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Research article

Spinal 5-HT1A, not the 5-HT1B or 5-HT3 receptors, mediates descending serotonergic inhibition for late-phase mechanical allodynia of carrageenan-induced peripheral inflammation

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HIGHLIGHTS

- Spinal 5-HT has an anti-allodynic effect in late-phase carrageenan inflammation.
- 5-HT1A receptor is involved in anti-nociceptive processing in spinal cord.
- Spinal 5-HT1B or 5-HT3 receptor plays a limited role in nociceptive processing.

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ABSTRACT

Previous electrophysiological studies demonstrated a limited role of 5-hydroxytryptamine 3 receptor (5-HT3R), but facilitatory role of 5-HT1AR and 5-HT1BR in spinal nociceptive processing of carrageenan-induced inflammatory pain. The release of spinal 5-HT was shown to peak in early-phase and return to baseline in late-phase of carrageenan inflammation. We examined the role of the descending serotonergic projections involving 5-HT1AR, 5-HT1BR, and 5-HT3R in mechanical allodynia of early- (first 4 h) and late-phase (24 h after) carrageenan-induced inflammation. Intrathecal administration of 5-HT produced a significant anti-allodynic effect in late-phase, but not in early-phase. Similarly, intrathecal 5-HT1AR agonist (8-OH-DPAT) attenuated the intensity of late-phase allodynia in a dose dependent fashion which was antagonized by 5-HT1AR antagonist (WAY-100635), but produced no effect on the early-phase allodynia. However, other agonists or antagonists of 5-HT1BR (CP-93129, SB-224289) and 5-HT3R (m-CPBG, ondansetron) did not produce any anti- or pro-allodynic effect in both early- and late- phase allodynia. These results suggest that spinal 5-HT1A, but not 5-HT1B or 5-HT3 receptors mediate descending serotonergic inhibition on nociceptive processing of late-phase mechanical allodynia in carrageenan-induced inflammation.

1. Introduction

Nociceptive processing in the spinal cord may be facilitated or inhibited by descending serotonergic projections, which depends on several factors, including receptor subtype activation in the spinal cord and the type of pain [16]. The spinal 5-hydroxytryptamine 3 receptor (5-HT3R) predominantly mediates excitatory modulation in a number of pain states, and 5-HT3R antagonists have been shown to attenuate pain [23]. However, in carrageenan-induced inflammatory pain, blocking the spinal 5-HT3R does not alter electrically evoked neuronal responses, suggesting that the serotonergic system via the 5-HT3R is not activated in this model of inflammatory pain [13]. In contrast, spinal 5-hydroxytryptamine 1A and 1B receptors (5-HT1AR and 5-HT1BR) facilitate the neuronal responses of the spinal cord, according

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to electrophysiological studies of carrageenan–injected rats [27]. However, these receptor agonists also have analgesic effects in some pain states, which seem to depend on various factors including the nature of the noxious stimuli [1,8,12].

In addition to the lack of information on which specific spinal 5-HT receptor subtype plays a crucial role in mediating descending serotonergic pain modulation in the carrageenan model, the overall effect of serotonergic pathway activation remains unclear. We previously found that depletion of spinal 5-HT induces a significant increase in the intensity of mechanical allodynia at 1–4 h after the initiation of carrageenan inflammation, suggesting descending serotonergic inhibition may be dominant over facilitation in early-phase carrageenan inflammation [26]. A previous study using microdialysis of the spinal cord showed that 5-HT release peaks in early-phase carrageenan inflammation, but returns to baseline in late-phase, indicating early activation but rapid loss of involvement of serotonergic inhibition in spinal nociceptive processing [28]. Therefore, the role of descending serotonergic pain modulation involving spinal 5-HT1AR, 5-HT1BR and 5-HT3R may differ depending on the time after initiation of carrageenan inflammation.

In the present study, we evaluated the differences in the role of descending serotonergic projections involving 5-HT1AR, 5-HT1BR and 5-HT3R in mechanical allodynia of early- (first 4 h) and late-phase (24 h after) carrageenan-induced inflammation.

