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Anti-nociceptive and anti-allodynic effects of a high affinity NOP hexapeptide [Ac-RY(3-Cl)YRWR-NH_2] (Syn 1020) in rodents

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Abstract

There has been a flurry of activity to develop agonists and antagonists for the member of the opioid receptor family, NOP receptor (also known as ORL1), in part to understand its role in pain. Modifications of a hexapeptide originally identified from a combinatorial library have led to the discovery of a high affinity hexapeptide agonist Ac-RY(3-Cl)YRWR-NH_2 (Syn 1020). In the following experiments we characterized the anti-nociceptive effects of Syn 1020 in the tail-flick model of acute pain and the diabetic neuropathy model of chronic pain in mice and rats, respectively. Acute antinociception was assessed using the tail-flick assay in mice in which animals received intracerebroventricular (i.c.v.) or subcutaneous (s.c.) injections of Syn 1020 alone or with morphine and were tested for tail-flick latencies. In the chronic pain model, diabetic neuropathy was induced by injections of streptozotocin in rats. Tactile allodynia was measured, with von Frey hair filaments, following intraperitoneal (i.p.) injections of Syn 1020 or gabapentin (positive control). In mice, i.c.v. injections of Syn 1020 did not have any pro- or anti-nociceptive effects, however, Syn 1020 reversed morphine antinociception with a similar potency as N/OFQ (the natural ligand to NOP). S.c. injections of Syn 1020 in mice also produced analgesic effects. In rats, i.p, injections of Syn 1020 produced anti-allodynic effects. Thus, Syn 1020, a NOP receptor directed peptide, administered systemically has anti-nociceptive activity in both acute and chronic pain models in mice and rats respectively.
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1. Introduction

The NOP receptor (formerly known as the Opioid Receptor Like receptor ORL1) was discovered simultaneously by several groups based upon homology with the δ-opioid receptor (Bunzow et al., 1994; Mollereau et al., 1994; Wang et al., 1994). Shortly thereafter the natural ligand to the receptor Nociceptin or Orphanin FQ (N/OFQ) was identified (Meunier et al., 1995; Reinscheid et al., 1995). Since this receptor is part of the opiate receptor family, the role of this NOP receptor in pain modulation has been studied (for reviews see (Calo et al., 2000; Mogil and Pasternak, 2001). Initially, N/OFQ administered *via* intracerebroventricular (i.c.v.) route resulted in hyperalgesia (Meunier et al., 1995; Reinscheid et al., 1995). Although studies have verified the pro-nociceptive effects of N/OFQ when administered supraspinally, some studies have reported that i.c.v. N/OFQ can produce analgesia or no analgesic effects (Mogil et al., 1996; Standifer et al., 1996; Tian et al., 1997). However, when N/OFQ is co-administered with morphine, it blocks morphine-induced antinociception consistently across studies (Mogil et al., 1996; Tian et al., 1997). When N/OFQ is given intrathecally (i.t.) the pain-modulatory role is clearer. Spinally

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administered N/OFQ does not act as an opioid antagonist, indeed it is completely ineffective or it can potentiate morphine-induced antinociception (Grisel et al., 1996; Tian et al., 1997). Indeed, the majority of studies report that high doses of N/OFQ produce antinociception and anti-allodynia (Courteix et al., 2004; King et al., 1997; Xu et al., 1996).

Peptide and small molecule agonists and antagonists have been developed to shed some light on the complicated profile of N/OFQ and its receptor, NOP, in pain. It is thought that if NOP receptor agonists can act as analgesics at the spinal level without affecting or even having functional opioid antagonistic activity in the brain, then there may be a therapeutic use for these drugs (Zeilhofer and Calo, 2003). Conversely, if there is an endogenous N/OFQ tone in the brain, then an NOP receptor antagonist might block this activity and have analgesic activity of its own. In fact when given i.c.v., some peptides that are high affinity NOP receptor antagonists act as potent analgesics (Calo et al., 2000, 2002; Judd et al., 2003). One lower affinity and less selective non-peptide antagonist, JTC-801, produces anti-nociceptive activity in acute pain models and decreases hyperalgesia in a neuropathic pain animal model (Suyama et al., 2003; Yamada et al., 2002).

Utilizing a combinatorial library strategy, Dooley et al. (1997) identified a series of basic hexapeptides with subnanomolar NOP binding affinities, similar to N/OFQ. These compounds showed partial agonist activity when tested for stimulation of [^{35}S]GTPγS binding or inhibition of forskolin-stimulated cAMP accumulation in CHO cells transfected with mouse and rat NOP receptors respectively. One of these hexapeptides, Ac-RYYRIK-NH_2, was tested *in vivo*, and found to inhibit spontaneous locomotor activity in mice to a similar extent as N/OFQ, however, it was approximately 15-fold more potent than N/OFQ (Berger et al., 2000). N-terminal modifications as well as insertion of unnatural amino acids of one of these hexapeptides [Ac-RYYRWR-NH_2], was found to produce very high affinity compounds with a broad range of *in vitro* efficacies for the NOP receptor (Judd et al., 2004).

