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Cortical Muscarinic Receptor Function following Quinolinic Acid-Induced Lesion of the Nucleus Basalis Magnocellularis

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Phosphatidylinositol metabolism, linked to muscarinic receptor activation, was studied in rat cortical slices after a unilateral quinolinic acid lesion to the nucleus basalis magnocellularis (nbM) or the cortex itself. The incorporation of [^{3}H]inositol into inositol phosphate was measured in the presence of LiCl, followed by anion exchange chromatography to separate inositol phosphates. Carbachol produced a dose-related increase in the amount of inositol phosphate in both the control and the cortex from rats with lesions to the nbM. No significant difference in the accumulation of inositol phosphate was observed between the two groups. Destruction of cortical cells with quinolinic acid almost completely eliminated the activation of phosphatidylinositol metabolism by carbachol. These results suggest that muscarinic receptors linked to phosphatidylinositol metabolism in the cortex are localized on intrinsic cortical neurons and not on afferent terminals from basal forebrain cholinergic neurons.

INTRODUCTION

There is now considerable evidence that the neocortex and hippocampus receive cholinergic input from large neurons located in the nucleus basalis magnocellularis (nbM) and the nucleus of the diagonal band of Broca, respectively (20–22). In experimental animals, focal injections of excitotoxins such as kainic acid, ibotenic acid, and quinolinic acid into these nuclei destroy cholinergic cell bodies and produce a presynaptic cholinergic deficit in their target areas (9, 16). Biochemical studies on the autopsy brain of patients with senile dementia of Alzheimer's type have revealed a presynaptic cholinergic deficit in the neocortex and the hippocampus which is thought to arise from a progressive loss of cholinergic cells in the basal forebrain and which contributes to the behavioral symptoms of this disorder (4, 6, 7).

Since denervation in some neuronal systems leads to receptor supersensitivity, a number of investigations have attempted to determine if the loss of cholinergic innervation in the brain leads to muscarinic receptor supersensitivity. In experimental animals 5 weeks after ibotenate lesions of the nbM, no significant change was seen in the affinity or density of the high affinity muscarinic receptor sites thought to represent postsynaptic receptors, although a small decrease in the low affinity sites representing presynaptic receptors was observed (14, 20). Studies in acetylcholine-deficient Alzheimer brain have shown that generally there is no decrease in the affinity or density of muscarinic binding sites. However, in a recent report on autopsied Alzheimer brain, Mash *et al.* (19) showed that while there was no decrease in the M_1-muscarinic receptor sites there is a significant decrease in the M_2-receptor sites.

At present little is known about the biochemical or physiological function of muscarinic receptors in the brain following cholinergic denervation. Early electrophysiological studies on the neuronally isolated cerebral cortex and the denervated hippocampus (3) failed to find muscarinic receptor supersensitivity. However, in a recent electrophysiological study, Lamour *et al.* (15) reported an increase in sensitivity of cortical cells to cholinergic agonists following an electrolytic lesion of the nbM. Thus, the status of muscarinic receptor function in the cortex following loss of cholinergic input remains unclear.

If muscarinic receptors in the cortex undergo a change in sensitivity after cholinergic denervation, then changes in the biochemical messenger systems linked to these receptors can be expected. It is now widely recognized that muscarinic receptors in the brain are linked to inositol phosphate metabolism and their activation by appropriate agonists results in hydrolysis of phosphatidylinositides (PtdIns) to inositol phosphate (2, 5, 13). In the presence of lithium the conversion of inositol phosphate (IP) to inositol is blocked, and under this condition its accumulation in response to muscarinic receptor stimulation can serve as a useful index of the receptor function (2). In the present study using carbachol-induced stimulation of PtdIns hydrolysis as an index of receptor function, we have investigated the status of cor-

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tical muscarinic receptors after an excitotoxic lesion of the nbM. Experiments have been conducted in rats at 1, 6, and 12 weeks after a single unilateral injection of an endogenous excitotoxin, quinolinic acid, into the nbM. In separate experiments the receptor function has been assessed after an injection of the excitotoxin into the cortex itself.

