Neurobiology of Aging, Vol. 6, pp. 325–330, 1985. © Ankho International Inc. Printed in the U.S.A. 0197-4580/85 $3.00 + .00

Functional and Neurochemical Cortical Cholinergic Impairment Following Neurotoxic Lesions of the Nucleus Basalis Magnocellularis in the Rat

S. R. EL-DEFRAWY,* F. COLOMA,* K. JHAMANDAS,*[1] R. J. BOEGMAN,* R. J. BENINGER† AND B. A. WIRSCHING†

Department of Pharmacology and Toxicology and Department of Psychology†*
Queen's University, Kingston, Ontario, Canada, K7L 3N6

Received 10 January 1984

EL-DEFRAWY, S. R., F. COLOMA, K. JHAMANDAS, R. J. BOEGMAN, R. J. BENINGER AND B. A. WIRSCHING. *Functional and neurochemical cortical cholinergic impairment following neurotoxic lesions of the nucleus basalis magnocellularis in the rat.* NEUROBIOL AGING 6(4)325–330, 1985.—The effect of kainic and quinolinic acid on cortical cholinergic function was examined following injections of these agents into the nucleus basalis magnocellularis (nbm) or into the fronto-parietal cortex. The release of cortical ^{3}H-acetylcholine (^{3}H-ACh), high affinity choline uptake (HACU) and acetylcholinesterase was measured 7 days following injections of saline (control), kainic acid (4.7 nmoles) and quinolinic acid (60, 150 and 300 nmoles) into the nbm. These cortical cholinergic parameters were also examined after injections of saline (control), kainic acid (9.4 nmoles) and quinolinic acid (300 nmoles) into the fronto-parietal cortex. The release of ^{3}H-ACh, HACU and AChE was significantly reduced in animals injected with kainic or quinolinic acid into the nbm. Histological examination of stained sections showed a loss of cell bodies in the region of the nbm and the globus pallidus. The size of the lesion produced by quinolinic acid was proportional to the dose injected into the nbm. In animals injected with kainic acid or quinolinic acid into the cerebral cortex, the release of ^{3}H-ACh, HACU and AChE was not significantly reduced when compared with control animals, although histological examination of stained cortical sections showed a marked loss of cortical neurons. The results show that quinolinic acid, an endogenous neuroexcitant, produces a deficit of cholinergic function similar to that described in the cortical tissue of patients with senile dementia of Alzheimer's type. The toxic effects of quinolinic acid on cortical cholinergic function are due to its action on cholinergic cell bodies in the nbm. The cortical slice preparation from quinolinic acid-treated animals showing impairment of ^{3}H-ACh release, may be useful in assessing the action of drugs designed to improve cholinergic function.

Quinolinic acid Kainic acid Nucleus basalis magnocellularis Cholinergic markers Acetylcholine release Alzheimer's disease

NEUROCHEMICAL studies on autopsy brain of patients suffering from senile dementia of the Alzheimer type (SDAT) have demonstrated a selective loss of cholinergic markers such as choline acetyltransferase (CAT) and acetylcholinesterase, enzymes responsible for the synthesis and degradation of the transmitter acetylcholine (ACh), in hippocampal and cortical areas [2]. The decline in these cholinergic markers is believed to be a major contributing factor in the cognitive dysfunction seen in patients with SDAT. Recent studies have demonstrated that the cortical cholinergic loss may be due to the degeneration of a specific cholinergic pathway that originates from the nucleus basalis of Meynert and projects to the cerebral cortex [2]. The biochemical pathology characteristic of SDAT can be mimicked in animals such as the rat by injection of the excitotoxin, kainic acid, into certain subcortical nuclei. Stereotaxic injections of this neurotoxin into the nucleus basalis magnocellularis (nbm), located in the ventral region of the globus pallidus, results in a decrease in CAT, AChE and high affinity choline uptake (HACU) in the frontal and parietal cortex [6,8]. This suggests that the nbm cholinergic neurons project to cortical areas. The failure of kainic acid to completely abolish cortical cholinergic markers indicates that some cortical cholinergic neurons may be intrinsic [3, 6, 7] or that cortical cholinergic projections may also arise in different subcortical regions [21].

