The involvement of dorsal hippocampus in dextromethorphan-induced state-dependent learning in mice

Mohammad-Reza Zarrindast a,b,c,d,e,⁎, Vahid Owneg a,b, Ameneh Rezayof f, Farid Owneg a,b

a Department of Neuroscience, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
b Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
c Iranian National Center for Addiction Studies, Tehran University of Medical Sciences, Tehran, Iran
d Institute of Cognitive Sciences, Institute for Research in Fundamental Sciences (IPM), Tehran, Iran
e Institute of Cognitive Science Studies (ICSS), Tehran, Iran
f Department of Animal Biology, School of Biology and Center of Excellence in Phylogeny of Living Organisms, College of Science, University of Tehran, Tehran, Iran

Abstract

In an effort to understand the effect of dextromethorphan (DM; 3-methoxy-17-methylmorphinan), a noncompetitive antagonist of the N-methyl-D-aspartate (NMDA) receptors on memory retrieval, male NMRI mice received intraperitoneal (i.p.) or intra-CA1 injection of this drug before or after training and before testing in passive avoidance task. Pre-training i.p. (20 mg/kg) or intra-CA1 (0.5 and 1 μg/mouse) administration of DM induced amnesia in a dose-dependent manner. Post-training i.p. (10 and 20 mg/kg) or intra-CA administration of DM (0.5 and 1 μg/mouse) however, did not affect the memory retrieval. Moreover, memory retrieval was impaired in animals receiving either i.p. (20 mg/kg) or intra-CA1 administration of DM (0.5 and 1 μg/mouse) prior to testing, suggesting the DM-induced amnesia. Interestingly, the amnestic effect of pre-training i.p. (20 mg/kg) or intra-CA1 administration of DM (1 μg/mouse) was restored in mice receiving pre-test i.p. (5 and 10 mg/kg) or intra-CA1 (0.25 and 0.5 μg/mouse) administration of the drug, indicating DM-induced state-dependent learning. Taken together, it can be concluded that DM administration impairs memory retrieval in a dose- and time-dependent manner. Moreover, DM can induce state-dependent learning. Dorsal hippocampus appears to play an important role upon DM influence of learning and memory processes.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Dextromethorphan (DM, 3-methoxy-17-methylmorphinan) is used as an active ingredient in many over-the-counter cough suppressant medications (Benn and Peck, 1992). Immediately after oral administration of DM, it can be absorbed from the gastrointestinal tract, enters into the bloodstream and crosses the blood–brain barrier (Wills and Martin, 1988; Marier et al., 2005). It is important to note that DM is a low affinity, non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist in the CNS. Evidence suggests DM inhibitory effects on glutamate-induced neuroexcitotoxicity which is central to neuronal death mechanisms (Choi, 1987). DM has neuroprotective properties, thus has been proposed for treating neuronal disorders (Werling et al., 2007). Several lines of evidence support the agonistic effect of DM on μ-opioid, sigma 1 (Klein and Musacchio, 1989) and sigma 2 (Zhou and Musacchio, 1991) receptors, and its antagonistic/blocking functions on alpha3beta4 neuronal nicotinic receptors (Hernandez et al., 2000) and calcium channels. Therefore, the unique potential of DM in mediating neuroprotection seems to depend on multiple mechanisms. In addition, since DM can potentially block serotonin transporters, its implications in treating bipolar, unipolar, major depression, psychotic, and treatment-resistant depressive disorders have widely been articulated (for a review see Lauterbach, 2011). On the other hand, DM retains a potential for abuse (300 mg/day or more) by individuals of all ages, however its abuse by adolescents and young adults is of particular concern (Darboe et al., 1996; Noonan et al., 2002; Wolfe and Caravati, 1995; Schwartz, 2005). Acute high dose administration of DM has been demonstrated to activate the reward dopaminergic mesolimbic pathway which possibly mediates the abusive property of the drug (Jahng et al., 2001; Zhang et al., 2001).

