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Effects of tetrahydroaminoacridine and nicotine in nucleus basalis and serotonin-lesioned rats

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Abstract

The present study was designed to investigate the hypothesis that concurrent degeneration of serotonin and acetylcholine cells may decrease the therapeutic effects of cholinergic drugs on cognitive functioning in Alzheimer dementia. Therefore, we compared the effecs of pretraining injections of a cholinesterase inhibitor, tetrahydroaminoacridine (1, 3 and 5 mg/kg i.p.), and nicotine (0.03, 0.1 and 0.3 mg/kg i.p.) on spatial navigation (water maze) and passive avoidance in nucleus basalis- and nucleus basalis + *p*-chlorophenylalanine-lesioned rats. Nicotine (0.1 and 0.3 mg/kg) promoted passive avoidance performance of nucleus basalis-lesioned rats, but nicotine did not improve performance of combined-lesioned rats. Tetrahydroaminoacridine (3 mg/kg) facilitated passive avoidance performance of nucleus basalis- and combined-lesioned rats. However, tetrahydroaminoacridine-treated nucleus basalis + *p*-chlorophenylalanine-lesioned rats were not performing better than vehicle-treated nucleus basalis-lesioned rats. Spatial navigation of nucleus basalis and nucleus basalis + *p*-chlorophenylalanine-lesioned rats was slightly impaired during the first training day and tetrahydroaminoacridine 3 mg/kg restored the performance of combined-lesioned rats. Combined-lesioned rats performed as well as the controls during the other training days. The present results suggest that, in Alzheimer's disease, combined degeneration of nucleus basalis cholinergic and brainstem serotonergic cells decreases the therapeutic effect of nicotine, but not that of tetrahydroaminoacridine.

Keywords: Tetrahydroaminoacridine; Nicotine; Nucleus basalis lesion; *p*-Chlorophenylalanine treatment; Alzheimer's disease

1. Introduction

The cholinergic cells of the nucleus basalis innervate basolateral amygdaloid area, reticular thalamus and dorsolateral frontal cortex (Mesulam, 1991). The clinical studies describing that the loss of cholinergic cells in the basal forebrain during Alzheimer's disease may to some extent be responsible for the decline in cognitive functioning kindled interest in the behavioral functions of cholinergic cells (Bowen et al., 1983). The result of a drug trial that showed a modest but significant alleviation of Alzheimer's dementia by the use of a cholinesterase inhibitor, tetrahydroaminoacridine, also supports the importance of cholinergic cell loss in the development of clinical symptoms in Alzheimer's disease (Eagger et al., 1991).

Recent experimental studies have elucidated the role of nucleus basalis cholinergic cells in the regulation of avoidance behavior, spatial memory, and attention (Dunnett et al., 1991). A logical approach to investigate a deficit in nucleus basalis cholinergic cells occurring during aging and Alzheimer's disease has been to lesion the basal forebrain cholinergic system, or its target areas in animals by infusing excitatory amino acids intracerebrally. Several studies have compared the neurochemical, neuroanatomical and behavioral consequences of nucleus basalis lesions induced by different amino acids (for a review see: Dunnett et al., 1991). The infusion of neurotoxins such as quisqualate or α-amino-3-hydroxy-5-methyl-4-isoaxole propionic acid into the nucleus basalis region decreases cortical choline acetyltransferase activity more severely than do ibotenic acid infusions, indicating that quisqualate and α-amino-3-hydroxy-5-methyl-4-isoaxole propionic acid infusions deplete cholinergic cells in the nucleus basalis more effectively than ibotenic acid treatment (Dunnett et al., 1987; Riekkinen Jr. et al., 1990a; Page et al., 1991). On the contrary,

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quisqualate and α-amino-3-hydroxy-5-methyl-4-isoaxole propionic acid nucleus basalis lesions produced less non-specific neuronal damage in adjacent non-cholinergic nuclei than ibotenic acid lesions (Dunnett et al., 1987; Page et al., 1991). Anatomically restricted and neurochemically effective nucleus basalis quisqualate or α-amino-3-hydroxy-5-methyl-4-isoaxole propionic acid lesions do not affect spatial navigation in the water maze test, but ibotenic acid nucleus basalis lesions greatly impair performance in the water maze test (Dunnett et al., 1987; Page et al., 1991). Therefore, it is likely that the spatial navigation defect following ibotenic acid nucleus basalis lesioning is not related to the loss of nucleus basalis cholinergic cells, but is caused by non-specific cell death in adjacent non-cholinergic nuclei. Interestingly, ibotenic acid, quisqualate and α-amino-3-hydroxy-5-methyl-4-isoaxole propionic acid nucleus basalis lesions all produce a marked performance defect in passive avoidance performance and attentional functioning (Dunnett et al., 1987; Robbins et al., 1989; Page et al., 1991; Riekkinen Jr. et al., 1993a,b). Therefore, Robbins et al. (1989) proposed that nucleus basalis cholinergic cells are important for attentional functions and may also regulate passive avoidance memory.

