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ORIGINAL INVESTIGATION

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Effects of MK-801 and nicotine combinations on memory consolidation in CD1 mice

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Abstract *Rationale:* Recent experiments have shown that pre-trial administrations of nicotine to rats tested in a 16-arm radial maze attenuated the MK-801-induced deficit in both working and reference memory performance. Memory consolidation can be influenced in laboratory animals, by post-training administration of drugs. *Objective:* In the present study we have investigated the effects on memory consolidation of CD1 mice exerted by: a) the non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo-[a,d]cyclo-hepten-5,10-imine-maleate] b) nicotine, and c) combinations of MK-801 and nicotine. *Methods:* Different groups of mice were injected intraperitoneally (IP) with the single drugs and with their combinations, immediately after training in a passive avoidance task. Additional groups of animals were also injected 2 h post-training with the highest effective dose of MK-801 (0.3 mg/kg), with the highest effective dose of nicotine (0.5 mg/kg) or with the combination of an otherwise ineffective dose of MK-801 (0.1 mg/kg) with the highest effective dose of nicotine, respectively. Their performances were compared with those of mice injected with saline, with the vehicle of nicotine and with the other treatment combinations, respectively *Results:* The results showed that MK-801 exerted deleterious effects, while nicotine exerted facilitatory effects on mice performances. Further, an otherwise ineffective dose of MK-801 (0.1 mg/kg) antagonized the facilitatory effects of nicotine (0.25 and 0.5 mg/kg). In the 2 h post-training injected groups the treatments were ineffective, showing that the immediate post-training drug administrations affected memory consolidation processes. *Conclusions:* In conclusion, from the present research, it is evident that NMDA glutamate and nicotinic acetylcholine receptor systems interact in modulating memory consolidation in CD1 mice.

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Introduction

Some researches have studied, in recent years, the effects of post-training administration of the noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801 on memory, with contrasting results. No effect of post-training MK-801 on retention has been, for example, observed in gerbils tested in a spatial maze task (Mondadori et al. 1989) and rats (Robinson et al. 1989). In other research in which avoidance learning has been employed, memory enhancing (Mondadori et al. 1989) or memory impairing effects of post-training MK-801 (Mele et al. 1995; Castellano et al. 1996; Mele et al. 1996; Cestari and Castellano 1997; Castellano et al. 1999; Ciamei et al. 2000) were observed. According to Packard and Teather (1997), methodological factors, such as the use of different tasks, training-retention intervals, doses of MK-801 and species of animals, might account for the discrepant results. Moreover, in the experiments in which the time-dependent nature of the effects of MK-801 was not considered, proactive effects of the drug on retention test performance might be present. The time-dependent nature of the effects of MK-801 on retention was examined by Packard and Teather (1997) in male Long-Evans rats tested in two water maze tasks. They showed a drug-induced impairment of retention performance in animals injected with MK-801 immediately after training. This effect was absent when the animals were injected 2 h post-training, suggesting that the effects of immediate post-training administrations of MK-801 were due to influence on memory consolidation and not to proactive effects of the drug on motivational, attentional, sensory or motoric processes (see McGaugh 1966, 1973).

Some studies can be found in which the effects of post-training nicotine administrations have been investigated in rats and mice tested in different tasks (see Levin 1992 for review). Memory improvements in various in-

bred strains of mice tested in shock avoidance or visual discrimination conditioning have been reported (Bovet et al. 1966; Bovet-Nitti 1969). Moreover, post-training application of nicotine improved retention performance in rats tested in a Hebb-Williams maze (Garg and Holland 1968; Garg 1969). In experiments carried out with C57BL/6 mice tested in a light-dark discrimination task (Y water maze) post-training injections of nicotine improved retention when the drug was given immediately after training. At 2 h post-training, treatments were ineffective, indicating that the effect of nicotine was not due to non-mnemonic factors, but that the drug enhanced memory consolidation (Castellano 1976).

It has been recently demonstrated that nicotinic acetylcholine and NMDA glutamate receptor systems have direct interaction via nicotine-induced glutamate release (McGehee et al. 1995). It has been postulated that this interaction might give an important basis for nicotine's reinforcing effects (McGehee et al. 1995). Experiments carried out with rats tested in a 16-arm radial maze have recently shown that pre-trial nicotine administration attenuates the MK-801-induced deficit in both working and reference memory performance, suggesting the existence of interactions between the behavioural effects of these drugs (Levin et al. 1998).

