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EXCITOTOXIC LESIONS OF RAT BASAL FOREBRAIN: DIFFERENTIAL EFFECTS ON CHOLINE ACETYLTRANSFERASE IN THE CORTEX AND AMYGDALA

R. J. BOEGMAN,*† J. COCKHILL,* K. JHAMANDAS* and R. J. BENINGER‡

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

Abstract—Previous studies have shown that basal forebrain lesions using different excitotoxins produce similar decreases in cortical choline acetyltransferase, but differential effects on memory. However, basal forebrain cholinergic neurons send efferents to the amygdala and cortex. The present studies compared the effects of several excitotoxins on choline acetyltransferase levels in both of these structures. Lesions of the basal forebrain were made in rats by infusing different doses of either α-amine-3-hydroxy-5-methyl-4-isoxazole propionic acid, ibotenic acid, quisqualic acid, quinolinic acid or *N*-methyl-D-aspartic acid and measuring choline acetyltransferase seven days later. All of the excitotoxins exerted a differential response on cholinergic neurons of the basal forebrain projecting to the cortex or amygdala. Quinolinic acid was a more potent neurotoxin to cholinergic neurons innervating the amygdala than those projecting to the cortex. In contrast, quisqualic acid and α-amine-3-hydroxy-5-methyl-4-isoxazole were more potent neurotoxins to the cortical projection. α-Amine-3-hydroxy-5-methyl-4-isozazole propionic acid was the most potent excitotoxin for destroying cholinergic neurons innervating either the cortex or amygdala. A parallel neurotoxic response was obtained in the cortex and amygdala following infusion of ibotenic acid or *N*-methyl-D-aspartic acid with little selectivity for choline acetyltransferase depletion in the cortex or amygdala. Histological analysis of the injection site revealed that acetylcholinesterase-positive neurons were destroyed by the excitotoxins in a dose-dependent manner.

Excitotoxins (ibotenic acid, quinolinic acid, *N*-methyl-D-aspartic acid) that produce the greatest impairments in memory were found to produce the greatest depletion of choline acetyltransferase in the amygdala. These results might suggest that cholinergic neurons projecting to the amygdala play an important role in memory.

Excitotoxic lesions of the magnocellular cholinergic neurons in the nucleus basalis (nbm) have been used extensively in studies to examine the effects of cholinergic deafferentation in neurochemical and behavioural experiments.[6] When infused into the nbm axon sparing neurotoxic glutamate receptor agonists such as kainic, ibotenic, quisqualic or quinolinic acid produce reductions in cortical choline acetyltransferase (ChAT) activity of up to 80%.[1,7,9,12] While these reductions are associated with decreases in performance on memory tasks,[2,7,8,14,22,25–27,30,31] the degree of cortical cholinergic deficit does not appear to correlate directly with the extent of mnemonic impairment observed.[8,22,26,30] Thus, involvement of non-cholinergic projections from nbm to the neocortex has been proposed to explain the discrepancy between the excitotoxin induced cholinergic and mnemonic deficits.

Besides projecting to the cerebral cortex, the cholinergic neurons of the nbm also project to the basolateral amygdaloid nucleus.[5,19] Since it has been shown that animals with electrolytic lesions of the basolateral amygdala display memory deficits,[17,28] it was of interest to determine whether excitotoxic nbm lesions disrupt cholinergic input to the amygdala. Several recent studies have indicated that quisqualic acid (Quis) produces less-severe impairments in memory tasks than ibotenic acid (Ibo) or quinolinic acid (Quin), in spite of similar depletions in cortical ChAT.[8,22,26,30] Thus, a second aim of this study was to determine whether excitotoxic lesions of the nbm differentially alter cortical and basolateral amygdaloid cholinergic innervation. Some of our observations have been published in abstract form.[3]

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats, obtained from Charles River Canada, weighing between 300 and 350 g were group housed under temperature- and light-controled conditions with free access to food and water.

Surgical procedures

Animals were anesthetized with 4% halothane and then maintained during surgery with 2% halothane. Lesions intended to destroy the cholinergic neurons of the nbm were placed in the ventral globus pallidus–substantia innominata

†To whom correspondence should be addressed.

