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The Effects of THA on Medial Septal Lesion-Induced Memory Defects

PAAVO RIEKKINEN, JR.,*[1] JOUNI SIRVIÖ AND PAAVO RIEKKINEN

*Department of Neurology and *Departments of Neurology and Pathology*
University of Kuopio, P.O. Box 6, SF-70211 Kuopio, Finland

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RIEKKINEN, P., JR., J. SIRVIÖ AND P. RIEKKINEN. *The effects of THA on medial septal lesion-induced memory defects.* PHARMACOL BIOCHEM BEHAV **36**(2) 237–241, 1990.—Electrolytic lesioning of the medial septum (MS) was used to assess the effectiveness of tacrine (THA) in reversing lesion-induced spatial memory deficits in a water-maze. Lesioned animals were injected with either 3 mg/kg or 5 mg/kg of THA intraperitoneally 15 min prior to daily behavioral training. One group of the lesioned and sham-operated animals received saline. All animals underwent two training trials each day for a period of ten days, after which a spatial probe trial was performed and assessed. The accurate placement of MS lesions resulted in lowered acetylcholinesterase (AChE) and cholineacetyltransferase (ChAT) activity within the hippocampus of lesioned rats. Lesioning of the MS also impaired the learning performance in locating the escape platform during training and decreased the spatial bias during the probe trial. A lower dose of THA (3 mg/kg) significantly reversed the path length increase and spatial bias decrease induced by MS lesioning, but had no effect on escape latency. However, comparison between the saline- and THA- (5 mg/kg) injected MS-lesioned rats showed no significant differences in either escape latency or spatial bias. The present results support the use of cholinesterase inhibitors in further treatment trials of geriatric memory disorders.

Cholinergic deficit Hippocampus THA Spatial learning

IN Alzheimer's disease (AD) and to lesser extent during normal aging, the basal forebrain cholinergic system deteriorates (2, 3, 17, 18). In AD, large and consistent reductions in the activities of ChAT and AChE in both the hippocampus and cortex have been reported (3,18) and these decreases appear to correlate with the degree of cognitive impairment (17). In the hippocampus and cortex of normal aged humans (4,16) and rodents (21,22), the activities of ChAT and AChE have been reported to decrease in association with a corresponding cognitive decline (1).

Some of the early clinical features in AD patients include complaints by patients of becoming lost in relatively unfamiliar places and forgetting where familiar objects have been placed (19). Recent work has shown that analogous cognitive and memory impairment are also seen in subpopulations of aged animals (9,10). Studies have sought to determine a neurobiological basis for the impaired spatial learning observed in aged rats, and these efforts have focused on brain areas presumed to be involved in processing spatial information. Lesioning of the hippocampal formation (14) or the MS (13), which is the origin of the septohippocampal cholinergic system, disrupts performance in several learning tasks which are also sensitive to age-related cognitive decline. Furthermore, the age-related loss of cholinergic cell bodies in the MS (6) is correlated with the severity of retention impairment, providing an interesting parallel to AD.

The increase in central cholinergic function by the use of anticholinesterase to prevent the breakdown of acetylcholine constitutes one pharmacological strategy for alleviating cognitive decline in AD patients. Recently, tacrine (THA, anticholinesterase) has been shown to rehabilitate memory disorders in AD patients (23,24). Furthermore, recent studies have reported the beneficial effect of THA on memory and learning in experimental animals (2,7). One logical approach in the study of pharmacologically active agents with the aim of alleviating geriatric cognitive deficits is to lesion those brain areas which are both involved in spatial learning and manifest an age or disease related degeneration. Therefore, in the present study, we examined the effectiveness of daily IP administration of THA in reversing spatial reference memory deficits in MS-lesioned rats.

METHOD

Animals

Male Kuo:Wistar rats (300–350 g) were used in this study. Ten animals were included in the control group and nine animals in all MS-lesioned groups.

