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# Picolinic acid modulates kainic acid-evoked glutamate release from the striatum in vitro

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Since picolinic acid, a tryptophan metabolite yielded by the kynurenine pathway, selectively attenuates quinolinic and kainic acid excitotoxicity that is dependent on the presence of a glutamatergic afferent input, it was hypothesized that this agent may inhibit the presynaptic release of glutamate Using superfused rat striatal slices, this study examined the potential of picolinic acid, and related pyridine monocarboxylic acids, to modify kainic acid-induced glutamate release Kainic acid (0 25, 0 5 and 1 0 mM) stimulated the release of glutamate, an effect which was calcium dependent and was attenuated in the presence of the kainate/AMPA receptor antagonist, 6,7-dinitroquinoxalene-2,3-dione (500 $\mu$M) Picolinic acid significantly decreased glutamic acid release evoked by exposure of striatal slices to 1 mM kainate in the presence of calcium The inhibitory action of picolinic acid on kainate-induced release was also shared by nicotinic and isonicotinic acid In the absence of external calcium, kainic acid-induced glutamate release was significantly reduced by approximately 65% Under this condition, picolinic acid (100 $\mu$M) failed to influence kainic acid-induced release Picolinic acid (100 $\mu$M) itself increased glutamate release by 35% over basal release While the ability of picolinic acid to inhibit excitotoxin-induced release supports the notion that it may act presynaptically to modify excitotoxicity, lack of structural specificity in its action tends to cast doubt on this mechanism of action

## INTRODUCTION

Recent studies have shown that certain products of tryptophan metabolism via the kynurenine pathway can interact with excitatory amino acid (EAA) receptors as agonists or antagonists[17] One such metabolite, quinolinic acid (QUIN), a pyridine dicarboxylic acid, activates NMDA receptors to produce neuronal excitation[16] and excitotoxicity[14] In contrast, another metabolite, kynurenic acid, acts as an NMDA and non-NMDA receptor antagonist and can block QUIN-induced excitation or excitotoxicity[1,8]

A recent study involving evaluation of the anti-excitotoxic potential of several kynurenine pathway-derived metabolites showed that picolinic acid, a pyridine monocarboxylate, can attenuate the neurotoxic action of QUIN on basal forebrain cholinergic neurons[9] This anti-excitotoxic action of picolinic acid is unusual in that this tryptophan metabolite does not appear to have a prominent effect on synaptic excitation In experiments on hippocampal slices, Robinson et al [12] found that, unlike kynurenic acid, picolinic acid was not very effective in blocking neuronal excitation driven by synaptic activation Thus, picolinic acid appears to influence excitotoxicity without significantly affecting excitation of neurons

The mechanism underlying the anti-QUIN action of picolinic acid is unknown A recent study[2] from this laboratory which evaluated the action of picolinic acid against the neurotoxic effects of several excitotoxins revealed that this agent was only effective in attenuating the effects of those excitotoxins (QUIN and kainic acid) which require the presence of intact glutamatergic afferents for the expression of their neurotoxic effects This observation led to the postulate that picolinic acid may exert its protective effect against the actions of certain excitotoxins through an interaction with the glutamatergic afferent input at a presynaptic level[2] Previous studies have demonstrated that kainic acid stimulates glutamatergic afferents and produces a calcium-dependent release of glutamate[3,4,6,7,19] Since glutamatergic deafferentation of certain brain areas blocks kainate neurotoxicity in these areas, it has been

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hypothesized that synaptic release of glutamate, or a related substance, from glutamatergic afferents, contributes to the neurotoxic action of this excitotoxin[13] A similar action of QUIN has also been postulated, although the evidence for a stimulatory action of QUIN on glutamate release is much less than that for kainate[4,5] In view of these observations, it would appear that picolinic acid may attenuate kainate and QUIN-induced neurotoxicity by inhibiting the presynaptic release of glutamate from nerve terminals The actions of picolinic acid on this release are unknown The present study was undertaken to determine if picolinic acid and certain structurally related pyridine monocarboxylates can influence excitotoxin-evoked glutamate release This communication describes the action of kainic acid on the release of endogenous glutamate from slices of the rat striatum and the effect of picolinic acid and related agents on this release