Neuropathological studies have shown that the brainstem serotonergic raphe nuclei also degenerate in Alzheimer's disease (Bowen et al., 1983). Therefore, it is possible that the concurrent degeneration of cholinergic cells of the basal forebrain and the serotonergic cells of the brainstem is to some extent responsible for the development of severe dementia and limits the therapeutic effects of cholinergic drugs to alleviate the cognitive defect in Alzheimer's disease. Recent studies have elucidated the behavioral and pharmacological interaction between the cholinergic and brainstem serotonergic systems in the regulation of cognitive functioning (Vanderwolf, 1987; Nilsson et al., 1988; Riekkinen Jr. et al., 1990a; Jäkälä et al., 1992; Riekkinen and Riekkinen Jr., 1994; Sirviö et al., 1994). Firstly, serotonin depletion induced by *p*-chlorophenylalanine or 5,7-dihydroxytryptamine treatment has no effect on water maze or passive avoidance behavior, but a combination of muscarinic or nicotinic acetylcholine receptor antagonists and *p*-chlorophenylalanine produced a more severe spatial navigation and avoidance defect than treatment with cholinergic antagonists alone (Riekkinen et al., 1993). Secondly, we observed that cholinesterase inhibitors (tetrahydroaminoacridine and physostigmine) and a nicotinic acetylcholine receptor agonist (nicotine) did not completely alleviate water maze spatial navigation or the passive avoidance defect induced by muscarinic or nicotinic acetylcholine receptor antagonists in *p*-chlorophenylalanine-treated rats (Riekkinen et al., 1993; Riekkinen Jr. et al., 1994). Therefore, it is possible that a combined cholinergic and serotonergic pathology may be at least partly responsible for the cognitive decline and ineffectiveness of cholinergic replacement therapies to alleviate cognitive dysfunctioning in Alzheimer's disease (Bowen et al., 1983).

The earlier studies investigating pharmacological consequences of single and combined cholinergic and serotonergic blocks have examined the behavioral effects of systemically injected acetylcholine receptor antagonists that indiscriminately block the activity of all the central cholinergic systems (Riekkinen et al., 1993; Riekkinen and Riekkinen Jr., 1994). Therefore, it is difficult to suggest that the cholinergic cells of the basal forebrain interact with the serotonergic cells to regulate avoidance and spatial behavior. This issue is important as in Alzheimer's disease the cholinergic cells of the basal forebrain degenerate severely, whereas striatal and brainstem cholinergic activities are relatively intact (Bowen et al., 1983).

The present study was designed to investigate the hypothesis that, in Alzheimer's disease, the concurrent degeneration of cholinergic cells of the nucleus basalis and the serotonergic system decreases the therapeutic effects of cholinesterase inhibitors and nicotinic acetylcholine receptor agonists. Therefore, we studied the effects of single and combined nucleus basalis and *p*-chlorophenylalanine lesions on passive avoidance and water maze behavior, and we tested whether the therapeutic effect of nicotine and tetrahydroaminoacridine is decreased following a combined lesion.

2. Material and methods

2.1. Animals

Male Han:Wistar rats were used in the present study (300–360 g). The rats were housed singly with food and water ad libitum. Room temperature was +22° C, humidity was 50–60% with a light period of 14 h (lights on from 7.00 to 21.00 h). We had the permission of the local ethical committee to perform this study.

2.2. Drugs

Tetrahydroaminoacridine (1, 3 and 5 mg/kg, 60 min pretesting, Sigma, i.p. 2 ml/kg) and nicotine ((±)-nicotine hydrogen tartrate) (0.03, 0.1 and 0.3 mg/kg, 25 min pretesting, Sigma, s.c. 1 ml/kg) were dissolved in physiological saline (NaCl 0.9%).

2.3. Lesioning

The rats were anesthetized carefully during surgery using chloral hydrate (325 mg/kg i.p.) and were placed in a stereotaxic frame with bregma and lambda in the same horizontal plane. Nucleus basalis (AP = 0.8 mm,

DV = −7.8 mm, ML = 2.9 mm, relative to the bregma) lesions were made by infusing for 5 min bilaterally quisqualate (0.8 M in phosphate-buffered saline, 0.8 μl; Sigma) using a minipump that accurately controls the speed of infusion (Riekkinen Jr. et al., 1993a). For control purposes vehicle solution was infused into the nucleus basalis. *p*-Chlorophenylalanine (Sigma) treatment (400 mg/kg i.p., three consecutive days, 4 ml/kg) has been shown to deplete 80% of cortical and hippocampal serotonin (Riekkinen and Riekkinen Jr., 1994). *p*-Chlorophenylalanine was mixed in saline containing a 0.5% suspension of gum arabic (mixed fresh every day). For control purposes, gum arabic was mixed in saline and injected i.p. (4 ml/kg). We started behavioral testing 10 days after nucleus basalis lesioning and 7 days after *p*-chlorophenylalanine treatment. A day before nucleus basalis lesions, *p*-chlorophenylalanine treatment was started for the combined lesioned rats. Behavioral testing of combined lesioned rats was started 7 days after the *p*-chlorophenylalanine treatment.

