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ADX71943 and ADX71441, novel positive allosteric modulators of the $GABA_B$ receptor with distinct central/peripheral profiles, show efficacy in the monosodium iodoacetate model of chronic osteoarthritis pain in the rat

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ABSTRACT

We tested novel positive allosteric modulators (PAMs) of the γ-aminobutyric acid receptor B ($GABA_B$), ADX71943 and ADX71441in the monosodium iodoacetate model of chronic osteoarthritis pain in rats with the objective to delineate the role of peripheral versus central $GABA_B$ receptor populations in modulation of chronic pain. Anesthetized Sprague-Dawley rats received an injection of monosodium iodoacetate into the knee and were tested for hyperalgesia starting post-MIA day 14. Effects of compounds on ipsilateral joint compression threshold were evaluated on post-MIA day 14 (after acute treatment), as well as after repeated, daily treatment on days 21 and 28 (ADX71943 only) and were compared to those of celecoxib (30 mg/kg, p.o.). The PAMs were also tested in the rat rotarod test for potential muscle-relaxant effects. Acutely, ADX71943 (1–30 mg/kg, p.o.), the peripherally restricted PAM, resulted in similar increases in pain threshold across the doses on day 14, while showing reduced efficacy on day 21 and no efficacy on day 28. A clear reduction in the efficacy of celecoxib across testing was also noted in this experiment. Acutely ADX71441 (0.3–15 mg/kg, p.o.), the central-peripheral PAM, resulted in over 2-fold increases in pain threshold at 15 mg/kg (but not at lower doses) on day 14, while causing more modest effects on day 21. Celecoxib increased pain threshold after both acute and daily treatment, showing overall similar efficacy. Thus, early, presumably more inflammatory phase of osteoarthritis pain in more sensitive to $GABA_B$ PAMs with peripherally restricted profile, while later, presumably more neuropathic phase is more sensitive to PAMs with central-peripheral profile.

1. Introduction

There is growing evidence that the γ-aminobutyric acid receptor B ($GABA_B$) is involved in regulation of acute and chronic pain (Enna, 2006; McCarson and Enna, 2014). Activation of the $GABA_B$ receptor with the orthosteric receptor agonist baclofen produces antinociception in a wide range of animal models of acute and persistent pain as well as in the clinic (McCarson and Enna, 2014), whereas pharmacological blockade of the receptor with an antagonist or deletion of either of its two subunits ($GABA_{B1}$ or $GABA_{B2}$) leads to hyperalgesia (Gassmann et al., 2004; Malan et al., 2002; Schuler et al., 2001).

The positive allosteric modulators (PAMs) of the $GABA_B$ receptor are an alternative tool to direct activation of the receptor. In the last decade several selective $GABA_B$ PAMs have been identified, that have no or minimal intrinsic agonist activity, but can enhance both potency and efficacy of orthosteric agonists in several in vitro assays (Guery et al., 2007; Malherbe et al., 2008; Urwyler et al., 2001). Thus far we know little about their effects in models of acute or chronic pain. According to Brusberg et al. (Brusberg et al., 2009) $GABA_B$ receptor PAM CGP7930 reduced mechanical nociception in a model of colorectal distention in the rat, albeit to a lesser degree than baclofen.

Recently we reported characterization of novel, potent and selective $GABA_B$ PAMs, ADX71943 (Kalinichev et al., 2014) and ADX71441 (Kalinichev et al., 2017). In vitro, in HEK293 cell line expressing human $GABA_B$ receptor, ADX71943 and ADX71441 enhanced an EC_{20} of GABA with an EC_{50} of 40 and 96 nM, respectively (Kalinichev et al., 2014; 2017). Both PAMs showed a good selectivity when tested at 10 μM in binding experiments against a panel of 71 targets at Cerep

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(Poitier, France), including receptors, transporters and ion channels (Kalinichev et al., 2014; 2017). ADX71943 has a peripherally restricted profile as its brain penetration is limited by interaction with P-glycoprotein (PgP; Haddouk et al. unpublished data). In vivo, ADX71943 reduces pain sensitivity in the acetic acid-induced writhing and formalin tests in rodents, while having no effect in the marble burying and elevated plus maze tests, relevant to anxiety-like reactivity (Kalinichev et al., 2014). ADX71441, the PAM with a balanced central-peripheral profile shows efficacy in the acetic acid-induced writhing test, an anxiolytic-like profile in the marble burying and elevated plus maze tests and muscle-relaxant properties in the rotarod test at higher doses (Kalinichev et al., 2017).

Here we tested ADX71943 and ADX71441 in the monosodium iodoacetate model of chronic osteoarthritis pain in the rat with the objective to delineate the role of peripheral versus central $GABA_B$ receptor populations in modulation of chronic pain. The model has a good face validity as monosodium iodoacetate-induced inflammation and degeneration of the knee joint cartilage closely mimic symptoms in human patients (Clarke et al., 1997; Pomonis et al., 2005). Here, we compared the effects of ADX71943 and ADX71441 on ipsilateral joint compression threshold after acute administration, on post-MIA day 7 as well as after daily treatment, on post-MIA days 14, 21,28 (ADX71943 only) and compared to those of a non-steroidal anti-inflammatory drug celecoxib (Mccormack, 2011).