## MATERIALS AND METHODS

All release experiments were performed using male Sprague–Dawley rats (225–300 g), housed in a light/dark environment (12 h light, 12 h dark) at a constant temperature (20°C), and given full access to food and water The animals were killed by decapitation, the brain removed and rinsed in ice-cold Krebs' Ringer bicarbonate (KRB) solution at pH 7 4 Dissection of the striatal region was carried out in a cold room (4°C) The brain was placed, ventral surface upward, in a caudal to rostral dissection, on a KRB-soaked filter paper resting on a cold surface A plexiglass, two-blade holder was placed on the brain such that the rear blade was positioned immediately rostral to the optic chiasm Using the holder, a 4-mm coronal tissue section was prepared The dissected tissue slab was laid flat with rostral surface facing down, and striatum from both sides was carefully removed using a microdissection knife The striata were rapidly transferred to a moistened filter paper resting on the circular stage of a McIlwain tissue chopper Using this chopper, 300-$\mu$m-thick sagittal slices of the striatum were prepared and transferred to a glass petri dish containing ice-cold KRB solution The slices prepared from striata of two rats were pooled and transferred to four chambers of a superfusion apparatus Each chamber (0 5 ml volume) was maintained at 37°C by circulating water through a jacket surrounding the chamber The tissues were placed on a polypropylene mesh screen in each chamber and superfused with oxygenated KRB solution (maintained at 37°C) using a flow rate of 0 3 ml/min

Following a 1 5-h superfusion period to equilibrate tissues, a specific experimental protocol was followed to investigate (a) baseline and stimulus-evoked glutamate release, (b) calcium dependence of evoked release, and (c) release of glutamate in the presence of specific agents under study Details of individual experiments are provided in the following section (see Results) Samples of superfusate were collected at 5-min intervals and reserved for the estimation of glutamate levels

Quantitation of glutamate was carried out by reverse-phase high-performance liquid chromatography (HPLC) using an automated Shimadzu system The analysis involved pre-column derivatization of the amino acid with *O*-phthaldehyde (OPA) and $\beta$-mercaptoethanol at pH 10 4 to form fluorescent isoindole derivative, separation on a octadecylsilane reverse-phase column (Supelcosil LC-18, 4 6 mm i d $\times$150 mm length, 5 $\mu$m particle size, Supelco, Oakville, Ontario, Canada), and detection of the fluorescence measurement The mobile phase for HPLC, comprised of 50 $\mu$M aqueous sodium acetate/methanol (72/28 v/v) at pH 7 5, pumped through the column at a flow rate of 3 0 ml/min The detection of the amino acid derivative was carried out using an excitation and omission wavelength of 345 and 470 nm, respectively The peak areas of the chromatographic signals were integrated and used to calculate the concentration of amino acid Aqueous working standards and three water blanks were analyzed with each set of superfusate samples The concentration of glutamate in each experimental sample was calculated from the respective standard curve (see below)

At the end of superfusion the slices were removed from the chamber, and protein content in the homogenate determined by the method of Lowry et al [11]

The release of glutamate from slices was calculated as pmol/mg protein/5 min The results of experiments involving evoked release were expressed as percent over baseline release This value was calculated as follows

$$\% \text{ Over baseline release} = \frac{\text{Total stimulated release} - \text{baseline release}}{\text{Baseline release}} \times 100$$

The baseline release refers to the average release in three collection periods immediately preceding the application of the stimulating agent The stimulated release refers to the total release occurring above the baseline value

*Drugs and chemicals*

Kainic acid, picolinic acid, isonicotinic acid, nicotinic acid, 6,7-dinitroquinoxalene-2,3-dione (DNQX), *O*-phthaldehyde, and $\beta$-mercaptoethanol were all obtained from Sigma Chemical Co (St Louis, Missouri, USA) Analytical grade methanol and sodium acetate were obtained from Canlab (Toronto, Ontario, Canada) and Fisher Scientific (Canada), respectively All solutions and buffers were freshly prepared using deionized water

*Statistical analysis*

Data were compared using a one-way analysis of variance (ANOVA) followed by a Newman–Keuls test, or a Student's *t*-test

