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Research article

Antinociception induced by galanin in anterior cingulate cortex in rats with acute inflammation

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HIGHLIGHTS

- Intra-ACC injecting galanin induced antinociception in rats with acute inflammation.
- Galanin receptor 2 is involved in galanin-induced antinonciception in ACC.
- The galanin mRNA level and content increased in ACC in rats with acute inflammation.
- The mRNA level and content of Gal R2 increased in ACC in rats with acute inflammation.

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ABSTRACT

The present study was performed to explore the role of galanin in nociceptive modulation in anterior cingulate cortex (ACC) of rats with acute inflammation, and the changes in galanin and galanin receptor 2 (Gal R2) expressions in rats with acute inflammation. Intra-ACC injection of galanin induced antinociception in rats with acute inflammation, the antinociceptive effects induced by galanin were attenuated significantly by intra-ACC injection of the Gal R2 antagonist M871, indicating an involvement of Gal R2 in nociceptive modulation in ACC in rats with acute inflammation. Furthermore, we found that both the galanin mRNA expression and galanin content increased significantly in ACC in rats with acute inflammation than that in normal rats. Moreover, both the mRNA levels of Gal R2 and the content of Gal R2 in ACC increased significantly in rats with acute inflammation than that in normal rats. These results demonstrated that galanin induced antinociception in ACC in rats with acute inflammation. And there were changes in the expression of galanin and Gal R2 in rats with acute inflammation.

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1. Introduction

It is known that the neuropeptide galanin is widely distributed in the central nervous system and peripheral nervous systems [12,17], and there are three subtypes of galanin receptor (Gal R1, Gal R2, Gal R3) [4,12,18]. Many studies have reported that galanin and galanin receptors play important roles in the transmission and/or modulation of nociception in the central nervous system, such as the spinal cord, hypothalamic arcuate nucleus, nucleus accumbens, periaqueductal grey, and the central nucleus of amygdala [5,7,9,11,22–25,27,29].

Recently, may studies demonstrated that anterior cingulate cortex (ACC) is involved in the nociceptive modulation in the brain [3,6,13,14,16,19,21,26,30]. The ACC is reported to be involved in nociceptive modulation in acute and chronic inflammation [3,8]. Yi et al. found lesions of rostral ACC completely abolished the anti-nociceptive effects of contra- but not ipsi-lateral electro-acupuncture in the formalin-induced inflammatory pain model of rats [28]. Harris-Bozer et al. found that there were significant changes in the lowest-frequency activities in the local field potential range in ACC of rats with acute inflammation [8]. Li et al. found that 7 days after injecting complete Freund's adjuvant into the hind paw of rats, potentiation of excitatory postsynaptic potentials could be observed in ACC neurons [13].

As there are galanin-ergic fibers and galanin binding sites in ACC [1,17], and ACC is involved in acute inflammation [3,8], the present study was performed to explore the role of galanin in nociceptive modulation in ACC in rats with acute inflammation, and further to

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explore the involvement of Gal R2 in nociceptive modulation in ACC in rats with acute inflammation.

2. Methods

2.1. Animal preparation

All experiments were carried out on freely moving male Sprague–Dawley rats weighing 220–260 g (Experimental Animal Center of Luye Pharmaceutical Company, Yantai, China). The rats were housed in cages with free access to food and water, and maintained in a room temperature of 20 ± 2 °C with a normal day/night cycle. All experiments were performed according to the guidelines of the International Association for the Study of Pain [31] and after obtaining a permit (20140901-01) conformed to The Guidelines for the Care and Use of Laboratory Animals of Yantai University. Every effort was made to minimize both the animal suffering and the number of animals used.

2.2. Nociceptive tests

Rats were accustomed to the test condition for 3 days before the experiment to minimize the stress induced by handling and measurements [11,22]. The hindpaw withdrawal latencies (HWLs) during thermal and mechanical stimulation were measured as described previously [2,11,22]. Briefly, the entire ventral surface of the rat hindpaw was placed manually on a hot plate (YLS–6B Intelligent Heat Panel Instrument, Jinan Yiyan Science & Technology Development Co., Ltd., Jinan, China), which was maintained at a temperature of 52 ± 0.2 °C. The time to hindpaw withdrawal was measured in seconds and referred to as the HWL to thermal stimulation. The Randall Selitto Test (Ugo Basile, Type 7200, Italy) was used to assess the HWL to mechanical stimulation. A wedge-shaped pusher at a loading rate of 30 g/s was applied to the dorsal surface of the hindpaw. The latency required to initiate the withdrawal response was assessed and expressed in seconds. Before intra-ACC injection, the HWLs were tested three times and regarded as the basal HWLs. The HWLs recorded during subsequent experiments were expressed as percentage changes of the basal level for each rat (% changes of the HWL). Each rat was tested by both types of stimulation. Every measurement of the HWL to both thermal and mechanical stimulation was finished within 1–2 min. A cut-off limit of 15 s was set up to avoid tissue damage.

