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Passive Avoidance Deficits In Mice Following Ethylcholine Aziridinium Chloride Treatment

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POPE, C. N., L. F. ENGLERT AND B. T. HO. *Passive avoidance deficits in mice following ethylcholine aziridinium chloride treatment.* PHARMACOL BIOCHEM BEHAV 22(2) 297–299, 1985.—High-affinity choline uptake (HACU) appears to be the rate-limiting step in the synthesis of the neurotransmitter acetylcholine. The present experiment was designed to examine the effects of irreversible inhibition of HACU by ethylcholine aziridinium chloride (ECA) on passive avoidance retention in mice. Animals were injected intracerebroventricularly, and one-trial passive avoidance retention evaluated 21 days later. A significant retention deficit was observed in ECA-treated animals upon retest 24 hours after training. ECA-induced changes in retention were accompanied by significant reductions in choline acetyltransferase (CAT) activity in only two of seven brain regions tested, hippocampus (48% of control) and cerebellum (76% of control). The results support the involvement of hippocampal cholinergic activity in mediation of passive avoidance learning.

Cholinergic Passive avoidance Mice Hippocampus Ethylcholine aziridinium

SODIUM-DEPENDENT, high affinity choline uptake appears to be the rate-limiting step of acetylcholine synthesis in cholinergic neurons [1, 12, 18]. Interference of such process should provide a very sensitive means for experimental modulation of cholinergic activity. Competitive inhibitors of high affinity choline uptake (HACU), e.g., hemicholinium 3, can transiently disrupt cholinergic function *in vivo* [7], and several studies have shown memory retention deficits in animals exposed to these inhibitors [5, 9, 15]. Certain mustard analogs of choline have been shown to irreversibly inhibit HACU *in vivo* and cause a specific reduction of presynaptic cholinergic parameters for up to 9 weeks after treatment [3, 4, 17], suggesting that cholinergic terminals degenerate as a result of treatment with these compounds. One of these compounds, ethylcholine aziridinium chloride (ECA), has recently been shown to cause passive avoidance deficits in rats after bilateral intrastriatal injection [17]. In the present study, we examined the effects of ECA on passive avoidance retention in mice, 3 weeks after intracerebroventricular administration of ECA. Choline acetyltransferase (CAT), the enzyme catalyzing the final step of acetylcholine synthesis and a specific marker for cholinergic cells, was assayed in brain regions as an indicator of cholinergic integrity.

METHOD

Acetylethylcholine mustard (AECM) was synthesized by the method of Fisher *et al.* [4]. ECA was prepared from AECM essentially as described by Rylett and Colhoun for choline mustard aziridinium [16]. A freshly prepared solution of AECM (5 mg/ml) in double-distilled water was allowed to sit at room temperature for 60 minutes. The pH was then adjusted to 11.5 with 1 N NaOH, and maintained at that pH for 10 minutes with additional base. The solution was then adjusted to pH 7.4 with dilute HCl. Final concentration was adjusted to 1.4 mg/ml for injection.

Male, CD-1 mice (Charles River Breeding Laboratories) weighing 24–30 g were used. All injections were made intracereroventricular (ICV) in a 5 μl volume into the left lateral ventricle. (Five μl of 1.4 mg/ml ethylcholine aziridinium is about 30 nmoles.) Control animals received vehicle only as stated above. Mice were lightly anesthetized with ether, and a small incision made to expose the reference point Bregma. The injection was made 1.5 mm lateral to Bregma with a 50-μl Hamilton syringe and 27 gauge needle, modified such that only 3 mm of the tip entered the cranium. The accuracy of injection was verified by injection of 5 μl of 1% cresyl violet and examination of the ventricle system for bilateral distribution.

