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Quinolinic Acid Neurotoxicity in the Nucleus Basalis Antagonized by Kynurenic Acid

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BOEGMAN, R. J., S. R. EL-DEFRAWY, K. JHAMANDAS, R. J. BENINGER AND S. K. LUDWIN. *Quinolinic acid neurotoxicity in the nucleus basalis antagonized by kynurenic acid.* NEUROBIOL AGING 6(4) 331–336, 1985.—Quinolinic acid, a metabolite of tryptophan, behaves as an excitotoxic amino acid. It has been proposed that quinolinic acid might be implicated in neurodegenerative diseases. The related metabolite, kynurenic acid, has been found to be a powerful antagonist of quinolinic acid. The ability of quinolinic acid, alone or in combination with kynurenic acid, to destroy cholinergic neurons projecting to the cortex was examined by morphological and biochemical criteria. The compounds were injected unilaterally into the nbm of the rat. Neuronal destruction of the basal forebrain occurred with quinolinic acid alone; however, no cell loss was observed when kynurenic and quinolinic acid were co-injected. Quinolinic acid lesions of the nucleus basalis caused significant decreases in cortical choline acetyltransferase, acetylcholinesterase, high affinity choline uptake and ^{3}H-acetylcholine release. These reductions in cortical cholinergic markers were prevented by co-injecting kynurenic with quinolinic acid. A significant decrease in cortical choline acetyltransferase activity was observed three months following quinolinic acid lesions of the nucleus basalis. The results indicate that quinolinic acid can be used as an endogenous neurotoxin to produce lesions of the nbm resulting in impaired cortical cholinergic function similar to that seen in Alzheimer's disease.

Quinolinic acid Kynurenic acid Neurotoxicity Choline acetyltransferase Acetylcholinesterase
High affinity choline uptake Acetylcholine release Morphology

A transmitter role for the dicarboxylic amino acids, glutamate and aspartate, which activate central neurons has been proposed [5, 7, 17]. Recently it has become apparent that multiple receptor types for excitatory amino acids exist [5,7], and in a number of studies a parallel has been drawn between the potency of a series of glutamate analogues and their neurotoxic properties [6,21]. The type of lesion produced involves destruction of local neuronal cell bodies while axons of passage are spared. Lesions produced by injecting conformationally restricted analogues of glutamic acid into the brain have been used as animal models of neurodegenerative conditions.

Quinolinic acid, a metabolite of tryptophan, behaves as a potent excitotoxin [22, 24, 25]. Based on morphological data, quinolinic acid has been shown to produce axon sparing lesions [24]. It has been shown to be present and unevenly distributed in the brain with the highest concentration in the cortex followed by the hippocampus and brain stem [19]. In rat cortex, the amount of quinolinic acid increases by more than 600% between birth and old age [20]. Quinolinic acid-induced depolarization of cortical neurons is antagonized by kynurenic acid, also a tryptophan metabolite [22]. Based on this report, Foster *et al.* [9] studied the toxicity of quinolinic acid in the striatum and the role of kynurenic acid as a modulator of neurodegenerative and seizure activity. They found that co-injections of kynurenic and quinolinic acid protects the striatum from quinolinate-induced toxicity. Following reports that many cortical cholinergic nerve terminals originate from neurons in the basal forebrain [18,23], we set out to investigate the role of the two tryptophan metabolites, kynurenic acid and quinolinic acid [11], in modulating cortical cholinergic function. The present paper describes the neurochemical and morphological responses obtained after directing injections of quinolinic acid to the nucleus basalis magnocellularis (nbm) and compares the effects of co-administering kynurenic with quinolinic acid. A preliminary account of part of this work has been published elsewhere [1].