2.4. Behavioral testing

A previous report described the water maze system and the training details (Riekkinen Jr. et al., 1990b). The swim paths of the rats in the circular pool were monitored by a videocamera linked to a computer through an image analyser. The computer calculated the total swim distance. A submerged platform was placed into the same quadrant during every training trial and five consecutive (3 × 70 s trials in a day) training days were assessed. The rats were allowed to stay 5 s on the platform after completion of the swimming trial. A 30-s recovery period was allowed for the rats before the next daily training trial. We used path length as a measure of acquisition performance, as it is not confounded by changes of swim speed.

The Plexiglas passive avoidance box had two compartments (dark and bright) divided by a sliding guillotine door. The dark compartment had a metal grid floor. The experimenter placed the rats on the bright side of the passive avoidance box and opened the door after 30 s (training trial). The latency to enter (s) was measured. Five seconds after the entry into the dark chamber a shock of 1.0 mA was initiated. The experimenter again placed the rats on the bright side 24 h later and after a 30-s adaptation interval the guillotine door was opened (retention trial; maximum retention 360 s). The latency to re-entry was measured (retention latency, the higher entry latency, the better retention performance).

2.5. Dissection and biochemistry

The rats were decapitated rapidly. We measured the levels of serotonin, 5-hydroxyindoleacetic acid, noradrenaline and dopamine (Jäkälä et al., 1992; Riekkinen and Riekkinen Jr., 1994) and choline acetyltransferase activity (Fonnum, 1975) in dorsolateral frontal cortex of all the single or combined lesioned rats. In earlier publications we have described the biochemical methods in detail (Jäkälä et al., 1992). Coronal forebrain slices containing the lesioned nucleus basalis area were taken for histological analysis. We stained the coronal slices with Cresyl violet to study the size and site of lesions (Riekkinen Jr. et al., 1993a).

2.6. Statistical analysis

The one-way analysis of variance (ANOVA) followed by Duncan's post-hoc multiple group comparison was used in the analysis of biochemical and water maze behavioral parameters. The Wilcoxon signed ranks test was used to analyse passive avoidance data. $P < 0.05$ was accepted as significant.

3. Results

3.1. Passive avoidance

Nucleus basalis lesions

Fig. 1 shows the effects of nucleus basalis lesioning and tetrahydroaminoacridine and nicotine treatment on passive avoidance. Nucleus basalis-lesioned rats were impaired during the passive avoidance retention trial (overall group effect: $F(7,72) = 33.1$, $P < 0.05$). In

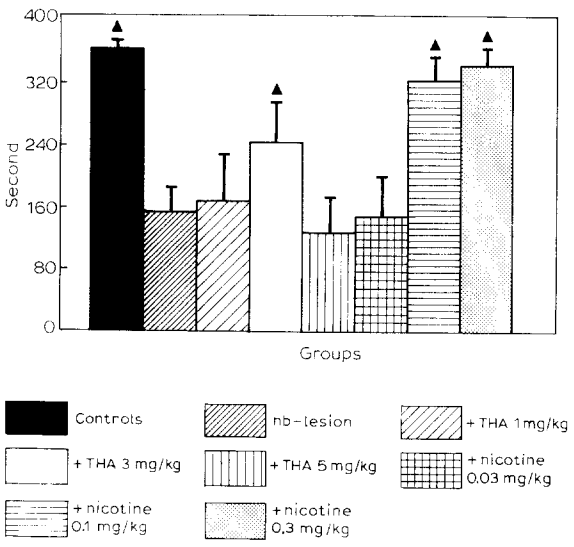

Fig. 1. The effects of tetrahydroaminoacridine (THA) and nicotine treatments on passive avoidance testing trial latencies (s) of quisqualic acid nucleus basalis-lesioned rats. THA (1, 3 and 5 mg/kg) was injected i.p. 60 min before the training trial. Nicotine (0.03, 0.1 and 0.3 mg/kg) was injected s.c. 25 min before the training trial. Note that nicotine and THA promote passive avoidance testing trial performance of nucleus basalis-lesioned rats, and that nicotine was more effective than THA to facilitate passive avoidance responding. ▲ $P < 0.05$ vs. nucleus basalis-lesioned rats (Wilcoxon signed ranks test). Values (s, *y*-axis) represent means ± S.D.

