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Involvements of galanin and its receptors in antinociception in nucleus accumbens of rats with inflammatory pain

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ABSTRACT

This study tested the hypothesis that antinociceptive effects of galanin and its receptors in nucleus accumbens (NAc) of rats with inflammatory pain provoked by subcutaneous injection of 0.1 ml of 2% carrageenin into the sole of the rat's left hindpaw. The hindpaw withdrawal latencies (HWLs) in response to thermal and mechanical stimulation significantly decreased in bilateral hindpaws at 3 and 4 hour after a subcutaneous injection of carrageenin. However intra-NAc injection of 2 and 3 nmol, but not 1 nmol of galanin markedly induced an increase in the HWLs in a dose-dependent way. Western blot also showed, that the expression of galanin receptor 1 (GalR1) and galanin receptor 2 (GalR2) were significantly upregulated in NAc at 3 hour after a subcutaneous injection of carrageenin. In addition, the rats were intra-NAc injected galanin, 5 min later following by intra-NAc injection of galanin receptor antagonist galantide, the galanin-induce antinociceptive effects were suppressed by galantide. The results demonstrated that galanin and its receptors might be involved in antinociception in the NAc of rats with inflammatory pain.

1. Introduction

Galanin is a 29-amino acid (30 in human) neuropeptide, which is widely distributed in peripheral and central nervous system (Tatemoto et al., 1983). Previous studies reported that galanin and its receptors might be involved in the transmission and modulation of nociceptive information in the central nervous system (Yu et al., 2001; Liu and Hökfelt, 2002; Hua et al., 2004; Wiesenfeld-Hallin et al., 2005; Gu et al., 2007; Xu et al., 2008; Jin et al., 2010). Our previous study showed that intra-nucleus accumbens (NAc) injection of galanin increased the hindpaw withdrawal latencies (HWLs) to thermal and mechanical stimulation in intact rats, indicating that galanin might play an antinociceptive role in the NAc (Xu et al., 2012a).

Inflammatory pain is very common in the clinic. A study demonstrated that galanin was upregulated after nerve injury or inflammation (Ma and Bisby, 1997). It has been reported that galanin plays an important role in the transmission of nociceptive information in the spinal cord of rats with inflammation, and opioid systems are involved in the galanin-induced antinociception (Xiong et al., 2005). Sun et al. reported that galanin had an antinociceptive role in the arcuate nucleus of hypothalamus of intact rats and rats with inflammation (Sun et al., 2003). The results indicated that galanin played a significant role in the transmission of nociceptive information in the spinal cord and brain of rats with inflammation.

Although a multitude of studies have proved that the NAc is an important limbic structure of the brain with roles in pain modulation (Gu and Yu, 2007; Jin et al., 2010; Li et al., 2010; Xu et al., 2012a), the underlying antinociceptive mechanism of galanin and especially galanin receptors in NAc remain largely unclear. Therefore, the present study investigated whether galanin and its receptors were involved in the modulation of nociceptive information in the NAc of rats with inflammatory pain.

2. Materials and methods

2.1. Animal preparation

All experiments were accomplished on freely moving male Sprague-Dawley rats weighing between 180 and 250 g (Experimental Animal Center of Kunming Medical University, Kunming, Yunnan, China). The rats were housed in cages with free access to food and water, and maintained in a temperature controlled ($24 \pm 1\,^{\circ}C$) room with a normal day/light cycle. All experiments were conducted according to the guidelines of the International Association for the Study of Pain (Zimmermann, 1983). All animal

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welfare and experimental procedures were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, 1996), and were approved by the Animal Ethics Committee of Kunming Medical University. Every effort was made to minimize both the number of animals used and the animal suffering.

2.2. Nociceptive tests

The HWLs during noxious thermal and mechanical stimuli were recorded as described before (Yu et al., 1996, 1999). Briefly, the response to noxious thermal stimulation was evaluated by the hot-plate (YLS-6B, Jinan YiYan Science and Technology Development Company, China) which maintained at a temperature of 52 °C (±0.2 °C) (Lindblom, 1994). The operator's one hand held the rat, another hand put the unilateral hindpaw on the hot-plate gently and made sure the entire plantar surface of the rat's left or right hindpaw was placed on the hot-plate. The time to hindpaw withdrawal was measured in seconds (s) and referred to as HWL to thermal stimulus.

The Randall Selitto Test (Ugo Basile, Type 7200, Italy) was used to assess the HWL to mechanical stimulation. A wedge-shaped pusher with a loading rate of 30 g/s was applied to the dorsal surface of a hindpaw and the mechanical stimulation required to initiate the struggle response was assessed and expressed in seconds.

All rats were accustomed to the testing conditions for 5 days before the experiment was run. The HWLs were usually between 3 and 6 s. Every measurement of the HWLs to both thermal and mechanical stimulation was finished within 2 minutes (min). A cut-off limit of 15 s was set up to avoid tissue damage.

