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Depletion of serotonin, dopamine and noradrenaline in aged rats decreases the therapeutic effect of nicotine, but not of tetrahydroaminoacridine

Minna Riekkinen *, Laura Aroviita, Miia Kivipelto, Kati Taskila, Paavo Riekkinen Jr.

Department of Neurology, Canthia Building, University of Kuopio, PO Box 1627, FIN-70211 Kuopio, Finland

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Abstract

The present study investigates the effects of nicotine (0.1 and 0.3 mg/kg) and tetrahydroaminoacridine (3 mg/kg) treatment on spatial navigation in aged control and *p*-chlorophenylalanine (a serotonin (5-hydroxytryptamine, 5-HT) synthesis inhibitor, 400 mg/kg on 3 successive days, i.p.)-treated rats. *p*-Chlorophenylalanine did not aggravate the water maze failure of aged rats. Nicotine (0.3 mg/kg) was more effective than tetrahydroaminoacridine (3 mg/kg) in promoting water maze navigation by aged control rats. *p*-Chlorophenylalanine blocked the therapeutic effect of nicotine (0.3 mg/kg), but did not decrease the effect of tetrahydroaminoacridine (3 mg/kg) in aged rats. Frontal cortex dopamine levels and choline acetyltransferase activity were lower in aged rats, but 5-HT and noradrenaline levels were unaltered. *p*-Chlorophenylalanine decreased selectively 5-HT levels in young rats, but in aged rats 5-HT, dopamine and noradrenaline levels were decreased. These results suggest that aged rats are neurochemically more sensitive to *p*-chlorophenylalanine treatment and that tetrahydroaminoacridine may more effectively than nicotine stimulate spatial learning if 5-HT, dopamine and noradrenaline systems are severely affected.

Keywords: Aging; Alzheimer's disease; Spatial navigation; 5-HT (5-hydroxytryptamine, serotonin); Nicotine; Tetrahydroaminoacridine

1. Introduction

Neurochemical studies have found that multiple transmitter systems are adversely affected in Alzheimer's disease (Bowen et al., 1983; Reinikainen et al., 1990). Clinical studies have described that the loss of cholinergic neurons in the basal forebrain correlates with the decline in cognitive functioning, supporting the 'cholinergic hypothesis' of age-related memory deficits (Whitehouse et al., 1982; Bowen et al., 1983; Reinikainen et al., 1990). The results of drug studies have shown modest but significant alleviation of the severity of clinical dementia and the impairment of neuropsychological performance in Alzheimer's disease after treatment with a cholinesterase inhibitor, tetrahydroaminoacridine, or a nicotinic acetylcholine receptor agonist, nicotine (Eagger et al., 1991; Jones et al., 1992). However, only some Alzheimer patients benefit from tetrahydroaminoacridine. Therefore, it is possible that the degeneration of other systems, such as the serotonin (5-hydroxytryptamine, 5-HT), noradrenaline or dopamine systems, may decrease the therapeutic effect of tetrahydroaminoacridine and nicotine (Bowen et al., 1983; Reinikainen et al., 1990).

Recently, several studies have elucidated the functions of the basal forebrain cholinergic and the brainstem 5-HT, noradrenaline and dopamine systems in the regulation of memory and attention (Dunnett et al., 1985; Nilsson et al., 1988; Verters, 1988; McGurk et al., 1989; Richter-Levin and Segal, 1989; Sirviö et al., 1991, 1994; Jäkälä et al., 1993; Riekkinen et al., 1993; Riekkinen and Riekkinen Jr., 1994; Steckler and Sahgal, 1995). Anatomical studies have revealed that the cholinergic basal forebrain nuclei have afferents from 5-HT, noradrenaline and dopamine cells, and the hippocampus and the cortex receive basal forebrain and brainstem afferents (Steinbusch, 1984; Jones and Yang, 1985; Verters, 1988; Sirviö et al., 1994). Several behavioral studies have studied the effects of concurrent cholinergic, 5-HT, noradrenergic and dopaminergic manipulations (Nilsson et al., 1988; Richter-Levin and Segal, 1989; Riekkinen Jr. et al., 1990b,1994; Riekkinen et al.,

* Corresponding author. Tel.: +358 71 162698; fax: +358 71 162048.