Drugs

Anticholinesterases have motor side effects which may be centrally mediated. Thus, the effects of THA on motor function

[1]Requests for reprints should be addressed to Paavo Riekkinen, Jr., Department of Neurology, University of Kuopio, P.O. Box 6, SF-70211 Kuopio, Finland.

were evaluated. In the test of elevated bridges, rats were singly placed in the middle of elevated horizontal bridges. The time taken for the rat to lose its balance and fall from the bridge was measured. The first bridge was flat with a width of 5 cm, the second was flat with a width of 2.5 cm and the third was tubular with a diameter of 2.5 cm. The drugs were dissolved in saline (5 ml/kg) and administered via IP route 15 min before testing. THA was shown to cause dose-dependent impairment. The motor side effects seen with the smaller dose selected for the learning test (3 mg/kg) were nonsignificant, but the higher dose selected for the learning test (5 mg/kg) produced some impairment. During the water-maze experiment drugs were injected 15 min before the first daily training trials.

Surgery

The animals were anesthetized with chloral hydrate (350 mg/kg IP) and placed in a stereotaxic frame with the bregma and lambda in the horizontal plane. MS lesions (a: 0.2 mm, m: 0.0 mm, d: −7 mm, relative to the bregma) (16) were performed with stainless steel electrodes (diameter 0.25 mm, 0.4 mm of the tip uninsulated) by passing anodal DC current (2 mA, 7 sec) through the electrode. The sham-operated group was treated identically, but no current was applied.

Histology

A piece of brain, cut frontally three millimeters anterior and posterior to the electrode track, was put into 3% formalin (phosphate-buffered saline, 0.1 M, pH 7.4) for ten hours and subsequently immersed in 30% sucrose. Serial sections (30 μm) were cut, and neighboring sections were stained with hematoxylin-eosin (H-E) and AChE histochemistry.

Biochemistry

Four days after the last injections of THA, the brains of the rats were dissected on ice and the hippocampus was stored at −75°C. For biochemical assays, hippocampal tissue was homogenized in 50 μM sodium phosphate buffer, pH 7.4, containing 0.32 M sucrose (10 v/w) using a Potter-Elvehjem homogenizer (1000 rpm, six strokes) in an ice bath. The homogenate (300 μl) was stored at −80°C for AChE, ChAT and total protein assays.

For AChE assays, the homogenate (100 μl) from the hippocampus was thawed and suspended in 1.0 ml of 10 mM sodium phosphate buffer, pH 7.4, containing 1 M NaCl, 1 mM EDTA and 0.5% Triton X-100. The suspensions were diluted in distilled water (1:9), and AChE activities were measured using a modification of the colorometric method of Ellman (5). In the present assays, the substrate used was acetyltiocholine iodide (0.50 mM), and AChE activity was inhibited using iso-OMPA (0.1 mM).

For ChAT assays, 100 μl homogenate was thawed and suspended in 2.9 ml of EDTA-NaOH solution, pH 7.4, containing 0.5% Triton X-100. Two microliters of diluted homogenate were needed for the assays of ChAT activity which were measured in triplicates according to the method of Fonnum (8). In the present assays, the substrate solution contained 41 mM sodium phosphate, 11 mM EDTA-NaOH, 10 mM choline bromide, 0.1 mM physostigmine (eserine), 300 mM NaCl and 0.28 mM Ac-CoA (^{14}C-Ac-CoA diluted with unlabelled Ac-CoA). Five microliters of substrate solution were needed for the measurements. Tubes containing substrate solution were incubated 40 minutes at +37°C.

Behavioral Testing

Water-maze apparatus. The water-maze pool was a circular black-painted fiberglass tank, 150 cm in diameter, 74 cm deep, and was filled to a height of 52 cm with water at room temperature. The platform was made of a Plexiglas tube and the top surface was composed of black rubber. The top surface was submerged 1.5 cm below the water line. The pool was divided into four quadrants and three annuli of equal surface area. The starting locations were labelled north, south, east and west and were located arbitrarily on the pool rim. The platform was located in the south-west quadrants during every training trial until the twentieth trial when it was removed. The swim paths were monitored by a videocamera linked to a computer through an image analyser. The computer calculated the total distance swum as well as the path lengths in all quadrants and annuli separately. The computer also calculated the swim speeds. The timing of the latency was started and ended by the experimenter.

