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Research report

Combined hypoxia and hypercapnia, but not hypoxia alone, suppresses neurotransmission from orexin to hypothalamic paraventricular spinally-projecting neurons in weanling rats

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ABSTRACT

Both orexin neurons in the lateral hypothalamus and spinally-projecting pre-sympathetic neurons (PSNs) in the paraventricular nucleus of the hypothalamus (PVN) play an important role in the regulation of cardiovascular function under normal conditions and during cardiovascular challenges such as hypoxia and/or hypercapnia. We have previously established, using selective optogenetic excitation of orexin neurons and pathways, there is a heterogeneous neurotransmission from orexin neurons to PSNs in the PVN. This study was undertaken to test whether this pathway is altered by acute exposure to hypoxia alone and/or combined hypoxia and hypercapnia (H/H). To test this hypothesis, we selectively expressed channelrhodopsin-2 (ChR2) in orexin neurons in the lateral hypothalamus and photoactivated ChR2-expressing fibers to evoke postsynaptic currents in spinally-projecting PSNs in an in vitro slice preparation in rats. In accordance with previously published data, two subpopulations of spinally-projecting PSNs were established, including those with glutamatergic or GABAergic inputs from orexin neurons. Hypoxia alone did not alter the peak amplitude of either glutamatergic or GABAergic neurotransmission, however, H/H significantly inhibited both glutamatergic and GABAergic neurotransmission from orexin neurons to SPNs. In conclusion, H/H may modulate cardiovascular function by affecting heterogeneous pathways from orexin neurons to spinally-projecting PSNs in the PVN.

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1. Introduction

We have previously established, using selective optogenetic excitation of orexin neurons and pathways, there is a heterogeneous neurotransmission from orexin neurons to pre-sympathetic bulbospinal neurons (PSNs) in the paraventricular nucleus of the hypothalamus (PVN) (Dergacheva et al., 2017). PSNs in the PVN project directly to the intermediolateral cell column of the thoracic spinal cord with collaterals to the rostral ventrolateral medulla and play an important role in the cardiovascular sympathoactivation (Hosoya et al., 1991; Kc and Dick, 2010; Ranson et al., 1998; Sawchenko and Swanson, 1982). Orexin neurons in the lateral hypothalamus are involved in regulation of many physiological functions including autonomic function, arousal, sleep, feeding, ventilatory chemosensitivity and breathing (Branch et al., 2016; Burdakov et al., 2003; Deng et al., 2007; Dergacheva et al., 2013; Dergacheva, 2015; Nattie and Li, 2012; van den Pol et al., 1998; Wu et al., 2002; Yamanaka, 2015; Young et al., 2005). The results from our previous study suggest that orexin neurons may exert sympathoexcitatory control of cardiovascular function upon activation of the direct projection from orexin neurons to PSNs in the PVN (Dergacheva et al., 2017).

It has been recently shown that orexin neurons are sensitive to hypoxia alone and a combination of hypoxia and hypercapnia (H/H) (Dergacheva et al., 2016). In addition, the neurotransmission from orexin neurons to parasympathetic cardiac vagal neurons is compromised with chronic exposure to H/H (Dergacheva, 2015). We propose that hypoxia and/or H/H may also affect the synaptic pathway from orexin neurons to PSNs in the PVN and these neurophysiological mechanisms may contribute to sympathetic and cardiovascular dysfunction associated with diseases characterized by repetitive breathing cessations resulting in H/H such as obstructive sleep apnea (OSA) (Bradley et al., 1986; Guilleminault et al., 1976).

Abbreviations: aCSF, artificial cerebrospinal fluid; ChR2, channelrhodopsin-2; hypoxia and hypercapnia, H/H EPSCs, excitatory postsynaptic currents; IPSCs, inhibitory postsynaptic currents; PSNs, pre-sympathetic neurons.

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Thus, this study was undertaken to test the hypothesis that acute hypoxia and/or H/H alters the synaptic neurotransmission from orexin neurons to PSNs in the PVN. To accomplish this goal, we used transgenic orexin Cre-rats to express channelrhodopsin-2 (ChR2) in orexin neurons and fibers. Postsynaptic currents in fluorescently identified PSNs were recorded upon photoactivation of ChR2 expressing orexin fibers that surrounded these neurons in an in vitro slice preparation before, during and post exposure to hypoxia alone and, in another set of experiments, to combined H/H.

2.Results

2.1. Monosynaptic projections from orexin-containing neurons to PSNs in the PVN

Photostimulation of ChR2-expressing fibers surrounding PSNs in the PVN generated fast postsynaptic responses in PSNs. The average latency of the responses were 4.1 ± 0.3 ms (ranging from 2.3 to 6.9 ms, n = 24 neurons) indicating a monosynaptic connection (Petreanu et al., 2009; Pinol et al., 2012). In agreement with our previous data (Dergacheva et al., 2017), these light-triggered responses included glutamatergic excitatory postsynaptic currents (EPSCs) which were blocked with CNQX (50 μM) and GABAergic inhibitory postsynaptic currents (IPSCs) which were abolished by application of gabazine (25 μM).

2.2. Acute hypoxia did not change synaptic neurotransmission from orexin neurons to PSNs

First we investigated the effects of acute hypoxia on the light-triggered postsynaptic neurotransmission to PSNs. Slices were exposed to hypoxia by changing control aCSF equilibrated with 95% O2–5% CO2, pH 7.4 to an identical solution equilibrated with 90% N2–5% O2–5% CO2, pH 7.4. During 10-min exposure to acute hypoxia no significant alterations occurred in either peak amplitude of glutamatergic EPSCs ($P > .05$, 1-way repeated-measures ANOVA and Dunnett's posttest, n = 6, Fig. 1, top) or peak amplitude of GABAergic IPSCs ($P > .05$, 1-way repeated-measures ANOVA and Dunnett's posttest, n = 6, Fig. 1, bottom).

