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

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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.

1. Introduction

Dextromethorphan (DM, 3-methoxy-17-methylmorphinan) is used as an active ingredient in many over-the-counter cough suppressant medications (Bem 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 concerns (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;

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Shulz et al., 2000). Previously, we also showed that NMDA receptors are involved in morphine state-dependent learning in mice (Jafari-Sabet et al., 2005; Zarrindast et al., 2006a, 2006b). NMDA receptors are tetrameric protein complexes comprising three subunits, NR1, NR2 and probably NR3 (Schuler et al., 2008). 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

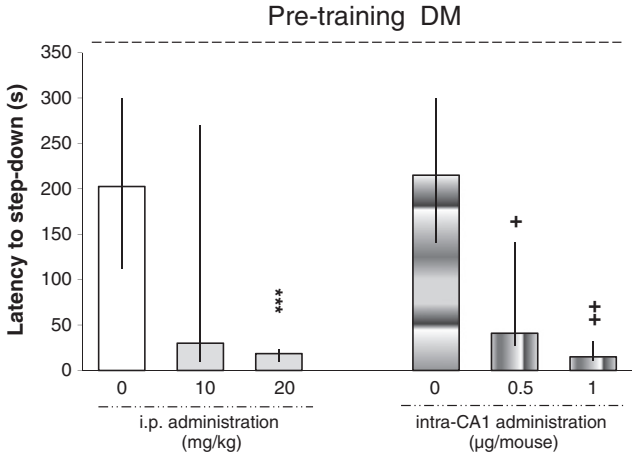

Fig. 1. The effect of pre-training i.p. or intra-CA1 administration of DM on memory retrieval. Groups of mice were trained 15 min before i.p. administration of DM (0, 10 and 20 mg/kg) or 5 min before intra-CA1 administration of DM (0.5 and 1 μg/mouse) and were tested 24 h later. Each value represents the median and interquartile ranges for 10 mice. ***P < 0.001, as compared to i.p. pre-training saline group. +P < 0.05, ++P < 0.01, as compared to intra-CA1 pre-training saline group.