METHOD

Male Sprague-Dawley rats (275–350 g) were lesioned with quinolinic acid or co-injected with different molar ratios of kynurenic and quinolinic acid. Details of the injection procedure are described in the previous paper [3]. The following

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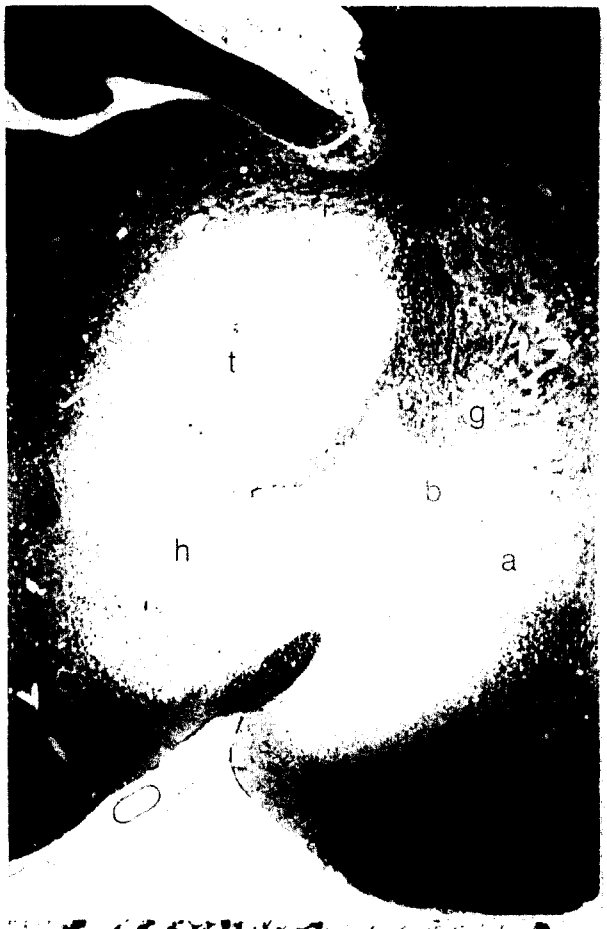
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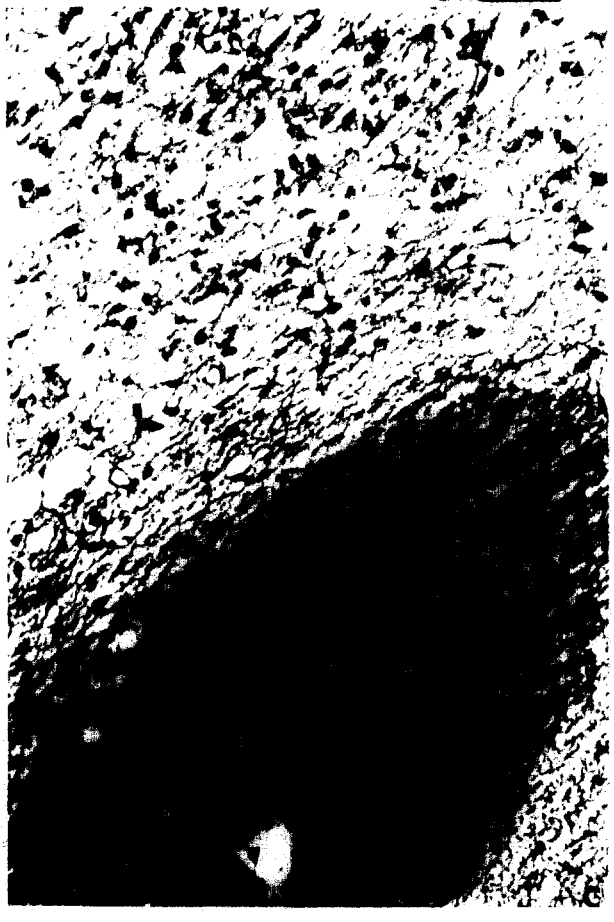


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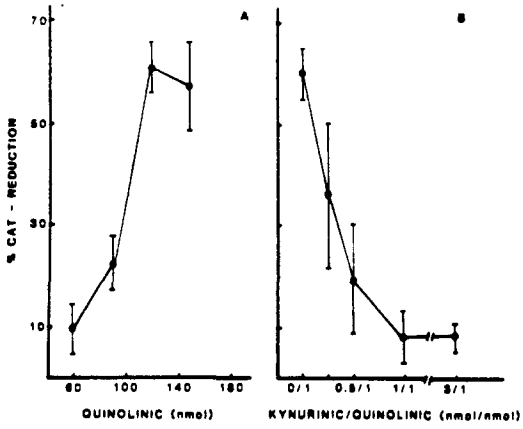

FIG. 2. Effect of quinolinic acid on cortical CAT activity. Unilateral injections (1 μl) of quinolinic acid alone or in combination with different molar concentrations of kynurenic acid were made into the area of the right nucleus basalis magnocellularis of adult rats. CAT activity was measured 7 days later in both the ipsilateral and contralateral cortex. The percent difference between the two values was graphed as a function of either quinolinic acid concentration (A), or as the molar ratio between kynurenic and quinolinic acid (B) with quinolinic acid being kept constant at 120 nmoles.

