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NOP RECEPTOR MEDIATES ANTI-ANALGESIA INDUCED BY AGONIST–ANTAGONIST OPIOIDS

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Abstract—Clinical studies have shown that agonist–antagonist opioid analgesics that produce their analgesic effect via action on the kappa-opioid receptor, produce a delayed-onset anti-analgesia in men but not women, an effect blocked by co-administration of a low dose of naloxone. We now report the same time-dependent anti-analgesia and its underlying mechanism in an animal model. Using the Randall–Selitto paw-withdrawal assay in male rats, we found that nalbuphine, pentazocine, and butorphanol each produced analgesia during the first hour followed by anti-analgesia starting at ~90 min after administration in males but not females, closely mimicking its clinical effects. As observed in humans, co-administration of nalbuphine with naloxone in a dose ratio of 12.5:1 blocked anti-analgesia but not analgesia. Administration of the highly selective kappa-opioid receptor agonist U69593 produced analgesia without subsequent anti-analgesia, and confirmed by the failure of the selective kappa antagonist nor-binaltorphimine to block nalbuphine-induced anti-analgesia, indicating that anti-analgesia is not mediated by kappa-opioid receptors. We therefore tested the role of other receptors in nalbuphine anti-analgesia. Nociceptin/orphanin FQ (NOP) and sigma-1 and sigma-2 receptors were chosen on the basis of their known anti-analgesic effects and receptor binding studies. The selective NOP receptor antagonists, JTC801, and J-113397, but not the sigma receptor antagonist, BD 1047, antagonized nalbuphine anti-analgesia. Furthermore, the NOP receptor agonist NNC 63-0532 produced anti-analgesia with the same delay in onset observed with the three agonist–antagonists, but without producing preceding analgesia and this anti-analgesia was also blocked by naloxone. These results strongly support the suggestion that clinically used agonist–antagonists act at the NOP receptor to produce anti-analgesia.

Key words: κ-opioids, anti-analgesia, nalbuphine, nociceptin/orphanin FQ receptor.

INTRODUCTION

Opioid analgesics, such as nalbuphine, pentazocine, and butorphanol, that have preferential action at kappa (κ)-opioid receptors with agonist–antagonist activity, are referred to as κ-type agonist–antagonists (Woods and Gmerek, 1985; Hoskin and Hanks, 1991; Craft and McNiel, 2003). They have been used clinically for decades, but are considered weak analgesics compared to those opioid agonists that have a much greater efficacy at the μ-opioid receptor, such as morphine or oxycodone (Hansen, 2000; Levine et al., 2000; Walker, 1995). To better understand the variables that control the efficacy of these drugs, we conducted a series of studies in patients with post-operative pain. We found that for all three agonist–antagonists, women experience greater analgesia than men (Gear et al., 1996a; Gear et al., 1996b; Gear et al., 1999; Gordon et al., 1995). And, in a placebo controlled study, men receiving nalbuphine actually experienced worse pain than those receiving placebo (Gear et al., 1999). Analgesia was observed in both men and women during the first hour after administration, but by ~90 min men reported increasing pain (i.e., anti-analgesia) (Gear et al., 1999).

In subsequent studies we found that co-administration of nalbuphine with the non-selective opioid receptor antagonist naloxone in a narrow range of dose ratios, centered around 12.5:1 (nalbuphine:naloxone), blocks anti-analgesia and produces enhanced and prolonged analgesia in men, similar to that observed in women (Gear et al., 2000; Gear et al., 2003). To explain these results, we proposed that in men nalbuphine acts at two distinct receptors, a κ-opioid ''analgesia'' receptor (Gutstein and Akil, 2001) and an ''anti-analgesia'' receptor, the identity of which remains to be determined. Pharmacodynamic modeling recently provided support for this hypothesis (Kshirsagar et al., 2008).

Investigating the mechanism of agonist–antagonist-induced anti-analgesia using human subjects is significantly hampered by the lack of clinically available receptor-selective pharmacological agents. Therefore, to

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Abbreviations: ANOVA, analysis of variance; EDTA, ethylenediaminetetraacetic acid; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; NOP, nociceptin/orphanin; FQ, receptor; norBNI, nor-binaltorphimine.

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take advantage of the broad range of receptor-selective agents only available for animal studies, we developed an animal model of agonist–antagonist anti-analgesia in male rats. The nociceptin/orphanin FQ (NOP) receptor was chosen because its activation at some brain sites has been associated with pain enhancement (Meunier et al., 1995; Mogil et al., 1996). The sigma receptor was chosen because sigma receptors have been suggested to have anti-analgesic effects (Chien and Pasternak, 1993; Chien and Pasternak, 1994).