Procedure. Rats were placed into the water, with the nose pointing toward the wall, at one of the four starting points that were ordered in a semi-random manner. The first swim of the day was always started from one of the points located farthest from the platform (north, east) and the starting location of the second swim of the day was a random choice between south and east. Testing consisted of five consecutive days of testing, followed by a break of two days, and then five additional testing days. The twentieth trial was a spatial probe test with no platform. On each trial, the rats were allowed a maximum of 90 seconds to find the hidden platform. If the rat found the platform it was allowed to stay there for 10 seconds. Rats that failed to find the platform within 90 seconds were placed on it for 10 seconds. A 30-second recovery period was used between daily trials. The twentieth trial was used as a spatial probe trial to measure the distance swum in the training quadrant. Spatial bias was expressed as the percentage of the total distance swum in the previous training quadrant during the probe trial.

Statistics. Behavioral data (escape latency, path length, swim speed and spatial bias) and results of the biochemical analyses were analysed using one-way ANOVA followed by a Scheffe post hoc multiple comparison.

RESULTS

Histology

Histological analysis of the hematoxylin-eosin-stained sections revealed that the electrolytic lesion destroyed the MS and the dorsal part of the vertical diagonal band of Broca. Figure 1 shows reconstructions of the minimum and maximum extent of the MS lesions.

Biochemistry

Results from biochemical analyses are shown in Table 1. Analysis of hippocampal AChE and ChAT activities revealed a significant overall group effect [AChE: $F(3)=21.1$, $p<0.01$; ChAT: $F(3)=17.1$, $p<0.01$]. Lesioned animals had an average AChE depletion of 51% and a ChAT depletion of 60% in the hippocampus (Scheffe, $p<0.05$). There were no differences in the levels of either AChE or ChAT depletions between the lesioned groups (Scheffe, $p>0.05$).

Behavior

The main group effect was significant in the analysis of escape latency, $F(3)=31.1$, $p<0.001$ (data not shown). Analysis of escape latencies revealed that all the MS-lesioned groups were slower than the controls in finding the escape platform (Scheffe, $p<0.05$ in all comparisons). Comparison between the saline- and

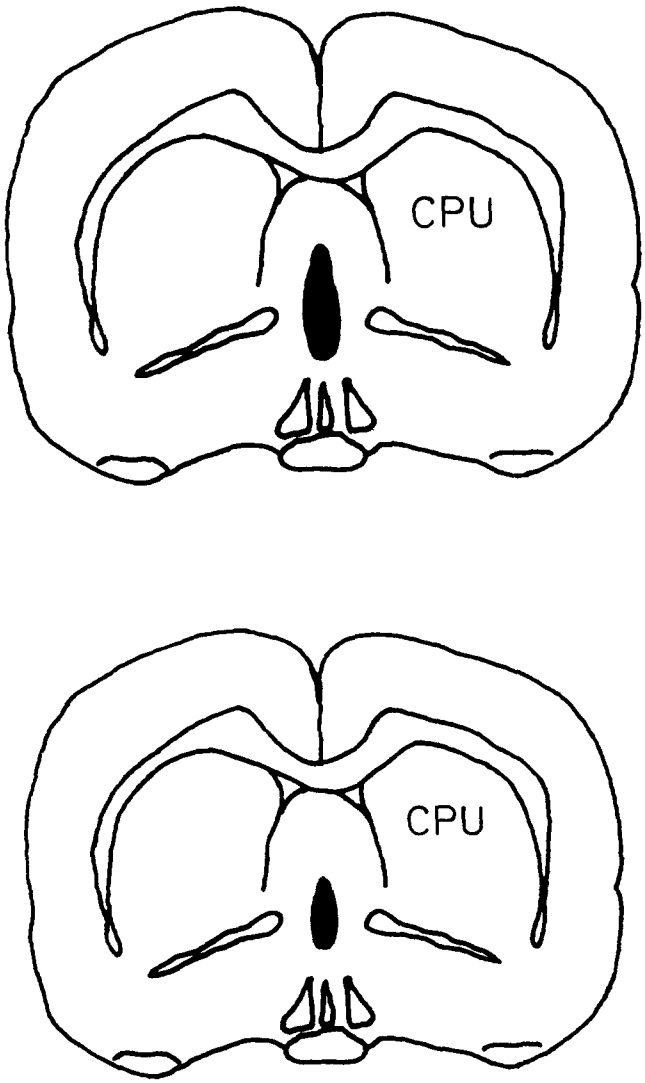

FIG. 1. Reconstruction of maximal (top) and minimal (bottom) extents of MS lesions. CPU = caudate putamen.