Abbreviations: AChE, acetylcholine esterase; AMPA, α-amino-3-hydroxy-4-isoxazole propionic acid; ChAT, choline acetyltransferase; Ibo, ibotenic acid; nbm, nucleus basalis magnocellularis; NMDA, *N*-methyl-D-aspartic acid; Quis, quisqualic acid; Quin, quinolinic acid.

region by inserting into the brain a cannula (0.31 mm diameter) at the following coordinates: 0.8 mm posterior to Bregma, 2.0 mm lateral and 8.0 mm ventral to the surface of the skull with the incisor bar set at −3.3 mm.[23] A volume of 0.5 μl of α-amino-3-hydroxy-4-isoxazole propionic acid (AMPA), Quis, Ibo, *N*-methyl-D-aspartic acid (NMDA) or Quin made up in 0.9% saline and adjusted to pH 7.0 with sodium hydroxide, was infused over 2.25 min. After completing the infusion, the canula was left in place for an additional 2 min before it was slowly withdrawn and the wound closed with sutures.

Neurochemical assessment of lesions

Seven days after the lesion animals were decapitated and the brains rapidly rinsed in ice-cold saline and placed on an ice-cold glass plate.

Samples of the frontal and paricetal cortex were obtained from a coronal tissue slice taken 3 mm caudal to the posterior aspect of the optic chiasm. The basolateral amygdala was prepared from a 2-mm-thick coronal slice cut caudal to the optic chiasm by a freehand dissection of the tissue lateral to the optic tract, medial to the extension of the corpus callosum, ventral to the rhinal fissure and dorsal to the piriform cortex. The tissue was immediately homogenized in ChAT homogenizing buffer[13] before being stored at −70°C. ChAT assays were carried out within a week using the procedure described by Fonnum.[13] All neurochemical results were expressed per mg protein[21] and calculated as specific enzyme activity (units/mg protein per h). The values for the lesion side were calculated as a percentage reduction compared to the intact side. For each excitotoxin a two-way mixed design analysis of variance (ANOVA), with the variables being structure (amygdala and cortex) and dose, was conducted. When a significant interaction was observed tests of simple main effects of structure at each dose were carried out.

Histology

Animals used for morphological analysis were pretreated with the irreversible acetylcholine esterase (AChE) inhibitor, di-isopropyl flurophosphate, 5 h before being killed in order to facilitate the selective staining of AChE in neurons.[4] The rats were killed under deep ketamine anesthesia by perfusion through the ascending aorta with 100 ml cold 0.9% saline followed by 500 ml cold 4% paraformaldehyde in 5 mM sodium phosphate buffer pH 7.4. After fixation the brain was removed and postfixed for 24 h at 6°C and then cryoprotected in 30% sucrose Sodium phosphate buffer (0.1 M) pH 7.4 for 24 h. Thin sections were cut and stained for AChE according to the procedure of Karnovsky and Roots.[16]

RESULTS

Choline acetyltransferase activity

The effect of different doses of Quin (10–180 nmol), Quis (15–180 nmol), AMPA (0.5–25 nmol), Ibo (5–50 nmol) or NMDA (15–120 nmol) when infused into the nbm on ChAT activity in the cortex and amygdala seven days post injection are shown in Figs 1–5, respectively. When saline ($n = 10$) was injected a 15 ± 3.6% decrease in ChAT activity was observed. Quin was significantly more toxic to the cholinergic neurons projecting to the amygdala than those projecting to the cortex, as reflected in a leftward shift of the dose–response curve (Fig. 1). At a Quin dose of 120 nmol a 60% reduction in ChAT activity in both the cortex and amygdala was detected. At doses of 20–80 nmol, Quin was less toxic to the cortical cholinergic projection. This was supported by the observation of a significant structure by dose interaction in the ANOVA [$F(4,34) = 4.78$, $P < 0.01$]. Tests of simple main effects revealed that depletions of ChAT in the amygdala were greater following doses of 20, 40 and 80 nmol.

