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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.

Keywords: NOP receptor; Antinociception; Nociception; Analgesia; Allodynia; Nociceptin; Thermal pain; Mechanical pain

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^{1}psi(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).

In all the experiments described above, the N/group was 10 for each drug dose/condition.

2.1.4. Statistical analyses

Antinociception (%Maximum Potential Effect; %MPE) was quantified by the following formula: %MPE = 100 * [(test latency − baseline latency) / (15 − baseline latency)].

If the animal did not respond prior to the 15-s cutoff, the animal was assigned a score of 100%.

Behavioral results were analyzed using ANOVAs with N/OFQ, Syn 1020, and morphine as between group variables and post-drug treatment time (10- and 20-min for i.c.v. injections and 10-, 20-, and 30-min for s.c. injections) as the repeated measure followed by Student Newman–Keuls *post-hoc* tests where appropriate. The level of significance was set at $P<.05$.

2.2. Tactile allodynia using the chronic diabetic neuropathy model

2.2.1. Animals

Male Sprague Dawley rats weighing 250–300 g at the beginning of the experiment were used. Animals were housed four/cage under standard laboratory conditions and were kept on a 12:12 h day–night cycle (lights on at 08:00). For the experimental day the animals were placed into separate cages supplied with food and water. Animals were tested in their home room.

2.2.2. Induction of diabetic neuropathy

Baseline values of paw withdrawal threshold were determined for each rat prior to streptozotocin administration using von Frey Filaments (as described in 2.2.3). Only rats with paw withdraw threshold greater than 7.0 g were used in the study. Rats received i.v. injections of 40 mg/kg Streptozotocin (Sigma) into the tail vein. Control animals received i.v. injections of PBS. After three–four days following the injection of streptozotocin, blood glucose levels were tested using Accucheck strips in a glucometer. Blood glucose levels for diabetic animals were ≥300 mg/dl. Animals that had lower blood glucose levels received another injection of streptozotocin (10–40 mg/kg depending on glucose levels). Blood glucose levels were re-tested after eight days and animals that were not diabetic were not included in the study. Animals were tested for tactile allodynia four–six weeks after streptozotocin injections. Only streptozotocin-treated rats that displayed a significant difference in paw withdraw threshold from control rats were used.

2.2.3. Von Frey hair testing

Each animal received an intraperitoneal (i.p.) injection of PBS, morphine (10 mg/kg, $N=8$), or Syn 1020 (10–100 mg/kg; $N=6$/group) and was placed back into its home cage. A group of animals received i.p. injections of Gabapentin (60 mg/kg; $N=9$) and served as the prototypic controls. Drugs were injected at a volume of 1 ml/kg. Animals were injected 5 min apart. Forty-five min after receiving their i.p. injection, animals were placed on a 16 by 16 in. wire mesh grid and allowed to acclimate for 15 min. After 60-min postinjection, tactile allodynia was measured with von Frey filaments using the modified up–down method (Chaplan et al., 1994; Dixon, 1991; Luo et al., 2001). Briefly, a von Frey filament that had a buckling weight of 2.0 g was applied to the left hindpaw of the animal. Continuous pressure with the filament was applied for about 5 s. If the animal lifted its paw, a positive response, then the next filament with less force was applied to the paw. If the animal did not lift its paw, a negative response, the next filament with increasing force was used. Each positive and negative response was recorded. This was continued until four more measurements following a positive response were collected or until the animal had made four consecutive positive responses or five consecutive negative responses. After testing, the animals were anesthetized with CO_2/O_2 and blood was collected to measure glucose levels. Animals were then returned to their original home cages.

2.2.4. Statistical analyses

The 50% paw withdrawal threshold was calculated (Chaplan et al., 1994; Luo et al., 2002) using the formula: $10^{(Xf+\kappa\delta)}$ / 10,000 where Xf is the final von Frey filament used (log units), κ is a value that analyzes the response pattern (taken from the table published by Chaplan et al., 1994), and δ is the mean difference between stimuli (log units). If the animal made four consecutive positive responses a score of 0.25 g was assigned, whereas if the animal had five consecutive negative responses then a score of 15.0 g was assigned.

Behavioral results were analyzed using a one way ANOVA with drug as the between group variable. Planned comparisons were used to compare the groups that received gabapentin (positive control), morphine, and Syn 1020 with the vehicle control group since it was hypothesized that these groups would produce a decrease in allodynia relative to controls. The modified Bonferroni Test was used for these planned comparisons (P value was set at $P<.05$ since we planned to compare the four drug groups to the vehicle control group).