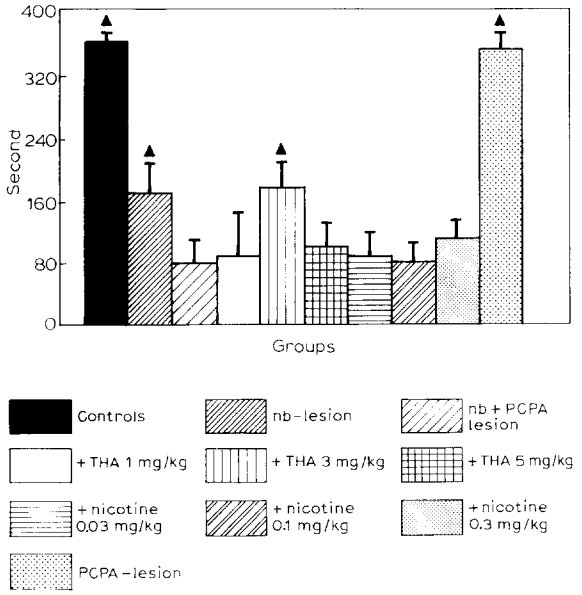

Fig. 2. The effects of tetrahydroaminoacridine (THA) and nicotine treatments on passive avoidance testing trial latencies (s) of combined quisqualic acid nucleus basalis + *p*-chlorophenylalanine (PCPA)-lesioned rats. THA (1, 3 and 5 mg/kg) was injected i.p. 60 min before the training trial. Nicotine (0.03, 0.1 and 0.3 mg/kg) was injected s.c. 25 min before the training trial. Note that *p*-chlorophenylalanine treatment aggravated nucleus basalis lesioning-induced passive avoidance failure and blocked completely the therapeutic effect of nicotine (Table 1, part B), respectively, on passive avoidance behavior. THA facilitated passive avoidance performance of combined-lesioned rats, but THA-treated combined-lesioned rats were not performing any better than vehicle-treated nucleus basalis-lesioned rats. ▲ $P < 0.05$ vs. nucleus basalis + *p*-chlorophenylalanine-lesioned rats (Wilcoxon signed ranks test). Values (s, *y*-axis) represent means ± S.D.

nucleus basalis-lesioned rats, tetrahydroaminoacridine 3 mg/kg to some extent improved the passive avoidance retention trial performance ($P < 0.05$), but the other doses tested (1 and 5 mg/kg) were ineffective ($P > 0.05$). Nicotine 0.1 and 0.3 mg/kg significantly improved the performance of nucleus basalis-lesioned rats ($P < 0.05$) and these two group of lesioned rats were not impaired compared with the controls ($P > 0.05$). The smallest dose (0.03 mg/kg) of nicotine did not facilitate passive avoidance behavior ($P > 0.05$). Nicotine 0.1 and 0.3 mg/kg-treated rats performed significantly better than tetrahydroaminoacridine (1, 3 or 5 mg/kg)-treated nucleus basalis-lesioned rats ($P < 0.05$).

Nucleus basalis + p-chlorophenylalanine lesions

Fig. 2 shows the effects of single and combined nucleus basalis and *p*-chlorophenylalanine lesions. The overall analysis revealed marked group differences ($F(8,81) = 55.1$, $P < 0.05$). We found that nucleus basalis lesions impaired passive avoidance behavior ($P < 0.05$) and that *p*-chlorophenylalanine treatment aggravated the passive avoidance defect of nucleus basalis-lesioned rats ($P < 0.05$).

Tetrahydroaminoacridine 3 mg/kg improved passive avoidance retention of nucleus basalis + *p*-chlorophenylalanine-lesioned rats ($P < 0.05$). Other doses of tetrahydroaminoacridine (1 and 5 mg/kg) did not alleviate the combined lesion-induced passive avoidance failure ($P < 0.05$). Tetrahydroaminoacridine 3 mg/kg-treated combined lesioned rats were not performing better than control-treated single nucleus basalis-lesioned rats ($P > 0.05$). Nicotine (0.03, 0.1 and 0.3 mg/kg) did not significantly improve the passive avoidance retention of nucleus basalis + *p*-chlorophenylalanine-lesioned rats ($P > 0.05$).

3.2. Water maze

Nucleus basalis lesions

Table 1A shows the water maze navigation performance of control and nucleus basalis-lesioned rats. Tetrahydroaminoacridine at 5 mg/kg produced a severe swimming defect and for this reason the effect of treatment with the highest dose was not tested in the water maze.