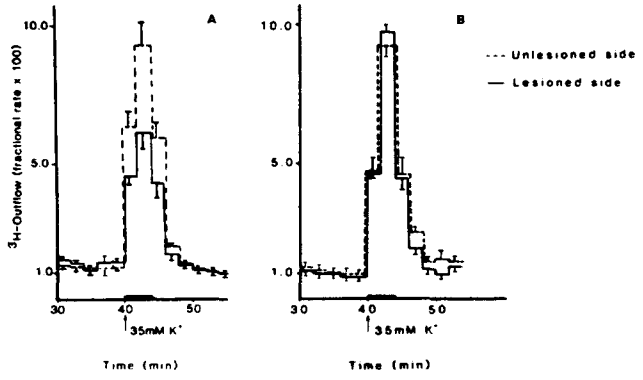

FIG. 3. K^+-Induced outflow of ^{3}H-acetylcholine from cortical slices. Unilateral injections (1 μl) of quinolinic acid (120 nmoles) alone (A), or quinolinic acid plus kynurenic acid (120 and 360 nmoles respectively) (B) were made into the area of the right nucleus basalis magnocellularis of adult rats. Slices (0.3 mm) were prepared 7 days later from both contralateral (unlesioned side) and ipsilateral (lesioned side) cortices and K^+-evoked release of radioactivity measured as a function of time.

cholinergic markers were assayed on a slab of fronto-parietal cortex dissected from each hemisphere. Choline acetyltransferase (CAT) activity was measured by the procedure of Fonnum [8] while acetylcholinesterase (AChE) was determined by the method of Ellman *et al.* [4]. Synaptosomal high affinity choline uptake (HACU) was carried out in the presence of 100 nM ^{3}H-choline [12,28] and K^+-evoked ^{3}H-acetylcholine (^{3}H-ACh) release was performed on 0.3 mm thick coronal slices [15,30].

For morphological studies, the brains were fixed in formalin and the hemispheres divided coronally into three pieces. These three pieces were embedded in a single paraffin block and step sections, each 5–7 μ in thickness, were cut through the block to provide a wide anatomical survey of the extent of the lesions. The sections were stained with hematoxylin and eosin and examined with the light microscope.

RESULTS

In animals injected with saline, the only microscopic abnormality seen was a small hemorrhagic tract containing hemosiderin-laden macrophages. Microscopic examination of the animals injected with quinolinic acid (120 nmoles) alone showed widespread destruction on the affected side (Fig. 1A). The lesions varied from frank cavitary and coagulative necrosis to areas in which the basic architecture of the neuropil was preserved but there was loss of neurons. Within the necrotic areas, numerous debris-laden macrophages were seen. At the periphery of the lesion, a rim of reactive capillary proliferation and some reactive astrocytes could sometimes be seen. The lesions affected mainly the grey matter nuclei, at times sparing the white matter strikingly (Fig. 1C).

The destruction appeared to involve a wide variety of nuclei (Fig. 1A); the distribution of involvement appeared to depend on the spread of quinolinic acid rather than on any selective vulnerability. The thalamus was commonly affected (Fig. 1D), as were the caudate nucleus, lateral preoptic area, central amygdyloid nucleus, basal nucleus magnocellularis, horizontal limb of the diagonal band, nucleus accumbens septi, substantia innominata, and the zona incerta. No abnormalities were seen in the rest of the brain away from the lesions; specifically the hippocampi appeared intact, as did the cortex. In animals injected with quinolinic acid plus kynurenic acid (120:360 nmoles), the only abnormality seen was the presence of a needle tract. The findings in these animals (Fig. 1B) were identical to those seen in the controls.

