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Neuromodulatory effects of the dorsal hippocampal endocannabinoid system in dextromethorphan/morphine-induced amnesia

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ABSTRACT

Dextromethorphan which is an active ingredient in many *cough medicines* has been previously shown to potentiate amnesic effect of morphine in rats. However, the effect of dextromethorphan, that is also a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, in combination with morphine on hippocampus-based long term memory has not been well characterized. The aim of the present study was to assess the possible role of endocannabinoid system of the dorsal hippocampus in dextromethorphan /morphine-induced amnesia. Our results showed that intraperitoneal (i.p.) injection of morphine (5 mg/kg) or dextromethorphan (5–15 mg/kg) before testing the passive avoidance learning induced amnesia. Combination of ineffective doses of dextromethorphan (7.5 mg/kg, i.p.) and morphine (2 mg/kg, i.p.) also produced amnesia, suggesting the enhancing effects of the drugs. To assess the effect of the activation or inhibition of the dorsal hippocampal cannabinoid CB_1 receptors on this amnesia, ACPA or AM251 as selective receptor agonists or antagonists were respectively injected into the CA1 regions before systemic injection of dextromethorphan and morphine. Interestingly, intra-CA1 microinjection of ACPA (0.5–1 ng/rat) improved the amnesic effect of dextromethorphan /morphine combination. The microinjection of AM251 into the CA1 region enhanced the response of the combination of dextromethorphan /morphine in inducing amnesia. Moreover, Intra-CA1 microinjection of AM251 inhibited the improving effect of ACPA on dextromethorphan /morphine-induced amnesia. It is important to note that intra-CA1 microinjection of the same doses of the agonist or antagonist by itself had no effects on memory formation. Thus, it can be concluded that the dorsal hippocampal endocannabinoid system, via CB_1 receptor-dependent mechanism, may be involved in morphine/dextromethorphan -induced amnesia.

1. Introduction

Endocannabinoids play critical roles in numerous physiological and pathophysiological processes (Katona and Freund, 2012). Endocannabinoids exert their effects through two types of receptors, namely CB_1 and CB_2 receptors which belong to $G_{i/o}$-protein-coupled receptors (Svízenská et al., 2008). CB_1 receptors are widely expressed in the major brain regions including the hippocampus (Liu et al., 2003) which is involved in learning and memory processes (Morris, 2006). Activation of CB_1 receptors in the dorsal hippocampal CA1 regions also inhibited long-term potentiation which may be associated with a G protein-dependent presynaptic inhibition of glutamate transmission (Sullivan, 2000). In view of the fact that there is an overlapping distribution of CB_1 and mu-opioid receptors in the hippocampus (Robledo et al., 2008), it has been suggested that a functional correlation between the endocannabinoid and opioid systems mediate hippocampus-based memory formation (Parolaro et al., 2010). Since integrity of hippocampal function is necessary for normal cognitive processes (Deng et al., 2010), the amnesic effect of morphine seems to be related to the hippocampus-based memory system in different animal learning models (Farahmandfar et al., 2010; Tirgar et al., 2014). It should also be considered that the neuromodulatory role of opioids within hippocampal formation circuits may be directly or indirectly associated with the high expression of mu opioid receptors in this brain site (Stumm et al., 2004).

Ample evidence suggests that dextromethorphan which is a non-opioid cough suppressant drug is frequently co-abused with other drugs (Wilson et al., 2011). Strong motivations for the co-abuse of dextromethorphan with morphine are assumed to lie in the high euphoric effect or the reduced side effects of the opiate (Mao et al., 1996). Although dextromethorphan has been shown to be effective in reducing the rewarding properties, the abuse liability of dextromethorphan is also reported among adolescents (Bryner et al., 2006). Drug abuse seems to reduce the populations of hippocampal neurons via

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attenuating neurogenesis (Eisch and Harburg, 2006) which is an important mechanism underlying memory formation (Aimone et al., 2006). It is well known that morphine plays a critical role in the modulation of hippocampal structure, physiology, and biochemistry (Simmons and Chavkin, 1996; Chavkin, 2000). Despite dextromethorphan's long clinical success, our recent study showed that the use of the drug alone or in combination with morphine induced amnesia via attenuating hippocampal calmodulin-dependent protein kinase II (CAMKII) and cAMP-response-element-binding protein (CREB) as critical mediators of memory formation (Ghasemzadeh and Rezayof, 2016). Considering that the effect of multi-drug abuse on learning and memory processes are not fully understood and that the dorsal hippocampal endocannabinoid system affects memory formation (de Oliveira et al., 2008), our aim was to investigate whether the CA1 endocannabinoid system via CB_1 receptors is involved in the effect of systemic co-administration of dextromethorphan /morphine on memory recall.

2. Materials and methods

2.1. Animals

The experiments were carried out on male Wistar rats (weighing approximately 200–220 g) obtained from the animal house of the School of Biology, University of Tehran. The animals were housed in groups of four per cage; they were maintained in a controlled temperature (22 ± 2 °C), and a 12:12-h light–dark cycle (lights on at 7:00 h am) with ad libitum access to food and water except during the test. All animals were allowed a week to adapt to the laboratory conditions prior to the experiments and were handled daily. 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 Institute of Health Guide for Care and Use of Laboratory Animals. Moreover, all efforts were made to minimize the number of animals used and their suffering.