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1993; Riekkinen and Riekkinen Jr., 1994; Riekkinen Jr. and Riekkinen, 1995). For example, treatment with a 5-HT synthesis inhibitor, *p*-chlorophenylalanine, or a 5-HT neurotoxin, 5,7-dihydroxytryptamine, did not impair passive avoidance behavior or spatial reference memory performance in the water maze test (Nilsson et al., 1988; Richter-Levin and Segal, 1989; Riekkinen Jr. et al., 1990b; Jäkälä et al., 1993; Riekkinen et al., 1993; Riekkinen and Riekkinen Jr., 1994), but aggravated the performance deficit induced by muscarinic or nicotinic acetylcholine receptor blockade (Richter-Levin and Segal, 1989; Riekkinen Jr. et al., 1990b; Riekkinen et al., 1993; Riekkinen and Riekkinen Jr., 1994). In our recent studies, we found that *p*-chlorophenylalanine treatment, which selectively decreased 5-HT levels in the cortex and hippocampus, completely blocked the therapeutic effect of nicotine on water maze and passive avoidance behavior in nucleus basalis-lesioned and in medial septal-lesioned young rats, but did not block the action of tetrahydroaminoacridine (Riekkinen Jr. et al., 1994; Riekkinen Jr. and Riekkinen, 1995). In contrast, Grigoryan et al. (1994) described that 6-hydroxydopamine-induced lesions of the noradrenergic bundle, which selectively depleted cortical and hippocampal noradrenaline levels, had no effect on the working memory-improving effect of nicotine in nucleus basalis + medial septal-lesioned rats. Thus, these data suggest that 5-HT projections may partly mediate the effects of nicotine, but not tetrahydroaminoacridine, on spatial memory in rats subjected to cholinergic lesions. However, the behavioral and pharmacological consequences of 5-HT depletion on spatial navigation in aged rats have not been studied previously.

The present study investigates in aged rats the effects of *p*-chlorophenylalanine on frontal cortex neurochemical systems (5-HT, noradrenaline and dopamine levels, choline acetyltransferase activity), on water maze spatial navigation and on the therapeutic action of nicotine and tetrahydroaminoacridine on water maze spatial navigation.

2. Materials and methods

2.1. Animals

Young (4 months old) and aged (26–28 months old) male Han-Wistar rats were used in the present study. The rats were singly housed with food and water ad libitum. Room temperature was +22°C, humidity was 50–60%, and a light period of 12 h (lights on from 07:00 to 19:00 h) was used. The study had the approval of the provincial government of Kuopio.

The following groups were used in the experiments:

2.1.1. Experiment 1 (intact rats)

Young saline-treated group, aged saline-treated group, aged tetrahydroaminoacridine 3 mg/kg-treated group, aged nicotine 0.1 mg/kg-treated group and aged nicotine 0.3 mg/kg-treated group. The number of rats in each group was 10.

2.1.2. Experiment 2 (p-chlorophenylalanine-pretreated rats)

Young *p*-chlorophenylalanine-treated group, aged saline-treated group, aged *p*-chlorophenylalanine-treated group, aged *p*-chlorophenylalanine + tetrahydroaminoacridine 3 mg/kg-treated group and aged *p*-chlorophenylalanine + nicotine 0.3 mg/kg-treated group. The number of rats in each group was 10–12.

2.2. Drugs

Tetrahydroaminoacridine (Sigma) (3 mg/kg, 60 min pretest) and (−)-nicotine hydrogen tartrate (Research Biochemicals) (0.1 and 0.3 mg/kg, 25 min pretest) were dissolved in physiological saline (0.9% NaCl) and injected intraperitoneally (i.p.) (2 ml/kg). Saline injections of an equal volume were used for control purposes (25 and 60 min before testing for controls; 25 and 60 min before testing for nicotine- and tetrahydroaminoacridine-treated rats, respectively). *p*-Chlorophenylalanine was mixed in saline containing a 0.5% suspension of arabic gum (mixed fresh every day) and injected i.p. (400 mg/kg/4 ml) on each of 3 successive days. For control purposes arabic gum was suspended in saline (mixed fresh every day) and injected i.p. on each of 3 consecutive days.