MATERIALS AND METHODS

Excitotoxic Lesions of the nbM and Cortex

Excitotoxic lesions and sham lesions of the nbM and cortex were made using the method previously described by El-Defrawy *et al.* (9). Briefly, the animals were anesthetized with halothane (2% halothane, 98% oxygen via inhalation; Halocarbon, Malton, Ontario) and positioned in a Narashighe small animal stereotaxic apparatus. The surface of the skull was exposed and the bregma was located. With the incisor bar set at 3.3 mm below the interaural line, the tip of the infusion cannula was aimed at the nbM on the right side of the brain, the coordinates from bregma being: 0.8 mm posterior, 2.6 mm lateral to the midline, and 8.0 mm ventral to the surface of the skull (23). The infusion apparatus consisted of a stainless steel cannula (o.d. = 0.35 mm; Small Parts, Inc., Florida) connected to a 10-μl Hamilton microsyringe via PE-20 polyethylene tubing (Intramedic). One microliter of quinolinic acid (QUIN) (120 nmol/μl 0.9% saline, pH 7.4) was infused over 2 min, 25 s via a Sage Instruments syringe pump (Model 341). The cannula was left in place for 3 min following infusion to allow diffusion of QUIN. Following removal of the cannula, the burr hole was covered with Bonewax, and the scalp was apposed with sutures. Sham lesioned animals received an injection of 1 μl of 0.9% saline (pH 7.4) over the same time period. The animals were allowed to recover for 1, 6, and 12 weeks.

The procedure for lesions of the frontoparietal cortex was similar to that used for the nbM except that two injections of 150 nmol of QUIN in 1 μl of 0.9% saline, pH 7.4, were administered. The coordinates for these injections were (a) 1.0 mm anterior to the bregma, 2.0 mm lateral to the midline, and 2.8 mm ventral to the surface of the skull; and (b) 1.3 mm posterior to the bregma, 3.0 mm lateral to the midline, and 2.8 mm ventral to the surface of the skull. The animals were used 1 week after the injections.

Tissue Preparation

(a) nbM experiments. At 1, 6, and 12 weeks postinjection the animals were killed by decapitation and the brains were rapidly removed and rinsed with ice-cold Krebs–Ringer bicarbonate (KRB), pH 7.2. The brains were placed, ventral surface up, on moistened filter paper (Whatman 50, hardened) on a chilled plexiglass plate kept on ice. A coronal slab of the whole brain was produced using two parallel stainless steel razor blades held 6.0 mm apart in a plexiglass holder. The slab was positioned, with the caudal surface up, on the filter paper and the frontoparietal cortex was dissected from each hemisphere. A small piece of each cortical section was removed and reserved for determination of choline acetyltransferase (CAT) activity (see below). A cortical mince was prepared by cross chopping (350 × 350 μm) the remainder of the dissected cortex on a McIlwain tissue chopper. The mince was immediately transferred to a 5-ml plastic beaker containing 1 ml KRB and dispersed using a fine artist's brush (size 000). The remainder of the coronal slab was fixed in 30% sucrose/10% formal saline for histological evaluation of the nbM lesion (see below).

(b) Cortical experiments. The procedure for removal and dissection of the rat brain was similar to that described above with two exceptions: (i) a portion of the cortex was not removed for CAT assay before mincing; and (ii) the coronal slab was not fixed for histological evaluation. The whole cortical section was minced and a small aliquot of this was removed for CAT assay.

Measurement of [^{3}H]Inositol Phospholipid Metabolism

The method of [^{3}H]inositol phospholipid metabolism, involving accumulation of inositol phosphates in the presence of lithium, was adapted from the procedure of Berridge *et al.* (1, 2).

