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Learning and Memory in Mice Treated with Choline Oxidase, a Hydrolytic Enzyme for Choline

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IKARASHI, Y., H. KURIBARA, T. SHIOBARA, A. TAKAHASHI, H. ISHIMARU AND Y. MARUYAMA. *Learning and memory in mice treated with choline oxidase, a hydrolytic enzyme for choline.* PHARMACOL BIOCHEM BEHAV **65**(3) 519–522, 2000.—Learning and maintenance of memory in mice intraperitoneally (IP) injected with choline oxidase (ChO, 6 units/g), a hydrolytic enzyme for choline (Ch), were assessed by means of a step-through passive-avoidance task. The ChO treatment induced a hydrolysis of free Ch in plasma, which in turn, induced a decrease in cerebral acetylcholine (ACh) release. In the learning test, the ChO-treated mice showed significant inhibition to learn the avoidance from electric shock. In the retention test, the impairment of the memory once established was not produced by posttreated ChO. We concluded that the decreased cerebral cholinergic neurotransmission induced by ChO retarded acquisition of passive-avoidance learning more readily than the maintenance of memory. © 2000 Elsevier Science Inc.

Acetylcholine Choline Choline oxidase Learning Memory Step-through passive-avoidance task Mice

RECENT evidence suggests that central cholinergic neuronal dysfunction may play an important role in learning impairments (7). The cholinergic neurotransmitter, acetylcholine (ACh), is synthesized from free Ch and acetyl-CoA in a reaction catalyzed by choline acetyltransferase (ChAT) (13). Central cholinergic neurons predominantly rely on the circulation as a primary source of free Ch (14), although Ch can be generated from Ch-containing phospholipids such as phosphatidyl Ch in the brain (9).

In our previous studies, we have demonstrated that free Ch in plasma is completely hydrolyzed by intravenous (IV) injection of choline oxidase (ChO), a hydrolytic enzyme for Ch (5), and that subsequent inhibition of the Ch supply to the brain produces a decrease in cerebral ACh release (6). These results suggest that ChO treatment might be a useful method for investigating learning and memory mechanisms associated with the central cholinergic system.

The purpose of the present study is to investigate the effects of ChO on learning and memory. For this purpose, a step-through passive-avoidance task (10), which has been proven to be appropriate for evaluation of learning and memory functions in rodents, was used.

METHOD

Reagents

ChO (Toyobo Co. Ltd., Osaka, Japan), 1000 units, dissolved in 1 ml of saline, was used in the present study. Ethylhomocholine iodine (EHC), an internal standard for determination of plasma Ch, was synthesized from 3-diamino-1-propernol (Sigma, St. Louis, MO) and iodoethane (Sigma).

Animals

Six-week-old male ddY mice weighing 28–30 g were obtained from Japan SLC, Inc. (Hamamatsu, Japan). They were kept at a temperature of 23 ± 1°C, relative humidity of 55 ± 5%, and controlled lighting, with lights on from 0700 to 1900 h daily. The animals were given standard laboratory food (Oriental Yeast, Tokyo, Japan) and water ad lib.

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Experimental protocols were approved by the Committee of Animal Experiments at Gunma University School of Medicine and met the "Guidelines for Animal Experimentation" of the Japanese Association of Laboratory Animal Science.

Step-Through Passive Avoidance

The apparatus for the step-through passive-avoidance test (10) consisted of two compartments: one illuminated [100 × 120 × 100 mm; light at the top of chamber (15 W)], and the other dark (100 × 170 × 100 mm). The compartments were separated by a guillotine door. In the acquisition test, the mouse was placed in the illuminated safe compartment. As the compartment was light, the mouse stepped through the opened guillotine door into the dark compartment. The time spent in the illuminated compartment until the animal entering into the dark compartment was measured as latency time. At 3 s after the mouse entered into the dark compartment, a foot shock (0.3 mA, 50 V, 50 Hz AC, for 3 s) was delivered to the floor grids in the dark compartment. The mouse could escape from the shock only by stepping back to the safe illuminated compartment. We judged that the mouse acquired avoidance from foot shock if it remained in the illuminated compartment for 300 s after being placed there.

