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Action of picolinic acid and structurally related pyridine carboxylic acids on quinolinic acid-induced cortical cholinergic damage

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Picolinic acid, a pyridine monocarboxylic acid derived from tryptophan metabolism by the kynurenine pathway, was shown to block cortical cholinergic neurotoxicity induced by quinolinic acid (QUIN), a pyridine dicarboxylic acid yielded by the same pathway. This study examined the specificity of the anti-toxic effect of picolinic acid by comparing its effect with several structurally related mono- and dicarboxylic acids, and by evaluating its potential to influence cholinergic neurotoxicity produced by kainic, ibotenic and quisqualic acid. Picolinic acid or related agents were injected alone or in combination with a fixed dose of QUIN into the right nucleus basalis magnocellularis (nbM) of rats anesthetized with halothane. Cholinergic neurotoxicity was assessed 7 days post injection by measuring choline acetyltransferase (ChAT) activity in the frontoparietal cortex on the injected and uninjected side. In picolinate experiments, the staining of nbM neurons by acetylcholinesterase (AChE) histochemistry was also examined. Focal injections of QUIN depleted cortical ChAT activity and staining of AChE in the nbM. Co-injection of picolinic acid with QUIN attenuated the decline in these two cholinergic neuron markers. Isonicotinate (4-pyridine monocarboxylate), but not nicotinate (3-pyridine monocarboxylate), significantly attenuated QUIN's effect on cortical ChAT activity. Among several dicarboxylic acids examined, only dipicolinate (2,6-pyridine dicarboxylate) showed activity. It produced a modest QUIN-like effect, but in co-injection experiments it attenuated the QUIN-induced decrease in cortical ChAT. When co-injected into the nbM with a neurotoxic dose of kainic, ibotenic or quisqualic acid which produced a neurotoxic response comparable to that of QUIN, picolinic acid attenuated kainic acid-induced neurotoxicity, but not ibotenate or quisqualate neurotoxicity. In contrast, kynurenic acid, which blocked QUIN's action, blocked the neurotoxicity of both kainate and ibotenate. The results suggest that actions of picolinic acid against QUIN show certain selectivity in regard to the chemical structure of picolinic acid. In addition, picolinic acid has selectivity against excitotoxins acting via different mechanisms. Its ability to influence only those excitotoxins which require an intact glutamatergic afferent input for their neurotoxic effect suggests that the protective effect of picolinic acid may involve an interaction with this input.

INTRODUCTION

In 1981, Stone and Perkins[23] showed that quinolinic acid (QUIN), a pyridine dicarboxylic acid which is a metabolite of tryptophan via the kynurenine pathway, acts as an excitant at the amino acid receptors. Subsequently, Schwarcz et al.[19] reported that intrastriatal injections of QUIN produced axon sparing lesions similar to those produced by excitotoxins such as kainic acid. A number of studies have now established that focal injections of QUIN into specific areas produce neurotoxicity and that this effect is due to the activation of NMDA receptors[17]. Localized infusions of QUIN into the nucleus basalis magnocellularis (nbM) destroy cholinergic neurons that project from this area to the frontoparietal cerebral cortex and reduce cortical levels of choline acetyltransferase (ChAT) activity[3]. This and other neurotoxic actions of QUIN can be blocked by metabolites that originate from the metabolic pathway that yields QUIN[22]. Foster et al.[8] showed that one such agent, kynurenic acid, blocks the seizures and neurotoxicity induced by QUIN injections. Focal injections of kynurenic acid, in combination with QUIN, into the nbM fully block the cortical cholinergic toxicity induced by QUIN injections into this region[1].

In a recent study, using the nbM model of QUIN-induced cholinergic neurotoxicity, we reported that several tryptophan metabolites — quinaldic acid, hydroxyquinaldic acid, anthranilic acid, picolinic acid (a pyridine monocarboxylate) — attenuated this neurotoxicity

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when co-injected with the excitotoxin[9]. The anti-excitotoxin effects of picolinic acid are of interest since in previous electrophysiological experiments this agent appeared not to depress synaptically evoked responses that are readily blocked by kynurenic acid[16]. While it is known that kynurenic acid blocks QUIN neurotoxicity by an interaction with the NMDA receptors, the site and mechanism underlying the antagonism by picolinic acid remain unknown. It is presently not known whether picolinic acid possesses specificity of action with regard to both its chemical structure and effectiveness against excitotoxins other than QUIN.

