Glutamatergic–cholinergic interaction on memory consolidation in mice

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NMRI mice were trained in a one-trial inhibitory avoidance task. They were injected immediately after training with the muscarinic cholinergic agonist oxotremorine, the muscarinic cholinergic antagonist atropine, the *N*-methyl-D-aspartate (NMDA) noncompetitive antagonist MK-801, or with a combination of MK-801 and one of the cholinergic agents. Oxotremorine improved, while atropine and MK-801 impaired, memory retention. In addition, oxotremorine attenuated, while atropine enhanced, the effect of MK-801. The results show the existence of a glutamatergic–cholinergic interaction in modulating memory consolidation of mice.

A number of studies have shown that manipulations of the muscarinic cholinergic system affect memory processes in animals tested in a variety of experimental conditions, including one-trial inhibitory avoidance. It has been reported, for example, that posttraining administration of cholinergic agonists improves memory consolidation (Flood, Landry, & Jarvik, 1981; Flood, Smith, & Cherkin, 1983, 1985; Gasbarri, Introini-Collison, Packard, Pacitti, & McGaugh, 1993; Gower, 1987; Introini-Collison & Baratti, 1992), while posttraining administration of cholinergic antagonists impairs it (Baratti, Introini-Collison, & Huygens, 1984; Flood et al., 1981; Gasbarri et al., 1993; Gower, 1987). Recent experiments have shown that glutamatergic drugs can affect memory consolidation in mice. Research carried out with the inbred strain of mice C57BL/6 (C57) has demonstrated, in particular, that posttraining administrations of the *N*-methyl-D-aspartate (NMDA) antagonists MK-801 and CPP impair, in a dose-dependent fashion, the one-trial inhibitory avoidance response (Mele, Castellano, Cestari, & Oliverio, 1995; Mele, Castellano, & Oliverio, in press). Furthermore, in the above experiments, the existence of an interaction between dopamine (DA) and glutamate in modulating one-trial inhibitory avoidance behavior has been observed. In particular, in C57 mice (Mele et al., 1995), posttraining subeffective doses of D1 and D2 DA agonists (SKF38393 and quinpirole) were able to antagonize the action of CPP, while subeffective doses of D1

and D2 selective DA receptor antagonists (SCH23390 and sulpiride) enhanced the effect of the NMDA antagonist. With NMRI mice (Mele et al., in press), subchronic blockade of NMDA receptors with MK-801 (0.25 mg/kg once a day for 14 days) did not change sensitivity to either competitive (CPP) or noncompetitive (MK-801) NMDA antagonists. The same treatment with MK-801 induced an increased response to both SKF38393 and quinpirole, suggesting that repeated administrations of MK-801 induced an upregulation of both D1 and D2 dopamine receptors.

A number of data exist showing that acetylcholine (ACh) and DA interact in influencing memory (see Levin, McGurk, Rose, & Butcher, 1990). Recently, particularly in posttraining experiments, the involvement of D2 receptors in the effects of oxotremorine and atropine on memory in mice tested in an inhibitory avoidance and a Y-maze discrimination test has been reported (Gasbarri et al., 1993). In fact, in the inhibitory avoidance task, quinpirole blocked the retention-impairing effect of the muscarinic cholinergic antagonist atropine, and sulpiride significantly attenuated the memory-enhancing effect of the muscarinic cholinergic agonist oxotremorine. In the Y-maze discrimination task, atropine blocked the memory-enhancing effect of quinpirole and oxotremorine attenuated the memory-impairing effect of sulpiride.