Tetrahydroaminoacridine at 3 mg/kg inhibits acetylcholinesterase activity and promotes the spatial navigation performance of aged rats, and only at a slightly higher (5 mg/kg) dose does it produce severe catalepsy, which inhibits performance in the water maze task (Riekkinen Jr. et al., 1991a,b). The nicotine doses used have been earlier shown to promote the passive avoidance behavior of nucleus basalis-lesioned rats and water maze learning of medial septal-lesioned rats (Riekkinen Jr. et al., 1993; Riekkinen Jr. and Riekkinen, 1993). Previously we have found that nicotine and tetrahydroaminoacridine at the doses used in the present study do not improve water maze performance or passive avoidance behavior of control or *p*-chlorophenylalanine-treated young rats (Riekkinen Jr. and Riekkinen, 1993; Riekkinen et al., 1993; Riekkinen and Riekkinen Jr., 1994). We did not assess the effects of *p*-chlorophenylalanine treatment on water maze behavior in young rats, because we have previously found that *p*-chlorophenylalanine treatment (400 mg/kg × 3) does not impair their water maze performance (Riekkinen Jr. and Riekkinen, 1993; Riekkinen et al., 1993).

2.3. Morris water maze

The rats were tested by using the water maze test of spatial navigation behavior. The water maze pool was a circular fiber-glass tank, painted black, 152 cm in diame-

ter, 74 cm deep, and filled to a height of 52 cm with water at room temperature (21 ± 2°C). The platform was made of Plexiglas tube and the top surface was composed of black rubber. The top surface was 1.5 cm below the water line. The pool was divided into 4 quadrants and 3 annuli of equal surface area. The starting locations were called north, south, east and west and they were located arbitrarily at equal distances on the pool rim. On the 1st day of training no platform was in the pool and the rats were allowed to swim freely for 50 s to habituate them to the pool. The platform was located in the south-west quadrant during every training trial of the next 5 consecutive days of testing (3 trials per day, maximum swim time: 70 s, intertrial interval 25 s). Again, on the 7th day the platform was removed (1 trial, swim time for all of the rats: 50 s) and spatial bias was tested. The swim paths were monitored by a video camera linked to computer through an image analyzer. The computer calculated the total distance swum (cm). On the last day of the experiments (spatial bias testing day) the spatial bias, which is the percent of time spent in the previous location of the training quadrant, was measured (the higher the spatial bias, the better the retrieval performance was considered to be). Rats were placed in the water, facing the wall, at one of the 4 starting points chosen in a semirandom manner, and the timer was started by a remote control connected to the computer. If the rat found the platform, it was allowed to stay there for 10 s. Rats that failed to find the hidden platform in the alloted time were placed on it for 10 s. A 30-s recovery period was allowed between daily trials. After the last trial of every day, the rats were dried with a towel and returned to the home cage.

2.4. Dissection, biochemistry and histology

The following groups of rats were decapitated 2 days after the end of the experiment:

Experiment 1: young controls;

Experiment 2: young *p*-chlorophenylalanine-treated group, aged controls, aged PCPA-treated group.

The brains of rats were removed. The brain samples were dissected on ice and stored at −72°C until assayed. Jäkälä et al. (1993) have previously demonstrated that the changes in the concentrations of monoamines and their metabolites induced by *p*-chlorophenylalanine did not differ between frontal cortex, parieto-occipital cortex and hippocampal samples. Therefore, we used only frontal cortex samples in this study. The levels of 5-HT, and its metabolite 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline and dopamine were measured in the frontal cortex according to the method previously described by Jäkälä et al. (1993). The frontal cortex was taken from all of the aged rats and from every second of the young rats. The young rats taken for analysis were selected randomly.

The method of Fonnum (1975) was used to analyze choline acetyltransferase activity. The choline acetyltransferase activity was measured in frontal cortex samples. Half of the frontal cortex brain samples of the young rats and all frontal cortex samples of the aged rats were taken for analysis. The young rats taken for choline acetyltransferase analysis were selected randomly.