To gain a better understanding of picolinic acid, the present study addressed the questions of the specificity of picolinic acid action against QUIN-induced damage. The structural selectivity of picolinic acid was investigated by comparing its action with several pyridine mono- and dicarboxylates in neurotoxicity experiments. Its effectiveness against other excitotoxins was examined by evaluating its ability to afford protection against three excitotoxins — kainic acid, ibotenic acid, quisqualic acid — that produce neurotoxicity by interaction with different excitatory amino acid receptors. These actions of picolinate were determined using the nbM model of QUIN neurotoxicity employed in a previous study[9].

MATERIALS AND METHODS

Stereotaxic injections

Male Sprague–Dawley rats (275–350 g) were anesthetized with 4% halothane in oxygen and maintained on 2% halothane during surgery. The rat's head was placed in a stereotaxic frame and a single injection of either saline or drug was delivered via a steel cannula into the nbM using the coordinates: 0.8 mm posterior to bregma, 2.6 mm lateral and 8.0 mm ventral to the surface of the skull, with incisor bar set at −3.3 mm. The injection was made over 2.5 min, and the injection cannula was left in place for an additional 3.0 min to allow for diffusion. All drugs were injected in a volume of 0.5 μl. Solutions of drugs were made with 0.9% saline and adjusted to pH 7.4 with NaOH. In the co-injection experiments, the drugs were dissolved in the same solvent and delivered to the nbM as a single injection (0.5 μl). After injection, the animals were observed for 24 h and then returned to their home cages. Seven days post injection the animals were killed and the frontoparietal cortex from each hemisphere was removed for the biochemical analysis of choline acetyltransferase activity (ChAT), a marker for cholinergic neurons. In some experiments, the entire brain was removed and sections were prepared for histological assessment of the injection site (see below).

Biochemical analysis

A plexiglass razor blade holder was used to dissect an 8 mm coronal slab of the frontoparietal cortex. Right and left portions of the frontoparietal cortex were removed from this slab, and immediately homogenized for biochemical assay. The homogenate was assayed for protein content by the method of Lowry et al.[13], and for ChAT activity by the radiochemical method of Fonnum[6]. Specific cortical ChAT activities for injected and un-injected hemispheres were then compared to determine the change in the enzyme activity produced by various treatments.

Histology

The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and perfused transcardially with 200 ml saline (0.9%), followed by 300 ml paraformaldehyde (4%). The brains were removed and placed in 4% paraformaldehyde overnight, after which they were immersed in 30% sucrose for 24 h. Transverse 45 μm coronal slices were prepared on a freezing microtome, and stored in 0.9% saline at 4°C. Brain slices were stained for cholinergic neurons by the method of Karnovsky and Roots[10]. In order to facilitate the visualization of these cells, all animals were pretreated with di-isopropylfluorophosphate (DFP, 1.5 mg/kg, i.m.) six hours before perfusion[15].

Drugs and chemicals

QUIN, kainic acid, ibotenic acid, isonicotinic acid, picolinic acid, nicotinic acid, dipicolinic acid and kynurenic acid were all obtained from Sigma Chemical Company, St. Louis, MO, USA. Dinicotinic acid, cinchomeronic acid, isocinchomeronic acid and lutidinic acid were obtained from Aldrich Chemical Company Inc., Milwaukee, Wisconsin. Quisqualic acid was supplied by Cambridge Research Biochemicals, Valley Stream, New York. Halothane was obtained from Benson Medical Industries, Markham, Ontario.

