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Antinociceptive, antiallodynic and antihyperalgesic effects of the 5-HT_{1A} receptor selective agonist, NLX-112 in mouse models of pain

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ABSTRACT

Background and purpose:: NLX-112 (a.k.a. befiradol, F13640) is a drug candidate intended for the treatment of L-DOPA-induced dyskinesia. It is a highly selective serotonin 5-HT_{1A} receptor full agonist which has been previously tested in a variety of models of CNS effects including analgesic activity in rat. Its activity in mouse models of pain has not been previously investigated.
Experimental approach: The activity of NLX-112 was tested in mouse models of acute pain (hot plate), tonic pain (intraplantar formalin test), in the oxaliplatin-induced neuropathic pain model of chemotherapy-induced peripheral neuropathy and in the streptozotocin (STZ)-induced model of painful diabetic neuropathy.
Key results: The main findings indicate that (i) NLX-112 was markedly active in the formalin test with potent reduction of paw licking in both phases of the test (minimal effective dose (MED) 0.5 mg/kg i.p. and p.o. in acute phase, and 0.1 mg/kg i.p. and 1 mg/kg p.o. in late phase). The effects of NLX-112 in this test were completely abolished by the selective 5-HT_{1A} receptor antagonist, WAY100635; (ii) NLX-112 was active in the hot plate test and in the oxaliplatin-induced neuropathic pain model of chemotherapy-induced peripheral neuropathy, but at markedly higher doses (MED 2.5 mg/kg i.p.); (iii) NLX-112 was least active in the STZ-induced model of painful diabetic neuropathy (MED 5 mg/kg i.p.); (iv) NLX-112 did not affect locomotor activity.
Conclusions and implications: NLX-112 may have significant potential for treatment of tonic pain but may be less promising as a candidate for treatment of chemotherapy-induced or diabetic neuropathic pain.

1. Introduction

Although painful conditions, including acute, tonic and neuropathic pains, constitute a major public health challenge and a large market opportunity, there remain large unmet medical needs for improved therapeutic options. For example, although morphine is often indicated for the relief of severe pain (e.g. post-operatively, in myocardial infarction, severe injury or in severe chronic pain associated with terminal cancer after non-narcotic analgesics have failed) it is associated with substantial limitations, including psychological and physical tolerance and dependence, respiratory depression, lethargy, euphoria and a short plasma half-life of 2–3 h (Trescot et al., 2008).

In the case of neuropathic pain, which affects between 3 and 8% of the world's population, tricyclic antidepressants, serotonin-noradrenaline reuptake inhibitors, pregabalin, gabapentin and tramadol, as well as lidocaine or high-concentration capsaicin patches, often have undesirable and dose-limiting adverse effects which makes pharmacotherapy of neuropathic pain even more challenging, and are ineffective in approximately 40% of neuropathic patients (Finnerup et al., 2005; Gagnon et al., 2003; Gilron et al., 2009).

There is, therefore, a strong medical need to explore novel drug targets and identify new, active analgesic compounds (Brederson et al., 2013). In this context, serotonergic mechanisms have been implicated in the control of pain states and specifically, 5-HT_{1A} receptors are attractive candidates because they are expressed

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throughout the pain neuroaxis. For example, 5-HT_{1A} receptors are expressed at high levels in the dorsal horn of the spinal cord (Daval et al., 1987), both centrally, where they regulate 5-HT release and inhibit descending serotonergic neurons, as well as on spinal cord spinothalamic neurons and on inhibitory interneurons where they regulate transmission of nociceptive signals (Bardin, 2011; Perrin et al., 2011). The prototypical 5-HT_{1A} receptor agonist, such as 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) influenced spinal-cord regulated pain responses, but with discordant results. Thus, intrathecal administration of 8-OH-DPAT was found to increase the A-delta and C-fiber responses and decrease tail-flick latency (Alhaider and Wilcox, 1993; Ali et al., 1994; Zhang et al., 2001), whereas other studies found that spinal administration of 5-HT_{1A} receptor agonists induced analgesia (Bardin and Colpaert, 2004; Nadeson and Goodchild, 2002; Xu et al., 1994). An important consideration is that 5-HT_{1A} receptor agonists may differentially influence pain responses depending on the type of nociceptive stimulus: when administered intrathecally, 8-OH-DPAT exhibited pronociceptive activity in the paw pressure test but analgesic activity in the formalin test (Bardin and Colpaert, 2004), for review see Bardin (2011).

Taken together, these considerations suggest that 5-HT_{1A} receptor agonists could constitute useful therapeutic agents, if their mechanisms could be better elucidated and if suitably selective and efficacious compounds were available. Indeed, investigation of the relevance of such findings to human subjects has been hampered by the lack of clinically-available serotonin 5-HT_{1A} agonists. The only approved drugs that possess 5-HT_{1A} receptor agonist properties are non-selective partial agonists such as buspirone (which also acts as a dopamine D_2 receptor antagonist (Peroutka, 1985)), antidepressants such as vilazodone and vortioxetine (which also inhibit 5-HT reuptake and interact with various other receptors) or some antipsychotics such as aripiprazole or cariprazine, which are 'promiscuous' drugs interacting with multiple targets (Newman-Tancredi and Kleven, 2011).

