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Hippocampal Muscarinic Receptor Loss Following Trimethyl Tin Administration

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LOULLIS, C C , R L DEAN, A S LIPPA, D E CLODY AND J. COUPET. *Hippocampal muscarinic receptor loss following trimethyl tin administration* PHARMACOL BIOCHEM BEHAV 22(1) 147–151, 1985 —The effects of trimethyl tin on passive and active avoidance behavior, hippocampal muscarinic receptors and hippocampal cell destruction were examined in male rats. The animals were intubated with 18 μmoles/kg (3 5 mg/kg) of TMT hydrochloride or vehicle When tested two weeks later treated animals exhibited marked deficits in retention of passive avoidance and extinction of active avoidance tasks Receptor binding analysis, using 3H-QNB, revealed a significant decrease (21%) in muscarinic receptor density in the hippocampus Histological examination of the hippocampus revealed a concomitant loss in pyramidal cells in these animals These results suggest that muscarinic receptors reside on the hippocampal pyramidal cells and that these cells and receptors may be involved in retention of passive avoidance behavior

Trimethyl tin Hippocampal pyramidal cells Retention Passive avoidance Muscarinic receptors

CONSIDERABLE evidence exists indicating that the hippocampus is involved in cognitive functions and that lesions in this area result in severe deficits in learning and memory [10]. It has also been demonstrated that changes in cholinergic neurotransmission through pharmacological manipulations, including muscarinic receptor loss [12], can disrupt the retention of learning tasks [2,3].

The effects of acute administration of trimethyl tin (TMT) are distinguished from other organotin compounds by selective and persistent neuropathological changes in hippocampal pyramidal cells [5, 8, 9, 14] This relatively selective cell destruction by TMT may provide a useful neurobiological probe for investigating neurochemical connections in the hippocampus. Furthermore, the cognitive deficits which persist following TMT administration, as evidenced by impaired radial arm maze and passive avoidance performance [17,18], may provide a useful model for assessing hippocampal involvement in these deficits. The purpose of the present experiment was to investigate the effects of a low dose of TMT administration on passive and active avoidance behavior, hippocampal muscarinic receptors and neuroanatomical changes in the hippocampus

METHOD

Subjects, Drug Delivery and Behavioral Testing

Adult male wistar (Royalhart Ind.) rats were intubated once with a 0 9% saline or 18 μmoles/kg (3.5 mg/kg or 2.9 mg/kg as base) TMT hydrochloride (ICN Pharmaceutical, NY). Two weeks later, all animals were trained on a single trial passive avoidance task. TMT treated animals were sluggish for 3 to 4 days following intubation and appeared normal afterwards. We observed no other behavioral changes, such as seizures, self mutilation, hyperactivity or difficulty during handling.

Passive Avoidance

The passive avoidance apparatus was modified from Bartus *et al.* [4] and consisted of two chambers, a smaller lighted and larger dark compartment. Access from one to the other compartment could be controlled through a vertically moving trap door.

On the training day, each animal was placed in the lighted compartment, the trap door was raised and when the animal fully entered the dark compartment, a 0.5 mA footshock was delivered for 3 seconds. The latency in sec to enter the dark compartment was recorded and the animal was removed from the chamber and returned to its home cage Twenty-four hours after training animals were tested for retention in the exact manner as above except that footshock was not delivered when the animal entered the dark compartment. The latency in sec to enter the dark compartment was recorded A 600 sec maximum latency criterion was selected.

Active Avoidance

Animals treated with TMT which exhibited both a passive avoidance deficit and hippocampal muscarinic receptor loss (data not reported) were also tested for shock sensitivity (seventeen days post intubation) and on active avoidance

[1]Requests for reprints should be addressed to Dr Costas C Loullis, Department of CNS Research, Building 56B, Lederle Laboratories, Pearl River, NY 10965

TABLE 1
SHOCK SENSITIVITY EVALUATION

Current Level (mA)	% Rats Responding: Jump Response, Saline	Jump Response, TMT	Vocalization Response, Saline	Vocalization Response, TMT
0 05	0	0	0	0
0 10	0	0	20	40
0 20	80	80	60	80
0 30	100	100	100	100
0 4	100	100	100	100
0 50*	100	100	100	100

*Current level used for passive avoidance task

(three weeks post intubation) The active avoidance apparatus [7] consisted of a large lower compartment with a grid floor and a smaller platform 5 inches above the grid floor which could appear or disappear by moving a shelf on the back wall Animals were placed on the grid floor, a light came on for 5 sec and the small platform was uncovered If the animal did not jump onto the small compartment within 10 sec the light went off and a 1 mA pulsed shock (1 per 2 sec 0 25 sec duration) was delivered through the grid floor for 20 sec. The animal was allowed to stay on the small platform for 10 sec and then pushed back to the grid floor by moving the shelf on the back wall forward Following a 10 sec intertrial interval this procedure was repeated. Twenty-five such trials were given each day for four days (acquisition phase) On days five and six (extinction phase) the same procedure was repeated except that no shocks were delivered