Statistical analysis

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

RESULTS

Pyridine monocarboxylic acids

The action of picolinic acid and two other pyridine monocarboxylates — isonicotinic and nicotinic acid — were tested after combining these agents with QUIN in different molar ratios. The dose of QUIN was fixed at 120 nmol as this dose produces a maximal decrease in cortical ChAT activity after injection into the nbM[9]. Fig. 1 illustrates the results obtained with the 3 agents. Combination of picolinic or isonicotinic acid with QUIN

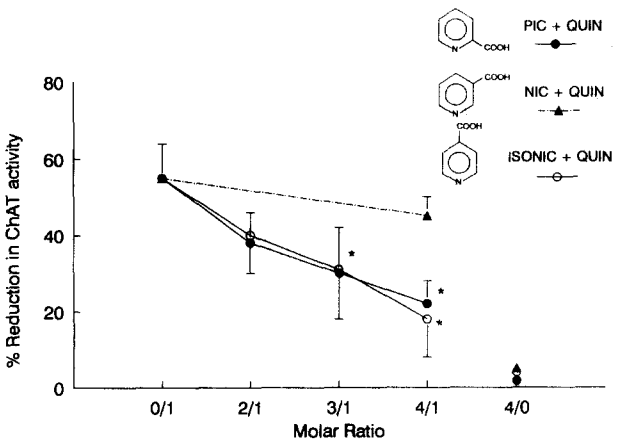


Fig. 1. Percent reduction (mean ± S.E.M.) in cortical ChAT activity after injection of different molar ratios of picolinic acid [PIC] (●), nicotinic acid [NIC] (▲), and isonicotinic acid [ISONIC] (○). ChAT activity in the ipsilateral and contralateral frontoparietal cortex was measured 7 days after drug infusion. The difference between the two values as a percentage is plotted as a function of molar ratios. The values on the far right represent picolinic acid, isonicotinic acid, and nicotinic acid alone (480 nmol) in the absence of QUIN. Each value is the mean ± S.E.M. from six to eight experiments. * Significantly different ($P \leq 0.05$) from control (molar ratio 0/1 and 4/0).

produced a dose-related attenuation of the decrease in cortical ChAT activity produced by QUIN alone. At the maximal dose of 480 nmol, picolinic (molar ratio 4:1) and isonicotinic acid (molar ratio 4:1) produced $22 \pm 6.0\%$ (mean $\pm$ S.E.M.) and $18 \pm 10.0\%$ decrease in cortical ChAT when combined with QUIN. These values were significantly lower ($p < 0.05$) than the value obtained with QUIN ($56 \pm 9.6\%$) or with picolinic acid alone. However, at 480 nmol, nicotinic acid co-injected with QUIN (molar ratio 4:1), produced a $45 \pm 5.0\%$ decrease in ChAT activity, a value not significantly different from that obtained with QUIN alone, indicating that nicotinic acid did not attenuate neurotoxicity. Thus, picolinic acid and isonicotinic acid produced a very comparable attenuation of the QUIN effect, while nicotinic acid was inactive. At the highest dose (480 nmol) tested, none of the 3 agents by themselves produced a significant decrease in cortical ChAT, indicating a lack of agonist activity.

Fig. 2 shows the action of picolinic acid on QUIN-induced neurotoxicity in nbM neurons stained for AChE. The tissue sections obtained from saline-injected animals (Fig. 2A) revealed the presence of large cholinergic neurons characteristic of this region. QUIN injections produced a severe depletion of the neurons that normally stained with AChE (Fig. 2B). In the tissue from the co-injected animals, the staining for AChE was still discernible, reflecting preservation of the neuronal perikarya. However, the density of these neurons appeared to be reduced in comparison with that of neurons in sections from the saline-injected animals. Thus, morphological experiments, like the biochemical experiments, showed that picolinate decreased QUIN-induced depletion of nbM cholinergic neurons.

