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Research report

Relationship between receptor occupancy and the antinociceptive effect of mu opioid receptor agonists in male rats

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ABSTRACT

The analgesic mechanisms of mu opioid receptor (MOR) agonists, including receptor occupancy at the site of action, are not completely understood. The aims of the present study were to evaluate: (i) receptor occupancy in the rat brain after administration of MOR agonists; (ii) the relationship between occupancy and the antinociceptive effect. Morphine (2 or 4 mg/kg) or oxycodone (1 or 3 mg/kg) was subcutaneously administered to rats. The antinociceptive effect of these drugs was measured by the hot-plate test. MOR occupancy in the thalamus was assessed by conducting an *ex vivo* receptor binding assay using [^{3}H][D-Ala^{2}, N-MePhe^{4}, Gly-ol]-enkephalin, followed by autoradiographic analysis. Both drugs produced antinociception in a dose-dependent manner, and these effects disappeared after the time point at which the maximal effect was elicited. Thalamic MOR occupancy was observed in a dose-dependent manner at the time point at which maximal antinociception was elicited, and relatively low occupancy was observed when the antinociceptive effect was decreasing. Good correlation between thalamic MOR occupancy and the antinociceptive effect was observed. These findings provide direct evidence for the receptor occupancy of MOR agonists at the site of action and its relationship with the analgesic effect.

1. Introduction

Mu opioid receptor (MOR) agonists such as morphine and oxycodone have been used for the treatment of pain associated with cancer and other disorders, including chronic inflammatory and postoperative pain. Several studies have investigated the pharmacological potency of MOR agonists in terms of the binding affinity (Chen et al., 1991; Nielsen et al., 2007; Peckham and Traynor, 2006) and signaling pathways after MOR stimulation, including inhibition of adenylate cyclase activity, stimulation of potassium channels, and inhibition of calcium channels (Childers, 1991; Standifer and Pasternak, 1997). In addition, it has been suggested that the analgesic effects of MOR agonists can be caused by inhibition of the ascending pain transmission system *via* the spinal dorsal horn and thalamus (Inturrisi, 2002; Jensen, 1997) and activation of the descending pain inhibitory system *via* periaqueductal gray matter (PAG), rostral ventromedial medulla, and nucleus raphe magnus (Behbehani and Fields, 1979; Basbaum and Fields, 1979). Dong et al. demonstrated that microinjection of morphine into the thalamic nucleus submedius produced antinociceptive effects in rats, and that these effects were antagonized by microinjection of naloxone, an opioid receptor antagonist, into the same region (Dong et al., 1999). These findings suggest that the region in the thalamus could be involved in opioid-induced analgesia as the site of action of MOR agonists. However, these analgesic mechanisms are not completely understood, partly because no direct evidences for *in vivo* receptor binding of MOR agonists at the site of action have been provided.

Evaluation of occupancy of opioid receptors has been approached by positron emission tomography (PET) using [^{11}C]carfentanil (selective MOR agonist radioligand) or [^{11}C]diprenorphine (non-subtype-selective antagonist radioligand). PET studies using these radioligands have revealed receptor occupancy in several brain regions after administration of opioid receptor antagonists at effective doses in animals (Hume et al., 2007; Saccone et al., 2016) and humans (Ingman et al., 2005). Hume et al. attempted to measure the occupancy of brain receptors using [^{11}C]diprenorphine after administration of opioid receptor agonists, including morphine and oxycodone, at effective doses in rats, but *in vivo* binding of [^{11}C]diprenorphine was not reduced significantly compared with that observed in control rats (Hume et al., 2007). A liquid chromatography/mass spectrometry-based assay using unlabeled ligands for opioid receptors has also been used for assessment of receptor occupancy by an opioid receptor

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antagonist, and has revealed the selectivity of opioid receptors *in vivo* (Need et al., 2007). However, MOR agonists have not been evaluated by this method. Thus, *in vivo* receptor binding of MOR agonists at the site of action and its relationship with pharmacological efficacy are not known.

As an alternative method to PET or liquid chromatography/mass spectrometry-based assays, an *ex vivo* receptor binding assay gives information about drug–receptor interaction *in vivo*. That is, tissues obtained from animals administered with the drug of interest are incubated with a radiolabeled ligand for the receptor. This method has been used for correlation analysis between MOR occupancy of an antagonist drug in the brain and its pharmacological effect (Landymore and Wilkinson, 1988). In addition, combined use with autoradiography allows region-specific analysis even in the tissues of small animals, such as the striatum and spinal cord of rats (Codd et al., 2010). However, no reports have analyzed receptor occupancy by MOR agonists using this method.

The aims of the present study were, therefore, to evaluate: (i) receptor occupancy in the brain after administration of MOR agonists to rats by conducting an *ex vivo* receptor binding assay; (ii) the relationship between receptor occupancy and the antinociceptive effect.

