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Involvement of protein kinase C in the galanin-induced antinociception in the brain of rats

Shi Jiao[a,b], Li-Bo Fu[a,c], Long-Chuan Yu[a,*]

[a] Neurobiology Laboratory and State Key Laboratory of Biomembrane and Membrane Biotechnology, College of Life Sciences, Peking University, Beijing 100871, PR China
[b] College of Animal Science and Veterinary Medicine, Shenyang Agricultural University, Shanyang 110866, PR China
[c] College of Life Science, Changchun Normal University, Jilin 130032, PR China

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ABSTRACT

Previous study in our laboratory demonstrates that microinjection of galanin into the arcuate nucleus of hypothalamus produced antinociceptive effects in rats. In the present study we investigated the involvement of protein kinase C (PKC) and PKC signaling pathways in the galanin-induced antinociception in the brain of rats. Intracerebroventricular injection of galanin produced antinociceptive effects in rats tested by hot-plate and Randall Selitto test. Interestingly, the galanin-induced antinociception was significantly attenuated by intracerebroventricular injection of the PKC inhibitor chelerythrine, indicating an involvement of PKC in the galanin-induced antinociception in rats. Taken together, the results demonstrate that galanin induces antinociceptive effects in the rat brain, and PKC is involved in the galanin-induced antinociception in the brain of rats.

The neuropeptide galanin is initially isolated from porcine intestine [19]. Galanin is widely expressed in the central and peripheral nervous systems and plays multiply physiological functions [7,11,15]. There are three types of galanin receptors, GalR1, GalR2 and GalR3, all of them are G-protein-coupled receptor (GPCR) [7]. Previous study in our laboratory demonstrated that galanin played an antinociceptive role in the arcuate nucleus of hypothalamus in intact rats, rats with inflammation and rats with chronic neuropathic pain [5,18].

It is known that protein kinase C (PKC) is important for intracellular signal transduction and plays critical roles in regulating neural plasticity and pains responses [1,2,10,13]. PKC has emerged as a critical second messenger in sensitization toward mechanical stimulation in models of neuropathic pain [13]. PKC epsilon and PKA mediate pain and sensitization of transient receptor potential vanilloid receptor 1 induced by agonists of rotease-activated receptor 2 [1]. PKC gamma expression in the trigeminal spinal subnucleus caudalis plays an important role in the mechanisms of orofacial static mechanical allodynia following trigeminal nerve injury [14]. Nerve growth factor mediates pancreatic inflammatory pain via PKC-dependent signaling of TRPV1 [2]. Recent study in our laboratory found that PKC is involved in morphine tolerance at the spinal level of rats, and intrathecal administration of a PKC inhibitor can block the development and maintenance of morphine tolerance [10].

The present study was performed to investigate the involvement of PKC and PKC signaling pathways in the galanin-induced antinociception in the brain of rats.

Experiments were performed on freely moving male Sprague–Dawley rats weighting between 200 and 250 g (Experimental Animal Center, Academy of Military Medical Sciences, Beijing, China). The rats were housed in cages with free access to food and water, and maintained in a room temperature of $20 \pm 4\,^{\circ}C$ with a 12 h light-dark cycle. All experiments were conducted according to the guideline of the International Association for the Study of Pain [22] and every effort was made to minimize both the animal suffering and the number of animals used.

Rats were accustomed to the test condition for 5 days before the experiment to minimize the stress induced by handling and measurements. The hind paw withdrawal latencies (HWLs) during thermal and mechanical stimulation were measured as described previously [9,11]. Briefly, the entire ventral surface of the rat hind paw was placed manually on a hot plate, which was maintained at a temperature of $52 \pm 2\,^{\circ}C$. The time to hind paw 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 hind paw. The latency required to initiate the withdrawal response was assessed and expressed in seconds. Before intrac-

* Corresponding author. Tel.: +86 10 6276 2099; fax: +86 10 6275 1526.
E-mail address: yulc@pku.edu.cn (L.-C. Yu).

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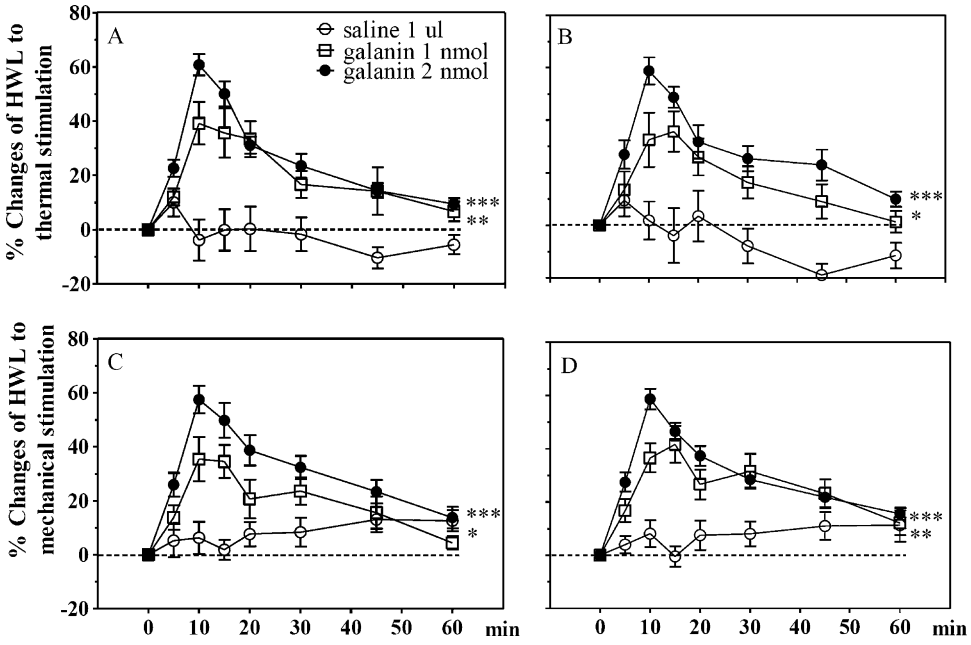