2.3. Intra-NAc injection

The rats were anesthetized by intraperitoneal injection sodium pentobarbital (45 mg/kg) and were placed on a stereotaxic instrument. A stainless steel guide cannula of 0.8 mm outer-diameter was directed to the NAc (B, +1.7 mm; L or R, 1.6 mm; V, 7.0 mm. B, anterior (+) or posterior (−) to Bregma; L or R, left or right to midline; V, ventral to the surface of skull) according to Paxinos and Watson (1998), and was fixed to the skull by dental acrylic. There were 3 days for the rats to recover from the operation before needle injection into the brain. On the experimental day a stainless steel needle with 0.4 mm out-diameter was directly inserted into the guide cannula with 1.5 mm beyond the tip of the latter. One microliter of solution was infused into the NAc over 1 min, and the injection needle was left in the place for 30 s after each injection.

2.4. Carrageenan-induced inflammation

On the day of the experiment, the inflammation was produced by unilateral subcutaneous injection of 0.1 ml of 2% carrageenin into the plantar region of the rat's left hindpaw (Yu et al., 1998; Sun et al., 2003; Xiong et al., 2005). Three hours (h) after injection of carrageenan, the HWLs were measured. Then each animal received an intra-NAc injection of solution. At 15 min after intra-NAc injection, the HWLs were assessed.

2.5. Western bolt assay of GalR1 and GalR2

Rats were anesthetized with 4% isofurane and decapitated, and the NAc were rapidly excised. The levels of GalR1 and GalR2 were analyzed by western blot assay and the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was also determined as an internal control. Tissues were homogenized in ice-cold lysis buffer and the whole tissue lysate homogenates were centrifuged at 12,000 × g for 20 min, and the supernatant was collected. The protein concentration was determined by a Bio-Rad (Hercules, CA) protein assay. For western blot analysis, an equal amount of protein (20 μg) was loaded in each well and separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, then transferred onto nitrocellulose membrane and blocked in 5% nonfat dry milk prepared in Tris Buffered Saline Tween (TBST). The membranes were incubated with the primary antibody overnight at 4 °C. The following primary antibodies were used: goat anti-GalR1 polyclonal IgG (1:500, Thermo), goat anti-GalR2 polyclonal IgG (1:500, Biorbyt) or mouse anti-GAPDH monoclonal IgG (1:500, Millipore). After having been washed three times for 15 min with TBST, the membranes were incubated with rabbit anti-goat IgG-Horse Reddish Peroxidase (HRP) (1:4000, Santa Cruz Biotechnology) or goat anti-mouse IgG-HRP (1:4000, Santa Cruz Biotechnology) for 1 h at room temperature. The bands were exposed to Fuji Medical X-Ray Film (Fuji Photo Film Co., Ltd., Karagawa, Japan) and the band density was determined by Image J software.

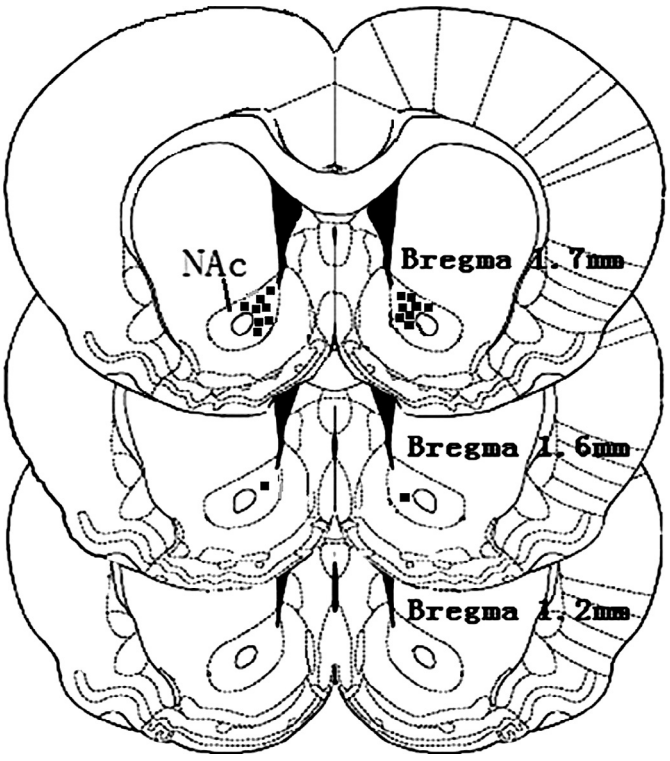

Fig. 1. Illustration of the location of the injection needle tips. NAc: nucleus accumbens.

2.6. Chemicals

Solutions for intra-NAc injection were prepared with 0.9% sterilized saline, each with a volume of 1 μl containing: (1) 1, 2 or 3 nmol of galanin (rat galanin, Tocris, UK); (2) 3 nmol of galantide (galanin (1–13)-substrate P (5–11) amide, Bachem, Switzerland).

2.7. Statistical analysis

At the end of the experiments, the location of the tip of the injection needle was verified and the injection points were shown in Fig. 1. Only the results from nociceptive tests that the tips of the injection needle were within the NAc were used for statistical analysis. The data from nociceptive tests were expressed as mean ± S.E.M. The difference between groups was determined by two-way ANOVA (repeated measures) followed by Bonferroni post