Because microinjections of the exogenous excitotoxin such as kainic acid into the nbm can produce some of the neurochemical changes seen in SDAT [1], it is possible that the cholinergic damage occurring in this disorder may arise from an endogenous neurotoxin generated in the brain. Recently, it was reported that quinolinic acid, a minor metabo-

[1]Requests for reprints should be addressed to Dr. K. Jhamandas, Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

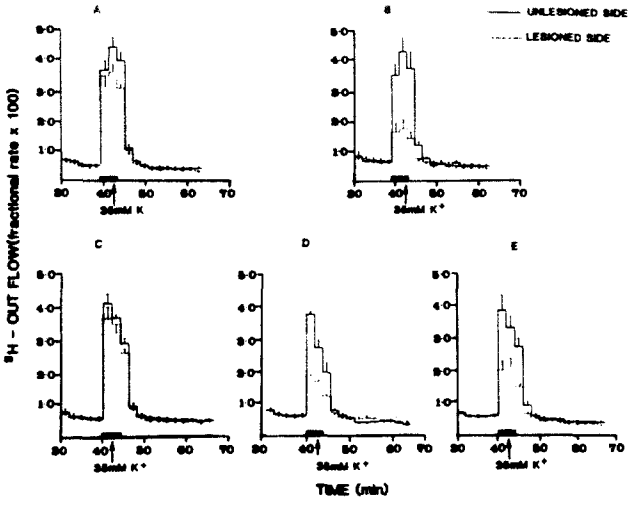

FIG. 1. The outflow of radioactivity from superfused frontoparietal cortical slices of animals that had received a unilateral 1 μl infusion of: (A) 0.9% saline; (B) 4.7 nmoles kainic acid; (C) 60 nmoles quinolinic acid; (D) 150 nmoles quinolinic acid; (E) 300 nmoles quinolinic acid into the nbm. After preincubation with ^{3}H-choline, slices were superfused with Krebs-Henseleit and stimulated by a 5-min exposure to medium containing 35 mM K^+. Points represent the mean outflow±SEM (n=4). Ordinate: outflow of radioactivity per time period as percent of total slice content. Solid and dashed lines represent release from the unlesioned and lesioned hemispheres respectively.

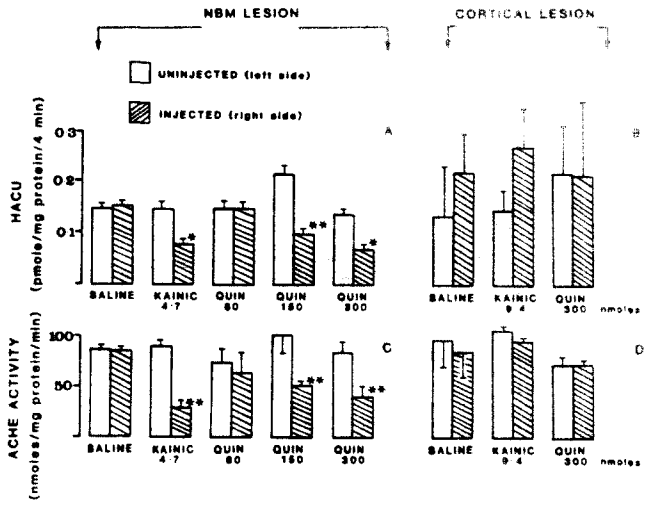

FIG. 2. High affinity choline uptake (HACU) and acetylcholinesterase activity (AChE) in the cerebral cortex following injections of saline, kainic acid and quinolinic acid into the nbm and the frontoparietal cortex. In the latter case an injection (1 μl) of kainate (4.7 nmoles) or quinolinic acid (150 nmoles) was made into the right frontal cortex and into the parietal cortex. n=3–5 animals. *Indicates the value is significantly different from the uninjected control side ($p<0.05$).

lite of the essential amino acid tryptophan, produces axon-sparing lesions of hippocampal and striatal neurons similar to those produced by kainic acid [16]. Thus, it is of interest to determine whether quinolinic acid produces a functional damage of cholinergic neurons projecting to the cortex.

The release of ACh from the cerebral cortex can be used as an indicator of the functional state of cholinergic neurons. Indeed, the synthesis and release of ACh in response to K^+ depolarization is reduced from cortical tissue obtained from patients with SDAT [18,19]. In the present study we tested whether focal administration of quinolinic acid into the brain of experimental animals produces a deficit in the spontaneous and the K^+-evoked release of ACh. AChE activity and HACU was compared to that of kainic acid. A preliminary account of this work has already been reported (El-Defrawy *et al., Soc Neurosci Abstr* **10**: 1187, 1984).

