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BRIEF COMMUNICATION

Amnesia Produced by Intracerebroventricular Injections of Hemicholinium-3 in Mice was Prevented by Pretreatment With Piracetam-Like Compounds

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FRANKLIN, S. R., V. H. SETHY AND A. H. TANG. *Amnesia produced by intracerebroventricular injections of hemicholinium-3 in mice was prevented by pretreatment with piracetam-like compounds.* PHARMACOL BIOCHEM BEHAV **25**(4) 925–927, 1986.—Intracerebroventricular (ICV) injections of hemicholinium-3 (HC-3) to mice before the training trial in a passive avoidance task produced an amnesic effect at the 24-hour retention test. Pretreatment by IP injection of piracetam, etiracetam, or pramiracetam, 30 minutes before HC-3 injections antagonized the amnesic effects of HC-3. Pretreatment with choline was not effective. The depletion of cerebral acetylcholine by the HC-3 injection was not prevented by piracetam or etiracetam.

Amnesia HC-3 Piracetam Cholinergic

NOOTROPIC agents, represented by piracetam, protect experimental animals from the amnesic effects produced by electroconvulsive shock, hypoxia, and injections of scopolamine [3, 6, 9, 10]. The mechanism of the anti-amnesic effect from this class of drugs is unknown, although the phenomenon is used as a model to infer therapeutic potential in clinical conditions with impaired cognitive functions. Senile dementia of the Alzheimer type (SDAT) is characterized by impaired memory/cognitive functions. Biochemical changes in brains of SDAT patients indicate reduced activity in most of the neurotransmitter and some neuropeptide systems. Among them, the central cholinergic system has been most extensively studied and is believed to play an important role in the pathophysiological condition of SDAT [1,4]. There is, therefore, considerable interest in experimental animal models of central cholinergic dysfunction for the evaluation of potential therapeutic agents for the treatment of SDAT. Central cholinergic blockade, resulting in impairment of memory functions, has been demonstrated with lesions to selective cholinergic pathways, or by the administration of cholinergic antagonists (e.g., scopolamine). A reversible depletion of acetylcholine (ACh) can be produced by the injection of hemicholinium-3 (HC-3), which is a selective blocker of high-affinity choline uptake (HACU) by cholinergic neurons. This study investigates the effects of several nootropic agents of the piracetam-type on amnesia produced by intraventricular injections of HC-3 in mice [2].

METHOD

Behavioral Test

Male albino mice of the Carworth-Farm strain were used in a one-trial passive avoidance task to study memory retrieval. The test chamber had dimensions of 25×12.5×12.5 cm with a metal grid floor through which electric shock could be delivered. The chamber was connected to a small platform (5×3 cm) by a circular opening (3 cm in diameter) with a sliding door. The wall of the chamber was not transparent, so that the inside was dark. A naive mouse placed on the platform would normally enter the chamber with a short latency. On the first experimental day, the mouse was placed

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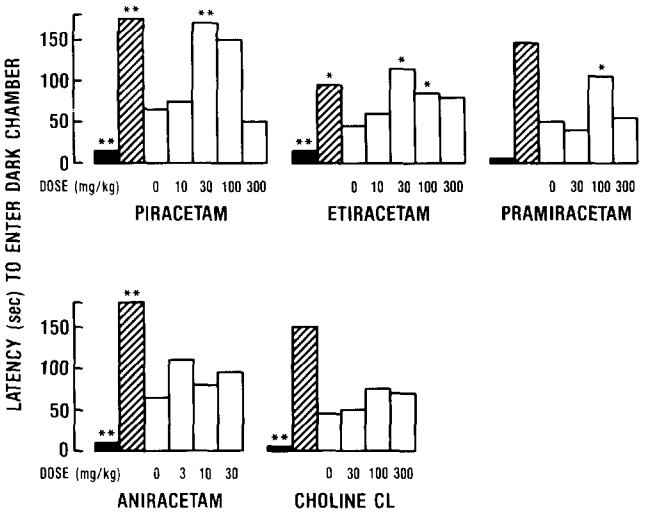

FIG. 1. Reversal of amnesia produced by ICV injections of HC-3 (1.5 μg/hemisphere) in mice (open bars). Values represent medians from groups of 8–10 mice each on the retention test (24 hours after training). Hatched bars represent control groups of mice receiving ICV injection of CSF and inhibitory conditioning. The solid bars represent control groups of mice receiving no injection or inhibitory conditioning. Both control groups were run on the same day as the experimental groups. $^{**}p<0.01$; $^{*}p<0.05$; compared to the group injected ICV with HC-3 only (Wilcoxon's Ranksum test). The differences between the solid and hatched columns are significant ($p<0.01$) in each experiment.

on the platform and, after entering the chamber, immediately received foot-shock (1 mA) for 2 seconds. The conditioned inhibition to re-enter the chamber was tested 24 hours later comparing the shocked animals to a group receiving no shock on the first day. A maximum of 180 seconds was used for cut-off on the second day if the animal failed to enter the chamber at that time. The median latency from each group was compared using Wilcoxon's Ranksum test.