The cortical minces prepared from quinolinic- or saline-injected animals were incubated in KRB, pH 7.2–7.4, for 1 h (with one change of the medium) in a 5-ml beaker placed in a shaking water bath at 37°C and aerated in an atmosphere of 95% O_2/5% CO_2. The slices were allowed to settle to the bottom of the beaker under gravity. Portions (50 μl) of the gently packed tissue were transferred to 5-ml beakers containing KRB, 8 μM LiCl, and 0.32 μM [^{3}H]inositol (12.5 Ci/mmol) in a total volume of 198 μl. The tissue was incubated for an additional 30 min to allow labeling of various intermediates in the PtdIns cycle. At this point PtdIns hydrolysis was stimulated by addition of 2 μl of vehicle (for baseline activity levels) or the appropriate concentration of carbachol (for receptor-stimulated activity). After 45 min the tissue was washed with 3 × 1 ml of KRB and again allowed to settle under gravity. A 150-μl aliquot of the gently packed tissue was transferred to a glass/glass homogenizer; 100 μl of KRB was added, followed by 930 μl of chloroform/methanol (1:2 (v/v)); and the tissue was homogenized. The homogenate was transferred to a 1.5-ml Eppendorff tube. Chloroform (310 μl) and 310 μl of water were added and the tubes were briefly mixed. After standing for 5 min to allow separation of the phases, the tubes were centrifuged at 2500*g* for 5 min at 4°C. The lower phase was evaporated to dryness overnight in an

oven at 37°C. The next day, 1.0 ml of 1 *N* NaOH was added, and the tube was mixed and left overnight for protein digestion. The following day the digest was centrifuged at 15,000*g* for 3 min. Duplicate aliquots (150 μl) of the supernatant were removed and assayed for protein according to the method of Lowry *et al.* (18).

An aliquot (0.75 ml) of the aqueous (upper) phase was removed and applied to a column of Dowex-1 (×8, 100 to 200 mesh; Sigma) in the formate form. The phosphate esters were eluted from the Dowex-1 anion exchange resin by the stepwise addition of solutions containing increasing concentrations of formate (1, 2).

To ascertain that the activity of carbachol on PtdIns hydrolysis was mediated by muscarinic receptors, the effect of a specific muscarinic receptor antagonist, atropine, on this turnover was tested. The method used was similar to that described previously except that 1 μl of atropine solution (final concentration in incubation media = 10^{-5} *M*) was added 25 min after the addition of [^{3}H]inositol.

Determination of Choline Acetyltransferase (CAT) Activity

CAT activity in the cerebral cortex of sham lesioned and quinolinic acid lesioned animals was measured by the procedure of Fonnum (12).

Determination of τ-Aminobutyrate (GABA) Content and Release

Biochemical assessment of cortical lesions induced by quinolinic acid was made by measuring the content and K^+-evoked release of GABA since injection of excitotoxins in this region destroys GABA neurons (16). These measurements were made in cortical minces prepared from the tissue that was also used for the measurement of PtdIns hydrolysis. The minced cortex was incubated at 37°C in 200 μl Krebs–Ringer bicarbonate buffer for 15 min. After this, the medium was removed and reserved for determination of basal release and replaced with 200 μl of high potassium (35 m*M*) medium. This was then removed and reserved for the estimation of K^+-evoked release of GABA. After this, the mince was homogenized in 1 ml 50% methanol and centrifuged at 15,000*g* for 10 min. The supernatant was frozen for determination of tissue GABA levels. GABA was estimated by high performance liquid chromatography using the procedure of Lindroth and Mopper (17).

Protein Determination

The protein content of tissue minces and homogenate samples was determined by the procedure of Lowry *et al.* (18).

Histology

After a minimum of 3 days of fixation in 30% sucrose/10% formal saline, the brain slabs were frozen on a cryostat chuck (−20°C) and 40-μm sections were cut and mounted on microscope slides. The slides were dried and stained for Nissl substance with cresyl violet.

For histological evaluation of cortical lesions, injections of neurotoxin were made in a separate group of animals since the cortical tissue needed for histology was the same as that required for biochemical tests.