The high affinity hexapeptide Ac-RY(3-Cl)YRWR-NH_2 (Syn 1020), has a similar affinity at human NOP receptors as N/OFQ (K_i values of 0.03 nM ± 0.02 and 0.04 nM ± 0.005, respectively; Judd et al., 2004), and is over 1000-fold selective for NOP over the opioid receptors (K_i values of 151 nM ± 13 and 130 nM ± 35 at μ- and κ-opioid receptors respectively). Syn 1020 is an extremely potent partial agonist, as determined by stimulation of [^{35}S]GTPγS binding to human NOP receptors (EC_{50} 0.30 ± 0.02 nM; % stimulation = 75.5 ± 9.4). Nevertheless, this compound acted as a NOP receptor antagonist in the mouse *vas deferens* (K_e 31.4 ± 4.2 nM), as it reversed the N/OFQ-mediated inhibition of electrically induced contractions of the smooth muscle (Judd et al., 2004). This is similar to the NOP partial agonist [Phe^1psi(CH_2-NH)Gly2]NC(1-13)NH_2, which is an antagonist in the mouse *vas deferens*, a partial agonist in CHO cells in culture, and a full agonist *in vivo* (Burnside et al., 2000; Butour et al., 1998; Grisel et al., 1998; Guerrini et al., 1998). Thus, in the following experiments we wanted to further characterize the anti-nociceptive effects of this hexapeptide, Syn 1020, *in vivo* following i.c.v. and systemic injections in the tail-flick model of acute pain and the diabetic neuropathy model of chronic pain in mice and rats, respectively.

2. Materials and methods

2.1. Acute thermal pain model

2.1.1. Animals

Male ICR mice, weighing 20–25 g at the start of the experiment, were used. Animals were group-housed under standard laboratory conditions and were kept on a 12:12 h day–night cycle (lights on at 08:00). Animals were handled for 1–2 days prior to conducting the experiments. On the day of the experiment, animals were transported to the testing room and acclimated to the environment for 1 h. Mice were maintained in accordance with the guidelines of SRI International and of the "Guidelines for the Care and Use of mammals in neuroscience and behavioral research" (National Research Council, 2003).

2.1.2. Drugs

The hexapeptide NOP receptor agonist, Syn 1020 (Judd et al., 2004), N/OFQ (Phoenix Pharmaceuticals) and morphine (obtained commercially from Lilly) were dissolved in phosphate buffered saline (PBS). Drugs were injected at a volume of 2 μl/injection (i.c.v.) or 0.1 ml/25 g (subcutaneous, s.c.).

2.1.3. Tail-flick assay

Acute nociception was assessed using the tail-flick assay with an analgesia instrument (Stoelting) that uses radiant heat. This instrument is equipped with an automatic quantification of tail-flick latency, and a 15-s cutoff to prevent damage to the animal's tail. During testing, the focused beam of light was applied to the lower half of the animal's tail, and tail-flick latency was recorded. Baseline values for tail-flick latency were determined before drug administration in each animal. The mean basal tail-flick latency was 5.3-s ± 0.1 S.E.M.

Following baseline measures, animals that received i.c.v. injections were lightly anesthetized with isoflurane and received a unilateral injection (2.0 mm caudal and 2.0 mm lateral with respect to Bregma, and −2.5 mm ventral from skull surface). Following i.c.v. injections, animals were tested for tail-flick latencies at 10- and 20-min postinjection. For animals receiving systemic administration of drugs, they received a s.c. injection of their assigned dose of drug and were tested for tail-flick latencies at 10-, 20-, and 30-min postinjection.

In the first experiment we examined the effects of i.c.v. injections of vehicle (PBS) and Syn 1020 alone (0.1–10.0 nmol). In the follow-up experiment, animals received i.c.v. injections of morphine (10.0 nmol) alone or co-administered with 0.1–10.0 nmol Syn 1020. The morphine/agonist dose-response curve was compared to that of i.c.v. injections of N/OFQ (0.1–10.0 nmol) and morphine (10.0 nmol). The dose of morphine was chosen such that it produced analgesic effects that were above 50% and yet not maximal effects (unpublished data, also see results). To examine the effects of systemically administered Syn 1020, animals received s.c. injections of Syn 1020 (10–100 mg/kg) alone or co-administered with morphine (10 mg/kg).

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