Neuroscience 261 (2014) 23–42

INVOLVEMENT OF CANNABINOID RECEPTORS IN PERIPHERAL AND SPINAL MORPHINE ANALGESIA

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Abstract—The interactions between the cannabinoid and opioid systems for pain modulation are reciprocal. However, the role and the importance of the cannabinoid system in the antinociceptive effects of opioids remain uncertain. We studied these interactions with the goal of highlighting the involvement of the cannabinoid system in morphine-induced analgesia. In both phases of the formalin test, intra paw and intrathecal morphine produced similar antinociceptive effects in C57BL/6, cannabinoid type 1 and type 2 receptor wild-type (respectively *cnr1*WT and *cnr2*WT) mice. In *cnr1* and *cnr2* knockout (KO) mice, at the dose used the antinociceptive effect of intra paw morphine in the inflammatory phase of the formalin test was decreased by 87% and 76%, respectively. Similarly, the antinociceptive effect of 0.1 µg spinal morphine in the inflammatory phase was abolished in *cnr1*KO mice and decreased by 90% in *cnr2*KO mice. Interestingly, the antinociceptive effect of morphine in the acute phase of the formalin test was only reduced in *cnr1*KO mice. Notably, systemic morphine administration produced similar analgesia in all genotypes, in both the formalin and the hot water immersion tail-flick tests. Because the pattern of expression of the mu opioid receptor (MOP), its binding properties and its G protein coupling remained unchanged across genotypes, it is unlikely that the loss of morphine analgesia in the *cnr1*KO and *cnr2*KO mice is the consequence of MOP malfunction or downregulation due to the absence of its heterodimerization with either the CB_1 or the CB_2 receptors, at least at the level of the spinal cord.

Key words: cannabinoid receptors, mu opioid receptors (MOP), morphine, pain, tail-flick test, formalin test.

INTRODUCTION

Among several pharmacological properties, analgesia is the most common feature shared by the cannabinoid and opioid systems (Manzanares et al., 1999; Massi et al., 2001). The cannabinoid and opioid receptors display similar properties. They both belong to the $G_{i/o}$ protein-coupled receptor family and are coupled to similar intracellular signaling mechanisms (Bidaut-Russell et al., 1990; Childers et al., 1992; Howlett, 1995). Indeed, the cannabinoids mediate their pharmacological effects through at least two types of receptors, namely CB_1 (Matsuda et al., 1990) and CB_2 (Munro et al., 1993). The anatomical distribution of the CB_1 receptor is consequent with its functions, including the modulation of pain perception at the central, spinal and peripheral levels (Hohmann, 2002; Walczak et al., 2005, 2006; Agarwal et al., 2007; Lever and Rice, 2007). By contrast, CB_2 receptor expression seems to be found predominantly in the peripheral tissues (Munro et al., 1993; Galiegue et al., 1995; Schatz et al., 1997; Jhaveri et al., 2007). However, the expression of this receptor has also been described on brainstem neurons (Van Sickle et al., 2005) and in microglial cell cultures (Beltramo et al., 2006). Opioids mediate their pharmacological effects mainly through three types of receptors: mu (MOP) (Yasuda et al., 1993), delta (DOP) (Evans et al., 1992; Kieffer et al., 1992) and kappa (KOP) (Chen et al., 1993). Although they are found throughout the central nervous system (CNS) and in the peripheral tissues, opioid receptors are primarily expressed at high levels in several brain areas involved in pain perception (Pol and Puig, 2004; Bodnar, 2012).

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Abbreviations: 2-AG, 2-arachidonoylglycerol; ANOVA, analysis of variance; A.U.C., area under the curve; CNS, central nervous system; cpm, counts per minute; DOP, delta opioid receptor; EDTA, ethylenediaminetetraacetic acid; HEPES, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid; KO, knockout; MOP, mu opioid receptor; MPE, Maximal Possible Effect; PFA, paraformaldehyde; PB, phosphate buffer; PBS, phosphate-buffered saline.

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http://dx.doi.org/10.1016/j.neuroscience.2013.12.030

Interactions between the two systems for pain modulation are reciprocal. Although the role of opioids in cannabinoid antinociceptive effects has been documented (Maldonado and Valverde, 2003; Cichewicz, 2004), there is little information regarding the involvement of the cannabinoid system in the antinociceptive mechanisms of opioids. Indeed, it was recently demonstrated that the CB_1 antagonist AM251 counteracts morphine-induced antinociception in an inflammatory pain model (da Fonseca Pacheco et al., 2008; Pacheco Dda et al., 2009) and in the tail-flick test in mice (Pacheco Dda et al., 2009). These observations led to the hypothesis that MOP activation could induce local release of endocannabinoids and that the subsequent peripheral (da Fonseca Pacheco et al., 2008) or central (Pacheco Dda et al., 2009) activation of the cannabinoid receptors CB_1 and/or CB_2 could contribute to the antinociceptive effects of morphine. A role for the endocannabinoid system in the inhibition of MOP mRNA expression and signaling was also recently described (Paldyova et al., 2008), demonstrating that intraperitoneal administration of the CB_2 antagonist SR144528 attenuates MOP activity through CB_2 cannabinoid receptors (Paldy et al., 2008; Paldyova et al., 2008).

