Neuroscience Letters 542 (2013) 21–25

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Peripheral group II and III metabotropic glutamate receptors in the knee joint attenuate carrageenan-induced nociceptive behavior in rats

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HIGHLIGHTS

- We evaluated effects of peripheral group II and III mGluR on arthritic pain of rats.
- Pain behavior is relevant with reduced weight bearing and mechanical hyperalgesia.
- Pre-treatment of group II and III mGluR agonist attenuated nociceptive behavior.
- Post-treatment of group II and III mGluR agonist attenuated nociceptive behavior.

ARTICLE INFO

Article history:
Received 30 October 2012
Received in revised form 15 February 2013
Accepted 1 March 2013

Keywords:
Arthritic pain
Metabotropic glutamate receptors
Weight bearing
Mechanical hyperalgesia
Carrageenan

ABSTRACT

This study sought to evaluate whether peripheral group II and III metabotropic glutamate receptors (mGluRs) in the knee joint have inhibitory effects on carrageenan-induced nociceptive behavior. To this end, changes in weight load and hind paw withdrawal threshold were measured in rats following the administration of specific peripheral group II and III mGluR agonists 30 min before (induction phase) and 4 h after (maintenance phase) the injection of carrageenan (1%, 50 μl).

During the induction phase of arthritic pain, a significant recovery of reduced weight load occurred after the administration of 500 μM APDC ((2R, 4R)-4-aminopyrrolidine-2,4-dicarboxylate; group II agonist) and 100 and 500 μM L-AP4 (L-2-amino-4-phosphonobutylate; group III agonist). Similarly, 100 and 500 μM APDC and 500 μM L-AP4 significantly reduced mechanical hyperalgesia during the induction phase. In the maintenance phase, APDC at all doses (10, 100 and 500 μM) and 100 and 500 μM L-AP4 significantly counteracted the reduction in weight load, and APDC and L-AP4 at all doses (10, 100 and 500 μM) inhibited mechanical hyperalgesia.

The current study suggests that peripheral group II and III mGluRs in the knee joint negatively modulates nociceptive behavior during both the induction and maintenance phases of carrageenan-induced arthritic pain.

1. Introduction

The knee joint is the largest hinge joint in the body and is vital in bearing the body's weight and supporting normal walking. It is a complex structure and consists of bones, cartilage, ligaments, and muscles. It also contains synovial fluid, which lubricates the joint. The peripheral afferent nerves that innervate the knee joint convey mechanical information such as weight load to the spinal cord, where glutamate is released into peripheral and central nerve terminals and initiates excitatory neurotransmission. Inflammation of or injury to the knee joint can increase the amount of glutamate released from nerve terminals and cause the subsequent sensitization of peripheral receptors (primary hyperalgesia) or spinal dorsal horn neurons (secondary hyperalgesia) [23].

Glutamate generates neuronal excitability and signal transmission by binding to G protein-coupled receptors (metabotropic glutamate receptors, mGluRs) [8] and ligand-gated receptors (ionotropic glutamate receptors, iGluRs). Based on sequence homology, signal transduction, and pharmacology, the 8 subtypes of mGluRs have been classified into 3 groups: group I (mGluR1 and 5), group II (mGluR2 and 3), and group III (mGluR4, 6, 7, and 8). Group I receptors increase neuronal excitability by coupling to $G_{q/11}$ proteins, which activate phospholipase C and mobilize

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http://dx.doi.org/10.1016/j.neulet.2013.03.006

Ca^{2+} from intracellular stores. By contrast, group II and group III decrease neuronal excitability by coupling to G_i/G_0 proteins, inhibiting adenylate cyclase and decreasing cyclic AMP production [2].

Group II and III mGluRs are localized to primary afferent fibers and dorsal root ganglia [5,17] as well as the spinal cord [10,15], and are responsible for regulating pain transmission via activity-dependent autoinhibition [6,11]. Intraplantar injection of peripheral group II and III mGluR agonists reduces capsaicin- or prostaglandin E_2-induced hyperalgesia in rodents [6,25]. However, it is less understood whether peripheral group II and III mGluRs in the knee joint influence pain-related behavior.

The purpose of the present study was to investigate whether peripheral group II and III mGluRs in the knee joint contribute to the alleviation of nociceptive behavior (reduction in weight load and mechanical hyperalgesia) during the induction and maintenance phases of carrageenan-induced arthritic pain.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (200–230 g; Sam, Korea) were housed (5 rats per cage) in a temperature- and light-controlled room (22–25 °C, 12 h light/dark cycle with lights on at 07:00). Food and water were available *ad libitum*. All experimental procedures were conducted in accordance with the guidelines set by the Korea University College of Medicine Animal Research Policies Committee.