Pyridine dicarboxylic acids

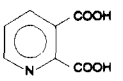
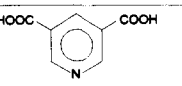
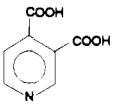
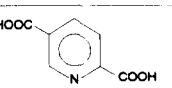
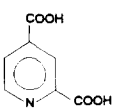
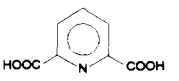
The comparative actions of several pyridine dicarboxylic acids — cinchomeronic, isocinchomeronic, dinicotinic, lutidinic and dipicolinic acid — on the viability of cortical cholinergic neurons were evaluated by single infusion into the nbM. These agents were infused alone or in combination with a fixed dose of QUIN (120 nmol) and Table I shows the effects of these combinations on cortical ChAT activity at 7 days post injection. The doses of dicarboxylic acids that were combined with QUIN were those which could be solubilized with 120 nmol QUIN at pH 7.4. As shown, only dipicolinic acid combined with QUIN reduced cortical ChAT activity, the decrease in this activity being significantly less ($P < 0.01$) than that produced by injection of QUIN alone. When injected without QUIN, none of the dicarboxylic acids, except dipicolinic acid, influenced cortical ChAT activity. Dipicolinic acid (240 nmol) produced a significant decrease ($P < 0.01$) of $33 \pm 7.4\%$ in cortical ChAT activity. Thus, dipicolinic acid exerted a dual effect: a weak QUIN-like decrease in cortical ChAT activity, and a picolinate-like attenuation of the QUIN-induced cholinergic toxicity.

Action of picolinic acid against different excitotoxins

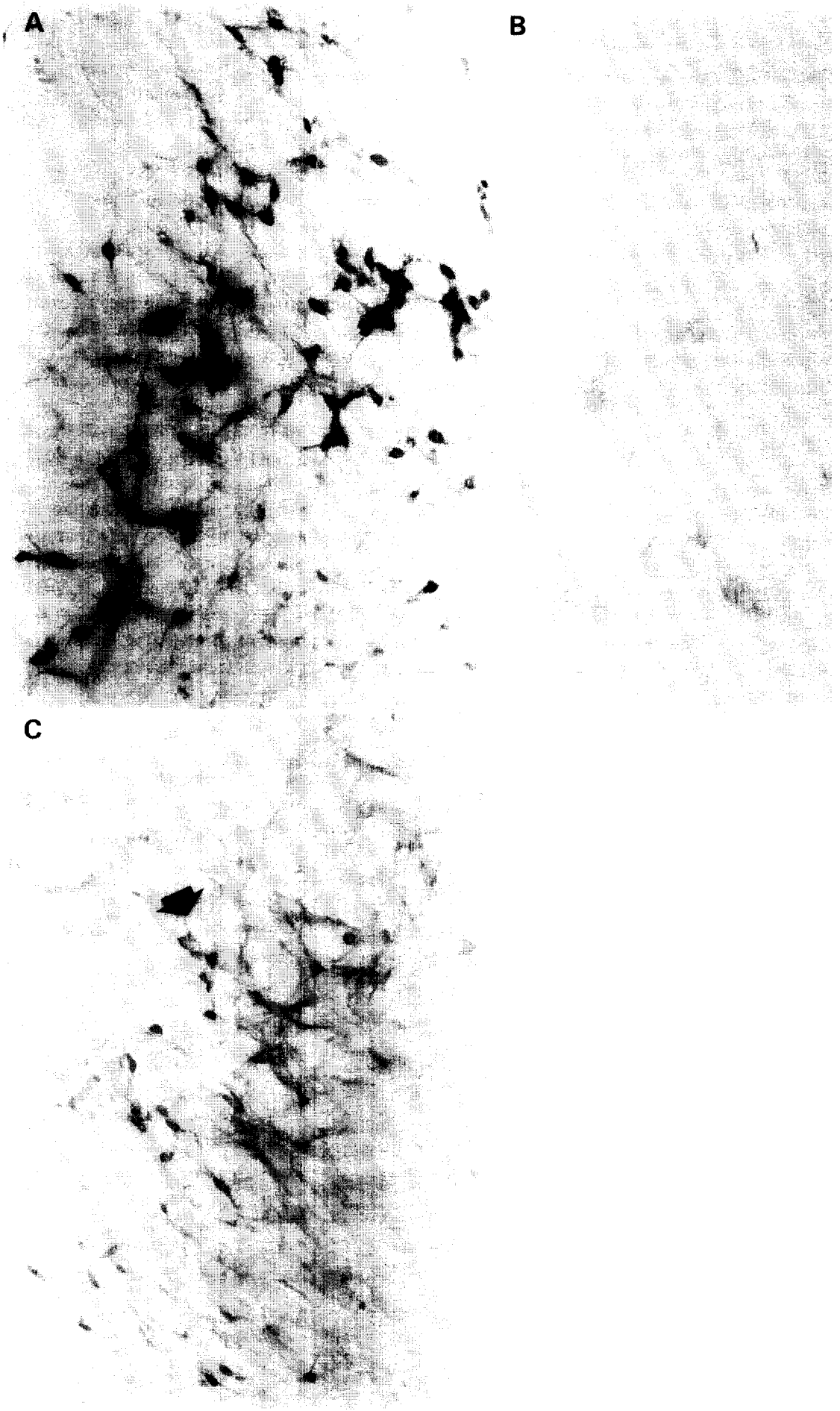
Previous experiments have demonstrated that nbM injection of kainic, ibotenic, and quisqualic acid, like those of QUIN, produce cholinergic neurotoxicity. To determine the selectivity of the antagonism of QUIN action by picolinate, picolinic acid was co-injected with kainic, ibotenic or quisqualic acid. In these experiments, the dose of picolinic acid (480 nmol) was that which produced maximal attenuation of QUIN (120 nmol) action, and the neurotoxic dose of each of the toxins — kainic acid (4.7 nmol), ibotenic acid (25 nmol), quisqualic acid (60 nmol) — was that which produced a cortical ChAT decrease comparable in size to that produced by QUIN (120 nmol). In parallel experiments, the action of kynurenic acid (240 nmol) was tested under similar conditions. Fig. 3 shows comparative effects of picolinic acid (480 nmol) as well as