Some experiments have finally shown an interaction between the glutamatergic and the cholinergic systems. It has, for example, been observed that ACh potentiated electrophysiologically induced stimulus responses to NMDA (Markham & Segal, 1990) and to glutamate (Cox, Metharate, & Ashe, 1994). Interactions between these systems have also been reported in behavioral ex-

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periments. In monkeys, it has been found that pretraining administrations of equivalent doses of MK-801 and scopolamine impaired performance on a delayed nonmatching-to-sample (DNMS) task. Moreover, the administration of MK-801 and scopolamine, at low doses that had no effect on performance when given alone, impaired DNMS performance significantly when given in combination. This finding demonstrates the existence of a synergistic interaction between the cholinergic and the glutamatergic systems in visual recognition memory (Aigner, 1995). Other experiments studied the interaction between these systems in mice tested in an elevated plus-maze. By using this test, based on the natural aversion of mice and rats to high and open spaces, Itoh, Nabeshima, and Kameyama (1990, 1991) reported that transfer latency (TL; i.e., the time in which animals move from the open arms of the maze to the enclosed ones) was shorter on Day 2 onward than on Day 1 and suggested that this shortened TL could be utilized as a parameter for learning and memory. In further experiments, Sharma and Kukarni (1992) explored, in mice, the mechanism of learning (acquisition) in these experimental conditions and, 30 min before the Day 1 trial, injected them with scopolamine, MK-801, or physostigmine. They observed that, with the first two drugs, the transfer latencies on Day 1 and Day 2 trials were significantly higher than those of control animals, suggesting a disruption by these agents of the process of learning. On the contrary, physostigmine induced a significant reduction of TL on Day 2, suggesting a facilitation of the acquisition mechanism. In combination studies, physostigmine reversed both scopolamine- and MK-801-induced acquisition deficits. This result, which was observed by comparing the training latencies on Days 1 and 2, suggested that (1) the acquisition deficit induced by scopolamine might be due to interference in cholinergic neurotransmission in the CNS, and (2) the noncompetitive NMDA-receptor antagonist MK-801 displayed its amnesic effect through the modulation of cholinergic neurotransmission.

In the present research, posttrial experiments were carried out to assess the existence of a possible interaction between cholinergic and glutamatergic systems in modulating memory consolidation in mice. For this purpose, NMRI mice were used. They were injected, after training in a one-trial inhibitory avoidance test, with the NMDA receptor antagonist MK-801, alone or in combination with a muscarinic cholinergic agonist (oxotremorine) or a muscarinic cholinergic antagonist (atropine).

METHOD

Subjects

Male NMRI mice (Plaisant, Roma, Italy), aged 8 weeks and weighing 25–30 g, were used as subjects in this experiment. Upon arrival, the mice were housed in groups of 8 in standard breeding cages (21 × 21 × 12 cm) and kept in a 12:12-h light:dark cycle (lights were on from 0700 to 1900 h), at a constant temperature

(22° ± 1°C), given food and water ad lib and tested during the second half of the light period (between 1400 and 1700 h) in a sound-insulated room. The research in this paper was conducted in accordance with Italian national laws and regulations on the use of animals in research and NIH guidelines on animal care.

Apparatus and Experimental Procedure

The mice were trained on a step-through inhibitory avoidance apparatus, as previously described by McGaugh and Landfield (1970). On the training day, each mouse was placed in the light compartment, facing away from the dark compartment. When the mouse turned around, the door leading to the dark compartment was opened. When the mouse had stepped with all four paws into the dark side, the door was closed, a footshock (0.2 mA, 50 Hz, 1 sec) was delivered, and the step-through latency was recorded. The mouse was removed from the apparatus and injected intraperitoneally with test compounds in a volume of 10 ml/kg. Retention was tested 24 h later following a similar procedure, except that no shock was administered. A maximum step-through latency of 180 sec was considered.

For the MK-801 dose-response experiment, the animals were injected with either vehicle or (+)MK-801 at doses of 0.1, 0.25, or 0.5 mg/kg immediately after training. The cholinergic antagonist and agonist were also injected immediately after the animals were removed from the apparatus on the training day. The doses used were 2, 3, and 4 mg/kg and 0.02, 0.03, and 0.04 mg/kg, respectively, for atropine and oxotremorine. To study the interaction between the NMDA antagonist and the cholinergic agents, the mice, immediately after being removed from the apparatus on the training day, were injected with either the vehicle or the ineffective dose of muscarinic cholinergic antagonist (atropine 2 mg/kg) or agonist (oxotremorine 0.02 mg/kg). Immediately after that, a dose of (+)MK-801 was administered. The doses of (+)MK-801 used were 0.1 and 0.25 mg/kg when the NMDA antagonist was injected in association with atropine and 0.25 and 0.5 mg/kg when it was used in combination with oxotremorine. All drugs were dissolved in saline, and the control groups were injected with saline. Each experimental group consisted of 7–8 mice.

