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Effects of THA on Passive Avoidance and Spatial Performance in Quisqualic Acid Nucleus Basalis-Lesioned Rats

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AALTONEN, M., P. RIEKKINEN, J. SIRVIÖ AND P. RIEKKINEN, JR. *Effects of THA on passive avoidance and spatial performance in quisqualic acid nucleus basalis-lesioned rats.* PHARMACOL BIOCHEM BEHAV **39**(3) 563–567, 1991.— Bilateral quisqualic acid nucleus basalis (NB) lesions impaired passive avoidance (PA) retention. NB lesions did not impair acquisition performance (stable platform location) in the water maze (WM). However, NB-lesioned rats were impaired in learning the new location of the escape platform in WM. Pretraining injections of tacridine (an anticholinesterase, THA) at 3 mg/kg, but not at 1 mg/kg, slightly improved PA retention performance in NB-lesioned rats. THA (1 or 3 mg/kg) did not alleviate NB lesion-induced WM defect. The results further suggest that loss of NB neurons impair PA acquisition and relearning of the new platform location in WM, and that cholinergic neuron loss may be at least partially involved in the NB lesion-induced performance defect.

Quisqualic acid Nucleus basalis Acetylcholine Passive avoidance Water maze relearning Alzheimer's disease THA

CENTRAL cholinergic transmission has been implicated in learning and arousal functions (3, 4, 7, 15, 21, 28). Drugs that block the activity of the cholinergic system impair learning, whereas stimulation of the cholinergic system may enhance cognitive performance (5, 6, 8, 15, 20). Interest in the functions of the basal forebrain cholinergic neurons was kindled by clinical studies demonstrating that the severity of cholinergic neuron loss in the NB is related to the degree of clinical dementia in Alzheimer's disease (AD) patients (13, 14, 30). These findings have led to the development of the "cholinergic hypothesis" of age-related memory deficits and have initiated the attempts to develop a cholinergic therapy for AD-related memory deficits (7, 13, 20, 23, 25, 27). The increase of central cholinergic activity by inhibiting the breakdown of acetylcholine by the administration of acetylcholinesterase (AChE) inhibitors [e.g., tacridine (THA)] (23, 25, 26) is a pharmacological strategy for restoring the activity of a damaged NB cholinergic system.

Interestingly, several studies have suggested that ibotenic acid (ibo) lesioning of the NB impairs performance in a large number of tasks used to assess learning and memory [e.g., passive avoidance (PA) and water maze (WM) reference memory performance] (2, 6, 8, 19). However, ibo is not selective for cholinergic neurons, and therefore, the contribution of non-cholinergic neuron loss to NB lesion-induced behavioral deficits cannot be excluded (3, 10, 18, 19, 21, 29). Indeed, recent studies have questioned the importance of cholinergic neuron loss for some of the learning defects induced by nonselective ibo NB lesioning (3, 10, 18, 19, 21, 29). For example, quisqualic acid (quis) NB lesions produce smaller nonspecific subcortical damage than do ibo lesions, but similar cholinergic neuron loss (3,18). Yet, in a WM, quis produced less of a behavioral defect than did ibo (3,18).

Parametric manipulations of task demand on memory are critical for both theoretical and conceptual analyses of mnemonic functions (12). Therefore, it would be interesting to determine whether quis NB lesioning impairs performance in a more difficult WM training paradigm than the reference memory paradigm previously used.

In the present investigation, we wanted to study the effects of bilateral quisqualic acid NB lesioning on PA retention and on a modified training paradigm (fixed location of the platform during the training period, reversal of the platform location during the subsequent trial blocks). Furthermore, the importance of cholinergic neuron loss for the quis NB lesion-induced performance deficits was investigated by studying the efficacy of THA in reversing lesion-induced performance defects.

METHOD

Animals and Surgery

Thirty-two male (8 controls, 8 NB-lesioned saline, 8 NB-lesioned THA 1 mg/kg, 8 NB-lesioned THA 3 mg/kg) Kuo:Wistar

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TABLE 1

CHOLINE ACETYLTRANSFERASE (ChAT) ACTIVITY (nmol/mg PROTEIN/MINUTE) IN THE FRONTAL CORTEX (FR) OF QUISQUALIC ACID NUCLEUS BASALIS (NB)-LESIONED AND CONTROL RATS

Groups	ChAT Activity in the FR
Controls (saline)	1.18 ± 0.2
NB-lesion (saline)	0.42 ± 0.2*
NB-lesion (THA 1 mg/kg)	0.50 ± 0.2*
NB-lesion (THA 3 mg/kg)	0.46 ± 0.1*

The results are expressed as mean ± S.D.
*$p<0.05$ vs. controls, Duncan's post hoc multiple group comparison.