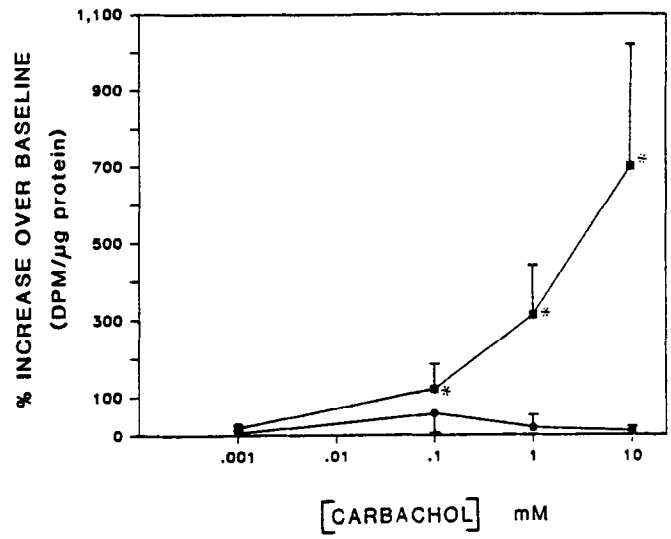

FIG. 1. Dose–response curve for carbachol-stimulated PtdIns hydrolysis in the absence (■) and presence (●) of atropine (10^{-5} *M*). Each point represents the mean ± SD of six determinations. The vertical axis represents increased accumulation of inositol 1-phosphate over baseline in the presence of lithium. *$P < 0.01$ indicates a significant increase in response over the baseline.

Data Analysis

(a) nbM lesion experiments. The data obtained for the carbachol dose–response curve were expressed as the percentage increase over control (carbachol absent). These results were analyzed by a one-way analysis of variance (ANOVA) for statistical significance with subsequent analysis by a Newman–Keuls studentized range test. The data obtained for carbachol-stimulated PtdIns hydrolysis in the time study were expressed as dpm/mg protein. These data were also analyzed by one-way ANOVA and Newman–Keuls tests.

(b) Cortical lesion experiments. The data obtained for GABA release and content, CAT assays, and PtdIns hydrolysis were analyzed using a Student *t* test to determine any significant differences.

RESULTS

Carbachol-Induced Accumulation of Inositol Phosphates

The effects of different doses of carbachol on the accumulation of IP_1 are represented in Fig. 1. A statistically significant action of carbachol on this response was apparent in the dose range 10^{-4} to 10^{-2} *M*. In the presence

TABLE 1

Choline Acetyltransferase Activity (pmol Acetylcholine Formed/h/mg Protein) in the Cerebral Cortex after Quinolinic Acid-Induced Lesion of the nbM

Time postinjection	Saline-injected group			Quinolinic-injected group		
	Contralateral	*n*	Ipsilateral	Contralateral	*n*	Ipsilateral
1 week	47.8 ± 5.3	8	46.6 ± 4.0	48.5 ± 16.3	16	17.0 ± 5.6*
6 weeks	63.4 ± 3.8	5	67.8 ± 6.1	52.2 ± 9.6	13	22.9 ± 8.8*
12 weeks	65.7 ± 6.9	6	65.8 ± 14.7	61.6 ± 16.8	11	26.7 ± 8.4*

* Significantly different ($P < 0.001$) from the value on the contralateral (uninjected) side in the same group.
Note. The data shown are means ± SD.

of atropine (10^{-5} *M*), the action of 10^{-2} *M* carbachol was virtually abolished, indicating that this effect was mediated through muscarinic receptors.

Carbachol Action after a Lesion of the nbM

To determine the action of carbachol after cholinergic denervation of the cortex, cholinergic neurons in the nbM were destroyed by a focal injection of quinolinic acid. At 1, 6, and 12 weeks postlesion, the action of carbachol on the accumulation of IP_1 was tested.

To verify the successful placement of the lesion, cortical CAT activity was determined in all animals used in the time study. The results are presented in Table 1. The injection of saline into the nbM (sham lesion) did not produce a significant change in cortical CAT activity when compared to the contralateral cortex at any of the postinjection times. Quinolinic acid injection (120 nmol), however, produced a significant decrease in CAT activity in the ipsilateral cortex when compared to the contralateral control cortex ($P < 0.001$). Although it appeared that the level of CAT activity was higher in both the injected and contralateral hemispheres at 12 weeks than it was at 1 or 6 weeks postlesion, the difference between the values was not significant. Microscopic examination of the brains of rats injected with the neurotoxin showed an area of cellular degeneration at the injection site. Damage, as indicated by destruction of perikarya and glial infiltration, was centered in the nbM and extended to involve most of the globus pallidus. There was very little damage to cells in the caudate putamen. In each case, the extent of the lesion was clearly demarcated by gliosis. The saline-injected animals showed no evidence of damage to the tissue in the vicinity of the injection site.

