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Activation of delta-opioid receptor contributes to the antinociceptive effect of oxycodone in mice

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ABSTRACT

Oxycodone has been used clinically for over 90 years. While it is known that it exhibits low affinity for the multiple opioid receptors, whether its pharmacological activities are due to oxycodone activation of the opioid receptor type or due to its active metabolite (oxymorphone) that exhibits high affinity for the mu-opioid receptors remains unresolved. Ross and Smith (1997) reported the antinociceptive effects of oxycodone (171 nmol, i.c.v.) are induced by putative kappa-opioid receptors in SD rat while others have reported oxycodone activities are due to activation of mu- and/or delta-opioid receptors. In this study, using male mu-opioid receptor knock-out (MOR-KO) mice, we examined whether delta-opioid receptor was involved in oxycodone antinociception. Systemic subcutaneous (s.c.) administration of oxycodone (above 40 mg/kg) could induce a small but significant antinociceptive effect in MOR-KO mice by the tail flick test. Delta-opioid receptor antagonist (naltrindole, 10 mg/kg or 20 mg/kg, i.p.) could block this effect. When oxycodone was injected directly into the brain of MOR-KO mice by intracerebroventricular (i.c.v.) route, oxycodone at doses of 50 nmol or higher could induce similar level of antinociceptive responses to those observed in wild type mice at the same doses by i.c.v. Delta-opioid receptor antagonists (naltrindole at 10 nmol or ICI 154,129 at 20 μg) completely blocked the supraspinal antinociceptive effect of oxycodone in MOR-KO mice. Such oxycodone antinociceptive responses were probably not due to its active metabolites oxymorphone because (a) the relative low level of oxymorphone was found in the brain after systemically or centrally oxycodone injection using LC/MS/MS analysis; (b) oxymorphone at a dose that mimics the level detected in the mice brain did not show any significant antinocieption effect; (c) oxycodone exhibits equal potency as oxymorphone albeit being a partial agonist in regulating $[Ca^{2+}]_I$ transients in a clonal cell line expressing high level of mu-opioid receptor. These data suggest that oxycodone by itself can activate both the mu- and delta-opioid receptors and that delta-opioid receptors may contribute to the central antinociceptive effect of oxycodone in mice.

1. Introduction

Oxycodone (14-hydroxy-7,8-dihydrocodeinone), a pure semi-synthetic opioid agonist derived from a naturally occurring alkaloid, thebaine, has been in clinical use since 1917 for the management of moderate and severe acute or chronic pain. With a methoxy group at the 3- position of the aromatic ring A, a position hypothesized by Beckett and Casy that must contain a hydroxy moiety for the receptor binding and analgesic potency of opioid drugs [1], it is surprising that oxycodone is more potent than morphine in vivo considering that mu-opioid receptor binding affinity of oxycodone is much less than that of morphine in the rat brain homogenate [2,3]. In humans, oral oxymorphone is 10-fold more potent than oral morphine based on effective dose [4]. Oxycodone has approximately 1.5 times more potent analgesic than morphine when administered intravenously or orally for the relief of post-operative and cancer pain [5,6]. In rats, oxycodone has an analgesic

Abbreviations: MOR-KO, mu-opioid receptor knock out; i.c.v., intracerebroventricular; s.c., subcutaneous.

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potency 2–4 times that of morphine after systemic administration [subcutaneous (s.c.) or intraperitoneal (i.p.)] [7]. However, oxycodone has been shown to induce significantly less antinociception compared with morphine when given intrathecally (i.t.) or intracerebroventricularly (i.c.v.) in rats [8–10]. Oxycodone is metabolized by cytochrome P450 in the liver into noroxycodone and oxymorphone, and the oxymorphone's affinity for mu-opioid receptor is higher than oxycodone [11,12]. Activation of mu-opioid receptor by oxymorphone could contribute to the observed oxycodone adverse effects associated with mu-opioid receptor activation such as respiratory depression, miosis, euphoria, constipation, tolerance, dependence and addiction [13]. However, only 10% of oxycodone is *O*-demethylated to oxymorphone [12]. Noroxycodone and oxymorphone undergo further *O*-demethylation and *N*-demethylation, respectively to form noroxymorphone [14]. Noroxycodone is the major metabolite of oxycodone in rats [15] and in humans [16]. Moreover, in pharmacokinetic studies, plasma concentrations of oxymorphone are either very low or absent following the systemic or oral administration of oxycodone in human [7,12,16,17]. The absence of detectable level of metabolites suggests oxycodone by itself or metabolites other than oxymorphone could activate one or multiple opioid receptor types.