Statistical Analysis

The results of the dose-response experiments were evaluated by one-way analysis of variance (ANOVA) in which the mean step-through latencies on the test day were compared. For the results of the acute association experiments, a two-factor (first injection and second injection) ANOVA was used. The levels were two for the first-treatment factor—vehicle and acetylcholine antagonist (atropine) or agonist (oxotremorine)—and three for the second-treatment factor—vehicle, MK-801 0.1, and MK-801 0.25 or vehicle, MK-801 0.25, and MK-801 0.5, respectively, for the two experiments. Individual between-group comparisons, when appropriate, were carried out by post hoc test (Newman-Keuls test).

RESULTS

The NMDA noncompetitive antagonist (+)MK-801 administered immediately after training impaired, in a dose-dependent fashion, memory retention in NMRI mice (Figure 1). ANOVA showed a main treatment effect [$F(3,27) = 74.59, p < .001$].

Posttrial injections of the cholinergic muscarinic antagonist, atropine, induced a dose-dependent impairment in the one-trial inhibitory avoidance response (Figure 2). One-way ANOVA revealed a main treatment effect [$F(3,27) = 72.156, p < .001$]. By contrast, oxotremorine

Figure 1. Mean step-through latencies on the retention trial of NMRI mice receiving, immediately after training, different doses of the NMDA noncompetitive antagonist MK-801. Vertical bars: SEM. Sal: saline-injected mice. * $p < .01$ versus saline (Newman-Keuls test).

enhanced memory retention, with the higher dose inducing the maximum response in all animals. Also in this case, ANOVA revealed a clear treatment effect [$F(3,27) = 97.03, p < .001$].

Figure 3 shows the effects of posttrial injections of ineffective doses of atropine (Figure 3A) or oxotremorine (Figure 3B) in combination with the NMDA antagonist MK-801. In the first case, there was a synergistic effect between the two drugs, with the ineffective dose of the muscarinic antagonist potentiating the effect of both doses of MK-801. Two-way ANOVA showed a first-treatment effect [$F(1,41) = 43.89, p < .001$], a second-treatment effect [$F(2,41) = 90.38, p < .001$], and an interaction between the two treatments [$F(2,41) = 9.8, p < .001$]. The ineffective dose of oxotremorine (0.02 mg/kg), however, antagonized the effects of MK-801. Also in this case, the two-way ANOVA revealed a first-treatment effect [$F(1,41) = 59.5, p < .001$], a second-treatment effect [$F(2,41) = 124, p < .001$], and an interaction between the two treatments [$F(2,41) = 12.67, p < .001$].

DISCUSSION

The results of the present research indicate three main points. The first concerns the memory-enhancing effect of the muscarinic cholinergic agonist oxotremorine and the impairment of memory exerted by the muscarinic cholinergic antagonist atropine. These results agree with those of previous investigations in showing that cholinergic enhancing drugs improve learning and memory in a number of tasks, while memory impairments can be induced by drugs blocking cholinergic neurotransmission (Brioni & Izquierdo, 1988; Deutsch, 1983; Flood et al., 1985; Gower, 1987; Pavone, Fagioli, & Castellano, 1993). The second point concerns the memory consolidation impairment observed following the posttraining administration of the NMDA noncompetitive antagonist MK-801. This result is in agreement with findings obtained

in the same experimental conditions (one-trial inhibitory avoidance) in NMRI mice (Mele et al., in press). It must be considered that the posttraining administration of CPP, an NMDA competitive antagonist, also impaired memory consolidation in C57BL/6 mice (Mele et al., 1995). The third and main point evident from the present research concerns the glutamatergic–cholinergic interaction on the modulation of memory consolidation in NMRI mice. In fact, a low and by itself ineffective dose of oxotremorine antagonized and a low and by itself ineffective dose of atropine enhanced the impairing effect observed following posttraining MK-801 administration.