TABLE 2A

PASSIVE AVOIDANCE TRAINING TRIAL ENTRY LATENCY (EL, SECOND, s), TOTAL SHOCK DURATION (TSD, SECOND, s) AND RE-ENTRY (RE) VALUES

Groups	EL (s)	TSD (s)	RE
Controls (saline)	32 ± 16	1.9 ± 1.0	0.8 ± 0.4
NB-lesioned (saline)	36 ± 12	1.7 ± 2.0	1.0 ± 0.4
NB-lesioned (THA 1 mg/kg)	41 ± 14	2.7 ± 1.1	1.3 ± 1.2
NB-lesioned (THA 3 mg/kg)	71 ± 22*	2.2 ± 1.3	0.6 ± 0.4

Values are expressed as mean ± S.D.
*$p<0.05$ vs. controls, Duncan's post hoc multiple group comparison.

rats (250–275 g) were used in the experiment. Quis lesions were made as following: 0.8 μl per hemisphere (quis 0.12 M in phosphate buffered saline, pH 7.4) was used to bilaterally lesion the NB (AP: −0.8 mm, DV: −7.7 mm, ML: ±2.7 mm). The control rats were subjected to a similar operation, but the infusion needle was lowered to only DV −6.0 mm relative to the bregma, and no infusions were made, in order to avoid damaging the NB. Two weeks after lesioning, behavioral testing was started.

Drugs

THA was dissolved in saline and injected IP 30 min before the PA training trial and 20 min before behavioral testing was started in the WM on the second training day. Previously, it has been shown that THA produces a long-lasting AChE inhibition (8,23). Therefore, it is reasonable to believe that THA inhibited AChE activity during the training period (3 h) on the second day of the WM experiment.

Histology and Biochemistry

After decapitation of the rats, a coronal slice containing the NB tissue was placed into 4% formalin for 10 hours. Then the tissue was immersed in 30% sucrose. Serial sections (40 μm) were stained with hematoxylin-eosin and acetylcholinesterase (AChE) to locate NB lesions. After collecting the histological sample, dorsal frontal neocortex (40–60 mg) was dissected on ice. Brain samples were stored at −72°C until assayed. ChAT activity was measured according to the method of Fonnum (1975).

Passive Avoidance

A Plexiglas PA box was divided into 2 compartments by a sliding guillotine door. The dark compartment had a metal grid floor. The rats were placed on the lighted side. After 10 s, the door opened into the dark compartment. Five s after the entry of the rats into the dark chamber, a 1.0-mA shock was initiated and maintained, until the rats escaped from the dark compartment and until a training criterion of avoiding the dark compartment for a 1-min interval was attained. Entry latency, number of reentries and cumulative shock duration were measured during the training trial. Seven days after training, the rats were placed in the lighted side of the apparatus, and the door was opened 10 s later. The latency to enter the dark chamber was measured.

Water-Maze

The WM testing system is described in detail elsewhere (15). 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 as well as the path lengths in all quadrants and annuli separately. The timing of the latency was started and ended by an experimenter. During the 1st training day, 3 trial blocks of 3 training trials were assessed (70-s maximum duration, 10-s reinforcement period on the platform, 20-s recovery period). The platform was located in the southwest quadrant during the 1st day. On the 2nd day, 3 trial blocks were assessed, and location of the platform was changed 3 times: trials 1–3 northeast, trials 4–6 southeast, trials 7–9 northwest. The training trials lasted for 70 s (10-s reinforcement period, 20-s recovery) during the 2nd day.

Statistics

Split-plot ANOVA was used to analyse group (1st day: mean values of the 3 training trials of a trial block; 2nd day: trial) effects on escape distance values. One-way ANOVA followed by Duncan's post hoc multiple group comparison were used to analyse group differences.