3. Results

3.1. Effects of i.c.v. injections of Syn 1020 on tail-flick latency in mice

i.c.v. injections of Syn 1020 did not produce any analgesic activity (Fig. 1). The overall ANOVA indicated a significant main effect of the repeated measure post-injection time [$F(1,76)=24.3$, $P<.05$] indicating that regardless of whether the animals received an injection of Syn 1020 or vehicle, a decrease in %MPE was observed (Student Newman–Keuls, $P<.05$). The positive control morphine alone (10 nmol) produced a significant increase in %MPE relative to vehicle controls [$F(1,46)=45$, $P<.0001$] which was evident at both 10 and 20 min postinjection (Fig. 2, leftmost symbols).

As seen in Fig. 2A, N/OFQ attenuated morphine-induced antinociception. The overall ANOVA indicated a main effect of N/OFQ dose [$F(4,61)=8.047$, $P<.0001$], where the 3.0–10.0 nmol doses of N/OFQ decreased morphine-induced antinociception (Student Newman–Keuls, $P<0.05$). Similarly, Syn 1020 co-administered with morphine, produced a dose-dependent attenuation in morphine-induced antinociception to the same extent as N/OFQ (Fig. 2B). The overall ANOVA indicated a significant

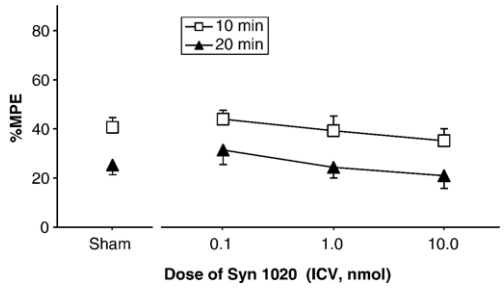

Fig. 1. Syn 1020 (0.1–10 nmol) did not have any analgesic effects in the tail-flick model of acute pain in mice 10 and 20 min after i.c.v. injection. Data are mean %MPE (±S.E.M.).

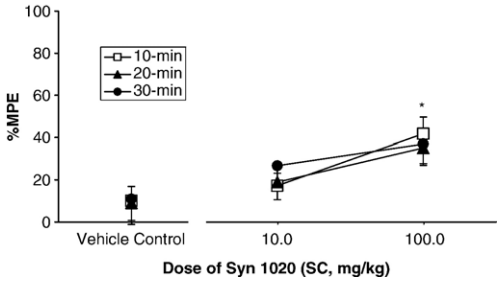

Fig. 3. Syn 1020 (10–100 mg/kg) produced an increase in tail-flick latency in mice 10, 20, and 30 min after s.c. injections. Data are mean %MPE (±S.E.M.). Asterisks represent significant differences from vehicle controls collapsed across both time points (Student Newman–Keuls test, $P<0.05$).

effect of dose [$F(3,82)=21.85$, $P<.0001$]. The two highest doses of Syn 1020 significantly attenuated morphine-induced antinociception at the 10- and 20-min time points (Student Newman–Keuls, $P<.05$). The 1.0 nmol dose of Syn 1020 attenuated morphine-induced %MPE by 18–25%, whereas the 10.0 nmol dose of Syn 1020 attenuated morphine-induced %MPE by 43–54% (Student Newman–Keuls, $P<.05$). The levels for %MPE produced by co-administration of 10.0 nmol Syn 1020 and morphine were similar to those observed in vehicle controls (see leftmost symbols, Fig. 1).

3.2. Effects of s.c. injections of Syn 1020 on tail-flick latency in mice

The effects of Syn 1020 (s.c.) on tail-flick latency are shown in Fig. 3. The overall ANOVA revealed a significant main effect of dose [$F(2,27)=3.57$, $P<.05$]. Only the 100 mg/kg dose of Syn 1020 exhibited analgesic effects that were evident at the first 10-min time point postinjection and was still evident after 30-min (Student Newman–Keuls, $P<.05$). This high dose of Syn 1020 increased tail-flick latency by a 2.5-fold difference from vehicle controls. Animals that received morphine exhibited an increase in %MPE that was 6-fold higher than that observed with vehicle controls (data not shown).