On the other hand, a variety of studies have postulated the critical role of hippocampal NMDA receptors in long-term potentiation which is a putative underlying physiological process in learning and memory (Liu et al., 2004; Berg et al., 2013). NMDA-receptor antagonists can therefore disrupt memory performance during learning tasks (McHugh et al., 2008; Matus-Amat et al., 2007). Furthermore, NMDA-receptor antagonists also induce state-dependent learning (Harrod et al., 2001; Jackson et al., 1992; Ceretta et al., 2008). State-dependent learning and the corresponding retrieval of the acquired information provides the subject with better memory retrieval in the same state where learning has already occurred (Bruins Slot and Colpaert, 1999;
was administered i.p. or injected into the hippocampal CA1 regions dissolved in sterile 0.9% saline, just before the experiments. The drug involved in morphine state-dependent learning in mice (Jafari-Sabet et al., 2005; Zarrindast et al., 2006a, 2006b). The NR1 subunit plays a central part in formation of functional channels and also modulates several properties of NMDA receptors (Atlason et al., 2007). In addition to the fact that DM prevents the induction of long-term potentiation in vivo (Krug et al., 1993), it has been shown to impair spatial learning in the Morris water maze in rats, in a dose-dependent manner (Bane et al., 1996). Moreover, the impairment of spatial memory and learning has also been induced by repeated administration of high doses of DM during the adolescent period in rats which is possibly due to an increased expression of a functional subunit of NMDA receptor (NR1) in the prefrontal cortex and hippocampus (Zhang et al., 2007). Given the wide use of DM in cough-treating medications and that it blocks NMDA receptors as an antagonist, it is important to note its possible untoward effects on learning and memory processes. Therefore its exact mechanism of action needs to be evaluated in different animal models. Considering the above, the current investigation pursued three main aims including: 1 — to investigate the effect of acute systemic administration of DM on memory retrieval in the passive avoidance learning; 2 — to examine whether DM can induce state-dependent learning and 3 — to evaluate the role of dorsal hippocampus in DM response in passive avoidance learning.

2. Materials and methods

2.1. Animals and substances

Male albino NMRI mice (Pasteur Institute, Iran), weighting 22–26 g at the time of surgery, were used. Animals were maintained in a temperature-controlled (22 ± 2 °C) room with a 12/12-h light–dark cycle (lights on 07:00 h). All experiments were carried out during the light phase of the cycle. Mice were allowed to acclimatize with the laboratory conditions for at least 1 week before surgery. Food and water provided ad libitum except for the periods of behavioral testing during the passive avoidance learning task. Each experimental group comprised 10 animals and each animal was tested only once. Behavioral tests and animal care were conducted in accordance with the standard ethical guidelines (NIH, publication no. 85-23, revised 2010; European Communities Directive 86/609/EEC) and approved by the local ethical committee.

2.2. Surgery

Animals were anesthetized by i.p. injection ketamine/xylazine mixture (50 and 5 mg/kg, respectively) and placed in a stereotaxic frame (Stoelting Instruments, USA) with flat-skull position. A midline incision was made to retract the skin and the underlying periosteum. Bilateral stainless steel guide cannulae (22 gauge) were implanted 1 mm above the CA1 regions of the dorsal hippocampi according to the stereotaxic coordinates; AP, −2 mm posterior to the bregma; L, ±1.6 mm from midline; V, −1.5 mm relative to dura (Paxinos and Franklin, 2001). The cannulae were anchored to the skull by means of dental cement after which stainless steel stylets (27 gauge) were inserted into the guide cannulae to maintain patency prior to microinjections.

2.3. Drugs and injections

Dextromethorphan (DM; Sigma St, USA), a noncompetitive antagonist of the N-methyl-D-aspartate (NMDA) receptors was used. DM was dissolved in sterile 0.9% saline, just before the experiments. The drug was administered i.p. or injected into the hippocampal CA1 regions (intra-CA1) at a total volume of 10 ml/kg or 1 µl (0.5 µl per each CA1 region), respectively. Intra-CA1 injections of the drug or saline were given by lowering a 27-gauge injector cannula to extend 1 mm beyond the tip of the guide cannula to the site of injection. The injector cannula was attached to a 2-µl Hamilton syringe via a polyethylene tubing. Injection time was 60 s, followed by an additional 60 s to facilitate diffusion of the drug from the tip of the injection cannula.

