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Pharmacological modulation of hypoxia-induced respiratory neuroplasticity

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ABSTRACT

Hypoxia elicits complex cell signaling mechanisms in the respiratory control system that can produce long-lasting changes in respiratory motor output. In this article, we review experimental approaches used to elucidate signaling pathways associated with hypoxia, and summarize current hypotheses regarding the intracellular signaling pathways evoked by intermittent exposure to hypoxia. We review data showing that pharmacological treatments can enhance neuroplastic responses to hypoxia. Original data are included to show that pharmacological modulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) function can reveal a respiratory neuroplastic response to a single, brief hypoxic exposure in anesthetized mice. Coupling pharmacologic treatments with therapeutic hypoxia paradigms may have rehabilitative value following neurologic injury or during neuromuscular disease. Depending on prevailing conditions, pharmacologic treatments can enable hypoxia-induced expression of neuroplasticity and increased respiratory motor output, or potentially could synergistically interact with hypoxia to more robustly increase motor output.

1. Hypoxia triggers respiratory neuroplasticity

Respiratory neuroplasticity is an experience-induced and persistent change in the neural system controlling breathing (Fuller and Mitchell, 2017). Accordingly, respiratory neuroplasticity is distinct from direct, "real time" stimulation of respiratory neural circuits, such as occurs during chemoreceptor stimulation. Hypoxia can be a powerful trigger of respiratory neuroplasticity, and the pharmacological approaches that have been used to study the underlying mechanisms are the focus of this article.

Much of our current knowledge regarding cellular and molecular mechanisms of hypoxia-induced respiratory neuroplasticity comes from selective application of agonists and/or antagonists of membrane–bound neurotransmitter/neuromodulator receptors on and near respiratory-related neurons, or through pharmacologic manipulation of downstream signaling molecules (e.g., kinases, phosphatases). These same pharmacologic approaches are useful for controlling the neuroplastic impact of hypoxia in the context of neurorehabilitation (Gonzalez-Rothi et al., 2015). This article provides an overview of pharmacological approaches that have been used to activate or inhibit hypoxia-induced respiratory neuroplasticity. Particular emphasis is placed on experimental methods including drugs and different routes of delivery used (Tables 1–3). Mechanisms of phrenic motor plasticity are highlighted since more is known about the underlying molecular pathways in comparison to other respiratory motor systems. We focus on acute exposure to single or multiple bouts of hypoxia, and conclude with a brief overview of how pharmacological strategies may enhance the development and/or optimization of neurorehabilitation protocols based on moderate hypoxia exposures. This article does not address chronic exposure to sustained hypoxia or intermittent hypoxia, and the reader is referred to several comprehensive reviews of these topics (Almendros et al., 2014; Bisgard, 1995; Fields and Mitchell, 2015; Navarrete-Opazo and Mitchell, 2014).

1.1. Hypoxia pattern and severity

Before discussing experimental methods and hypoxia-induced molecular signaling pathways, we first briefly comment regarding the importance of the hypoxia paradigm. The most salient point is that hypoxia-induced respiratory plasticity is sensitive to the pattern of hypoxia exposure. Indeed the exposure pattern rather than the total hypoxic "dose" is the primary determinant of the signaling pathways that are activated (Baker and Mitchell, 2000; Mitchell et al., 2001). For example, moderate acute *intermittent* hypoxia (AIH) evokes a sustained

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Table 1
Summary of drugs which reveal respiratory neuroplasticity following a single exposure to hypoxia. Moderate acute sustained hypoxia (mASH) describes exposures with PaO_2 values of 35–45 mmHg; severe acute sustained hypoxia (sASH) describes studies with PaO_2 in the range of 25–35 mmHg. I.T. = intrathecal drug delivery, I.P. = intraperitoneal drug delivery, PMF = phrenic motor facilitation, A2A = adenosine 2A, 5-HT = serotonin, AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, R = receptor.

