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RESEARCH NOTE

Lack of Recovery of Cortical Cholinergic Function following Quinolinic or Ibotenic Acid Injections into the Nucleus Basalis Magnocellularis in Rats

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Cortical cholinergic markers fail to recover following injection of quinolinic or ibotenic acid into the rat nucleus basalis magnocellularis.

The use of excitotoxins to produce animal models of human neurodegenerative disorders such as Huntington's disease and temporal lobe epilepsy has been proposed (17). Neurotoxic (7, 9, 11) or electrothermic (15, 16) lesions of the nucleus basalis magnocellularis (nbM) produce marked reductions in all presynaptic cholinergic markers in the neocortex, indicating that a major component of the cholinergic innervation to the cortex originates from this site in the basal forebrain. Spontaneous recovery of two cholinergic markers (choline acetyltransferase and high-affinity choline uptake) in the neocortex following unilateral injection of the excitotoxin, ibotenic acid, into the nbM has recently been reported (20). Considerable evidence indicates that central cholinergic systems play an important role in learning and memory (12, 14). Thus, the implication of recovery of cortical cholinergic function after lesions

Abbreviations: AChE—acetylcholinesterase, [^{3}H]AChR—[^{3}H]acetylcholine release, CAT—choline acetyltransferase, HACU—high-affinity [^{14}C]choline uptake, nbM—nucleus basalis magnocellularis.

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to the basal forebrain on behavior becomes important. The present studies were designed to evaluate the extent to which cortical cholinergic function recovers after quinolinic acid and ibotenic acid lesions of the nbM. Contrary to previous reports (16, 20), our results indicate that recovery of cortical cholinergic markers does not occur. A preliminary report of these studies has appeared (4).

Stereotaxic lesions of the nbM with quinolinic acid (120 nmol/1 μl saline) or ibotenic acid (25 nmol/1 μl saline) were carried out on male Sprague–Dawley rats (275 to 350 g) under halothane anesthesia as described elsewhere (3). Coordinates for the injection were 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. Animals were killed by decapitation and the frontoparietal cortex dissected on ice. Tissue slices (0.3 mm thick) to be used for K^+ (35 mM)-evoked [^{3}H]acetylcholine release ([^{3}H]AChR) after incubation in [^{3}H]choline (2, 3, 19); synaptosomes prepared for measurement of high-affinity [^{14}C]choline uptake (HACU) (2, 18), or tissue homogenates for the assays of choline acetyltransferase (CAT) employing [^{14}C]acetyl CoA (2, 6) or acetylcholinesterase (AChE) (2, 5), were immediately prepared from both the lesion and contralateral control hemisphere of each animal. Protein was measured according to Lowry *et al.* (13). The remainder of the brain was fixed in 10% Formalin/20% sucrose and 40-μM-thick frozen sections were prepared and stained with cresyl violet for histological verification of the lesion (3). Isotopes were purchased from New England Nuclear.

In our experiments, control values (mean ± SE) of cholinergic markers obtained from the contralateral cortex or sham-lesion animals were: K^+-evoked [^{3}H]AChR, 19.5 ± 1.4 (total % release above baseline); HACU, 0.2 ± 0.02 (pmol choline/mg protein/4 min); CAT, 58.2 ± 2.1 (nmol ACh formed/mg protein/h); AChE, 63.5 ± 4.2 (nmol ACh hydrolyzed/mg protein/min). These values are similar to those reported in the literature (7, 8, 10).

No significant decrease in cortical cholinergic markers was obtained in sham (saline)-injected animals, except for a 12% reduction in CAT activity which was significant ($P < 0.005$). In contrast, 1 week after a lesion of the nbM with 120 nmol quinolinic acid, a 36 to 55% reduction in cholinergic markers was obtained (Table 1). A decrease in all cholinergic markers was evident at 3, 6, and 12 weeks after the lesion with no significant difference (one-way ANOVA) between the values obtained at the different time intervals. Regression analysis revealed no statistically significant correlation between time and reduction in any of the markers studied. Seven days following a unilateral ibotenic acid lesion of the nbM, cortical cholinergic markers were reduced by 30 to 50%; again no significant recovery was observed 12 weeks after the lesion (Student's t test).

