Quinolinate (QUIN) is an endogenous neurotoxic product of the kynurenine pathway of tryptophan metabolism. Quinolinate is a selective agonist of the NMDA family of glutamate receptors and, as such, may play a role in the pathology of a number of major central nervous system (CNS) disorders. These include both chronic neurodegenerative disorders such as Alzheimer’s, Parkinson’s, and Huntington’s diseases and the AIDS-dementia complex, as well as the acute and delayed neuropathology resulting from traumatic, hypoxic, ischemic or hypoglycemic CNS insults. The work described herein sought to 1.) Compare the neurotoxicity of quinolinate to that of glutamate and to 2.) Explore the structural requirements for quinolinate neurotoxicity by evaluating substituted analogs of quinolinate with a goal of possible discovery of compounds of potential therapeutic value against glutamate receptor-mediated neurotoxicity. Quinolinate has been reported to excite neurons of the CNS ubiquitously, but with variable potency across regions of the mammalian brain, although in a manner consistently sensitive to blockade by specific pharmacological antagonists of NMDA receptor activation. We have compared the neurotoxic effects of exposure to glutamate and quinolinate and the combined effects of glutamate and quinolinate exposure on primary cultures of rat cerebellar granule cells in vitro, by measuring the release of the
cytoplasmic enzyme lactate dehydrogenase (LDH) from cerebellar granule neurons. We also examined the effects of the novel quinolinate analogs: 2-nitropyridine-3-carboxylic acid (2NPC), 3-nitropyridine-2-carboxylic acid (3NPC) and 2-thiopyridine-3-carboxylic acid (2TPC). The results indicate that for cerebellar granule cell cultures at day 13 in vitro (13 DIV) 1.) Glutamate induces significant neurotoxicity with an EC$_{50}$ for LDH release of 76.2 (95% C.I.: 46.8-124.2) $\mu$M but 2.) Quinolinate, at concentrations as high as 1 mM effects no measurable neurotoxicity. 3.) Quinolinate, however, potentiates the neurotoxicity of 400 $\mu$M glutamate in a dose-dependent manner with an EC$_{50}$ of 152.4 (95% C.I.: 57.6-403.1) $\mu$M, and at a dose of 1 mM enhances 400 $\mu$M glutamate toxicity 2.5-fold. 4.) Both the NMDA receptor-channel blocker, MK-801 and the strychnine-insensitive, glycine site antagonist 5,7-dichlorokynureninic acid (5,7-DCKA) are able to fully block the neurotoxicity of 400 $\mu$M glutamate + 1 mM quinolinate with IC$_{50}$ values of 6.7 (95% C.I.: 0.7-69.0) nM and 14.2 (95% C.I: 10.8-18.7) nM, respectively. 5.) Quinolinate surmounts protection against neurotoxicity afforded by 32 nM 5,7-DCKA in a dose-dependent manner and appears capable of completely reversing the protective effects of 5,7-DCKA when concentration was increased to 1 mM. These findings provide evidence that quinolinate potentiates glutamate-mediated neurotoxicity through NMDA receptor activation, and that this potentiation cannot be readily explained by simple additivity to the neurotoxicity induced by glutamate at the NMDA-receptor agonist site. Quinolinate potentiation of glutamate toxicity may involve the glycine site of NMDA receptor. Saturating concentrations of the quinolinate analogs 2NPC, 3NPC and 2TPC, however, provide partial protection against glutamate-mediated neurotoxicity.
Effects of Quinolinate and Nitro-, or Thio- Substituted Analogs of Quinolinate on Glutamate Receptor-Mediated Neurotoxicity in Cerebellar Granule Cell Cultures

by

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1. INTRODUCTION

Endogenous excitatory amino acids such as L-glutamate and L-aspartate, exert important physiological and pathological roles in the mammalian central nervous system (CNS) through activation of elements of a family of glutamate receptors which includes 
\(\alpha\)-amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, kainate receptors and N-methyl-D-aspartate (NMDA) receptors (Sugiyama et al., 1987; Olney, 1989; McDonald and Johnson, 1990; Choi, 1992; Nakanishi, 1992). The NMDA receptors are the best characterized among glutamate receptors and have several unique characteristics that distinguish them from their ionotropic "non-NMDA" counterparts: 1). The NMDA receptor requires both glutamate and glycine for efficient activation (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988); 2). The NMDA receptor channel is subject to a voltage-dependent Mg\(^{2+}\) blockade at resting membrane potential and opens only upon simultaneous depolarization and agonist binding (Mayer et al., 1984); 3). The NMDA channel possesses high permeability to Ca\(^{2+}\) ions in addition to Na\(^{+}\) and K\(^{+}\). NMDA receptors are involved in synaptic transmission, synaptic plasticity, embryonic CNS development and long-term potentiation, which is a neuronal representation of learning (MacDermott et al., 1986; Mayer and Westbrook, 1987). NMDA receptor dysfunction, however, results in persistently elevated intracellular Ca\(^{2+}\)
levels following excessive or prolonged NMDA receptor activation, and leads to a syndrome of neuronal injury or death which appears to be a pathological event common to many chronic and acute central nervous system diseases (Choi, 1988; Stone and Burton, 1988; Morris, 1989; McDonald et al, 1990; Olney, 1990; Nakanishi, 1992).

Studies suggest NMDA receptors may represent the primary site through which neurotoxicity is mediated by L-glutamate (Goldberg et al., 1987; Choi et al., 1988; Novelli et al., 1988). Quinolinate (2,3-pyridine-dicarboxylic acid), is a metabolite of tryptophan from the kynurenine pathway, which also exerts a potent, neuroexcitatory action mediated by NMDA receptor activation (Stone, 1993). Quinolinate has been identified in a variety of mammalian tissues including liver and brain (Lombardi et al., 1983a, b; Stone, 1993). In recent years quinolinate has received great attention as a possible etiologic factor in the pathology of several major CNS disorders including Huntington’s and Alzheimer’s diseases, AIDS related dementia, as well as hypoxic, ischemic, traumatic and hypoglycemic insults (reviewed by Stone, 1993). Therefore, it is required to completely elucidate the mechanism of quinolinate action.

