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BRIEF COMMUNICATION

Complementary Memory Storage Sites in Mice: Their Development as Affected by the Competitive NMDA Receptor Antagonist, CPP

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CHURCH, A. C., J. B. FLEXNER AND L. B. FLEXNER. *Complementary memory storage sites in mice: Their development as affected by the competitive NMDA receptor antagonist, CPP.* PHARMACOL BIOCHEM BEHAV **52**(1) 237-240, 1995. – Bitemporal injections of puromycin consistently induce amnesia of aversive maze learning in mice when administered within 3 days of training. These bitemporal puromycin injections lose their amnestic effectiveness if the latency between training and injection is extended beyond 6 days. Consistent with other evidence, we conclude that in our experimental paradigm, complementary memory storage sites normally develop in additional cerebral areas within 6 days following training. Previous experiments have indicated that the central adrenergic and cholinergic systems are critically involved in this process. We now present evidence that administration of the NMDA receptor antagonist, CPP, blocks the development of these complementary memory storage sites. As suggested by studies of long-term potentiation, NMDA receptor-dependent postsynaptic calcium appears to be essential for the development of these storage sites and indeed to trigger their development.

Complementary memory storage Development Maze learning NMDA receptor antagonist
Long-term potentiation

DAMAGE to the hippocampal area of man (12,19), monkey (13), cat (18), and rodents (7,9,15,16) has been found to severely impair recent memory whereas remote memory is largely spared. This observation has led to the concept that memory storage sites, initially limited to the hippocampal-entorhinal area, increase with time to involve additional cerebral areas. Consistent with this view is the finding that bitemporal intracerebral injections of the amnesic agent, puromycin, that affect the hippocampal-entorhinal area, consistently induce amnesia in mice if administered within 3 days of aversive Y-maze training. Production of amnesia at times longer than 6 days after training requires a combination of bitemporal plus biventricular plus bifrontal (T + V + F) injections of puromycin that affect widespread neocortical sites in addition to the hippocampal-entorhinal area (7).

Using this change in reaction to puromycin, we have reported that the central adrenergic (4,5) and cholinergic systems (6) are essential for the development of these complementary memory storage sites. The experiments reported here were designed to test the effect of SC injections of the competitive NMDA receptor antagonist, 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP). Specifically, we have investigated: 1) the effect of CPP administered posttraining on the normal development of complementary memory storage sites, and 2) the duration of CPP's effect on the development of complementary memory storage sites.

METHOD

As previously detailed (4), 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

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out of 10 correct responses. During both training and testing sessions, intermittent foot shocks from a DC source were given for failure to leave the stem of the Y-maze 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). With this schedule, mice ran normally in the maze with 90% of the testing errors being errors of discrimination (as opposed to errors resulting from failure to start or complete the maze). Errors were summed until mice reached the criterion. The median number of trials to reach criterion was 4.0. 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 training (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. Thus, percentage savings equals $(T - R)/T \times 100$. Negative savings were scored as zeroes. Because the Mann-Whitney U-test was used for statistical comparisons between groups, median scores are used in presentation of data along with range values.

Based on the experience of others (1,14,17), CPP (Research Biochemicals, Natick, MA) was dissolved in saline and administered SC at a dose of 5 mg/kg. All mice survived this treatment in excellent condition.

The puromycin injection procedure has been fully described (4). Prior to the puromycin injections, mice were lightly anesthetized with sodium hexobarbitone (Evipal, 150 mg/kg, IP). Each injection in the bitemporal procedure contained 90 μg of puromycin HCl (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 of puromycin so that in either procedure the subject received 180 μg of puromycin.

Experiment 1

The purpose of this experiment was to determine if CPP (5 mg/kg) affects learning or relearning. Mice were injected 1 day before training with either CPP (four mice) or saline (four mice) and then tested for memory 7 days later. Median errors on training in both groups was five trials. Retention in both groups was perfect (median savings 100%, range 0, $U = 5.5$, $p = 0.293$).

Experiments 2a, 2b, and 2c

The purpose of the following experiments was to test whether CPP might affect the amnesic properties of puromycin and so lead to misinterpretation of our results.

In Experiment 2a, control groups were treated with CPP 1 day after training and then 1 day later injected bitemporally with puromycin. As in untreated mice, the bitemporal puromycin induced profound amnesia in all subjects (median savings 0%, range 0%, $U = 6$, $p = 0.343$, $n = 4$).

