ELSEVIER

Physiology & Behavior 88 (2006) 124 – 131

PHYSIOLOGY & BEHAVIOR

Influence of nitric oxide on morphine-induced amnesia and interactions with dopaminergic receptor agents

Ameneh Rezayof [a], Rana Amini [a], Yassaman Rassouli [a], Mohammad-Reza Zarrindast [b,c,d,*]

[a] *School of Biology, University College of Science, University of Tehran, Tehran, Iran*
[b] *Department of Pharmacology, School of Medicine and Iranian National Center for Addiction Studies, Tehran University of Medical Sciences, P. O. Box 13145-784, Tehran, Iran*
[c] *School of Cognitive Science, Institute for Studies in Theoretical Physics and Mathematics, Tehran, Iran*
[d] *Institute for Cognitive Science Studies, Tehran, Iran*

Received 5 December 2005; received in revised form 12 March 2006; accepted 15 March 2006

Abstract

The interactions of dopaminergic receptors and nitric oxide (NO) with morphine-induced memory of passive avoidance have been investigated in mice. Pre-training administration of morphine (1, 3 and 5 mg/kg, s.c.) dose-dependently decreased the learning of a one-trial passive avoidance task. Pre-training administration of L-arginine, a nitric oxide precursor (50, 100 and 200 mg/kg, i.p.), alone did not affect memory formation. The drug (100 and 200 mg/kg) decreased significantly amnesia induced by pre-training morphine (5 mg/kg). Pre-training administration of L-NAME (N^G-nitro-L-arginine methyl ester), a nitric oxide synthase (NOS) inhibitor (20 and 30 mg/kg, i.p.), dose-dependently impaired memory formation. In addition, co-pretreatment of different doses of L-NAME (10, 20 and 30 mg/kg) with lower dose of morphine (1 mg/kg), which did not induce amnesia by itself, caused inhibition of memory formation. Pre-training administration of apomorphine, a dopaminergic receptor agonist (0.25, 0.5 and 1 mg/kg, i.p.), alone also did not affect memory formation, but morphine-induced amnesia was significantly inhibited by pretreatment with apomorphine (0.5 and 1 mg/kg, 5 min, i.p.). On the other hand, the inhibition of morphine-induced amnesia by L-arginine (200 mg/kg, i.p.) was significantly decreased by pretreatment with different doses of dopamine D_1 receptor antagonist, SCH 23390 (0.001, 0.01 and 0.1 mg/kg, i.p.) or D_2 receptor antagonist, sulpiride (12.5, 25, 50 and 100 mg/kg, i.p.). However, the dopamine receptor antagonists could not affect memory formation by themselves. It may be concluded that the morphine-induced impairment of memory formation can be prevented by nitric oxide donor and, in this effect, dopaminergic mechanism is involved.

Keywords: Morphine; Passive avoidance learning; Dopaminergic agents; L-Arginine; L-NAME; Mouse

1. Introduction

Several lines of evidence indicate that opiates modify learning and memory processes [1–4]. Animal studies show that morphine and related opioid drugs exert amnesic effects in different models of memory [5–7]. It has been also reported that morphine impaired memory acquisition in the shuttle avoidance test [8] and radial maze [9,10]. Pretreatment with the opiate receptor antagonist, naloxone antagonizes the disruptive effect of opiates such as morphine on working memory [3]. Moreover, the opiate receptor antagonist improves spatial memory in both young and adult rats [1]. It seems likely that μ-opioid receptors play an important role in the morphine response. Our previous studies have shown that pre-training administration of morphine impairs retention of memory, dose- and time-dependently in the step-down passive avoidance task, which was antagonized with naloxone [11–13]. It is also well known that learning and memory are critically involved in the morphine dependence and relapse [14].

Furthermore, a variety of neurotransmitters are considered to be involved in the processes of learning and memory in the brain. Some investigators have suggested a functional inter-relationship between μ-opioid and dopamine receptors in

* Corresponding author. Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran. Tel./fax: +98 21 6640 2569.
E-mail addresses: zarinmr@ams.ac.ir, zrrndst@yahoo.ca (M.-R. Zarrindast).

0031-9384/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2006.03.017

modulating of the brain functions, such as dependence [15] and reward-related learning [16–18]. Adriani et al. [19] also reported that dopamine receptors are able to alter the capability to learn and store information.