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (250–350 g), purchased from Harlan (Indianapolis, IN, USA), were used in the study unless indicated otherwise. Upon arrival to the animal facility of Algos Preclinical Services Inc. (Roseville, MN, USA), they were group-housed (3 per cage) in type III cages (22×37×18 cm) and maintained on a 12-h light/dark cycle (lights on from 07:00 to 19:00 h) under constant temperature (22 ± 2 °C) and humidity (> 45%) conditions with food and water available *ad libitum*. Animals were acclimated for at least 5 days before experimentation. All experimental procedures and conditions were approved by the Ethical Committees of Addex Therapeutics and Algos Preclinical Services Institutional Animal Care and Use Committee. They were performed in full compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), the French National Committee (décret 87/848), The International Association for the Study of Pain (Zimmermann, 1983) and National Institutes of Health (NIH).

2.2. Induction of chronic osteoarthritis pain

Rats were anaesthetized with isoflurane before 2 mg monosodium iodoacetate (in saline) was injected in a 50 µl volume into the synovium of the left knee using a syringe with a 27 gauge needle inserted through the patellar tendon.

2.3. Primary mechanical hyperalgesia testing

Mechanical hyperalgesia was evaluated using a digital Randall-Selitto device (IITC Life Sciences, Woodland Hills, CA, USA) by investigators that were blind to treatment group assignment. Animals were acclimated to the testing room for a minimum of 15 min, after which they were suspended with the aid of a restraint sling, leaving the hind limbs available for testing. The joint compression threshold was measured once at each time point for the ipsilateral and contralateral knee joints. Pressure was applied gradually over approximately 10 s to the medial and lateral parts of the knee joint. Measurements were taken from the first observed nocifensive behavior, including vocalization, struggle or withdrawal. A cut-off value of 500 g was used to prevent injury to the animal.

2.4. Selection of doses of ADX71943 and ADX71441

The doses of ADX71943 and ADX71441 were selected based on a series of experiments performed in our laboratory. Our goal was to obtain a full range of responses with both compounds. As in the experiment involving ADX71943, a full effect was seen at the lowest dose (1 mg/kg), in the experiment involving ADX71441 a lower dose range was used.

2.5. Effects of ADX71943 on primary mechanical hyperalgesia

Rats (n=10/group) were tested for primary mechanical hyperalgesia before monosodium iodoacetate administration (day −1) and on post-administration days 14, 21, and 28. On each day tests were performed at baseline, as well as 1 and 2 h after animals were treated orally, via gavage (p.o.) with vehicle (plasdone), ADX71943 (1, 3, 10, 30 mg/kg), or celecoxib (30 mg/kg), which was suspended in 1% carboxymethyl cellulose (CMC). The once-daily treatment regimen continued for 14 days (days 14–28). Blood was collected via tail vein from all experimental animals at the end of behavioural assessment (approximately 2 h following treatment) on days 14 and 21. On day 28, following behavioural testing (approximately 2 h following treatment), terminal brain and blood samples were collected from a subset of experimental animals (n=4/group). Concentrations of ADX71943 in the brain and plasma were analysed as described below.

2.6. Effects of ADX71441 on primary mechanical hyperalgesia

Rats (n=10/group) were tested for primary mechanical hyperalgesia before monosodium iodoacetate administration (day −1) and on post-administration days 14 and 21. On each day, tests were performed at baseline as well as 1, 2, and 4 h after p.o. treatment with vehicle (1% CMC), ADX71441 (0.3, 1, 3, 15 mg/kg) or celecoxib (30 mg/kg) administered. The once-daily treatment regimen continued for 7 days (days 14–21). Blood was collected via the tail vein from a subset of ADX71441-treated animals (n=4/group) at the end of behavioural assessment (approximately 4 h following treatment) on day 14 and again, as terminal samples from the same animals, on day 21. Plasma was analysed as described for the pharmacokinetic studies.

2.7. Rotarod

Male Sprague-Dawley rats (250–350*g*) were purchased from Charles River (L'Arbresle, France) and maintained in the animal facility of Addex Therapeutics under standard laboratory conditions. A rat rotarod apparatus (Ugo Basile, Camerio, Italy) with constant speed of 15 rotations per minute was used in these experiments. Rats were given 6 training sessions (180 s each), with an intersession interval of 4–10 min, the day before the experiment. Those animals that were able to stay on the rod without falling for the entire session in at least 3 out of the 6 sessions were selected for the study. On the testing day, rats (n=10/group) were treated p.o. with vehicle (plasdone), ADX71943 (10, 30, 100 mg/kg) or baclofen (1, 3, 6 mg/kg) 60 min before being placed on the rod. Animals were tested on the rotarod in three sessions performed 1, 2, and 4 h following treatment (cut-off time 180 s). In a second experiment, rats (n=10/group) were treated p.o. with vehicle (1% CMC), ADX71441 (1, 3, 10 mg/kg) or baclofen (10 mg/kg). Animals were tested on the rotarod 1, 2, and 3 h following treatment, again with a cut-off of 180 s.

2.8. Data analysis

The ipsilateral and contralateral joint compression threshold responses (s) at baseline and following vehicle treatment were analysed