Shock Sensitivity Measurement

Possible differences in shock threshold were also evaluated with the same shock grid and shock source used for the passive avoidance tasks. Six different current levels (see Table 1) were applied to the foot grids in ascending order (duration=0.5 sec), with a 15 second interval separating presentations. At each current level, an observer recorded whether the shock elicited a jump response (defined as a minimum of 2 paws leaving the grid) or a vocalization (any auditory response)

Dissection

Following passive avoidance or active avoidance testing all animals were sacrificed by decapitation and the brain was quickly removed from the cranium The left hemisphere was immersed in 10% buffered formalin for histological examination The hippocampus was quickly dissected, on ice, from the right hemisphere and stored at −20°C for neurochemical analysis

Muscarinic Receptor Binding

Hippocampal tissues were homogenized in 10 volumes (w/v) of ice-cold 0.32 M sucrose in glass-teflon homogenizers. 3H-QNB parameters for hippocampal tissues from each animal were determined as described elsewhere [19] Hippocampal membranes (final protein concentration in incubation medium was 0 05–0 07 mg per 2 ml final assay volume) were incubated for 1 hr at 25°C with varying concentrations of (−) 3H-QNB (33 1 Ci/mMole, New England Nuclear) in 50 mM sodium phosphate buffer, pH 7 4 Specific binding was defined as total binding minus binding in the presence of 10 μm atropine sulphate Incubation was terminated by collection of particulate protein and bound 3H-ligand by filtration on Whatman GF/B glass fiber filters, which were then washed 3 times with 3 ml of ice-cold buffer The filters were placed in glass scintillation vials, and following the addition of 10 ml Beckman Ready solv-HP scintillation fluid, the radioactivity was measured by liquid scintillation spectrometry at 55% efficiency for tritium

Protein Determination

Protein concentrations were determined by the Bio-Rad assay procedure (Bio-Rad, CA)

Histological Evaluation

The left hemisphere was serially sectioned in the sagittal plane at 10 mμ Sections were mounted on slides, stained with cresyl violet and cover-slipped for examination under a light microscope

RESULTS

In the passive avoidance procedure, the training latencies for the control and TMT groups were not significantly different The mean±S E M for the control group was 10 2±1 5 sec and for the TMT treated group 8 5±1 5 sec Retention latencies of the two groups, however, differed significantly on the test day The TMT treated group mean±S E M was 22±8 1 sec as compared to 347±108 sec for the control group (Fig 1)

The results of TMT treatment on active avoidance are illustrated in Fig 2 The control and TMT treated groups did not differ during the acquisition phase, on days 1 through 4, and attained criterion of 95–100% avoidance at the same rate During extinction, however, the TMT treated group differed significantly from the control group on the first day The difference is also apparent on the second day but it did not reach statistical significance

Jump and vocalization thresholds were performed on animals treated with vehicle and TMT Treated animals were not different from controls in their response to shock at the intensities used in the testing procedures These results are presented in Table 1

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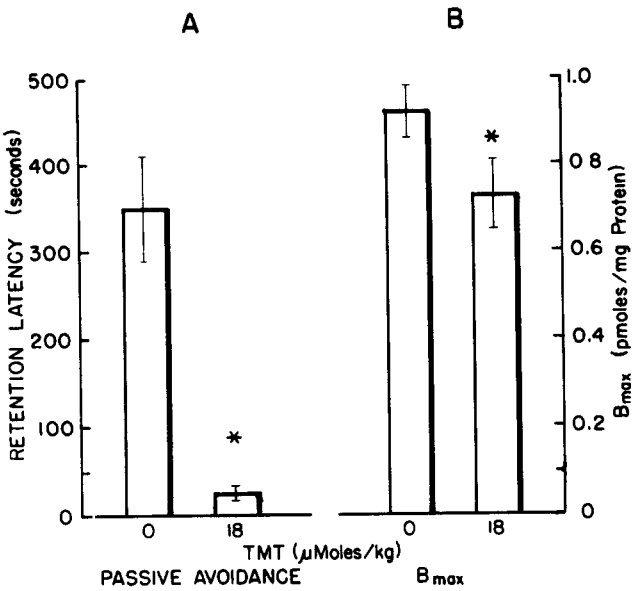