Fig. 1. Effects of intracerebroventricular injection of galanin on HWLs to thermal (A and B) and mechanical stimulation (C and D) in rats. HWL, hindpaw withdrawal latency. Data are presented as mean ± S.E.M., and the difference between groups was determined by two-way analysis of variance (ANOVA).

erebroventricular injection, the HWL was 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 2 min. A cut-off limit of 15 s was set up to avoid tissue damage.

Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) and mounted on a stereotaxic instrument. A stainless steel guide cannula of 0.8 mm outer-diameter was directed into the intracerebroventricular (B, −0.8 mm; L or R, 1.5 mm; V, 3.6 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 [16]. 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 diameter was directly inserted into the guide cannula with 1 mm beyond the tip of the latter. One microliter of solution was thereafter infused into the brain over 1 min. The measurement was performed on the left and right hindpaw at each time point. The HWLs recorded during subsequent measurements (measured at 5, 10, 15, 20, 30, 45 and 60 min after the injection) were expressed as percentage changes from the basal level for each rat. Each rat was tested with both types of stimulation.

Solutions for intracerebroventricular injection were prepared with sterilized saline (0.9%), each volume of 1 μl containing: (1) 1 and 2 nmol of galanin (galanin, Tocris, UK); (2) 1, 5 and 10 nmol of chelerythrine (Sigma, St. Louis, Mo). The doses of galanin and PKC inhibitor were determined according to the published papers of our laboratory [5,9,10,20,21].

At the end of the experiments, rats were killed by a high dose of pentobarbital (80 mg/kg), and the heads were fixed in 4% formalin for 24 h with the injecting tube in situ before section. The location of the tip of the injecting tube was verified. In the present study, all the tips of the injection needle were located in the cerebral ventricle of rats. Data from the experiment were expressed as mean ± S.E.M., statistical difference between groups was determined by two-way analysis of variance (ANOVA) for repeated measurements ($F_{\text{left/left}}$ is the F value of the two groups: the left HWL of the first group compared with the left HWL of the second group). $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ are considered as significant differences.

First, we investigate the influence of intracerebroventricular injections of galanin on the nociceptive responses to thermal and mechanical stimulation in rats. Three groups of rats received intracerebroventricular injections of 1 nmol ($n = 8$) and 2 nmol of galanin ($n = 8$), or 1 μl of 0.9% saline ($n = 8$) as a control. As shown in Fig. 1, the HWLs to both thermal and mechanical stimulation increased significantly after intracerebroventricular injection of 1 nmol of galanin (thermal test: $F_{\text{left/left}} = 14.15$, $P < 0.01$; $F_{\text{right/right}} = 8.41$, $P < 0.05$; mechanical test: $F_{\text{left/left}} = 4.76$, $P < 0.05$; $F_{\text{right/right}} = 14.62$, $P < 0.01$) or 2 nmol of galanin (thermal test: $F_{\text{left/left}} = 43.81$, $P < 0.001$; $F_{\text{right/right}} = 27.64$, $P < 0.001$; mechanical test: $F_{\text{left/left}} = 26.85$, $P < 0.001$; $F_{\text{right/right}} = 36.77$, $P < 0.001$). The results demonstrated that galanin induced antinociceptive effects in the brain of rats.

Furthermore, the involvement of PKC and PKC signaling pathways in the galanin-induced antinociception in the brain of rats were investigated. Four groups of rats received intracerebroventricular injection of 2 nmol of galanin, followed 5 min later by 1 nmol ($n = 8$), 5 nmol ($n = 8$) and 10 nmol of chelerythrine ($n = 8$), or 1 μl of 0.9% saline as the control group ($n = 8$). The HWL to thermal and mechanical stimulation increased significantly after intracerebroventricular injection of 2 nmol of galanin. Interestingly, after intracerebroventricular injection of 5 nmol (thermal test: $F_{\text{left/left}} = 11.82$, $P < 0.01$; $F_{\text{right/right}} = 20.51$, $P < 0.01$; mechanical test: $F_{\text{left/left}} = 11.13$, $P < 0.01$; $F_{\text{right/right}} = 10.26$, $P < 0.01$) and 10 nmol of chelerythrine (thermal test: $F_{\text{left/left}} = 14.92$, $P < 0.01$; $F_{\text{right/right}} = 30.41$, $P < 0.001$; mechanical test: $F_{\text{left/left}} = 12.37$, $P < 0.01$; $F_{\text{right/right}} = 9.46$, $P < 0.01$) the HWLs to thermal and mechanical stimulation decreased significantly compared with the control group. While in rats received intracerebroventricular injection of 1 nmol of chelerythrine, the HWLs to thermal and mechanical stimulation showed no significant changes (thermal test: $F_{\text{left/left}} = 0.00$, $P = 0.97$; $F_{\text{right/right}} = 0.89$, $P = 0.35$; mechanical test: $F_{\text{left/left}} = 0.37$, $P = 0.55$; $F_{\text{right/right}} = 0.15$, $P = 0.70$) compared to the control group, as shown in Fig. 2.

Another group of rats received intracerebroventricular injections of 1 μl of 0.9% saline, followed 5 min later by intracerebroven-