While experiments using pharmacological tools to modify cannabinoid signaling suggested that endocannabinoids are clearly involved in the antinociceptive effects of opioids, studies using transgenic mice are not conclusive. Thus, the role and the importance of the cannabinoid system in the antinociceptive effects of opioids remain uncertain. The aim of this study was therefore to investigate whether opioid and cannabinoid systems can interact at various levels of the neuraxis. We evaluated the role of the cannabinoid system in peripheral (i.e. local injection), spinal and systemic antinociception induced by the activation of MOP following morphine administration in C57BL/6, *cnr1*WT, *cnr1*KO, *cnr2*WT and *cnr2*KO mice.

EXPERIMENTAL PROCEDURES

Animals

Male C57BL/6, *cnr1*WT, *cnr1*KO, *cnr2*WT and *cnr2*KO mice (25–30 g at the time of testing) were used in the current study. They were housed in groups of two to four in standard plastic cages with sawdust bedding in a climate-controlled room. The mice were maintained under a 14-h light/dark cycle (light period 06:00–20:00 h). All experiments were conducted between 07:00 and 12:00 h. The mice were allowed free access to food pellets and water. The C57BL/6 mice were purchased from Charles River, St-Constant, Quebec, Canada, whereas the *cnr1* and *cnr2* transgenic mice were obtained from Pr. Beat Lutz (Institute of Physiological Chemistry and Pathobiochemistry, University of Mainz, Germany) and Jackson Laboratory (Bar Harbor, ME, USA), respectively. These colonies were maintained in-house. This research protocol was approved by the Local Animal Care Committees at the Université de Montréal and Université de Sherbrooke and all procedures conformed to the directives of the Canadian Council on Animal Care and guidelines of the International Association for the Study of Pain. All animal experiments were designed to minimize the number of animals used and their suffering.

Drugs

Morphine sulfate (Morphine HP® 50, lot #151034; Sandoz, Boucherville, QC, Canada) was diluted in a sterile saline solution (0.9% NaCl). Drugs were administered into the dorsal surface of the left hind paw (i.paw), intrathecally (i.t.) or subcutaneously (s.c.) before intradermal (i.d.) formalin injection into the plantar surface of the left hind paw. Morphine was administered i.paw (1 μg/10 μL), i.t. (0.1 μg/5 μL), and s.c. (3 mg/kg for the formalin test or 1, 3 and 10 mg/kg for the tail-flick test). Intrathecal injections were performed in non-anesthetized mice as described previously (Fairbanks, 2003; Gendron et al., 2007). Briefly, a 30-G ½ needle mounted on a 10-μL Luer-tip Hamilton syringe (VWR) was inserted into the L5–L6 intervertebral space, and 5 μL of morphine was injected. Saline was used as vehicle control. The appropriate placement of the needle was confirmed by the observation of a light flick of the tail.

Behavioral studies

Formalin test. The formalin test is a well-established model of tonic pain that is characterized by a transient, biphasic nociceptive response (Tjolsen et al., 1992). The first phase is characterized by the acute activation of sensory receptors. The second phase involves an inflammatory reaction in the peripheral tissue and the development of CNS sensitization. The mice were acclimatized to the testing environment (a clear Plexiglas box 30 × 30 × 30 cm) for 15–20 min or until the cessation of explorative behavior. Thereafter, drugs were injected i.paw, i.t., or s.c. with saline or morphine 5 or 10 min before a 10-μL i.d. injection of a 2% formaldehyde solution (i.e., 5.4% formalin, Fisher Scientific, Montreal, QC, Canada) into the plantar surface of the left hind paw. The experimenter was blind to the drug treatments during testing. Following each injection, the mice were immediately placed in the observation chamber. Nociceptive behaviors were observed for 60 min with the help of a mirror angled at 45° below the observation chamber to allow for an unobstructed view of the hind paws.

The nocifensive behaviors were assessed using a weighted score, as described previously (Dubuisson and Dennis, 1977; Coderre et al., 1993). Following an injection of formalin into the left hind paw, the nociceptive mean score was determined for each 3-min block during the 60-min recording period. In each 3-min bin, the total time the animal spent in four different behavioral categories was recorded: (0), the injected paw is comparable to the contralateral paw and is used normally by the animal; (1), the injected paw has little or no weight placed on it; (2), the injected paw is elevated and is not in contact with any surface; and (3), the