Memory retention deficit was evaluated by passive avoidance in a light-dark apparatus similar to that described by Jarvik and Kopp [11], 21 days after treatment. Entry into the dark chamber on Trial 1 was followed immediately by shock (1.0 mA for 3 seconds), after which the animal was returned to his home cage. After 24 hours, the procedure was repeated and latency to enter the dark chamber on Trial 2 was compared between treatment groups. Difference scores, i.e., Trial 2 (seconds) minus Trial 1 (seconds), were tested

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TABLE 1

EVALUATION OF PASSIVE AVOIDANCE RETENTION IN MICE 21 DAYS AFTER ICV INJECTION OF 30 NMOLES ECA*

	Median Entry Time (Sec)		
	T1	T2-T1	Number "Avoiding"†
Control	21	600+	15/20
ECA	22	244‡	5/20§

*Conditions of test are described in text.
†Values represent number of animals in a group of 20 whose retest difference score was greater than 600 seconds.
‡$p<0.01$ (Mann-Whitney U-test).
§$p<0.005$ (Chi Square).

TABLE 2

CHOLINE ACETYLTRANSFERASE ACTIVITY IN BRAIN REGIONS 21 DAYS AFTER ICV INJECTION OF 30 NMOLES ECA

	Activity*	
Tissue	Control (n=10)	ECA (n=10)
Cortex	12.3 ± 0.3	11.9 ± 0.4
Striatum	42.3 ± 1.3	42.1 ± 1.4
Hippocampus	12.7 ± 0.4	6.1 ± 0.5†
Cerebellum	1.5 ± 0.06	1.2 ± 0.06‡
Midbrain	12.7 ± 0.3	12.0 ± 0.4
Medulla	13.7 ± 0.3	13.2 ± 0.3
Hypothalamus	9.8 ± 0.7	8.4 ± 0.5

*Values represent umole acetylcholine synthesized/hr/gram of tissue at 37°C ±standard error of mean.
†$p<0.001$ (Student's t-test).
‡$p<0.01$ (Student's t-test).

for significance between groups with the Mann-Whitney U-test. Differences in number of animals "avoiding" (i.e., those with retest difference scores greater than 600 seconds) were tested for significance with Chi Square analysis.

After completion of Trial 2, animals were sacrificed by decapitation. Brains were rapidly removed and regions dissected essentially as described by Glowinski and Iversen [10] for rat brain. Activity of choline acetyltransferase (CAT) was measured by the method of Fonnum [6], and differences in regional activity were tested for significance by Student's t-test.

RESULTS

ECA treatment did not affect entry time in Trial 1 (median time in seconds: control, 21; ECA-treated, 22). However, a significant difference in entry time between treatment groups was observed upon retest 24 hours later (Table 1). In the ECA-treated animals, these changes in passive avoidance retention were accompanied by significant reduction of CAT activity in only two brain regions, hippocampus (48% of control) and cerebellum (76% of control) (Table 2). A tendency toward reduction was seen in hypothalamus (85% of control) but the effect was not statistically significant. All other brain regions had at least 94% of control activity.

DISCUSSION

This study has shown that irreversible inhibition of HACU by ECA can cause deficits in passive avoidance retention in mice, and that these behavioral changes are associated with persistent alterations in regional CAT activity. Sandberg *et al.* [17] found impairment of acquisition and retention of a passive avoidance response in rats after intrastriatal injection of ECA. While similar behavioral deficits were observed in both studies, respective biochemical analyses implicated involvement of different brain regions in mediation of the behavioral changes. Intrastriatal injection of ECA resulted in striatal-specific cholinergic dysfunction [17], while in the present study, ICV administration produced persistent CAT reduction in hippocampus and cerebellum only. The significance of ECA-induced changes in the cerebellum remains to be determined. However, considerable evidence suggests a vital role for the hippocampus in memory processing in rodents [2, 8, 13, 14, 19]. The results reported here support the involvement of hippocampal cholinergic activity in mediation of passive avoidance learning. Further work is needed to evaluate the effects of ECA-induced cholinergic dysfunction in additional paradigms for assessing memory function.

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