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Role of hippocampal and prefrontal cortical signaling pathways in dextromethorphan effect on morphine-induced memory impairment in rats

Zahra Ghasemzadeh, Ameneh Rezayof *

Department of Animal Biology, School of Biology and Center of Excellence in Phylogeny of Living Organisms, College of Science, University of Tehran, Tehran, Iran

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ABSTRACT

Evidence suggests that dextromethorphan (DM), an NMDA receptor antagonist, induces memory impairment. Considering that DM is widely used in cough-treating medications, and the co-abuse of DM with morphine has recently been reported, the aims of the present study was (1) to investigate whether there is a functional interaction between morphine and DM in passive avoidance learning and (2) to assess the possible role of the hippocampal and prefrontal cortical (PFC) signaling pathways in the effects of the drugs on memory formation. Our findings indicated that post-training or pre-test administration of morphine (2 and 6 mg/kg) or DM (10–30 mg/kg) impaired memory consolidation and retrieval which was associated with the attenuation of the levels of phosphorylated Ca^{2+}/calmodulin-dependent protein kinase II (p-CAMKII) and cAMP responsive element-binding protein (p-CREB) in the targeted sites. Moreover, the memory impairment induced by post-training administration of morphine was reversed by pre-test administration of the same dose of morphine or DM (30 mg/kg), indicating state-dependent learning (SDL) and a cross-SDL between the drugs. It is important to note that the levels of p-CAMKII/CAMKII and p-CREB/CREB in the hippocampus and the PFC increased in drugs-induced SDL. In addition, DM administration potentiated morphine-induced SDL which was related to the enhanced levels of hippocampal and PFC CAMKII-CREB signaling pathways. It can be concluded that there is a relationship between the hippocampus and the PFC in the effect of DM and/or morphine on memory retrieval. Moreover, a cross SDL can be induced between the co-administration of DM and morphine. Interestingly, CAMKII-CREB signaling pathways also mediate the drugs-induced SDL.

1. Introduction

Dextromethorphan (DM) is increasingly prescribed as an antitussive drug which can readily cross the blood brain barrier to affect the central nervous system (for a review, see Siu & Drachtman, 2007). A bulk of literature has been published on analgesic (Tao, Chen, & Huang, 2011), antidepressant (Lauterbach, 2011) and neuroprotective (Cheng et al., 2015) properties of DM. Despite its safety and efficacy, DM might have the potential of being abused (Schwartz, 2005) because it modulates the dopaminergic mesolimbic pathways (Steinmiller, Maisonneuve, & Glick, 2003). The rewarding/reinforcing effects of DM may be due to over-expression of tyrosine hydroxylase gene in the midbrain dopaminergic neurons (Zhang, Jahng, & Kim, 2001). On the other hand, DM reduces withdrawal symptoms and craving in individuals with opioid dependence (Bisaga, Gianelli, & Popik, 1997). Glick, Maisonneuve, Dickinson, and Kitchen (2001) have also reported that systemic administration of DM decreases morphine self-administration in rats.

Much of the current literature on DM pays particular attention to its inhibitory effect on *N*-methyl-D-aspartate glutamate receptors (NMDARs) as a low-affinity noncompetitive antagonist (for a review, see Siu & Drachtman, 2007). In view of the critical role of NMDARs in hippocampal synapse formation (Lisman, 2003), it has been suggested that the NMDAR antagonists can impair hippocampal-dependent learning (de Lima, Laranja, Bromberg, Roesler, & Schröder, 2005). Increase of Ca^{2+} influx results in NMDARs stimulation which, in turn, leads to the activation of Ras-ERK1/2 and nuclear Ca^{2+}-calmodulin (CaM) kinase signalling pathways underlying synaptic plasticity (Hardingham & Bading, 2003). Activation of these pathways phosphorylates cAMP-

* Corresponding author at: Department of Animal Biology, School of Biology, College of Science, University of Tehran, P.O. Box 4155-6455, Tehran, Iran. Fax: +98 (21) 66405141.
E-mail address: rezayof@khayam.ut.ac.ir (A. Rezayof).

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response-element-binding protein (CREB; Naqvi, Martin, & Arthur, 2014) on serine 133 to increase the transcription of CREB-dependent genes (Silva, Kogan, Frankland, & Kida, 1998). Inhibition of the phosphorylation of CREB (p-CREB) by the NMDAR antagonist has been reported to induce impairment of memory consolidation and long-term potentiation (LTP), suggesting the critical role of CREB in memory formation (Pittenger et al., 2002). On the other hand, calcium calmodulin-dependent kinase II (CaMKII) is one of the elements which may be necessary for LTP expression and the consolidation of various forms of memory (for a review, see Lisman, Schulman, & Cline, 2002). In a study conducted by Tan and Liang (1997), it was shown that training in the passive avoidance task led to the activation and phosphorylation of CaMKII (p-CAMKII) in the amygdala.