The retention test using the mice acquired the avoidance (given the foot shock on day 8) were carried out on days 15 and 22. The step-through latency was measured for up to 300 s without delivering foot shock. We judged that the mouse maintained the avoidance memory when it stayed for 300 s in the illuminated compartment.

Ambulatory Activity

Ambulatory activity of mice was measured using a tilting-type ambulometer with 10 bucket-like Plexiglas cages (20 cm in diameter and 15 cm in height, SMA-10: O'Hara & Co., Tokyo, Japan). This apparatus was designed to selectively record ambulation of the mouse in the cage by detecting slight tilts of the cage generated by horizontal movements, but not any vertical movements or turning, of the mouse by means of microswitches attached to the cage (8).

Experimental Schedules

Twenty mice were divided into two groups: ChO-treated, and control. The animals were intraperitoneally (IP) injected with ChO (6 units/g body weight) in ChO-treated group (n = 10) or with saline (6 μl/g of body weight) in the control group (n = 10), for 8 days. Ambulatory activity and passive-avoidance learning of all mice were measured at 80 and 120 min, respectively, after injection of ChO or saline every day for an initial 7 days. On day 8, an unavoidable foot shock for 3 s was given to all mice in the dark compartment, and then the animals in each group were split into two subgroups. Half of the animals (n = 5) in each group were injected with ChO (6 units/g, IP) and the other half (n = 5) were injected with saline (6 μl/g, IP) for 14 days from day 9 to day 22. Ambulatory activity and retention of passive avoidance of all mice in these four subgroups were measured on days 15 and 22.

Determination of Plasma Ch levels in ChO-Treated Mice

In an other set of experiments, ChO (6 units/g, IP) was injected in 5 mice. Blood was collected at 3 h prior to injection, 0 (immediately before injection), 0.5, 2, 6, 12, 17, 20, 24, 27, and 30 h after ChO injection from the orbital sinus by using a heparinized hematocrit capillary (Iuch Co. Ltd., Tokyo, Japan), under 4.0% halothane anesthesia performed by a small-animal anesthetizer (model TK-4, Bio Machinery, Funabashi, Japan). After centrifugation of the collected blood at 3000 rpm for 10 min, 10 μl of plasma was mixed with an equal volume of 1 M $HClO_4$. EHC (0.5 nmol/10 μl) dissolved in 0.5 M $HClO_4$ was added to it as internal standard. The mixture was passed through a 0.45-μm millipore filter. The filtrate, 5 μl, was injected into a liquid chromatograph equipped with an electrochemical detection (LC-ECD) system for determination of the Ch level (3).

RESULTS

Changes in Plasma Ch levels in ChO-Treated Mice

Changes in plasma Ch levels after IP injection of ChO (6 units/g) in mice are shown in Fig. 1. The normal Ch level in mice plasma was 11.0 ± 0.8 (mean ± SE) nmol/ml. Plasma Ch could not be detected immediately after the IP injection of ChO. The disappearance of plasma Ch continued for up to 20 h. Thereafter, the Ch level recovered approximately 22 and 72% of the normal level at 24 and 30 h, respectively, after ChO injection.

Step-Through Passive-Avoidance and Ambulatory Activity

The results of passive-avoidance learning in ChO-treated and control mice are shown in Fig. 2A (percentage of mice that learned avoidance) and B (latency time of step-through). In the first acquisition trial, all mice in the ChO-treated and control groups entered into the dark compartment immediately after being placed in the illuminated compartment. No significant difference was observed in the latencies between the groups on the first day. The latency time of control group was increased by repeating the acquisition trial. On the fifth day, all control mice (100%) stayed in the illuminated compartment over 300 s. On the other hand, the latency time of the ChO-treated group was significantly lower when compared with the controls, on the third ($p < 0.05$) and on fourth days ($p < 0.01$). It took 7 days until all ChO-treated mice stayed in the illuminated compartment over 300 s.

The ambulatory activities of the ChO-treated and control mice during avoidance learning processes (days 1 to 7) are shown in Fig. 3. Although irregular inhibition of the activity was observed on days 2 and 7 in the ChO-treated group, no significant differences were observed between the two groups on other days.