METHOD

Stereotaxic Injections

Male Sprague-Dawley rats (275–350 g) were anesthetized with halothane (Halocarbon, Malton, Ontario; 2% halothane, 98% oxygen via inhalation) and positioned in a Narashighe small animal stereotaxic apparatus. For injections into the nbm, the following coordinates were used: 0.8 mm posterior to bregma; 2.6 mm lateral and 8.0 mm ventral to the surface of the skull with the incisor bar set at −3.3 mm [13]. Cortical lesions were made by injecting neurotoxin at two sites along the fronto-parietal cortex (1.7 mm anterior to bregma, 2.0 mm lateral, 2.6 mm ventral; and 1.8 mm posterior to bregma, 3.0 mm lateral, 2.3 mm ventral).

Kainic acid (Lot 32F-0867, Sigma) or quinolinic acid (Lot 72F-0283, Sigma) was dissolved in 0.9% saline and titrated to pH 7.4 with 1 N NaOH. Kainic acid (1 μl of 4.7 nmoles/μl) or quinolinic acid (1 μl of 60, 150 or 300 nmoles/μl) was infused at the specific site over a period of 2 min, 25 sec via a Hamilton cannula (0.35 mm o.d.). After the infusion, a period of 3 min was allowed for diffusion. The cannula was withdrawn and the scalp was apposed with sutures. Sham-lesioned rats received an infusion of 1 μl of 0.9% saline. To minimize or prevent damage to remote areas 20 mg/kg pentobarbital was administered to animals immediately after recovery from anesthesia. The animals were killed 7 days post-lesioning for ACh release experiments, assessment of AChE activity and HACU, and for histology.

Release Experiments

The release of ^{3}H-acetylcholine (^{3}H-ACh) from slices of frontoparietal cortex was investigated using a modification of the methodology described by Somogyi and Szerb [20] and Lehmann and Scatton [9]. The rats were decapitated, the brain rapidly removed and hemisected. A slab of frontoparietal cortex was dissected from each hemisphere and sliced (0.3 mm thick) coronally with a McIllwain tissue chopper. Dispersed brain slices were preincubated for 5 min in Krebs-Henseleit buffer (KRB) at 37°C in a Dubonoff metabolic shaking incubator under an atmosphere of 95% O_2–5% CO_2. Unless otherwise specified, the KRB was of the following composition (mM): NaCl, 118; KCl, 4.8; KH_2PO_4, 1.2; $CaCl_2$, 1.3; $NaHCO_3$, 25; glucose, 10 and physostigmine, 0.01. The preincubation medium was exchanged for medium containing 5×10^{-8} M methyl-^{3}H-choline chloride (specific activity 80 Ci/mmole) and the incubation continued for a further 15 min. The slices were rinsed twice and four slices (total weight 8.5±0.5 mg) were transferred into a superfusion chamber.

Brain slices were superfused from the top of the chamber at a rate of 0.75 ml/min. After a 30-min period (t=30 min), samples of superfusate were collected at 2.0-min intervals

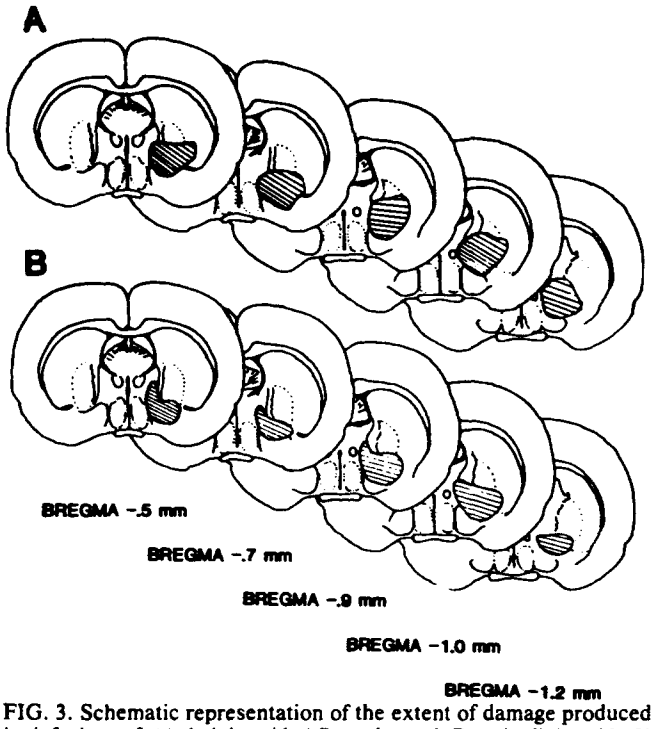

FIG. 3. Schematic representation of the extent of damage produced by infusions of: (A) kainic acid, 4.7 nmoles and (B) quinolinic acid 150 nmoles (right side) into the nbm, as assessed by cresyl violet histology. Five coronal sections are depicted with numbers below referring to distance (mm) posterior to bregma.