2.4. Passive avoidance apparatus

Animals were submitted to the behavioral procedure. The apparatus was a (30 × 30 × 40 cm high) wooden box the floor of which consisted of parallel stainless steel bars (0.3 cm in diameter, spaced 1 cm apart). A wooden platform (4 × 4 × 4 cm) was placed on the center of the grid floor. Upon the training session, animals were placed on the platform and their latency to step down on the grid with all four paws was measured. Immediately after stepping down on the grid, the animals received an electric shock (1 Hz, 0.5 s, 45 V DC) continuously for 15 s. The shocks were delivered to the grid floor by an isolated (Borj Sanat, Iran) stimulator. If any animal stayed on the platform for over 20 s or stepped up to the platform before the 15 s of electric shock ended, it was excluded from the experiments. Retention test session was carried out 24 h after the training and was procedurally identical to training, except that no shock was delivered. Step-down latency was used as a measure of memory retrieval. An upper cut-off time of 300 s was set. The retention test was carried out between 8:00 a.m. and 2:00 p.m.

2.5. Behavioral study

2.5.1. Experiment 1: the effect of pre-training i.p. or intra-CA1 administration of DM on memory retrieval

In this experiment, the effect of pre-training i.p. or intra-CA1 injection of DM on inhibitory avoidance response was examined using six groups of mice (n = 10/group). Three groups received i.p. injection of saline (10 ml/kg) or DM (10 and 20 mg/kg) 15 min before the training (pre-training). The other three groups received pre-training intra-CA1 administration of saline (1 µl/mouse) or DM (0.5 and 1 µg/mouse) 5 min before the training. On the test day, step-down latency (as an index for memory retrieval) was measured 24 h after training in all groups (Fig. 1).

2.5.2. Experiment 2: the effect of post-training i.p. or intra-CA1 administration of DM on memory retrieval

In order to examine the effects of post-training i.p. or intra-CA1 administration of DM on memory retrieval, i.p. (10 and 20 mg/kg) or
intra-CA1 (0.5 and 1 µg/mouse) DM was injected immediately after a successful training session in four groups (n = 10/group). In this experiment, two control groups received post-training i.p. (10 ml/kg) or intra-CA1 (1 µl/mouse) administration of saline. In 24 h time, all animals were tested for memory retrieval and the step-down latency during retrieval was recorded (Fig. 2).

2.5.3. Experiment 3: the effect of pre-test i.p. or intra-CA1 administration of DM on memory retrieval

In this experiment, the effect of pre-test i.p. or intra-CA1 administration of DM on inhibitory avoidance response was examined using six groups of mice (n = 10/group). All groups of animals were trained in the passive avoidance task. On the test day, three groups of animals received i.p. saline (10 ml/kg) or DM (10 and 20 mg/kg) 15 min before testing (pre-test). The remaining three groups received intra-CA1 administration of saline (1 µl/mouse) or DM (0.5 and 1 µg/mouse) 5 min prior to the step. Step-down latency (as an index for memory retrieval) was measured 30 min post injection in all groups (Fig. 3).

2.5.4. Experiment 4: the effect of pre-test i.p. or intra-CA1 administration of DM in mice trained under saline or DM

In this experiment, we examined the effects of pre-training and pre-test administration of DM on memory during the passive avoidance task. Two control groups received i.p. (10 ml/kg) or intra-CA1 (1 µl/mouse) administration of saline before training and testing sessions. Three groups of animals received pre-training i.p. injection of DM (20 mg/kg) 15 min before training, followed by the pre-test DM (5 and 10 mg/kg, i.p.) 15 min before testing. The other three groups of animals received a pre-training intra-CA1 DM at 1 µg/mouse, 5 min before training, followed by pre-test administration of DM (0.25 and 0.5 µg/mouse, intra-CA1) 5 min prior to the testing session (Fig. 4).

2.6. Verification of cannulae placements

When behavioral testing sessions were concluded, animals were killed by anesthetic overdose and received microinjection of Methylene Blue (1%) at the same volume as drug microinjections. This was to mark the site of the drug injection. Mice brains were removed and injection sites were histologically verified according to the atlas of Paxinos and Franklin (2001). Data from the animals with injection sites located outside the CA1 regions were not used in the analysis.