2.2. Carrageenan-induced arthritis

To induce arthritis, 1% lambda-carrageenan (50 μl, in saline) was injected into the right knee joint of each animal under sodium enflurane (0.5–2%) after baseline behavioral testing. The injected leg was flexed manually for approximately 5 min. To ensure that the carrageenan injection caused arthritis, we measured the diameter of the right and left knee joints before and after the injection of carrageenan. Knee joint diameter was defined as the distance between the lateral and medial collateral ligament regions.

2.3. Behavioral studies

Weight loaded onto the side of the inflamed joint can generate pain. Using a weight-bearing device, we assessed arthritic pain in freely walking rats [18]. The bottom of a path was equipped with a load cell sensor (CB1-K2, DACELL, Korea), and output signals were fed to a digital amplifier (DN-AM 300, DACELL, Korea) for appropriate amplification and filtering. The signal was digitized via an analog-digital converter (1716, DACELL, Korea) and plotted as a time–weight curve on a personal computer (WBT, Korea Univ., Korea). The investigator identified the plates that the rat stepped on, and output signals were selected for plotting the time–weight curves. The test was repeated 3 or 4 times to obtain at least 8 time–weight curves for a given limb.

To measure the mechanical threshold for hind paw withdrawal, a series of von Frey filaments (0.41–15.10 g, Stoelting, Wood Dale, IL, U.S.A.) were applied. Under a transparent plastic dome (28 cm × 28 cm × 10 cm) on a metal mesh floor, a von Frey filament was applied to the plantar surface of the ipsilateral or contralateral hind paw of the rats. The 50% withdrawal threshold was determined using the up-down procedure, and stimuli were presented at intervals of several seconds. A brisk foot withdrawal to the von Frey application was regarded as a positive response. Interpolation of the 50% threshold was performed according to the method of Dixon [9].

To examine whether motor impairment induced by APDC and L-AP4 treatment might lead to changes in the behavioral signs of arthritic pain, a modification of the combined behavioral score (CBS) of Gale et al. [12] was performed. The CBS represents the degree of motor impairment and consists of motor scores, toe spread, righting reflex, extension withdrawal reflex, placing reflex and inclined plane tests. Neurological function was evaluated using a scoring system that ranged from 0 (normal rat) to 90 (completely paralyzed rat).

2.4. Drug administration

The group II mGluR agonist APDC ((2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate) and group III agonist L-AP4 (L-2-amino-4-phosphonobutylate) were purchased from Tocris Cookson Ltd. (Ellisville, MO, U.S.A.). Stock solutions of these drugs were made by dissolving them in saline or 10% dimethyl sulfoxide.

In the pre-treatment paradigm, APDC (10, 100, and 500 μM/50 μl) or L-AP4 (10, 100, and 500 μM/50 μl) injections were administered 30 min before the carrageenan injection, and pain-related behavioral tests were performed 4 h after the carrageenan was administered. In the post-treatment paradigm, mGluR agonists were injected 4 h after the carrageenan injection, and behavior were assessed 90 min after group II and III agonist injection. The group II and III agonists were administered under sodium enflurane (0.5–2%) anesthesia. The investigator conducting the behavioral tests was blind to the injected drug.

2.5. Statistical analysis

The weight load measurements were normalized by expressing them as a percentage of body weight on a given day. Repeated-measures analysis of variance (ANOVA) was used to compare the data relative to the time course of pain-related behavior. One-way ANOVA with post hoc Bonferroni test was used to compare the data obtained from different experimental groups at corresponding time points after drug injection. For all data, $p < 0.05$ was considered statistically significant.

3. Results

3.1. Time course of behavior related to carrageenan-induced arthritic pain

The weight load value and paw withdrawal threshold for mechanical stimulation were measured at various time intervals following carrageenan injection into the right knee joint.

Intra-articular injection of carrageenan resulted in a significant reduction of weight bearing that began approximately 3 h after injection and was maintained for approximately 3 days after the injection (Fig. 1A). At 4 h after the injection, the weight load values of the affected (right) leg showed the most notable decrease: from 56 ± 1.9% to 18.3 ± 1.2%. The threshold of paw withdrawal also decreased to the greatest extent at 4 h post-injection (from 14.6 ± 1.9 g to 2.6 ± 1.2 g). In the saline-injected group, weight bearing and paw withdrawal thresholds for the ipsilateral hind limb were unchanged.

3.2. Effects of APDC and L-AP4 on weight bearing and paw withdrawal thresholds during the induction of arthritic pain

To evaluate the inhibitory effects of APDC and L-AP4 during the induction of arthritic pain, the drugs were administered 30 min