Neuroscience Research 69 (2011) 51–59

Contents lists available at ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

Influence of three-day morphine-treatment upon impairment of memory consolidation induced by cannabinoid infused into the dorsal hippocampus in rats

Mohammad Reza Zarrindast[a,b,c,d,e,*], Majid Navaeian[f], Mohammad Nasehi[g]

[a] Department of Neuroscience, School of Advanced Medical Technologies, Tehran University of Medical Sciences, Tehran, Iran
[b] Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[c] School of Cognitive Sciences, Institute for Research in Fundamental Sciences (IPM), Tehran, Iran
[d] Iranian National Center for Addiction Studies, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[e] Institute for Cognitive Sciences Studies, Tehran, Iran
[f] Department of Biology, Faculty of Basic Sciences, Islamic Azad University, Shahre-rei Branch, Tehran, Iran
[g] Department of Biology, Faculty of Basic Sciences, Islamic Azad University, Garmsar Branch, Semnan, Iran

ARTICLE INFO

Article history:
Received 28 January 2010
Received in revised form
19 September 2010
Accepted 24 September 2010
Available online 1 October 2010

Keywords:
Cannabinoids
Hippocampus
Sensitization
Morphine
Naloxone
SCH23390
Sulpiride
Step-through inhibitory avoidance task

ABSTRACT

In the present study, the effects of morphine treatment upon reduction of memory consolidation by post-training administration of the non-selective cannabinoid CB_1/CB_2 receptor agonist, WIN55,212-2, into the dorsal hippocampus (intra-CA1) have been investigated in rats. Step-through inhibitory avoidance apparatus was used to test memory retrieval, which was made of two white and dark compartments. In training day, electric shocks were delivered to the grid floor of the dark compartment. On the test day, the animal was placed in the white compartment and allowed to enter the dark compartment. The latency with which the animal crossed into the dark compartment was recorded as memory retrieval. Morphine was injected subcutaneously (S.C.), once daily for three days, followed by a five day morphine-free period before training. Bilateral post-training intra-CA1 infusions of WIN55,212-2 (0.25 and 0.5 μg/rat) shortened the step-through latency, which suggested impaired memory consolidation. The deleterious effect of WIN55,212-2 (0.5 μg/rat) was prevented in rats previously injected with morphine (10 mg/kg/day × 3 days, S.C.). Prevention of the WIN55,212-2-induced amnesic-like effect was counteracted by the mu-receptor antagonist, naloxone, and the dopamine D_2 receptor antagonist, sulpiride, but not by the D_1 receptor antagonist, SCH 23390, when administered prior to each morphine injection. The results have suggested that subchronic morphine treatment may cause mu-opioid and D_2 receptor sensitization, which in turn prevents impairment of memory consolidation induced by WIN55,212-2.

1. Introduction

For several years, psychoactive drugs such as *Cannabis sativa* derivatives (e.g., marijuana) as well as opiates have been used for recreational and medicinal purposes. Both endogenous cannabinoids and opiate substances have high levels of expression in the brain and may play important neuromodulatory functions (Fride et al., 2003). The modulatory role of cannabinoids may be achieved through membrane receptors, fatty acid-derived neurotransmitters and the enzymes involved in generation and degradation of these neurotransmitters (Pacher et al., 2006). Cannabinoids mediate their pharmacological effects through three different receptor subtypes known as CB_1, CB_2, and CB_3. CB_1 and CB_3 subtypes are located in the central nervous system (Devane et al., 1988; Vaccani et al., 2005), whereas CB_2 is mainly localized in peripheral tissues (Munro et al., 1993) and the brain (Van Sickle et al., 2005). Three different subtypes of opioid receptors: mu, delta and kappa have been identified (Kieffer, 1995). There are similarities and interactions between opioids and cannabinoids. It has been shown that the CB_1 receptor is involved in conditioned place preference, memory, anxiety-like behavior and development of physical dependence by opioids (Ledent et al., 1999). The receptors of both these drugs belong to the G-protein that couples to the G_i/G_o GTP-binding proteins (Cichewicz, 2004). Cannabinoid and opioid receptor activation may cause an increase in the mitogen-activated protein kinase pathway activity (Bouaboula et al., 1995; Fukuda et al., 1996), modulate potassium conductance through protein kinase C signaling and inhibit calcium influx through voltage-gated calcium channels

* Corresponding author at: Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran.
Tel.: +98 21 66402569; fax: +98 21 66402569.
E-mail address: zarinmr@ams.ac.ir (M.R. Zarrindast).