In marked contrast to the dose–response curve of Quin, injection of Quis into the nbm was significantly less toxic to the cholinergic projection to the amygdala (Fig. 2). A Quis dose of 60 nmol which produced approximately a 20% reduction in ChAT activity in the amygdala gave a maximal reduction of 75% in cortical ChAT activity. AMPA, an agonist which is more selective than Quis for AMPA-receptors and which does not act on the metabotropic receptor, essentially gave similar results to that obtained with Quis; i.e. cortically projecting cholinergic neurons were more sensitive than those projecting to the amygdala (Fig. 3). AMPA, however, was significantly more potent in destroying basal forebrain cholinergic neurons than either Quis or Quin as indicated by a significant shift in the dose–response curves to the left. For both Quis and AMPA the interactions in the ANOVA were significant [$F(3,20) = 8.90$, $P < 0.01$ and $F(4,23) = 28.67$, $P < 0.01$, respectively]. Tests of simple main effects showed that Quis doses of 30, 60, 120 and 180 and AMPA doses of 1 and 5 nmol produced greater depletions of ChAT in the amygdala.

The dose–response curves for Ibo and NMDA are shown in Figs 4 and 5. Neither Ibo nor NMDA appeared to be selectively toxic to only one population of basal forebrain cholinergic neurons with

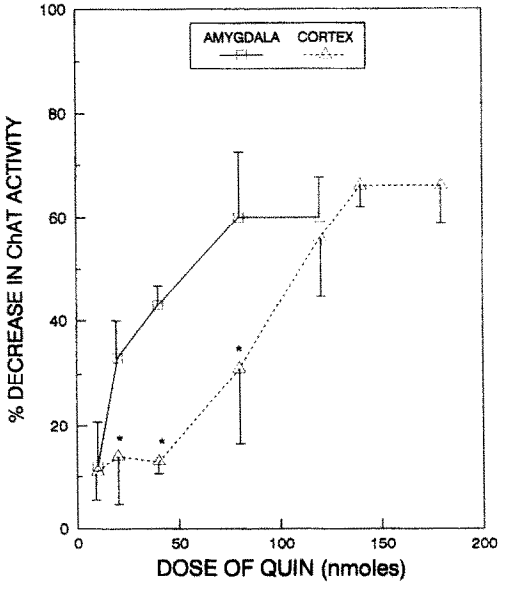

Fig. 1. The dose–response depletion of ChAT activity in the cortex and amygdala following an infusion of different doses of Quin into the nbm of adult rats. Each point represents the mean ± S.D. of $n = 4$–12 animals; $^*P < 0.05$, Student's *t*-test.

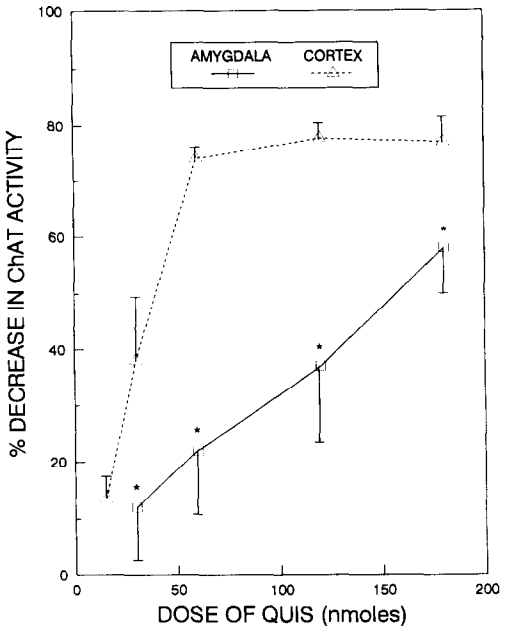

Fig. 2. The dose–response depletion of ChAT activity in the cortex and amygdala following an infusion of different doses of Quis into the nbm of adult rats. Each point represents the mean ± S.D. of $n = 4$–8 animals; $^*P < 0.05$, Student's t-test.

