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Research Report

Sympathetic regulation of vascular tone via noradrenaline and serotonin in the rat carotid body as revealed by intracellular calcium imaging

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ABSTRACT

Hypoxia-induced chemosensory activity in the carotid body (CB) may be enhanced by the sympathetic regulation of vascular tone in the CB. In the present study, we recorded cervical sympathetic nerve activity in rats exposed to hypoxia, and examined noradrenaline (NA)- and serotonin (5-HT)-induced intracellular Ca^{2+} ($[Ca^{2+}]_i$) responses in smooth muscle cells and pericytes in isolated blood vessels from the CB. Multifiber electrical activity recorded from the cervical sympathetic trunk was increased during the inhalation of hypoxic gas. NA induced $[Ca^{2+}]_i$ increases in smooth muscle cells in arteriole specimens, whereas 5-HT did not cause any $[Ca^{2+}]_i$ responses. However, NA did not induce $[Ca^{2+}]_i$ increases in pericytes in capillaries, whereas 5-HT did and this response was inhibited by the 5-HT_2 receptor antagonist, ketanserin. In conclusion, cervical sympathetic nerves enhanced by hypoxia may reduce blood flow in the CB in order to increase chemosensitivity. Thus, hypoxic chemosensitivity in the CB may involve a positive feedback mechanism via sympathetic nerves.

Abbreviations: ASMA, alpha-smooth muscle actin; CB, carotid body; CSNA, cervical sympathetic nerve activity; DBH, dopamine beta-hydroxylase; HR, HEPES-buffered Ringer's solution; LEL, *Lycopersicon esculentum* lectin; NA, noradrenaline; PBS, phosphate-buffered saline; ROI, region of interest; RT-PCR, reverse transcriptase-polymerase chain reaction; SCG, superior cervical ganglion; SYN, synaptophysin, TPH, tryptophan hydroxylase; 18S rRNA, 18S ribosomal RNA; 5-HT, 5-hydroxytryptamine, serotonin; $[Ca^{2+}]_i$, intracellular Ca^{2+}

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1. Introduction

The carotid body (CB) is a peripheral chemoreceptor that is responsible for monitoring the arterial blood levels of pO_2, pCO_2, and pH. Hypoxia stimulates glomus cells within the CB to trigger chemosensory discharges in the carotid sinus nerve (Gonzalez et al., 1994). Afferent signals from the CB are carried to the nucleus solitary tract, leading to an increase in ventilation (Lahiri et al., 2006). The CB also receives sympathetic innervation via postganglionic nerves from the superior cervical ganglion (SCG) (Claps and Torrealba, 1988), and electrical stimulation of the cervical sympathetic trunk was previously shown to evoke discharges in the carotid sinus nerves of cats (Eyzaguirre and Lewin, 1961). Furthermore, hypoxic ventilatory responses are known to be reduced by CB sympathetic denervation or removal of the SCG in the goat (Ryan et al., 1995). Thus, hypoxia-induced activity in the carotid sinus nerve may be enhanced by sympathetic nerve fibers to the CB. Sympathetic nerve fibers innervate both arterioles and capillaries in the CB of the rat (McDonald, 1983). Moreover, electrical stimulation of the cervical sympathetic trunk was shown to cause a reduction in blood flow in the CB, and this effect was attributed to vasoconstriction within the CB in the cat (Daly et al., 1954; Acker and O'Regan, 1981). These findings suggested that sympathetic nerve fibers regulate vascular tone to reduce blood flow in the CB.

The main neurotransmitter of the sympathetic nervous system, noradrenaline (NA), is known to be released from postganglionic nerve fibers (Burnstock, 2002). Furthermore, we previously reported that immunoreactivities for the serotonin (5-hydroxytryptamine; 5-HT) biosynthetic enzyme, tryptophan hydroxylase 1 (TPH1), and 5-HT plasma membrane transport protein, 5-HT transporter, were detected in sympathetic nerve fibers in the CB of the rat (Yokoyama et al., 2013). Our previous findings suggested that not only NA, but also 5-HT is released from sympathetic nerve fibers in the CB. Morphologically, arterioles and capillaries are surrounded by contractile cells including smooth muscle cells and pericytes in the CB of the rat, respectively (McDonald and Larue, 1983). Therefore, the contractility of smooth muscle cells and pericytes are expected to be regulated by NA and 5-HT from sympathetic nerve fibers in the CB.