2. Methods

2.1. Animals and intrathecal catheter implantation

The experimental protocol was in accordance with the International Association for the Study of Pain guidelines for the use of animals in research, and was approved by the Institutional Animal Care and Use Committee. Male Sprague–Dawley rats weighing 225–250 g were housed in a room at a constant temperature of 22–23 °C with an alternating 12 h light/dark cycle with free access to food and water. All of the experimental agents were administered via an intrathecal (i.t.) catheter, which had been implanted according to previous studies [11]. An i.t. PE-10 catheter was inserted into the i.t. space through the atlanto-occipital membrane, and moved in a caudal direction along the i.t. space until it reached the lumbar enlargement. The external PE-10 catheter was exteriorized to the top of the head for i.t. administration of the agents. Following implantation, the animals received a subcutaneous injection of 5 mL lactated Ringer's solution containing 0.3 mg/kg ketorolac, and were allowed to recover in individual cages. When the animals showed neurological deficits, they were immediately sacrificed with an overdose of sevoflurane anesthetics. After the experiment was completed and the animals were euthanized, the lumbar spine of each animal was cut and dissected to ensure correct placement of the i.t. catheter.

2.2. Experimental agents

The following agents were used: 5-HT hydrochloride (nonspecific 5-HTR agonist), 8-OH-DPAT (5-HT1AR agonist), WAY-100635 (5-HT1AR antagonist), CP-93129 (5-HT1BR agonist), SB-224289 (5-HT1BR antagonist), 1-(3-chlorophenyl) biguanide hydrochloride (m-CPBG; 5-HT3R agonist), ondansetron (5-HT3R antagonist). These agents were dissolved in dimethyl sulfoxide and diluted with saline before i.t. injection. All of the drugs were delivered in a volume of 10 μL followed by flushing of the catheter with 10 μL saline.

2.3. Carrageenan-induced inflammation and von Frey test

Animals were given a 30-min acclimation period in a cage with a wire mesh floor. After this period, the von Frey test was performed to obtain a baseline of 50% paw withdrawal threshold (PWT), which was determined using the up–down method [10]. Thirty minutes after measuring baseline PWT, and after brief inhalation of sevoflurane, carrageenan (100 μL, 2% degraded λ-carrageenan, Wako Pure Chemical Industries, Japan) was injected subcutaneously into the center of the hind paw.

To measure the intensity of the allodynic responses to mechanical stimuli, von Frey filaments were applied perpendicularly to the middle of the plantar surface. The forces of the filaments were between 0.41 g and 15.2 g, starting with a 2 g force through the wire mesh floor. Each application was maintained for 5 s or until paw lifting or licking, which were considered positive responses in the up–down method. The investigator who performed the von Frey test was blinded to the drug used in the i.t. treatment.

2.4. Study protocol

Effects of i.t. nonspecific 5-HTR agonist, and specific agonists and antagonists of 5-HT1AR, 5-HT1BR, and 5-HT3R on mechanical allodynia were tested in two separate sessions. First, the test was performed on the day of carrageenan injection (early-phase allodynia), in which animals were intrathecally administered one of the experimental drugs 10 min before carrageenan injection, after which PWT was measured every hour for 4 h. The second session was performed 24 h after carrageenan injection (late-phase allodynia). After measuring the PWT at 24 h, the change in the PWT was observed every hour for 4 h after i.t. injection of the experimental agents. An antagonism study was performed on the experimental agents that showed anti-allodynic effect, which involved i.t. pretreatment with WAY-100635 (5-HT1AR antagonist) 10 min before i.t. 8-OH-DPAT (5-HT1AR agonist).

2.5. Statistical analysis

Data are expressed as the means ± SEM for each group. The early-phase allodynia was compared using the hyperalgesic area under the curve (AUC), which is the sum of the %hyperalgesic effect (=[(baseline PWT–post-carrageenan PWT)/baseline PWT] × 100) below the baseline over the study. For late-phase allodynia, the PWT were converted into %MPE using the following formula: %MPE = [(post-drug threshold–PWT at 24 h)/(cutoff threshold–PWT at 24 h)] × 100. Differences among the groups or treatments were analyzed using one-way analysis of variance (ANOVA) followed by the Bonferroni correction. A value of $p < 0.05$ was taken to indicate statistical significance.

3. Results

As reported previously, the decrease in PWT started as early as 1 h after injection (early-phase allodynia), and lasted more than 24 h (late-phase allodynia) after the induction of carrageenan inflammation [2]. After i.t. catheterization, 18 of 240 rats exhibited motor abnormalities or had misplaced i.t. catheters. The doses presented in this study were the maximal doses at which adverse effects were not observed.

The i.t treatment with 5-HT hydrochloride had no effect on early-phase allodynia (Fig. 1A and 1B) but significantly reduced the intensity of late-phase allodynia in a dose-dependent manner (Fig. 1C and 1D). The magnitude of the i.t. 5-HT agonist effect was modest with %MPE being around 40% (Fig. 1D).

No significant differences in allodynic responses were observed among animals treated with i.t. vehicle, 5-HT1AR, 5-HT1BR, or 5-