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BRIEF COMMUNICATION

Long-Term Suppression of the Development of Complementary Memory Storage Sites in Mice: Functional Interdependence of Acetylcholine and Dopamine

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FLEXNER, J. B., A. C. CHURCH AND L. B. FLEXNER. *Long-term suppression of the development of complementary memory storage sites in mice: Functional interdependence of acetylcholine and dopamine.* PHARMACOL BIOCHEM BEHAV 43(2) 617–619, 1992.—Bitemporal injections of puromycin consistently induce amnesia of aversive maze learning in mice when given within 3 days after training. These injections consistently fail to induce amnesia when given 6 or more days after training. Consistent with the evidence from other laboratories, we interpret these results to indicate that the initial, temporal memory storage sites are supplemented 6 days after training by the development of complementary storage sites in other cerebral areas. Previous experiments have shown that this process is suppressed for 30–60 days by a single SC injection of scopolamine, a muscarinic antagonist. We now find that this suppressive action of scopolamine can be completely nullified by haloperidol, a dopaminergic antagonist. This finding supports the view that there may be a therapeutic role for dopamine antagonists in the treatment of cognitive dysfunction associated with cholinergic loss.

Memory storage Acetylcholine Dopamine Muscarinic receptors Scopolamine Haloperidol

IN mice, bitemporal injections of puromycin that primarily affect the hippocampal–entorhinal area consistently induce amnesia of aversive Y-maze learning for 3 days after training but are consistently ineffective 6 or more days after training. At these later times, additional puromycin injections covering widespread forebrain areas are necessary to induce amnesia (6). Consistent with the evidence and views of others (10,14, 16,18,19,20), we conclude from our results that the storage of recent memory is limited to the hippocampal area and that after 5 days additional, independent storage sites appear in the neocortex.

We have reported that after treatment with the muscarinic receptor antagonist, scopolamine (SCO), the duration of amnesia produced by bitemporal puromycin was increased from the normal period of 5 days after training to 30–60 days post-training (5). We interpreted this finding to indicate that SCO suppressed the development of complementary memory storage sites for 30–60 days. The purpose of the present experiments was to test the possibility that this suppressive effect of SCO could be prevented by simultaneous treatment with the dopaminergic receptor antagonist, haloperidol (HALO). This approach is based upon the knowledge that blockade of dopamine (DA) receptors increases the evoked release of acetylcholine (ACh) in the limbic system (3,15). HALO blocks both D_2 and, to a lesser degree, D_1 receptors (9).

METHOD

As previously detailed (2), male and female Swiss-Webster mice from our closed colony were randomly selected and then trained in a single session in a Y-maze to a criterion of 9 of 10 correct responses. During both training and testing sessions,

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intermittent foot-shock from a DC source was given for failure to leave the stem of the Y within 5 s and for errors of left–right discrimination. Retention tests were administered 10 days following puromycin injections to ensure recovery from the acute effects of the antibiotic (lethargy, aphagia, adipsia, excitability on handling but no convulsions). By using this schedule, mice ran normally in the maze with 90% of the testing errors being errors of discrimination. Errors were summed until the mouse reached the criterion. The median number of trials needed to reach criterion was seven. Memory was evaluated in the retention tests in terms of the percentage savings in errors. This percentage was calculated by obtaining the difference between the number of errors to criterion in the training tests (T) and the number of errors to criterion in the retention tests (R) and dividing that difference ($T - R$) by the number of errors to criterion in the training tests (T). Thus, percentage savings equals $[(T - R)/T] * 100$. Any negative savings were scored as zero. Because the Mann–Whitney U-test was used for statistical comparisons between groups, median scores are used in the presentation of data along with range values.

The puromycin injection procedure has been fully described (2). Prior to puromycin injections, mice were lightly anesthetized with sodium hexobarbitone (Evipal, 150 mg/kg, IP). Each injection in the bitemporal procedure contained 90 μg puromycin H_2Cl (ICN Pharmaceuticals) dissolved in 12 μl distilled water and brought to pH 6 with NaOH. Each injection in the bitemporal, biventricular, and bifrontal procedure (T + V + F) contained 30 μg puromycin.

Scopolamine MeBr (SCO, 1.5 mg/kg) and HALO (Sigma Chemical Co., St. Louis, MO) were dissolved in water and a single injection of 0.1 to 0.2 ml (containing both agents) was administered SC. All mice survived these treatments in excellent condition.

DESIGN AND RESULTS

A test was conducted to determine whether HALO would prevent the memory storage site suppressive actions of SCO. A mixture of SCO (1.5 mg/kg) and HALO was injected SC 2 days after maze training. Ten days after drug administration, puromycin was injected bitemporally. As shown in Fig. 1, bitemporal puromycin induced profound amnesia in all mice that received 0.75 or 1.50 mg/kg HALO and in four of six mice treated with HALO at the 2.25-mg/kg dose (median savings 25%). In contrast, bitemporal puromycin was ineffective in mice treated with 3.00 or 3.75 mg/kg HALO (median savings, respectively, 83 and 86%).