TABLE 1.

Effects of quinolinic acid alone and in combination with structurally related pyridine dicarboxylic acids on cortical ChAT activity.

AGENT	STRUCTURE	% REDUCTION IN CORTICAL ChAT ACTIVITY*
SALINE	----	5.6 ± 4.2
QUINOLINIC ACID(QUIN) (120 nmol)		56.0 ± 9.7 **
QUIN + DINICOTINIC ACID (180 nmol)		50.0 ± 9.5
QUIN + CINCHOMERONIC ACID (480 nmol)		56.0 ± 4.5
QUIN + ISOCINCHOMERONIC ACID (240 nmol)		57.0 ± 10.2
QUIN + LUTIDINIC ACID (480 nmol)		55.0 ± 15.7
QUIN + DIPICOLINIC ACID (180 nmol)		28.5 ± 12.7 **,†

* Measured 7 days after injection (0.5ul) into the right nucleus basalis magnocellularis
** Significant when compared to saline injection $p < 0.001$;
† Significantly less when compared to quinolinic acid injection; $p < 0.01$.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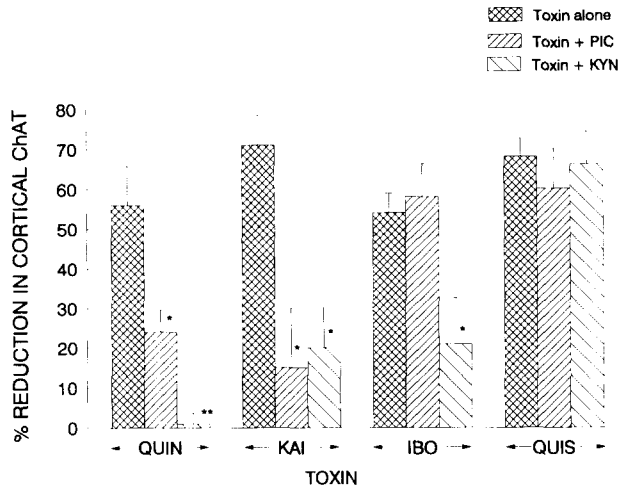

Fig. 3. Cortical ChAT activity after injections of picolinic acid (PIC, 480 nmol) or kynurenic acid (KYN, 240 nmol) in combination with quinolinic acid (QUIN, 120 nmol), kainic acid (KAI, 4.7 nmol), ibotenic acid (IBO, 25 nmol), or quisqualic acid (QUIS, 60 nmol). Injections were made into the right nbM and ChAT activity in the frontoparietal cortex from injected and uninjected hemispheres was measured 7 days later. Each value is the mean ± S.E.M. of assays carried out in triplicate from 4 to 8 animals. * $P \leq 0.01$, ** $P \leq 0.001$ when compared with injection of corresponding excitotoxin alone.

