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Research Report

Morphine can produce analgesia via spinal kappa opioid receptors in the absence of mu opioid receptors

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Abbreviations:
i.c.v., intracerebroventricular
i.t., intrathecal
s.c., subcutaneous
ED_{50}, median effective dose
nor-BNI, nor-binaltorphimine
DPDPE, [D-Pen2,D-Pen5]enkephalin

ABSTRACT

Previous studies have demonstrated the virtual lack of analgesia in mu opioid receptor knockout mice after systemic administration of morphine. Thus, it has been suggested that analgesic actions of morphine are produced via the mu opioid receptor, despite its ability to bind to kappa and delta receptors in vitro. However, it is not clear whether the results of these studies reflect the effect of morphine in the spinal cord. In the present study, we report study of the analgesic actions of spinally-administered morphine and other opioid receptor agonists in mu opioid receptor knockout and wild type mice. Morphine produced a dose-dependent antinociceptive effect in the tail flick test in the knockout mice, although higher doses were needed to produce antinociception than in wild type mice. The antinociceptive effect of morphine was completely blocked by naloxone (a non-selective opioid antagonist) and nor-binaltorphimine (nor-BNI, a selective kappa-opioid receptor antagonist), but not by naltrindole (a selective delta-opioid receptor antagonist). U-50,488H (a selective kappa-opioid receptor agonist) also produced a dose-dependent antinociceptive effect in knockout mice but presented lower analgesic potency in knockout mice than in wild type mice. Analgesic effects of [D-Pen2,D-Pen5]enkephalin (DPDPE, a selective delta-opioid receptor agonist) were observed in wild type mice but abolished in knockout mice. SNC80 (a selective delta-opioid receptor agonist) was not antinociceptive even in wild type mice. The present study demonstrated that morphine can produce thermal antinociception via the kappa opioid receptor in the spinal cord in the absence of the mu opioid receptor. Lower potency of U50,488H in mu opioid receptor knockout mice suggests interaction between kappa and mu opioid receptors at the spinal level.

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1. Introduction

Morphine is a prototypical opioid agonist most frequently used clinically to produce analgesia. Morphine is weakly selective to the mu opioid receptor and also possesses affinity to the delta and kappa opioid receptors. Thus, morphine was long thought to act on all three receptor types to produce analgesia. This has been supported by in vivo pharmacological studies (Heyman et al., 1987; Takemori and Portoghese, 1987; Omote et al., 1991). Pharmacological studies also suggested possible interactions among opioid receptors, in particular, those between the mu and delta receptors in producing analgesia (Larson et al., 1980; Vaught et al., 1982; Malmberg and Yaksh, 1992). The generation of mu opioid receptor knockout mice approximately a decade ago and those of the other opioid receptors that followed led to better understanding of the mechanism of opioid analgesia. Surprisingly, the analgesic effects of morphine were abolished or strongly reduced in mu receptor knockout mice, indicating that the mu receptor was mandatory for the analgesic effects of morphine and that the delta or kappa receptor alone could not mediate morphine's analgesic actions (Matthes et al., 1996; Sora et al., 1997b, 2001; Loh et al., 1998; Schuller et al., 1999; Hosohata et al., 2000; Hall et al., 2003). In the initial studies, morphine was administered systemically most probably with the assumption that systemic morphine acts both in the brain and the spinal cord. Studies utilizing the intracerebroventricular (i.c.v.) route also showed the ineffectiveness of morphine in mu receptor knockout mice reflecting the results of studies with systemic administration (Loh et al., 1998; Schuller et al., 1999; Hosohata et al., 2000). On the other hand, there have been indications by studies that when morphine is administered systemically in animals, its direct action is predominantly in the brain (Manning and Franklin, 1998; Heinricher et al., 2001). In these studies, the analgesic effects of systemic morphine were completely reversed by blocking pathways within the brain. Furthermore, Honda et al. (2004) showed that the analgesic effects of systemic and i.c.v. morphine were attenuated by muscarinic antagonists, while the antagonist had no effect on the actions of intrathecal morphine. Thus systemic administration may not reflect the actions of morphine in the spinal cord, and morphine needs to be administered intrathecally (i.t.) to specifically observe its spinal actions. Although some studies have suggested the ineffectiveness of intrathecal morphine in mu receptor knockout mice (Schuller et al., 1999; Hosohata et al., 2000), there have been no studies that fully looked at the effect of intrathecal morphine on acute thermal pain in these mice. The present study was designed to examine the analgesic effects of spinally administered morphine in mu receptor knockout mice and to determine the possible molecular target of morphine in the absence of the mu opioid receptor.