2.7. Statistics

The step down latencies are expressed as the median and interquartile range. Since the data is not normally distributed, the Kruskal–Wallis non-parametric one-way analysis of variance (ANOVA) followed by a two-tailed Mann–Whitney U-test has been used. In all statistical evaluations P < 0.05 indicated a statistical significance.

3. Results

3.1. The effect of pre-training i.p. or intra-CA1 administration of DM on memory retrieval

Fig. 1 (left panel) shows the effects of pre-training i.p. administration of DM on the step-down latency in passive avoidance learning task.

Fig. 2. The effect of post-training i.p. or intra-CA1 administration of DM on memory retrieval. Groups of mice were trained immediately after i.p. administration of DM (0, 10 and 20 mg/kg) or intra-CA1 administration of DM (0.5 and 1 µg/mouse) and were tested 24 h later. Each value represents the median and interquartile ranges for 10 mice.

Fig. 3. The effect of pre-test i.p. or intra-CA1 administration of DM on memory retrieval. Groups of mice were tested 15 min before i.p. administration of DM (0, 10 and 20 mg/kg) or 5 min before intra-CA1 administration of DM (0.5 and 1 µg/mouse). Each value represents the median and interquartile ranges for 10 mice. ***P < 0.001, as compared to i.p. pre-test saline group. +P < 0.01, as compared to intra-CA1 pre-test saline group.

Fig. 4. The effect of pre-test i.p. or intra-CA1 administration of DM in mice trained under saline or DM. Five groups of animals (left panel) received pre-training i.p. administration of saline (1 ml/kg) or DM (20 mg/kg). On the test day, mice received saline or DM (5 and 10 mg/kg, i.p.) 15 min before the test. The other five groups of animals (right panel) received intra-CA1 pre-training injection of a high dose DM (1 µg/mouse) and pre-test injection of different doses of DM (0.25 and 0.5 µg/mouse, intra-CA1). Each value represents the median and interquartile ranges for 10 mice. ***P < 0.001, as compared to i.p. pre-training saline/pre-test saline group. ++P < 0.01, as compared to i.p. pre-training DM/pre-test saline group. ###P < 0.001, as compared to intra-CA1 pre-training saline/pre-test saline group. *P < 0.05, ^P < 0.01, as compared to intra-CA1 pre-training DM/pre-test saline group.
Kruskal–Wallis non-parametric ANOVA indicated that the step-down latency in the passive avoidance task was reduced in DM-treated animals (0, 10 and 20 mg/kg, i.p., before the training) [H(2) = 11.9, P < 0.01], showing an amnestic impact of DM. The maximum response was obtained with 20 mg/kg of DM (P < 0.001).

Fig. 1 (right panel) indicates that memory retrieval was impaired in animals receiving intra-CA1 DM (0, 0.5 and 1 μg/mouse, 5 min before the training) [Kruskal–Wallis non-parametric ANOVA: H(3) = 12.1, P < 0.01]. Post-hoc analysis showed that 1 μg/mouse of DM produced the maximum amnestic effect (P < 0.01).

3.2. The effect of post-training i.p. or intra-CA1 administration of DM on memory retrieval

Fig. 2 (left panel) indicates the effects of post-training i.p. administration of DM on the step-down latency in passive avoidance learning task. Kruskal–Wallis non-parametric ANOVA revealed that post-training i.p. injection of DM (0, 10 and 20 mg/kg) does not alter memory retrieval in the passive avoidance task [H(2) = 0.9, P > 0.05].

Fig. 2 (right panel) shows that memory retrieval had no change in the animals treated with intra-CA1 injection of DM (0, 0.5 and 1 μg/mouse), 5 min before the training [Kruskal–Wallis non-parametric ANOVA: H(2) = 1.5, P > 0.05].

3.3. The effect of pre-test i.p. or intra-CA1 administration of DM on memory retrieval

Fig. 3 (left panel) demonstrates the effects of pre-test i.p. injection of DM on memory retrieval in passive avoidance learning task. Kruskal–Wallis non-parametric ANOVA indicated that the step-down latency in the passive avoidance task is decreased in DM-treated animals (0, 10 and 20 mg/kg, i.p., 15 min prior to testing) [H(2) = 14.4, P < 0.001], suggesting the DM-induced amnesia. The maximum response was obtained with 20 mg/kg of DM (P < 0.001).

