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BRAIN RESEARCH

Research Report

Role of nitric oxide in the rat hippocampal CA1 in morphine antinociception

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ABSTRACT

In the present study, the effects of intra-hippocampal CA1 injections of L-arginine, a nitric oxide (NO) precursor and N^G-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor, on morphine-induced antinociception in rat formalin test were investigated. To induce inflammation pain, formalin (50 μl at 2.5%) was injected into the right hind-paw of male Wistar rats prior to testing. Morphine (3–9 mg/kg) was injected intraperitoneally (i.p.) 10 min before injection of formalin. The present study shows that administration of L-arginine (0.08, 0.15, 0.3, 1.0 and 3.0 μg/rat), but not L-NAME (0.15, 0.3 and 1.0 μg/rat), 5 min before formalin injection reversed morphine-induced antinociception at the early phase of formalin test. However, both drugs blocked morphine antinociception at the late phase of the test, but none of these drugs elicited any response by themselves at the tonic phase when injected alone. Moreover, the response to L-arginine was potentiated by L-NAME pre-treatment. It should be noted that a single injection of both L-arginine and L-NAME showed nociceptive effect at the early phase of the test. The present study reveals an expression of NADPH-diaphorase in the rat brain samples administered by L-arginine. Expression of NADPH-d is decreased in the samples which were pre-injected with L-NAME. This study suggests NO participation in the rat hippocampal CA1 area in morphine-induced antinociception.

1. Introduction

Nitric oxide (NO) is a molecule of great pharmacological interest and physiological importance (Fukuto and Mayer, 1996). The retrograde neurotransmitter, NO, has been shown to produce postsynaptically in response to activation of central excitatory amino acids (Garthwaite, 1991). The molecule, NO, plays a role in the regulation of behavior (Moncada et al., 1991). It has been implicated in the actions of opioids (Kivastik et al., 1996).

Recent behavioral lines of evidence suggest that the hippocampal formation is involved in nociception (Soleimannejad et al., 2006, 2007). According to previous experiments the pyramidal cells and interneurons in the dorsal hippocampal CA1 respond to persistent noxious activation (Khanna, 1997; Khanna and Zheng, 1999). Injection of local anaesthetic into the dentate gyrus of the hippocampal formation produces an analgesic effect in the formalin test (McKenna and Melzack, 1992). Although partial hippocampectomy is used to treat chronic pain (Gol and Faibish, 1967), electrical stimulation of

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the hippocampal formation has been shown to evoke painful sensations in humans (Delgado, 1955; Gloor et al., 1981). Furthermore, blocking of neural transmission along the major afferent (McKenna and Melzack, 1992) or efferent (Vaccarino and Melzack, 1992) hippocampal pathways have been implicated in pain relief. Nociception-driven decreased induction of Fos protein, the transcription protein that is expressed in neurons following synaptic excitation (Aloisi et al., 1997) in CA1 has also been demonstrated (Khanna et al., 2004).

A few studies demonstrated that intrathecal (ith) administration of L-NAME elicits a slight antinociception or enhances morphine antinociception in rats (Przewtocki et al., 1993). Pretreatment with L-NAME (20 mg/kg) in animals repeatedly injected with morphine (30 mg/kg for 3 days) increased morphine-induced antinociception in the formalin test (Zarrindasta et al., 2007). L-Arginine, according to previous studies, reduces morphine antinociception (Bhargava and Bian, 1998; Bhargava et al., 1997; Bian and Bhargava, 1998).

Despite the large amount of evidence showing the antinociceptive effect of morphine in animal models, but the role of the hippocampus or NO agents is controversial. At present the precise modulatory system of pain is unclear. In case of hippocampal CA1 region, it is only postulated that the region might modulate the morphine antinociceptive effect by the activation of the NMDA receptors, which in turn, modulates the effects of morphine through a mechanism dependent on NO.

In this study we investigated the role of NO at the hippocampal CA1 region on morphine-induced antinociception using the formalin model of persistent pain. For this purpose, intra-CA1 injections of NO agents were used. Since a high neuronal activity of NADPH-diaphorase has been marked by NOS isoforms (Thomas and Pearse, 1964; Tracey et al., 1993), NADPH-d activity is used as a marker for NOS (Gabbot and Bacon, 1993). NADPH-d histochemical reaction is now commonly used for visualization of NOS protein (Andronowska et al., 2005). In the present experiments the participation of NO in the rat hippocampus in morphine-antinociception was examined both behaviorally and by using NADPH-d histochemistry, a marker of NOS activation.

