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# Selectivity profiling of NOP, MOP, DOP and KOP receptor antagonists in the rat spinal nerve ligation model of mononeuropathic pain

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ABSTRACT

Agonists selectively acting at NOP, MOP, DOP and KOP receptors as well as mixed opioid receptor agonists are known to exert anti-hypersensitive efficacy in the rat spinal nerve ligation (SNL) model of neuropathic pain. To investigate the relative contribution of individual opioid receptor activation to the overall efficacy of mixed opioid receptor agonists, selective doses of respective opioid receptor antagonists have to be employed. In order to identify such selective antagonist doses, doses of the selective NOP, MOP, DOP and KOP receptor agonists Ro65–6570, morphine, SNC-80 and U50488H, that produced maximum efficacy without apparent side effects, were challenged by each of the receptor antagonists J-113397 (NOP receptor), naloxone (MOP receptor), naltrindole (DOP receptor) and nor-binaltorphimine (KOP receptor). J-113397, naloxone, naltrindole and nor-binaltorphimine at intraperitoneal doses of 4.64, 1, 10, and 10 mg/kg, respectively, inhibited anti-hypersensitive effects mediated by the corresponding cognate NOP, MOP, DOP and KOP receptor selective agonists. Selectivity could be demonstrated for MOP, DOP and NOP receptor antagonists, as they did not attenuate effects mediated by agonists acting on non-cognate receptors, whereas the KOP receptor antagonist nor-BNI demonstrated partial cross-antagonism of the DOP receptor agonist SNC-80. Thus, specific doses of opioid receptor antagonists that completely but still selectively attenuate full anti-hypersensitive efficacy of corresponding opioid receptor agonists were identified in the rat SNL model.

## 1. Introduction

Opioids remain an important option in the treatment of pain. Agonists of all three classical opioid receptors (mu opioid peptide [MOP], delta opioid peptide [DOP], kappa opioid peptide [KOP]) as well as of the nociceptin/orphanin FQ peptide (NOP) receptor have been shown to produce anti-nociceptive and/or anti-hypersensitive/anti-hyperalgesic effects in a variety of different animal pain models (e.g. Bie and Pan, 2007; Khroyan et al., 2009; Pradhan et al., 2012; Schröder et al., 2014). In humans, among the selective opioid receptor agonists, only MOP receptor ligands are used for the treatment of pain. However, a number of compounds with a mixed opioid receptor profile is also in development (for review see: Bird and Lambert, 2015). In fact, mixed opioid receptor ligands may hold additional therapeutic potential, since in preclinical pain models selective opioid receptor agonists have been shown to interact synergistically. For example, synergistic interactions have been described between MOP-DOP (Negus et al., 2009; Rossi et al., 1994; Sutters et al., 1990), MOP-NOP (Courteix et al., 2004), MOP-KOP (Sutters et al., 1990) and KOP-DOP (Miaskowski et al., 1990) receptor agonists.

For the development of mixed opioid receptor agonists it is important to determine whether and to what degree the different receptor activities (as shown in vitro) contribute to the overall effect. Two principle tools for this characterization do exist, receptor knockout mice and receptor-selective antagonists. Both approaches however, have their shortcomings.

Although knockout mice are generally regarded as very valuable tools, caution is warranted regarding their pharmacological specificity. In a comprehensive pharmacogenomic study, our group has previously shown (Rutten et al., 2014) that there are marked interdependencies between the different opioid receptors such that knockout of one opioid receptor type also affected the response to agonists for other opioid receptor types in a mouse model of diabetic hyperalgesia (Rutten et al., 2014).

The alternative approach to the use of knockout mice is the use of selective pharmacological tools, i.e. receptor-specific antagonists. Antagonists for each of the opioid receptors exist. However, none of these compounds shows a complete selectivity for its respective target

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receptor. In particular at higher doses/exposures, these antagonists may also hit the other, non-cognate opioid receptors.