Fig. 3 (right panel) indicates an impaired memory retrieval in animals injected with intra-CA1 DM (0.5 and 1 μg/mouse), 5 min before the training [Kruskal–Wallis non-parametric ANOVA: H(2) = 9.2, P < 0.05]. As post-hoc analysis pointed out, the pre-test intra-CA1 administration of 1 μg/mouse of DM produces the maximum amnestic effect (P < 0.01).

3.4. The effect of pre-test i.p. or intra-CA1 administration of DM in mice trained under saline or DM

The effects of pre-test i.p. administration of DM in animals trained under saline or DM on step-down latency have been shown in Fig. 4 – left panel. According to Kruskal–Wallis non-parametric ANOVA, animals with pre-training DM-induced amnesia, had their retrieval restored to the control level following pre-test administration of DM (5 and 10 mg/kg) [H(3) = 29.8, P < 0.001]. The above observation is in favor of DM state-dependent learning. The maximum restoration was observed with 5 mg/kg of DM (P < 0.01).

The right panel of Fig. 4 shows the effects of pre-test intra-CA1 administration of DM in animals trained under saline or DM on memory retrieval in passive avoidance learning task. Kruskal–Wallis non-parametric ANOVA [H(3) = 17.4, P < 0.01] revealed that the amnesia induced by the pre-training intra-CA1 administration of DM was restored in animals which received pre-test administration of the DM (0.25 and 0.5 μg/mouse) as compared to the pre-training DM (1 μg/mouse)/pre-test saline-treated control group.

4. Discussion

Using a one-trial passive avoidance task in mice, the present results showed that pre-training or pre-test, but not post-training i.p. administration of DM potentially impairs memory retrieval in a dose-dependent manner. It can consequently be suggested that DM, as an NMDA receptor antagonist, exerts a significant effect on the acquisition and memory retrieval, but not consolidation when tested 24 h later. A few previously published reports examining the effect of DM on learning and memory processes have shown that the drug can affect memory formation in animal models. For example, Sierocinska et al. (1991) indicated that systemic administration of different doses of DM disrupts long, but not short-term memory retrieval in the passive avoidance task. In a similar investigation using different types of NMDA receptor antagonists such as MK-801 and DM, Murata and Kawasaki (1993) reported that intracerebroventricular injection of these antagonists impairs the learning process in rats. Moreover, DM is shown to impair the spatial learning using the Morris water maze in a dose-dependent manner (Bane et al., 1996). Findings from a response-acquisition procedure have demonstrated that DM can disrupt the initial response acquisition (i.e., learning) through positive reinforcement (Morgan et al., 2006). Having noted these, although DM is somehow considered a safe and effective antitussive in children and adults, the potential risk of DM-related memory impairment as one of its under-sighted side effects should be refocused. On the other hand, previous studies have shown that DM can reduce pain (Siu and Drachtman, 2007) or stress (Kamei et al., 1996), since both pre-training and pre-test, but not post-training administration of the drug can induce amnesia, the effect may not be due to drug influence on pain or stress. In addition, Plesan et al. (1998) reported that systemic administration of 45 mg/kg of DM had no antinociceptive effect in a hot plate test. It should be considered that the doses used in our study were 10 and 20 mg/kg of DM.

In the second phase of the present study, the effects of pre-training, post-training and pre-test intra-CA1 microinjection of DM in the passive avoidance task were also examined in mice. Interestingly, the present data obtained from intra-CA1 administration of different doses of DM were similar to its systemic administration. This shows that the dorsal hippocampal NMDA receptors are possibly involved in mediating DM-induced amnesia. In other investigations using intra-CA1 administration of different NMDA-receptor antagonists, the decrease in memory retention of inhibitory learning (Jafari-sabet, 2006) and the disruption in spatial discrimination reversal learning (Watson and Stanton, 2009) were similarly observed in rats. Moreover, using NMDA-receptor antagonists, the critical role of dorsal hippocampal NMDA receptors in the induction of long term potentiation (LTP) has been well established (Bashir et al., 1991; Harris et al., 1984; Mulkey and Malenka, 1992). Since hippocampal NMDA receptors are shown to critically involve in synaptic plasticity, for the prescription drugs which can cross the blood–brain barrier such as DM, the untoward effects and possible interference with learning and memory processes should be taken into account.