Several characteristics of quinolinate distinguish it from L-glutamate and L-aspartate: 1) Quinolinate appears to be a selective agonist of the NMDA receptor, while endogenous L-glutamate and L-aspartate act relatively non-selectively on glutamate receptor subtypes including the NMDA receptor, kainic acid receptor and AMPA receptors etc. (Collingridge and Lester, 1989; Stone, 1993); 2) In contrast to L-glutamate or L-aspartate, quinolinate lacks neuronal or glial uptake mechanisms for its removal from the extracellular space. Labeled quinolinate injected into the rat brain has been shown to remain in the region of the injection for up to 2 hours (Foster et al., 1984); 3)
QUIN is produced by macroglial and microglial cells of CNS but not by neurons (Lombardi et al., 1983a, b; Stone, 1993); Furthermore, although glutamate and quinolinate are distributed in various brain sites, there are marked variations in neuronal sensitivity to quinolinate in different regions of the CNS which is different from glutamate and aspartate (Perkins and Stone, 1983; McLennan, 1984; Wolfensberger et al., 1983; Choi, 1988). Thus, it is necessary to answer the following questions: does quinolinate act in a manner equivalent to L-glutamate at the molecular level or do these differences suggest different molecular mechanisms? Is there a functional interaction that exists between glutamate and quinolinate to regulate NMDA receptor activation?

Because quinolinate may play an important role in brain disorders, structural analogues of quinolinate could be of therapeutic value in treatment of acute and chronic pathologies of the CNS which involve excess NMDA receptor activation if they act as pharmacological antagonists of the NMDA receptor, or act metabolically to prevent quinolinate formation or accumulation in brain. 2-nitropyridine-3-carboxylic acid (2NPC), 3-nitropyridine-2-carboxylic acid (3NPC) and 2-thiopyridine-3-carboxylic acid (2TPC), are novel synthetic compounds, which are structural analogs of quinolinate (Figure 1). We are interested first in the possible role they might play as ligands of glutamate receptors and then in their possible capacity to regulate quinolinate metabolism or availability.

The hypothesis and objective of these experiments was to investigate the effects of glutamate and quinolinate, alone, on neuronal toxicity mediated by NMDA receptor activation, as well as to characterize possibly unique features of an interaction between glutamate and quinolinate in cultured cerebellar granule cells. We further evaluated the
capacity of the quinolinate structural analogs to produce excitotoxic cell death in the cerebellar granule cells through activation of glutamate receptors, or to block this excitotoxic cell death mediated by glutamate in cultured cerebellar granule cells.

Figure 1 Structure of quinolinate and its analogs.
2. LITERATURE REVIEW

Glutamate receptor activation mediate important excitatory and neurotoxic functions in the vertebrate central nervous system (CNS) (Monaghan and Cotman, 1989). With the development of potent and selective agonist and antagonists, two major subtypes of glutamate receptors were suggested: ionotropic glutamate receptors and G-protein-coupled metabotropic glutamate receptors. The former has been further classified into three pharmacological subtypes: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors, kainate receptors and N-methyl-D-aspartate (NMDA) receptors. Of these glutamate receptors, the NMDA receptors have received great attention because of a wide range of both neurophysiological and pathological processes it involves such as synaptic plasticity, learning, CNS development and neurotoxicity (Watkins et al., 1990; Tanabe et al., 1992).

As endogenous excitatory amino acid, neither glutamate nor aspartate acts selectively at any of the pharmacologically defined receptor populations. Quinolinate, another endogenous excitatory substance, has been found to be a selective agonist at NMDA receptors, and has been implicated in the etiology of a number of brain disorders (reviewed by Stone, 1993). The following sections will review the NMDA receptor literature and then review studies illuminating the relationship of quinolinate to NMDA receptor function.
2.1. Characteristics of N-methyl-D-aspartate (NMDA) receptor

Receptors activated by glutamate, aspartate and other acidic amino acids are the primary mediators of neuronal excitation in the vertebrate CNS. Among excitatory amino acid receptors, NMDA receptors, the most abundant species, are thought to have important roles in synaptic transmission throughout the CNS (Stone and Burton, 1988). NMDA receptors have been found to play a central role in synaptic plasticity, CNS development, the establishment of long-term potentiation and in experimental models of learning (Kennedy, 1989; Morris, 1989; McDonald and Johnson, 1990; Udin and Scherer, 1990; Nakanishi, 1992). On the other hand, NMDA receptor dysfunction, which follows the persistently elevated intracellular Ca\(^{2+}\) levels after excessive or prolonged NMDA receptor activation, may result in the activation of destructive enzymes such as phospholipases, proteases, and protein kinases, which eventually lead to neuronal injury or death. This process, coined "excitotoxicity" by Olney (1989), appears to be an important element in the neuronal pathology of many chronic and acute CNS diseases such as stroke, hypoglycemia, Huntington's disease, epilepsy, Alzheimer's disease and AIDS-related dementia (Mayer and Westbrook, 1987; Rothman and Olney, 1987; Choi, 1988; Stone and Burton, 1988; Morris, 1989; Olney, 1989; McDonald and Johnson, 1990; Olney, 1990; Nakanishi, 1992).

Many studies suggest that the NMDA receptor represents the primary site through which glutamate induces neurotoxicity, since it can be prevented or reduced by treatment with NMDA receptor antagonist such as the channel blockers - MK-801, ketamine, or the competitive antagonist 2-amino-5-phosphonopentanoic acid (AP5) (Goldberg et al., 1987; Choi et al., 1988; Novelli et al., 1988). NMDA receptors have several unique
characteristics compared to other subtypes of glutamate receptors: first, occupancy of
two pharmacologically distinct recognition sites by glutamate and glycine are necessary
for efficient activation of NMDA receptors (Johnson and Ascher, 1987; Kleckner and
Dingledine, 1988); second, NMDA receptor-operated channels are highly permeable to
Ca\(^2+\) in addition to Na\(^+\) and K\(^+\) (Mayer and Westbrook, 1987) and third, are regulated by
extracellular Mg\(^2+\) in a voltage-dependent fashion (Mayer et al., 1984; Nowak et al.,
1984).

In addition to having the unique characteristics of gating by ligands as well as
sensitivity to voltage, NMDA receptors possess a number of regulatory sites which have
important role in modulating receptor function: an NMDA recognition domain for
endogenous agonists such as L-glutamate and exogenous substances such as NMDA; a
strychnine-insensitive co-agonist domain which is activated by glycine or structurally
similar amino acids; a polyamine recognition domain activated by spermine; and, a
cation channel domain allowing translocation of calcium as well as sodium and
potassium ions in which resides some distinct channel inhibitory sites, modulated by
Mg\(^2+\) and blockers such as phencyclidine (PCP), dizocilpine (MK-801) and dextrophan
(Mayer et al., 1989; MacDonald and Nowak, 1990; Franklin and Murray, 1992).