2.5. Statistical analysis

Statistical analysis were done by using the SPSS/PC + programs. The multivariate analysis test for repeated measurements (MANOVA) was used to analyze the behavioral data for water maze training days. The one-way analysis of variance (ANOVA) test followed by Duncan's post-hoc multiple group comparisons was used in the statistical analysis of data for water maze spatial bias and biochemical parameters. $P < 0.05$ was accepted as significant.

3. Results

3.1. Behavior

3.1.1. Experiment 1 (intact rats)

Analysis of water maze escape distance values showed a significant overall Group effect ($F(4,45) = 19,17$, $P <$

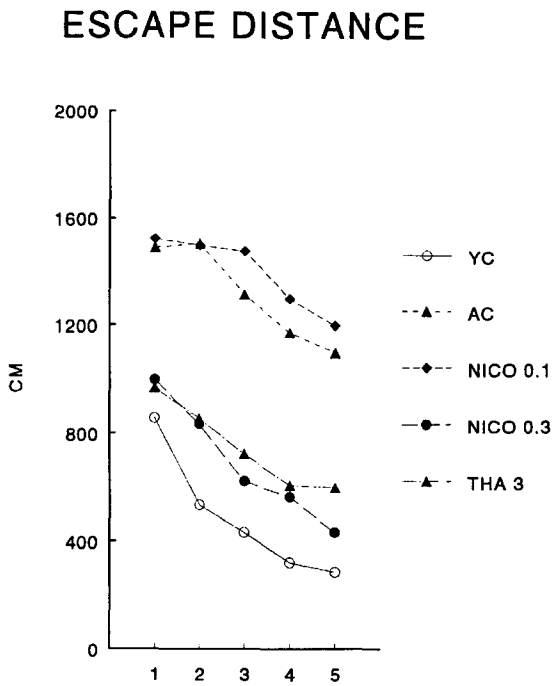

Fig. 1. The effects of tetrahydroaminoacridine and nicotine on water maze escape distance values (cm) in aged rats. Tetrahydroaminoacridine (3 mg/kg i.p.) was injected 60 min before the daily training trials. Training days 1–5 are shown on the *X*-axis. Nicotine (0.1 and 0.3 mg/kg i.p.) was injected 25 min before the daily training trials. Note that tetrahydroaminoacridine (3 mg/kg) and nicotine (0.3 mg/kg) reversed the performance deficit in aged rats. Abbreviations: YC, young control; AC, aged control; NICO 0.1, aged control + nicotine 0.1 mg/kg; NICO 0.3, aged control + nicotine 0.3 mg/kg; THA 3, aged control + tetrahydroaminoacridine 3 mg/kg.

Table 1
Retention of water maze task (spatial bias = % of total time spent in the previous location of the escape platform)

Experiment 1 (intact rats)	
Treatment groups	Spatial bias (%)
Young controls	34 ± 9 [a]
Aged controls	17 ± 7
+ Tetrahydroaminoacridine 3 mg/kg	20 ± 12
+ Nicotine 0.1 mg/kg	21 ± 8
+ Nicotine 0.3 mg/kg	31 ± 8 [a]

Values represent group means ± S.D. Note that the promoting effect of nicotine (0.3 mg/kg) on spatial bias was blocked by *p*-chlorophenylalanine pretreatment in aged rats.
[a] $P < 0.05$ vs. aged controls (Duncan's post-hoc multiple group comparisons).

0.001) (Fig. 1). Further analysis revealed that aged rats performed worse than young rats ($F(1,18) = 32,88$, $P < 0.001$). Tetrahydroaminoacridine (3 mg/kg) improved significantly the performance of aged rats ($F(1,18) = 11.82$, $P = 0.003$). Furthermore, also nicotine (0.3 mg/kg) improved the performance of aged rats ($F(1,18) = 15.41$, $P = 0.001$). However, nicotine 0.1 mg/kg-treated aged rats performed slightly worse than the aged controls ($F(1,18) = 5.01$, $P = 0.04$). Tetrahydroaminoacridine 3 mg/kg-treated aged rats had significantly longer escape distance values than young controls ($F(1,18) = 5.49$, $P = 0.03$). Aged rats treated with nicotine (0.3 mg/kg) did not differ significantly from the young controls.