THA- (3 mg/kg) treated MS-lesioned rats showed no significant difference in escape latency (Scheffe, $p>0.05$); however, the group of MS-lesioned rats receiving THA 5 mg/kg were slower than any of these groups (Scheffe, $p<0.05$ in both comparisons). Analysis of the path lengths revealed a marked overall group effect, $F(3)=33.1$, $p<0.01$ (Fig. 2). Compared to the controls only the MS-lesioned groups injected with saline and THA 5 mg/kg were impaired. The MS-lesioned group injected with THA 3 mg/kg did not differ significantly from the control group (Scheffe, $p>0.05$).

TABLE 1

ACETYLCHOLINESTERASE (AChE) AND CHOLINEACETYLTRANSFERASE (ChAT) ACTIVITIES IN THE HIPPOCAMPUS OF CONTROLS AND LESIONED RATS

Group	AChE	ChAT
Controls	63 ± 5	1.20 ± 0.2
Medial septal lesioned	32 ± 8*	0.46 ± 0.3*
Medial septal lesioned (THA 3 mg/kg)	33 ± 7*	0.48 ± 0.3*
Medial septal lesioned (THA 5 mg/kg)	32 ± 9*	0.49 ± 0.2*

AChE activities (nmol/min/kg protein) are expressed as a mean ± SD.
*$p<0.05$ vs. controls, Scheffe post hoc multiple comparison.

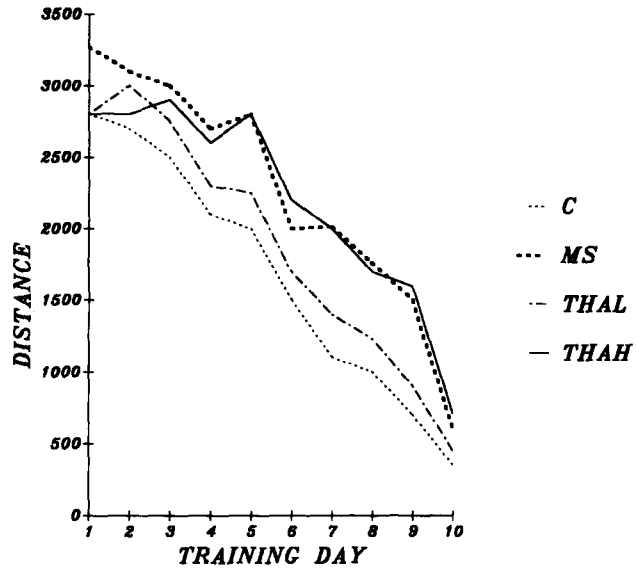

FIG. 2. Path lengths of the training trials. The Y-axis indicates escape distance given by the computer system (arbitrary units). The X-axis indicates training days (1–10). Group abbreviations: C = Controls, MS = medial septal lesioned receiving NaCl 0.9%, THAL = medial septal lesioned receiving THA 3 mg/kg, THAH = medial septal lesioned receiving THA 5 mg/kg. The Scheffe post hoc multiple group comparison revealed that the MS and THAH groups were impaired compared to the controls.

In the spatial probe trial, the percentage of the total distance swum in the quadrant of the previous platform location is used as an index of memory. The results of the spatial probe trial are shown in Fig. 3. The main group effect was significant in the spatial bias analysis, $F(3)=25.1$, $p<0.01$. Group comparison revealed that the controls and the MS-lesioned rats injected with THA 3 mg/kg did not differ significantly. However, the two other MS-lesioned groups (saline and THA 5 mg/kg injected) showed impairment ($p>0.05$).

The main group effect was significant in the swim speed analysis, $F(3)=34.1$, $p<0.001$ (data not shown). Group comparisons revealed that the MS-lesioned rats injected with saline swam faster and that rats injected with THA 5 mg/kg swam slower than the controls (Scheffe, $p<0.05$). The group of MS-lesioned rats injected with THA 3 mg/kg and the controls did not differ significantly (Scheffe, $p<0.05$).