The aim of the present research was to study the effect of the interaction between MK-801 and nicotine on memory consolidation. CD1 mice were used which were tested in a passive avoidance task. The animals were injected with the drugs, alone or in combination, immediately after training in these experimental conditions, and were tested for retention 24 h after training. In addition, the highest effective dose of MK-801 and of nicotine, and combination of an otherwise ineffective dose of MK-801 with the highest effective dose of nicotine, were administered 2 h after training, to assess whether the effects observed following immediate post-training administrations involved non-mnemonic factors or the consolidation of memory (McGaugh 1989).

Materials and methods

Subjects

Male CD1 mice (River Labs, Como, Italy) weighing about 30 g at the beginning of the experiments were used throughout. All mice were maintained upon their arrival in the laboratory (2 weeks before the experiments) in groups of four for each cage, with food and water available ad libitum, and kept at a constant temperature of 21°C. In all the experiments the animals were tested during the second half of the light period (between 1400 and 1700 hours) in a sound insulated room. All animals were tested once. All experimental groups consisted of eight animals.

Care and handling of the animals were in accordance with NIH ethical regulations. The experimental protocol was approved by the Italian Ministry of Health on October 5th, 1998 (Decree 118/98B).

One-trial inhibitory avoidance apparatus and experimental procedure

Mice were trained on a step-through inhibitory avoidance apparatus, as previously described (McGaugh and Landfield 1970). A straight alley was divided into two compartments, one 7.5 cm long and the other 14 cm long. The floor was 2.5 cm wide and the top 10 cm wide. The smaller compartment was made of white Plexiglas. The larger one was made of black Plexiglas and was equipped with a removable cover of the same material to allow the compartment to be in darkness. The two compartments were separated by a sliding door. A tensor lamp (60 W, positioned 80 cm above the apparatus) illuminated the small compartment. The floor of the larger compartment consisted of two oblique stainless steel plates folded at the bottom, through which scrambled constant current could be delivered. In particular, the two plates were separated at the bottom by a small space (0.5 cm). The shape of the electrified floor ensured the mouse had to make contact with both plates simultaneously in order to receive the shock (0.1 mA, 50 Hz, 1 s).

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, the footshock was delivered and the step-through latency was recorded. The mouse was then removed from the apparatus and injected.

Retention was tested 24 h later following a procedure similar to that of training, except that no footshock was administered. On the test day, no time limit for the step-through latencies was set.

Three sets of experiments were carried out. In the first experiment different groups of mice were injected intraperitoneally (IP) immediately after training with saline or MK-801 at doses of 0.1, 0.2 and 0.3 mg/kg. Two additional groups of mice were injected with saline and MK-801 (0.3 mg/kg), respectively, 2 h after training.

In the second experiment, different groups of mice were injected IP immediately after training with nicotine vehicle and nicotine at the doses of 0.1, 0.25 and 0.5 mg/kg. Two additional groups of mice were injected with the vehicle of nicotine and nicotine (0.5 mg/kg), respectively, 2 h after training.

In the third experiment, different groups of mice received, immediately after training, a first injection of saline or of an ineffective dose of MK-801 (0.1 mg/kg) immediately followed by a second injection of nicotine vehicle or of one of two effective doses of nicotine (0.25 and 0.5 mg/kg). Four additional groups of mice were first injected with saline or with MK-801 (0.1 mg/kg) and immediately after with the nicotine vehicle or nicotine (0.5 mg/kg) 2 h after training.

Drugs

MK-801 (RBI, Natick, Mass., USA) was dissolved in saline and injected at a volume of 4 ml/kg. Control groups were injected with saline (0.9% NaCl). Nicotine bitartrate (RBI) was dissolved in distilled water and the pH of the solution was adjusted to 7 with NaOH and injected at a volume of 4 ml/kg. Control groups were injected with the vehicle. Both control and drug treatments were administered IP.

Statistics

The results were statistically evaluated by analysis of variance (one- and two-way ANOVA), in which mean step-through latencies of the groups on the test day were compared. Further analyses for individual between-groups comparisons were carried out with post-hoc tests (Duncan multiple range test).

Results

In the first set of experiments, immediately post-training IP administration of MK-801 significantly impaired retention in mice in a dose-dependent way (Fig. 1).