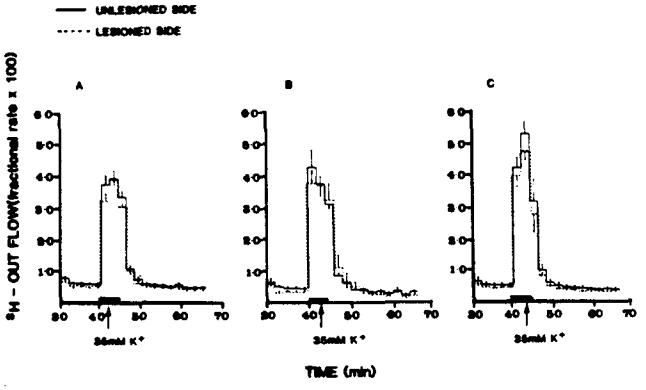

FIG. 4. The outflow of radioactivity from superfused frontoparietal cortical slices of animals that had received a 1 μl infusion of saline; kainic acid, 4.7 nmoles or quinolinic acid, 150 nmoles at each of two points along the frontoparietal cortex. (A) 0.9% saline; (B) kainic acid; (C) quinolinic acid. After preincubation with ^{3}H-choline, slices were superfused with Krebs-Henseleit and stimulated by a 5-min exposure to medium containing 35 mM K^+. Points represent mean outflow±SEM (n=4). Ordinate: outflow of radioactivity per time period as percent of total slice content. Solid and dashed lines represent release from unlesioned and lesioned hemispheres respectively.

for the duration of the experiment. At 40 min (t=40 min), the slices were exposed to KRB medium containing 35 mM K^+ for 5 min (when elevated K^+ medium was used, isosmolarity was maintained by an equimolar reduction in Na^+). At the end of this period the slices were superfused with normal medium for another 20 min. The tissues from each chamber were solubilized and the radioactivity counted. Two samples from empty superfused chambers were collected for residual radioactivity. Radioactivity in all samples was counted in a liquid scintillation counter using the external standards channel ratio method and quench correction. Efficiency of counting was 30–35%.

The outflow of radioactivity from the tissue was calculated from the radioactivity released during each sample period and expressed as a percentage of total slice content at the time of collection of that sample.

Biochemical Analyses

Measurements of HACU and AChE in each animal were made on the uninjected side and injected side. Since each animal served as its own control the influence of variability between different groups of animals was minimized.

The rate of HACU was measured according to modifications of the methods of Simon *et al.* [17] and Hartgraves *et al.* [5]. Synaptosomes were prepared by centrifuging cortical homogenates at 1000×g and the supernatant at 17,000×g. ^{3}H-Choline (100 nM) was added to the resuspended pellet of synaptosomes and uptake was allowed to proceed for 4 min at 37°C. Blanks were run in the same fashion except the incubation medium contained no Na^+. The reaction was stopped by filtration through nitrocellulose-cellulose-acetate filters (pore size 0.45 μm) and the radioactivity retained on the filter counted. AChE activity was determined by the method of Ellman *et al.* [4] while protein was measured according to Lowry *et al.* [12].

Histology

After 10–20 days of fixation in 10% formalin, the brains were frozen on a cryostat chuck and sectioned (40 μm thickness) to reveal the lesion site. Tissue sections were stained for Nissl substance with cresyl violet and serial sections were used to map the size of the lesion.

RESULTS

nbm Injections

^{3}H-Acetylcholine release. The release of ^{3}H-ACh from cortical slices prepared from animals with sham injections into the nbm is shown in Fig. 1A. No significant difference in spontaneous or K^+-evoked release of radioactivity was observed between injected and uninjected sides.