Multiple brain regions such as the hippocampus (for a review, see Eichenbaum, 2004) and the prefrontal cortex (PFC; for a review, see Ridderinkhof, van den Wildenberg, Segalowitz, & Carter, 2004) are involved in cognitive functions. In 1993, Markowitsch et al. demonstrated that a deficit in the memory was observed in frontal-damaged patients. Because of the direct projection from the hippocampal formation to the PFC, high frequency stimulation in the hippocampus could induce LTP in the PFC (Laroche, Jay, & Thierry, 1990). Hippocampo-prefrontal cortex communications which are mediated by the activation of NMDA receptors play a critical role in learning procedures (for a review, see Godsil, Kiss, Spedding, & Jay, 2013). Excitatory amino acids such as glutamate and/or aspartate have also been reported to mediate this functional interaction (Jay, Thierry, Wiklund, & Glowinski, 1992).

To date, there are few studies investigating the association between morphine and DM in learning and memory processes. In addition, the neurobiological basis of this interaction is poorly understood. Since DM is a common antitussive medication which is widely available and is commonly co-used with opiates (Bryner et al., 2006), this study set out to identify (1) the effect of systemic administration of DM on morphine-induced memory impairment, and (2) whether there is a functional correlation between morphine and DM in memory performance. To explore the mechanisms underlying the effects of morphine and DM during memory formation, we also investigated the CAMKII/CREB signaling pathways in the hippocampus and the PFC. This study helps us advance our knowledge of drug-induced state dependent learning (SDL). In this kind of learning, memory retrieval of newly acquisitioned information is most efficient when a subject (humans or laboratory animals) is in the same state during both the encoding and *retrieval* phases (Bruins Slot & Colpaert, 1999). Thus, the other aim of the present project is to assess the possible role of the signaling pathways of the targeted sites in the induction of SDL by the co-administration of DM and morphine in the passive avoidance task.

2. Materials and methods

2.1. Animals

Male Wistar rats obtained from the vivarium of the School of Biology, University of Tehran, weighting between 200 and 220 g, at the start of the experiment were used. The animals were housed in groups of four in standard cages with a 12-h light/12-h dark cycle (lights on at 7:00 am) and under controlled temperature (22 ± 2 °C). Water and food were available at all times except during the training and testing phases. The animals were allowed at least 1 week of adaptation to the laboratory conditions before any procedures. All procedures for the treatment of animals were approved by the Research and Ethics Committee of the School of Biology, University of Tehran and were done in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Drugs

The drugs used in this study included morphine sulfate (Temad, Tehran, Iran) and dextrometorphan hydrobromide monohydrate (DM; Sigma, USA). Antibodies directed against total CREB, P-CREB, total CAMKII, p-CAMKII and β-actin were obtained from Cell Signaling Technology (Beverly, MA, USA). Poly vinylidene fluoride membrane (PVDF) was obtained from Millipore (Billerica, MA, USA). Electrochemiluminescence (ECL) kit was taken from Amersham Bioscience (Piscataway, NJ, USA). It should be considered that morphine and DM were dissolved in sterile 0.9% saline and delivered intraperitoneally in a volume of 1 ml/kg before use. Considering that the changes of locomotor activity may affect the measurement of memory formation, the doses of morphine (Ma et al., 2009; Rezayof, Zarrindast, Sahraei, & Haeri-Rohani, 2003) and DM (Redwine & Trujillo, 2003) were chosen based on our pilot study and some previous investigations which had shown that these doses have no effect on locomotor activity.

2.3. Passive avoidance apparatus

To evaluate memory retrieval, the step-through passive avoidance apparatus (Borj Sanat, Tehran, Iran) was used. The apparatus was a (20 × 20 × 30 cm high) Plexiglas box and consisted of two compartments: black (shock) and white (safe). The floor of black compartment consisted of parallel stainless steel rods (2.5 mm in diameter, spaced 1 cm apart) through which a foot shock was delivered. The apparatus was located in a sound-attenuated room.

2.4. Behavioral testing

2.4.1. Training phase

The animals were allowed to habituate in the experimental room for 1 h prior to the experiments. During training sessions, each animal was placed in the safe compartment and after 5 s, the guillotine door separating the compartments was opened, and their latency to enter the black compartment was measured. The rats usually entered the shock compartment within 10–20 s after the door opening. If any animal stayed on the white compartment for over 120 s, it was excluded from the experiments. After 30 min, the animal was placed in the safe compartment again and after 5 s, the guillotine door was opened. Immediately after the crossing of the animal to the dark (shock) compartment, the guillotine door was closed; then the animal received an electric shock (1 Hz, 3 s, 50 mA). After 2 min, the rat was transferred to the white compartment and their latency to enter the black compartment was recorded. If the animal did not enter the dark compartment during 120 s, successful acquisition of passive avoidance response was recorded. Since pre-training administration of the drugs may alter animals' sensitivity to shock, or the degree of arousal during the original training rather than directly modifying memory storage processes (Castellano, Cestari, & Ciamei, 2001; Jafari-Sabet, Khodadadnejad, Ghoraba, & Ataee, 2014), we administered the drugs after training in all the experiments.

2.4.2. Testing phase

Retrieval test session was carried out 24 h after the training and the animal was tested for memory retrieval. Step-through latency was used as a measure of memory retrieval. Retrieval test was done by placing the animal back in the safe compartment and measuring its latency to enter the shock compartment. No foot shock was delivered on the retrieval tests, and the cut-off time of 300 s was set.