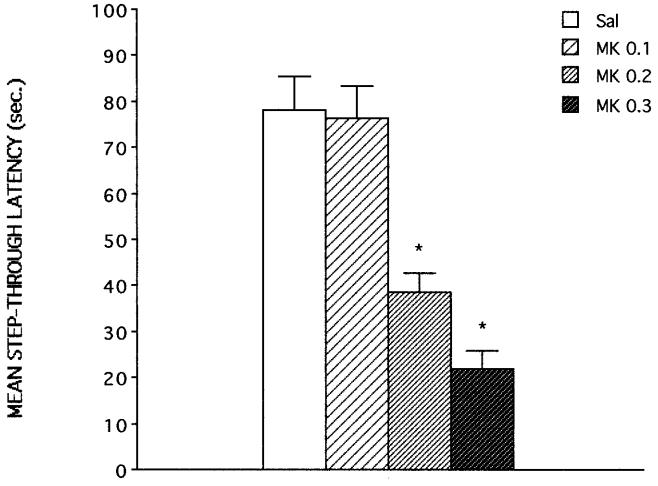

Fig. 1 Mean step-through latencies of mice injected with saline or MK-801 (0.1, 0.2 and 0.3 mg/kg). *Vertical bars*: SEM. *P<0.001 versus all the other groups

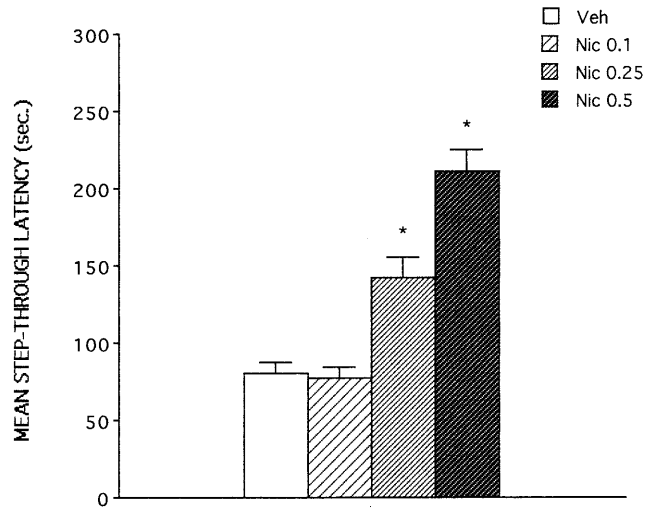

Fig. 2 Mean step-through latencies of mice injected with vehicle or nicotine (0.1, 0.25 and 0.5 mg/kg). *Vertical bars*: SEM. *P<0.001 versus all the other groups

ANOVA (one-way) showed significant differences between groups [F(3,29)=22,26, P<0.001]. Individual between-treatments comparisons revealed significantly lower mean step-through latencies, when compared with saline-injected controls, for those groups that were injected with MK-801 at the doses of 0.2 and 0.3 mg/kg (P<0.001), while no difference was observed between the performances of controls and MK-801 (0.1 mg/kg)-injected animals.

The mean step-through latencies (±SEM) of the groups injected 2 h after training with saline or MK-801 (0.3 mg/kg) were 79.37±3.28 and 78.62±3.88 seconds (s), respectively. ANOVA (one-way) showed no significant differences [F(31,14)=0,02, NS] between the retention performances of the groups.

In the second set of experiments, immediately post-training IP administration of nicotine significantly enhanced retention in mice in a dose-dependent way (Fig. 2). ANOVA (one-way) showed significant differences between groups [F(33,28)=33.54, P<0.001]. Individual between treatments comparisons revealed significantly higher mean step-through latencies, when compared with saline-injected controls, for those groups which were injected with nicotine at doses of 0.25 and 0.5 mg/kg (P<0.001), while no difference was observed between controls and nicotine (0.1 mg/kg) injected animals.

