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Activation of galanin receptor 1 inhibits locus coeruleus neurons via GIRK channels

Yun-Fei Bai [a], Hai-Tao Ma [b], Li-Na Liu [c], Hui Li [d], Xiao-Xiao Li [a], Yu-Tao Yang [a], Bing Xue [c], Di Wang [b], Zhi-Qing David Xu [a, e, *]

[a] Department of Neurobiology, Beijing Key Laboratory of Neural Regeneration and Repair, Beijing Laboratory of Brain Disorders (Ministry of Science and Technology), Beijing Institute for Brain Disorders, Capital Medical University, Beijing, 100069, China
[b] Department of Physiology, Liaoning Medical University, Jinzhou, 121000, China
[c] Medical Experiment and Test Center, Capital Medical University, Beijing, 100069, China
[d] Department of Anatomy, Capital Medical University, Beijing, 100069, China
[e] Department of Neuroscience, Karolinska Institutes, 17177, Stockholm, Sweden

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ABSTRACT

The noradrenergic neurons of the locus coeruleus (LC) are associated with various brain functions and psychiatric disorders, such as addiction and depression. It has been shown that neuropeptide galanin (GAL) inhibits neuronal excitability in LC, but the mechanisms remain unclear. In the present study, we investigated the ionic and signal transduction mechanisms underlying inhibitory effect of GAL on LC neurons using whole-cell patch clamp recording in rat brain slices. Bath application of GAL decreased the spontaneous firings and induced a dose-dependent hyperpolarization of LC neurons and this effect was attenuated by knockdown of Galr1, but not Galr2, confirming that mainly GALR1 mediates the inhibition effect of GAL. The inhibitory effect of GAL was also blocked by treatments of pertussis toxin (PTX), GTP-γ-s or GDP-β-s, respectively, indicating that the functions of PTX sensitive $G_{i/o}$ protein are required for GAL-induced hyperpolarization. Moreover, the blockers of GIRK (tertiapin-Q or SCH2 3390 hydrochloride) attenuated the GAL response while blocker of BK/SK/K_{ATP} channels or TASK-1/3 channels did not affect it significantly, suggesting that GIRK channels play an important role in GAL-induced hyperpolarization in LC neurons. Taken together, the inhibitory effect of GAL on LC neurons is mediated by GALR1 via PTX-sensitive $G_{i/o}$ proteins, which activate GIRK channels.

1. Introduction

The locus coeruleus (LC) neurons are the most prominent noradrenergic nucleus in the central nervous system and play a key role in numerous physiological functions including attention, sleep/arousal and stress responses [1–3], as well as psychiatric disorders, such as addiction and depression [4]. The pacemaker properties (spontaneous firings) observed in homogeneous LC neurons are modulated by several neurotransmitters including neuropeptides [5,6]. For example, orexins are demonstrated to depolarize membrane potential of LC neurons by activating nonselective cationic channels, and by inhibiting K^+ channels [7]. In contrast, LC neurons are subjected to inhibitory effects by noradrenaline (NE), which activates G-protein coupled inwardly rectifying K^+ (GIRK) channels via α2-adrenoceptors [8].

Galanin (GAL) is a 29 amino acid (30 in human) neuropeptide expressed widely in the brain [9], and is associated with the regulation of addiction, depression, epilepsy and Alzheimer's disease [4,10]. The various GAL actions are mediated via three G-protein coupled receptor subtypes, GAL receptor 1–3 (GALR1-3) [11]. The levels of GAL mRNA and its three receptors are differentially expressed in LC neurons among species, as high levels of Galr1/2 mRNA are found in rats [12], while Galr3 mRNA is undetectable [13]. The GAL receptors exert their effects via different signaling pathways. Studies from various cell lines have shown that GALR1 and GALR3 couple with a pertussis toxin (PTX)-sensitive $G_{i/o}$ protein to open GIRK channels in CHO cells [14]. Unlike GALR1 and GALR3, GALR2 predominantly couples with a PTX-resistant $G_{q/11}$ protein to induce an activation of phospholipase C, which

* Corresponding author. Department of Neurobiology, Capital Medical University, Beijing 100069, China.
E-mail address: zhiqingx@ccmu.edu.cn (Z.-Q.D. Xu).

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stimulates of Ca^{2+} release and opens large conductance Ca^{2+}-activated K^+ (BK) channels [15]. Moreover, GAL is shown to co-express with NE in the LC and serotonin in the dorsal raphe nucleus [16,17].

Early electrophysiological studies have reported that GAL decreases spontaneous firings, hyperpolarized membrane potential and induces outward currents of LC neurons in brain slices, presumably via GALR1 and an increase in K^+ conductance [18,19]. However, the exact intracellular signaling pathway underlying the actions of GAL on noradrenergic LC neurons remains to be elucidated. In the present study, we demonstrated that GAL inhibits LC neurons by activating GALR1-$G_{i/o}$ proteins-GIRK channels pathway.

2. Materials and methods

2.1. Preparation of brain slices

Animal experiments were performed under the ethical guidelines of the Ethics Committee of Capital Medical University, conducted in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats (weight 200–350 g) were used for brain slices preparation in electrophysiology experiments. Brain slices containing LC neurons were prepared and processed using procedures previously described [18]. Briefly, the rat was anesthetized with chloral hydrate (60 mg/kg, i. p.) and quickly decapitated. The brain was rapidly removed and submerged into ice-cold and oxygenated (95% O_2/5% CO_2) cutting solution that contained (in mM): 2.5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 2 $MgSO_4$, 2 $CaCl_2$, 10 glucose and 213 sucrose. Coronal slices (300 μM thick) were sectioned using a vibratome (VT1200 S, Leica, Wetzlar, Germany) and then incubated in artificial cerebrospinal fluid (ACSF) which contained (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 2 $MgSO_4$, 2 $CaCl_2$, 25 glucose and saturated with 95% O_2/5% CO_2. Slices were incubated at 34.5 °C for 45 min in ACSF, and were then equilibrated at room temperature (22–26 °C) for at least 30 min prior to recording.

