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Research article

Serotonin-mediated modulation of hypoxia-induced intracellular calcium responses in glomus cells isolated from rat carotid body

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HIGHLIGHTS

- We examined serotonin-induced intracellular Ca^{2+} response in rat carotid body glomus cells.
- Serotonin did not change intracellular Ca^{2+} levels in glomus cells during normoxia.
- Serotonin enhanced hypoxia-induced intracellular Ca^{2+} responses in glomus cells.
- Ketanserin inhibited an elevation of hypoxia-induced Ca^{2+} responses in glomus cells by serotonin.
- Hypoxic chemosensitivity of glomus cells may be modulated by serotonin in carotid body.

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ABSTRACT

In the present study, we examined serotonin (5-HT)-induced intracellular Ca^{2+} ($[Ca^{2+}]_i$) responses to hypoxia in glomus cells isolated from carotid body (CB) of the rat. 5-HT did not induce any $[Ca^{2+}]_i$ responses in clustered glomus cells during normoxia (21% O_2), whereas, the perfusion of hypoxic solution (1% O_2) induced repetitive increases in $[Ca^{2+}]_i$ in the same specimens. The frequency and magnitude of hypoxia-induced $[Ca^{2+}]_i$ changes observed in the glomus cells were enhanced in the presence of 5-HT, and this response was inhibited by the 5-HT2 receptor antagonist, ketanserin. Furthermore, RT-PCR analysis detected the expression of 5-HT1A, 5-HT1B, 5-HT1D, 5-HT1F, 5-HT2A, 5-HT2B, 5-HT3A, and 5-HT3B receptor mRNAs in extracts of the CB. These results suggest that 5-HT increases hypoxia-induced $[Ca^{2+}]_i$ responses in glomus cells. 5-HT may elevate hypoxic responses in glomus cells in order to increase chemosensory activity of the CB.

1. Introduction

The carotid body (CB) is a peripheral chemoreceptor that is responsible for monitoring arterial blood levels of partial pressures of oxygen (pO_2) and carbon dioxide (pCO_2), and pH. Hypoxia has been shown to stimulate glomus cells within the CB in order to release excitatory transmitter, adenosine 5′-triphosphate (ATP), and triggers chemosensory discharges in the afferent nerve terminals of the carotid sinus nerve [1,2]. Afferent signals from the CB are carried to the nucleus solitary tract, leading to an increase in ventilation [3]. Previous studies also detected dopamine in glomus cells, and showed that it plays an inhibitory role in hypoxic chemotransduction in the CB [4,5]. In addition to these transmitters and modulators, carotid sinus nerve discharge and ventilatory responses were increased by an intracarotid injection of serotonin (5-hydroxytryptamine; 5-HT), and the 5-HT-evoked ventilatory effect was abolished by CB denervation in the rat [6]. Thus, chemosensory activity in the CB may be enhanced by 5-HT. In our previous study, immunoreactivity for the 5-HT biosynthetic enzyme, tryptophan hydroxylase 1, and 5-HT plasma membrane transporter was detected in glomus cells and perivascular sympathetic nerve fibers in the CB of the rat [7]. Our previous findings suggest that 5-HT is endogenously synthesized and released from glomus cells and sympathetic nerve fibers in the CB. In the CB of the rat, 5-HT2A and 5-HT5A receptors were previously shown to be expressed in the glomus cells by reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry [8,9]. Moreover, 5-HT2A receptor was shown to be involved in elevation of

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membrane potential in the glomus cells isolated from the rat [9]. Therefore, the hypoxic chemosensitivity of glomus cells is expected to be modulated by 5-HT from these cells in the CB.

In the present study, we examined intracellular Ca^{2+} ($[Ca^{2+}]_i$) changes as an index of hypoxic chemosensitivity in glomus cells isolated from the CB of the rat following the application of 5-HT and its antagonist. We also investigated the mRNA expression of 5-HT receptors in the CB by RT-PCR.

2. Materials and methods

2.1. Animals

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 #A201326).

2.2. Intracellular calcium imaging

Rats were anesthetized with diethyl ether and euthanized by exsanguination from the abdominal aorta. CBs were immediately removed bilaterally and immersed in ice-cold Dulbecco's modified Eagle's medium-F-12 (DMEM/F12; GIBCO, Tokyo). Each CB was cut into four pieces, placed in DMEM/F12 containing 2.0 mg/ml collagenase P (Roche Applied Science, Mannheim, Germany) and 2.0 mg/ml dispase (Roche Applied Science), and incubated for 30 min at 37 °C. Partially digested CBs were plated onto poly-L-lysine-coated coverslips. Modified DMEM/F12 solution (DMEM/F12 containing 1% glucose, 50 U/ml penicillin, and 50 μg/ml streptomycin) was placed as drops on coverslips, and then incubated for 2–3 h at 37 °C in humidified air.