Figure 1B shows the release of ^{3}H-ACh from the cerebral cortex of animals injected with kainic acid into the nbm. The spontaneous release from the cortex ipsilateral to the injection (ipsilateral cortex) did not differ significantly from the release occurring from the cortex contralateral to the injected side (contralateral cortex). The K^+-evoked release of ^{3}H-ACh from the ipsilateral cortex was reduced by about 70% when compared to the contralateral cortex. No difference was apparent in the spontaneous release from the two cortices during the post-stimulus recovery phase. The results of release experiments performed on quinolinic acid-lesioned animals are shown in Fig. 1C–E. The spontaneous and K^+-evoked release occurring from the ipsilateral and contralateral cortex in animals injected with 60 nmoles of quinolinic acid did not differ significantly. However, following injection of a higher dose of quinolinic acid (150 or 300 nmoles), the K^+-evoked release from the ipsilateral cortex was significantly reduced when compared to the contralateral cortex. The spontaneous release occurring before or after K^+ application was not significantly different. Although the two

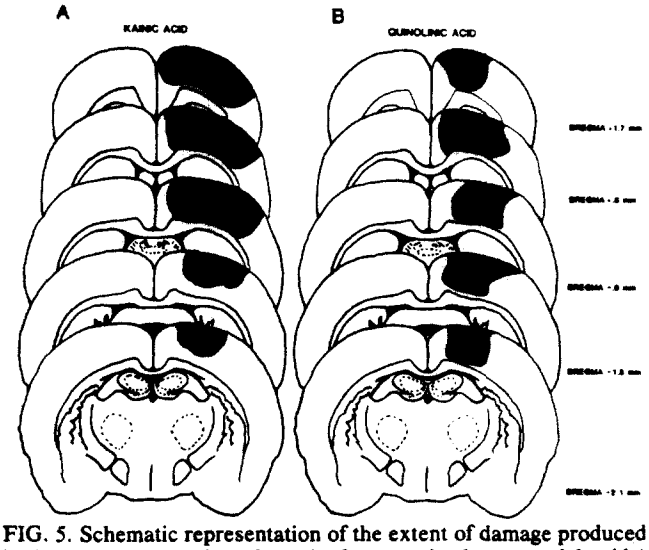

FIG. 5. Schematic representation of the extent of damage produced by infusions at two sites along the frontoparietal cortex (right side) of: (A) kainic acid, 4.7 nmoles/site; (B) quinolinic acid, 150 nmoles/site as assessed by cresyl violet histology. Five coronal sections are depicted with numbers referring to the distance (mm) anterior (+) or posterior (−) to bregma.

doses of quinolinic acid that reduced K^+-evoked release differed by 100%, their effect on the evoked release was comparable.

AChE activity and HACU. The effects of kainate and quinolinic acid injections were also investigated on AChE activity and HACU, two markers of cholinergic function. The tissue samples used in this part of the study were obtained from the tissue block prepared for the ^{3}H-ACh release experiments described above. The results are shown in Fig. 2A and C. In saline-injected animals there was no difference in these cholinergic markers when the ipsilateral and contralateral cortices were compared. Following injection of kainic acid, the HACU and AChE activity in the ipsilateral cortex decreased by 47% and 70% respectively. Quinolinic acid injections (150 and 300 mmoles) produced a similar effect on these markers. Injection of 60 nmoles, the lowest dose employed, did not produce a significant decrease in HACU or AChE. However, following 150 nmoles, the HACU and AChE activity decreased by 64% and 58% respectively. A higher dose of quinolinic acid, 300 nmoles, produced comparable effects on these two biochemical markers. Thus, as was observed in the preceding release experiments, these two doses of quinolinic acid produced effects of comparable magnitude on HACU and AChE activity.

Histology. Figure 3 represents the extent of kainic acid and quinolinic acid (150 nmoles) induced neuronal damage in the nbm and adjacent areas. In saline-injected animals there was no evidence of damage to the tissue in the vicinity of the injection site (not shown). Kainic acid (Fig. 3A) produced a sphere of cellular degeneration with a diameter of approximately 1.5 mm. Damage, as indicated by destruction of perikarya and glial infiltration, was apparent in the ventral pallidal area and often extended to cover two-thirds of the globus pallidus. Little damage to the cells in the caudate-putamen was seen. The lateral boundary of the lesion tended to follow the border between the globus pallidus and the striatum. Some degeneration of perikarya in the lateral hypothalamus and pre-optic area was also apparent. Quinolinate (150 nmoles) produced a sphere of degeneration (Fig. 3B) which was 1–1.5 mm in diameter. Gliosis was apparent in the area of destruction. At this dose quinolinic acid was neurotoxic to some of the perikarya in the globus pallidus. The dorsolateral boundary of the lesion did not extend past the border between the globus pallidus and the striatum.