2.2. Electrophysiological recordings

The slice was transferred into the recording chamber (volume was 1.5 ml) and the flow rate of an oxygenated (95% O_2/5% CO_2) ACSF was approximately 2 ml/min. The LC neurons were identified by differential interference contrast infrared video microscopy (BX51WI, Olympus, Tokyo, Japan) with distinct anatomical properties. Whole-cell patch clamp recordings were conducted at holding potential of −60 mV using an EPC-10 amplifier and PatchMaster software (HEKA Elektronic Inc., Lambrecht, Germany). In current clamp mode, the spontaneous APs were measured, and only one neuron was recorded in each slice if drugs were applied. The signals were filtered at 2.9 kHz, digitized at 10 kHz. Patch pipettes with the resistance of 3–5 MΩ were made in two stages on a vertical pipette-puller (PC-10, Narishige, Tokyo, Japan). The pipettes were filled with internal solution containing (in mM): 143 KCl, 8 NaCl, 1 $MgCl_2$, 10 HEPES, 2 Na_2ATP, and 0.4 Na_2GTP (pH adjusted to 7.2 with KOH). In GTP analog experiments, GTP was replaced with 200 μM GTP-γ-s or 4 mM GDP-β-s in the internal solution. Other drugs were all bath applied with a peristaltic pump or bath perfusion, and the time required for complete exchange of the solution was about 30 s. All drugs were applied after recording for at least 5 min of control events. Electrophysiological recordings were carried out at room temperature (22–26 °C).

2.3. Construction of siRNA via lentiviral vector

The rat knockdown lentivirus of Galr1 and Galr2 were purchased from Genechem (Shanghai, PR China). The anti-Galr1 siRNA sequence was 5′-AAGGTTCTCAATC ATCTGCAT-3′, and a lentivirus vector containing the scrambled sequence 5′-GATC CTCTTATGG-CACTTA-3′ was used as a negative control. The sequence of anti-Galr2 is 5′-AGGTGACACGGATGATCAT-3′, and the scrambled sequence 5′-CAAAGGCTACGACATTGTA-3′ was used as a negative control. The GFP was used as a reporter gene for the lentivirus vector encoding the siRNA sequence for Galr1/2 and its respective scrambled sequences.

2.4. Intracerebroventricular injection

The rats were anesthetized with chloral hydrate (60 mg/kg, i. p.) and mounted onto a stereotactic frame. The injection was performed with a 10 μl Hamilton syringe (29 gauge). The intracerebroventricular injection site was the right lateral ventricle (1.5 mm lateral, 0.8 mm posterior and 4.5 mm deep from bregma). PTX (0.5 μg) was administered in the lateral ventricle 24–48 h prior to recording. 3 μl of lentiviral vectors expressing Galr1 or Galr2 siRNA were intracerebroventricular injected into the rat over a period of 10 min. After the injection was complete, the needle was left in place for 10 min to allow the lentiviral solution to sufficiently diffuse into the cerebrospinal fluid prior to withdrawal. The lentiviral vectors expressing the scrambled siRNA (3 μl) of Galr1 and Galr2 were injected as negative controls. 20–30 days post lentiviral transfection, the LC neurons with GFP-fluorescence were recorded.

2.5. Drugs and chemicals

GAL was obtained from ChinaPeptids Co., Ltd (Shanghai, China) and dissolved in water into a 1 mM stock solution, and stored at −80 °C. AR-M1896, tertiapin-Q, SCH 23390 hydrochloride, ML365 and PK-THPP were obtained from Tocris Bioscience (Ballwin, MO, USA). PTX, charybdotoxin, apamin, glibenclamide, GTP-γ-s, GDP-β-s and $BaCl_2$ were purchased from Sigma Chemical (Saint Louis, MO, USA). All drugs were prepared as stock solution and stored at −20 °C. The drugs were dissolved in bath ACSF to obtain the experimental concentration before use and bath-applied for 5–10 min before GAL was applied for 2 min.

2.6. Data analysis

Data were analyzed using Igor (WaveMetrics, Lake Oswego, OR, USA) and GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA) software. The properties of APs were calculated by Mini-Analysis 6.0.1 (Synaptosoft, Inc., Decatur, GA, USA). The concentration-response curve of GAL-induced hyperpolarization was fitted by the Hill equation with GraphPad Prism 5. All results were presented as mean ± SEM. The Student's paired or unpaired *t*-test was used for statistical analysis and $p < 0.05$ was regarded as significant.

3. Results

3.1. Mediation of GAL response via GALR1

In the present study, the noradrenergic LC neurons were recorded using whole-cell patch clamp technique. The basal frequency of spontaneous firing was 0.76 ± 0.49 Hz (n = 42) and the average membrane potential was −54.07 ± 2.72 mV (n = 42). Bath application of 250 nM GAL reversibly decreased the spontaneous discharge and induced a hyperpolarizing response (9.36 ± 0.78 mV, n = 13, Supp. 1 A/C) in all tested LC neurons. Statistical analysis showed that membrane potential decreased from −56.22 ± 0.67 mV to −64.46 ± 1.41 mV after GAL application (n = 6, $p < 0.001$, Supp. 1 B). We also applied tetrodotoxin (TTX) to

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