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Research report

Peripheral interactions between cannabinoid and opioid receptor agonists in a model of inflammatory mechanical hyperalgesia

Q-Schick Auh[b], Yang Hyun Chun[b], Ohannes K. Melemedjian[a], Youping Zhang[a], Jin Y. Ro[a,b,*]

[a] University of Maryland School of Dentistry, Department of Neural and Pain Sciences, 650 W. Baltimore St., Baltimore, MD 21201, USA
[b] Kyung Hee University, School of Dentistry, Department of Oral Medicine, 1 Hoegi Dong, Dongdaemun Gu, Seoul, Republic of Korea

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ABSTRACT

Activation of opioid and cannabinoid receptors expressed in nociceptors induces effective antihyperalgesia. In this study, we examined whether combinations of opioid and cannabinoid receptor agonists directed at the injured site would enhance therapeutic effectiveness. Behavioral pharmacology experiments were performed to compare the effects of DAMGO, a selective agonist for μ-opioid receptor (MOR), ACPA, a specific agonist for CB1, and combinations of DAMGO and ACPA in attenuating complete Freund's adjuvant (CFA)-induced mechanical hyperalgesia in the rat hindpaw. DAMGO (1 μg–1 mg) or ACPA (1 μg–2 mg) was administered into the inflamed paw when mechanical hyperalgesia was fully developed. When administered individually, DAMGO and ACPA dose-dependently reversed the mechanical hyperalgesia. DAMGO displayed a lower ED50 value (57.4 ± 2.49 μg) than ACPA (111.6 ± 2.18 μg), but ACPA produced longer lasting antihyperalgesic effects. Combinations of DAMGO and ACPA also dose-dependently attenuated mechanical hyperalgesia, but the antihyperalgesic effects were partial and transient even at high doses. Using isobolographic analysis, we determined that combined treatment with DAMGO and ACPA produced antagonistic effects with the observed ED_{50} of 128.4 ± 2.28 μg. Our findings showed that MOR and CB1 agonists directed at the inflamed site effectively attenuate mechanical hyperalgesia when administered individually, but exert opposing effects when administered together. The antagonistic interactions between the two classes of drugs at the inflamed site suggest distinct mechanisms unique to peripheral nociceptors or inflamed tissue, and therefore require further studies to investigate whether the therapeutic utility of the combined drug treatments in chronic pain conditions can be optimized.

1. Introduction

Classical opioid receptors such as μ, δ and κ receptors and cannabinoid receptor type 1 (CB1) and type 2 (CB2) are a family of metabotropic receptors coupled to $G_{i/o}$ protein. It is well known that activation of both receptor systems invokes intracellular signaling cascades that inhibit adenylyl cyclase (Howlett and Fleming, 1984), decrease Ca^{2+} channel conductance (Caulfield and Brown, 1992; Seward et al., 1991), and activate inward rectifying and A-type potassium channels (Takeda et al., 2004; Wacnik et al., 2008). Activation of opioid or cannabinoid receptors produces similar pharmacological outcomes, including antinociceptive effects (Bushlin et al., 2010). Potent analgesic effects of both opioids and cannabinoids are, however, offset by serious side effects mediated by their receptors within the CNS.

Preclinical and clinical studies continue to provide strong justification that opioid and cannabinoid receptors localized in primary afferent neurons are viable targets for effective pain management. Recent development of peripherally restricted opioids and cannabinoids (Arendt-Nielsen et al., 2009; Bileviciute-Ljungar et al., 2006; Yu et al., 2010), and novel gene-based therapies to increase peripheral opioid receptor (Raja, 2012) and opioid peptides (Machelska et al., 2009) attest to ongoing efforts to garner maximum therapeutic advantages of peripheral receptors without producing centrally mediated side effects. Interestingly, MOR and CB1 in primary afferent neurons also share remarkable similarities in the transcriptional regulation of their expression. Peripheral inflammation increases μ-opioid receptor (MOR) expression in dorsal root ganglia (DRG) and trigeminal ganglia (TG) (Mousa, 2003; Pol

* Corresponding author at: University of Maryland Dental School, Department of Neural and Pain Sciences, 650 W. Baltimore St Baltimore, Maryland 21201, USA.
E-mail address: jro@umaryland.edu (J.Y. Ro).

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and Puig, 2004; Puehler et al., 2004). Available data show inflammatory cytokines such as interleukin (IL)-1β, IL-4, IL-6, and TNFα induce MOR expression in neuronal as well as in non-neuronal cell lines (Borner et al., 2004; Kraus et al., 2001). We have recently demonstrated that the same inflammatory cytokines induce MOR upregulation in TG (Zhang et al., 2014). Similarly, peripheral inflammation increases CB1 expression in TG, and inflammatory cytokines such as IL-1β and IL-6 induce CB1 expression in TG (Niu et al., 2012). These findings imply that inflammatory cytokines concurrently regulate both CB1 and MOR transcription.

Since the increase in MOR and CB1 densities has been proposed as one of the major mechanisms underlying pronounced antihyperalgesic effects of peripheral opioids and cannabinoids under inflammatory conditions (Niu et al., 2012; Zollner et al., 2003), it is reasonable to assume that targeting both receptor systems in the periphery would lead to greater antihyperalgesic effects in treating inflammatory pain and hyperalgesia. Synergistic or additive interactions between MOR agonist and CB1 agonist have been described for systemic effects mediated primarily by the receptors in the CNS (Cox et al., 2007; Maguire et al., 2013; Tham et al., 2005). However, similar studies evaluating interactions between the peripheral MOR and CB1 under pathological pain conditions have not been conducted.