The present study aimed to examine the sympathetic regulatory mechanism underlying vascular tone in the CB for chemosensory activation. We recorded multifiber electrical activity dissected from the cervical sympathetic trunk in rats exposed to hypoxia in order to determine the effects of hypoxia on sympathetic nerve input to the CB. We also evaluated intracellular Ca^{2+} ($[Ca^{2+}]_i$) changes as an index of contractile responses by smooth muscle cells and pericytes in isolated CB blood vessels following the application of NA or 5-HT and its antagonist. We also confirmed the mRNA expression and immunohistochemical localization of TPH1 and TPH2 in the SCG by reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry.

2. Results

2.1. *Alpha-smooth muscle actin (ASMA)-immunoreactive vasculature and dopamine beta-hydroxylase (DBH)-immunoreactive nerve fibers in CB*

Free-floating sections stained with ASMA and the major synaptic vesicle membrane protein, synaptophysin (SYN), were shown in Fig. 1A. ASMA immunoreactivity was observed in the walls of large-sized blood vessels (diameter >30 μm, arrow) located outside the region of the CB that was comprised of clustered glomus cells immunoreactive to SYN. Within the CB, ASMA immunoreactivity was observed in the walls of several small-sized blood vessels (diameter <20 μm, arrowheads). Small-sized blood vessels with ASMA immunoreactivity were closely surrounded by clustered glomus cells immunoreactive to SYN. Cryostat sections stained with the NA biosynthetic enzyme, DBH, were shown in Fig. 1B. Varicose nerve fibers with DBH immunoreactivity encircled the smooth muscle layer of arterioles (diameter >20 μm) in the CB (Fig. 1B). DBH-immunoreactive varicose nerve fibers also ran along the small-sized blood vessels (diameter <10 μm). In addition to varicose nerve fibers, parts of glomus cells were also immunoreactive for DBH.

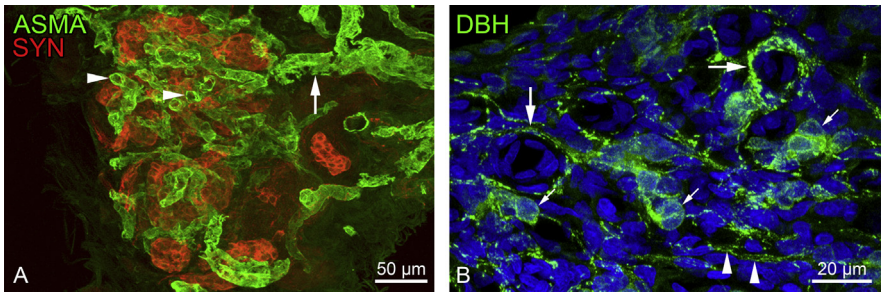

Fig. 1 – (A) Confocal projection view of free-floating sections stained with ASMA and SYN. ASMA immunoreactivity is observed in large-sized blood vessels (arrow) outside the CB and characterized by clusters of glomus cells immunoreactive to SYN. ASMA immunoreactivity is also observed in small-sized blood vessels (arrowheads) between SYN-immunoreactive clustered glomus cells within the CB. (B) Cryostat sections stained with DBH. Nuclei is stained with DAPI (blue). DBH immunoreactivity is observed in the varicosities of nerve fibers (arrows) associated with the smooth muscle layers of arterioles. DBH immunoreactive varicose nerve fibers (arrowheads) also run along small blood vessels with an external diameter of less than 10 μm. Parts of glomus cells are immunoreactive for DBH (small arrows).