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Immunohistochemical localization of dopamine D2 receptor in the rat carotid body

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ABSTRACT

Dopamine modulates the chemosensitivity of arterial chemoreceptors, and dopamine D2 receptor (D2R) is expected to localize in the glomus cells and/or sensory nerve endings of the carotid body. In the present study, the localization of D2R in the rat carotid body was examined using double immunofluorescence for D2R with various cell markers. D2R immunoreactivity was mainly localized in glomus cells immunoreactive to tyrosine hydroxylase or dopamine β-hydroxylase (DBH), but not in S100B-immunoreactive sustentacular cells. Furthermore, D2R immunoreactivity was observed in petrosal ganglion cells and nerve bundles in the carotid body, but not in the nerve endings with P2X2 immunoreactivity. In the carotid ganglion, a few punctate D2R-immunoreactive products were detected in DBH-immunoreactive nerve cell bodies. These results showed that D2R was mainly distributed in glomus cells, and suggested that D2R plays a role in the inhibitory modulation of chemosensory activity in a paracrine and/or autocrine manner.

1. Introduction

The carotid body (CB) is an arterial chemoreceptor that contains glomus cells (type I cells), which exhibit sensitivity to O_2, CO_2, and H^+, and sustentacular cells (type II cells) (Dasso et al., 2000; Nurse, 2005, 2014). Glomus cells receive carotid sinus nerve (CSN), which are sensory afferent nerves, from the petrosal ganglion of the glossopharyngeal nerve (Iturriaga and Alcayaga, 2004; Lahiri et al., 2006). When glomus cells sense changes in arterial blood gas levels, they depolarize and release excitatory neurotransmitters such as acetylcholine and ATP. It has been reported that ATP excites glossopharyngeal afferent terminals *via* postsynaptic P2X2/3 receptors (Piskuric and Nurse, 2013), and induce hyperventilation by transmitting information concerning pO_2 and pCO_2/H^+ to the nucleus solitary tract *via* CSN (Fitzgerald et al., 2006; Gourine, 2005; Nurse, 2005; Zhang and Nurse, 2004).

In addition to excitatory neurotransmitters, catecholamine such as dopamine and noradrenaline is synthesized and released by glomus cells in response to hypoxia (Bairam et al., 2001; Fidone and Gonzalez, 1982; Fidone et al., 1982; Vicario et al., 2000). Dopamine has been shown to decrease the hypoxia-evoked firing rate of CSN in an isolated preparation of the cat CB with CSN (Iturriaga and Alcayaga, 2004), and the inhibition of peripheral D2R by domperidone, a D2R antagonist, enhanced CSN activity (Iturriaga et al., 1996). On the other hand, Zapata (1975) reported that dopamine sometimes increased the firing rate of CSN in an isolated preparation of the cat CB with CSN. Dopamine receptors are G protein coupling receptors that have been classified into five subtypes; D1 and D5 are coupled with Gαs, while D2, D3, and D4 are coupled with Gαi (Jaber et al., 1996). The mRNAs of the D1 receptor and D2 receptor (D2R) have been detected in the rat CB by *in situ* hybridization or RT-PCR (Bairam et al., 1998; Czyzyk-Krzeska et al., 1992; Gauda et al., 1996). At the protein level, D2R immunoreactivity has been detected in the CB of the mouse and human (Fagerlund et al., 2010; Kåhlin et al., 2010; Lazarov et al., 2009). Moreover, D2R mRNA was also shown to be expressed in the rat petrosal ganglion (Bairam et al., 2003; Czyzyk-Krzeska et al., 1992; Gauda et al., 1996). Although it is expected dopamine bind both glomus cells and nerve endings from petrosal ganglion neurons, it is unknown exact mechanism of dopamine-mediated modulatory action on signal transduction between glomus cells and sensory nerve endings.

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On the other hand, it has been reported that sympathetic and parasympathetic nerve fibers were widely distributed in the CB (Kummer, 1997). Postganglionic sympathetic nerve fibers in the CB are derived from the superior cervical ganglion and the carotid ganglion that diffusively distributed in the periphery of the CB. Functionally, electrical stimulation of cervical sympathetic nerve trunk induces increase of CSN activity (Eyzaguirre and Lewin, 1961). Because D2R mRNA is expressed in the superior cervical ganglion cells (Czyzyk-Krzeska et al., 1992; Gauda et al., 1996), it is suspected that D2R is distributed in the autonomic nerves in the CB to modulate chemosensory function.

In the present study, we determined the exact histological localization of D2R in CB, petrosal ganglion and carotid ganglion using double-immunofluorescence for D2R with various immunohistochemical markers for the glomus cells, sustentacular cells, sensory nerve endings, and autonomic nerve fibers. We tried to discuss D2R-mediated dopamine modulation for chemosensory activity in rat carotid body.

2. Materials and methods

Animal experimental protocols were approved by the Committee on the Use of Live Animals in Teaching and Research of Iwate University (approval number: A201326).

Male Wistar rats (8–10 weeks old, $n=6$) were anesthetized with pentobarbital sodium (50 mg/kg; intraperitoneal injection) and perfused transcardially with Ringer's solution (200 ml) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4, 200 ml). The bifurcations of carotid arteries containing CB and petrosal ganglion were then removed under a dissecting microscope and immersed in the same fixative for an additional 6–8 h at 4 °C. After three washes in phosphate buffered saline (PBS, pH 7.4) for 10 min, tissue samples were soaked in 30% sucrose in PBS for 8 h at 4 °C and frozen. The CB was sectioned at a thickness of 10 μm on the cryostat and mounted on glass slides coated with chrome alum-gelatin.