kynurenic acid (240 nmol) on the neurotoxic effects of four excitotoxins. Picolinic acid produced a significant attenuation of the QUIN and kainate but not ibotenate and quisqualic acid effect on cortical ChAT activity. Kynurenic acid blocked QUIN's effect and also significantly attenuated the action of both kainic and ibotenic acid. Thus, while picolinate was inactive against ibotenic acid, kynurenic acid attenuated the action of this excitotoxin. Neither agent influenced the action of quisqualate. These experiments indicate that picolinic acid exerted a selective effect against the neurotoxic effect of certain excitotoxins.

DISCUSSION

The results of the present study showed that picolinic acid, a pyridine monocarboxylic acid, attenuated the neurotoxic effect of QUIN, a pyridine dicarboxylic acid, on cortical cholinergic neurons. This action of picolinic acid was revealed by biochemical assessment involving measurement of cortical ChAT activity, and by histochemical assessment involving staining of AChE positive neurons in the nbM region. Injection of QUIN into the nbM clearly depleted these cholinergic markers, and co-injection of picolinic acid with QUIN reduced the effect of this excitotoxin on both markers. This study also showed that the neuroprotective action of picolinic acid was not shared by all structurally related pyridine carboxylates, and that picolinic acid was not universally effective against all glutamate receptor agonists. These observations suggest that picolinic acid exhibits a certain selectivity in regard to its protective action against QUIN-induced neurotoxicity.

The selectivity of picolinic acid action was apparent in toxicity experiments that compared its effects with those of structurally related pyridine mono- and dicarboxylates. Of the two monocarboxylates examined, only isonicotinic acid, in which the carboxyl group is located at the para position in the pyridine ring, closely mimicked the action of picolinic acid, the potency and efficacy of the anti-QUIN effect being similar for both agents. In contrast, nicotinic acid, which bears the carboxyl group at the meta position, failed to attenuate QUIN's effect. Thus, the location of the carboxyl group in the pyridine ring appears to be an important determinant of the anti-QUIN effect of these monocarboxylic acids. The action of a pharmacological agent usually reflects its interaction with a specific macromolecule site or receptor. In view of the structural selectivity observed here, it is possible that both picolinic and isonicotinic acid influence QUIN's action through an interaction with such a site on cholinergic neurons in the nbM. Since QUIN produces neurotoxicity by acting through the NMDA receptor[8], picolinic acid may simply have influenced its action by blocking this receptor. However, a differential effect of picolinic acid against different excitotoxins which act on the NMDA receptor tends to argue against this mechanism (see Discussion below).

In the present study, kynurenic acid antagonized the effect of QUIN, kainate and ibotenate. In previous studies, kynurenic acid had no effect on ibotenate-induced damage in the striatum[8] or in the nbM[24]. The reason for this discrepancy is unknown, but it may be related to regional differences and/or methodological differences in the assessment of neuronal damage[24].

Among the several pyridine dicarboxylic acids tested, only dipicolinic acid (2,6-pyridine dicarboxylic acid) exerted a significant anti-QUIN effect. When it was co-injected with QUIN, the decrease in cortical ChAT activity produced by the combination was lower than that elicited by the QUIN injection. However, unlike

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Fig. 2. Photomicrographs of rat basal forebrain sections stained for AChE 6 h after animals were treated with diisopropylfluorophosphate (DFP, 1.5 mg/kg, i.m.). (A) saline 0.9%, (B) quinolinic acid (QUIN, 120 nmol); (C) QUIN (120 nmol) plus picolinic acid (480 nmol). Note the presence of large cells in (A) and (C) indicated by arrows.