## RESULTS

### *Effect of kainate on glutamate release*

The effect of kainate on the release of glutamate from striatal slices are shown in Fig 1A When tissue slices were exposed to kainic acid (1 mM) for a 5-min period, there was an immediate increase in the release of glutamate The release evoked by kainate gradually returned to the original baseline level over the following 30-min superfusion period Fig 1B illustrates the dose–response relationship for the stimulatory action of kainic acid on glutamate release An increase in the release of glutamate was apparent at 0 25 mM kainate concentration and a maximal increase in release (200% above baseline) was observed at 1 mM kainate A higher concentration of kainate also induced release, but the recovery to pre-kainate baseline levels was inconsistent (data not shown)

To determine if the stimulatory effect on kainate involved activation of EAA receptors, the action of DNQX (100, 250 and 500 $\mu$M), a kainate/AMPA receptor antagonist, on this release was tested In these and subsequent experiments, the release protocol used to study kainate effect (Fig 1A) was slightly modified

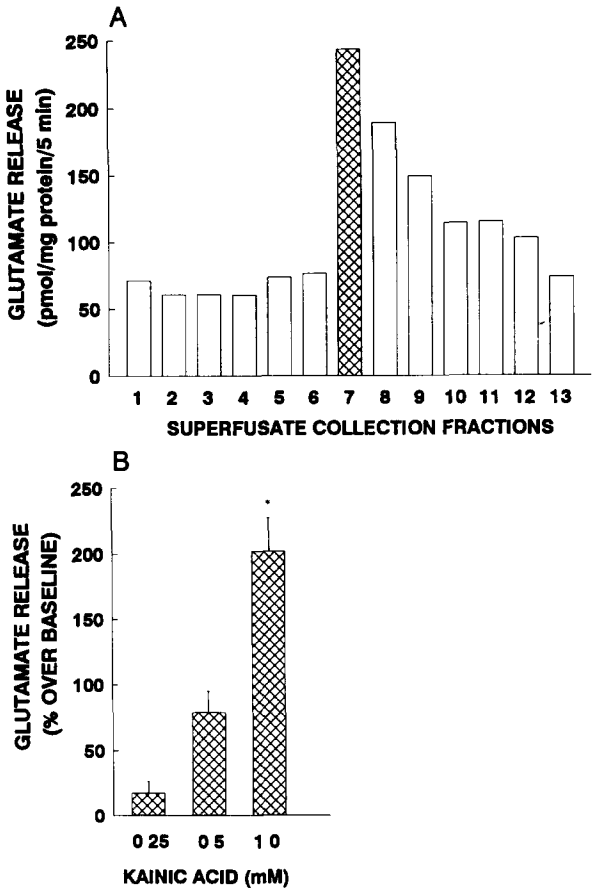


Fig 1 A representative experiment showing the action of kainic acid on endogenous glutamate release from rat striatal slices The tissues were superfused with Krebs' bicarbonate for 1 5 h prior to the start of sample collection Superfusate samples (1–13, horizontal axis) were collected at 5-min periods Slices were exposed to Krebs' bicarbonate containing kainic acid (1 mM) during the period designated by crossed bars B effect of different concentrations of kainic acid on the release of glutamate from rat striatal slices The vertical axis represents the total release of glutamate expressed as a percent of baseline value The baseline release (128 1 ± 17 0 pmol/mg protein/5 min) represents average release during the six collection periods preceding exposure to kainate (see panel A) Each point represents an average of 10 experiments ± S E M * Significantly above baseline ($P < 0$ 05)

After three baseline sample collections, the tissues were superfused with a medium containing a fixed concentration of DNQX, and exposure to this agent was maintained for the subsequent seven collection periods Following this, the tissues were superfused with normal medium Fig 2 shows the results from experiments with DNQX At the two lower concentrations (100 and 250 $\mu$M), DNQX appeared to attenuate the kainate-stimulated glutamate release, but this effect was not statistically significant ($P > 0$ 05) However, at a higher dose (500 $\mu$M), the antagonist produced a significant inhibition of the kainate effect In the presence of this concentration of DNQX, the kainate-evoked release was only about 25% above baseline release, representing a 75% inhibition of this