Syn 1020 administered s.c. attenuated morphine-induced increase in tail-flick latency in a dose-dependent manner (Fig. 4). The overall ANOVA revealed a significant main effect of dose [$F(3,31)=7.14$, $P<.001$]. *Post-hocs* revealed that although there was a trend for the 30 mg/kg dose to inhibit morphine-induced antinociception (reduction by 20%) at the 10-min time point, only the 100 mg/kg dose significantly inhibited morphine's effects by 32% at the 10-min time point, 35% at the 20-min time point, and by 41% at the 30-min time point (Student Newman–Keuls, $P<0.05$).

3.3. Effects of i.p. injections of Syn 1020 on tactile allodynia in rats in the diabetic neuropathy model

One month following streptozotocin injections, animals that were diabetic had blood sugar levels at 516.5±13.2 mg/dl, whereas age-matched controls that received streptozotocin vehicle had blood sugar levels at 111.8±2.1 mg/dl. The weights for the diabetic animals were 76% of vehicle controls (313 g±6.8

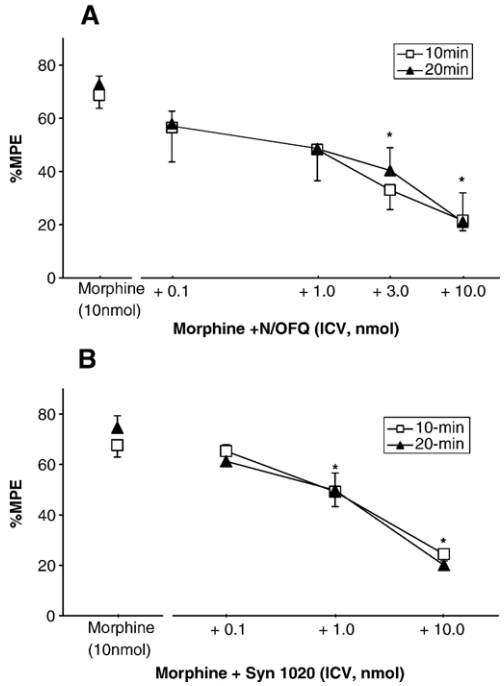

Fig. 2. I.c.v. injections of the N/OFQ (A) and Syn 1020 (B) dose-dependently reversed antinociception induced by morphine (10 nmol) at the 10 and 20 min time points. Data are mean %MPE (±S.E.M.). Asterisks represent significant differences from morphine alone collapsed across both time points (Student Newman–Keuls test, $P<0.05$).

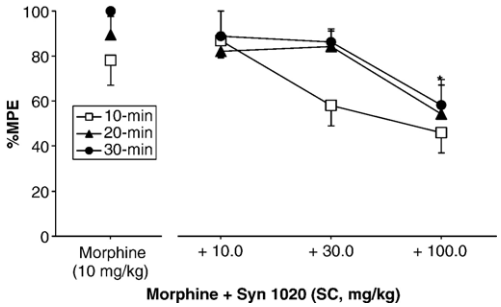

Fig. 4. Syn 1020 (100 mg/kg) dose-dependently reversed antinociception induced by morphine (10 mg/kg) 10, 20, and 30 min after s.c. injections. Data are mean %MPE (±S.E.M.). Asterisks represent significant differences from morphine alone collapsed across all time points (Student Newman–Keuls test, $P<0.05$).

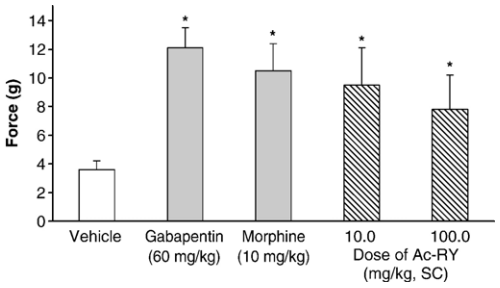

Fig. 5. Syn 1020 (10 and 100 mg/kg) produced anti-allodynic effects in the diabetic neuropathy model in rats, similar to that observed in rats that received gabapentin, 1 h after i.p. injections. Data are mean Paw Withdrawal Threshold. Asterisks represent significant differences from vehicle control (Bonferroni test, $P<0.05$).

versus 412.2 g±9.5). Diabetic animals did not tend to themselves relative to controls and looked pale with ruffled fur.