TABLE 1

Changes in Cholinergic Markers in the Cerebral Cortex of Rats following Unilateral Quinolinic Acid (120 nmol) or Ibotenic Acid (25 nmol) Lesions of Nucleus Basalis Magnocellularis[a]

Toxin	Time after lesion (weeks)	[^{3}H]AChR (total % release above baseline)	HACU (pmol choline/mg protein/ 4 min)	CAT (nmol ACh formed/mg protein/h)	AChE (nmol ACh hydrolyzed/mg protein/min)
Quinilinic[b] acid	1	40.7 ± 7.8	36.1 ± 4.7	55.1 ± 2.6	37.8 ± 4.6
	3	37.0 ± 4.3	43.8 ± 5.6	40.3 ± 5.2	44.5 ± 2.5
	6	31.8 ± 8.2	49.9 ± 14.0	39.3 ± 0.9	60.2 ± 8.9
	12	44.5 ± 8.8	27.3 ± 5.7	37.2 ± 12.5	39.9 ± 7.7
Ibotenic[c] acid	1	47.0 ± 6.6	40.6 ± 3.9	35.5 ± 6.0	48.3 ± 7.2
	12	33.4 ± 5.8	34.9 ± 9.4	32.4 ± 6.3	39.9 ± 7.7

[a] Each value is the mean percentage decrease (±SE) of the lesion hemisphere compared with the contralateral hemisphere from groups of three to eight animals.

[b] No significant difference (one-way ANOVA) was observed between the percentage decrease at the different time points after lesion in any of the markers. Regression analysis showed no correlation between percentage decrease and time in any of the markers.

[c] No significant difference (Student's *t* test) observed between the percentage decrease at 1 and 12 weeks in any of the markers.

At the light microscopic level, the brain showed cell loss with gliosis in the ventral globus pallidus at 1 and 12 weeks after lesion with quinolinic acid (Fig. 1). Lesions were centered in the nbM and displayed a spheroid pattern of degeneration extending 2.4 mm in a medial–lateral direction and 1.3 mm in a dorsal–ventral direction.

The reduction in cortical cholinergic markers that follows a neurotoxic lesion of the nbM confirms reports of a major cholinergic projection to the cortex (9, 11). The literature regarding recovery of cholinergic markers in the cortex following lesions of the basal forebrain is controversial (1, 15, 16, 20). We have consistently found significant reductions in CAT, HACU, AChE, and [^{3}H]AChR, 1 to 12 weeks after unilateral neurotoxic lesions of the nbM. These findings do not support earlier work showing total recovery of CAT within 12 weeks and HACU within 4 weeks of a unilateral ibotenic acid lesion of the nbM (20). The magnitude of the changes in CAT and HACU induced by lesion of the nbM in the present study was similar to that observed by Wenk and Olton (20), suggesting that the reported recovery of cholinergic markers was not a result of an inadequate lesion of the nbM. A similar lack of recovery of cortical CAT was observed by Pedata *et al.* (16)