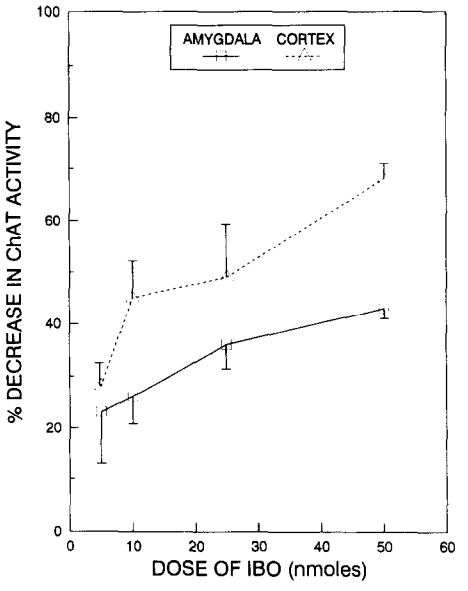

Fig. 4. The dose–response depletion of ChAT activity in the cortex and amygdala following an infusion of different doses of Ibo into the nbm of adult rats. Each point represents the mean ± S.D. of $n = 3$–6 animals. There was a main effect of structure in the ANOVA indicating that the effect of Ibo was greater in the cortex than amygdala.

projections to the cortex and amygdala responding in parallel to these neurotoxins. In each case however, the projection to the amygdala was apparently less responsive to the excitotoxin. ANOVA revealed a small but significant interaction [$F(3,20) = 3.04$, $P = 0.05$] for the NMDA experiment and no interaction for the Ibo experiment. In both cases the main effect of structure was significant indicating that these two toxins had a significantly greater effect on the cortex than amygdala.

The apparent ED_{50} value for the neurotoxins used in this study on the cholinergic projection to the amygdala or cortex is shown in Table 1. If destruction of all nbm cholinergic neurons resulted in an 80% reduction in ChAT activity of the cortex and amygdala then the apparent ED_{50} for the toxins will be

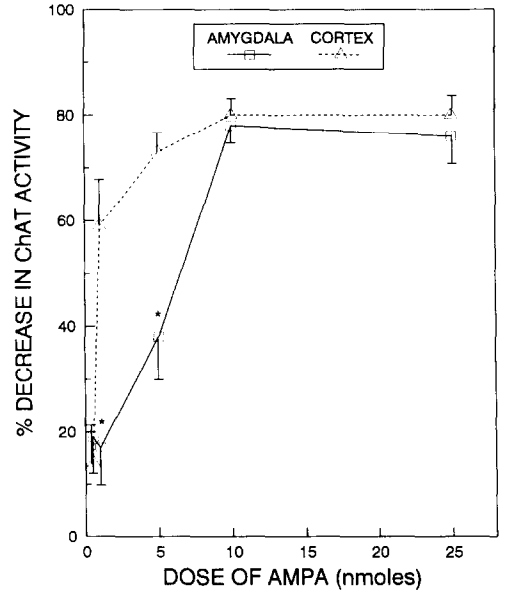

Fig. 3. The dose–response depletion of ChAT activity in the cortex and amygdala following an infusion of different doses of AMPA into the nbm of adult rats. Each point represents the mean ± S.D. of $n = 4$–8 animals; $^*P < 0.05$, Student's t-test.

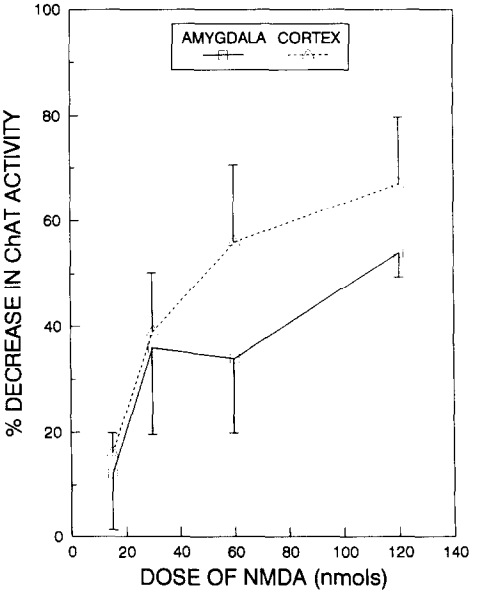

Fig. 5. The dose–response depletion of ChAT activity in the cortex and amygdala following an infusion of different doses of NMDA into the nbm of adult rats. Each point represents the mean ± S.D. of $n = 6$ animals.