Discovery of new compounds acting at these sites is important not only for the
provisional research tools to evaluate fundamental central nervous system mechanisms,
but also as potential therapeutic agents for CNS diseases.

In the search for substances with therapeutic potential, ligands which interact with
the NMDA recognition domain regulatory site, and the PCP binding site have been most
studied. Both competitive NMDA receptor antagonist such as AP5 and 2-amino-7-
phosphonoheptanoic acid (AP7), 3-(2-carboxypiperazin-4-yl) propyl-1-phosphonic acid (CPP) and noncompetitive NMDA antagonists (e.g. phencyclidine and MK-801) are able to attenuate neurotoxicity and convulsions induced by NMDA in vivo, (McDonald et al., 1989), but the clinic use of these compounds has been diminished due to their psychotomimetic effects and abuse potential (Willetts et al., 1990).

The glycine site of the NMDA receptor complex has gained attention since marked the potentiation by glycine of the NMDA response in mouse cortical neurons was first reported by Johnson and Ascher (1987). Studies of NMDA receptors expressed in Xenopus oocytes after injection of rat brain mRNA unequivocally demonstrated that occupation of the glycine site by an agonist such as glycine or structurally similar amino acids is an absolute requirement for receptor activation (Kleckner and Dingledine, 1988). In agreement with this, other electrophysiological studies indicate that the noncompetitive blockers gain access to the binding site within the ion channel of NMDA receptor only when the receptor is occupied by NMDA agonists and glycine agonist (Huettner and Bean, 1989). Glycine accelerates the association and dissociation of \([^3]H\)TCP (N-[1-(2-thienyl)cyclohexyl]-piperidine) and \([^3]H\)MK-801 to and from NMDA receptors (Reynolds et al., 1987; Benavides et al., 1988). In addition, kynurenate and several structurally related bicyclic compounds of the quinoline and quinoxaline type which are selective antagonists at the glycine site of NMDA receptor not only block \([^3]H\)glycine binding as well as inhibit the glutamate stimulated association and dissociation of \([^3]H\)TCP to and from the NMDA receptor (Kessler et al., 1989).

Subtypes of NMDA receptors appear to exist in the brain. There are different ways to classify the subtypes of NMDA receptor. Dansyz et al. (1990) reported that
strychnine-insensitive glycine site antagonists of NMDA receptor have different sensitivities in different brain areas: 7-chlorokynurenate and 1-hydroxy-3-amino-2-pyrrolidone (HA-966) displace glycine or structurally similar amino acids at glycine site in the forebrain, while only HA-966 was effective in the pons and spinal cord. This heterogeneity of the strychnine-insensitive glycine site may be associated with the subtypes of NMDA receptor. Studies also showed that the medial stratum and septum are preferentially labeled by glutamate, while cortex and thalamus are preferentially labeled by NMDA receptor competitive antagonist 3-(2-carboxypiperazin-4-yl) propyl-1-phosphonic acid (CPP) (Monaghan, 1991; Reynolds and Palmer, 1991). In addition, it was reported that glutamate, aspartate, cysteine sulphinate, or quinolinate (but not homocysteate) activate amino acid receptors with the Hill coefficient greater than one. This implies there are at least two different kinetic forms of NMDA receptor, one of which may require two or more molecules of agonist for activation (Curra and Dingledine, 1992). Furthermore, quinolinate, an endogenous metabolite of tryptophan, was shown to excite NMDA receptors in some regions of CNS and has an interesting possibilities for distinguishing regional heterogeneity (Stone, 1993). Molecular cloning studies have demonstrated that rodent NMDA receptor is made up of a heterologous assembly of subunits and provided a molecular basis for NMDA receptor heterogeneity. Each NMDA receptor complex is composed of at least one NMDAR1 and at least one of four classes of highly homologous NMDAR2 (NR2A-NR2D) subunits (Moriyoshi et al., 1991; Monyer et al., 1992; Priestley et al., 1995). Therefore, the identification of pharmacologically distinct NMDA receptor subtypes and the investigation of the possible different functions among receptor subtypes are likely to be important to design and
target novel agents for maximizing therapeutic intervention while minimizing the interference of normal function.

2.2. Quinolinate - an endogenous selective NMDA receptor agonist

Quinolinate (2,3-pyridine dicarboxylic acid) is considered a rigid analog of glutamate and aspartate. Quinolinate is derived from tryptophan via the kynurenine pathway, which was identified in mammalian liver in the 1940s (Stone and Burton, 1988; Stone, 1993). The detection of quinolinate in the rodent brain using chromatographic and mass fragmentographic methods was first reported in 1983, and it was soon also shown to be present in the human CNS (Lombardi et al., 1983a, b; Wolfensberger et al., 1983). In several different brain areas of rat, different amounts of quinolinate were detected. The concentration of quinolinate in the cortex was highest, striatum contained the lowest concentration, and intermediate concentrations were found in the hippocampus, cerebellum and brain stem of rats (Moroni et al., 1984). Results similar to those of rat and other species were found in normal postmortem human brain that quinolinate level in cerebral cortex was 2- to 4-fold greater than those present in the striatum (Wolfensberger et al., 1983). Interestingly, the concentrations of quinolinate in the rat cerebral cortex increase progressively with age from 3 days to 30 months (Moroni et al., 1984). Much interest has been generated in the possibility of quinolinate involvement in the neuronal damage seen in neurodegenerative diseases. Studies suggested that neuronal damage associated with Huntington’s disease, ischemia, hypoglycemia, infection, AIDS-associated dementia and Alzheimer’s disease etc. may involve quinolinate or other
tryptophan metabolites of the kynurenine pathway (Stone, 1993; Huang et al., 1995; Power and Johnson, 1995; Power, C., and R.T. Johnson, 1995. Sardar et al., 1995; Sei et al., 1995; Bordelon et al., 1997).