2. Results

2.1. The antinociceptive effect in the hot-plate test

Fig. 1 shows the antinociceptive effects of morphine and oxycodone in the hot-plate test. Morphine exhibited a maximal effect 40 min after administration at doses of 2 and 4 mg/kg, and the effect disappeared within 80 min after administration (Fig. 1A). Oxycodone exhibited a maximal effect 20 min after administration at doses of 1 and 3 mg/kg, and the effect disappeared within 80 min after administration (Fig. 1B).

2.2. MOR occupancy in the thalamus

MOR occupancy in the thalamus after administration of morphine or oxycodone was evaluated at two time points for each dose; when the antinociceptive effect reached a maximum and when the effect was decreasing. Occupancy was measured by autoradiographic analysis of brain sections after *ex vivo* receptor binding using [^{3}H] [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin (DAMGO). Fig. 2A shows representative autoradiograms of brain sections from morphine-dosed rats. Thalamic occupancies after administration of morphine (2 mg/kg) were 43.6 ± 3.0% and 31.1 ± 3.4% at 40 min and 80 min, respectively. Occupancies after administration of morphine (4 mg/kg) were 59.4 ± 1.8% and 30.2 ± 2.7% at 40 min and 80 min, respectively (Fig. 2B). Fig. 3A shows representative autoradiograms of brain sections from oxycodone-dosed rats. Thalamic occupancies after administration of oxycodone (1 mg/kg) were 51.5 ± 1.4% and 32.1 ± 4.6% at 20 min and 60 min, respectively. Occupancies after administration of oxycodone (3 mg/kg) were 73.5 ± 1.3% and 57.2 ± 5.3% at 20 min and 60 min, respectively (Fig. 3B).

2.3. Relationship between thalamic MOR occupancy and the antinociceptive effect in the hot-plate test

Fig. 4 shows the relationships between thalamic MOR occupancy and the antinociceptive effect in the hot-plate test in morphine- and oxycodone-dosed rats. There was good correlation with the square of the correlation coefficient of 0.997 in morphine-dosed rats and 0.910 in oxycodone-dosed rats, respectively.

3. Discussion

Opioid-induced analgesia has been investigated for decades in terms of biochemistry, neuropharmacology, and behavioral pharmacology. However, the analgesic mechanism of opioid receptor agonists is not fully understood, partly due to the lack of direct evidences for *in vivo* receptor occupancy of these drugs at the site of action and its relationship with the analgesic effect. Therefore, in the present study, MOR occupancy in the rat brain after subcutaneous administration of morphine or oxycodone was assessed. Both drugs are known to have a potent analgesic effect because they act selectively on MORs (Lemberg et al., 2006a; Narita et al., 2008), and have been widely used in clinical settings. We measured their antinociceptive effects in rats by the hot-plate test. Both drugs produced antinociception in a dose-dependent manner, and the effects disappeared after the time point at which the maximal effect was elicited. These findings were in accordance with studies in rats using similar doses to those employed in the present study (Lemberg et al., 2006b; Pöyhiä and Kalso, 1992).

Next, we evaluated MOR occupancy at two time points after administration; when the antinociceptive effect reached a maximum and when the effect was decreasing. The thalamus was selected as the site for the analysis of occupancy because it has been suggested that antinociception in the hot-plate test reflects analgesia at the supraspinal level predominantly (Dennis et al., 1980; Irwin et al., 1951; Langerman et al., 1995; Ramabadran and Bansinath, 1986) and that the thalamus is one of the sites of

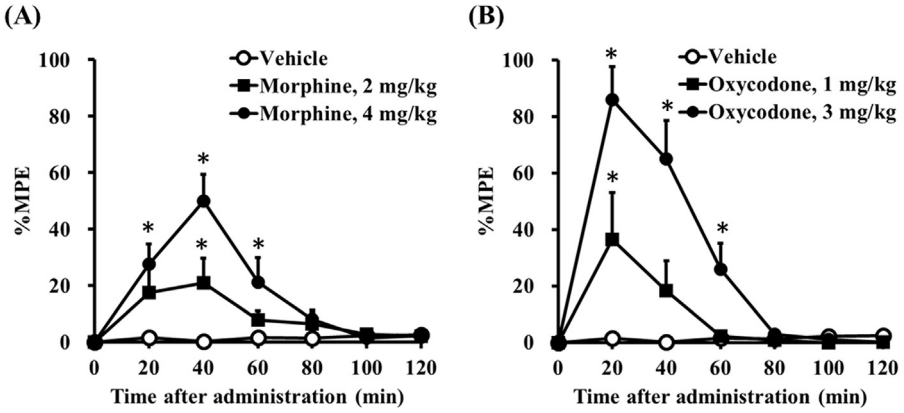

Fig. 1. Antinociceptive effect in rats after subcutaneous administration of morphine or oxycodone. Antinociceptive effects of morphine (A) and oxycodone (B) were evaluated by the hot-plate test. The effect was expressed as %MPE. Data are the mean ± SEM of 6–7 rats. $^*P < .05$ versus vehicle-treated group (ANOVA followed by Dunnett's test).