Therefore, the aim of the present study was to establish receptor-selective doses of the NOP receptor antagonist J-113397 (Kawamoto et al., 1999; Ozaki et al., 2000), the MOP receptor antagonist naloxone, the DOP receptor antagonist naltrindole, and the KOP receptor antagonist nor-binaltorphimine (nor-BNI) in the rat spinal nerve ligation (SNL) model of mononeuropathic pain. To this end, in a first step, different doses of each antagonist were tested against a maximally efficacious dose of the corresponding reference agonist (Ro65–6570 for the NOP receptor, morphine for the MOP receptor, SNC-80 for the DOP receptor, and U50488H for the KOP receptor; doses derived from previous pilot studies) to determine the antagonist dose needed to fully block the agonist effect. In a second step, it was then tested whether the antagonist dose determined in step one would interfere with the anti-hypersensitive effects of the non-cognate agonists.

## 2. Material and methods

### 2.1. Animals

A total of 609 male Sprague Dawley rats (140–160 g body weight) from a commercial breeder (Janvier, Genest St. Isle, France) were used. The animals were housed under standardized conditions: light/dark cycle (06.00–18.00 h light, 18.00-06.00 h dark), room temperature 20–24 °C, relative air humidity 35–70%, 15 air changes per hour, air movement < 0.2 m/sec, tap water and standard diet ad libitum, macrolon type 4 cages with 5 animals per cage. There were at least 5 days between delivery and start of the experiment. All efforts were made to reduce the number of animals used. Therefore, animals were repeatedly tested between the first and fifth week after surgery, with a maximum of three tests and a washout phase of at least one week between tests. Animal testing was performed in accordance with the recommendations and policies of the International Association for the Study of Pain (Zimmermann, 1983) and the German Animal Welfare Law. All study protocols were approved by the local government committee for animal research, which is advised by an independent ethics committee. Animals were assigned randomly to treatment groups. Different doses and vehicles were tested in a randomized fashion. Although the operators performing the behavioral tests were not formally 'blinded' with respect to the treatment, they were not aware of the study hypothesis or the nature of differences between drugs. At the end of the experiment animals were killed by slow infusion of $CO_2$ in an enclosed anesthesia chamber (flowrate: 10–20% of the cage volume/min).

### 2.2. Drugs

The following drugs were used: J-113397 (*CAS no.: 2177461-40-0;* Grünenthal GmbH, Aachen, Germany), morphine HCl (*CAS no.: 52-26-6;* Merck AG, Darmstadt, Germany), morphine sulfate (*CAS no.: 6211-15-0;* Baxter, Cherry Hill, NJ, USA), sodium pentobarbital (*CAS no.: 57-33-0;* Narcoren®), naloxone HCl (*CAS no.: 51481-60-8;* Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), naltrindole HCl (*CAS no.: 111469-81-9*;Tocris), nor-Binaltorphimine (nor-BNI) (*CAS no.: 105618-26-6*; Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), Ro65–6570 (*Grünenthal GmbH*, Aachen, Germany), SNC-80 (*CAS no.: 156727-74-1*; Enzo Life Sciences GmbH, Lörrach, Germany), and U50488H (*CAS no.:114528-79–9*; Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). The following chemicals were used: cremophor EL, DMSO, 5% glucose (Sigma-Aldrich Co., St Louis, MO, USA; Sigma-Aldrich Chemie GmbH, Munich, Germany), saline (Baxter, Cherry Hill, NJ, USA; Baxter, Unterschleißheim, Germany). Ro65–6570 was dissolved in 10% Cremophor EL/0.9% NaCl. J-113397, morphine HCl, naloxone HCl, naltrindole HCl, nor-BNI, SNC-80 and U50488H were dissolved in 0.9% NaCl. Administration volume was 5 ml/kg for intravenous (i.v.) and intraperitoneal (i.p.) and 2 ml/kg for subcutaneous (s.c.) administration, respectively.

