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Research Report

Central glucocorticoid receptors modulate the expression of spinal cannabinoid receptors induced by chronic morphine exposure

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Abstract

Central cannabinoid receptors (CBRs) have been implicated in the opioid analgesic effects. However, it remains unclear as to whether the expression of central CBRs would be altered after repeated morphine exposure. Here, we show that chronic intrathecal treatment with morphine (10 μg, twice daily for 6 days) induced a time-dependent upregulation of both CB-1 and CB-2 receptors within the spinal cord dorsal horn. This morphine-induced CB-1 and CB-2 upregulation was dose-dependently attenuated by the intrathecal co-administration of morphine with the glucocorticoid receptor (GR) antagonist RU38486 (0.25, 0.5, or 2 μg). The intrathecal RU38486 treatment regimen also attenuated the development of morphine tolerance. These results indicate that the expression of spinal CBRs was altered following repeated morphine exposure and regulated by the activation of central GRs.

Theme: Sensory systems
Topic: Pain modulation: pharmacology

Keywords: Cannabinoid receptor; Morphine; Tolerance; Glucocorticoid receptor

1. Introduction

Cannabinoids are a known class of analgesics, which produce antinociception mainly through spinal and supraspinal mechanisms [4,11,12,22,30,36,48,52,54,57,59]. In addition, cannabinoids have been shown to be effective in attenuating neuropathic pain as well [1,10,17,27,32,42–44,61]. Of interest is that recent evidence suggests that central cannabinoid receptors (CBRs) may also be involved in the opioid analgesic effects [40,60] as well as the development of morphine tolerance [13,47]. These data suggest that central CBRs may play a significant role in the mechanisms of morphine antinociception and tolerance.

Two major cannabinoid receptor subtypes, namely cannabinoid-1 (CB-1) and cannabinoid-2 (CB-2) receptors, have been cloned [7,31,33]. Both CB1R and CB2R, with 44% sequence homology, belong to the superfamily of G protein-coupled receptors [4]. Although CB-2 receptors are generally regarded as peripheral receptors mainly involved in the immune system [24], both CB-1 and CB-2 receptors have been located in the central nervous system including the superficial laminae of the spinal cord dorsal horn and are responsible for the pharmacological properties of central cannabinoid actions [4,9,16,19,38,49,56]. Recent studies have demonstrated that CB-1 receptors are upregulated in the contralateral thalamic region and the ipsilateral spinal cord dorsal horn after a unilateral nerve injury [32,51]. Since neurochemical changes within the spinal cord following nerve injury have many characteristics in common with those after morphine exposure [25], it is possible that the expression of central CBRs would also be altered following repeated morphine exposure.

Glucocorticoid receptors (GRs) are an active regulator in inflammatory responses through interactions with intracellular elements such as activating protein-1 as well as transcrip-

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tional and posttranscriptional regulation [34]. In addition, GRs have been located within the spinal cord dorsal horn [5,6] and activation of neuronal GRs contributes to neural plasticity related to neuronal injury [2] and the process of learning and memory [35,39,45,46]. Moreover, activation of GRs has been shown to modulate morphine-induced antinociception [3,37], locomotor activity [53], and dopamine-dependent responses [29,50]. Since both CBRs and GRs play a role in the cellular mechanisms of morphine antinociception and tolerance, it would be of interest to examine whether central GRs would regulate the expression of central CBRs that may occur following chronic morphine exposure.

In a rat model of morphine tolerance induced by repeated morphine exposure, we examined the hypotheses that (1) the expression of central CBRs (CB-1 and CB-2 receptors) would be altered after repeated morphine exposure and (2) co-administration of a GR antagonist with morphine would regulate an altered expression of CBRs.

2. Methods and materials

2.1. Experimental animals

Adult male Sprague–Dawley rats (Charles River) weighing 300–350 g were used. Animals were housed in cages with water and food pellets available ad libitum. The animal room was artificially illuminated from 7:00 to 19:00 h. The experimental protocol was approved through our Institutional Animal Care and Use Committee.