2.3. Surgical procedures and intra-ACC injection

Rats were anaesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg, Xudong Chemical Factory, Beijing, China) and mounted on stereotaxic frame, a stainless steel guide cannula of 0.8 mm outer diameter was directed to the ACC (1.6 mm anterior to Bregma; 0.7 mm lateral to midline; 2.0 mm ventral to the surface of skull) according to Paxinos and Watson [20], and was fixed to the skull by dental acrylic. There were more than 3 days for rats to recover from the operation. On the day of experiment, a stainless steel needle with 0.4 mm outer diameter was directly inserted into the guide cannula with 1.5 mm beyond the tip of the latter. One microliter of solution was thereafter infused into ACC over 1 min. The injection needle was left in the site for 1 min after each injection before removal.

2.4. The model of acute inflammation

Animals received a unilateral injection of carrageenan (2 mg in 0.1 ml saline, Sigma) into the left hindpaw. The contralateral paw was untreated. Three hours after injection of carrageenan, the HWLs were measured by the hot-plate test and the Randall Selitto Test as the basal HWL. Then each animal received an intra-ACC injection of either vehicle or drug. After intra-ACC injection, the HWLs of each animal were assessed.

2.5. RT-PCR

For PCR test, normal rats (n = 3, as a control) and rats with acute inflammation (n = 3) received injection of over dose of 10% trichloroacetaldehyde monohydrate and the brain was removed immediately. The regions of the ACC were dissected on ice and then frozen at −80 °C. The total RNA was isolated with UNIQ-10 Column Trizol Total RNA Extraction Kit (Sangon Biotech, Shanghai, China). Total RNA was reverse-transcribed using AMV First Strand cDNA Synthesis Kit (Sangon Biotech, Shanghai, China). PCRs were done using SG Fast qPCR Master Mix (Sangon Biotech, China). Sequences of primers for the experiments were rat galanin: sense 5′- CACATGCCATTGACAAC-CAC – 3′ and antisense 5′- AACTCCATTATAGTGCGGACG −3′; rat Gal R2: sense 5′- GCCGCCATCGGGCTCATCTG −3′ and antisense 5′- GTCGAGGTGCGCTCCATGCT −3′); rat GAPDH: sense 5′-GACCACCCAGCCCAGCAAGG −3′ and antisense 5′- TCCCCAGGCCC-CTCCTGTTG −3′. The qRT-PCR assay was performed in 20 μl final reaction mixture according to the instructions for SG Fast qPCR Master Mix (Sangon Biotech, China) using StepOne^{TM} Real-Time PCR System and software (Thermo Fisher Scientific, USA) under the following parameters: 95 °C for 3 min and 45 cycles of 95 °C for 7 s, 57 °C for 10 s, and 72 °C for 15 s. The unigene expression levels were calculated with the 2 (-delta delta C (T)) method [15]. The GAPDH was amplified as an internal control. The qRT-PCR analyses were performed thrice with independent RNA samples.

2.6. Western blot

For Western blot, normal rats (n = 3, as a control) and rats with acute inflammation (n = 3) received injection of over dose of 10% trichloroacetaldehyde monohydrate and the brain was removed immediately. The regions of the ACC were dissected on ice and then frozen at −80 °C. The brain tissue samples were homogenized in RIPA lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China) containing phenylmethanesulfonyl fluoride (Beyotime Institute of Biotechnology, Shanghai, China) and centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatant was collected and measured by BCA Protein Assay Kit (Beyotime Institute of Biotechnology, Shanghai, China). Whole protein extracts (80 μg) of ACC samples were subject to 10% SDS–PAGE (for Gal R2 and beta-actin) or 15% SDS-PAGE (for galanin). Then the proteins were transferred to PVDF membranes (Millipore, MA, USA) for 1 h at 106 V. The membranes were incubated in blocking solution (5% non-fat milk in Tris-buffered saline containing 0.1% Tween-20, TBST) for 2 h at room temperature, and sequentially in TBST containing primary antibodies to the polyclonal goat anti-galanin antibody (1:1000; SAB2501407, Sigma–Aldrich, St. Louis, MO, USA), polyclonal rabbit anti-Gal R2 antibody (1:1000; ab203072, Abcam, Cambridge, UK), or beta-actin antibody (1:1000; AA1128, Beyotime Institute of Biotechnology, Shanghai, China) at 4 °C overnight. The membranes were washed 3 times with TBST for 10 min each and then probed with HRP-conjugated donkey anti-goat secondary antibody (1:1500; A0181, Beyotime Institute of Biotechnology, Shanghai, China), HRP-conjugated goat anti-rabbit secondary antibody (1:1500; A0208, Beyotime Institute of Biotechnology, Shanghai, China), HRP-conjugated goat anti-mouse secondary antibody (1:1500; A0216, Beyotime Institute of Biotechnology, Shanghai, China) for 1 h at room temperature. The membranes were washed 3 times with TBST for 10 min each again. Brands were visualized by enhanced chemiluminescence (ECL) detection reagents (Beyotime Institute of Biotechnology, Shang-

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