Neuroscience Letters 584 (2015) 168–172

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

GABA-mediated modulation of ATP-induced intracellular calcium responses in nodose ganglion neurons of the rat

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HIGHLIGHTS

- ATP increased intracellular Ca^{2+} levels in nodose ganglion neurons via P2X receptor.
- ATP increased intracellular Ca^{2+} levels in satellite cells via P2Y receptor.
- GABA inhibited ATP-induced intracellular Ca^{2+} responses in isolated neurons.
- Bicuculline enhanced ATP-induced Ca^{2+} responses in neurons with satellite cells.
- Neuronal excitability may be modulated by GABA from satellite cells in nodose ganglion.

ARTICLE INFO

Article history:
Received 16 September 2014
Received in revised form 6 October 2014
Accepted 7 October 2014
Available online 17 October 2014

Keywords:
Nodose ganglion
Sensory neuron
Satellite cell
ATP
GABA

ABSTRACT

We examined ATP-induced intracellular Ca^{2+} ($[Ca^{2+}]_i$) responses in the neurons and satellite cells from one of the viscerosensory ganglia, the nodose ganglion (NG), as well as the GABA-mediated modulation of ATP-induced neuronal $[Ca^{2+}]_i$ responses using intracellular calcium imaging. In neurons with satellite cells, ATP induced $[Ca^{2+}]_i$ increases in both the neurons and satellite cells. The P2X receptor agonist, α,β-meATP, induced $[Ca^{2+}]_i$ increases in neurons and this response was inhibited by the P2X receptor antagonist, PPADS. On the other hand, the P2Y receptor agonist, ADP, induced $[Ca^{2+}]_i$ increases in satellite cells, and this response was inhibited by the P2Y receptor antagonist, MRS2179. RT-PCR detected the expression of P2X2, P2X3, P2Y1, and P2Y2 receptor mRNAs in NG extracts. Immunohistochemistry revealed that NG neurons and satellite cells were immunoreactive to P2X2 and P2X3, and P2Y1 and P2Y2 receptors, respectively. In isolated neurons, the ATP-evoked $[Ca^{2+}]_i$ increase was inhibited by GABA. However, in neurons with satellite cells, the $GABA_A$ receptor antagonist, bicuculline, enhanced the ATP-induced $[Ca^{2+}]_i$ increase in neurons. These results suggest that viscerosensory neuronal excitability may be modulated by GABA from satellite cells in NG.

1. Introduction

The nodose ganglion (NG) is the distal sensory ganglion of vagus nerve and contains viscerosensory neurons related to the vagus reflex for gastrointestinal, respiratory, and cardiovascular functions [1–3]. NG contains the cell bodies of pseudounipolar neurons, which transmit sensory information from visceral organs to the medulla oblongata. NG neurons are surrounded by satellite cells, a type of glial cell in the peripheral nervous system. Although neurons in the sensory ganglia are devoid of synaptic contacts, neurons and satellite cells possess receptors for various neurotransmitters, and previous studies have suggested that neurotransmitters non-synaptically act within sensory ganglia [4,5].

Adenosine 5′-triphosphate (ATP) has been shown to mediate interactions between neurons and satellite cells in sensory ganglia. ATP induced an increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) in both the neurons and satellite cells of the trigeminal ganglion (TG) in the mouse [6,7]. Furthermore, electrical stimulation evoked the release of ATP from the neuronal cell bodies of the dorsal root ganglion (DRG), which, in turn, induced $[Ca^{2+}]_i$ increases in the adjacent satellite cells [8]. ATP is known to act on two families of purinoreceptors, ionotropic P2X and G protein-coupled P2Y receptors,

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http://dx.doi.org/10.1016/j.neulet.2014.10.008
0304-3940/

which consist of seven (P2X1-7) and eight (P2Y1, 2, 4, 6, 11–14) individual receptor subunits, respectively [9,10]. In the sensory ganglia, functional P2X2 and P2X3 receptors were expressed in the neurons of the DRG and TG [11,12], and ATP evoked $[Ca^{2+}]_i$ increases in TG neurons in the mouse via P2X3 receptor [7]. On the other hand, ATP induced $[Ca^{2+}]_i$ increases in the satellite cells of the TG via multiple P2Y receptors [6]. Although P2X2 and P2X3 receptor immunoreactivities were detected in NG neurons of the rat [13], the presence and function of P2 purinoreceptors in NG neurons and satellite cells remain unclear.