Table 1. Calculated ED_{50} values for excitotoxins injected into the nucleus basalis magnocellularis on the cholinergic projection to the cortex and amygdala

Excitotoxin	Cortex (ED_{50} M)	Amygdala (ED_{50} M)
Quin	0.0900	0.0424
Quis	0.0335	0.1276
AMPA	0.0009	0.0046
Ibo	0.0123	0.0405
NMDA	0.0458	0.0745

the dose producing a 40% depletion. These values were calculated from linear regression analysis of the dose–response curves.

Of all the toxins used, Quin was unique because the cholinergic projection to the amygdala responded to lower doses of the agonist than the cholinergic projection to the cortex. While the other toxins were more selective to the cortical cholinergic projection there was a differential response among the toxins with AMPA being the most selective, followed by Quis, Ibo and NMDA.

Histology

The injection tract could be visualized in AChE-stained sections and was in all cases close to the large AChE-positive neurons of the nbm. At doses of Quin or Quis, which produced a partial lesion to either the cortex or amygdala, some AChE-positive neurons located close to the injection site were spared (Fig. 6). In contrast doses of either toxin which destroyed both projections to the cortex and amygdala resulted in a complete loss of neurons throughout the injection area (Fig. 6).

DISCUSSION

A large number of studies have demonstrated that infusions of excitotoxins, which interact with excitatory amino acid receptors, into the nbm of the rat produce a depletion of cholinergic neurons projecting to the frontoparietal cortex.[1,9,12,18,20] The present results, showing a reduction in cortical ChAT following various excitoxins, are consistent with these findings. The ChAT-positive neurons in the nbm also give rise to a dense cholinergic projection to the amygdala, but the actions of excitotoxins on this projection have received little attention. Since the amygdala plays an important role in memory, it is important to determine the extent to which excitotoxin infusions into the nbm influence cholinergic input into this area and if significant differences exist with regard to the sensitivity of cortical and amygdaloid projections to excitotoxins acting on different EAA receptor subtypes. The present study addressed this question by evaluating the effects of five excitotoxins (AMPA, Quis, Ibo, NMDA and Quin) on the activity of ChAT, a biochemical marker for cholinergic neurons, in the frontoparietal cortex and amygdala seven days following a single unilateral injection into the nbm. The results showed that excitotoxins exerted a differential action on cholinergic neurons in these two brain areas. The apparent potency of excitotoxins, that activate EAA receptors in the nbm, on ChAT, in the cortex differed from that seen in the amygdala.

In a previous study, Dunnet *et al.*[8] demonstrated differences between the potency of four excitotoxins (kainic acid, Ibo, NMDA and Quis) with regard to their action on the cortex after injection into the nbm. They found that Quis produced less subcortical damage than the other toxins at doses that produced a comparable loss of cortical cholinergic markers. The present study shows that the five excitotoxins used here differ in regard to the potency of their action on cholinergic neurons projecting to the cortex and amygdala. The rank order of apparent potency of AMPA and Quis in the frontoparietal cortex differs from that in the amygdala. In the cortex the order of apparent potency of the toxins employed in this study was AMPA > Ibo > Quis > NMDA > Quin, while in the amygdala this rank order was AMPA > Ibo = Quin > NMDA > QUIS. Examination of the dose–response curves for AMPA and QUIS revealed two significant differences: the dose–response curves for the cortex showed a remarkably steep rise, and at lower doses both AMPA (less than 5 nmol) and QUIS (less than 60 nmol) produced a much greater reduction in cortical ChAT than in the amygdala. These findings are indicative of greater sensitivity of the cortically projecting cholinergic neurons to these two excitotoxins. In contrast, Quin appeared to produce reductions in ChAT in both areas. Both NMDA and Ibo, which act on the NMDA receptor, also produced similar reductions in ChAT in the two areas, although the dose–response curves for these two agonists were shallower than those for other toxins. Thus in the present study, certain doses of toxins activating AMPA receptors produce a significant cholinergic deficit in the cortex while relatively sparing the cholinergic neurons to the amygdala. In contrast, toxins which activate NMDA receptors produce deficits of comparable magnitude in the two areas.