DISCUSSION

In the present study, MS-lesioned rats were impaired in both the acquisition and retention of the platform location. This finding is in good agreement with earlier data (20). Furthermore, MS lesioning increased motor activity, as revealed in the higher swim speeds of the lesioned rats. Since the learning performance in locating the platform depends on extra-maze cues that are kept

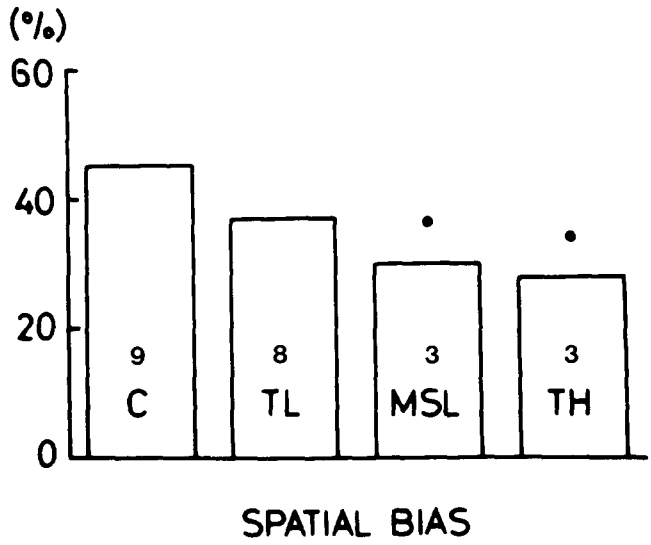

FIG. 3. The relative distance swum in the training quadrant during the spatial probe trial. Values are expressed as percentages of the total distance swum (mean ± S.D.). Group abbreviations: C = Controls, TL = medial septal lesioned receiving THA 3 mg/kg, MSL = medial septal lesioned, TH = medial septal lesioned receiving THA 5 mg/kg. • $p<0.05$ vs. controls, Scheffe post hoc multiple comparison.

unchanged during the course of training, the level of performance can be interpreted as an index of spatial reference memory. Therefore, the main findings of the present study, partial restorations by THA 3 mg/kg of path length increase and spatial bias decrease induced by MS-lesioning can be interpreted in terms of improved spatial memory function. Moreover, motorhyperactivity was reversed by THA 3 mg/kg. Interestingly, the higher dose of THA used (5 mg/kg) further increased escape latencies and did not restore either the path length or the spatial bias deficits induced by MS lesions. Moreover, the swim speed was lowest in this group.

The electrolytic method used to lesion the MS partly denervated the hippocampus from the cholinergic innervation, but due to the nonspecificity of the lesioning method used, the destruction of noncholinergic neurons is evident. The present results demonstrating a partial recovery of spatial learning deficits induced by nonspecific MS lesions with the anticholinesterase, THA, support the importance of cholinergic cell loss in the behavioral defects observed. Indeed, it has previously been shown that spatial learning impairments induced by fimbria fornix transsection, which would destroy both cholinergic and glutamatergic fibers, is partly alleviated by a muscarinic agonist, oxotremorine (18).

The failure of THA to completely reverse the spatial navigation impairment induced by MS lesioning could be explained in several ways. THA might possibly overstimulate peripheral and other central cholinergic pathways and may have induced side effects detrimental to the task performance. However, only THA 5 mg/kg decreased the swim speed and resulted in an impairment in the test of motor coordination. In contrast, the 3 mg/kg dose restored the swim speed to that of the controls and induced no deficits in the test of motor coordination. Another explanation might be that the doses used did not completely restore cholinergic activity within the hippocampus. Finally, since the MS lesioning method used in the present study may have possibly destroyed both cholinergic and noncholinergic cells, restoration of septohippocampal cholinergic transmission could have possibly reversed only that component of the memory impairment resulting from the loss of cholinergic neurons.

These results are interesting in view of the recent reports demonstrating a rehabilitation of memory function in AD patients by THA (23,24). The improved reference memory of MS-lesioned rats due to THA suggests that the model in the present study could be effective in screening potential treatments for age-related memory impairments. Furthermore, these data and the results of Kwo-on-Yuen and Thal (11), demonstrating the amelioration by THA of the nucleus basalis lesion-induced behavioral defect in a water maze, lend additional empirical support for the use of THA and other cholinomimetics in clinical trials of treatment strategies for Alzheimer's disease.

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