Cortical Injections

In separate experiments kainic acid and quinolinic acid were injected directly into the frontal and parietal cortex at doses that were damaging to the nbm. In each animal kainic acid (4.7 nmoles) or quinolinic acid (150 nmoles) was injected into the frontal as well as the parietal cortex. Thus, the total dose of kainic acid and quinolinic acid injected into the fronto-parietal cortex was 9.4 and 300 nmoles respectively.

^{3}H-Acetylcholine release. The spontaneous and K^+-evoked release of ^{3}H-ACh from the cortical slices obtained from saline-injected rats was similar to that from the non-injected side (Fig. 4A). In animals receiving kainate into the cortex, the spontaneous and K^+-evoked release of radioactivity from the injected side was not significantly different from that of the uninjected side (Fig. 4B). Similarly, no significant difference was seen in animals injected with quinolinic acid (Fig. 4C).

HACU and AChE activity. Cortical HACU and AChE activity following focal injections of saline, kainic acid or quinolinic acid into the fronto-parietal cortex are shown in Fig. 2B and D. In contrast with earlier observations on the nbm injected rats (Fig. 2A and C), the cortically injected animals did not exhibit a decrease in these two markers. The values of HACU in cortically injected animals were more variable than in subcortically injected rats, and in some groups (saline and kainic) the injected side in fact showed a higher choline uptake than the uninjected side. However, this increase in HACU was not statistically significant ($p>0.05$, Student's t-test).

Histology. Injections of kainic acid into the fronto-parietal cortex resulted in a rectangular area of damage extending approximately 5 mm rostro-caudally and 4 mm medio-laterally (Fig. 5A). The ventral aspect of the lesion was demarcated by the corpus callosum. Most of the perikarya in the area of the lesion had degenerated. Cortical lesions induced with quinolinic acid were almost identical to the kainic acid lesions except they extended 2 mm medio-laterally (Fig. 5B).

DISCUSSION

This study examined the effects of an endogenous excitant, quinolinic acid and an exogenous neurotoxin, kainic acid, on cortical cholinergic function following injection in the nbm. This region provides ipsilateral cholinergic projections to frontal and parietal cortical areas [6,8]. The effect of a focal injection of quinolinic and kainic acid into the cerebral cortex was also examined. Seven days following micro-injections of quinolinic acid or kainic acid into the nbm, the K^+-induced release of radiolabelled acetylcholine from slices of the frontal-parietal cortex was significantly reduced. The impairment of transmitter release observed in the cortex was due to the toxic action of quinolinic acid on the cholinergic cell bodies of these neurons located in the nbm. This interpretation is supported by a parallel reduction in two markers of cholinergic function, AChE and HACU, and by histological evidence of damaged cells in the nbm. A previous study has demonstrated that quinolinic acid injected into the rat

striatum reduces AChE and HACU, as well as CAT activity [16]. This cholinergic enzyme marker was not measured in the present study, however, it has been measured in a following study and was reduced by quinolinate injections (see following report).

As was observed by Schwarcz *et al.* [16], who reported a dose related reduction in striatal CAT activity after injections of 60–600 nmoles of quinolinic acid, the lowest dose (60 nmoles) injected into the nbm study failed to modify the two cholinergic markers or the release of ^{3}H-ACh from the cerebral cortex in this study. However, two higher doses (150 and 300 nmoles) clearly produced significant reductions in all presynaptic markers of cholinergic function. In the present study the maximal neurotoxic effect of quinolinic acid on ^{3}H-ACh release and cholinergic markers occurred at 150 nmoles. In contrast, the peak effect of quinolinic acid on striatal cholinergic neurons reported by Schwarcz *et al.* [16] occurred at 600 nmoles. This difference may be due to differences in the sensitivity of cholinergic neurons to the action of quinolinic acid in the striatum and the nbm. Variations in regional sensitivity of brain neurons to the excitant action of quinolinic acid after microinotophoretic application has been reported by Perkins and Stone [14,15]. It may also be due to the use of halothane anesthesia in this study and barbiturate anesthesia, which is known to restrict the degree of neurotoxicity, in the Schwarcz study [16].

Quinolinic acid in the present study was considerably less potent than kainic acid. Injections of 150 nmoles quinolinic acid produced effects on cholinergic function comparable in size to the effects produced by 4.7 nmoles kainic acid. This difference in potency could be related to different receptors activated by these two agents. Evidence from electrophysiological [14, 15, 22] and transmitter release [9] experiments indicates that quinolinate preferentially activates Mg-sensitive NMDA (N-methyl-D-aspartate)-type amino acid receptors, while kainate preferentially activates Mg-insensitive kainate-type receptors to produce neuronal excitation.