In retention tests on days 15 and 22, the latency times of mice in the four subgroups, posttreated with ChO and saline

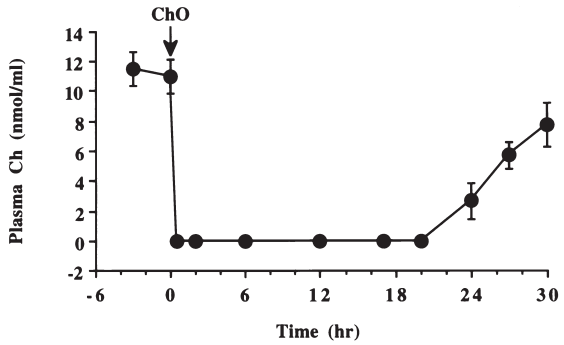

FIG. 1. Changes in free Ch levels in mice plasma after IP injection of ChO (6 units/g). Each value represents the mean ± SE (n = 5).

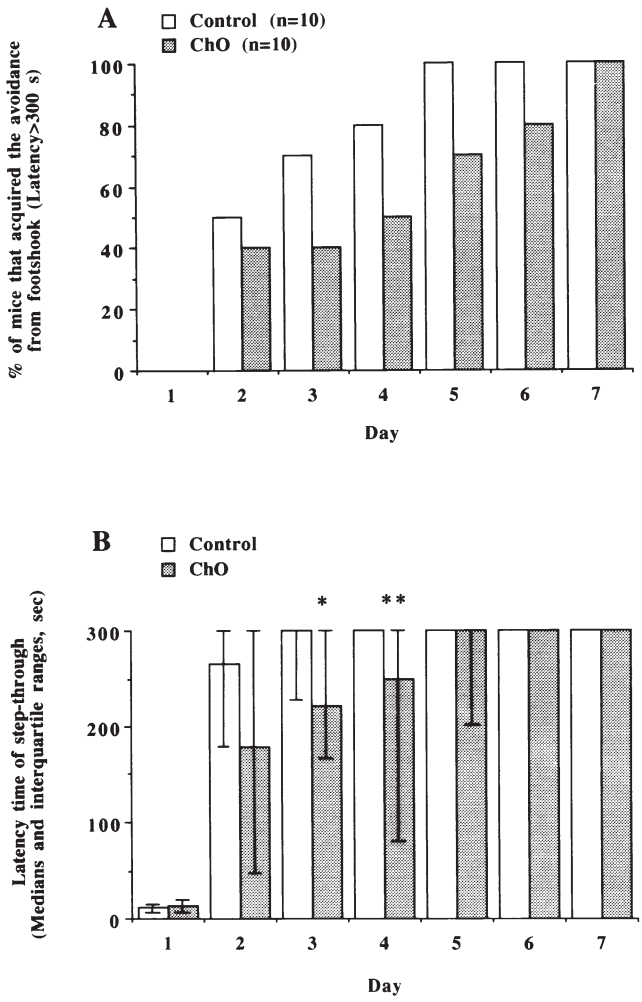

FIG. 2. Effect of ChO on passive-avoidance learning. (A) Indicates percentage of mice that learned avoidance (n = 10). (B) Indicates latency time of step-through passive avoidance. The data are expressed as medians and interquartile ranges (n = 10). Statistical significance was assessed by using Mann–Whitney U-test ($^*p < 0.05$ and $^{**}p < 0.01$, to the corresponding control group).

in the saline pretreated group, and posttreated with ChO and saline in the pretreated ChO group, were over 300 s. No significant differences in the ambulatory activity were observed among these four subgroups on days 15 and 22, as shown in Table 1.

DISCUSSION

By the determining plasma Ch, IP injection of ChO induced a long-lasting decrease in the free Ch level in the mouse plasma. This result suggests, as demonstrated in rats IV injected with ChO (5,6), that long-lasting decrease in plasma Ch level may induce not only a 70% decrease in extracellular concentration of free Ch but also a 40% decrease in ACh release in the brain of ChO-treated mouse. It implies that ChO treatment induces a decrease in cerebral cholinergic neurotransmission.