No significant overall group effect was observed in the analysis of water maze escape distance values mea-

Table 1
Effects of tetrahydroaminoacridine (1 and 3 mg/kg i.p.; 60 min before daily training) and nicotine (0.03, 0.1 and 0.3 mg/kg s.c.; 25 min before daily training) on water maze navigation performance of nucleus basalis- and nucleus basalis + *p*-chlorophenylalanine-lesioned rats

Effects of tetrahydroaminoacridine and nicotine on spatial navigation									
Treatment (mg/kg)	C vehicle	NB vehicle	P vehicle	NB + P vehicle	THA 1	THA 3	Nic .03	Nic .1	Nic .3
Part A: Nucleus basalis-lesioned rats									
Distance (cm)	612 ± 100	601 ± 70			634 ± 91	579 ± 67	588 ± 10	622 ± 70	620 ± 100
Part B: Nucleus basalis + p-chlorophenylalanine-lesioned rats									
Distance (cm)	581 + 91	571 + 76	601 + 77	619 + 83	600 + 93	587 + 73	570 + 110	612 + 79	627 + 66

Tetrahydroaminoacridine and nicotine did not facilitate spatial navigation of lesioned rats. The mean path lengths of single or combined lesioned rats measured during a 5-day training period were not increased compared with the controls. Distance = mean distance value of 5 training days; treatments are expressed as mg/kg and the treatment group abbreviations are as follows: C = control-lesioned (vehicle-treated); NB = nucleus basalis-lesioned (vehicle-treated); P = *p*-chlorophenylalanine-lesioned (vehicle-treated); NB + P = nucleus basalis + *p*-chlorophenylalanine-lesioned (vehicle); THA 1 and 3 = tetrahydroaminoacridine 1 and 3 mg/kg; Nic .03, .1 and .3 = nicotine 0.03, 0.1 and 0.3 mg/kg.

Table 2
The effects of quisqualic acid nucleus basalis and *p*-chlorophenylalanine (PCPA) lesioning on frontal cortical choline acetyltransferase activity and monoamine and metabolite levels

	5-HT	5-HIAA	NA	DA	ChAT
Nucleus basalis lesions					
Control	243 ± 34	266 ± 56	198 + 43	247 ± 6	1.1 ± 0.1
NB-l	233 ± 55	276 ± 55	187 ± 44	252 ± 11	0.4 ± 0.1 [a]
+ nicotine 0.03 mg	212 ± 32	256 ± 55	191 ± 34	265 ± 8	0.4 ± 0.1 [a]
+ nicotine 0.1 mg	255 ± 55	266 ± 54	211 ± 33	273 ± 10	0.4 ± 0.2 [a]
+ nicotine 0.3 mg	212 ± 44	256 ± 66	187 ± 44	240 ± 6	0.4 ± 0.1 [a]
+ THA 1 mg	211 ± 33	244 ± 55	180 ± 33	255 ± 7	0.5 ± 0.2 [a]
+ THA 3 mg	245 ± 55	277 + 66	201 ± 23	260 ± 6	0.4 ± 0.1 [a]
+ THA 5 mg	223 ± 33	267 ± 55	221 ± 65	276 ± 5	0.4 ± 0.1 [a]
Nucleus basalis + p-chlorophenylalanine lesions					
Control	255 ± 55	245 ± 55	212 ± 56	267 ± 7	1.1 ± 0.2
PCPA	38 ± 9 [a]	18 ± 7 [a]	170 ± 55	301 ± 69	1.1 + 0.1
PCPA + NB-l	41 ± 8 [a]	16 ± 11 [a]	198 ± 55	270 ± 42	0.4 ± 0.1 [a]
+ nicotine 0.03 mg	42 ± 7 [a]	20 ± 6 [a]	170 ± 66	271 ± 55	0.4 ± 0.1 [a]
+ nicotine 0.1 mg	39 ± 5 [a]	21 ± 4 [a]	173 ± 55	260 ± 51	0.4 ± 0.1 [a]
+ nicotine 0.3 mg	44 ± 6 [a]	18 ± 6 [a]	180 ± 44	245 ± 44	0.5 ± 0.1 [a]
+ THA 1 mg	37 ± 4 [a]	16 ± 11 [a]	190 ± 66	249 ± 66	0.4 ± 0.1 [a]
+ THA 3 mg	40 ± 8 [a]	22 ± 12 [a]	187 ± 55	276 ± 66	0.4 ± 0.1 [a]
+ THA 5 mg	38 ± 7 [a]	20 ± 6 [a]	180 ± 66	298 ± 60	0.5 ± 0.1 [a]

5-HT, 5-HIAA, DA and NA levels (ng/g of brain tissue) and ChAT activity (nmol/mg protein/min) are expressed as group means ± S.D. The one-way ANOVA followed by Duncan's post-hoc multiple group comparison was used to analyse neurochemical data. [a] $P < 0.05$ vs. controls. Abbreviations: ChAT = choline acetyltransferase activity; DA = dopamine; 5-HIAA = 5-hydroxyindole acetic acid; 5-HT = 5-hydroxytryptamine, serotonin; NA = noradrenaline; NB-l = nucleus basalis; PCPA = *p*-chlorophenylalanine; THA = tetrahydroaminoacridine.

sured during the five consecutive training days of nucleus basalis-lesioned rats treated with NaCl 0.9%, tetrahydroaminoacridine (1 and 3 mg/kg) or nicotine (0.03, 0.1 and 0.3 mg/kg) (overall effect: $F(7,72) = 1.0$, $P > 0.05$). Furthermore, comparisons of the different study groups showed no marked differences between any of the groups ($P > 0.05$).