Specimens were loaded for 60 min at 37 °C with 5 μM Fura-2 AM (Dojin, Kumamoto, Japan) and 0.05% cremophor (Fluka, Buchs, Switzerland) diluted in HEPES-buffered Ringer's solution (HR). HR contained 136 mM NaCl, 5.6 mM KCl, 1 mM $MgCl_2$, 2.2 mM $CaCl_2$, 11 mM glucose, and 10 mM HEPES, and was adjusted to pH 7.4 with NaOH while continuously bubbling with 5% CO_2–21% O_2 balanced in N_2. Specimens were rinsed with HR and then transferred to a closed-bath perfusion chamber (RC-20; total volume 48 μl, Warner Instruments, Hamden, CT, USA) placed on the stage of an inverted microscope (IX70; Olympus, Tokyo). Preparations were continuously perfused with the HR at a constant flow rate of 0.4 ml/min. Fura-2 fluorescence images were collected approximately every 5 s with a CCD camera (C4742-95; Hamamatsu Photonics, Hamamatsu, Japan), and analyzed with computer software (AquaCosmos; Hamamatsu Photonics). KCl solution (50 mM; 30 s) was prepared by simply adding KCl to HR, and was perfused at the end of each experimental run to test for cell viability. Hypoxic solution was made by aerating HR with 5% CO_2–1% O_2 balanced in N_2. The drug 5-HT hydrochloride (H9523; Sigma, St. Louis, MO, USA) was dissolved in distilled water. Ketanserin (S006; Sigma) was dissolved in dimethyl sulfoxide. Each solution was diluted to the desired final concentration in HR. The perfusion period of 5-HT and hypoxic solution in the present study was range from 1–10 min and 3–12 min, respectively.

2.3. RT-PCR

RT-PCR analysis was performed to investigate the mRNA expression of 5-HT receptors in CB. For RT-PCR analysis, the CB of either the left or right side was removed and frozen in liquid nitrogen. Total RNA from the CB was extracted using a magnetic bead method (MagExtractor; TOYOBO, Osaka, Japan). RT-PCR was performed using a QIAGEN One Step RT-PCR Kit (Qiagen, Tokyo, Japan) with gene-specific primers for 5-HT receptors 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,

Table 1
Primers for RT-PCR.

mRNA (accession #)	Primer sequences	Position	Productlength
5-HT1A	5′-GGTCACCTGCGACCTGTTTA-3′(sense)	318–337	381 bp
(NM_012585)	5′-ACCTTCCTGACAGTCTTGCG-3′ (antisense)	679–698	
5-HT1B	5′-CCGGCTAACTACCTGATCGC-3′ (sense)	235–254	311 bp
(NM_022225)	5′-AAGAAGGGTGGCAGCGAAAT-3′ (antisense)	526–545	
5-HT1D	5′-CGGAGTCGAATCCTGAACCC-3′ (sense)	664–683	253 bp
(NM_012852)	5′-CCCCCAGAATGATGCCCAAT-3′ (antisense)	897–916	
5-HT1F	5′-CTGTCGGCTATAGCGTTGGA-3′ (sense)	381–400	319 bp
(NM_021857)	5′-TCCGACTTGCTTGTCTCTTGT-3′ (antisense)	679–699	
5-HT2A	5′-CTGGTCATCATGGCAGTGTC-3′ (sense)	339–358	259 bp
(NM_017254)	5′-GGTTCTGGATGGCGACATAG-3′ (antisense)	578–597	
5-HT2B	5′-AAATGAAGCAGACTGCCGAGA-3′ (sense)	348–368	373 bp
(NM_017250)	5′-CACTGATTGGCCTGAATTGGC-3′ (antisense)	700–720	
5-HT2C	5′-GACTACTTGTCATGCCCCTGT-3′ (sense)	654–674	294 bp
(NM_012765)	5′-ACACTTTGCTTTCGTCCCTCA-3′ (antisense)	927–947	
5-HT3A	5′-TGTGCAGAACTGCTCTCTGAC-3′(sense)	625–645	354 bp
(NM_024394)	5′-GAGTATCCCAGAAGGAGCGTG-3′ (antisense)	958–978	
5-HT3B	5′-GTCTACCTGGACCTTTGCGT-3′ (sense)	195–214	483 bp
(NM_022189)	5′-CCGGATGTGGTAAGTGGAGG-3′ (antisense)	658–677	
5-HT4	5′-CCCCTCTACGCATCGCATT-3′ (sense)	421–439	237 bp
(NM_012853)	5′-AATAGGCCAGCACCATGAGG-3′ (antisense)	638–657	
5-HT5A	5′-TTACAAGGCTGCGAAGTTCC-3′ (sense)	813–832	332 bp
(NM_013148)	5′-TGAAGAAGGAGTTGGAATAGCC-3′ (antisense)	1123–1144	
5-HT5B	5′-ATCGCTACTGGACTATCACGC-3′ (sense)	751–771	368 bp
(NM_024395)	5′-CGCGGTGAATACCGTCTCA-3′ (antisense)	1100–1118	
5-HT6	5′-GTGCCATCTGCTTCACCTACT-3′ (sense)	923–943	294 bp
(NM_024365)	5′-GCCAGGTGACAAAGAACATTCC-3′ (antisense)	1141–1162	
5-HT7	5′-TGGTGATCTCGGTGTGCTTC-3′ (sense)	429–448	499 bp
(NM_012765)	5′-TTCCTGGCGGCCTTGTAAAT-3′ (antisense)	908–927	
β-actin	5′-CCCTGAAGTACCCCATTGAA-3′ (sense)	275–294	242 bp
(NM_031144)	5′-ACCAGAGGCATACAGGGACA-3′ (antisense)	497–516	

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