EXPERIMENTAL PROCEDURES

Animals

All experiments were performed on 250–300 g (58–68 days old) adult male and female Sprague–Dawley rats (Charles River Laboratories, Hollister, CA, USA). Animals were housed in a controlled environment at the animal care facility of the University of California, San Francisco, under a 12-h light/dark cycle. Food and water were available *ad libitum*. Experiments were approved by the Institutional Animal Care and Use Committee at UCSF and adhered to guidelines of the American Association of Laboratory Animal Care, the National Institutes of Health, and the Committee for Research and Ethical Issues of the International Association for the Study of Pain. Effort was made to minimize the number of animals used and their suffering.

Mechanical nociceptive threshold testing

Nociceptive testing was performed using an Ugo Basile Analgesymeter (Stoelting, Chicago, IL, USA), which applies a linearly increasing mechanical force on the dorsum of the hind paw. Nociceptive threshold was defined as the force in grams at which the rat withdrew its paw; experimenters were not blinded to the treatment. The starting pressure is 0 g and the rate of increase is 32 g/s; cut-off is 200 g, but this was not reached for any of the animals used in the current study. Each paw was treated as an independent measure and each experiment performed on a separate group of rats. Nociceptive threshold scores are obtained from a mean of three measurements for each time point, taken at 5-min intervals immediately before (baseline) and after drug administration.

Prior to experiments, rats were trained in the paw-withdrawal test at 5-min intervals for 1 h/d for 3 d. On the day of the experiment, baseline paw-withdrawal threshold was measured before intravenous (i.v.) drug administration. Post-administration thresholds were recorded 30 min later and thereafter at 15-min intervals for a total of 3 h. Changes in paw-withdrawal threshold are presented as percent change from baseline.

Drugs

Nalbuphine hydrochloride, an agonist–antagonist, naloxone hydrochloride, a non-selective opioid receptor antagonist, U69593, a selective κ-opioid receptor agonist, and nor-binaltorphimine (norBNI), a selective κ-opioid receptor antagonist were obtained from Sigma–Aldrich (St. Louis, MO, USA). Pentazocine lactate (Talwin 30 mg/ml) was obtained from Hospira (Lake Forest, IL, USA). Butorphanol was obtained from Bedford Laboratories (Bedford, OH, USA). J113397 and JTC-801, non-peptide (NOP, ORL1) receptor-selective antagonists, BD 1047, a selective sigma (σ)-receptor antagonist ($\sigma_1 > \sigma_2$), and NNC 63-0532, a selective NOP receptor agonist, were obtained from Tocris Bioscience (Ellisville, MO, USA). SB 612111, another non-peptide selective (NOP, ORL1) receptor antagonist, was obtained from Axon Medchem BV (Groningen, The Netherlands). Nalbuphine and naloxone were dissolved in physiological saline (0.9%); pentazocine was diluted with physiological saline to 5 mg/ml; U69593 was dissolved in 45% aq 2-hydroxypropyl-β-cyclodextrin; J-113397, SB 612111, and JTC801 were dissolved in dimethyl sulfoxide (DMSO); and BD 1047 was dissolved in water. NNC 63-0532 was dissolved in 100% ethanol and then diluted with 0.9% saline so that the final injection was 50% ethanol; the concentration of this solution was adjusted so that the injection volume was 250 μl. Nalbuphine, pentazocine, butorphanol, naloxone, saline, U69593, or NNC 63-0532 were administered intravenously (i.v.) into a lateral tail vein with a 25-gauge infusion catheter; animals were briefly anesthetized with 2.5% isoflurane to facilitate this procedure. To allow time for absorption J-113397, JTC801, SB 612111, and BD 1047 were administered subcutaneously (s.c.) in the nape of the neck without anesthesia 45 min prior to nalbuphine administration (Uchiyama et al., 2008).

Receptor binding

Binding assays for nalbuphine, pentazocine, and naloxone at σ_1, σ_2, and NOP receptors were performed by MDS Pharma Services (Bothell, WA, USA; assay services now acquired by Eurofins (https://www.eurofinspanlabs.com/Panlabs)). Details of assay binding conditions are given in Table 1.

Statistical analysis

Group data (all groups $n = 6$) are presented as mean ± SEM; data were analyzed using one-way or two-way analyses of variances (ANOVAs) as appropriate. Significance (alpha level) was set at $p \leqslant 0.05$). Two-way ANOVAs demonstrating a significant interaction were further analyzed with one-way ANOVAs to determine the basis of the interaction. For within-subject effects, one-way repeated measures ANOVAs were performed to determine if individual groups changed significantly over time. If so, simple contrasts were employed to determine which time points differed significantly from baseline. Because simple contrasts analysis requires multiple comparisons, a Bonferroni-type correction was applied to adjust the alpha level by dividing 0.05 by the number of comparisons. Scheffé post hoc analysis was employed to determine the basis of significance for between-subject main effects involving more than two groups.