Cortical CAT, AChE, HACU and ^{3}H-ACh in sham-injected animals are summarized in Table 1. There were significant reductions in CAT on the lesioned side, whereas AChE, ACh release and HACU were not significantly different. Rats injected unilaterally with quinolinic acid alone into the nbm showed a dose-dependent reduction in cortical CAT when compared to the uninjected contralateral side (Fig. 2A). A steep dose-response curve was obtained between 60 and 120 nmoles with a maximal decrease in CAT of 57%. A

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FIG. 1. (A) Brain from a rat injected 7 days previously with quinolinic acid (120 nmoles). The pale circumscribed area of necrosis extends widely throughout the subcortical nuclei, but relatively spares white matter tracts. a=amygdaloid nuclei, b=nucleus basalis, c=caudate putamen, g=globus pallidus, h=lateral hypothalamic area, i=internal capsule, t=thalamic nuclei. Hematoxylin and eosin ×7. (B) Brain from a rat injected 7 days previously with quinolinic acid plus kynurenic acid (120:360 nmoles). The section is at the same level as in (A). No necrosis is seen. Hematoxylin and eosin ×7. (C) High power view of supra-optic area from an animal injected 7 days previously with quinolinic acid. The supra-optic grey matter (top) shows necrosis, with vacuolation, pyknotic nuclei and no evidence of viable neurons, whereas the optic tract (bottom) appears spared. Hematoxylin and eosin ×70. (D) Thalamus from a similar animal to that seen in (C). The normal neurons and neuropil (top) contrast with the necrotic tissue (bottom). Hematoxylin and eosin ×70.

TABLE 1

THE EFFECT OF LESIONS PLACED IN THE NBM ON CORTICAL CAT, AChE, HACU AND ^{3}H-ACh RELEASE

	CAT (nmoles ACh formed/mg protein/hr)	AChE (nmoles ACh hydrolyzed/mg protein/min)	HACU (pmoles choline/mg protein/4 min)	^{3}H-ACh Release (% release over base)
Saline (sham)				
Uninjected	58.2 ± 2.1	63.5 ± 4.2	0.2 ± 0.02	19.5 ± 1.4
Injected	51.1 ± 2.4*	58.5 ± 2.5	0.2 ± 0.02	17.8 ± 2.0
% Change	12.3	8.6	0	10.0
Quinolinic Acid (120 nmoles)				
Uninjected	49.4 ± 6.2	69.2 ± 2.0	—	17.9 ± 2.1
Injected	20.2 ± 4.7†	36.7 ± 0.8†	—	10.6 ± 2.1
% Change	60.1	44.1		40.7
Quinolinic Plus Kynurenic (120:360 nmoles)				
Uninjected	47.8 ± 3.5	60.3 ± 3.9	0.3 ± 0.04	19.9 ± 4.4
Injected	46.5 ± 5.2	62.0 ± 4.5	0.3 ± 0.04	17.8 ± 2.1
% Change	2.6	2.7	0	10.5

Unilateral 1 μl injections of either 0.9% saline, 120 nmoles quinolinic acid or 120 nmoles quinolinic plus 360 nmoles kynurenic acid were made into the right nbm of adult rats. Frontoparietal cortical cholinergic markers were measured 7 days later in both the injected and contralateral side. Values are mean ± SEM from 4–9 animals.

*$p<0.005$, †$p<0.0005$.

dose of quinolinic acid (120 nmoles) which gave a reduction in CAT activity of 55% was injected alone or in combination with different molar ratios of kynurenic acid (Table 1 and Fig. 2B). From Fig. 2B it was estimated that a kynurenic: quinolinic ratio of 0.35:1 afforded 50% protection in CAT activity. Kynurenic acid alone (360 nmoles), when infused into the nbm, resulted in a decrease of 11% in cortical CAT when compared to the uninjected side. This decrease was similar to that obtained following a saline injection and represents non-specific tissue damage. Similarly, co-injections of kynurenic plus quinolinic acids (3:1) completely prevented the decrease in AChE, HACU or ^{3}H-ACh release found in quinolinic acid-treated animals (Table 1). Thus, kynurenic acid afforded complete protection against the neurotoxic action of quinolinic acid on cholinergic neurons projecting from the nbm to the cerebral cortex.

The spontaneous release of ^{3}H-ACh from cortical slices prepared from animals with quinolinic acid lesions (120 nmoles) of the nbm did not differ significantly from that of the contralateral cortex (Fig. 3A). However, a 41% reduction ($p<0.005$) in K^+-evoked release of ^{3}H-ACh, expressed as total percent release over baseline, was seen from the lesioned side. In marked contrast, there was no significant difference in K^+-evoked release of ^{3}H-ACh from cortical slices from animals which received a co-injection of quinolinic plus kynurenic acids (120 and 360 nmoles respectively). The release profile appeared the same as that of the contralateral uninjected side (Fig. 3B).