Since quinolinate appears to be involved in the etiology of several major CNS disorders (Stone, 1993), it is important to investigate the neurotoxic mechanism of quinolinate. It was reported that quinolinate excited cerebral cortex neurons of anesthetized rats when applied locally by microiontophoresis; AP5 or AP7, as NMDA competitive antagonists, antagonized the excitatory effects of quinolinate without blocking excitation elicited by application of quisqualic acid or kainate (Stone and Perkins, 1981; Stone, 1986). Binding studies showed quinolinate displaced glutamate from hippocampal membrane with an IC₅₀ of 180 μM and displaced AP5 with an IC₅₀ of 350 μM, but could not displace kainic acid at concentration of 1 mM (French et al., 1984; Olverman et al., 1984). In addition, glutamic acid diethyl ester (GDEE), which is thought to block quisqualic acid responses, was not effective in antagonizing the responses evoked by quinolinate (Stone and Burton, 1988). These studies suggest that the excitations were mediated through the glutamate recognition site of NMDA receptors by quinolinate (Perkins and Stone, 1983a; Stone, 1993).

None of these preferential agonists of prototypical excitatory amino acid receptor ligands such as NMDA, AMPA and kainate are found in the mammalian CNS. L-glutamate and L-aspartate have been extensively recognized as endogenous ligands but neither of them acts selectively on any of the proposed receptor populations (Collingridge and Lester, 1989; Stone, 1993). Quinolinate not only selectively acts at NMDA receptors but also exhibits marked variations in neuronal sensitivity to its effects
in different regions of the brain. Electrophysiological studies have shown that a much smaller fraction of rat cerebellar and spinal cord neurons are sensitive to quinolinate, while cerebral cortical, hippocampal, and neostriatal neurons are almost uniformly sensitive to quinolinate. Thus, two pharmacological subtypes of NMDA receptor were suggested by the effects of quinolinate: A NMDA 1 receptor which responds to NMDA in spinal cord and cerebellum, and a NMDA 2 receptor sensitive to both NMDA and quinolinate in other CNS regions (Perkins and Stone, 1983a, b; Monaghan and Beaton, 1991). Therefore not only is quinolinate involved in the etiology of some important brain disorders but it may also be useful as a tool to identify subtypes of the NMDA receptor.

Another distinguishing characteristic of quinolinate is the absence of active uptake mechanisms for its removal from the extracellular space in contrast to L-glutamate or L-aspartate, which are actively transported by specific glial and neuronal transporters. Labeled quinolinate injected into the rat brain remains in the region of the injection for up to 2 hours (Foster et al., 1984). Quinolinate therefore can not possibly function in a manner similar to the traditional neurotransmitters L-glutamate and L-aspartate. Furthermore, glutamate and quinolinate are differentially distributed in many brain areas (Wolfensberger et al., 1983; Choi, 1988) and it is unknown if they interact or regulate the activity of one another in the same region. Questions thus raised are here: Does quinolinate act the exact same way as L-glutamate on NMDA receptors or are other mechanisms involved? Do these differences suggest different molecular sites of action as well?

Rat cerebellar granule cells are a widely used primary neuronal culture system. It provides a much less complex model of the CNS and yield > 90% neurons, the vast
majority of which express glutamate receptors (Kingsbury et al., 1985). In addition, studies showed the functions of NMDA receptors in the cerebellum are important in neuronal development and excitatory synaptic transmission (Rabacchi et al., 1992; Silver et al., 1992; D'Angelo et al., 1993; Komuro and Rakic, 1993). As such, cerebellar granule cell culture is an excellent model system for the investigation of the functional consequences of glutamate receptor activation (Gallo et al., 1982; Taniwaki et al., 1997). Assay of leakage of the cytoplasmic enzyme lactate dehydrogenase (LDH) from damaged neurons has been reported to be a reliable, quantitative index of the extent of neuronal cell death (Goldberg et al., 1987; Shalaby et al., 1989; Priestley, 1990; Berman and Murray, 1996). We chose cerebellar granule cells to address the question of the mechanism as well as functional interaction between glutamate and quinolinate in the first section of the present study.

If quinolinate contributes to excitotoxic neuronal death then structural analogs of quinolinate could be of therapeutic value if they act as pharmacological antagonists of the NMDA receptor, or act metabolically to prevent quinolinate formation or accumulation in brain. As such, quinolinate analogs may represent a novel therapeutic avenue in treatment of acute and chronic pathologies of the CNS, which involve excess NMDAR activation. 2-nitopyridine-3-carboxylic acid (2NPC), 3-nitopyridine-2-carboxylic acid (3NPC) and 2-thiopyridine-3-carboxylic acid (2TPC), are novel synthetic compounds, which are structural analogous to quinolinate; we are interested in the possible role they might play as ligands for glutamate receptors or as metabolic inhibitors of the kynurenine pathway. In the second section of this work, we examined the ability of the 2NPC, 3NPC and 2TPC to produce excitotoxic cell death in the cerebellar granule cells through
activation of glutamate receptors, or to block glutamate mediated neuronal toxicity in cultured cerebellar granule cells.
3. MATERIALS AND METHODS

The experiments are based on quantitative comparisons between control groups of cells and those treated with experimental compounds at the indicated concentrations. As described in the experimental procedures (Figure 2), the cerebella of 8 day old Sprague Dawley rats were extracted and cells cultured for 13 days. Following 2 hours of exposure of cells to the indicated compounds, spectroscopic measurements were taken to detect and quantify the treatment effects.

Following sections describe the material we used, cell culture, measurement and data analyses.
3.1. Materials

Chemicals were purchased from the following companies: L-glutamate, glycine, bovine serum albumen (BSA), trypsin, DNAse, poly-L-lysine (MW > 393,000), and soybean trypsin inhibitor (SBTI) from Sigma (St. Louis, MO); Basal Eagle's medium (BME) from Gibco (Grand Island, NY); Quinolinate from Aldrich Chem. Co.; 5,7-Dichlorokynurenic acid from RBI. Quinolinate analogs were synthesized by Ms. H. Qi in the Lab of Dr. Mark Zabriskie.

3.2. Cell culture

Cerebellar granule neurons were prepared from 8-day old Sprague-Dawley rats as described previously (Novelli et al., 1988). Briefly, isolated cerebella were put in Kreb's buffer containing 3 mg/ml bovine serum albumin and 1.2 mM MgSO₄ (KB/BSA), and were dissociated by trypsinization (312.5 µg/ml of trypsin, 15 min., 37°C). The digestion was terminated by adding 15 ml KB/BSA containing soybean trypsin inhibitor (166 µg/ml) and DNAse (26 µg/ml). Cerebellar granule cells were then dissociated by triturating in solution containing 80 µg/ml DNAse, 500 µg/ml SBTI. The supernatant was then collected and centrifuged for 5-7 min. at low speed. The cellular pellet was resuspended in Basal Eagle's medium containing 25 mM KCl, 2 mM glutamine, 10% fetal calf serum and 100 µg/ml gentamycin. The cells were plated in poly-L-lysine-coated 35 mm polystyrene culture dishes at 2.5 - 3 x 10⁵ cells/cm² and incubated at 37°C in a humidified 5% CO₂ atmosphere. To attenuate the proliferation of non-neuronal cells, 10 µM of cytosine arabinoside was added after 24 hours of culture. At 8 days and 12
days in culture, 100 μL of a 25 mg/ml D-glucose solution was added to each plate to replenish energy resources.