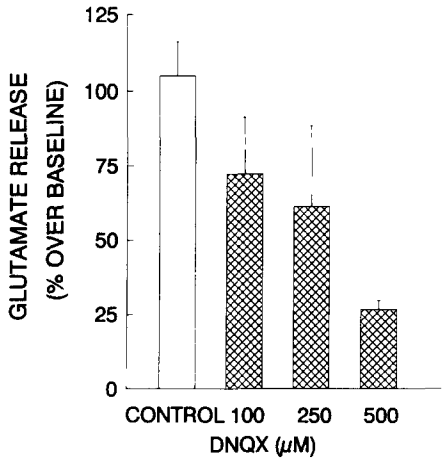


Fig 2 Effect of the kainate/AMPA receptor antagonist, 6,7-dinitroquinoxalene-2,3-dione (DNQX), on striatal glutamate release induced by 1 mM kainate Vertical axis represents total glutamate release expressed as a percent of baseline value (100 0 ± 9 0 pmol/mg protein/5 min) Control release represents that induced by exposure of slices to kainate alone (1 mM) Each bar represents the average of eight experiments ± S E M * Significantly lower than control value ($P \leq 0$ 05)

release by the antagonist At the three concentrations used here, DNQX alone did not alter the baseline release of glutamate (data not shown)

In subsequent tests, using the experimental procedure employed in the DNQX experiments, the action of picolinic acid (100 $\mu$M) on the kainate-stimulated glutamate release was tested In the first part of these experiments, the effect of picolinic acid alone on glutamate release was investigated As shown in Fig 3, when several doses of this agent were tested, a significant increase in glutamate release – 35% above baseline – was observed in the presence of 100 $\mu$M picoli-

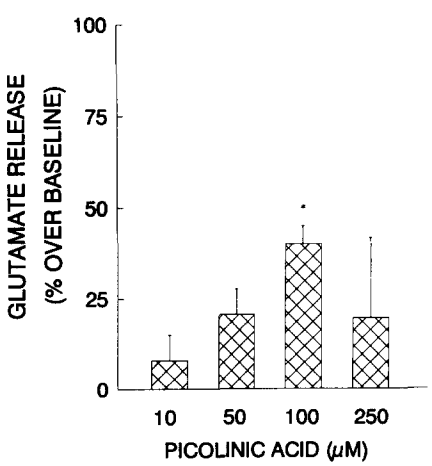


Fig 3 Effect of picolinic acid alone on the spontaneous release of glutamic acid from striatal slices Vertical axis represents total release in the presence of picolinic acid expressed as a percent of baseline value (94 0 ± 16 0 pmol/mg protein/5 min) Each value is an average of eight experiments ± S E M * Significantly different from release in the absence of picolinic acid ($P < 0$ 05)

nate In subsequent experiments, the effect of picolinate on kainate-induced glutamate release was investigated Picolinate produced a dose-related decrease in the kainate-induced response, with maximal inhibition of 70% occurring at a concentration of 100 $\mu$M picolinate (Fig 4) In previous experiments, other monocarboxylates have been found to share the anti-excitotoxic action of picolinate[2] To determine if their action is also exerted on glutamate release, the effects of nicotinic and isonicotinic acid on the release of glutamate were tested At a dose of 250 $\mu$M, both agents inhibited the kainic-acid evoked release to the same extent as did picolinic acid (Fig 4) Both agents also mimicked the stimulatory effect of picolinic acid alone on glutamate release (data not shown)

The persistence of a picolinate-resistant component of kainate-induced glutamate release (about 30% of total release response) suggested that picolinic acid may only be attenuating the calcium-dependent component of release To determine the calcium-dependent component of glutamate release, the action of kainate was tested in the absence of external calcium and the presence of EGTA In this part of the study, the release protocol (Fig 1A) was modified to exclude calcium ions from the superfusion medium After three baseline collections in normal medium, the striatal slices were exposed to calcium-free medium containing EGTA (1 mM) This superfusion condition was maintained until the end of the experiment As shown in

