some of the evidence supporting the role of NO in the NMDA receptor-mediated toxicity is derived from studies demonstrating protection against this response with inhibitors of NO synthesis. Thus, L-NAME, which inhibits neuronal and non-neuronal forms of the enzyme, and 7-nitroindazole, which selectively inhibits the neuronal enzyme, have been reported to reduce or prevent this phenomenon. However, we found that these inhibitors augment neuron loss in the dopaminergic neuron (Connop *et al.*, 1994, 1995) and the NADPH diaphorase neuron (Kalisch *et al.*, 1999) models of excitotoxicity, a finding that suggests a neuroprotective role of NO in the CNS. This is not unusual in view of reports that NO can scavenge free radicals, has the ability to inhibit the NMDA receptor ion conductance and gating, and has the potential to produce vasodilatation following neuronal activation (see Iadecola, 1997). The activity-dependant localized vasodilation produced by NO could meet the energy demand of neurons that become excited following stimulation of the NMDA receptor. Interestingly, the neurotoxicity produced by indirect activation of NMDA receptors, through use of agents inhibiting energy metabolism, is not augmented by NOS inhibitors partly because this toxicity is not preceded by the neuronal excitation that is a hallmark of toxins directly activating the NMDA receptor (Connop *et al.*, 1996, 1997). Also, we have observed that while the excitotoxic response in certain neurons is augmented by NOS inhibition, this is not observed in other neurons, such as the striatal enkephalinergic neurons, possibly due to differences in endogenous defence mechanisms (Kalisch *et al.*, 1999).

The ability of NO to inhibit the NMDA receptor-mediated excitotoxic response could be exploited by use of agents that produce NO but that do not yield toxic peroxynitrate. Lipton and colleagues (1993) have demonstrated that nitroso-compounds such as nitroglycerin protect cultured neurons against a toxic insult, an effect attributed to action at the redox modulatory site on the NMDA receptor. The ability of nitroglycerin, a clinically used compound, to produce neuroprotection *in vitro* prompted us to determine if it influences the excitotoxic response *in vivo*. Indeed, in preliminary studies systemically administered nitroglycerin has been found to reduce the NMDA receptor-mediated neurotoxicity in the dopaminergic model, an effect that is not explained by its well recognized peripheral vasodilatory action (Kard *et al.*, 1998). Whether mechanisms producing endogenous nitric oxide in the brain can be harnessed to influence neuronal pathology remains to be investigated in future studies.

CONCLUDING REMARKS

The existence of a metabolic pathway in the brain which generates EAA antagonists offers possibilities for endogenous neuroprotection. Experimental studies on models in which neuron damage is produced by an excitotoxin injection or by ischemia have already shown that agents raising levels of the endogenous KYNA exert neuroprotective effects. To date, the evaluation of such agents has focused on modulation of acute neuronal damage and their potential to influence chronic neuron loss, seen in neurodegenerative disorders, remains to be explored. However, the modulation of chronic neuron loss will entail sustained brain exposure to elevated levels of endogenous KYNA and, therefore, the implications of this for physiological responses of neurons that depend on EAA receptor activity become an important issue. As KYNA blocks NMDA as well as non-NMDA receptors, the blockade of these on a long term basis could impair the numerous physiological functions mediated by such receptors. One of the most important roles, of NMDA receptors is in memory and learning and these could be adversely affected by the endogenous KYNA. Bannerman *et al.* (1997) demonstrated that intraventricular 7-chloro-kynurenic acid impairs performance of experimental animals in the water maze experiments and produces sensory motor deficits. Although chronic effects of the endogenous KYNA on learning and memory remain unknown, similar effects are predictable on the basis of its ability to produce NMDA receptor blockade. The agents that elevate brain endogenous KYNA also produce sedation. Another problem pertains to the potential neurotoxic effects of the endogenous KYNA itself. Although generally regarded as a neuroprotectant, one study has reported that chronic intrastriatal infusion of KYNA produce a loss of glutamic acid decarboxylase immunoreactive neurons (Rieke, 1992). However, our experiments, involving continuous infusions of KYNA, picolinic acid or QUIN, showed that while QUIN clearly destroyed striatal NADPH diaphorase neurons, neither KYNA nor picolinate produced this effect (Kalisch, 1994). Thus, more studies will be needed to clarify effects of sustained endogenous KYNA exposure on neuronal viability, especially in cell populations that show high vulnerability to EAA-based damage.

The development of agents that effectively inhibit acute and chronic loss of neurons without compromising their physiological responses, would constitute an attractive and ideal neuroprotective strategy. An agent such as picolinic acid would be of interest in this respect since it has the ability to attenuate the neurotoxic but not neuroexcitatory response. However, this metabolite has not been identified in the brain, it is significantly less effective than KYNA, and the precise mechanism of its effect remains unclear. The chelation of zinc may underlie the anti-toxic action of picolinate. In view of the presence of zinc in neuronal pathways and increasing appreciation of its involvement in cell loss, the role of endogenous zinc chelators such as picolinic acid and hydroxyquinaldic acid (Suzuki *et al.*, 1957) in the modulation of neurototxicity merits attention.

Lastly, NO, widely implicated as a mediator of the NMDA receptor-linked physiological and pathophysiological phenomena, appears to have neuroprotective potential. Indeed, the potential of this mediator might be exploited through use of agents that are able to produce a form of NO lacking neurotoxic property. However, given multiple roles of NO in physiological functions, the potential of interference with such functions remains a possibility. These problems not withstanding, the endogenous factors which modulate neuron viability provide important leads for the development of effective neuroprotectant agents of the future.

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