The action of carbachol was examined on the accumulation of IP_1 in the frontoparietal cortex of the sham- and quinolinic acid-injected animals. Three doses of carbachol (10^{-4}, 10^{-3}, and 10^{-2} *M*) were used as these doses produced a significant action in preceding tests on intact animals. Figure 2 represents the action of carbachol on the cortex of the uninjected and injected hemispheres of animals with sham and excitotoxic lesions. In all ani-

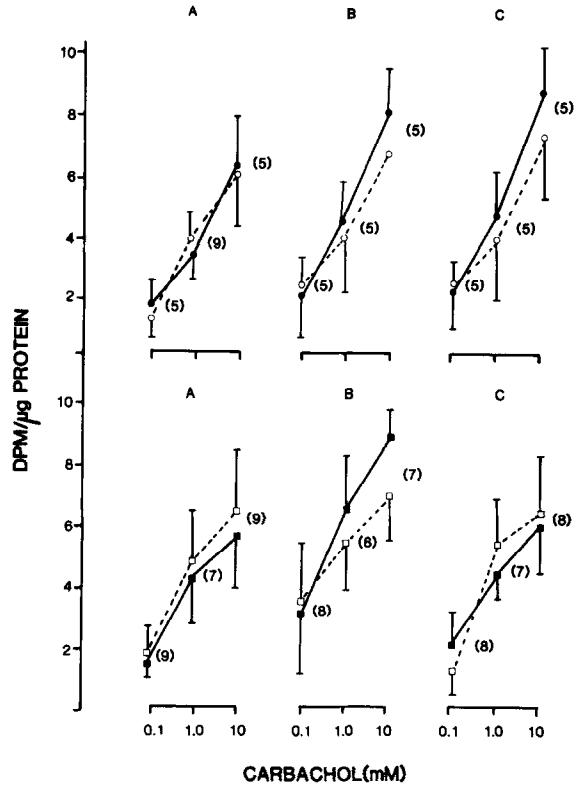

FIG. 2. Carbachol-stimulated PtdIns hydrolysis in the frontoparietal cortex in animals with 1-week-old (A), 6-week-old (B), and 12-week-old (C) excitotoxic lesions of the nbM. Upper panel: data from sham lesion animals. Lower panel: data from quinolinic lesion animals. Open symbols (○, □) indicate response in the cortex from injected hemisphere and closed symbols (●, ■) indicate response from the contralateral uninjected hemisphere. Figures in brackets represent number of experiments at each dose of carbachol. Each point is the mean ± SD.

TABLE 2

Changes in Markers for Cortical GABA after a Quinolinate-Induced Lesion of the Frontoparietal Cortex

Rat No.	GABA content[a] (nmol/hemisphere) C	I	Percentage decrease	GABA release[b] (nmol/hemisphere) C	I	Percentage decrease
1	19.50	1.80	90.8	2.86	0.37	87.1
2	18.72	0.87	95.4	3.47	0.65	81.3
3	19.71	5.34	72.9	2.05	0.24	88.3
4	27.33	4.14	84.9	2.53	0.84	66.8
5	22.53	3.90	82.7	3.57	0.90	74.8
Means ± SD	21.56 ± 3.53	3.21 ± 1.83*	85.3	2.90 ± 0.64	0.6 ± 0.29*	79.8

Note. C, contralateral hemisphere; I, ipsilateral (quinolinic-injected) hemisphere.
[a] Tissue levels of GABA (nmol/hemisphere).
[b] GABA release evoked by 35 mM potassium (nmol/hemisphere).
* Indicates significant difference from values in the contralateral hemisphere ($P < 0.01$).

mals, carbachol produced a dose-related increase in the accumulation of IP_1 and statistical analysis revealed a significant difference between the action of carbachol doses. Comparison between the carbachol action on the ipsilateral and contralateral cortices in the sham lesioned animals showed no significant differences. A similar comparison of the carbachol effect in the quinolinic acid-injected groups also showed no significant differences between hemispheres. Thus, injection of QUIN into the nbM, resulting in a significant loss of CAT activity in the cortex, did not influence the action of carbachol on the muscarinic receptor-mediated response.