Nucleus basalis + p-chlorophenylalanine lesions

Table 1B shows the water maze spatial navigation performance of control-, nucleus basalis- and nucleus basalis + *p*-chlorophenylalanine-lesioned rats. Analysis of path length values measured during the first training day showed that nucleus basalis + *p*-chlorophenylalanine-lesioned rats had longer path length values than the controls or any other group (overall effect: $F(8,81) = 7.1$, $P < 0.05$; $P < 0.05$) (path length on the first day: controls = 1098 cm, nucleus basalis = 1134 cm, nucleus basalis + *p*-chlorophenylalanine = 1398 cm). Tetrahydroaminoacridine 3 mg/kg-treated nucleus basalis + *p*-chlorophenylalanine-lesioned rats did not have increased path length values during the first day of training ($P > 0.05$) (path length on the first day: 978 cm).

The path length values of nucleus basalis + *p*-chlorophenylalanine-lesioned rats measured during the rest of the training period did not differ from the control values (overall effect of days 2, 3, 4 and 5: $F(8,81) < 1.1$, $P > 0.05$; $P > 0.05$ for all post-hoc multiple group comparisons).

3.3. Biochemistry

Table 2 shows the results of biochemical analyses. *p*-Chlorophenylalanine treatment decreased selectively the serotonin and 5-hydroxyindoleacetic acid levels measured from the frontal cortical sample (overall effect: $F(8,81) = 67.1$, $P < 0.05$). The serotonin and 5-hydroxyindoleacetic acid levels of all the rats treated with *p*-chlorophenylalanine were as severely affected ($P > 0.05$). *p*-Chlorophenylalanine treatment decreased the serotonin levels of control and nucleus basalis-lesioned rats as effectively ($P > 0.05$). Dopamine and noradrenaline levels were not affected by single *p*-chlorophenylalanine or combined *p*-chlorophenylalanine + nucleus basalis lesions (overall effect: $F(8,81) < 0.8$, $P > 0.05$; for all comparisons).

All the of nucleus basalis ($F(7,82) = 65.1$, $P < 0.05$) and nucleus basalis + *p*-chlorophenylalanine ($F(8,81) = 70.1$, $P < 0.05$) lesions decreased frontal cortical choline acetyltransferase activity. Cortical choline acetyltransferase activity of nucleus basalis-lesioned and combined *p*-chlorophenylalanine + nucleus basalis-lesioned rats was as severely decreased ($P > 0.05$).

4. Discussion

The novel result of the present study is that combined degeneration of brainstem serotonergic and nu-

cleus basalis cholinergic neurons has important pharmacological consequences. Firstly, serotonin depletion blocked the passive avoidance behavior promoting action of nicotine. Secondly, treatment with an optimal tetrahydroaminoacridine dose facilitated the performance of nucleus basalis- and combined-lesioned rats. However, tetrahydroaminoacridine-treated combined-lesioned rats were not performing better than single-lesioned or the vehicle-treated nucleus basalis-lesioned group. On the contrary, our study confirmed previous evidence showing that nucleus basalis cholinergic projections do not importantly regulate spatial navigation (Dunnett et al., 1987, 1991; Riekkinen Jr. et al., 1990b, 1993a,b) and demonstrated that combined degeneration of nucleus basalis cholinergic and serotonergic fibers only temporarily and slightly during the first training day impaired water maze spatial navigation.

The finding that nucleus basalis quisqualate lesion markedly impaired passive avoidance behavior supports previous evidence indicating that cholinergic cells of the nucleus basalis may be important for avoidance behavior (Dunnett et al., 1987; Riekkinen Jr. et al., 1993a). Recent studies have suggested that the nucleus basalis cholinergic projection may, via the basolateral amygdala, regulate passive avoidance performance (Riekkinen Jr. et al., 1993a). Firstly, a lesion involving the basolateral nucleus of the amygdala markedly impaired passive avoidance responding (Riekkinen Jr. et al., 1993a). Secondly, muscarinic and nicotinic acetylcholine receptor antagonists less effectively impaired passive avoidance behavior of amygdala-lesioned rats than of the controls (Riekkinen Jr. et al., 1993a). Thirdly, nicotinic and muscarinic acetylcholine receptor agonists facilitated the passive avoidance behavior of nucleus basalis-lesioned rats, but amygdaloid lesioning blocked the therapeutic effect of cholinergic drugs (Riekkinen Jr. et al., 1993a). Therefore, it is likely that cholinergic drugs may modulate passive avoidance behavior via the basolateral amygdala. The lack of an effect of a nucleus basalis lesion on water maze behavior is in good agreement with earlier results showing that quisqualate or α-amino-3-hydroxy-5-methyl-4-isoaxole propionic acid infusions produce anatomically selective and neurochemically effective lesion, and no marked effect on spatial navigation behavior (Dunnett et al., 1987; Page et al., 1991).