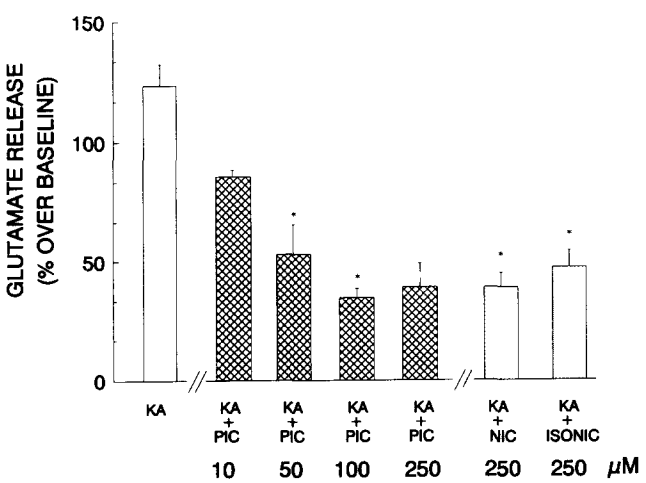


Fig 4 Effect of pyridine monocarboxylates, picolinic (PIC), nicotinic (NIC) and isonicotinic (ISONIC) acid, on the kainic acid (KA)-evoked release of glutamic acid from rat striatal slices In release experiments involving pyridine monocarboxylates, the agent under investigation was added to the superfusion medium 15 min pre-kainate exposure and maintained for 15 min post-kainate (see Fig 1) Vertical axis represents total release of glutamate expressed as a percent of baseline value (94 0 ± 16 0 pmol/mg protein/5 min) Each value represents an average of six experiments ± S E M * Significantly different from control release (evoked by 1 mM kainic acid) ($P <$ 0 05)

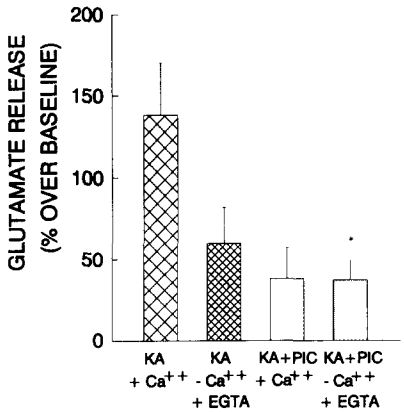


Fig 5 Action of picolinic acid (PIC) (100 $\mu$M) on the kainate-evoked release of glutamate (see Fig 1) from superfused striatal slices in the presence (1 3 mM) and absence of external calcium In experiments involving omission of calcium, the slices were exposed to calcium-free medium containing 1 mM EGTA for 30 min prior to the start of collection and throughout the subsequent superfusion Vertical axis represents total release expressed as a percent of baseline glutamate release (124 6 ± 6 0 pmol/mg protein/5 min) Each value represents an average of 12 experiments ± S E M * Significantly different from control release (evoked by 1 mM kainic acid)

Fig 5, in the absence of external calcium the kainate-evoked release was approximately 50% over baseline, representing a 65% decrease in the kainate effect obtained in the presence of calcium Picolinic acid did not significantly alter kainic acid-evoked glutamate release in the absence of external calcium (Fig 5) Thus, the ability of picolinate to inhibit kainate-induced glutamate release required the presence of calcium in the superfusate

## DISCUSSION

The present study, which examined the influence of picolinic acid on kainic acid-induced glutamate release from rat striatal slices, yielded four major observations (a) kainic acid produced a $Ca^{2+}$-dependent release of glutamate, a response that was sensitive to the kainate/AMPA receptor antagonist, DNQX, (b) picolinic acid significantly attenuated the calcium-dependent, but not $Ca^{2+}$-independent, kainate-induced glutamate release, (c) picolinic acid alone augmented the baseline release of glutamate, and (d) the inhibitory action of picolinic acid on evoked glutamate release was shared by the other pyridine monocarboxylates nicotinic and isonicotinic acid