Carbachol Action after a Lesion of the Cortex

To determine whether the muscarinic receptor-stimulated accumulation of IP_1 was due to activation of receptors located on cell bodies in the cortex, the action of carbachol was tested following injection of quinolinic acid into the frontoparietal cortex.

To verify the success of the lesion, the tissue content of GABA and K^+-stimulated release of GABA were measured. These values were decreased by 85.3 and 79.8%, respectively, after quinolinic acid injection (Table 2). Histological examination of the cortex showed a rectangular area of extensive damage extending approximately 2.5 mm mediolaterally. The ventral aspect of the cortical lesion was demarcated by the corpus callosum. Most of the perikarya in the area of the lesion had degenerated 1 week after the injection of the neurotoxin.

The action of 10^{-4} *M* carbachol on the accumulation of IP_1 was tested 1 week after the cortical injection. This dose of carbachol was chosen since it was at the lower end of the dose–response curve in earlier tests and produced consistent action in these experiments. An attempt was made to test the action of higher doses of carbachol, but the results were very variable. Table 3 shows the action of 10^{-4} *M* carbachol in individual experiments. The accumulation of radioactivity was expressed as the change in dpm per hemisphere. Each value reflects the carbachol-evoked increase in radioactivity over the basal level (carbachol absent). It was not possible to express the results as per milligram protein since the cortical lesion produced a very large decrease in protein and expression on this basis masked the decrease in the accumulation of IP_1 apparent in the actual dpm values. The technique employed for the dissection of the cortex was standardized so that exactly the same cortical region (tissue block) was removed from each brain, and in each experiment all of the dissected tissue was used. As shown in Table 3, injection of quinolinic acid into the cortex reduced the carbachol response by about 85%. Thus, destruction of cortical cells by the excitotoxin almost eliminated the action of carbachol on muscarinic receptors,

TABLE 3

The Effect of 10^{-4} M Carbachol on the Accumulation of [^{3}H]Inositol 1-Phosphate in the Frontoparietal Cortex after a Quinolinate-Induced Cortical Lesion

Rat No.	δ dpm/hemisphere Contralateral	Ipsilateral[a]	Percentage decrease
1	1209	219	82
2	2301	339	85
3	2940	414	86
4	1846	297	84
5	2445	345	86
Means ± SD	2148.2 ± 654.2	322.8 ± 71.6**	85

[a] Quinolininc acid was injected 1 week prior to sacrifice.
** Significantly different ($P < 0.001$) from the value on the contralateral (saline) injected hemisphere.

suggesting a predominant localization of receptors on these cells.

DISCUSSION

This study examined whether muscarinic receptor function in the rat cerebral cortex is altered following removal of a major cholinergic projection to this region from the basal forebrain. The cholinergic cell bodies in the nbM were destroyed by a focal injection of the endogenous excitotoxin, quinolinic acid, and receptor function in the cortex evaluated 1, 6, and 12 weeks postlesion by measuring the carbachol-induced hydrolysis of phosphoinositides. The results showed that lesions of the cholinergic basocortical pathway produced no significant change in the sensitivity of muscarinic receptors to carbachol. In contrast, quinolinate-induced lesions of the cortex almost abolished the biochemical response to carbachol.

In accordance with a number of previous reports, carbachol produced a dose-related increase in the accumulation of inositol 1-phosphate in the presence of lithium (2.5). That this effect could be abolished by atropine confirmed the involvement of muscarinic cholinergic receptors in the carbachol response. However, there was considerable variance between carbachol effects in different animals. To minimize this the cortical tissue dissection was standardized such that the same region was removed and the amount of protein per incubation vessel was kept below 600 μg (13).