RESULTS

Histology and Biochemistry

Quis infusions produced gliosis and loss of AChE-positive neurons in the ventromedial globus pallidus (Fig. 1). ChAT activity (Table 1) was decreased in the frontal cortex, $F(3,28)=12.1$, $p<0.05$. No significant differences were found in the analysis of the ChAT activity of quis NB-lesioned groups ($p>0.05$).

Passive Avoidance

Analysis of the PA acquisition trial results (Table 2A) revealed that NB-lesioned rats treated with 3 mg/kg THA ($p<0.05$)

TABLE 2B

PASSIVE AVOIDANCE RETENTION TRIAL ENTRY LATENCY (SECOND, s) VALUES

Groups	Latency (s)
Controls (saline)	343 ± 23
NB-lesioned (saline)	67 ± 54*
NB-lesioned (THA 1 mg/kg)	90 ± 34*
NB-lesioned (THA 3 mg/kg)	167 ± 44*†

Values are expressed as mean ± S.D.
*$p<0.05$ vs. controls and †$p<0.05$ vs. NB-lesioned saline-treated rats, Duncan's post hoc multiple group comparison.

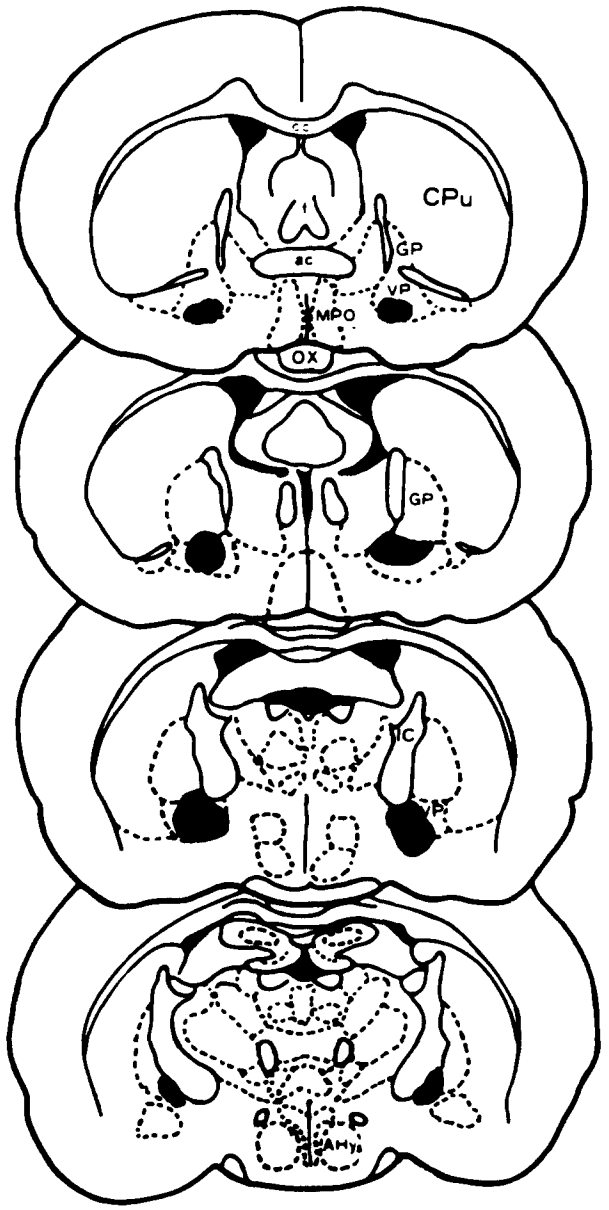

FIG. 1. A coronal reconstruction of a typical quisqualic acid nucleus basalis lesion. Black area indicates cell loss. Abbrevations: AC = anterior commissure, AHy = anterior hypothalamus, CPU = caudate putamen, GP = globus pallidus, IC = internal capsule, MPO = medial preoptic area, VP = ventral pallidum, OX = optic chiasm.

entered the dark compartment more slowly than the controls, $F(3,28) = 6.0$, $p<0.05$. No significant group effect was observed in the analysis of the total shock duration, $F(3,28) = 0.1$, $p>0.05$, and number of reentries, $F(3,28) = 0.4$, $p>0.05$, measured during the acquisition trial.