The failure of quinolinate or kainate to abolish cortical cholinergic function following nbm injection suggests that the residual function might be due to intrinsic cortical cholinergic neurons [3]. This provided the basis for intracortical injections. In previous work injections of kainate into the cerebral cortex yielded conflicting results. Johnston *et al.* [7] reported a 30% reduction in CAT activity after injecting 9.4 nmoles into rat cortex. In contrast, following an intracortical kainate injection, Lehmann *et al.* [8] failed to observe a significant reduction in CAT activity although the level of glutamic acid decarboxylase was reduced. In the present study injections of quinolinic acid or kainic acid into the fronto-parietal cortex, at twice the dose that produced damage in the nbm, failed to decrease ^{3}H-ACh release, AChE activity or HACU. An apparent increase in choline uptake observed after intracortical injections might be due to factors such as mechanical tissue irritation resulting from such injections.

A lack of effect of cortically administered toxins on cholinergic neurons further confirms the axon-sparring neurotoxic action of these agents. Despite a relatively high dose of kainate (9.4 nmoles) injected into the fronto-parietal cortex, the presynaptic cholinergic function in the injected side was not reduced. Histological assessment, however, provided clear evidence of damage to the cerebral cortex. Thus, failure of intracortical injections to induce cholinergic damage was not due to the lack of delivery of an adequate dose of the neurotoxin. The results of the present study would suggest that intrinsic cholinergic neurons either are resistant to the action of these toxins or that such elements make a modest contribution to the total release of ^{3}H-ACh evoked by K^+-depolarization. The contribution for such neurons to HACU and AChE activity also may be quite small and difficult to detect considering the intergroup variance in these two markers. Eckenstein and Thoenen [3] have recently demonstrated cholinergic cell bodies in the cerebral cortex. The sensitivity of such intrinsic cholinergic neurons to neurotoxins remains to be examined in future experiments.

Injections of quinolinic acid into the nbm in this study produced the functional cholinergic deficit which previously has been observed in the biopsied cerebral cortex of human patients with Alzheimer's disease. Sims *et al.* [17,18] demonstrated that K^+-stimulated (31 mM) release of radiolabelled acetylcholine from slices of biopsied neocortex of Alzheimer's patients was reduced when compared with non-disease controls. In the present study, 35 mM K^+-evoked release of acetylcholine from cortical slices of quinolinic or kainic acid treated animals was also reduced when compared to contralateral controls. The spontaneous release of ^{3}H-acetylcholine from slices was not significantly affected in lesioned rats. LoConte *et al.* [10] observed an 18% reduction in the *in vivo* release of cortical acetylcholine, over a 20 minute period, in rats following electrolytic lesions of the forebrain. A lack of effect on the spontaneous release in the present study could be due to the fact that the release from slices is already low, owing to a lack of impulse flow from the subcortical cell bodies, and small changes in this release may not be readily apparent. Measurements of this release over extended periods may reveal a deficit as is observed in the K^+ release. The observation that quinolinate causes cholinergic dysfunction raises the possibility that damage to cholinergic neurons in Alzheimer's disease might result from an abnormal production or disposition of an endogenous neurotoxin such as quinolinic acid, or from an abnormal sensitivity to its synaptic actions. Quinolinic acid is found in human and animal brain [23] and its concentration is apparently increased by administration of tryptophan or in aging [11].

ACKNOWLEDGEMENTS

This research was supported by a grant from the Ontario Mental Health Foundation. R. J. Beninger and S. R. El-Defrawy were supported by the Ontario Ministry of Health and the Canadian Geriatrics Research Society. The authors wish to thank Paul Payson and Brenda Scarth for their technical assistance, and Ms. D. Browne for typing the manuscript.

REFERENCES

1. Coyle, J. T., D. L. Price and M. R. Delong. Alzheimer's disease: A disorder of cortical cholinergic innervation. *Science* **219**: 1184–1190, 1983.

2. Davies, P. Neurotransmitter-related enzymes in senile dementia of the Alzheimer's type. *Brain Res* **171**: 319–327, 1979.