In addition to ATP, immunoreactivity to the major inhibitory neurotransmitter gamma-amino butyric acid (GABA) was detected in the neuronal cell bodies of DRG, TG, and NG in the rat [14]. $GABA_A$ receptor immunoreactivity was also localized in the DRG neuronal cell bodies of the rat [15]. In the TG of the rat, functional $GABA_A$ receptors were expressed in neuronal cell bodies, and the surrounding satellite cells were immunoreactive for GABA [16]. These findings suggested that an endogenous GABAergic modulatory system may exist in the sensory ganglia. Furthermore, GABA was shown to inhibit the excitatory effects of ATP on the DRG neurons of the rat via $GABA_A$ receptors [17]. Therefore, ATP-induced neuronal excitability is expected to be modulated by endogenous GABA in the sensory ganglia.

In the present study, we investigated $[Ca^{2+}]_i$ changes in NG neurons and satellite cells using NG neurons with satellite cells as well as isolated neurons to determine whether ATP- and GABA-mediated neuron-satellite cell interactions existed in NG. We measured the $[Ca^{2+}]_i$ responses of neurons and satellite cells to ATP and the agonists of P2X and P2Y receptors to examine the expression and function of those receptors. We also examined the mRNA expression and immunohistochemical localization of P2X2, P2X3, P2Y1, and P2Y2 receptors in NG by RT-PCR and immunohistochemistry. We evaluated $[Ca^{2+}]_i$ changes in NG neurons and satellite cells following the application of ATP and GABA or their antagonists.

2. Materials and methods

2.1. Animals

Eighteen male Wistar rats (8–10 weeks old; 180–200 g) were purchased from Japan SLC (Hamamatsu, Japan). All animal experiments in the present study were approved by the Local Animal Ethics Committee of Iwate University (accession number #A201325).

2.2. Intracellular calcium imaging

The methods of intracellular calcium imaging were performed as previously reported [18], with some modifications. To prepare the specimens of NG neurons with satellite cells, NGs were placed in Dulbecco's modified Eagle's medium-F-12 (DMEM/F12; GIBCO, Tokyo) containing 0.4 mg/ml collagenase P (1213857; Roche Applied Science, Mannheim, Germany), and incubated for 25 min at 37 °C. Partially digested ganglia were dropped onto coverslips, and then incubated at 37 °C in a humidified atmosphere of 95% air–5% CO_2. For the preparations of isolated NG neurons, several pieces of NG were placed in DMEM/F12 containing 2.0 mg/ml collagenase P and 0.5 mg/ml trypsin (Difco, Detroit, MI, USA), and incubated for 1 h at 37 °C. The suspension was subjected to centrifugation at 1500 rpm for 5 min, and the supernatant was aspirated. The pellet of ganglion neurons was plated onto coverslips. Cells were cultured under the same conditions as neurons with satellite cells. KCl solution was prepared by simply adding KCl to HEPES-buffered Ringer's solution (HR). The drug ATP (A2383; Sigma, St. Louis, MO, USA), α,β-methylene ATP (α,β-meATP; M6517; Sigma), pyridoxal phosphate-6-azophenyl-2,4-disulfonic acid (PPADS; P178; Sigma), adenosine 5′-diphosphate (ADP; A2754; Sigma), 2′-deoxy-N^6-methyladenosine-3′,5′-diphosphate (MRS 2179; M3808; Sigma), and GABA (A2129; Sigma) were prepared from stocks at the desired concentrations in HR. (+) Bicuculline (14340; Fluka) was dissolved in DMSO and diluted to the final concentration in HR.