0168-0102/$ – see front matter © 2010 Elsevier Ireland Ltd and the Japan Neuroscience Society. All rights reserved.
doi:10.1016/j.neures.2010.09.007

(Skinner et al., 2000). Activation of presynaptic cannabinoid and opioid receptors may inhibit the release of several neurotransmitters (Schlicker and Kathmann, 2001) and play a role in learning and memory (Hampson and Deadwyler, 1999; Hernandez-Tristan et al., 2000; Lichtman, 2000; Nasehi et al., 2009). Receptor mRNAs of both drugs have an overlapping distribution in several areas of the brain, including the limbic system, mesencephalon, brain stem and spinal cord (Rodriguez et al., 2001; Salio et al., 2001). It has been reported that CB_1 cannabinoid and mu-opioids receptors colocalize in striatal GABAergic neurons (Hohmann and Herkenham, 2000; Pickel et al., 2004; Rodriguez et al., 2001). Therefore, a potential coupling similar to second messenger mechanism in their interactions may be involved (Rios et al., 2006). Other similarities exist between both cannabinoids and opioids, such as in learning and memory (Nasehi et al., 2009), antinociception (Suplita et al., 2008), hypothermia, sedation, hypotension, inhibition of intestinal motility, motor depression and reward (Maldonado et al., 2006; Manzanares et al., 1999).

Some investigations have indicated that repeated administration of opiates may either result in behavioral sensitization (Contet et al., 2008; Dong et al., 2006). Behavioral sensitization refers to the enhanced behavioral response to indirect-acting dopamine psychostimulant agonists (e.g., amphetamine and cocaine) or direct dopamine agonists (quinpirole and apomorphine) (Trezza et al., 2009; Vezina et al., 2002). Examples of sensitization also exist within the nervous system, including kindling, long term potentiation (LTP) and locomotor activity (Kuribara, 1995; Shippenberg et al., 1996). Extensive preclinical studies have been undertaken in order to investigate the neurobiological mechanisms of addictive processes that underlie the development of sensitization to morphine (Ashton et al., 2004; Cador et al., 1995; Rueda et al., 2002). Many studies have focused on the role of the mesolimbic dopamine system as a mediator of behavioral sensitization produced by sub-chronic injections of psychostimulants (Henry et al., 1998; Shippenberg et al., 1996; Zarrindast and Rezayof, 2004). It has been reported that morphine-induced impairment of spatial memory can be improved in morphine sensitization (Farahmandfar et al., 2010). We have also shown that morphine induced amnesia can be reversed by previous repeated administration of morphine followed by morphine withdrawal, due to sensitization which may be mediated through the dopamine receptor mechanism (Zarrindast et al., 2005a, 2008; Zarrindast and Rezayof, 2004). The hippocampus which is involved in long term potentiation (Bliss and Collingridge, 1993) is an essential site for learning and memory, and is rich in CB_1 and dopamine receptors (Izquierdo and Medina, 1995; Nguyen et al., 1994). Dopamine receptors of this site are also involved in morphine sensitization (Zarrindast et al., 2006b).

In the present study, the effects of morphine treatment upon reduction of memory consolidation by post-training administration of the non-selective CB_1/CB_2 receptor agonist, WIN55,212-2, into the dorsal hippocampus (intra-CA1) have been investigated in rats. The possible roles of mu-opioid and D_1 and D_2 dopamine receptors on this phenomenon were also investigated.