The present results showing that combined nucleus basalis lesion and *p*-chlorophenylalanine treatment more severely impaired passive avoidance behavior support previous evidence indicating that basal forebrain cholinergic and brainstem serotonergic systems interact to regulate behavioral functioning (Sirviö et al., 1994). Initially, Vanderwolf (1987) reported that combined treatment with *p*-chlorophenylalanine and scopolamine severely disrupted navigation in 'a swim to a platform' and passive avoidance tasks. More recently, Riekkinen and Riekkinen Jr. (1995) studied the effects of combined treatment with muscarinic or nicotinic acetylcholine receptor antagonists and serotonin depletor *p*-chloroamphetamine or *p*-chlorophenylalanine on water maze navigation behavior. They reported that combined blockade of nicotinic or muscarinic acetylcholine receptors and serotonin systems severely impaired water maze acquisition, but not consolidation of spatial memory or performance of well-trained rats (Riekkinen and Riekkinen Jr., 1995). Nilsson et al. (1988) reported that intracerebroventricular infusion of 5,7-dihyroxytryptamine, a serotonin neurotoxin, aggravated the medial septal lesion-induced water maze defect, and that only combined grafting of fetal cells rich in developing cholinergic and serotonergic cells alleviated the lesion-induced spatial navigation defect (Nilsson et al., 1990). Furthermore, serotonin depletion blocked the therapeutic effect of nicotine on medial septal lesion-induced passive avoidance and water maze defects, but did not block the therapeutic effect of tetrahydroaminoacridine (Riekkinen Jr. et al., 1994). Finally, Markowska and Wenk (1991) showed that partial serotonin depletion diminished the behavioral recovery of ibotenic acid nucleus basalis-lesioned rats in a complex non-spatial maze. The present study confirmed previous evidence indicating that a combined serotonin depletion and quisqualate nucleus basalis lesion only slightly and transiently (during the first training day) impaired spatial memory functioning in the water maze test, suggesting that spatial memory acquisition was not markedly impaired (Jäkälä et al., 1993). The present results are in good agreement with previous evidence from Sahgal and Keith (1993) that cholinergic cells of the nucleus basalis and brainstem serotonin cells do not synergistically regulate memory. Sahgal and Keith (1993) reported that in a test designed to accurately measure spatial working memory function, namely a 'delayed non-matching to position' task, nucleus basalis quisqualate lesion only transiently impaired non-mnemonic aspects of performance and 5,7-dihydroxytryptamine raphe dorsalis lesion did not modulate the performance of control- or nucleus basalis-lesioned rats. Interestingly, Jäkälä et al. (1992) reported that serotonin and cholinergic systems may jointly regulate attentional behavior. *p*-Chlorophenylalanine treatment impaired attentional function in a 5-choice serial reaction-time test and the combination of scopolamine treatment and serotonin depletion produced a greater attentional defect than did *p*-chlorophenylalanine or scopolamine alone. Therefore, it is possible that a combined lesion impairs attentional function more severely than nucleus basalis and *p*-chlorophenylalanine lesions alone, and causes a non-mnemonic performance defect in the water maze during the first training day. However, spatial memory function was not impaired and combined-lesioned rats

learned the platform location during the training period. The finding that tetrahydroaminoacridine alleviated the water maze defect observed after the combined lesion suggests that the loss of cholinergic cells of the nucleus basalis is, at least partly, responsible for the transient water maze navigation defect.

It is possible that the dose of nicotine was too small and that a higher dose would have improved passive avoidance and water maze behavior of nucleus basalis + *p*-chlorophenylalanine-lesioned rats. However, at a slightly higher dose (0.9 mg/kg) than used in the present study, nicotine produced peripheral and central side-effects (Decker et al., 1992; Riekkinen Jr. et al., 1993a,b), so that we did not use higher doses. Indeed, several studies have shown that cholinergic drugs have inverted U-shaped dose-response curves, and stimulate memory function only at optimal doses (Brammer, 1982; Flood et al., 1985). Furthermore, following nicotine treatment, nucleus basalis-lesioned rats were performing in passive avoidance tests almost as well as the control group. The lack of a sufficiently high dose of tetrahydroaminoacridine may not explain the failure to completely alleviate passive avoidance failure in nucleus basalis-lesioned rats, as tetrahydroaminoacridine improved passive avoidance behavior of nucleus basalis-lesioned rats at 3 mg/kg, and had an inverted U-shaped dose-response curve. It is possible that the cholinergic side-effects (diarrhea, salivation, lacrimation) caused by acute tetrahydroaminoacridine 5 mg/kg may have inhibited passive avoidance behavior and caused the inverted U-shaped dose-response curve. Indeed, in our previous study we found, during a repeated treatment schedule, tolerance to tetrahydroaminoacridine treatment-induced side-effects and broadening of the dose window (Riekkinen Jr. et al., 1991). The effect of serotonin depletion on the recuperative effect of tetrahydroaminoacridine on nucleus basalis lesion-induced performance defect was less marked than the effect on the therapeutic effect of nicotine. We found that tetrahydroaminoacridine 3 mg/kg treatment improved passive avoidance and water maze behavior of combined-lesioned rats as effectively as it did the performance of nucleus basalis-lesioned rats. Neverthless, since the passive avoidance performance of combined-lesioned rats was markedly impaired, nucleus basalis + *p*-chlorophenylalanine-lesioned rats treated with tetrahydroaminoacridine 3 mg/kg did not have longer entry latencies than vehicle-treated nucleus basalis-lesioned rats during the testing trial in passive avoidance.