2. Results

Baseline tail flick latencies of wild type and mu receptor knockout mice were 2.93 ± 0.26 s (n = 58) and 3.00 ± 0.26 s (n = 70), respectively, and were not significantly different.

2.1. *The antinociceptive effect of spinal morphine in mu receptor knockout mice and its blockade with naloxone*

Morphine given i.t. produced a dose-dependent antinociceptive effect in both wild type mice (Fig. 1A) and mu receptor knockout mice (Fig. 1B). The dose required to produce a significant effect was higher in knockout mice than in wild type mice. The median effective doses (ED_{50}) of morphine were 0.17 μg (0.07–0.35 μg, 95% confidence limits) and 5.00 μg (3.01–9.00 μg, 95% confidence limits) in wild type and knockout mice, respectively (Fig. 2), and were significantly different. Doses of morphine at 10 μg and above could not be examined in knockout mice because they yielded a paradoxical pain behavior (intermittent bouts of biting and scratching of affected dermatomes), which has previously been described in rats (Yaksh et al., 1986; Yaksh and Harty, 1988). This pain behavior has been shown to be non-reversible by naloxone (a non-selective opioid antagonist), thus is not mediated by opioid receptors. The concomitant spinal administration of naloxone (10 μg) completely blocked the antinociceptive effect of morphine in knockout

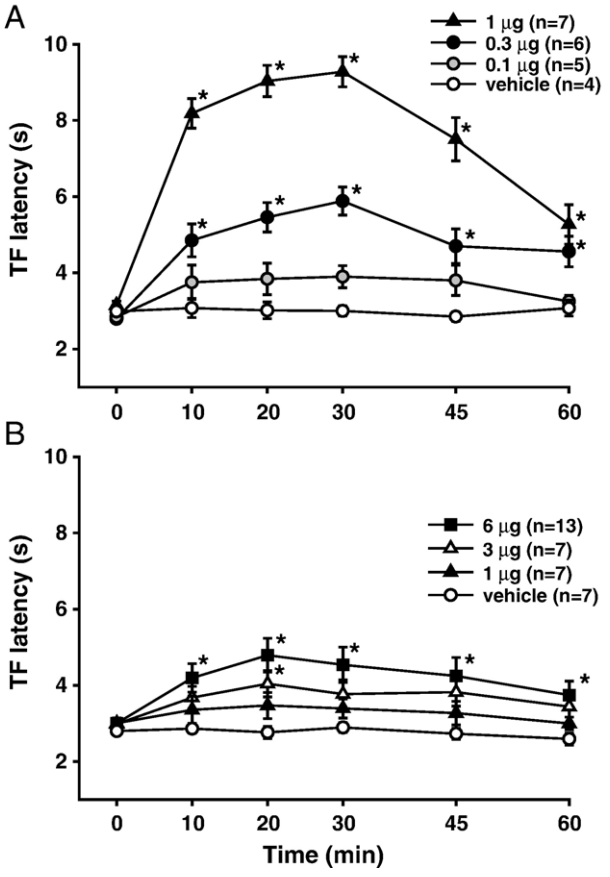

Fig. 1 – Time courses of the antinociceptive effect of morphine in wild type (A) and mu opioid receptor knockout (B) mice. Tail flick latencies were measured prior to and every 10–15 min during a period of 60 min after intrathecal administration of morphine. Values are expressed in mean ± SE. *Significantly different from vehicle control.