During the retention trial it was observed that spatial bias was decreased in the aged control group when compared with the young controls ($P < 0.05$, Duncan's analysis). Nicotine (0.3 mg/kg) increased the spatial bias of aged rats ($P < 0.05$, Duncan's analysis) to the level of young controls. Nicotine (0.1 mg/kg) and tetrahydroaminoacridine (3 mg/kg) had no effect on the spatial bias of aged rats (Table 1).

3.1.2. Experiment 2 (p-chlorophenylalanine-pretreated rats)

Analysis of water maze escape distance values showed a significant overall Group effect ($F(4,51) = 35.43$, $P < 0.001$) (Fig. 2). Further analysis revealed that young *p*-chlorophenylalanine-pretreated rats performed significantly better than aged controls ($F(1,22) = 83.04$, $P < 0.001$). *p*-Chlorophenylalanine treatment had no effect on the escape distance values of aged rats. Tetrahydroaminoacridine 3 mg/kg improved the performance of aged *p*-chlorophenylalanine-pretreated rats ($F(1,21) = 29.19$, $P < 0.001$), but nicotine (0.3 mg/kg) did not promote the performance of *p*-chlorophenylalanine-pretreated rats. Young *p*-chlorophenylalanine-treated rats were significantly better than aged tetrahydroaminoacridine (3 mg/kg) + *p*-chlorophenylalanine-treated aged rats ($F(1,21) = 6.38$, $P = 0.02$).

Aged control rats had a lower spatial bias than young *p*-chlorophenylalanine-pretreated rats ($P < 0.05$, Duncan's analysis). *p*-Chlorophenylalanine pretreatment had no effect on the spatial bias of aged rats. Tetrahydroaminoacridine (3 mg/kg) or nicotine (0.3 mg/kg) was unable to restore this deficit in aged *p*-chlorophenylalanine-pretreated rats (Table 2).

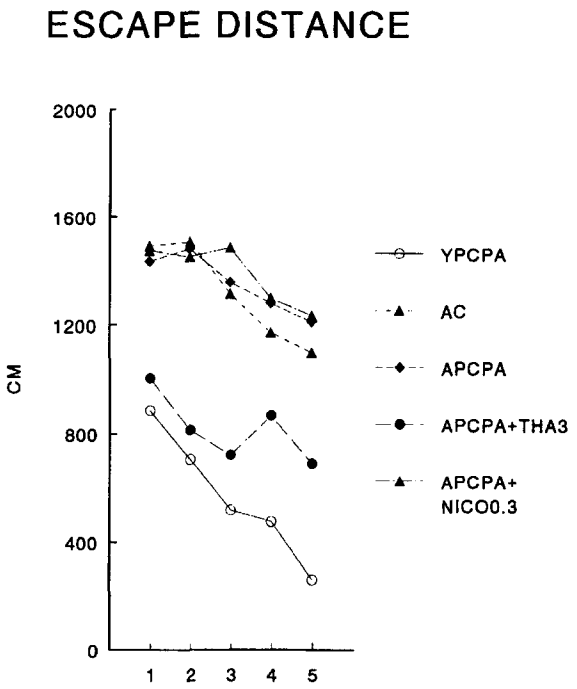

Fig. 2. The effects of tetrahydroaminoacridine and nicotine treatments on water maze escape distance values (cm) in *p*-chlorophenylalanine-pretreated rats. Training days 1–5 are shown on the *X*-axis. Tetrahydroaminoacridine (3 mg/kg i.p.) was injected 60 min before the daily training trials. Nicotine (0.1 and 0.3 mg/kg i.p.) was injected 25 min before the daily training trials. Note that serotonin depletion decreased the performance-improving effect of nicotine (0.3 mg/kg), but not that of tetrahydroaminoacridine (3 mg/kg), in aged rats. Abbreviations: YPCPA, young *p*-chlorophenylalanine; AC, aged control; APCPA, aged *p*-chlorophenylalanine; APCPA + THA 3, aged *p*-chlorophenylalanine + tetrahydroaminoacridine 3 mg/kg; APCPA + NICO 0.3, aged *p*-chlorophenylalanine + nicotine 0.3 mg/kg.