Since the initial observation by Ferkany and Coyle[7], several studies have demonstrated that kainic acid stimulates release of glutamate from slice preparations of the striatum and other brain regions[3,6,19] A study by Young et al [19] showed that kainic acid also stimulates glutamate release from the striatum in vivo The pre-

sent study on striatal slices demonstrated two components of kainate action on glutamate release a calcium-dependent component of release accounting for approximately 70% of the total release induced by kainate, and a calcium-independent component accounting for the remaining 30% release The calcium-dependent component most likely originates from terminals of the glutamatergic neurons that project from the cerebral cortex to the neostriatum[15] This possibility is suggested by the study of Young et al [19] who reported that following decortication kainic acid-evoked glutamate release from the rat striatum was reduced by approximately 60%, a value that closely approximates the calcium-dependent release in this study The kainate/AMPA antagonist, DNQX, also reduced the kainate effect on glutamate release by nearly 60% Thus, it appears from these findings that the kainate-evoked calcium-dependent release of striatal glutamate may originate largely from nerve terminals, and that kainate may act on a presynaptic receptor to produce this release The origin of the calcium-independent component of release is less clear This release may originate from glutamatergic nerve terminals via a cellular mechanism that does not require calcium, possibly inhibition of glutamate reuptake, or it may originate from non-neuronal elements such as striatal glial cells

Picolinic acid produced a dose-related inhibitory effect of the kainate-evoked glutamate release from striatal slices The magnitude of the maximal picolinate effect was very similar to the inhibitory effect produced by the absence of calcium in the superfusion medium Under both conditions, approximately a two-thirds reduction of the total glutamate release evoked by kainic acid was observed When the action of picolinic acid on the calcium-independent release was examined, it failed to inhibit this release It would appear from these results that picolinic acid eliminated the component of glutamate release that is calcium-dependent Thus, picolinic acid may exert its inhibitory action on glutamate release by inhibiting translocation of external calcium ions to the release mechanism However, this possibility remains to be tested in future experiments

An alternate possibility is that picolinic acid interacts with an antagonist-sensitive site to influence the kainate-evoked glutamate release DNQX, an AMPA/kainate receptor antagonist, produced inhibition of release comparable to that induced by a maximal dose of picolinic acid, but this antagonist was less potent than the pyridine monocarboxylic acid In other tissue models, however, the action of picolinic acid apparently does not correspond to that of DNQX Thus, in electrophysiological studies on hippocampal slices, DNQX very effectively blocks synaptically evoked excitatory responses[10], but picolinic acid apparently exerts little influence on these responses[12] In view of this discrepancy, a simple interaction of picolinic acid with DNQX-sensitive sites is unlikely to explain its inhibitory action on glutamate release observed in the striatum

Although picolinic acid inhibited the evoked release of glutamate, by itself it produced a weak stimulatory action A statistically significant increase in release was only apparent at one concentration of picolinic acid The significance of this effect is presently unknown In separate experiments, we observed that a dose of picolinate which induced a significant release of glutamate from striatal slices did not influence high affinity glutamate uptake by striatal synaptosomes (unpublished observations) The possibility that picolinic acid exerts its stimulatory action, and indeed its inhibitory action, on kainate-evoked release by influencing the release of another neurotransmitter or neuromodulator in the striatum cannot be excluded Additional studies are therefore needed to explore the mechanism of action of picolinic acid

On the basis of a previous report[2] that picolinic acid attenuates the excitotoxic action of only those agents that require an intact glutamatergic afferent input, and the present finding that this agent inhibits kainate-evoked presynaptic release of glutamate, it is suggested that picolinic acid attenuates excitotoxicity by depressing glutamate release Its weak but discernable stimulatory action on baseline glutamate release may explain its low potency and efficacy in excitotoxicity tests when its neuroprotective action is compared with other tryptophan metabolites such as kynurenic acid[2,9] However, other observations concerning picolinate tend to cast some doubt on this explanation of the anti-neurotoxic effect of picolinic acid Two pyridine monocarboxylates, nicotinic and isonicotinic acid, that were found to exert differential effects on cholinergic excitotoxicity[2], did not exert a similar differential action on the excitotoxin-evoked glutamate release in the present study Additionally, while picolinic acid clearly attenuates QUIN neurotoxicity, the role of glutamate release as a contributory factor in this neurotoxicity is not clear[18] The evidence that QUIN induces a calcium-dependent release of glutamate comparable to that induced by kainate is not convincing, although a stimulatory effect of QUIN on amino acid release from the cortex in vivo has been demonstrated[4] Since both kainate and QUIN have been found to induce glutamate release from brain regions in vivo, it may be useful in future to test the action and specificity of picolinic acid on this release Such studies may reveal a better correlation

between the actions of picolinic acid on transmitter release and neurotoxicity

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## REFERENCES


1 Boegman, R J , El-DeFrawy, S R , Jhamandas, K , Beninger, R J and Ludwin, S K , Quinolinic acid neurotoxicity in the nucleus basalis antagonized by kynurenic acid, *Neurobiol Aging*, 6 (1985) 331–336