FIG 1 The means±S E M. of the retention latencies, in sec, for the control (n=10) and TMT (18 μMoles/g) treated (n=10) are depicted in part A The Bmax values, in pMoles/mg protein, in the hippocampus for the same groups (n=5) are shown in part B *$p<0$ 05, one-tailed t-test)

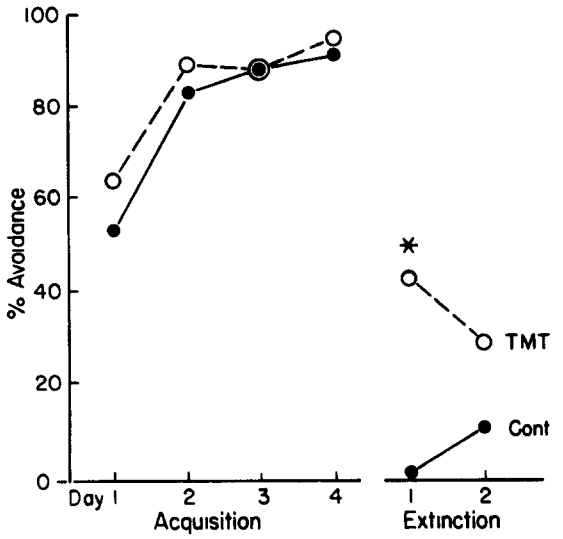

FIG 2 The means±S E M of % avoidance for the control (●—●) and TMT (18 μMoles/kg (○—○) groups (n=5) are illustrated as a function of days (25 trials/day) during the acquisition and extinction phases *$p<0$ 05, Mann-Whitney U test

3H-QNB Scatchard analysis revealed a significant decrease in muscarinic receptor density (Bmax) for TMT treated animals with no change in the affinity constant Group means±S E M for control and treated animals are presented in Fig 1

Light microscope examination of serially mounted sections of the hippocampus revealed a decrease in number and size of hippocampal pyramidal cells in the TMT (18 μMoles/kg) treated animals Representative sections, taken approximately 2 mm lateral to the mid-sagittal plane, for a control (Fig 3A) and an experimental animal are presented in Fig 3

DISCUSSION

Animals treated with TMT exhibited a significant deficit in retention of passive avoidance as evidenced by retention scores significantly lower than those of the control In contrast, the training day scores for the two groups were not different, indicating that the retention deficit cannot be accounted for by any gross motor impairments induced as a result of TMT administration Furthermore, this deficit could not be attributed to changes in shock sensitivity for animals receiving TMT, since jump and vocalization thresholds were not different from controls The results of the active avoidance testing suggest that learning deficits may not be present at this dose, since both groups behaved similarly during acquisition On the other hand, the differences between the groups during extinction indicate that these animals behave similarly to animals with hippocampal damage and exhibit perseverative behavior [11,13] It is unlikely that the effects of TMT on either passive or active avoidance are confounded by hyperactivity, since a previous study [15] using higher doses (5 or 6 mg/kg, base), as compared to this study (2.9 mg/kg, base), revealed no hyperactivity.

TMT treated animals exhibited a significant decrease in hippocampal muscarinic receptors and a concomitant loss of hippocampal pyramidal cells as revealed by 3H-QNB binding and neuroanatomical examination. These results indicate that the loss of hippocampal pyramidal cells observed in this and other studies [5, 8, 9, 14], is associated with the loss in muscarinic receptor density The dose of TMT used in the present study was considerably lower compared to doses used in previous studies and is below the dose (5 mg/kg, base) previously reported to spare damage to the dentate gyrus [8] It is therefore unlikely that changes in muscarinic receptor density in the dentate gyrus are responsible for the changes in behavior. Furthermore, histological examination of the hippocampus (Fig 3) illustrates that, at this low dose, damage is restricted to the CA3 pyramidal cell line where approximately 43% of hippocampal muscarinic receptors are located [1] This represents a substantial loss of muscarinic receptors in a small area of the hippocampus and may explain the large behavioral deficit as compared to the apparently small (21%) receptor loss observed when the entire area is assayed

During the preparation of this manuscript, data from a recent abstract [16] indicated that high doses of TMT (7.0 mg/kg) administered to rats resulted only in short-term changes in hippocampal muscarinic receptors and small long-term changes in the amygdala. These results are at variance with the present study where long-term changes in hippocampal muscarinic receptors have been demonstrated at half the TMT dose. The data from this abstract are also difficult to interpret with respect to the dose dependency of neuropathological changes which have been shown to occur across different brain regions [5] More specifically, it appears that at lower doses neuropathology is observed primarily in the hippocampus whereas with increasing doses cortical and then amygdaloid neuropathology occurs Thus it would be expected that changes in muscarinic binding would occur in the hippocampus before they were detected in the amygdala The discrepancy between the present data and