3.2. Biochemistry and histology

Table 3 describes the effects of aging and 5-HT depletion on the values for serotonergic and cholinergic biochemical markers measured in frontal cortex samples.

Table 2
Retention of water maze task (spatial bias = % of total time spent in the previous location of the escape platform)

Experiment 2 (*p*-chlorophenylalanine-pretreated rats)	
Treatment groups	Spatial bias (%)
Young *p*-chlorophenylalanine-treated	30 ± 11 [a]
Aged controls	21 ± 6
Aged *p*-chlorophenylalanine	24 ± 6
+ Tetrahydroaminoacridine 3 mg/kg	24 ± 12
+ Nicotine 0.3 mg/kg	22 ± 7

[a] $P < 0.05$ vs. aged controls (Duncan's post-hoc multiple group comparisons).

Table 3
The effects of *p*-chlorophenylalanine treatment and aging on frontal cortex levels of monoamines and serotonin metabolite and choline acetyltransferase activity

Frontal cortex					
	5-HT	5-HIAA	NA	DA	ChAT
Young controls	212 ± 31	270 ± 41	223 ± 20	194 ± 84	0.91 ± 0.09
Young PCPA	48 ± 20 [a,b]	39 ± 7 [a,b]	217 ± 47	172 ± 60	1.01 ± 0.09
Aged controls	233 ± 51	265 ± 56	199 ± 58	128 ± 40 [a]	0.71 ± 0.08 [a]
Aged PCPA	30 ± 11 [a,b]	15 ± 4 [a,b]	931 ± 5 [a,b]	20 ± 8 [a,b]	0.71 ± 0.16 [a]

Monoamines and metabolite levels (ng/g of brain tissue) and choline acetyltransferase activity (nmol/mg protein/min) are expressed as group means ± S.D. Abbreviations: ChAT, choline acetyltransferase; DA, dopamine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; NA, noradrenaline; PCPA, *p*-chlorophenylalanine. [a] $P < 0.05$ vs. young controls; [b] $P < 0.05$ vs. aged controls (Duncan's post-hoc multiple group comparisons).

p-Chlorophenylalanine treatment decreased 5-HT and 5-HIAA levels of young (5-HT: −77%; 5-HIAA: −86%) and aged rats (5-HT: −87%; 5-HIAA: −95%) in the frontal cortex (overall effect: $P < 0.05$, Duncan's analysis). No significant difference was found between *p*-chlorophenylalanine-treated young and aged groups in 5-HT levels, but 5-HIAA levels of aged *p*-chlorophenylalanine-treated rats were lower than those of young *p*-chlorophenylalanine-treated rats ($P < 0.05$, Duncan's analysis). Dopamine levels were not decreased in brain samples from young rats following *p*-chlorophenylalanine treatment. However, dopamine levels were decreased in aged control rats when compared with young control rats (−35%) ($P < 0.05$, Duncan's analysis). Furthermore, *p*-chlorophenylalanine treatment further decreased the dopamine levels in aged rats (−84%) ($P < 0.05$, Duncan's analysis). Noradrenaline levels remained unaltered in young *p*-chlorophenylalanine-treated groups. Aging did not have a significant effect on noradrenaline levels, but *p*-chlorophenylalanine treatment decreased noradrenaline levels in aged rats (−54%) ($P < 0.05$, Duncan's analysis).

The choline acetyltransferase activity in the frontal cortex was decreased in aged rats when compared with young rats (−22%) ($P < 0.05$, Duncan's analysis). Choline acetyltransferase activity was not altered following *p*-chlorophenylalanine treatment in young and aged rats.

4. Discussion

The present study shows that: (A) *p*-chlorophenylalanine treatment in aged, cognitively impaired rats more severely affected 5-HIAA, dopamine and noradrenaline markers than in young rats; (B) *p*-chlorophenylalanine did not further aggravate the age-related deficit of water maze performance; and (C) *p*-chlorophenylalanine completely blocked the therapeutic effect of nicotine, but not of tetrahydroaminoacridine, on spatial navigation behavior in aged rats.