Mechanical hyperalgesia is a prominent symptom in most chronic pain conditions, especially those associated with deep tissues. Mechanical hyperalgesia is characterized by pain upon touch, palpation, stretching or even movement, all of which could result from sensitization of nociceptors (Mense, 1993). Joseph and Levine showed that most nociceptors paly a role in mechanical hyperalgesia and that MOR on nociceptors attenuate mechanical hyperalgesia (Joseph and Levine, 2010). In trigeminal nociceptors, TRPV1 neurons that mediate inflammatory mechanical hyperalgesia also express MOR (Lee et al., 2016), and that the administration of an MOR agonist at the inflamed tissue effectively attenuate mechanical hyperalgesia (Zhang et al., 2014). Similarly, treatment with a CB1 agonist at the inflamed tissue blocks inflammation-induced mechanical hyperalgesia in a receptor specific manner (Niu et al., 2012). The objective of the present study was to evaluate whether the combination of MOR and CB1 agonists administered directly into the inflamed tissue would lead to additive, synergistic, or antagonistic antihyperalgesic effects on inflammatory mechanical hyperalgesia.

2. Materials and methods

2.1. Subjects

Male Sprague Dawley rats (8 weeks old; 250–300 g, Harlan, Indianapolis) were used in all experiments. Animals were housed in a temperature-controlled room under a 12:12 light–dark cycle with access to food and water ad libitum. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and under a University of Maryland approved Institutional Animal Care and Use Committee protocol.

2.2. Induction of inflammation

Inflammation was induced by the injection of complete Freund's adjuvant (CFA, 50 μl; 1:1 isotonic saline) into the plantar surface of the right hindpaw with a 27-gauge needle over 5–10 s.

2.3. Mechanical sensitivity test

Mechanical sensitivity of the hindpaw was assessed with the Randall–Selitto test, an established rodent model for testing mechanical hypersensitivity of the paw. Experiments were conducted according to the procedure described previously (Auh and Ro, 2012). Briefly, animals were first allowed to habituate to the experimental room for 30 min for three consecutive days. The withdrawal response to noxious paw pressure was assessed using a digital paw pressure Randall–Selitto applicator for rodents (IITC Life Science, Woodland Hills, CA). Each rat was placed in a cloth holder suspended in a sling, and the probe of the pressure applicator was placed under the plantar surface of the hindpaw. A gradually increasing pressure was applied until the rat withdrew its hindpaw. The pressure applicator captures and stores the pressure upon reaction. The lowest pressure necessary to elicit the withdrawal response prior to inflammation was considered as the baseline mechanical threshold.

Antihyperalgesic effects of DAMGO ([D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin, Sigma Aldrich, St. Louis, MO, USA), a highly selective MOR agonist, ACPA (Arachidonyl-cyclopropylamide, Tocris, Bristol, United Kingdom), a specific agonist for CB1, or combination of DAMGO and ACPA were measured on day 3 after intraplantar (i.pl) injection of CFA, during which mechanical hyperalgesia was most profound. On day 3, DAMGO (1, 30, 100 μg and 1 mg) or ACPA (1, 30, 100 μg, 1 and 2 mg) or combinations of the two agonists dissolved in phosphate buffer solution (PBS; 20 μl) was administered into the plantar surface of the inflamed hindpaw. The same volume of vehicle control was administered in the identical manner. A pre-drug treatment mechanical threshold for evoking a hindpaw withdrawal response was determined 15 min prior to drug injection. Changes in mechanical sensitivity of the hindpaw were assessed 30, 60, 120 and 180 min after the administration of each drug. The specificity of DAMGO and ACPA for MOR and CB1, respectively, has been well documented in the literature, including our previous studies that confirmed their specificity against selective antagonists in inflammatory muscle pain models (Niu et al., 2012; Nunez et al., 2007). All experimental and control groups consisted of 5 animals per group.

2.4. Statistical analysis

One-way ANOVA was used to compare the differences in baseline mechanical thresholds before and after CFA-induced inflammation for each drug treatment groups. The antihyperalgesic effects of drug treatments were analyzed with a two-way ANOVA with repeated measures. For each treatment, the percent maximum possible effect (%MPE) was calculated using the following formula: [test threshold (g) – baseline (g)/cut off threshold (250 g) – baseline (g)] x 100. We chose the cut off threshold as 250 g since the average mechanical threshold for adult rats under normal condition was around 250 g %MPE was calculated at the time point at which the greatest antihyperalgesic effects were observed. The ED_{50} (the dose that caused 50% of maximum antihyperalgesia) was generated from standard non-linear regression analysis of the log dose-response curve (Prism 6.0, Graphpad Software, San Diego, CA).

Interactions of agonist combinations were analyzed using fixed ratio design isobolograms whereby combinations of two drugs in known ratio were administered as fractions of their respective ED_{50} (Tallarida, 2002). The analysis of isobologram was adapted from a published study (Tham et al., 2005). Briefly, the isobologram was constructed by connecting $ED_{50DrugA}$ on the vertical axis to $ED_{50DrugB}$ on the horizontal axis. We then calculated the theoretical dose required for a purely additive interactions using the following formula: $Z_{add} = (f)\ ED_{50DrugA} + (1-f)\ ED_{50DrugB}$, where f is the fraction of drug A. Z_{add} was compared to the actual dose (Z_{mix} determined from the ED_{50} of the combination dose-response curve) required to achieve the same effect experimentally via the Student's t-test (Tallarida, 2002). The variance for Z_{add} was calculated as $Var(Z_{add}) = (f)^2\ Var(ED_{50DrugA}) + (1-f)_2\ Var(ED_{50DrugB})$. All