In the passive-avoidance test, ChO-treated mice showed significantly lower latency times on days 3 and 4, i.e., impairment of learning. However, no significant differences in ambulatory activity between ChO and the control groups were observed on these days, suggesting that the shorter latency times in ChO-treated mice were not a consequence of changes in ambulatory activity. Taken together, these results indicate that ChO treatment inhibits leaning of a passive-avoidance task. This hypothesis may be supported by the findings that rats fed a Ch-deficient diet (4,12) and senescence-accelerated mice (10) show impaired learning with decreased brain ACh content. Interestingly, even if cerebral cholinergic neurotransmission was inhibited by ChO treatment, all ChO-treated mice could learn to avoid the shock by taking a longer time (7 days) than the control mice (5 days). This finding may suggest the importance of repeated training to learn the passive-avoidance task in the mice with decreased cholinergic neurotransmission.

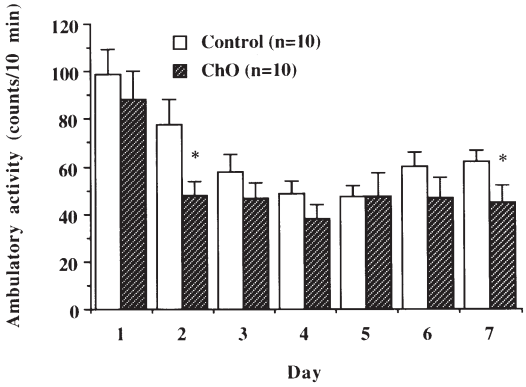

FIG. 3. Ambulatory activity in the ChO-treated and control mice during the acquisition period of passive avoidance. Each value represents the mean ± SE (n = 10). Statistical significance was assessed by using a two-way ANOVA with Students' t-test ($^*p < 0.05$, to the corresponding control group).

Although all mice treated with ChO or saline acquired avoidance learning at least within 7 days, the number of electric shocks required for learning differed among mice. Therefore, to avoid the effect of the difference in the number of shocks on extinction, on day 8, all mice were given an unavoidable electric shock to securely acquire the memory, and subsequently the retention tests were held on days 15 and 22 to evaluate the effects of ChO on the maintenance of memory. Thus, from the comparison among the four subgroups, we could evaluate that the effects of posttreated ChO on the memory once established under normal condition or under abnormal conditions lowered ACh release induced by the ChO treatment. The results (all mice in four subgroups stayed

TABLE 1

AMBULATORY ACTIVITY IN THE ChO- AND SALINE-TREATED MICE DURING THE PERIOD OF RETENTION TESTS OF PASSIVE AVOIDANCE

Days 1–8	Control (Saline)		ChO	
Days 9–22	Saline	ChO	Saline	ChO
Day 15	66.4 ± 8.1	49.8 ± 8.8	51.6 ± 11.5	65.2 ± 11.0
Day 22	63.2 ± 9.7	62.6 ± 13.3	65.8 ± 18.1	66.0 ± 15.5

Each value represents the mean ± SE (counts/10 min) for five animals. Statistical significance was assessed by using a two-way ANOVA with Student's t-test. No significant differences were observed among the four groups.

over 300 s in the illuminated compartment) of the retention test suggested that the memory, once established, may be scarcely influenced by ChO treatment even under the condition of ChO-induced lower cholinergic neurotransmission.

We have demonstrated in a step-down passive-avoidance task in rats that the maintenance of memory was impaired by neuronal degeneration induced by the intraventricular infusion of a protease inhibitor, leupeptin (1). The impairment of memory has also been reported in the amnesic models induced by scopolamine, a muscarinic receptor antagonist (2), and destruction of the basal forebrain (11). These results suggest that impairment of memory might occur when other factors, in addition to decreased ACh release, are involved in the degeneration of cholinergic neurotransmission.

In conclusion, the decreased cerebral cholinergic neurotransmission induced by ChO impaired the acquisition of passive-avoidance learning more readily than the maintenance of memory. These findings might be useful for further investigation of the effects of the cholinergic mechanism in learning and memory processes.

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