The question that inevitably arises is whether the *p*-chlorophenylalanine treatment aggravates the passive avoidance failure of nucleus basalis-lesioned rats and decreases the therapeutic effect of cholinergic drugs via further dysregulation of damaged nucleus basalis cholinergic activity or independently of cholinergic mechanisms. Firstly, several studies have investigated the effects of combined acetylcholine and serotonin lesions on neurochemical parameters, such as transmitter levels or enzyme activity. For example, the decrease of cortical and hippocampal choline acetyltransferase activity induced by nucleus basalis or medial septal lesions, respectively, was not aggravated by i.p. *p*-chlorophenylalanine or intraventricular 5,7-dihydroxytryptamine treatments (Nilsson et al., 1988; Riekkinen Jr. et al., 1990a, 1994). These studies also showed that the levels of serotonin in hippocampus and cortex of combined basal forebrain (nucleus basalis or medial septal) and serotonin-lesioned rats were not lower than the levels in serotonin-lesioned rats (Nilsson et al., 1988; Riekkinen Jr. et al., 1990a, 1994). Furthermore, Dekker and Thal (1992) recently reported that 5,7-dihydroxytryptamine raphe and ibotenic acid nucleus basalis lesion independently decreased cortical serotonin and acetylcholine levels as detected by in vivo microdialysis. However, the present and previous studies (Nilsson et al., 1988; Riekkinen Jr. et al., 1990a, 1994; Dekker and Thal, 1992) may have missed dynamic and short-duration neurochemical interactions between cholinergic and serotonergic brain lesions. Indeed, pharmacological stimulation of cholinergic systems by peripherally injected nicotine or cholinesterase inhibitors increases the activity of the serotonergic system (Ribeiro et al., 1993), suggesting that an acute increase in functioning of cholinergic cells and release of acetylcholine may also increase serotonin activity. Hence, it is possible that some of the therapeutic effects of tetrahydroaminoacridine and nicotine in nucleus basalis-lesioned rats may be mediated via serotonin projections. Finally, it is also possible that the *p*-chlorophenylalanine treatment-induced presynaptic serotonin depletion blocks the increase in the functioning of postsynaptic cortical serotonin receptors and second messenger systems that normally develops in nucleus basalis-lesioned rats as a compensatory response (Erfurth et al., 1993). Indeed, Erfurth et al. (1993) showed that, ipsilateral to an ibotenic acid nucleus basalis lesion the sensitivity of cortical acetylcholine and serotonin receptors increased, and the increase of agonist-induced second messenger response was correlated with the degree of cholinergic neuron loss (Erfurth et al., 1993). Furthermore, it is also possible that the upregulation of acetylcholine receptors developing as a compensatory response to the cholinergic cell loss is blunted by the concurrent serotonin lesion. Indeed, Alonso and Soubrie (1991) found that intracerebroventricular 5,7-dihydroxytryptamine, that nearly completely depleted forebrain serotonin, did not affect the cortical density of muscarinic receptors or of muscarinic M_1 and non-M_1 acetylcholine receptor subtypes. On the contrary, serotonin depletion blocked the upregulation of muscarinic receptors caused by re-

peated scopolamine, a muscarinic acetylcholine receptor antagonist, treatment. Therefore, it is possible that the upregulation of muscarinic acetylcholine receptors is less effective following combined nucleus basalis + *p*-chlorophenylalanine lesion than single nucleus basalis lesion, and that the severe behavioral defect of combined-lesioned rats is caused by a further dysregulation of cholinergic function.

In conclusion, the present results suggest that the integrity of presynaptic serotonin systems is important for the therapeutic effects of nicotine to reverse functional defects following cholinergic brain lesion. On the contrary, tetrahydroaminoacridine stimulated the avoidance behavior of combined-lesioned rats as effectively as that of nucleus basalis-lesioned rats.

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