The absence of cortical denervation supersensitivity was indicated by the similar response to carbachol obtain in the excitotoxin-injected hemisphere, in the contralateral side, and in the saline-injected control animals. Neither the dose of the agonist employed nor the age of the lesion influenced the action of carbachol. In a previous study involving receptor binding, McKinney and Coyle (20) reported that the affinity and density of [^{3}H]QNB binding sites (representing postsynaptic cholinergic receptor sites) was not significantly influenced by a 5-week-old ibotenate lesion of the nbM in rats. Recently, Mash *et al.* (19) showed that the density of M_1-muscarinic receptors in autopsied Alzheimer brain did not significantly differ from that in the age-matched control brain. Thus, there is a close agreement between the results of the preceding binding studies and the present study which measured a functional index of muscarinic receptor activity. Both approaches point to a lack of muscarinic receptor supersensitivity after cholinergic deafferentation.

The results of these studies, however, conflict with those obtained in a recent electrophysiological study conducted in rats bearing an electrolytic lesion of the nbM (15). In that study the excitant action of microiontophoretically applied cholinergic agonists on cortical neurons was altered by the lesion; a greater percentage of cells was excited by acetylcholine 2 and 3 weeks after the nbM lesion and by carbachol 2 weeks after the lesion. An increase in the sensitivity to acetylcholine was also observed by these investigators. However, Lamour *et al.* (15) reported that an increase in cholinergic sensitivity was more obvious after a 2-week lesion than after a 3-week lesion, suggesting that this phenomenon may have been temporary. It should be noted that in earlier electrophysiological studies no true denervation supersensitivity of central muscarinic receptors was observed (3). In the Bird and Aghajanian (3) investigation, a surgical lesion of the fimbria, which produced cholinergic loss in the hippocampus, increased the sensitivity of cells in this region to acetylcholine but not to the more stable choline ester, carbachol. These investigators attributed this apparent muscarinic supersensitivity to a reduced degradation of acetylcholine in the denervated hippocampus. Thus, it appears that cholinergic denervation of the cerebral cortex does not produce an increase in the muscarinic receptor sensitivity. The possibility that receptor supersensitivity does occur but is reversed by a presynaptic cholinergic recovery (25) is ruled out by the observation that the activity of CAT, a marker of presynaptic cholinergic function, was reduced in all three groups of animals with an excitotoxic lesion of the nbM. This observation confirms an earlier study showing that presynaptic cholinergic markers do not recover to control levels by this time after a quinolinate-induced lesion to the nbm (8).

In contrast to the subcortical injection of quinolinic acid, injection into the frontoparietal cortex almost abolished the action of carbachol on PtdIns hydrolysis. This lesion resulted in uniform neuronal destruction of cortical cell bodies in all layers. The large loss of protein resulting from the lesion made it difficult to express the carbachol data on the basis of protein content. However, examination of the results of individual experiments showed a large decrease in the carbachol-induced response. This decrease paralleled the decrease in GABA content and potassium-induced release of GABA, both of which served as biochemical markers for the cortical lesion. This loss of GABA function is in agreement with results of a previous study which reported a decrease in glutamate decarboxylase activity in the cortex following cortical kainic acid injections (16). The ability of cortical excitotoxic lesions to produce a decrease in the carbachol-induced response suggests that the muscarinic receptors mediating the PtdIns hydrolysis were predominantly postsynaptic. It is unlikely that atrophy of basal forebrain cholinergic neurons 7 days following cortical lesions was responsible for the 85% reduction in inositol phosphate labeling since cortical damage only leads to a 20% reduction in basal forebrain ChAT activity 10 days after the lesions (24).

The present findings on inositol metabolism in the rat cerebral cortex after a subcortical excitotoxic lesion and

a local lesion closely parallel those of Fisher *et al.* (10, 11) in the guinea pig hippocampus after a fimbrial lesion. These investigators reported that cholinergic denervation of the hippocampus did not alter the muscarinic agonist-mediated changes in inositol metabolism measured in the absence of lithium. However, direct injections of ibotenate into the hippocampus, causing cell loss in this region, produced a marked reduction in the cholinergic response. The results of the present cortical study carried out in the presence of lithium and the preceding Fisher study involving a different anatomical locus suggest that cortical muscarinic receptors, which are innervated by the cholinergic input and linked to inositol metabolism, are postsynaptic.

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