The differential actions of excitotoxins activating AMPA and NMDA receptors on the two cholinergic projections may have implications for the involvement of nbm cholinergic neurons in learning and memory. Several studies have demonstrated that excitotoxin injection in the nbm produces impairments of memory which have been attributed to the loss of cholinergic neurons in the frontoparietal cortex.[1,2,7,8,14,22,25–27,30,31] However, recent studies have shown that the depletion of cholinergic markers in the cortex does not correlate with behavioural deficits following excitotoxin injection in the nbm.[2,8,22,26,30] Quis injection into the nbm, while producing a greater decrease in cortical ChAT than similar Ibo injections, produced a lesser impairment of memory. Similarly, AMPA- and Ibo-induced deficits in cortical ChAT have been found to exert differential actions on acquisition and retention. Thus, these studies have

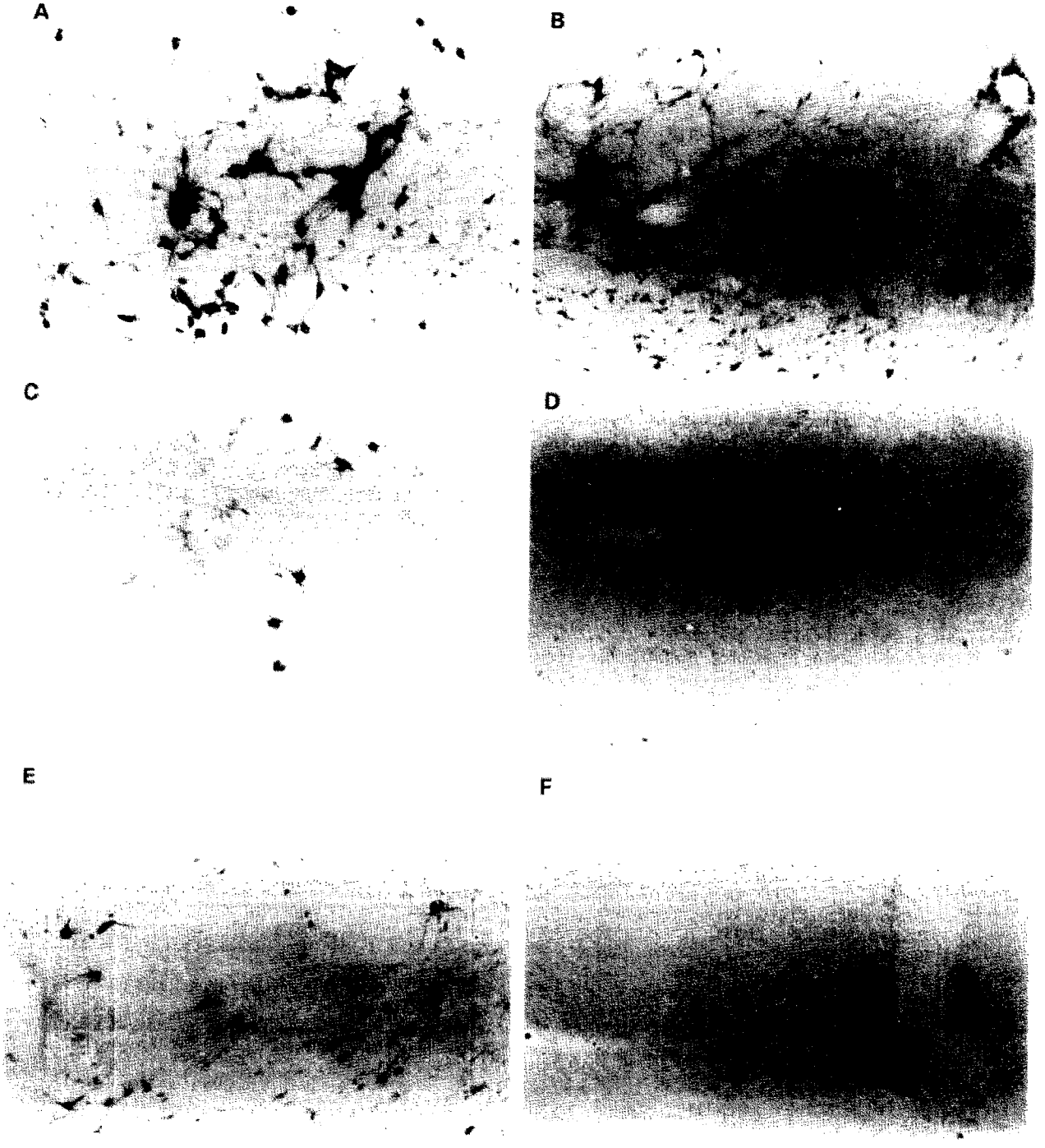

Fig. 6. Photomicrographs of AChE-positive cells in the nbm of saline (A), 60 nmol Quis (B), 40 nmol Quin (C), 120 nmol Quin (D), 1 nmol AMPA (E) and 10 nmol AMPA (F) lesioned rats. High doses of Quin and AMPA destroy all AChE-positive cells while some cells survive exposure to lower doses.

argued that cholinergic neurons in the nbm may not be involved in mechanisms subserving memory and have proposed the possible involvement of unidentified, non-cholinergic projections in the mnemonic deficits induced by the excitotoxins. Although possible damage to nbm GABA neurons has been proposed as a potential mechanism,[24] only in the cat is there evidence that these neurons project to the cortex.[11] In addition EAA-induced lesions of the nbm show no depletions of GABA markers in the cortex.[15] We have found that injections of the GABA agonist muscimol into the nbm produce mnemonic deficits (in preparation). Perhaps this effect occurs through suppression of cholinergic pathways, as these injections depress cholinergic activity in the cortex.[29]

The results of the present study would suggest that the discrepancy between cortical cholinergic and mnemonic deficits reported by others may arise from differences in the sensitivity to excitotoxins of cortical and amygdaloid projections from the nbm. At the doses of Quis (up to 120 nmol/μl) or AMPA (4.5 nmol/0.3 μl) that have been employed in previous behavioural studies,[8,24,26] there may have been a large depletion of