2.3. H/H inhibited synaptic neurotransmission from orexin neurons to PSNs

We next investigated whether H/H, elicited by equilibrating aCSF with 85% N2, 6% O2, and 9% CO2, pH 7.1, affected the amplitude of light-triggered postsynaptic currents in PSNs. H/H produced a significant depression of the peak amplitude of glutamatergic EPSCs (from 56.2 ± 11 pA to 27.9 ± 4.7 pA, H/H at 9–10 min, n = 6, $P < .05$, 1-way repeated-measures ANOVA and Dunnett's posttest, n = 6, Fig. 1, top). Similarly, the peak amplitude of GABAergic IPSCs was significantly diminished by H/H (from 83.8 ± 14 pA to 52.5 ± 15 pA, H/H at 9–10 min; $P < .05$, 1-way repeated-measures ANOVA and Dunnett's posttest, n = 6, Fig. 2, bottom). The amplitude of both glutamatergic and GABAergic postsynaptic currents recovered upon 9–10 min of reoxygenation (56.2 ± 11 pA vs. 51.2 ± 14 pA, $P > .05$ n = 6 and 83.8 ± 14 pA vs. 81.8 ± 22 pA, $P > .05$, n = 6, respectively, see Fig. 2, top and bottom, respectively).

3. Discussion

There are two major findings in this study: 1) Photoactivation of ChR2-expressing orexinergic fibers in the PVN yields short-latency heterogeneous responses in PSNs including glutamatergic EPSCs and GABAergic IPSCs, which are not changed by hypoxia. 2) However, combined H/H significantly diminishes both glutamatergic and GABAergic postsynaptic neurotransmission from orexin neurons to PSNs in the PVN.

Systemic hypoxia (10% O2) does not change activity of putative PSNs in the PVN that project to the rostral ventrolateral medulla (RVLM) (Hirooka et al., 1997). Similar results were obtained by other authors, which have shown that acute hypoxia (10%) activates neuroendocrine, but not spinally-projecting PSNs in the PVN (Coldren et al., 2017). These previous results are in consensus with the current findings demonstrating that hypoxia alone does not affect synaptic pathway from orexin to PSNs. In contrast, combined H/H inhibits neurotransmission from orexin neurons to PSNs. We have previously shown that H/H has more profound depressor effects on the firing activity of orexin neurons than hypoxia alone (Dergacheva et al., 2016). We propose that profound depression of firing activity of orexin neurons with H/H may contribute to the inhibition of neurotransmission from orexin neurons to PSNs. Consistent with the results from this study, both orexin receptor expressions (orexin receptors 1 and 2) are increased in the PVN after intermittent exposure to H/H (Hunt et al., 2013). The authors speculated that upregulation of these orexin receptors is the result of decreased level of orexin release and receptor density compensation due to H/H exposures (Du et al., 2016). Similar results have been obtained with acute exposure to hypercapnia alone. Orexin mRNA levels are decreased whereas the mRNA levels of orexin receptor 1 are increased in the PVN after 3 h exposure to hypercapnia (Wang et al., 2013).

Hypoxia or H/H typically simulate neurons in the brain regions involved in cardiorespiratory regulation (Kramer et al., 1999). For instance, neurons in caudal hypothalamus are activated by hypoxia, hypercapnia, as well as combination of H/H (Dillon and Waldrop, 1992; Dillon and Waldrop, 1993). Similarly, the firing rates of neurons in the periaqueductal gray are increased by hypoxia or hypercapnia (Kramer et al., 1999). Combined H/H elicits a significant increase in the firing discharge of more than 50% neurons in the ventrolateral medulla (Nolan and Waldrop, 1993). Neuronal activation in these brain regions, involved in cardiorespiratory regulation, likely contributes to the generation of cardiorespiratory activation in response to hypoxia or hypercapnia. In contrast, orexin neurons in the lateral hypothalamus are depressed by acute H/H (Dergacheva et al., 2016). Moreover, the results from this study indicate that the pathway from orexin neurons to PSNs in the PVN is inhibited by H/H. Interestingly, another important pathway from hypoxia-sensitive neurons in the caudal ventrolateral medulla to PVN neurons is likely activated by hypoxia (King et al., 2013). Taking the results from previous studies and data from this work together, we speculate that H/H-inhibited neurotransmission from orexin to PSNs in the PVN could be a path-specific alteration.

The results from this study indicate that both light-triggered glutamatergic EPSCs and GABAergic IPSCs in PSNs are inhibited by H/H exposure. Glutamatergic neurotransmission tends to decrease as early as at 2 min of H/H exposure, however a significant decrease only occurs at 10 min of the exposure. In contrast, very small alteration in GABAergic IPSCs occurs at 2 min of H/H. Orexinergic signaling has been shown to facilitate glutamatergic, but not GABAergic, neurotransmission from orexin neurons to PSNs (Dergacheva et al., 2017). Inhibition of orexinergic transmission with H/H may contribute to the effects of H/H on glutamatergic EPSCs in PSNs. It is worth mentioning that these in vitro results were obtained at room temperature, which may increase the delay of the responses. It is possible that in a whole-animal model other mechanisms (including peripheral chemoreceptors) contribute to the effects of H/H that build more rapidly to evoke responses. It is likely that different subpopulations of PSNs, including those with

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