2. Materials and methods

2.1. Animals

Male Wistar rats (Pasteur Institute, Tehran, Iran) weighing 220–250 g at the start of the experiments were used. The animals were housed four/cage, in a colony room with a 12/12 h light/dark cycle (7:00–19:00 lights on), at $22 \pm 2\,^{\circ}C$. They had free access to food and tap water, except during the limited periods of the experiments. All animals were allowed to adapt to laboratory conditions for at least one week before surgery and were handled for five minutes/day during this adaptation period. Each animal was used once only. Eight animals were used for each group of experiments, which were carried out during the light phase of the cycle. All procedures were carried out in accordance with the Institutional Guidelines for Animal Care and Use.

2.2. Surgery

For surgical procedures (anesthesia) ketamine and xylazine (Alfasan Chemical Co., Woerden, Holland) were injected intraperitoneally. Rats anesthetized with xylazine (20 mg/ml) and ketamine (65 mg/ml) were placed in a stereotaxic apparatus with the incisor bar positioned −3.3 mm. Two stainless-steel, 22-gauge guide cannula were placed (bilaterally) 1 mm above the intended injection site according to Paxinos and Watson (2007). Stereotaxic coordinates for the CA1 regions of the dorsal hippocampus were −3 to −3.5 mm (depending on body weight) posterior to the bregma, ±1.8 to 2 mm lateral to the sagital suture and −2.8 to −3 mm ventral of the dorsal surface of the skull. Cannulae were secured to anchor jewelers' screws with dental acrylic. Stainless steel styles (27-gauge insect pins) were inserted into the guide cannula to keep them free from debris. All animals, after a brief anesthetic clearing period, were allowed a one week recovery period from surgery.

2.3. Drugs

Drugs used in this study were: the non-selective CB_1/CB_2 receptor agonist WIN55,212-2 mesylate (Tocris Cookson, Bristol, UK), morphine sulfate (Temad, Tehran, Iran) and naloxone hydrochloride (Tolid-Daru, Tehran, Iran). The D_1 receptor antagonist, SCH23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride) and D_2 receptor antagonist, sulpiride, were purchased from Sigma Chemical Co (St. Louis, CA, USA). All compounds were tested at three doses: WIN55,212-2 (0.1, 2.5 and 0.5 μg/rat), morphine (2.5, 5, 10 mg/kg), naloxone (0.25, 0.5, 1 mg/kg), SCH23390 (0.04, 0.08 and 0.12 mg/kg) and sulpiride (0.3, 0.6 and 0.9 mg/kg). WIN55,212-2 was dissolved in a vehicle (water/dimethylsulfoxide, DMSO, (9:1) solvent and one drop of Tween 80). Morphine, naloxone and SCH23390 were dissolved in 0.9% physiological saline, just prior to the experiments. Sulpiride was dissolved in a minimal volume of diluted acetic acid (1 drop, 5 μl, pH 6.3, with a Hamilton micro-syringe, 10 μl) and made-up to a volume of 5 ml with 0.9% physiological saline which was then diluted to the required volume with saline (0.9% w/v NaCl solution).

2.4. Drug treatment

Eight animals were used in each experimental group. Control groups received either two saline (1 ml/kg) or vehicle (for sulpiride, 1 ml/kg) and for WIN55,212-2, 1 μl/rat) injections. WIN55,212-2 was injected intra-CA1 in volume of 1 μl/rat and the remaining drugs were injected subcutaneously (S.C.) in a volume of 1 ml/kg. The timing of the pre-test drug administration was selected based on pilot and previous studies (Nasehi et al., 2009; Rezayof et al., 2006; Zarrindast and Rezayof, 2004). The protocol is summarized in Table 1.

2.5. Intra-CA1 injections

For drug infusions, the animals were gently restrained by hand. The stainless steel styles were removed from the guide cannula and replaced by 27-gauge injection needles (1 mm below the tip of the guide cannula). Injection solutions were administered manually in a total volume of 1 μl/rat (0.5 μl per side) over a 60 s period and injection needles were left in place for an additional 60 s to facilitate