cortical ChAT, but only a modest depletion of amygdaloid ChAT. In contrast, doses of Ibo (approximately 27 nmol) used previously[8,24,26] may have produced comparable cholinergic deficits in the two regions. Since the amygdala is important in memory,[17,28] our findings would suggest that the relative sparing of the amygdaloid projection by Quis may contribute to its lesser effect on memory. Conversely, the greater impairment of these tasks by Ibo may occur because it damages both projections and this combined effect outweighs the apparently greater depletion of cortical ChAT by Quis injections. Indeed we have recently observed that Ibo lesions producing greater depletions of amygdaloid ChAT than Quis lesions led to greater deficits in memory in a double-Y maze task (in preparation).

The basis for the differential sensitivity of the two nbm cholinergic projections to excitotoxins interacting with AMPA and NMDA receptors is unknown. Possible differences in the distribution or affinity of the receptors associated with these projections may contribute to the observed differences in sensitivity. The cortical and amygdaloid projecting cholinergic neurons appear to be of distinct origin.[5,10] Anatomical evidence shows that the projection to the amygdala originates from ChAT-positive neurons that overlap with the subset of cholinergic neurons projecting cortically. The reduced sensitivity of these neurons to AMPA or Quis may reflect lower affinity or density of AMPA receptors on the amygdaloid projecting neurons. An alternate explanation for this reduced sensitivity is that the modest depletions of ChAT produced by lower dose ranges of AMPA and Quis reflect their action on cortically projecting neurons that may send collateral projections to the amygdala. The existence of such projections remains unknown, and thus the most parsimonious explanation for reduced sensitivity is a relative lack of AMPA receptors or their reduced sensitivity on cholinergic neurons projecting to the amygdala.

CONCLUSIONS

The present study suggests that populations of forebrain cholinergic neurons differ in regard to their sensitivity to excitotoxins acting on different EAA receptor subtypes. They further suggest that in functional studies in intact animals, careful consideration should be given to the specific excitotoxin and the dose that is infused focally to induce neurochemical deficits.

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