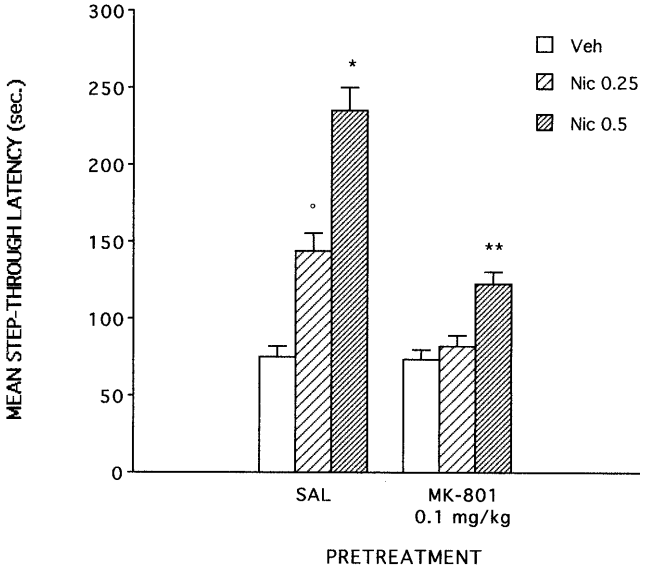

Fig. 3 Mean step-through latencies of mice pretreated with saline (*left columns*) or MK-801 (0.1 mg/kg; *right columns*) and treated with vehicle or nicotine (0.25 and 0.5 mg/kg). *Vertical bars*: SEM. *P<0.001 versus all the other groups. °P<0.001 versus control groups. **P<0.01 versus MK-801+nicotine 0.25 group

The mean step-through latencies (±SEM) of the groups injected 2 h after training with vehicle or nicotine (0.5 mg/kg) were 76.50±3.43 and 75.50±3.53 s, respectively. ANOVA (one-way) showed no significant differences [F(31,14)=0,04, NS] between the retention performances of the groups.

In the third set of experiments (Fig. 3), MK-801 (0.1 mg/kg) antagonized the effects of nicotine (0.25 and 0.5 mg/kg). Two-way ANOVA revealed a significant effect for both pretreatment [F(31,42)=48.42, P<0.001] and treatment [F(32,42)=60.00, P<0.001], and also evidenced the existence of an interaction between these two factors [F(32,42)=16.32, P<0.001]. Individual between-treatment comparisons revealed that the performance of saline pretreated mice was improved by nicotine (0.25 and 0.5 mg/kg) treatment when compared with the performance of vehicle-treated animals (P<0.001). For the experiments in which combined administrations of MK-801 and nicotine were carried out, in MK-801-pretreated animals no difference was recorded between vehicle and nicotine 0.25 mg/kg-treated animals, while the mean step-through latency of the group which received MK-801 and nicotine (0.5 mg/kg) was significantly higher in

comparison with that of MK-801 and nicotine (0.25 mg/kg)-treated groups ($P<0.01$), and significantly lower as compared with the group which received saline and 0.5 mg/kg nicotine ($P<0.001$). No differences were observed between the performances of mice pretreated with saline and treated with nicotine (0.25 mg/kg) and those of mice pretreated with MK-801 (0.1 mg/kg) and 0.5 mg/kg nicotine.

The mean step-through latencies (±SEM) of the groups injected 2 h after training with combinations of saline and the nicotine vehicle, saline and nicotine (0.5 mg/kg) or combinations of MK-801 (0.1 mg/kg) with nicotine vehicle or with nicotine were 77.38±3.67, 79.88±3.37, 80.00±4.15 and 77.75±3.12 s, respectively. ANOVA (two-way) did not reveal a significant effect for the pretreatment [$F(31,28)=4.83$, NS], the treatment [$F(31,28)=1.21$, NS] and for the interaction between these two factors [$F(31,28)=0.44$, NS].

One-way ANOVA showed no significant differences [$F(33,28)=0.70$, NS] among the training latencies of the groups of mice injected immediately after training with different doses of MK-801 or saline, with mean training latencies ranging from 9.37±0.59 to 10.25±0.50 s. In addition, no significant differences [$F(33,28)=0.08$, NS] were found among the training latencies of the groups of mice injected post-training with different doses of nicotine or with nicotine vehicle, with mean training latencies ranging from 9.37±0.49 to 9.75±0.59 s. The training latencies of the different groups of mice injected immediately after training with the combinations of saline and nicotine vehicle or saline and different doses of nicotine (0.25 or 0.5 mg/kg) or with the combinations of MK-801 (0.1 mg/kg) and nicotine vehicle or different doses of nicotine (0.25 or 0.5 mg/kg) did not differ significantly [$F(35,42)=0.08$, NS]. The mean training latencies ranged from 9.25±0.45 to 9.62±0.56 s.