In contrast to these drugs, the novel, serotonergic ligand, NLX-112 (a.k.a. befiradol or F13640) exhibits a different profile. NLX-112 specifically targets 5-HT_{1A} receptors, with a 'full agonist' activity, and exhibits no measurable affinity at a wide range of other receptors, binding sites or enzymes (Colpaert et al., 2002). The properties of NLX-112 have previously been investigated in a variety of rat models of pain with pronounced activity in the formalin test (Bardin et al., 2003), in models of intraoperative and postoperative pains (Kiss et al., 2005) and in models of chronic pain, such as ischemic injury of spinal cord or ligature of the infraorbital nerve (Deseure et al., 2002, 2004; Wu et al., 2003). These data suggest that NLX-112 may be a promising pharmacotherapeutic agent for treatment of a variety of chronic pain conditions. However, (i) information is not available concerning the analgesic activity of NLX-112 in other species, notably mouse, which is commonly used in preclinical testing; and (ii) no preclinical data are available characterizing the activity of NLX-112 in models of some common chronic pain conditions, such as chemotherapy-induced peripheral neuropathy (CIPN) and painful diabetic neuropathy (PDN). Lack of preclinical data supporting activity of NLX-112 in PDN is surprising because NLX-112 was previously tested in an 8-week, multicenter, randomized, double-blind and placebo-controlled Phase 2 clinical trial for this indication. The trial was completed in 2010 and it did not confirm efficacy of NLX-112 in reducing pain related to PDN (https://www.clinicaltrialsregister.eu/ctr-search/trial/2009-012123-28/FR).

The present study therefore used mouse models of pain to assess the antinociceptive, antiallodynic and antihyperalgesic properties of NLX-112 as a potential new analgesic to treat pain of various origins, including acute pain, tonic pain and neuropathic pain related to PDN and CIPN.

2. Materials and methods

2.1. Animals

Adult male Albino Swiss (CD-1) mice weighing between 18 and 22 g were used in this study. Mice were housed in groups of 10 per cage at constant ambient temperature of 22 ± 2 °C, and humidity (50 ± 10%) under a light/dark (12:12) cycle. The animals had free access to food and water before experiments. Each experimental group consisted of 8–10 animals selected randomly. The experiments were performed between 8 a.m. and 2 p.m. and immediately after completion of the assay the animals were euthanized via cervical dislocation. All procedures for animal maintenance and treatment were performed in compliance with the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes and were approved by the Local Ethics Committee of the Jagiellonian University in Krakow (39/2016).

2.2. Chemicals used in in vivo tests

NLX-112 (a gift from Neurolixis, Inc., California, US) was dissolved in 0.9% saline (Polfa Kutno, Poland). STZ and oxaliplatin were purchased from Sigma Aldrich (Poland) and (Activate Scientific GmbH, Germany), respectively. NLX-112, STZ and oxaliplatin were administered at a constant volume of 10 ml/kg. For the experiments STZ was prepared by dissolving in 0.1 N citrate buffer (Polskie Odczynniki Chemiczne, Poland). Oxaliplatin was prepared in a 5% glucose solution (Polfa Kutno, Poland). Formalin (37% formaldehyde solution) was purchased from Polskie Odczynniki Chemiczne (Poland). For pain tests, it was diluted in distilled water (Polskie Odczynniki Chemiczne, Poland) to obtain 5% solution. The selective 5-HT_{1A} receptor antagonist, WAY100635, (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide maleate, Tocris UK), was dissolved in distilled water immediately before administration in a volume of 2 ml/kg.

2.3. Streptozotocin-induced diabetic neuropathic pain model

2.3.1. Induction of diabetes and painful diabetic neuropathy

To induce type I diabetes and diabetic neuropathic pain, mice were administered streptozotocin (STZ), an alkylating antitumor drug which destroys insulin-secreting islet cells. Mice were administered a single injection of STZ (200 mg/kg i.p.). Age-matched control mice received an equal volume of citrate buffer. Blood glucose levels were measured 1 day before (referred to as 'day 0') and repeatedly 1, 2 and 3 weeks after STZ injection using a blood glucose monitoring system (AccuChek Active, Roche, France). Blood samples (5 μl) for the measurement of glucose concentration were obtained from the tail vein of the mice. The animals were considered to be diabetic when their blood glucose concentration exceeded 300 mg/dl (Tanabe et al., 2008). Approx. 70% of mice developed diabetes and were used in subsequent pain tests.

2.3.2. Assessment of tactile allodynia in STZ-treated mice – von Frey test

The electronic von Frey unit (Bioseb, France) is supplied with a single flexible filament applying increasing force (from 0 to 10 g) against the plantar surface of the hind paw. In this assay the nocifensive paw withdrawal response automatically turns off the stimulus and the mechanical pressure that evoked the response was recorded. On the day of the experiment, the mice were placed individually in test compartments with a wire mesh bottom and