In Experiment 2b, a group of mice was injected with CPP 9 days after training to allow ample time for the development of complementary memory storage sites. On the following day this group received puromycin via bitemporal injections. As in untreated mice, the bitemporal puromycin failed to produce amnesia (median savings 100%; range = 83–100%, $U = 6$, $p = 0.343$, $n = 4$).

In Experiment 2c, a group of mice was injected with CPP 9 days after training to allow ample time for the development of complementary memory storage sites. On the following day this group received puromycin via six injections of puromycin (T + V + F). As in untreated mice, the T + V + F procedure induced profound amnesia (median savings 0%; range 0%; $n = 4$).

Experiment 3

The purpose of this experiment was to determine the effect of CPP (5 mg/kg) on the development of complementary memory storage sites. The schedule of training, drug treatment, and testing was identical to that used with adrenergic and cholinergic ligands (5,6) (i.e., CPP was given 2 days after training and then, to allow ample time for the normal process of memory spread, puromycin was injected bitemporally 10 days later). As shown in Table 1, group a, all mice treated in this way were amnesic, consistent with failure of development of complementary memory storage sites.

Experiment 4

In Experiment 3, the posttraining administration of CPP (5 mg/kg) prolonged the normal period during which bitemporal puromycin induced amnesia. The purpose of the present experiment was to determine if a single administration of CPP produces a long-lasting effect (i.e., to determine if the development of complementary memory storage sites recovers with time). The results are presented in Table 1.

With CPP (5 mg/kg) being given 2 days after training, puromycin was injected bitemporally up to 90 days later. As indicated in group b of the table, puromycin consistently induced profound amnesia 30 days after CPP. In contrast, with puromycin injections delayed until 60–90 days after training (groups c and d), four of six mice at each of these times retained memory. Although this development of puromycin-insensitive memory is substantial, it is still significantly less than that of the saline controls (groups e and f; $U = 5.5$, $p = 0.036$)

DISCUSSION

Our experience with CPP leads to the following conclusions:

1. In the absence of puromycin, CPP had no effect on the learning of the Y-maze or on its relearning.
2. CPP had no effect on the amnesic properties of puromycin.
3. CPP given 2 days after training inhibited the development of complementary memory storage sites for at least 30 days with partial recovery evident at 60 and 90 days.

Our interest in the NMDA receptor was stimulated by studies of hippocampal long-term potentiation (LTP). In hippocampal LTP the increase in synaptic strength that persists for hours or months follows brief repetitive activation of excitatory synapses. This phenomenon has been widely studied as a model for cellular mechanisms related to memory (3,11). Hippocampal LTP is believed to be dependent upon an increase in postsynaptic calcium that, with some exception (2,8,10), is NMDA receptor dependent. Similarly, the results of the present study indicate that the development of complementary memory storage sites can be blocked by an NMDA receptor antagonist. In agreement with previous studies from

TABLE 1

INHIBITION OF THE DEVELOPMENT OF COMPLEMENTARY MEMORY STORAGE SITES BY CPP (5 mg/kg)

Drug Treatment and Timing	N	Median Errors	
		Train	Test
a. Train (2)→CPP (10)→puromycin	6	4.0	7.5
Retained memory	0		
Amnesic	6		
b. Train (2)→CPP (30)→puromycin	6	4.0	6.0
Retained memory	0		
Amnesic	6		
c. Train (2)→CPP (60)→puromycin	6	3.5	3.0
Retained memory	4		
Amnesic	2		
d. Train (2)→CPP (90)→puromycin	6	4.0	1.0
Retained memory	4		
Amnesic	2		
e. Train (2)→Saline (60)→puromycin	6	4.0	0.0
Retained memory	5		
Amnesic	1		
f. Train (2)→Saline (90)→puromycin	6	5.0	0.0
Retained memory	6		
Amnesic	0		

Note: Time between procedures is indicated in days in parentheses. Puromycin was administered bitemporally.

this laboratory of other neurotransmitter antagonists, the effect of the antagonist persists for at least 1 month following administration. An important question that remains unanswered in these studies is what kind of neural mechanism might be responsible for this month-long inhibition.

As others have reported, not all cases of LTP are NMDA receptor dependent (2,8,10). By extension to the present study, it may also be the case that the development of some memory storage sites is NMDA receptor independent.

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