Intracerebroventricular (ICV) injections of HC-3 were done free-hand by inserting the tip of a 10 μl Hamilton micro-syringe through the skull to a depth of 2.5 mm at points approximately 2 mm posterior to an imaginary line intersecting the posterior extent of the orbits of the eyes and 1 mm lateral to midline. HC-3 (Aldrich Chemical Co.) was dissolved in artificial cerebrospinal fluid and injected in a volume of 1 μl per hemisphere. Control animals were injected with CSF. Two hours after the ICV injections, mice were given the training trial in the passive avoidance task. The retrieval test was conducted 24 hours later with no further treatment.

Analysis of Cerebral Acetylcholine Content

The effect of ICV injection of HC-3 on brain acetylcholine content was studied in a separate group of mice. At treatment times corresponding to the behavioral studies, mice were sacrificed by a beam of microwave radiation focused at the skull for 0.2 seconds. The brain was quickly removed from the skull and homogenized in 0.4 N perchloric acid. Acetylcholine was extracted and assayed by high pressure liquid chromatography [8].

TABLE 1

PRETREATMENT WITH PIRACETAM AND ETIRACETAM ON THE ACETYLCHOLINE LEVEL IN WHOLE BRAINS OF MICE AFTER ICV INJECTIONS OF HC-3 (1.5 mg/HEMISPHERE)

Treatment	N	ACh Conc. (nmol/g)
CSF (ICV)	4	18.61 ± 1.64
Saline + HC−3 (ICV)	4	2.29 ± 0.21*
Piracetam 100 mg/kg + HC−3 (ICV)	4	2.26 ± 0.21*
Etiracetam 100 mg/kg + HC−3 (ICV)	4	5.30 ± 1.41*

*$p<0.001$ compared to the CSF-only group; there is no significant difference among the other three groups.

Drugs

Piracetam, etiracetam, and pramiracetam were dissolved in physiological saline; aniracetam was suspended in 0.25% methylcellulose, and all drugs were injected IP 30 minutes before the ICV injection of HC-3. Choline chloride was dissolved in water and administered by oral gavage.

RESULTS

For each drug tested for the antagonism of HC-3 in the one-trial passive avoidance task, there was always a control group receiving no shock and a group receiving shock conditioning and ICV injection of artificial CSF (solid and hatched columns in Fig. 1).

ICV injections (CSF or HC-3) produced a transient behavioral depression in mice. The animals appeared completely recovered at the time of the conditioning trial (2 hours later). ICV injections of CSF produced no amnesic effect as the control groups (shocked vs. non-shocked groups) always exhibited a significant conditioned inhibition (passive avoidance) effect. Compared to the CSF-injected mice, HC-3 (1.5 μg/hemisphere) produced a significant, but seldom complete, reversal of passive avoidance, suggesting an amnesic effect. In a preliminary study, HC-3 was injected, one, two and four hours before the training session. The amnesic effect was most consistently produced at the two-hour pretreatment time. This treatment-interval for HC-3 was used to study the interaction with nootropic agents. Pretreatment with piracetam, etiracetam, or pramiracetam, 30 min before ICV injection of HC-3, returned the avoidance response to a level comparable to that of the group not treated with HC-3. The dose-response relation was biphasic for each of the nootropic agents. Aniracetam and choline chloride produced no significant reversal of the amnesic effects from HC-3.

An identical protocol was used to study the interaction of the nootropic agents and ICV injections of HC-3 on whole brain ACh concentration. Mice sacrificed 2 hours after HC-3 injections had a very low level of ACh compared to control mice injected with artificial CSF (Table 1). Pretreatment with piracetam or etiracetam at a dose which effectively reversed the behavioral effects of HC-3 had no effect on the depletion of brain ACh.

DISCUSSION

We have demonstrated in mice that three nootropic agents had anti-amnesic activity against an ICV injection of HC-3 which also greatly reduced brain ACh levels. A fourth nootropic agent, aniracetam, was ineffective, possibly due to poor bioavailability of the compound. The anti-amnesic effect of this class of compounds is therefore demonstrated in an animal model with a biochemical lesion characteristic of the clinical condition in SDAT. The dose-response relation of the anti-amnesic effect by the nootropic agents has an inverted U-shape. Similar biphasic dose-response relations have been reported in different models of memory functions with these compounds [3, 6, 10].

Although the amnesia produced by HC-3 is presumed to be the result of ACh-depletion in the brain, the mode of antagonism by the nootropic agents is not clear. Using an identical dosing protocol as in the behavioral study, the depletion of ACh in whole brains by HC-3 was not reversed by piracetam of etiracetam (Table 1). Pedata *et al.* [7] reported that pre-treatment with piracetam had no effect on the reduction of ACh level by ICV injection of HC-3 in the cerebral cortex of rats, while the reduction in the hippocampus was further enhanced. No enhanced depletion of ACh is observed in the brains of mice in this study, possibly due to a reduced sensitivity by assaying ACh in whole brain samples. Enhanced depletion of ACh by the combination of HC-3 and piracetam, as reported in the Pedata study, may be due to an increase in activities of the cholinergic neurons by the nootropic drugs. Although the piracetam-like nootropic agents are not known to have direct cholinomimetic properties, they have been shown to increase high-affinity uptake of choline in the hippocampus of rats, suggesting an increase in the local turnover of ACh [7,11]. Since the hippocampus has long been implicated in memory functions, an increase in hippocampal neuronal activity for the otherwise deficient cholinergic system may account for the anti-amnesic effect of the nootropic agents in both animal and clinical studies [5].

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