2.3. RT-PCR

RT-PCR analysis was performed to investigate the mRNA expression of the P2X2, P2X3, P2Y1, and P2Y2 receptors in NG. For RT-PCR analysis, rats were anesthetized using diethyl ether and euthanized by exsanguination from the abdominal aorta. NG was removed and frozen in liquid nitrogen. Total RNA from NG was extracted using a magnetic bead method (MELT total RNA extraction kit, Ambion, Austin, TX, USA). RT-PCR was performed using a QIAGEN One Step RT-PCR Kit (Qiagen, Tokyo, Japan) with gene-specific primers for P2X2, P2X3, P2Y1, P2Y2, and β-actin as internal controls. Details of the primers used in the present study are shown in Table 1. Reverse transcription was performed for 30 min at 50 °C and initial PCR activation was incubated for 15 min at 95 °C. Following reverse transcription, PCR amplification was performed 40 times as follows: 30 s at 94 °C for denaturation, 30 s at 60 °C for annealing, and 1 min at 72 °C for extension. After PCR amplification, a final extension was performed for 10 min at 72 °C. PCR end products were visualized on 2% agarose gels using ethidium bromide. The mRNA templates were omitted for a negative control.

2.4. Immunohistochemistry

The methods of immunohistochemistry have been described previously [18]. The following antibodies were used: polyclonal guinea pig antibody against P2X2 (1:100; GP14106, Neuromics, Edina, MN, USA), and polyclonal rabbit antibody against P2X3 (1:1000; RA10109, Neuromics), P2Y1 (1:100; APR-009, Alomone Labs, Jerusalem, Israel), or P2Y2 (1:100; APR-010, Alomone Labs). As secondary antibodies, Alexa Fluor 488 conjugated donkey antibody against either guinea pig IgG (1:200; 706-545-148, Jackson ImmunoResearch, West Grove, PA, USA), or rabbit IgG (1:200; A21206, Invitrogen, Carlsbad, CA, USA) were used. After incubation with secondary antibodies, sections were then counterstained with DAPI and coverslipped with mounting medium (Fluoromount, Diagnostic Biosystems, Pleasanton, CA, USA). Sections were examined with a confocal laser scanning microscope (C1; Nikon, Tokyo, Japan).

3. Results

3.1. ATP- and P2 purinoreceptors agonist-induced $[Ca^{2+}]_i$ responses

In NG neurons with satellite cells, the application of ATP (2.5 μM) for 10 s induced transient $[Ca^{2+}]_i$ increases in both the neurons and satellite cells ($n = 8$; Fig. 1A–E). KCl (60 mM) for 30 s evoked a $[Ca^{2+}]_i$ increase in NG neurons, but did not change $[Ca^{2+}]_i$ in the satellite cells (Fig. 1D and E). The P2X receptor agonist, α,β-meATP (200 μM), for 5 s evoked a transient $[Ca^{2+}]_i$ increase in the neurons with satellite cells (Fig. 2A–E). However, the α,β-meATP-induced $[Ca^{2+}]_i$ response in the same neurons was reversibly inhibited by preincubating with the P2X receptor antagonist, PPADS (10 μM), for 30 s ($n = 4$; Fig. 2C–E). The P2Y receptor agonist, ADP (10 μM), for 5 s did not cause any $[Ca^{2+}]_i$ response in NG neurons, whereas an increase in $[Ca^{2+}]_i$ was observed in the same neurons with the application of KCl (60 mM) for 30 s (Fig. 2E). On the other hand, ADP (10 μM) for 5 s induced a transient $[Ca^{2+}]_i$ increase in the satellite cells surrounding NG neurons (Fig. 2F–J). The ADP-evoked