3. Eckenstein, F. and H. Thoenen. Cholinergic neurons in the rat cerebral cortex demonstrated by immunohistochemical localization of choline acetyltransferase. *Neurosci Lett* **36**: 211–215, 1983.
4. Ellman, G. L., K. Courtney, S. Andres and R. Featherstone. A new rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* **7**: 88–95, 1961.
5. Hartgraves, S. L., P. L. Mensah and P. H. Kelly. Regional decreases of cortical choline acetyltransferase after lesions of the septal area and in the area of nucleus basalis magnocellaris. *Neuroscience* **7**: 2369–2376, 1982.
6. Johnston, M. V., M. McKinney and J. T. Coyle. Evidence for a cholinergic projection to the neocortex from neurons in the basal forebrain. *Proc Natl Acad Sci USA* **76**: 5392–5396, 1979.
7. Johnston, M. V., M. McKinney and J. T. Coyle. Neocortical cholinergic innervation: A description of extrinsic and intrinsic components in the rat. *Exp Brain Res* **43**: 159–172, 1981.
8. Lehmann, J., J. I. Nagy, S. Atmadja and H. C. Fibiger. The nucleus basalis magnocellularis: The origin of a cholinergic projection to the neocortex of the rat. *Neuroscience* **5**: 1161–1174, 1980.
9. Lehmann, J. and B. Scatton. Characterization of the excitatory amino acid receptor mediated release of ^{3}H-acetylcholine from rat striatal slices. *Brain Res* **252**: 77–89, 1982.
10. LoConte, G., L. Bartolini, F. Casamenti, I. Marconcini-Pepeu and G. Pepeu. Lesions of cholinergic forebrain nuclei: Changes in avoidance behaviour and scopolamine actions. *Pharmacol Biochem Behav* **17**: 933–937, 1982.
11. Lombardi, G., R. Corradetti, V. Carla, C. Aldinio and F. Moroni. Mass-fragmentographic identification of the excitotoxin quinolinic acid: Ontogenetic changes and modulation of its concentration in the rat brain by tryptophan and by p-chlorophenylalanine. *Soc Neurosci Abstr* **9**: 1184, 1983.
12. Lowry, O. H., N. J. Rosenbrough, A. L. Farr and R. S. Randall. Protein measurements with the folin phenol method. *J Biol Chem* **193**: 265–275, 1951.
13. Paxinos, G. and C. Watson. *The Rat Brain in Stereotaxic Coordinates*. Toronto, Canada: Academic Press, 1982.
14. Perkins, M. N. and T. W. Stone. Pharmacology and regional variations of quinolinic acid-evoked excitations in the rat central nervous system. *J Pharmacol Exp Ther* **226**: 551–557, 1983.
15. Perkins, M. N. and T. W. Stone. Quinolinic acid: Regional variations in neuronal sensitivity. *Brain Res* **259**: 172–176, 1983.
16. Schwarcz, R., W. O. Whetsell and R. M. Mangano. Quinolinic acid: An endogenous metabolite that produces axon-sparing lesions in rat brain. *Science* **219**: 316–318, 1983.
17. Simon, J. R., S. Atweh and M. J. Kuhar. Sodium-dependent high affinity choline uptake: A regulatory step in the synthesis of acetylcholine. *J Neurochem* **26**: 909–922, 1976.
18. Sims, N. R., O. M. Bowen, C. C. T. Smith, R. H. A. Flack, A. N. Davison, J. S. Snowden and D. Neary. Glucose metabolism and acetylcholine systhesis in relation to neuronal activity in Alzheimer's disease. *Lancet* **1**: 333–336, 1980.
19. Sims, N. R., D. M. Bowen and A. N. Davison. ^{14}Acetylcholine synthesis and ^{14}C carbon dioxide production from (U-^{14}C) glucose by tissue prisms of human neocortex. *Biochem J* **196**: 867–876, 1981.
20. Somogyi, G. T. and J. C. Szerb. Demonstration of acetylcholine release by measuring labelled choline efflux from cerebral cortical slices. *J Neurochem* **19**: 2667–2677, 1972.
21. Vincent, S. R., K. Satoh, D. M. Armstrong and H. C. Fibiger. Substance P in the ascending cholinergic reticular system. *Nature* **306**: 688–691, 1983.
22. Watkins, J. C. Pharmacology of excitatory amino acid receptors. In: *Glutamate: Transmitter in the Central Nervous System*, edited by P. J. Roberts, J. Storm-Mathisen and G. A. R. Johnston. Chichester, U.K.: John Wiley, 1981, pp. 1–24.
23. Wolfensberger, M., U. Amsler, M. Cuenod, A. C. Foster, W. D. Whetsell and R. Schwarcz. Identification of quinolinic acid in rat and human brain tissue. *Neurosci Lett* **41**: 247–252, 1983.