3.3. Measurement of neurotoxicity

Cell injury was quantitated by spectrophotometric measurement of the cytoplasmic enzyme lactate dehydrogenase (LDH) which is released from damaged neurons after exposure to toxic substances and has previously been demonstrated to be an index of neuronal injury in cell cultures (Koh and Choi, 1988; Shalaby et al., 1989; Patel et al., 1990).

Because the neurotoxicity of glutamate varies in cerebellar granule cells with pH, temperature and the age of the cultured cells (Choi, 1988; Berman and Murray, 1996), the following protocol was used in our experiments: Rat cerebellar granule cells (13 days in vitro) in 6-well plates were washed twice with HEPES-Buffered Locke’s buffer (154 mM of NaCl; 5.6 mM of KCl; 1 MgCl₂; 2.3 CaCl₂; 5.6 mM glucose; 8.6 mM HEPES; pH 7.4) and then cells of each well were exposed to 1 ml of fresh Locke’s solution containing glutamate and / or other compounds at pH 7.4 and maintained for 2 hours at 22°C. 0.5 ml of solution from each well of the culture plate was then transferred to microcentrifuge tubes. Aliquots (0.2 ml) of incubation buffer were added to 2.3 μmol sodium pyruvate and 0.2 mg NADH, each in 0.5 ml of 0.1 M KHPO₄ buffer (pH 7.5), for spectroscopic quantification of the formation of NAD from NADH by lactate dehydrogenase (LDH) using pyruvate as substrate at 340 nm as described previously (Koh and Choi, 1988).
3.4. Data analysis

For the LDH release, least squares nonlinear regression analysis was performed with Graphpad Prism software to fit the following four parameter logistic equation:

\[
Y = A + \frac{B - A}{1 + \left(\frac{EC_{50}}{X}\right)^D}
\]

where \(Y\) equals the level of response, \(A\) and \(B\) are the minimum and maximum levels of the concentration-response curve, \(D\) the slope factor, \(X\) the compound concentration. In the nonlinear regression analyses, when assessing the toxicity of glutamate(GLU) or quinolinate(QUIN) alone, parameter "A" was set to a constant value equal to the mean of all control determinations. When assessing potentiation or inhibition of glutamate-mediated toxicity parameters \(A\) or \(B\) were, respectively, set to a constant value equal to the level of toxicity associated with an Emax concentration of glutamate(i.e., 400 \(\mu M\)). These analyses were thus simplified to fitting the data to a three parameter logistic equation.
4. RESULTS

4.1. Effects of quinolinate on glutamate-mediated neurotoxicity in cultured cerebellar granule neurons

For comparing the effects of glutamate and quinolinate on cultured cerebellar granule neurons, cells were exposed to the indicated concentrations of glutamate, quinolinate, and glutamate/quinolinate etc. in Locke’s buffer solution as shown in Figure 3 and Figure 4. LDH release was significantly increased in 400 μM glutamate-treated cells compared to that in untreated cells (P < 0.01) and 85% of glutamate-mediated LDH release could be blocked by inclusion of 10 μM MK-801 (Figure 4). In contrast, LDH release remained unchanged in quinolinate treated cells even at concentration as high as 1 mM compared to untreated cells (Figure 3). Dramatically, when 1 mM quinolinate was combined with 400 μM glutamate, the release of LDH was increased 2.5 fold that of cells treated with the same concentration of glutamate alone (p < 0.01) (Figure 4).

To characterize the potentiation by quinolinate of glutamate toxicity, quinolinate concentration-response experiments were performed in the presence of 400 μM glutamate. Figure 5 shows that quinolinate potentiates the neurotoxicity of glutamate in a dose-dependent manner with an EC₅₀ of 152.4(95% C.I.: 57.6-403.1) μM. To evaluate the role of glutamate in the quinolinate mediated potentiation, we performed glutamate concentration-response experiments in the presence of 1 mM quinolinate. Error! Reference source not found. indicates that no significant toxicity is exerted by 1 mM quinolinate unless the concentration of glutamate ≥ 100 μM. At glutamate concentrations ≥ 100 μM, LDH release in glutamate/quinolinate treated cells is increased greatly over
that of cells treated with glutamate alone, although LDH release is increased in a dose dependent manner in both groups.

![Figure 3](image)

Figure 3 Effect of quinolinate on LDH release from cerebellar granule cell cultures. Cells were exposed to indicated concentrations of quinolinate (QUIN) or glutamate (GLU) in Locke's solution. QUIN-treated cells showed no increase in LDH release from cells even at a concentration of 1 mM compared to control. GLU-treated cells showed a significant increase in LDH release from cells compared to control (P < 0.01). Results were normalized to control glutamate concentration (400 μM). Values are means of triplicate determinations ± standard error.
Figure 4 Effect of glutamate and quinolinate on LDH release from cerebellar granule cell cultures. LDH release from cells after 2-hour exposure to indicated compounds in Locke's solution at 22°C. The results revealed that 400 μM glutamate (GLU) caused a significant increase in LDH release from rat cerebellar granule cells (**P < 0.01) and that 85% of this release can be blocked by 10 μM MK-801. LDH release in cells treated with quinolinate (1 mM) (QUIN) alone was unchanged from that of untreated cells. Quinolinate at a concentration of 1mM, however, significantly increased LDH release in the presence of 400 μM glutamate compared to (**P < 0.01 as compared to LDH in glutamate-treated cells). Results were normalized to LDH release by control glutamate concentration (400 μM). Values are means of triplicate determinations ± standard error.
Figure 5  Dose-dependent potentiation by quinolinate (QUIN) of glutamate (GLU)-mediated toxicity of in cerebellar granule cell cultures. LDH release from rat cerebellar granule neurons after 2 hour exposure to indicated concentrations of quinolinate in the presence of 400 µM GLU. QUIN-mediated a potentiation of maximal GLU-induced LDH release with an EC₅₀ of 152.4 (95% C.I.: 57.6-403.1) µM. Results were normalized to that of LDH release from control glutamate concentration (400 µM). Values are means of triplicate determinations ± standard error.