Ross and Smith [18] reported that oxycodone is a putative kappa-opioid agonist based on studies in which i.c.v. pretreatment of rats with the kappa-opioid selective antagonist, nor-binaltorphimine (nor-BNI) abolished i.c.v. oxycodone but not morphine antinociception. In contrast, Nozaki et al. [19] reported that systemic antinociceptive effect of oxycodone is mainly mediated by mu-opioid receptor in nondiabetic mice, whereas in diabetic mice, these effects were mediated by mu- and kappa-opioid receptors. However, the involvement of receptors other than mu-opioid receptor in oxycodone antinociceptive activity has been controversial. Recent animal studies have failed to show the reduction of the antinociceptive effect of oxycodone with selective kappa- and delta-opioid receptor antagonists [3,10]. Nielsen and colleagues [2] also suggested that the observed oxycodone pharmacology may represent the binding and activation of an opioid receptor dimer, such as the delta/kappa. Whether delta-opioid receptor is involved in oxycodone antinociception remains unresolved. However, because oxycodone produces effects typical of mu-opioid agonists, the central role of mu-opioid receptor in oxycodone activity is unequivocal.

Therefore, the aim of the present study is to examine the contribution of the central delta-opioid receptor in the oxycodone's antinociceptive activity and to also investigate whether oxymorphone, the metabolite of oxycodone, plays an important role in this effect of oxycodone in mice. We evaluated the antinociceptive effects of systemic or supraspinal administered oxycodone in MOR-KO mice [20] in order to determine the possible central molecular target(s) of oxycodone in the absence of mu-opioid receptor. Selective delta-opioid receptor antagonists were used to investigate the possible role of delta-opioid receptor for the antinociceptive effect of oxycodone. Except determination of the brain levels of oxycodone and its active metabolite oxymorphone following systemic or supraspinal administration of oxycodone, we also injected oxymorphone directly in its pure form into the brain, in order to evaluate the role of oxymorphone in the antinociceptive response of oxycodone.

2. Materials and methods

2.1. Ethical statement

All experimental procedures were approved by the Institutional Animal Care and Use Committee at an AAALAC international-accredited animal facility: the Animal Center of National Defense Medical Center, Taiwan, R.O.C. (Approval Number: IACUC-09-070).

2.2. Animals

In order to remove the factor of mu-opioid receptor on the effect of oxycodone and investigate the role of delta-opioid receptor on the effect of oxycodone, we used both MOR-KO mice and selective delta-opioid receptor antagonists in our study.

MOR-KO mice [20] were kindly provided by Dr. John Pintar at Robert Wood Johnson Medical School, Piscataway, New Jersey, USA. These mice derived from heterozygote/heterozygote mating were viable and fertile. Knockout mice used in the present study were littermates derived from mating of homozygous MOR-1 knockout mice. Wild-type mice were of the parental C57BL/6J strain. Adult male wild-type and MOR-KO mice, weighing 20–30 g (8–12 weeks) were used in this study. All mice were kept in an animal room with a 12 h light/dark cycle, at a temperature of $25 \pm 2\,^{\circ}C$ and humidity of 55% at an AAALAC international-accredited animal facility: the Animal Center of National Defense Medical Center, Taiwan, R.O.C. Standard diet and water were provided ad libitum during the experiment. The care of animals were carried out in accordance with institutional and international standards (Principles of Laboratory Animal Care, NIH) and the protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the National Defense Medical Center, Taiwan, ROC. All studies involving animals are reported in accordance with the ARRIVE guidelines [21,22]. In order to minimize the animals used, the sample sizes usually were 6–10 in our study. For dose response study of the systemic administered saline or drug, we only used 6 animals per group. On the other hand, for those experiments to see the effects of antagonist on oxycodone or i.c.v. injection of drug(s), we have used 10 animals per group in order to minimize the variation or loss of animals due to the i.c.v. injection. In some later studies, we only used 4 animals per group due to the limited number of male MOR-KO mice we could get. Male mice after weaning were randomly housed in 4 per polypropylene cage in clean convention facility. One week before experiment, mice were randomly assigned into different experimental groups.

2.3. Drug administrations for antinociception study

2.3.1. Systemic administration

S.c. injections of saline or oxycodone (1–30 mg/kg for wild type mice; 30–80 mg/kg for MOR-KO mice) were administered. Systemic antagonists (naloxone or naltrindole) were administered i.p. 30 min before test drug administration.

2.3.2. Intracerebroventricular (i.c.v.) injections

Different doses of oxycodone (10–100 nmol) were acutely administered into the right lateral ventricle of mice by i.c.v. injection using the method of Laursen et al. [23]. The procedure included cutting the scalp of the mouse, locating bregma and injecting (saline or drug) 2 mm lateral to bregma at a depth of 2 mm by using a 50-μl Hamilton microliter syringe (Hamilton Co., Reno, NV) in a volume of 5-μl with a 30-gauge needle of appropriate length. The syringe was held manually at an approximate 45° angle to the skull, with the bevel of needle facing up and pointing toward the tail of the animal. Each mouse received one injection only.

2.4. Determination of the antinociceptive effect of drugs

Drug-induced antinociception was evaluated by the tail-flick test [24]. The light intensity of tail-flick apparatus (Model: Ugo Basile, Italy) was set to allow the basal tail-flick latencies of most mice were within the range of 2.5–3.5 s. A cut-off time of 10 s was