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Spinal activation of the NPY Y1 receptor reduces mechanical and cold allodynia in rats with chronic constriction injury

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ABSTRACT

Neuropeptide tyrosine (NPY) and its associated receptors Y1R and Y2R have been previously implicated in the spinal modulation of neuropathic pain induced by total or partial sectioning of the sciatic nerve. However, their role in chronic constrictive injuries of the sciatic nerve has not yet been described. In the present study, we analyzed the consequences of pharmacological activation of spinal Y1R, by using the specific Y1R agonist $Leu^{31}Pro^{34}$-NPY, in rats with chronic constriction injury (CCI). CCI and sham-injury rats were implanted with a permanent intrathecal catheter (at day 7 after injury), and their response to the administration of different doses (2.5, 5, 7, 10 or 20 µg) of $Leu^{31}Pro^{34}$-NPY (at a volume of 10 µl) through the implanted catheter, recorded 14 days after injury. Mechanical allodynia was tested by means of the up-and-down method, using von Frey filaments. Cold allodynia was tested by application of an acetone drop to the affected hindpaw. Intrathecal $Leu^{31}Pro^{34}$-NPY induced an increase of mechanical thresholds in rats with CCI, starting at doses of 5 µg and becoming stronger with higher doses. Intrathecal $Leu^{31}Pro^{34}$ also resulted in reductions in the frequency of withdrawal to cold stimuli, although the effect was somewhat more moderate and mostly observed for doses of 7 µg and higher. We thus show that spinal activation of the Y1R is able to reduce neuropathic pain due to a chronic constrictive injury and, together with other studies, support the use of a spinal Y1R agonist as a therapeutic agent against chronic pain induced by peripheral neuropathy.

1. Introduction

Neuropathic pain induced by peripheral nerve injury is a serious public health concern [1,2]. Patients undergoing neuropathic pain typically suffer allodynia (pain induced by normally innocuous stimuli), hyperalgesia (an exaggerated response to painful stimuli), and paresthesia (tingling, tickling, pricking, numbness or burning sensations) [2–4]. In addition, neuropathic pain patients also manifest progressive alterations in quality of life, including severe depression, alterations of sleep, eating and memory, and functional limitations [5–7]. Unfortunately, and despite the existence of a number of analgesic drugs available against neuropathic pain, a large percentage remains refractory to treatment [2,8,9]. Moreover, most of these drugs also cause some type of adverse effect, limiting their use in high doses or for prolonged periods of time [8].

Neuropeptide tyrosine (NPY), a 36 aa peptide [10], is broadly distributed across the central [11] and peripheral nervous systems [12], and is strongly conserved through evolution, including in humans [13]. NPY acts through 5 different receptors known so far [14–16]. However, types 1 and 2 receptors (Y1R, Y2R) seem to be the most relevant in the mechanisms of pain [16–23]. Only very few dorsal root ganglion (DRG) neurons normally express NPY, but its expression is strongly upregulated by peripheral nerve injury. In contrast, both Y1R and Y2R are regularly expressed in DRG neurons where they exhibit a complementary expression, the former primarily in small neurons, and the latter in medium-sized and large ones. Finally, at the spinal cord level, an abundant NPY-expressing neuropil is normally detected, and produced primarily by local superficial dorsal horn interneurons, but also contributed by primary afferent neurons and descending inputs. Y1R and Y2R expression is also detected in the spinal cord, both in pre- (Y1R, Y2R) and postsynaptic (Y1R) locations (for a detailed description of the expression and distribution patterns of NPY, Y1R and Y2R in DRGs and the spinal cord, see [16,17] and references therein).

An increasing number of studies strongly supports the pain-modulating role of NPY [19,20,24–30], suggesting that along with its associated receptors, they could be attractive targets for the develop-

Abbreviations: AUC, area under the curve; CCI, chronic constriction injury; CFA, complete freund's adjuvant; DRG, dorsal root ganglion; NPY, neuropeptide tyrosine; SLNC, single ligature nerve constriction; SNI, spared nerve injury; VGLUT2, vesicular glutamate transporter type 2; Y1R, NPY receptor type 1; Y2R, NPY receptor type 2

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ment of drugs against pain. In fact, the analgesic impact of the spinal activation of Y1- and Y2Rs in rats has been analyzed using a variety of pain models such as sciatic nerve axotomy [31] or spared nerve injury (SNI) [22], skin incision [32], and acute [22,33] or chronic [21,33,34] inflammation. However, no study has yet established the potential antiallodynic role of NPY or the selective activation of its associated receptors in rats with neuropathic pain induced by a compressive injury of the sciatic nerve.

In the present study, we explored the potential analgesic role of the pharmacological activation of spinal Y1Rs in rats with CCI [35]. Mechanical and thermal (cold) allodynia were used as parameters of change in rats with CCI treated either with vehicle or the specific Y1R agonist Leu^{31}, Pro^{34}-NPY.

2. Materials and methods

2.1. Animals

All experiments were performed in 109 male Sprague-Dawley rats (weight 180–280 g). Animals were maintained in 12 h day/night cycles (lights on from 7 a.m. to 7 p.m.), with water and food ad libitum. All experiments were performed according to the recommendations of the International Association for the Study of Pain (IASP) and the Society for Neuroscience (SFN) on the use of animals in research, and were approved by the IACUC of the IRTM (CICUAL-IIMT-12-04).