In aged rats, choline acetyltransferase activity (−22%) and dopamine levels (−35%) were significantly decreased in the frontal cortex when compared with those of young rats. Several studies have investigated the effects of aging on choline acetyltransferase activity and monoamine levels in the brain (for reviews: Pradhan, 1980; Decker, 1987). These studies suggest that the degree of degeneration of cholinergic and monoaminergic cells depends on rat strain, age and cognitive function (Pradhan, 1980; Decker, 1987; Fischer et al., 1989; Riekkinen Jr. et al., 1990a). For example, Gallagher et al. (1990) reported that choline acetyltransferase activity was decreased in the frontal cortex of 28–29-month-old Long-Evans rats that were cognitively impaired in water maze tests. Further, Lee et al. (1994) found a decrease (−24%) in frontal cortex dopamine levels in a subpopulation of aged Sprague-Dawley (20–22 months old) rats that had impaired water maze learning.

A more interesting and novel result was that *p*-chlorophenylalanine had a greater effect on 5-HT, noradrenaline and dopamine systems in aged than in young rats. The 5-HT depletion produced by *p*-chlorophenylalanine (400 mg/kg × 3) was selective for the serotonergic (5-HT: −77%; 5-HIAA: −86%) system in young rats, and these results are in line with previous studies (Jäkälä et al., 1993; Riekkinen et al., 1993; Riekkinen and Riekkinen Jr., 1994). However, in aged rats, in addition to the depletion of 5-HT (−87%) and 5-HIAA (−95%) in the frontal cortex, *p*-chlorophenylalanine treatment also decreased noradrenaline (−54%) and dopamine (−84%) levels. Earlier experiments have reported that the catecholamine systems of aged rats are more sensitive than those of young rats to the actions of neurotoxins. For example, Riekkinen Jr. et al. (1992) and Sirviö et al. (1991) have shown that a noradrenergic neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP-4) produced more severe biochemical and functional deficits in aged than in young rats. Riekkinen Jr. et al. (1992) showed that DSP-4 depleted noradrenaline more severely in aged (−85%) than in young rats (−68%) and caused a greater increase in neocortical high-voltage spindles in aged rats. However, in this present study the reduction in catecholamine concentrations after *p*-chlorophenylalanine treatment in aged rats was not severe enough to impair water maze behavior, as *p*-chlorophenylalanine treatment did not aggravate the wa-

ter maze defect of aged rats. It is possible that, due to the ceiling effect caused by poor performance of aged controls, no aggravation of age-related water maze failure was observed after *p*-chlorophenylalanine treatment. Nevertheless, *p*-chlorophenylalanine treatment did block the therapeutic effect of nicotine on spatial navigation: nicotine facilitated acquisition (decreased escape distance) and retrieval (increased bias) more effectively than tetrahydroaminoacridine did in aged controls, but in aged *p*-chlorophenylalanine-treated rats only tetrahydroaminoacridine stimulated water maze navigation.

It could be argued that the dose of nicotine used was inadequate and that at a higher dose nicotine would have promoted the water maze behavior of *p*-chlorophenylalanine-treated aged rats. However, nicotine at a slightly higher dose than that used in this study causes marked peripheral and central side-effects (Riekkinen Jr. et al., 1993; Riekkinen Jr. and Riekkinen, 1993) and therefore we did not test the effects of higher doses. Furthermore, nicotine at the dose used in this study (0.1 mg/kg) has been shown to improve performance in the working memory task in animals with lesions of the forebrain cholinergic projection systems (Hodges et al., 1992). In our own studies, we also found that nicotine (0.1 and 0.3 mg/kg) improved the passive avoidance performance of nucleus basalis-lesioned rats (Riekkinen Jr. et al., 1993). Indeed, several studies have shown that cholinergic drugs have an inverted U-shaped dose-response curve and are able to stimulate memory function only at optimal doses (Flood et al., 1985; Riekkinen Jr. et al., 1991b). Furthermore, tetrahydroaminoacridine improved water maze navigation in controls as well as in *p*-chlorophenylalanine-lesioned aged rats. Therefore, it is likely that *p*-chlorophenylalanine treatment markedly reduces (see also Riekkinen Jr. et al., 1994; Riekkinen Jr. and Riekkinen, 1995) the therapeutic effect of nicotine. This conclusion is consistent with the findings of other studies. Previously, Hodges et al. (1990) have shown that chronic treatment with low doses (0.3 or 3 mg/kg) of tetrahydroaminoacridine improved cognitive function in rats with a nucleus basalis lesion or after treatment with alcohol in drinking water; however, the acetylcholine content was not significantly affected in any of the brain areas investigated. Thus, they concluded that the effect on cognitive function did not appear to be mediated by inhibition of acetylcholinesterase and that alternative mechanisms of action of tetrahydroaminoacridine are possible.