A significant group effect was observed in the analysis of the PA retention trial entry latency data, $F(3,28) = 7.8$, $p<0.05$ (Table 2B). NB quis-lesioned saline- or THA- (1 or at 3 mg/kg) treated rats were impaired ($p<0.05$) compared with the controls. THA at 3 mg/kg ($p<0.05$), but not at 1 mg/kg ($p>0.05$), slightly improved retention in quis NB-lesioned rats.

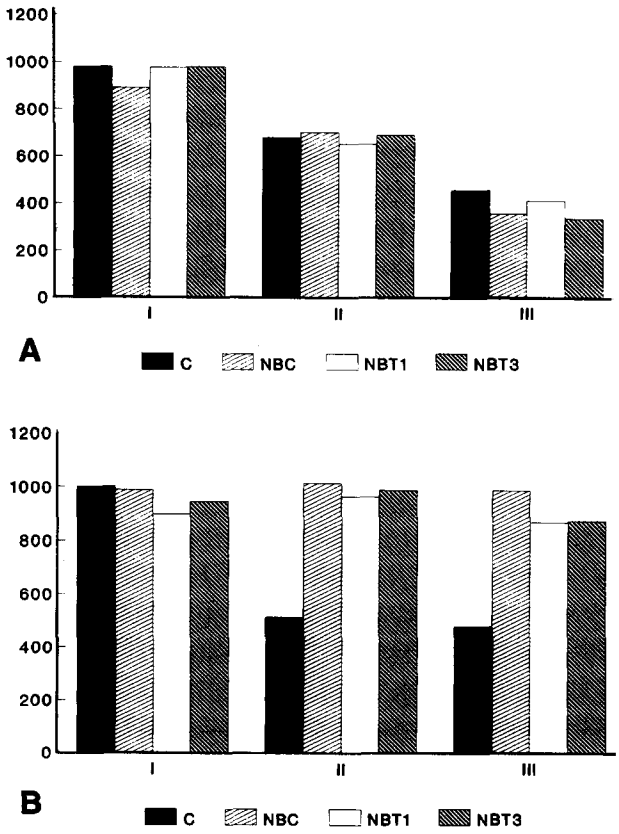

FIG. 2. Effects of THA on the water maze performance of quisqualic acid nucleus basalis-lesioned rats. The values shown on the Y axis represent distance units given by the computer system (arbitrary pixels). The X axis represents the group mean values of daily trial blocks (I, II and III = 1st, 2nd and 3rd trial blocks). Abbrevations: C = controls, NBC = nucleus basalis lesioned (saline), NBT1 = nucleus basalis lesioned (THA 1 mg/kg), NBT3 = nucleus basalis lesioned (THA 3 mg/kg). (A) Escape distance values measured during the first training day. (B) Escape distance values measured during the second training day.

Water Maze

No significant group effect, $F(3,28) = 0.9$, $p>0.05$, was observed in the analysis of the escape distance values measured during the trial blocks of the 1st training day (Fig. 2A).

No significant group effect, $F(3,28) = 0.2$, $p>0.05$, was observed in the analysis of the escape distance values measured during the 1st trial block in the 2nd training day (Fig. 2B).

In the analysis of the escape distance data measured during the 2nd trial block, a significant group effect, $F(3,28) = 12.1$, $p<0.05$, was observed. Quis NB lesioned rats treated with saline were impaired compared with the controls ($p<0.05$). THA at 1 or 3 mg/kg did not improve quis NB lesion-induced performance defect ($p>0.05$).

A significant group effect was observed in the analysis of the 3rd trial block escape distance data, $F(3,28) = 13.1$, $p<0.05$. Quis NB-lesioned rats treated with either saline or THA at 1 or 3 mg/kg were impaired ($p<0.05$).

Analysis of the swimming speed data measured during the 1st training day revealed no significant differences [group: $F(3,28) = 0.3$, $p>0.05$] (Table 3). During the 1st [group: $F(3,28) = 14.2$, $p<0.05$], 2nd [group: $F(3,28) = 16.1$, $p<0.05$] and 3rd

TABLE 3

THA AT 1 AND 3 mg/kg INDUCED CHANGES IN THE SWIMMING SPEED IN WATER-MAZE TEST

Group	Relative Swimming Speed (%)
Controls (saline)	100 ± 12
NB-lesion (saline)	104 ± 10
NB-lesion (THA 1 mg/kg)	94 ± 9
NB-lesion (THA 3 mg/kg)	70 ± 7*

Mean ± S.D. are shown. Control level = 100%.
*$p<0.05$ vs. controls, Duncan's post hoc multiple group comparison.