2 Cockhill, J , Jhamandas, K , Boegman, R J and Beninger, R J , Action of picolinic acid and structurally related pyridine carboxylic acids on quinolinic acid-induced cortical cholinergic damage, *Brain Res* , 599 (1992) 57–63

3 Collins, G G S Anson, J and Surtees, L , Presynaptic kainic acid and NMDA receptors regulate excitatory amino acid release in the olfactory cortex, *Brain Res* , 265 (1983) 157–159

4 Connick, J H and Stone, T W , Quinolinic acid effects on amino acid release from the rat cerebral cortex in vitro and in vivo, *Br J Pharmacol* , 93 (1988) 868–876

5 Fedele, E and Foster, A C Changes in striatal extracellular amino acids measured by intracerebral dialysis following injection of a neurotoxic dose of quinolinate into the rat striatum, *Br J Pharmacol* , Suppl 104 (1991) 336P

6 Ferkany, J W and Coyle, J T , Kainic acid selectively stimulates the release of endogenous excitatory amino acids *J Pharmacol Exp Ther* , 225 (1983) 399–406

7 Ferkany, J W Azcaek, R and Coyle, J T , Kainic acid stimulates excitatory amino acid neurotransmitter release at presynaptic receptors, *Nature*, 298 (1982) 757–759

8 Foster, A C , Vezzani, C A , French, E D and Schwarcz, R , Kynurenic acid blocks neurotoxicity and seizures induced in rats by the related brain metabolite quinolinic acid, *Neurosci Lett* , 48 (1984) 273–278

9 Jhamandas, K , Boegman, R J , Beninger, R J and Bialik, M , Quinolinate induced cortical cholinergic damage modulation by tryptophan metabolites, *Brain Res* , 529 (1990) 185–191

10 Lambert, N A , Borroni, A M , Grover, L M and Teyler, T J , Hyperpolarizing and depolarizing $GABA_A$ receptor-mediated dendritic inhibition in area CA1 of the hippocampus, *J Neurophysiol* , 66 (1991) 1538–1548

11 Lowry, O H , Rosenbrough, N S , Farr, A L and Randall, R S , Protein measurement with the folin phenol reagent, *J Biol Chem* , 193 (1951) 265–275

12 Robinson, M B , Schults, M K , Freund, R K , Johnson, R L and Koerner, J F , Structure-function relationships for kynurenic acid analogues at excitatory pathways in the rat hippocampal slice, *Brain Res* , 361 (1985) 19–24

13 Schwarcz, R , Foster, A C , French, E D and Whetsell, W O , Excitotoxic models of neurodegenerative disorders, *Life Sci* , 35 (1984) 19–32

14 Schwarcz, R , Whetsell, W O and Mangano, R M , Quinolinic acid an endogenous metabolite that produces axon-sparing lesions in rat brain, *Science*, 219 (1983) 316–318

15 Spencer, H J , Antagonism of cortical excitation of striatal neurons by glutamic and diethyl ester Evidence for glutamic acid as an excitatory transmitter in the striatum, *Brain Res* , 102 (1976) 91–101

16 Stone, T W and Perkins, N M , Quinolinic acid a potent endogenous excitant at amino acid receptors in CNS, *Eur J Pharmacol* , 72 (1981) 411–412

17 Stone, T W and Connick, J H , Quinolinic acid and other kynurenines in the central nervous system, *Neuroscience*, 15 (1985) 597–617

18 Vezzani, A , Ungersted, V , French, E D and Schwarcz, R , In vivo brain dialysis of amino acids and simultaneous EEG measurements following intrahippocampal quinolinic acid injection evidence for a dissociation between neurochemical changes and seizures, *J Neurochem* , 45 (1985) 335–344

19 Young, A M J , Crowder J M and Bradford, H F , Potentiation by kainate of excitatory amino acid release in striatum complimentary in vivo and in vitro experiments, *J Neurochem* , 50 (1988) 337–345