picolinic acid, which lacked intrinsic activity, dipicolinic acid by itself produced a significant decrease in cortical ChAT activity. In a previous study on the rat striatum[12], dipicolinic acid was found to produce an NMDA receptor-mediated stimulatory effect on the in vitro release of acetylcholine, but when injected focally, it did not produce a neurotoxic depletion of striatal ChAT activity. In that study, intrastriatal injections of dipicolinic acid also failed to decrease glutamic acid decarboxylase (GAD) activity, suggesting that it lacked a neurotoxic action on both striatal GABAergic and cholinergic neurons. The difference between the neurotoxic action of dipicolinic acid seen in the striatal study of Lehmann et al.[12] and the present study may arise from differences in the sensitivity of the two populations of cholinergic neurons to excitotoxins. However, Foster et al.[7] have reported that at a higher dose picolinic acid produced a small lesion at the injection site and a very modest decrease in striatal ChAT activity. The dual action of dipicolinic acid observed in the present study suggests that in the nbM this dicarboxylic acid acts as a partial agonist, although it is not clear whether the agonist and antagonist components of its action share a common site or mechanism. However, the dual action of dipicolinic acid appears to be consistent with the structural features that this agent shares with both QUIN (presence of two carboxyl groups in a pyridine ring) and picolinic acid (proximity of a carboxyl group to the pyridine nitrogen). It is possible that dipicolinic acid also shares the mode of action of these two agents.

Although the exact site or mechanisms that underlie the picolinic acid effect cannot be ascertained from the present study, the results of experiments which evaluated the effect of picolinate against different excitotoxins yields important information in this respect. Because picolinic acid did not antagonize all the excitotoxins tested, it excludes the possibility that picolinic acid influences QUIN's neurotoxicity through a nonspecific depressant action (e.g. local anesthetic action) on nbM cholinergic neurons. Indeed, in the hippocampal slice experiments, picolinic acid behaves as a poor depressant of the synaptically driven responses[16]. Its lack of effectiveness against ibotenate, which, like QUIN, exerts toxicity through activation of the NMDA receptor, tends to argue against NMDA receptor blockade as the mechanism for the anti-QUIN effect. However, the possibility that picolinic acid blocks the NMDA receptor subtype which is selectively activated by QUIN[5,22,24] cannot be excluded. Possibly, the most significant clue regarding the mechanism by which picolinate acts is the observation that picolinic acid influences the neurotoxic action of QUIN and kainic acid but not that of ibotenic and quisqualic acid. This observation suggests that picolinic acid may be interacting with a process that is common to the neurotoxic action of both QUIN and kainic acid. It is now recognized that the neurotoxicity produced by QUIN and kainic acid is dependent on the presence of an intact glutamatergic afferent input to the neurons targeted by these two agents[11,14,17]. In contrast, the neurotoxicity of ibotenate and quisqualate can occur in the absence of a glutamatergic input[20,21]. It has been hypothesized that the presynaptic release of L-glutamate, or a related compound, by kainic acid and QUIN is an important factor in the expression of their excitotoxic action. Indeed, there is evidence that kainic acid induces glutamate release in vitro[4], and one study has demonstrated similar release in vivo by QUIN[2]. In view of these distinguishing characteristics of QUIN and kainate toxicity, a tentative explanation of the protective action of picolinate is that it depresses the glutamatergic afferent input into the nbM by a presynaptic mechanism and thus reduces the effectiveness of these two toxins. It remains to be determined in future work whether picolinic acid indeed influences presynaptic glutamate release.

Regardless of the mechanism involved, the activity profile of picolinic acid differs from that of kynurenic acid. Picolinic acid is both less potent and efficacious than kynurenic acid as an antagonist, and it does not block the neurotoxicity of ibotenate. Unlike kynurenic acid, picolinic acid does not effectively block the excitatory response driven by synaptic activation[16]. Thus, its site and mechanism of action may differ from that of kynurenic acid. In certain areas of the brain, regional differences are known to exist in regard to both the action of quinolinic acid[19] and kynurenic acid[8]. In view of this, it is essential in future to characterize the action of picolinic acid in other brain regions. As both picolinic acid and kynurenic acid are derived from the tryptophan metabolic pathway that yields QUIN, it is possible that a synergism between these two anti-neurotoxic metabolites serves to restrain the damaging effects of an endogenous excitotoxin such as QUIN.

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