[group: $F(3,28)=13.3$, $p<0.05$] trial blocks of the 2nd day, significant group-induced changes were observed in the analysis of swimming speed. THA at 3 mg/kg decreased swimming speed compared with all the other groups ($p<0.05$).

DISCUSSION

The present results demonstrating that THA slightly improved the PA retention defect may further support the importance of cholinergic neuron loss in the NB lesion-induced PA performance defect. Indeed, previous studies have shown that muscarinic and nicotinic antagonists impair PA retention in a similar way to that observed after NB lesions (5,16). Infusion of fetal ventral forebrain grafts rich in developing cholinergic cell bodies into the frontal cortex stabilizes PA retention defect induced by NB destruction (2). Finally, the age-related loss of NB ChAT-positive neurons correlates with the severity of PA retention defect (17). However, with the available nonselective lesioning methods, the importance of lesion-induced noncholinergic neuron loss in PA retention defects cannot be excluded. Indeed, THA only slightly stabilized PA retention impairment. Importantly, it is also possible that THA may have improved PA retention performance by stimulating some other cholinergic system(s). However, THA at 1 or 3 mg/kg (Riekkinen, Jr. et al., unpublished) did not improve PA or WM performance in intact rats, further suggesting that the PA-performance-stabilizing action of THA in NB-lesioned rats may be mediated by increased activity of the NB cholinergic system.

The present study also agrees with previous experiments demonstrating that quis NB lesions do not impair, or produce only slight defects, in WM spatial performance if the platform location is stable during training (3,18). Interestingly, quis NB lesioning did not impair performance during the first platform reversal, but during the second and third platform reversals, the spatial performance of quis NB-lesioned rats was impaired. However, it is not clear to what extent the reversal learning defect in WM is due to memory deficit. For example, quis NB lesioning impairs selective attention and acquisition of conditional discrimination (4,21). Furthermore, NB regulates cortical electrical arousal: Slow-wave and high-voltage spindle activity are increased in rats subjected to NB lesioning (15,18). Therefore, it is tempting to speculate that impaired arousal/attentional functions or conditioning to relevant cues may contribute to the reversal learning deficit of quis NB-lesioned rats.

The lack of effect of THA on quis NB lesion-induced WM defect may be interpreted as suggesting that the cholinergic neuron loss may not be involved in the reversal learning defect. Another explanation for our failure to demonstrate THA-induced recovery of reversal learning WM defect or complete stabilization of PA failure may be the lack of an optimal dose. Firstly, THA at 1 or 3 mg/kg may have insufficiently increased acetylcholine within the cholinergic synapses of the cortex, and therefore, the doses selected may not have restored cholinergic activity in the NB target areas (25). Secondly, since THA is not selective for NB cholinergic neurons, it may have induced side effects detrimental to performance. Indeed, THA at only slightly higher concentrations than used in the present study severely impaired swimming performance and increased escape path lengths of medial septal-lesioned rats in the WM (20). Furthermore, THA impaired motor performance in PA (increase in training trial escape latency values) and WM (decrease in swimming speed) tests in the present study also. Thirdly, tonic stimulation of acetylcholine receptors by the THA-induced increased levels of acetylcholine may not be able to completely alleviate the functional deficit of the NB cholinergic system. Indeed, previously it has been shown that the NB neurons have spontaneous discharge rates with highly variable patterns and wave shapes, and many neurons undergo rapid changes in activity in relation to various stimuli and behaviors (1, 9, 11, 22).

These results may have relevance for aging and AD, which are associated with a NB cholinergic neuron loss and cognitive defects (7, 13–15, 24–26). The present results provide further support for the importance of the cholinergic defect in AD-related cognitive disorders and suggest that THA may be effective in enhancing the activity of the cholinergic NB.

In conclusion, our results demonstrate that quis NB lesioning impairs PA retention and WM reversal learning, but only the PA deficit is stabilized by THA.

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