Figure 6 Potentiation of glutamate (GLU)-mediated toxicity by QUIN (1mM) in cerebellar granule cell cultures. LDH release after 2 hour exposure to indicated concentrations of glutamate in the absence or presence of quinolinate (1 mM). Glutamate/quinolinate-treated group (filled triangle with an EC₅₀ 71.0 (95% C.I.: 53.0-95.0) µM. Glutamate-treated group (filled squares with an EC₅₀ 76.2 (95% C.I.: 46.8-124.0) µM). *P<0.05. **P<0.01. QUIN potentiation was is normalized
to glutamate concentration response LDH release values. Values are means of triplicate determinations ± standard error.
The experiments suggest that quinolinate potentiates the neurotoxicity of glutamate in cultured cerebellar granule cells at concentrations where quinolinate alone exerts no neurotoxicity. In subsequent experiments, different concentrations of MK-801 (an NMDA receptor channel blocker, Monaghan, 1991) and 5,7-Dichlorokynurenic acid (5,7-DCKA, an antagonist selective at the strychnine-insensitive glycine binding site of the NMDA receptor, Baron et al., 1990) were used to investigate if quinolinate potentiates neurotoxicity of glutamate through NMDA receptor. Figure 7 and Figure 8 showed MK-801 and 5,7-DCKA completely reverse neurotoxicity to basal line level with IC\textsubscript{50} of 6.7 (95% C.I.: 0.7-69.0) nM and IC\textsubscript{50} of 14.2 (95% C.I.: 10.8-18.7) nM, respectively (LDH level in untreated group as a baseline). These results suggest that potentiation of GLU toxicity by QUIN depends ultimately on NMDA receptor activation.

Figure 7 Protection by the NMDA receptor channel blocker-MK-801 against toxicity induced by GLU/QUIN in cerebellar granule cell cultures. Inclusion of MK-801 at the indicated concentrations inhibited LDH release with an IC\textsubscript{50} 6.7 (95% C.I.: 0.7-69.0)nM from cultures exposed to 400 µM GLU and 1 mM QUIN for 2 hours.
Inhibition was normalized to control LDH release induced by GLU+QUIN. Values are means of triplicate determinations ± standard error.

Figure 8 Protection by the NMDA receptor, glycine site antagonist 5,7-DCKA against toxicity induced by GLU/QUIN in cerebellar granule cell cultures. Inclusion of 5,7-DCKA at the indicated concentrations inhibited LDH release with an IC<sub>50</sub> 14.2 (95% C.I.: 10.8-18.7) from cultures exposed to 400 µM glutamate and 1 mM quinolinate for 2 hours. Values for 5,7-DCKA inhibition of LDH release were normalized to control LDH release induced by GLU/QUIN. Values are means of triplicate determinations ± standard error.

To address the possibility that potentiation by QUIN of GLU toxicity might involve activity at sites other than the agonist site of the NMDA receptor complex, the sensitivity of QUIN-mediated toxicity to 5,7-DCKA, a competitive antagonist of the strychnine-insensitive glycine site of the NMDAR, regulation of the effect of QUIN was assessed. The data of Figure 9 indicate that the concentration-response curve for potentiation by QUIN of GLU-mediated toxicity appears to be shifted rightward by increasing concentrations of 5,7-DCKA. Although the full extent of these concentration-response curves could not be determined because of the limiting solubility of QUIN, it
appears that 5,7-DCKA antagonism of QUIN-mediated toxicity was, surmountable by increasing QUIN concentrations. Depression of the level of toxicity induced by GLU at its $E_{\text{max}}$ concentration can be seen in Figure 9, by the progressively lowered baseline level of LDH release upon which QUIN potentiation is observed. If fixed concentrations of 5,7-DCKA can be surmounted by increasing concentrations of QUIN but shift the concentration-response curves for QUIN potentiation of GLU toxicity rightward, it is possible that QUIN, under these conditions, interacts as an agonist at the glycine site.

Figure 9 Antagonism by 5,7-DCKA of QUIN potentiation of GLU toxicity in cerebellar granule cell cultures. Data reflect LDH release from 2 hour exposure of cultures to indicated concentrations of QUIN in the presence of 400 μM glutamate and 10 μM (filled circle), or 100 nM (filled triangle) or 32 nM (open triangle) or absence of (filled square) 5,7-DCKA. Results are normalized to LDH release from control exposure to maximally toxic levels of GLU(400 μM). Values are means of triplicate determinations ± standard error.
4.2. Effects of quinolinate analogs on glutamate-mediated toxicity in cerebellar granule cells

We then examined the effects of nitro- or thio- substituted analogs of QUIN on cerebellar granule cells. None of the QUIN analogs showed significant cytotoxicity as indicated by increases in LDH release even at millimolar concentrations (Figure 10). The LDH release data are consistent with the absence of any sign of cytopathology by microscopic evaluation. To maximize the probability of NMDA receptor activation and thereby optimize the signal to noise ratio of the LDH assay, saturating concentrations of glycine(GLY; 100 μM) were added to all determinations of excitotoxicity or neuroprotection of QUIN analogs. None of the nitro-, or thio- analogs of QUIN showed any signs of cytotoxicity at concentrations equal to the upper limits of their solubilities in Locke’s solution, while each was able to provide limited protection to cells against GLU-mediated cytotoxicity. LDH release was maximally reduced by less than 30% of maximal GLU/GLY-mediated LDH release. At concentrations of 2 mM, 4 mM, and 1 mM, respectively, 2NPC, 3NPC and 2TPC significantly inhibit LDH release induced by 400 μM glutamate and 100 μM glycine and do so in a dose-dependent manner (Figure 11 and Figure 12).
Figure 10 Effects of QUIN analogs on LDH release from cerebellar granule cell cultures. LDH release after 2 hour exposure to the indicated concentrations of the indicated compounds: a. 2NPC; b: 3NPC; c: 2TPC. Results were normalized to maximal LDH release from control GLU/GLY treatment. Values are means of triplicate determinations ± standard error.
Figure 11. Effect of QUIN analogs on LDH release from cerebellar granule cell cultures mediated by 400 µM GLU and 100 µM GLY. LDH release after 2 hour exposure to the indicated compounds at the indicated concentrations. Results are normalized to maximal LDH release from control GLU/GLY treatment. Values are means of triplicate determinations ± standard error.
Figure 12 Dose-response curves of neuroprotection by 2NPC(a), 3NPC(b) and 2TPC(c) against GLU/GLY(400 µM/100 µM) mediated neurotoxicity in cerebellar granule cell cultures. LDH efflux after 2 hour exposure to indicated concentrations of quinolinate analogs. The rank order of potency of neuroprotective potency is as follows: 2TPC(IC₅₀ of 53.2 [95% C.I.: 6.4-442.3] µM) > 3NPC(IC₅₀ of 413.3 [95% C.I.: 27.4-6230.0] µM) > 2NPC (IC₅₀ of 3.4 [95% C.I.: 0.8-14.0] mM). Results are normalized to control levels of maximal LDH release mediated by GLU/GLY. Values are means of triplicate determinations ± standard error.
5. DISCUSSION:

Rodent cerebellar granule cell cultures provide populations which are > 90% neuronal; these neurons express NMDA, non-NMDA and metabotropic glutamate receptors (Kingsbury et al., 1985). NMDA receptors activation plays a critical role in development and plasticity of the central nervous system and in excitatory synaptic transmission (Rabacchi et al., 1992; Silver et al., 1992; D'Angelo et al., 1993; Komuro and Rakic, 1993). Primary cultures of cerebellar granule cells provide a useful model system for in vitro investigation of the mechanisms and functional consequences of glutamate receptor activation (Kingsbury et al., 1985; Gallo et al., 1982; Taniwaki et al., 1997). The assay of leakage of the enzyme lactate dehydrogenase (LDH) from the damaged neurons has been shown to be a reliable quantitative index of the extent of neuronal cell death (Goldberg et al., 1987; Shalaby et al., 1989; Priestley, 1990; Berman and Murray, 1996). On the basis of these data, we examined the possible neurotoxic or neuroprotective effects of structural analogs of QUIN. In agreement with the findings of Lysko et al. (1989), our initial experiments indicated that at a concentration of 400 \( \mu \text{M} \) glutamate maximally induces significant neurotoxicity and that > 85% of this glutamate-mediated neurotoxicity on cerebellar granule cells can be blocked by 10 \( \mu \text{M} \) of MK-801 (Figure 3 and Figure 11). These data suggest that glutamate neurotoxicity in cerebellar granule cell cultures is primarily mediated by NMDA receptor activation. In contrast, quinolinate by itself did not show any significant toxicity even at concentrations as high as 1 mM. This is consistent with the data of Monaghan and Beaton (1991) which indicate that QUIN is a much weaker competitor for glutamate binding in cerebellum than in
forebrain. Under the assay conditions we employed, at concentrations of QUIN which approached the limits of its solubility in physiological solution, our studies provide no evidence of direct toxicity of QUIN on cultured cerebellar granule cells; no other published reports exist to the contrary. In situ hybridization studies of the differential distribution of NMDA receptor mRNA in brain suggest a molecular basis for the pharmacological heterogeneity of sensitivity to quinolinate in brain. The NR2A subunit is distributed uniformly across brain, whereas NR2C is predominantly localized in cerebellum; NR2B, in contrast, is nearly exclusively distributed to the forebrain in mature animals (Moriyoshi et al., 1991). Thus, forebrain sensitivity, as well as insensitivity of cerebellum, to QUIN is likely a consequence of NMDAR subtype distribution (Perkins and Stone, 1983; McLennan, 1984; Monyer et al., 1992).

Because QUIN alone exerted no toxicity to cerebellar granule cells, this model system presented an ideal opportunity to visualize the potentiation of GLU toxicity by QUIN; our data provide the first evidence that glutamate-mediated neurotoxicity can be greatly potentiated by quinolinate. This potentiation is dose-dependent manner and is characterized by an EC50 of 152.4 (95% C.I.: 57.6-403.1) µM (Figure 5). Potentiation of GLU toxicity by QUIN had a threshold for GLU of 100 µM (Figure 13). Our data indicate that potentiation of GLU-mediated neurotoxicity by QUIN requires NMDA receptor activation because both the NMDA receptor channel blocker - MK-801 and 5,7-DCKA, a selective competitive antagonist of the strychnine-insensitive glycine site of the NMDA receptor, afford full protection against the neurotoxicity mediated by 400 µM glutamate combined with 1 mM quinolinate (Figure 7 and Figure 8). These findings
suggest that quinolinate positively modulates the neurotoxicity of glutamate in cerebellar granule cell cultures through NMDA receptor activation.

Although all published reports support characterization of QUIN as an NMDA receptor agonist, a number of other modulatory sites govern receptor activation; these sites are functionally regulated by several endogenous and exogenous compounds (Mayer et al., 1989; MacDonald and Nowak, 1990; Yoneda and Ogita, 1991). To gain insight into the molecular basis of potentiation by QUIN of GLU toxicity, we evaluated the capacity of specific pharmacological modulators of NMDA receptor functional domains to influence the response to QUIN. Full activation of NMDA receptor populations requires occupancy by agonists of the strychnine-insensitive glycine site; this functional significance of this site is indicated by its depiction not as a modulatory site but as a 'co-agonist' site (Kleckner and Dingledine, 1988). Kynurenic acid, which is a product of an alternate branch of the tryptophan metabolism, has antagonist activity at the glycine co-agonist site of the NMDA receptor (Kessler et al., 1989; McNamara et al., 1990; Patel et al., 1990). 5,7-Dichlorokynurenic acid (5,7-DCKA), is a derivative of kynurenic acid and among the most potent and selective antagonists of the strychnine-insensitive glycine binding site of the NMDA receptor (Baron et al., 1990). Surmountability of the neuroprotective effects of 5,7-DCKA by QUIN, and its capacity to induce a rightward shift in the QUIN dose-response relationship suggests that QUIN potentiation of glutamate neurotoxicity may involve modulation of the glycine site of the NMDA receptors (Figure 9, open triangles).

Functional significance for the observed potentiation by quinolinate of glutamate neurotoxicity will depend upon whether the potentiation occurs in mature neurons, and in
vivo. Moreover, the data we present here may represent the biology of subtypes (NR2A, NR2C) of NMDA receptors (McLennan, 1984; Moriyoshi et al., 1991; Monyer et al., 1992). Since NR2C is predominantly localized to the cerebellum in mature animals, potentiation by QUIN is possibly a characteristic of NR2C assembled with NR2A if potentiation of glutamate toxicity by quinolinate can not occur in other brain regions where other NMDA receptor subtypes (NR2B/NR2A) predominate (Perkins and Stone, 1983; McLennan, 1984; Monyer et al., 1992).