Results of cortical CAT assays carried out three months after lesioning animals with 120 nmoles quinolinic acid showed a 40.5±4.7% reduction (57.1±3.6 uninjected, 34.2±3.0 injected, $p<0.001$), while both sham and co-injected groups showed no significant change.

DISCUSSION

The nbm provides cholinergic projections to the frontal and parietal cortex [18,23]. We examined the effect of quinolinic acid injected into the nbm on brain morphology, cortical cholinergic markers and the ability of kynurenic acid to antagonize the neurotoxicity produced by quinolinic acid.

The morphological results showed destruction of the subcortical structures of the forebrain in animals injected with quinolinic acid alone, and in contrast, at light microscopic level, complete sparing in animals treated with both quinolinic acid and kynurenic acid. There appears to have been no selectivity for particular neurons or nuclei; within a given lesion there was destruction of large numbers of neurons of differing morphological types.

Small changes in biochemical markers (Table 1) can be expected following surgical manipulation of the animals. These values formed the baseline above which drug effects were evaluated. A certain amount of variability in the biochemical assays between different control groups is evident; a similar degree of variability can be calculated from control data present in the literature [13,14]. Since each animal served as its own control, our data represents differences between the two hemispheres in the same animal. The differences between groups of control animals could be due to biological variation or sample preparation. Our experiments showed that 120 nmoles quinolinic acid produced a decrease of approximately 55% in CAT activity. This dose corresponds with that used by Schwarcz *et al.* [26] in the hippocampus to precipitate seizures and to produce well defined morphological changes in various regions of rat brain. Our dose, however, was less than half that found to give a 50% reduction in striatal CAT. It is possible that the striatum

requires larger doses of quinolinic acid or that the barbiturate anesthetic used by Foster *et al.* [9] protected striatal neurons from toxicity [10].

In experiments in which kynurenic and quinolinic acids were co-injected, the cholinergic neurotoxicity of quinolinic acid was completely prevented without any evidence that kynurenic acid acted as a partial agonist. This is in agreement with electrophysiological data which show that kynurenic acid has no effect on the spontaneous activity of cortical neurons [22]. Based on cortical CAT activity, complete protection against quinolinic acid was obtained at an equimolar concentration of kynurenic acid, with half maximal protection at 0.35:1. This is in contrast to a dose ratio of 0.7:1 which was required to protect striatal CAT activity by 50% in barbiturate anesthetized animals [9]. Kynurenic acid could play a role *in vivo* as a natural modulator of the possible neurotoxicity [27] associated with increasing amounts of quinolinic acid which accompany aging [20]. The presence of kynurenic acid in the brain has not been demonstrated; however, the enzymes responsible for its synthesis have been identified [11].

A decrease in the K^+-evoked release of radioactive ACh from the cortex of patients with Alzheimer's disease has been documented [29]. A similar reduction in the release of ACh from rats injected with quinolinic acid was obtained in this study. Since the nbm receives glutamatergic input [2], overstimulation of NMDA receptors on nbm cholinergic neurons by quinolinic acid [15,16] may result in the neurotoxicity.

In marked contrast to the spontaneous recovery in cortical cholinergic markers reported by Wenk and Olton [31] following ibotenic acid lesions of the nbm, our animals showed significant reductions in cortical CAT three months after lesioning with quinolinic acid. The reason for this difference is not known since we were unable to obtain recovery of cortical cholinergic function after quinolinic or ibotenic acid lesions of rat nbm (manuscript in preparation).

Excitotoxins such as kainic acid and ibotenic acid are not present in the mammalian central nervous system. However, quinolinic acid is not only present in mammalian brain, but increases by more than 600% with age [20]. Quinolinic acid, though less potent, is capable of producing the same degree of damage to cholinergic neurons of the nbm projecting to the cortex as that obtained with kainic acid [3]. In addition, quinolinic acid does not produce the remote neuronal damage that has been found to occur with kainic acid [27]. Currently, quinolinic acid is one of the most promising compounds known to occur in the brain with excitotoxic properties.

ACKNOWLEDGEMENTS

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