When HALO (3.0 mg/kg) was used alone in the same treatment regimen, bitemporal puromycin also failed to elicit amnesia (median savings 90%; range 72–100%; $n = 8$). This performance did not differ significantly ($p > 0.1$) from that of animals that received saline injections 2 days after training and bitemporal puromycin 10 days following the saline (median savings 100%; range 75–100%; $n = 8$).

Additional groups of mice were also tested to determine if HALO (3 mg/kg) in combination with SCO (1.5 mg/kg) might affect the amnesic properties of puromycin and so lead to a misinterpretation of our results.

The first of these control groups was treated with SCO + HALO 1 day after training and then injected bitemporally with puromycin 1 day later. As in untreated mice, the bitemporal puromycin induced profound amnesia in all subjects (median savings 0%; range 0; $n = 4$).

In the second group, mice received the SCO + HALO combination 9 days after training to allow ample time for the development of additional memory storage sites. After a 10-day interval, puromycin was injected bitemporally. Again, as in untreated mice, the bitemporal puromycin failed to produce amnesia in these mice (median savings 90%; range 75–100%; $n = 4$).

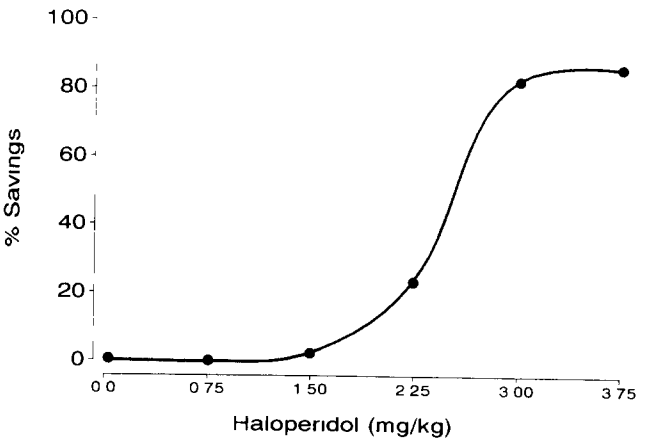

FIG. 1. Dose-response curve of haloperidol's (HALO) effect on scopolamine's (SCO) suppression of complementary memory storage sites. Mice were injected SC with SCO alone (1.5 mg/kg) or in combination with HALO 2 days after training. Bitemporal puromycin was then administered 10 days after drug treatment. Mice were tested for retention about 12 days after puromycin. The values presented are medians. Ten mice were tested in the 3.0-mg/kg group, while 6 mice were tested in all other groups. Range of variation in percentage savings of errors at consecutive points: (0), (0–17), (0–100), (67–100), and (75–100). See text for additional details.

In the third group of controls, the same time intervals (9 + 10 days) and drug dosages of SCO + HALO were used except six injections of puromycin (T + V + F) were made. Again, as in untreated mice, the T + V + F procedure induced profound amnesia (median savings 0%; range 0; $n = 4$).

DISCUSSION

Our experiments with a mixture of SCO (1.5 mg/kg) plus HALO (3.0 mg/kg) and with HALO alone lead to the following conclusions:

1. SCO + HALO did not affect the amnesic properties of puromycin.
2. As we have previously reported (5), SCO suppresses the development of complementary memory storage sites for 30–60 days. When mice are treated with SCO + HALO, this suppressive effect of SCO is completely eliminated.
3. Treatment with HALO alone did not significantly affect this process, which increases storage sites.

There is now considerable evidence that DA and ACh interact not only in motor but also in cognitive function. In the case of motor function, lesions in animals of the substantia nigra (17), for example, cause diminished striatal DA activity and Parkinsonian-like symptoms (1,8). Improvement in these motor deficits can be obtained with either DA agonists or ACh antagonists (4,11). The earliest observations relating to cognitive function are relatively recent (12,13). They demonstrated that SCO's inhibition of performance in an eight-arm

radial maze was significantly attenuated by treatment with HALO. It is pointed out that one likely site for a role of DA and ACh in cognitive function is the medial septum (12). The medial septum is the source of a major cholinergic pathway that innervates the hippocampus, while the DA innervation of the septum arises in the tegmentum (15). Lesions of hippocampal DA afferents produced by injections of 6-hydroxydopamine into the septum or into the ventral tegmentum resulted in a sustained increase of hippocampal cholinergic activity (15). These DA lesions also led to improved performance of mice in the eight-arm radial maze (7). Our studies extend this pattern of results to another cognitive process, namely, development of complementary memory storage sites. Although a cholinergic antagonist suppresses this cognitive process, these suppressive affects can be eliminated by administering a DA antagonist.

As has been suggested (12,13), these findings indicate that there may well be a therapeutic role for DA antagonists in the treatment of cognitive dysfunction that is associated with cholinergic loss.

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