Interestingly, our earlier data suggest that intact 5-HT fibers are a prerequisite for nicotine treatment to be effective in improving maze performance in young rats subjected to basal forebrain lesions. We observed that in young, medial septal- and nucleus basalis-lesioned rats, selective depletion of 5-HT by *p*-chlorophenylalanine blocked the effects of nicotine on passive avoidance and water maze behavior (Riekkinen Jr. and Riekkinen, 1995). Therefore, it is possible that a nicotine treatment-induced increase in the release of 5-HT (Ribeiro et al., 1993) is a prerequisite for any water maze performance improvement to occur in young rats with lesions of basal forebrain nuclei containing cholinergic projection cells. Several previous studies support the view that serotonergic and cholinergic afferents to the hippocampus may interact and that this interaction has cognitive relevance (Jeltsch et al., 1994; Sirviö et al., 1994). Following combined cholinergic and serotonergic denervation of the hippocampus, grafts providing the denervated hippocampus with new cholinergic and seroronergic innervations induce a better recovery than grafts providing the hippocampus with only one or the other of these innervations (Jeltsch et al., 1994). Noradrenaline and dopamine cells also modulate cognitive functioning, and it could be argued that the loss of the therapeutic effect of nicotine following *p*-chlorophenylalanine treatment is mediated, at least partly, via degeneration of noradrenaline and/or dopamine neurons. In contrast, Grigoryan et al. (1994) concluded that the enhancing effect of nicotine on water maze performance in rats with lesions of the forebrain cholinergic neurons was not mediated by an interaction with the noradrenergic system. This finding may imply that the partial noradrenaline loss observed in aged *p*-chlorophenylalanine-treated rats in this study may not adversely affect the therapeutic response to nicotine. However, in aged rats the compensatory responses following noradrenaline lesions may be impaired and functional defects are more easily observed. Indeed, Sirviö et al. (1991) described that in aged rats DSP-4 treatment induced a selective and partial noradrenaline (−79%) lesion and impaired water maze navigation, but a near complete noradrenaline lesion in young rats had no effect on water maze behavior. This result indicates that noradrenaline lesions in aged rats may have a greater impact on the functioning of mechanisms underlying water maze spatial navigation than they have in young rats. The marked loss of dopamine (−84%) may also contribute to the lack of any therapeutic effect of nicotine to stimulate spatial navigation in *p*-chlorophenylalanine-treated rats. For example, the impairment in radial arm maze spatial memory performance elicited by a nicotinic acetylcholine receptor antagonist was potentiated by a dopamine$_2$ receptor antagonist (McGurk et al., 1989) and was reversed by a dopamine$_2$ receptor agonist in rats (Levin et al., 1989). Therefore, partial noradrenaline and dopamine depletion may have adversely affected the function of brain areas involved in spatial navigation and contributed in concert with 5-HT depletion (Riekkinen Jr. et al., 1994; Riekkinen Jr. and Riekkinen, 1995) to the lack of an effect of nicotine treatment to enhance spatial navigation.

In conclusion, the present results show that *p*-chlorophenylalanine produces a larger 5-HT, noradrenaline and dopamine depletion in aged than in young rats, and completely blocks the therapeutic effect of nicotine on spatial navigation. In contrast, the performance-improving effect of tetrahydroaminoacridine was unaltered. This indicates

that tetrahydroaminoacridine may more effectively than nicotine stimulate behavioral functions if 5-HT, noradrenaline and dopamine activity is reduced.

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