The current study also showed that the animals in which memory retrieval was impaired due to the pre-training of systemic or intra-CA1 administration of DM, pre-test administration of the drug restored the retrieval to the control level. This finding may be related to DM-induced state-dependent learning. With regard to the state-dependent learning, new information which has been acquired while the animal is under the effect of a certain drug, can only be recalled when the animal is in the same state as during the encoding phase (Izquierdo and Dias, 1983; Koek, 2011; Shulz et al., 2000). Therefore, it might be inferred that the memory retrieval in animals under DM administration depends on the congruence between the states of learning and corresponding information retrieval. We have shown in our previous studies the similar induction of state-dependent learning by morphine (Ardjmand et al., 2011; Darbandi et al., 2008; Khajehpour et al., 2008). Our earlier findings revealed that morphine-related state-dependent learning may be pertained to the opiatereward (Zarrindast and Rezayof, 2004). In addition, it has been shown that pre-test administration of NMDA receptor antagonists, N-AP5 (Jafari-Sabet et al., 2005) or MK-801 (Zarrindast et al., 2006a, 2006b) decreased morphine state-dependent learning, suggesting a functional interaction between NMDA and μ-opioid receptors. With the view of the fact that DM has a
tendency to be abused in humans (Boyer, 2004) and also has a high affinity binding potential for the sigma-1 receptors (Klein and Musacchio, 1989; Werling et al., 2007), one might speculate that the ability of DM to provide state-dependent learning depends on its rewarding effect. Some other studies have declared that the systemic administration of NMDA receptor antagonists including ketamine, phencyclidine, MK-801 and CGS 19755 produce a state-dependent recall (Harrod et al., 2001; Jackson et al., 1992). Furthermore, Ceretta et al. (2008) reported that aracaine, a competitive antagonist of the polyamine binding site at the NMDA receptor, can also induce memory impairment in a state-dependent manner. On the other hand, it should be considered that the amnesia induced by pre-training injection of DM was significantly reversed by pre-test intraperitoneal or intra-CA1 administration of the lower dose of the drug in the present study. However, state-dependent learning can usually be induced with the same doses during the training and memory retrieval phases (Bruins Slot and Colpaert, 1999; Khavandgar et al., 2002; Zarrindast et al., 2006a, 2006b), but the results obtained in our investigation revealed that pre-test administration of a lower dose of the drug can also produce state-dependent learning. This is in agreement with our previous studies which pre-test administration of a lower dose of ethanol induced state-dependent memory retrieval of the memory acquired under pre-training administration of a higher dose of ethanol (Rezayof et al., 2007, 2008). Therefore, if a lower dose of the drug can produce the same state during memory retrieval, it is possible to induce state-dependent learning. However, this hypothesis should be more clarified further.

In summary, our findings re-emphasize on the DM-induced impairment of memory formation in a time- and dose-dependent manner and that the CA1 region of dorsal hippocampus may be involved in mediating this effect. Moreover, to our knowledge, this is the first study showing that DM can induce state-dependent memory retrieval. Therefore, memory impairment and the potential for abuse (state-dependent recall) may be considered as important side effects of DM. Pharmacovigilance may consider addition of these warnings into DM leaflet when clinically supported. Nevertheless, the underlying mechanism for the above is not fully clear and further studies are needed.

Acknowledgments

The authors thank the Iran National Science Foundation (INSF) for providing the financial support for this project.

References


Bruins Slot LA, Colpaert FC. Recall rendered dependent on an opiate state. Behav Neurosci 1999;113:337–44.


Watson DJ, Stanton ME. Spatial discrimination reversal learning in weanling rats is impaired by striatal administration of an NMDA-receptor antagonist. Learn Mem 2009;16:564–72.