The effects of Syn 1020 on tactile allodynia are shown in Fig. 5. The one-way ANOVA indicated a significant effect of dose [$F(4,34)=4.11$, $P<.001$]. As expected, the positive control, Gabapentin, reversed allodynia 60 min following administration, as evidenced by the increase in response threshold relative to control animals (Bonferroni Test, $P<.05$). The μ-opioid receptor agonist morphine also produced a robust anti-allodynic effect (Bonferroni Test, $P<.05$). Similar to morphine, both 10 mg/kg and 100 mg/kg Syn 1020 had anti-allodynic effects and produced a significant increase in response threshold relative to controls (Bonferroni Test, $P<.05$).

4. Discussion

Syn 1020 was identified as a high affinity ligand at NOP receptors (Judd et al., 2004). *In vitro*, Syn 1020 has high intrinsic activity at NOP receptors with respect to stimulation of [^{35}S]GTPγS, whereas, it acted as an NOP receptor antagonist in electrically stimulated mouse *vas deferens* by blocking N/OFQ-mediated inhibition of electrically induced contractions of the smooth muscle (Judd et al., 2004). In the present study, Syn 1020 attenuated morphine-induced antinociception following both i.c.v. and s.c. administration in mice, whereas alone it produced mild anti-nociceptive effects when administered s.c. but not following i.c.v. administration. Furthermore, in rats, anti-allodynic effects of Syn 1020 were evident. Taken together, the data indicates that this NOP-directed peptide is not only active supraspinally but can produce peripheral and/or potentially centrally mediated effects when administered systemically *via* the s.c. and i.p. routes of administration in both mice and rats using different models of nociception.

In the present study, i.c.v. injections of Syn 1020 alone did not have any pro- or anti-nociceptive effects. Although the i.c.v. injection itself causes a small amount of stress-induced analgesia, it is unlikely this masks a modest anti-nociceptive effect of i.c.v. injected Syn 1020. In fact, Syn 1020 attenuated morphine antinociception with a similar potency to N/OFQ itself. These latter findings are similar to what has been previously reported with N/OFQ analogues discussed below. Previous studies have examined the effects of N/OFQ fragments and analogues. i.c.v. injections of N/OFQ-NH_2 and N/OFQ(1-13)NH_2 both have hypoalgesic effects and can inhibit morphine-induced antinociception in mice, whereas N/OFQ (1-9)NH_2 is not active in acute pain assays (Calo et al., 1998). Structural modifications made to the N/OFQ peptide to increase binding affinity to the receptor and increase resistance to enzymatic degradation resulted in the agonists [Arg(14), Lys (15)] N/OFQ and [(pF)Phe(4)] N/OFQ(1-13)NH_2. These two compounds behaved as potent NOP receptor agonists at human recombinant NOP receptors and in the mouse *vas deferens in vitro* and produced hypoalgesia and anti-morphine effects *in vivo* that were longer lasting relative to N/OFQ (Bigoni et al., 2002; Rizzi et al., 2002b,c). The hexapeptide Ac-RYYRIK-NH_2 and its analog Ac-RYYRIK-ol both are partial agonists *in vitro*, but both exhibit full agonist activity when tested *in vivo* (Berger et al., 2000; Gunduz et al., 2006; Janik et al., 2003). Similar to N/OFQ, Ac-RYYRIK-ol had pro-nociceptive effects when administered ICV, but anti-nociceptive effects when given i.t (Gunduz et al., 2006).

Although [Arg(14), Lys(15)] N/OFQ and [(pF)Phe(4)] N/OFQ(1-13)NH_2 are potent NOP receptor agonists with long half-lives, they are unable to penetrate into the central nervous system due to their large size. This led to the development of smaller peptide and non-peptide molecules. The partial agonist Ac-RYYRIK-NH_2 developed from a combinatorial library was just as potent as N/OFQ in inhibiting spontaneous locomotor activity (Berger et al., 2000; Dooley et al., 1997). ZP120, derived from the hexapeptide Ac-RYYRIK-NH_2 also inhibited spontaneous locomotor activity and produced hypoalgesic effects when administered i.c.v. where it was 10-fold more potent than N/OFQ, although it had no activity when it was administered i.v. (Rizzi et al., 2002a). Non-peptide small molecule agonists including Ro64-6198 and NNC 63-0532 possess anxiolytic activity but no analgesic activity when administered systemically (for recent review see Zaveri, 2003). Although the non-peptide small molecules can penetrate into the central nervous system with ease, in general, they lack the high selectivity for the NOP receptor relative to the other opioid receptors (Zaveri, 2003).