2.2. Chronic constriction injury (CCI)

CCI was induced in rats previously anaesthesized using Isoflurane (5% induction, 3.0% maintenance, 0.8 L/min O_2 flow rate; Piramal Healthcare, UK), according to the method by Bennett and Xie [35]. In brief, the right sciatic nerve was exposed at the midthigh level, and carefully dissected from the surrounding tissue. The nerve was ligated using 4 loose 5.0 silk ligatures, followed by suture in layers of the surgical wound using Vicryl 5.0 (muscle) and mononylon 5.0 (skin) (Ethicon, Livingston, Scotland). After a single subcutaneous dose of dexketoprofen trometamol (5 mg/kg; Lab Argentia, Bs As, Argentina) and the topical application of 2% lidocaine hydrochloride gel (Astra-Zeneca, Buenos Aires, Argentina) on the surgical wound, the animals were left to recover in a warm environment, before being returned to the animal house.

2.3. Intrathecal catheterization implantation and agonist administration

A chronic intrathecal (i.t.) catheter (PE 10, o.d. 0.61 mm; Intramedic, Clay Adams, Becton Dickinson and Company, New Jersey, USA) was implanted under anesthesia (as above), according to the method by Storkson and cols. [36], between the L5 and L6 vertebrae, with its tip at the lumbar enlargement, in sham rats and rats 7 days after induction of CCI. The proper location of the tip of the catheter was tested 24 h before the pharmacological experiments by assessing sensory and motor blockade after i.t. injection of 10 µl of lidocaine (50 mg/ml; Xylocaína, AstraZeneca, Buenos Aires, Argentina). All animals failing to show signs of sensory and motor blockade, or that manifested signs only in the left leg (contralateral to the CCI) were not included in the study. The pharmacological experiments were conducted on day 14 after CCI.

We used the Y1R agonist Leu^{31}, Pro^{34}-NPY (Tocris Bioscience, Bristol, UK). Leu^{31}, Pro^{34}-NPY, dissolved at a concentration of 1 mg/ml in 0.25% acetic acid and diluted to working concentrations in vehicle (sterile saline), was tested at doses of 2.5, 5, 7, 10 and 20 µg, in all cases to a final volume of 10 µl. The effect of the agonist was exposed by analysis of pain-like behavior in sham and injured rats (see below).

2.4. Control experiments

Two types of control experiments were performed: (1) Rats (n = 6) where the sciatic nerve was exposed but not ligated (sham rats), and with catheterization and injection of 10 µg of Leu^{31}, Pro^{34}-NPY; (2) Rats (n = 8) with CCI, and with catheterization and injection of vehicle (0.25% acetic acid).

2.5. Pain-like behavioral testing

2.5.1. Measurement of mechanical threshold

Mechanical threshold was evaluated using von Frey filaments (1.4, 2, 4, 6, 8, 10, 15 y 26 g; Stoelting, Inc., Wooddale, IL, USA). The medial aspect of the plantar surface of the ipsilateral hindpaw was mechanically stimulated, following the modified up-down method of Dixon, as described by Chaplan and cols. [37], to establish the 50% withdrawal threshold. Mechanical withdrawal threshold was tested previous to the injection of the agonist (0 min; basal response), and 5, 15, 30, 45, 60, 75 and 90 min after the application of the agonist. A paw withdrawal reflex obtained with 4.0 g force or less was considered an allodynic response.

2.5.2. Assessment of cold allodynia

Cold allodynia was assessed using a modified version of the method established by Choi and cols. [38]. After acclimatization in individual cubicles for 15 min, a drop of acetone was gently brought in contact with the plantar surface of the ipsilateral hindpaw. Applications were made four times every four minutes, for a total of 108 min from the beginning of the pharmacological experiment. Foot withdrawal was scored as positive (1) and lack of withdrawal as negative (0). The frequency of withdrawal was evaluated in 16 min bins (totaling 7 bins), each bin consisting of the average obtained from 4 consecutive stimulations. The first 16 min bin represents basal response, previous to agonist injection.

After all behavioral testing, animals were deeply anesthetized using an overdose of chloral hydrate (1.5 g/kg, intraperitoneal) followed by cervical dislocation.

2.6. Statistical analysis

All data is expressed as mean ± SEM, and presented as curve graphs and area under the curve (AUC) bar graphs. Statistical analysis was performed using Two-way repeated measures ANOVA followed by the Bonferroni posthoc test (curve graphs), or One-way ANOVA followed by the Tukey posthoc test (AUC graphs).

In all cases, levels of significance were established as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3. Results

All rats with CCI showed changes in the position of the injured leg, including retraction and protection (pain-like behavior). In contrast, injured or sham rats virtually never showed signs of altered pain-like behavior in the contralateral paw.

Sham rats treated with 10 µg intrathecal Leu^{31}, Pro^{34}-NPY presented no significant changes in mechanical thresholds, as tested in the ipsilateral hindpaw (0 min: 13.41 ± 1.75; 5 min: 15.05 ± 0.12; 15 min: 15.0 ± 0.0; 30 min: 15.0 ± 0.0; 45 min: 15.0 ± 0.0; 60 min: 15.05 ± 0.12; 75 min: 15.0 ± 0.0; 90 min: 15:00 ± 0.0). In contrast, rats with CCI plus intrathecal injection of vehicle showed clear signs of mechanical (Figs. 1 and 3) and cold allodynia (Figs. 2 and 3). Conversely, as it will be described in more detail in the following sections, intrathecal injection of Leu^{31}, Pro^{34}-NPY in rats with CCI resulted in dose-dependent antiallodynic effects.

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