2. Results

Fig. 1 shows the verification of the placement of the cannula in CA1 field of the dorsal hippocampus. Location of the tip of the cannula in the CA1 field (Fig. 1A) was verified (Fig. 1B) by the atlas of Paxinos and Watson (1987). Diffusion distance for agents which were microinjected into the site of interest as evidenced by dye injection into the CA1 area through this cannula confirms that the distance remains localized to the CA1 region.

2.1. *Dose–response induced by morphine in formalin injected rats*

Fig. 2 shows the effect of morphine on pain induced by formalin in rats. Administration of morphine (3–9 mg/kg, i.p.) 10 min before the formalin test resulted in a significant decrease in the overall pain behavior scores of rats compared to the saline group. This effect of opioid was apparent in both the acute (0–5 min, $F_{5,30}=9.254$; $p<0.0001$) and tonic (15–60 min, $F_{5,30}=43.528$; $p<0.0001$) intervals of the formalin test. In view of the results, morphine (6 mg/kg, i.p.) was used for subsequent studies.

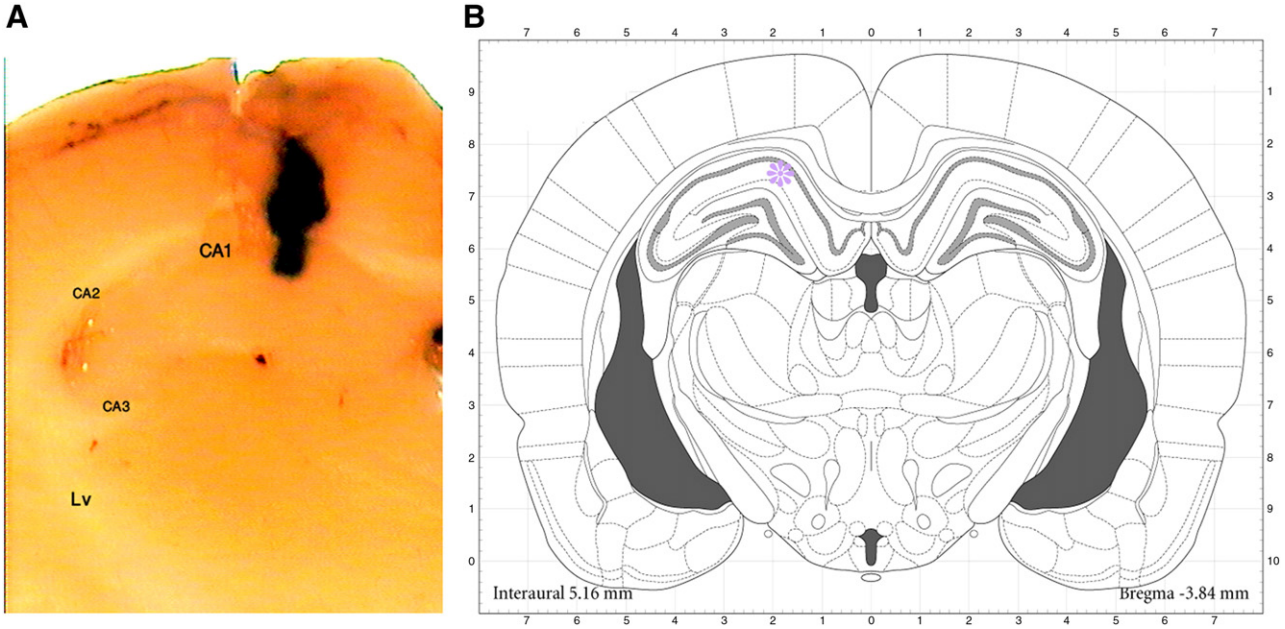

Fig. 1 – (A) Cannulae placements in CA1 evidenced by ink injection in a volume of 1 μl/rat, intra-hippocampal (AP: – 3.8). (B) Verification from atlas of Paxinos and Watson (1987).