2.2. Intrathecal catheter implantation and drug delivery

An intrathecal (i.t.) catheter (PE 10) was implanted in each rat under the pentobartital (50 mg/kg, intraperitoneal) anesthesia according to our previously published method [28]. Those animals exhibited neurological deficits (e.g., paralysis) after i.t. catheter implantation was excluded from the experiments. Drugs were delivered via an i.t. catheter in a total volume of 10 μl followed by a saline flush. RU38486 and morphine were purchased from Sigma (St. Louis, MO). Morphine was dissolved in normal saline and RU38486 in 10% ethanol solution. The 10% ethanol solution was used as vehicle control.

2.3. Induction of morphine tolerance and behavioral test

Tolerance to the antinociceptive effect of morphine was induced using an i.t. treatment regimen in that 10 μg morphine was given twice daily for 6 days. Differences in morphine antinociception among treatment groups were assessed using the tail-flick test by generating cumulative dose–response curves in that increment log doses of morphine were given to the same rats until no additional analgesia was demonstrated or the cut-off time was reached in response to a higher dose [26]. The routine tail-flick test was used with baseline latencies of 4–5 s and a cut-off time of 10 s. At least two trials were made for each rat with an intertrial interval of 1 min and with changes of the tail position receiving radiant heat stimulation at each trial.

The percent of maximal possible antinociceptive effect (%MPAE) was determined by comparing the tail-flick latency before (baseline, BL) and after a drug injection (TL) using the equation: % MPAE = [(TL − BL) / (10 − BL)] × 100% (the constant 10 refers to the cut-off time). The experiments were conducted with the experimenters being blinded to treatment conditions.

2.4. RT-PCR

Animals were sacrificed after being decapitated under the pentobartital (100 mg/kg, intraperitoneal) anesthesia. Total RNA was isolated from the lumbar spinal cord dorsal horn samples, obtained through a laminectomy, by using TRIZOL Reagent (TEL-TEST, Friendswood, TX). After incubation for 15 min at 4 °C, chloroform was added for the phase separation. The upper aqueous phase was collected and RNA was precipitated after being mixed with isopropyl alcohol. The RNA pellet was washed once with 75% ethanol and air-dried, which was finally redissolved in RNase-free water. A260/A280 ratios were between 1.6 and 1.8.

RT-PCR was performed within the linear range of reaction using the Titan One Tube RT-PCR System (Roche, Indianapolis, IN). Each PCR amplification with a volume of 25 μl contains 100 ng of total RNA, 0.4 μM of each primer, 0.2 mM of each deoxynucleotide (dNTP), 5 mM dithiothreitol, 5 U RNase-Inhibitor, 1.5 mM $MgCl_2$, and 0.5 μl enzyme mix. The reactions were carried out in an MJ research thermocycler using the following programs: CB-1: forward primer (CATCATCATCCACACGTCAG), reverse primer (ATGCTGTTGTCTAGAGG CTG), program (94 °C 1 min, 60 °C 1 min, 72 °C 2 min, 35 cycles); CB-2: forward primer (CGGCTTGGAGTTCAA CCCTA), reverse primer (ACAA-CAAGTC CACCCC ATGAG), program (94 °C 1 min, 60 °C 1 min, 72 °C 2 min, 37 cycles). Every PCR was accompanied by one negative control reaction without template RNA. PCR products were analyzed by gel electrophoresis on an ethidium bromide-stained 1% agarose gel (Sigma) in tris-borate-EDTA buffer. The amount of RNA per RT-PCR sample was normalized using PCR with primers specific for β-actin: forward primer (TAC AAC CTC CTT GCA CC), reverse primer (ACA ATG CCG TGT TCA ATG G), program (95 °C 1 min, 55 °C 1 min, 72 °C 1 min, 32 cycles). Each band was then measured with a computer-assisted imaging analysis system and normalized against the loading control. Differences were compared using a one-way ANOVA followed by post hoc Newman–Keuls' tests.

2.5. Western blot

Animals were sacrificed after being decapitated under the pentobartital anesthesia. Fresh lumbar spinal cord dorsal