Although physiological levels of QUIN in the CNS are likely below the threshold for toxicity mediated by QUIN alone, our data indicate that potentiation of GLU toxicity occurs at QUIN concentrations well below those required for it to be toxic alone. QUIN, therefore may play an important role in potentiation of GLU released from CNS insult or trauma. Moreover, central nervous system QUIN levels are increased in a number of pathological states and in aging. QUIN has been reported to increase in concentration in brain several-fold in aged rats (Moroni et al., 1984), and QUIN concentration in brain was found to be 2- to 3-fold greater than that of normal individuals, and 5- to 6-fold higher in the CSF of comatose patients dying from liver failure. In addition, QUIN levels are increased in the CSF of patients infected with HIV (see review by Stone, 1993). It is possible that QUIN may accumulate sufficiently to activate NMDA receptors through potentiation of glutamate in some pathological states.

It has been shown that occupation of the glycine site is necessary for efficient NMDA receptor activation (Johnson and Asher, 1987; Kleckner and Dingledine, 1988; Patel et al., 1990). Glycine has also been shown to increase the NMDA agonist-induced binding of NMDA receptor channel blockers (Reynolds et al., 1987; Franklin and
Murray, 1992). Data arising from in our experiments are in agreement with these studies since 5,7-DCKA competed with endogenous glycine and is able to completely reverse the neurotoxic effects of glutamate combined with quinolinate (Figure 8 and Figure 9). In addition, even though glycine may be present in neuronal medium, the neurotoxicity of glutamate on cerebellar granule cells frequently can be increased by addition of exogenous glycine (Figure 11) which is consistent with the report by Simpson et al. (1994). Further investigations will be carried out to elucidate the relationship between glycine and QUIN. We have found (data not shown) that QUIN also potentiates the glutamate-mediated toxicity in the presence of saturating concentrations of glycine. One possibility is that QUIN may influence cooperativity among multiple glycine and/or glutamate binding sites residing on subunits of this oligomeric receptor-ion channel complex.

Since quinolinate has been suggested to be involved in the etiology of several major CNS disorders (Stone, 1993), analogs of QUIN may provide valuable experimental tools or reveal novel therapeutic strategies against this pathology. We find 2NPC, 3NPC and 2TPC, which are, respectively, nitro- or thio- substituted analogs of QUIN, were, even at millimolar concentrations, not toxic to cultured cerebellar granule neurons but could reduce the neurotoxicity of maximally effective concentrations of glutamate/glycine. Experiments need to be designed to investigate whether they have capacity to regulate QUIN metabolism or availability.

In conclusion, Our data show that at concentrations as high as 1mM, QUIN is unable to cause neurotoxicity in rat cerebellar granule neurons, alone. Our data are the first to provide evidence that QUIN potentiates the neurotoxicity of maximally effective
concentrations of glutamate. We further demonstrated this potentiation is through NMDA receptor. We also provide data which imply the potentiation by QUIN may involve the glycine site of NMDA receptor and that this potentiation cannot be readily explained by simple additvity to the neurotoxicity induced by glutamate acting at the NMDA-receptor agonist site. As such, all these data may provide evidence of another mechanism of glutamate receptor-mediated neurotoxicity which will be of interest to further investigations. Nitro- or thio- quinolinate analogs showed partial protection against neurotoxicity induced by glutamate/glycine at millimolar concentration.
6. SUMMARY

Little is known of the influence of QUIN on CNS function. QUIN possesses agonist activity at the NMDA receptor, and may be involved in the pathology of brain disorders such as Huntington’s diseases, AIDS-related dementia, and Alzheimer’s diseases, as well as the pathology of hypoxic, traumatic CNS insults. The possible participation of QUIN in excitotoxic CNS pathologies has attracted great attention in recent years. It is important to completely elucidate the neurotoxic mechanism of quinolinate and to explore the possible therapeutic value of structural analogs of QUIN, which may potentially act as pharmacological antagonists of NMDA receptor activation. We have compared the neurotoxic effects of exposure to GLU alone and QUIN alone, exposure to GLU/QUIN on rat cerebellar granule cells in vitro by measuring the release of LDH from dying cells. We further evaluated the capacity of structural analogs of QUIN to produce excitotoxic cell death in the cerebellar granule cells through activation of glutamate receptors, or to block this excitotoxic cell death mediated by glutamate.

Our data shows that GLU induces significant neurotoxicity on cultured cerebellar granule cells but quinolinate is unable to cause neurotoxicity on cells even at concentration as high as 1mM; however, our data are the first provide evidence that QUIN potentiates glutamate-mediated neurotoxicity. Furthermore, our data provide evidence that potentiation by quinolinate of glutamate-mediated neurotoxicity occurs through activation of the NMDA receptor since both the NMDA receptor-channel blocker-MK801 and strychnine-insensitive, glycine site antagonist 5,7-DCKA are able to fully block the neurotoxic effect mediated by GLU/QUIN. Moreover, our experiments
present data which imply that the potentiation by QUIN may involve the glycine site of NMDA receptor because QUIN surmounts the protection of 5,7-DCKA against cytotoxicity, but appears to be decreased in potency by this selective competitive antagonist of the NMDAR glycine site. This phenomenon cannot be readily explained by simple additivity of QUIN to the neurotoxicity induced by glutamate acting at the NMDA-receptor agonist site. These data may provide evidence of another mechanism of glutamate receptor-mediated neurotoxicity, which will be of interest in the future.

Our results suggest future studies to investigate whether the observed QUIN potentiation of glutamate neurotoxicity occurs in mature neurons, and in vivo; and whether it may represent the biology of specific subtypes (NR2A, NR2C) of NMDA receptor or it is generally true of NMDA receptors.

Nitro- and thio- substituted analogs of QUIN showed partial protection against neurotoxicity induced by GLU/GLY(400μM/100μM) at millimolar concentrations. Substitutions for the acidic elements of positions 2 or 3 of pyridine ring of QUIN appears to represent important determinants of neurotoxicity or neuroprotective function of QUIN at NMDA receptors. Further experiments need to be designed to investigate whether these QUIN analogs have capacity to regulate QUIN metabolism or availability.
BIBLIOGRAPHY