Discussion

From the first set of experiments it is evident that immediate post-training administration of the non-competitive NMDA receptor antagonist MK-801 dose-dependently impaired retention test performance of CD1 mice tested in a passive avoidance task. Contrasting results exist in literature concerning the effects of MK-801 on retention. No effect, enhancing or impairing effects have been reported (Mondadori et al. 1989; Robinson et al. 1989; Mele et al. 1996), depending on task, dose of MK-801 used, animal species, training-retention test intervals (see Packard and Teather 1997). Mainly, lack of data concerning delayed post-training injections effects could not exclude that the effects of MK-801 were due to influences on non-mnemonic factors. In the present study, it was thus decided to administer MK-801 also 2 h after training. Similarly to what observed by Packard and Teather (1997) in rats tested in two water maze tasks, no effect was observed following the delayed administration of the drug. Thus, our results show, as in the study of Packard and Teather (1997), that the impairing effect observed following immediate post-training MK-801 administration was due to a direct influence of the drug on memory consolidation of mice (McGaugh 1989).

In the second set of experiments post-training immediate, but not 2 h, administration of nicotine improved retention performance of mice. Again, lack of effect following 2 h post-training administration of the drug demonstrates that it affected memory consolidation of the animals. This result is in agreement with results obtained in previous research (Castellano 1976) in which nicotine improved memory of C57BL/6 mice tested in a Y water maze involving light-dark discrimination following immediate but not 2 h post-training administration.

The third set of experiments showed the existence of an interaction between nicotine and MK-801 in modulating memory consolidation of CD1 mice. In fact, an otherwise ineffective dose of MK-801 (0.1 mg/kg) completely antagonized the memory improving effects of 0.25 mg/kg nicotine and partially antagonized the effect of the highest dose of nicotine tested (0.5 mg/kg). The effects were absent when the drugs were administered 2 h after training, suggesting the existence of an effect on the memory consolidation of the animals.

Some behavioural studies have recently shown the existence of nicotine and MK-801 interactions. Levin et al. (1998) have observed that, in rats tested in a 16-arm radial maze, pre-trial administration of nicotine attenuated the deficit induced by MK-801 on both working and reference memory performance. They postulate that nicotine might have counteracted the MK-801-induced memory deficit, since it stimulates glutamate release (McGehee et al. 1995). However, they make also the hypothesis that crossreactivity of ligands for nicotine and NMDA receptors might be involved in their results, since these receptors are similar in structure and nicotine has effects at the NMDA receptor, and MK-801 has effects on nicotine receptors (Aizenman et al. 1991; Amador and Dani 1991). The present research demonstrates the existence of an interaction between glutamatergic and nicotine cholinergic function in memory consolidation. The results might be interpreted in terms of interactions involving nicotinic and NMDA receptors in some brain structures, such as, for example, hippocampus, which plays an important role in memory and contains high densities of both NMDA and nicotinic receptors (Monaghan and Cotman 1985; Brioni and Arneric 1993).

Some researches have demonstrated that nicotine exerts its effects by releasing several neurotransmitters, including acetylcholine, dopamine and glutamate, and that interactions with these neurotransmitters are at the basis of its cognitive effects (Wonnacott et al. 1989; McGehee et al. 1995). In particular, scopolamine antagonises nicotine-induced improvement of performance in rats tested in a radial maze (Levin and Rose 1991). Moreover, nicotine administration potentiates the memory-improving effect of dopaminergic agonists in rats tested in the same experimental conditions (Levin and Eisner 1994; Levin

1995) and facilitates retention of avoidance responses in CD1 mice tested in a passive avoidance task acting through dopaminergic mechanisms (Brioni and Arneric 1993).

Finally, glutamatergic-cholinergic interaction on memory consolidation has been reported (Castellano et al. 1996) and dopaminergic mechanisms are involved in the memory deficit induced by MK-801 on memory, in CD1 mice tested in the same experimental conditions (Mele et al. 1996). Involvement of the above-cited neurotransmitters must be taken into consideration in further attempts to explain the mechanisms at the basis of the results of the present research. It must, however, be clearly pointed out that, in absence of further investigations, the types and location of the receptor systems that are critical for the behavioural effects observed are for the moment matter of conjecture.

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