### 2.3. Spinal nerve ligation model of mononeuropathic pain

Under pentobarbital anesthesia (Narcoren® 60 mg/kg i.p.; Merial GmbH, Hallbergmoos, Germany), the L5/L6 spinal nerves were tightly ligated according to the method by Kim and Chung (Kim and Chung, 1992). The left L5 and L6 spinal nerves were exposed by removing a small piece of the paravertebral muscle and a part of the left spinous process of the L5 lumbar vertebra. The L5 and L6 spinal nerves were then carefully isolated and tightly ligated with silk (NC-silk black, USP 5/0, metric 1, Braun Melsungen AG, Melsungen, FRG). After checking hemostasis, the muscle and the adjacent fascia were closed with sutures and the skin was closed with sutures. After surgery, the animals were allowed to recover for 1 week. Animals developed tactile hypersensitivity which was stable for at least five weeks and animals were repeatedly tested (maximally 3 times) with a washout period of at least one week, animals that did not develop tactile hypersensitivity were exluded from the experiments (5–10%). For the assessment of tactile hypersensitivity the rats were placed on a metal mesh covered with a plastic dome and were allowed to habituate until the exploratory behavior diminished.

The threshold for tactile hypersensitivity was measured with an electronic von Frey (EVF) anesthesiometer (Somedic, Malmö, Sweden). Animals randomly assigned to groups of 9–10 for each test dose and vehicle, were tested 0.5 h before administration and on several time points after i.v., s.c. and i.p. administration. The median withdrawal threshold for each animal at a given time was calculated from five individual stimulations with the EVF filament. Withdrawal thresholds of the ipsilateral paw are expressed as % MPE by comparing the BL threshold of the L5/L6-ligated animals (0% MPE) and the control threshold of the sham animals (= 100% MPE). A cut off was set at 100% MPE: values above 100% were considered as 100%.

The effect of each compound and vehicle is calculated (n = 10 per group) for each testing time point (i.e. 30, 60 and 180 min post administration for morphine, SNC-80 and U50488H and 10, 30 and 50 min post administration for Ro65–6570 as interindividual % MPE value.

### 2.4. Testing procedures

#### 2.4.1. Test for full antagonism

Different doses of each antagonist were tested against a maximally efficacious dose of the corresponding reference agonist until a full antagonism was observed. As such, Ro65–6570 (veh or 0.215 mg/kg i.v.) was combined with J-113397 (veh or 0.215 mg/kg i.p.) and J-113397 (veh or 4.64 mg/kg i.p.). Morphine (veh or 10 mg/kg i.v.) was combined with naloxone (veh or 0.1 mg/kg i.p.), naloxone (veh or 0.3 mg/kg i.p.), and naloxone (veh or 1 mg/kg i.p.). SNC-80 (veh or 10 mg/kg s.c.) was combined with naltindole (veh or 0.1 mg/kg i.p.), naltindole (veh or 0.3 mg/kg i.p.), and naltindole (veh or 1 mg/kg i.p.). And U50488H (veh or 10 mg/kg i.v.) was combined with nor-BNI (veh or 1 mg/kg i.p.), nor-BNI (veh or 3.16 mg/kg i.p.), and nor-BNI (veh or 10 mg/kg i.p.).

#### 2.4.2. Test for selectivity/ cross-specificity

Full antagonistic doses (see above) were tested in comparison to their other non-cognate agonists. As such, the antagonists, J-113397 (4.64 mg/kg i.p.), naloxone (1 mg/kg i.p.), naltrindole (10 mg/kg i.p.), nor-BNI (3.16 mg/kg i.p.) or vehicle were administered 5 min before the selective NOP, MOP, DOP and KOP receptor agonists Ro65–6570 (0.215 mg/kg i.v.), morphine (10 mg/kg i.v.), SNC-80 (10 mg/kg s.c.), and U50488H (10 mg/kg i.v.), respectively or vehicle, except in the case of Ro65–6570, where antagonists were administered 25 min before administration of the agonist.