When Syn 1020 was administered systemically, it produced mild analgesic effects in the tail-flick assay relative to vehicle controls. Experiments examining systemic effects of N/OFQ have not been assessed because it is a larger peptide, has limited site of action, and is quickly degraded. Peripheral effects of N/OFQ have been reported, however, following local application only. For example, local injections of N/OFQ in the tail vein of the mouse have shown that it is analgesic for a short duration using the tail-flick assay with peak activity at 5 min (Kolesnikov and Pasternak, 1999). In the present study, analgesia was seen at 100 mg/kg Syn 1020, and Syn 1020 produced an increase in tail-flick latency, relative to controls, that was evident throughout the 30-min test period. However, the level of analgesia produced by Syn 1020 was much lower than what was observed with 10 mg/kg morphine. These findings are in concordance with what has been reported following both local peripheral and i.t. injections of N/OFQ (Kolesnikov and Pasternak, 1999; Tian et al., 1997). It is possible that systemic

administration of Syn 1020 produced its effects by acting at both peripheral and also at central (at the level of the spinal cord) sites of nociception.

When Syn 1020 was co-administered with morphine using the s.c. route of administration, it attenuated morphine-induced antinociception. Given that Syn 1020 induces a mild anti-nociceptive response and yet attenuates the actions of a higher efficacy compound, these findings suggest that Syn 1020 is acting as a partial agonist. Partial agonists because of their low intrinsic activity, may act predominantly as antagonists under conditions of high receptor tone and as weak agonists under conditions of low receptor tone (*i.e.*, Ariens, 1983). Thus, it is possible, that the activity of Syn 1020 is modulated by the level of μ-opioid receptor tone such that under basal conditions, where the stimulation of μ-opioid receptors is low, Syn 1020 functions as an agonist and has analgesic effects, whereas when co-administered with the prototypic μ-opioid receptor agonist morphine, there is high μ-opioid receptor tone and Syn 1020 may act as an antagonist to block morphine antinociception.

Systemically administering Syn 1020 into rats produced anti-allodynic effects that mimicked the effects observed by gabapentin. The effects of NOP ligands alone in chronic neuropathic pain have been examined. Spinal injections of N/OFQ also produce anti-allodynic effects in rats following chronic constriction injury and this effect can be blocked by the NOP receptor antagonist [Nphe]N/OFQ(1-13)NH_2 (Courteix et al., 2004; Hao et al., 1998; Yamamoto et al., 1997). A recent study has also reported that intraplantar and i.t. injections, but not s.c. administration, of Ro 64-6198 were able to attenuate tactile and thermal allodynia in a neuropathic pain model in rats (Obara et al., 2005). Ro 64-6198 was less potent relative to N/OFQ after i.t. injections but similar when injected intraplantarly and was reversed by NOP receptor antagonists. Interestingly, in normal rats and mice, N/OFQ induces allodynia when injected i.t. at very low (fmol–pmol) doses, but not at higher (nmol) doses (Hara et al., 1997; Okuda-Ashitaka et al., 1996). The allodynic actions of N/OFQ may be due to N/OFQ-induced substance P release from primary afferents, which only occurs at very low doses (Inoue et al., 1998). The anti-allodynic actions that we see are consistent with both the i.t. administration of NOP antagonists, and the phenotype found for NOP and preproN/OFQ knockout mice, which display increased nociceptive responses to prolonged stimuli such as formalin (Depner et al., 2003; Rizzi et al., 2006). The findings that NOP receptors in the spinal cord or the dorsal root ganglia are upregulated in chronic pain states (Andoh et al., 1997; Briscini et al., 2002) and that injections of N/OFQ and Syn 1020 can produce anti-allodynia following induction of neuropathy indicates that NOP receptors may be an important receptor system to consider for development of more effective treatment of neuropathic pain.

In summary, Syn 1020 was an active agonist when administered i.c.v. and had a similar potency as N/OFQ. When Syn 1020 was administered systemically it functioned as a partial agonist potentially by activating receptors peripherally as well as at the level of the spinal cord. Lastly, Syn 1020 possessed anti-allodynic activity, when administered i.p. in a model of neuropathic pain in rats suggesting that the NOP system may be an important target for chronic pain.

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