We performed double staining for D2R and tyrosine hydroxylase (TH), S100B, dopamine β-hydroxylase (DBH), the P2X2 receptor (P2X2), or vesicular acetylcholine transporter (VAT) in order to clarify the localization of D2R in the rat CB. TH and S100B are used as general markers of glomus cells and type II cells, respectively. DBH serves as a marker for sympathetic nerve fibers (Chow et al., 2001; Dickson et al., 1981). DBH immunoreactivity has also been detected in some glomus cells (Kato et al., 2013). P2X2 was previously shown to be expressed in nerve terminal enclosed glomus cells, and, thus, is a marker for sensory nerve terminals (Prasad et al., 2001). VAT is used as a marker for parasympathetic nerve fibers (Dixson et al., 2000; Schäfer et al., 1998). Details on the antibodies used in the present study are shown in Table 1, while those on antibody combinations are summarized in Table 2. Sections were rinsed in PBS (3 × 5 min) and incubated for 30 min at room temperature with non-immune donkey serum (1:50). After being incubated, sections were washed in PBS (3 × 5 min), incubated for 12 h with primary antibodies, and then rinsed in PBS (3 × 5 min). The sections were then incubated for 2 h with secondary antibodies and washed in PBS (3 × 5 min). They were counterstained with DAPI and coverslipped with Fluoromount (Diagnostic BioSystems, Pleasanton, CA).

According to D2R antibody, rabbit anti-D2R (Frontier Science) was raised against a synthetic polypeptide of 270–370 amino acid residues of the mouse D2R (Narushima et al., 2006). D2R antibodies have been used in immunohistochemical analyses of brain tissue (Narushima et al., 2006; Uchigashima et al., 2007). In the present study, we performed a pre-absorption test of D2R antibody for immunohistochemical control. The antibody for D2R (2 μg/ml) was incubated for 24 h at 4 °C with an antigen polypeptide for D2R (≥2.0 μg/ml, G015-D2R-AG, Frontier Science, Sapporo, Japan). Carotid body sections were then stained using of this preabsorbed antibody in place of a primary antibody by immunofluorescence as described above.

Table 1
Antibodies used in the present study.

	Catalog number	Host	Dilution	Source
Primary antibodies				
Dopamine D2 receptor (D2R)	D2R-Rb-Af	Rabbit	1:200	A
Tyrosine hydroxylase (TH)	MAB318	Mouse	1:2000	B
Dopamine â-hydroxylase (DBH)	ab31126	Mouse	1:4000	C
S100B	SH-B1	Mouse	1:2000	D
P2X2 receptor (P2X2)	GP14106	Guinea pig	1:1000	E
Vesicular acetylcholine transporter (VAT)	BML-SA109	Goat	1:10,000	F
Secondary antibodies				
Cy3, anti-rabbit IgG	711-165-152	Donkey	1:200	G
Alexa488, anti-mouse IgG	711-545-150	Donkey	1:200	G
Alexa488, anti-guinea pig IgG	706-545-148	Donkey	1:200	G
Alexa488, anti-goat IgG	A11055	Donkey	1:200	G

A, Frontier Science, Sapporo, Japan; B, Merck Millipore, Billerica, MA, USA; C, Abcam, Cambridge, UK; D, Sigma, Saint Louis, CA, USA; E, Neuromics, Edina, MN, USA; F, Enzo Life Sciences, Farmingdale, NY, USA; G, Jackson ImmunoResearch, West Grove, PA, USA.

Table 2
Combinations of antibodies for double immunofluorescence.

Primary antibody 1	Secondary antibody 1	Primary antibody 2	Secondary antibody 2
D2R	Cy3, anti-rabbit IgG	TH	Alexa488, anti-mouse IgG
D2R	Cy3, anti-rabbit IgG	DBH	Alexa488, anti-mouse IgG
D2R	Cy3, anti-rabbit IgG	S100B	Alexa488, anti-mouse IgG
D2R	Cy3, anti-rabbit IgG	P2X2	Alexa488, anti-guinea pig IgG
D2R	Cy3, anti-rabbit IgG	VAT	Alexa488, anti-goat IgG

The sections were observed by use of a confocal laser microscope (C2, Nikon, Tokyo), and the z-stacks of confocal images were obtained. Projection images were made from 3–5 series at 0.5–1 μm intervals with the computer software, NIS-elements (Nikon, Tokyo, Japan). All images were analyzed with the use of Photoshop CS5 (Adobe Systems, San Jose, CA) in addition to NIS-Elements.

3. Results

In the CB, D2R immunoreactivity was mainly observed in a cluster of glomus cells (Fig. 1A). Punctate D2R-immunoreactive products were detected in the perinuclear region of the cytoplasm of glomus cell (Fig. 1B). In sections of the preabsorption control, no immunoreactive products were observed in any region of the CB (Fig. 1C).

In sections stained by double immunofluorescence for D2R with TH, punctate structures immunoreactive to D2R were observed in TH-immunoreactive glomus cells (Fig. 1D). D2R immunoreactivity was widely distributed in the cytoplasm including perinuclear region and extending cytoplasmic processes. Punctate D2R immunoreactivity was also observed in the DBH-immunoreactive