Glutamatergic–cholinergic interactions have been demonstrated in recent years by neurochemical and electrophysiological research. However, contrasting results are present in the literature, indicating both a facilitatory and an inhibitory action of the NMDA system on cholinergic activity. In fact, while microdialysis data suggest an inhibitory effect of NMDA receptors on Ach release in the hippocampus (Belfiore et al., 1992; Giovannini, Mutolo, Bianchi, Michelassi, & Pepeu, 1994), it has been shown that Ach enhances EPSPs evoked by NMDA in the same structure (Markham & Segal, 1990). More-

Figure 2. Mean step-through latencies on the retention trial of NMRI mice receiving, immediately after training, different doses of the cholinergic muscarinic antagonist atropine (A) or of the cholinergic muscarinic agonist oxotremorine (B). Vertical bars: SEM. Sal: saline-injected mice. * $p < .01$ versus saline (Newman-Keuls test).

Figure 3. (A) Mean step-through latencies on the retention trial of NMRI mice receiving, immediately after training, saline (first injection) in association with different doses of the NMDA noncompetitive antagonist MK-801 (second injection). The performance of these mice is compared with that of animals receiving, immediately after training, different doses of the cholinergic antagonist atropine (2 mg/kg, first injection) in association with different doses of MK-801 (second injection). Vertical bars: SEM. Sal: saline. (B) Mean step-through latencies on the retention trial of NMRI mice receiving, immediately after training, saline (first injection) in association with different doses of the NMDA noncompetitive antagonist MK-801 (second injection). The performance of these mice is compared with that of animals receiving, immediately after training, different doses of the cholinergic agonist oxotremorine (0.02 mg/kg, first injection) in association with different doses of MK-801 (second injection). Vertical bars: SEM. Sal: saline. * $p < .01$ versus saline-injected mice, ‡ $p < .01$ versus control mice injected with the same dose of MK-801 (Newman-Keuls test).

over, it has been shown that glutamatergic fibers ending in the lateral septal area appear to be involved in the reduction of septohippocampal cholinergic activity following radial-arm maze discrimination training in the mouse (Marighetto, Micheau, & Jaffard, 1994). In this regard, it must be pointed out that, pharmacologically, posttraining inhibition of the septohippocampal cholinergic path has been shown to influence spatial learning but not inhibitory avoidance (Nagahara, Brioni, & McGaugh, 1992). Moreover, with regard to inhibitory avoidance, amnesic effects have been shown following posttraining infusion of NMDA-receptor antagonists into amygdala

and entorhinal cortex in rats (Ferreira, Da Silva, et al., 1992; Ferreira, Wolfman, et al., 1992). Neurochemical investigations concerning glutamatergic-cholinergic interaction in different brain structures seem thus to be needed.

With regard to behavior, clear interactions between glutamatergic and cholinergic systems have recently been demonstrated. Pretraining administration of physostigmine reversed scopolamine and MK-801 acquisition deficits in mice tested in an elevated plus-maze, indicating participation of cholinergic and NMDA receptors in the learning process (Sharma & Kulkarni, 1992). Experiments carried out in rats with the Morris water-maze test have also shown that normal functioning of glutamatergic and cholinergic systems is necessary for spatial learning, as blockade of NMDA receptors and cholinergic hypofunction prevents spatial learning but does not impair recall (McNamara & Skelton, 1993). Finally, in mice tested in a passive-avoidance step-down-task paradigm in which short-term memory was investigated, MK-801 showed an additive or potentiating influence on scopolamine-induced deficits, indicating that cholinergic and NMDA antagonism might play a hand-in-hand role in short-term memory disturbances (Sharma & Kulkarni, 1991).

In conclusion, the present experiments clearly show the existence of a glutamatergic-cholinergic interaction also in modulating memory consolidation in mice. This phenomenon might also be considered in light of the interactions recently demonstrated between glutamatergic and dopaminergic (Mele et al., 1995) and dopaminergic and cholinergic systems (Gasbarri et al., 1993). Further experiments will be needed to better clarify the brain mechanisms and the structures involved in the observed effects.

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