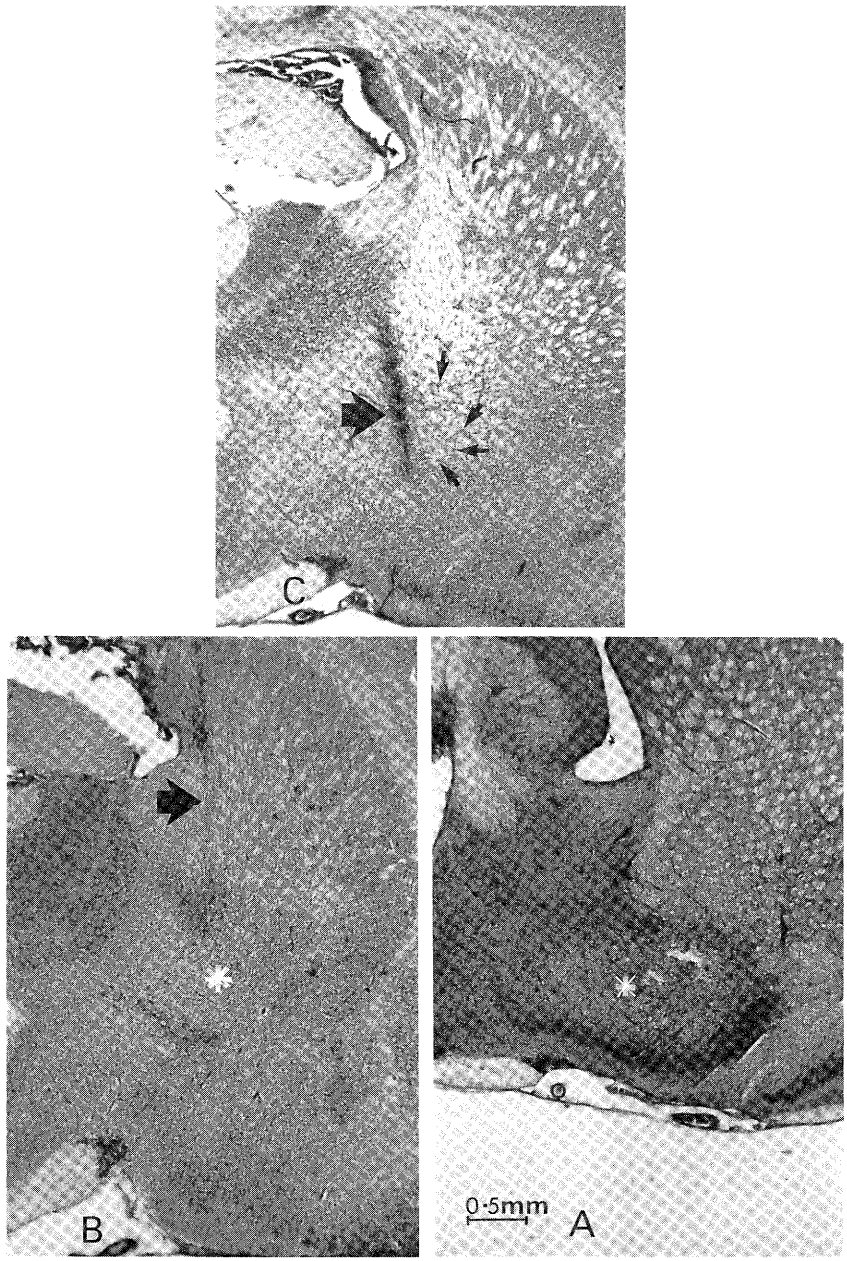

FIG. 1. Histologic appearance of the nucleus basalis magnocellularis 1 week (A) and 12 weeks (B) after infusing 1 μl quinolinic acid (120 nmol). Large neurons (small arrows) seen in saline-injected animals (C) were absent at the lesion site (*) which shows the cannula tract (large arrow) and glial infiltration.

20 days after electrolytic lesions of the magnocellular forebrain nuclei; however, in this and a subsequent study (15) recovery of HACU did occur. Bartus *et al.* (1) recently reported no difference between the reduction in cortical CAT and HACU activity observed at 2 weeks or 6 months after injection of ibotenic acid into the nbM. The reason for the discrepancy in the recovery of HACU could be due to the procedure used to induce a lesion; Pedata *et al.* (15, 16) used an electrolytic procedure whereas this study and that of Bartus *et al.* (1) relied on excitotoxins. The reported plasticity of the basal forebrain cholinergic system (20) is difficult to reconcile with recent data (8) showing a 56% reduction in cortical CAT 8 weeks after bilateral ibotenic acid lesions of the nbM, by which time an almost complete recovery should have occurred. Our data and those summarized above do not support the idea that extensive cholinergic recovery occurs in the cortex after maximal lesions of the basal forebrain.

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