2.2. Surgery and microinjection procedures

Rats were anesthetized with an intraperitoneal injection of a mixture of ketamine hydrochloride (50 mg/kg) plus xylazine (5 mg/kg). Using standard stereotaxic equipment, the CA1 regions of the dorsal hippocampi were bilaterally implanted with guide cannulas (22 gauges) according to the atlas of Paxions and Watson (antero-posterior: −3 to −3.5 mm posterior to the bregma, lateral: ± 1.8–2 mm from midline, ventral: −2.8 to −3 mm relative to the dura; Paxinos and Watson, 2007). The guide cannulas were fixed to the skull with dental cement (1 mm above the CA1 region). During the 1-week recovery period, the animals were habituated to the experimental room by being transferred to the experimental room and handled every day. The microinjections into the CA1 regions were bilaterally performed with 2-μl Hamilton microsyringe attached to the injection cannula via polyethylene tubing in a total volume of 1 μl/rat (0.5 μl/each side). The solution was injected slowly (over 1 min) and the cannula was left in place for an additional 60 s to reduce the backflow of the solution.

2.3. Drugs

The compounds used in this study were morphine sulfate (Temad, Tehran, Iran), Dextrometorphan hydrobromide monohydrate (Sigma, USA), ACPA (arachidonylcyclopropylamide; N-(2-cyclopropyl)−5Z, 8Z, 11Z, 14Z-eicosatertraenanmide; Tocris, Bristol, UK) and AM 251 (N-(piperidin-1-yl)−5-(4-isodophenyl)−1-(2,4-dichlorophenyl)−4-methyl-1H-pyrazole-3-arboxamide; Tocris, Bristol, UK). dextromethorphan and morphine were dissolved in sterile 0.9% saline before use. ACPA was dissolved in Tocrisolve™ (a soya oil and water emulsion) and was diluted with sterile 0.9% saline. In experiments where ACPA was applied, the control solution contained Tocrisolve™ with the same concentration as in the experimental solution (vehicle). AM251 was dissolved in dimethyl sulphoxide (DMSO; up to 10% v/v) and sterile 0.9% saline and a drop of Tween 80, which also was used as DMSO (Mohammadmirzaei et al., 2016; Naghdi and Asadollahi, 2004). Morphine and dextromethorphan were delivered intraperitoneally at a volume of 1 ml/kg. ACPA and AM251 were injected into the CA1 regions (intra-CA1) at a volume of 1 μl/rat.

2.4. Passive avoidance apparatus

A step-through passive avoidance apparatus (Borj Sanat, Tehran, Iran) was used for the evaluation of memory performance. The apparatus consisted of two chambers (illuminated and dark) separated by a guillotine door (20×20×30 cm high). The floor of the dark chamber contained stainless steel rods (2.5 mm in diameter, 1 cm apart) that could deliver foot shocks.

2.5. Behavioral testing

2.5.1. Training

Animals were transported to the experimental room and allowed to habituate to the experimental room for 60 min prior to the experiments. During the training trial, each animal was placed in the illuminated compartment; After 5 s, the guillotine door separating the chambers was open. When the rat crossed into the black chamber, its latency to enter the black chamber was measured. If any animal stayed on the illuminated chamber for over 120 s, it was excluded from the experiments. After 30 min, the animal was placed in the illuminated compartment again and after 5 s, the guillotine door was opened. As soon as it entered the dark compartment, the door was closed and the rat received an inescapable shock. After 2 min, the rat was transferred to the illuminated compartment and the latency times for entering the dark compartment were measured. An identical shock was delivered to animals entering the dark compartment before 120 s. If the animal did not enter the dark compartment during 120 s, successful acquisition of passive avoidance response was recorded.

2.5.2. Recall test

One day after the training trial, testing trial was done by placing the animal back in the illuminated compartment and measuring its latency to enter the shock compartment. Foot shock was not delivered on the testing trial, and the cut-off time limit was 300 s (Douma et al., 2011; Tajik et al., 2016).

2.6. Experimental design

2.6.1. Experiment 1. Dose-response curve of dextromethorphan, morphine or dextromethorphan/morphine

In this experiment, six groups of animals were used for evaluating the effect of dextromethorphan injection with or without morphine on memory recall. On the training day, each animal was trained in a passive avoidance task. On the test day, six groups of animals received morphine (0, 2 and 5 mg/kg) or dextromethorphan (5, 7.5 and 15 mg/kg). The other three groups received the same doses of dextromethorphan plus an ineffective dose of morphine (2 mg/kg) with 60 min interval. In all groups, the step-through latency was measured 30 min after the last injection as an indicator of memory recall (Fig. 1).

2.6.2. Experiment 2. The effect of intra-CA1 microinjection of ACPA (before the testing phase) with or without systemic injection of dextromethorphan / morphine on memory recall

In this experiment, eight groups of animals were successfully trained. On the test day, the animals received intra-CA1 microinjections of the different doses of a CB_1 receptor agonist, ACPA (0, 0.5, 0.75