Nitric oxide (NO) is a neural messenger molecule in the central nervous system, which is generated from L-arginine by nitric oxide synthase (NOS) [20]. There are considerable evidence for NO involvement in certain forms of long-term potentiation and expression [21,22]. NO as a neural messenger molecule in the central nervous system is known to be involved in many important opioid-induced effects [23,24] and there is also some evidence that NO may be involved in the rewarding properties of morphine [25–29]. It has been reported that morphine administration can modulate the expression of NOS [30,31]. NOS is closely associated with long-term potentiation (LTP), a persistent increase in the synaptic activity implicated in certain forms of learning and memory [32–34]. In addition, interactions between NO and dopamine (DA) actions have been investigated and bidirectional relations have been shown between NO synthesis and DA release [35,36]. Considering the functional interactions between the opioidergic system and NO, and also dopamine-related modulation of morphine effects, in the present study, the influence of pretreatment of dopaminergic agents with or without L-arginine (as precursor of NO) and L-NAME (NOS inhibitor) on morphine-induced amnesia in mice has been investigated.

2. Materials and methods

2.1. Animals

Male albino NMRI mice (Pasteur institute; Tehran, Iran) weighing 22–30 g were used. The animals were housed 10 per Plexiglas cage, in a room with controlled photoperiod (a 12-h light/dark cycle) and temperature (22±2 °C). They had food and water available ad lib and were allowed to adapt to the laboratory conditions for at least 1 week before the experiments. Each animal was used once only. All procedures were carried out in accordance with the institutional guidelines for animal care and use.

2.2. Apparatus

The passive avoidance apparatus consisted of a wooden box (30×30×40 cm high) with a steel-rod floor (29 parallel rods, 0.3 cm in diameter, set 1 cm apart). A wooden platform (4×4×4 cm) was set in the center of the grid floor. Intermittent electric shocks (1 Hz, 0.5 s, 40 V DC) were delivered to the grid floor by an insulated stimulator (Grass S44, USA).

2.3. Training

A single-trial step-down passive avoidance task was used. Each mouse was gently placed on the wooden platform. When the mouse stepped down from the platform and placed all its paws on the grid floor, intermittent electric shocks were delivered continuously for 15 s [37]. This training procedure was carried out between 10:00 and 15:00 h. Each mouse was placed on the platform again at 24 h after training and the step-down latency was measured with a stopwatch as passive avoidance behavior. An upper cut-off time of 180 s was set. The retention test was also carried out between 10:00 and 15:00 h.

2.4. Drugs

The drugs used in the study were morphine sulfate (Temad Co., Tehran, Iran), apomorphine, L-arginine and L-NAME (N^G-nitro-L-arginine methyl ester), SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride) and sulpiride (Sigma, St Louis, CA, USA). All drugs were dissolved in sterile 0.9% saline just before the experiment, except for sulpiride that was dissolved in one drop of glacial acetic acid with a Hamilton microsyringe and made up to a volume of 5 ml with sterile 0.9% saline and was then diluted to the required volume. Control animals received either saline or vehicle.

2.5. Drug treatment

Ten animals were used in each experimental group. In experiments where animals received two or three injections, the control groups also received two or three saline or vehicle injections. The intervals of drug administration were based on previous studies in order to obtain a maximum response [12,38].

2.5.1. Experiment 1: effect of morphine on memory retention

Experiment 1 examined the effects of pre-training administration of morphine on memory formation. One control group received saline (10 ml/kg) 30 min before training and 30 min before testing. Three groups of animals received pre-training morphine (1, 3 and 5 mg/kg, s.c.) 30 min before training, followed by pretest saline 30 min before testing (Fig. 1).

2.5.2. Experiment 2: effects of L-arginine on memory formation with or without morphine

Eight groups of 10 animals were used. Four groups of animals were pretreated with either saline (10 ml/kg) or L-

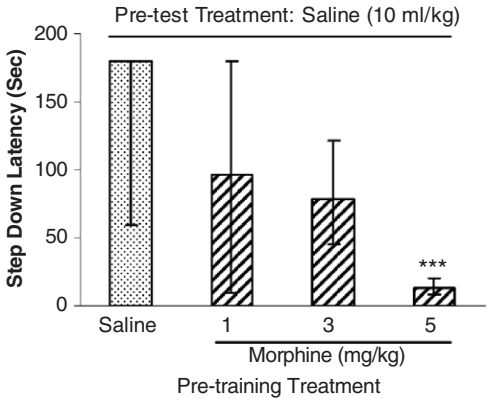

Fig. 1. The effects of pre-training administration of morphine on the step down latencies in mice. The animals were trained 30 min after either saline (10 ml/kg, s.c.) or morphine (1, 3 and 5 mg/kg, s.c.) and were tested 30 min after receiving saline. Each value represents the median and quartile of 10 animals. ***$P<0.001$, compared to control group.