Drug/Delivery	Primary action	Hypoxia paradigm	Outcome	Interpretation	Citation
Vehicle/I.T.	n/a	mASH	No Facilitation	mASH is not sufficient for PMF	(Devinney et al., 2016)
MSX-3/I.T.	A2A-R block	mASH	PMF	Spinal A2A-R block reveals PMF	
Methysergide/I.T.	5-HT-R block	mASH	No Facilitation	Spinal 5-HT-R block does not reveal PMF	
MSX-3 and methysergide/I.T.	Spinal A2A-R + 5-HT-R block	mASH	No Facilitation	PMF after spinal A2A-R blockade is 5HT-dependent	
Vehicle/I.T.	n/a	sASH	PMF	sASH is sufficient for PMF	
MSX-3/I.T.	A2A-R block	sASH	No Facilitation	sASH-induced PMF is dependent on spinal A2A-R	
Methysergide/I.T.	5-HT-R block	sASH	*Enhanced* PMF	Spinal 5-HT-R activation constrains, but does not abolish, A2A-dependent PMF	
Vehicle/I.T.	n/a	mASH	No Facilitation	mASH is not sufficient for PMF	(Wilkerson et al., 2008)
Okadaic Acid/I.T.	Protein phosphatase inhibition (PP1, 2, 5)	mASH	PMF	Spinal protein phosphatases constrain PMF	
Okadaic acid and methysergide/I.T.	Protein phosphatase inhibition + 5-HT-R block	mASH	No Facilitation	Spinal protein phosphatase inhibition reveals 5-HT-dependent PMF	
Ampakine CX717/I.P.	Positive allosteric modulation of AMPA-R	15% inspired O_2 for 1-min	Hypoglossal Motor Facilitation	Amapkines enable a single hypoxic exposure to evoke sustained increases in XII motor output	Fig. 2

increase in respiratory motor output called long term facilitation (LTF). However, an acute, *sustained* bout of moderate hypoxia of similar cumulative duration used to induce LTF does not produce sustained increases in respiratory motor output (Baker and Mitchell, 2000; Devinney et al., 2016; Mitchell et al., 2001; Wilkerson et al., 2008). The relative degree of hypoxemia within bouts of intermittent hypoxia also has a profound impact on the molecular pathways leading to facilitation. It follows that there is not a single unique molecular pathway that leads to LTF; moderate AIH paradigms (i.e., PaO_2 values of ~35–45 mmHg) induce LTF via serotonergic mechanisms whereas severe AIH paradigms (i.e., PaO_2 in the range of 25–35 mmHg) activate adenosinergic mechanisms of LTF (Devinney et al., 2013). The serotonergic and adenosinergic pathways actively inhibit one another, such that only one pathway prevails in specific conditions − this interplay is termed “cross-talk inhibition” (Devinney et al., 2013). On the other hand, when serotonergic and adenosinergic mechanisms are “balanced”, the pathways offset one another, and plasticity is no longer observed (Devinney et al., 2013, 2016). Lastly, when considering hypoxia exposure paradigms, it is important to keep in mind that respiratory plasticity itself adapts based on experience. This sustained change in the capacity to express plasticity after triggering experiences (e.g., chronic intermittent hypoxia) is known as metaplasticity (Fields and Mitchell, 2015). Thus, background experiences of hypoxia (e.g., sleep apnea) may facilitate or undermine plasticity elicited by AIH (reviewed in (Mateika, 2015; Mateika and Syed, 2013)).

2. Acute responses and short term potentiation

Mechanisms driving the rapid, acute increase in respiratory motor output (i.e., acute hypoxic ventilatory response (Powell et al., 1998)) during hypoxia are well-established and were recently reviewed (Pamenter and Powell, 2016). The acute hypoxic response is typically followed by a more gradual increase of respiratory output. Once hypoxia is terminated, respiratory activity typically drops rapidly, followed by a slow “roll off” to pre-hypoxia levels. Short term potentiation (STP) of the respiratory motor response to hypoxia includes both the gradual increase in output during hypoxia and the “roll off” in bursting after normoxic conditions are restored (Powell et al., 1998). STP may not be directly caused by hypoxia *per se*, but rather may be a response driven by non-specific increases in synaptic activity in respiratory neurons or networks. It is nevertheless a robust phenomenon having been described in a range of anesthetized animal preparations (Hayashi et al., 1993; Lee and Fuller, 2010a,b; Lee et al., 2015) and also in unanesthetized humans (Fregosi, 1991; Georgopoulos et al., 1995). Respiratory-related outcome measures showing STP include recordings of nerve activity (Hayashi et al., 1993; Lee and Fuller, 2010a,b; Lee et al., 2015), muscle EMG (Mateika and Fregosi, 1997) or direct quantification of ventilation (Fregosi, 1991; Georgopoulos et al., 1995).

Early work in anesthetized cats found that STP is unaffected by vagal nerve stimulation (Eldridge and Gill-Kumar, 1978), is affected by subthreshold respiratory drive (Eldridge, 1980), and does not require input from inspiratory neurons in the medulla (Eldridge, 1980; Eldridge and Gill-Kumar, 1980). Pharmacological methods have also been used to examine the mechanisms of respiratory STP. Early studies by Eldridge and colleagues used intravenous delivery of serotonin (5HT) antagonists to show that STP is 5HT independent (Millhorn et al., 1981). Thus, when methysergide, parachlorophenylalanine, or 5,7-dihydroxytryptamine were given, there was no change in the phrenic nerve response to carotid sinus nerve stimulation, and no effect on STP in anesthetized cats. Moreover, no effect on STP was observed following intravenous administration of antagonists for multiple other neurotransmitter receptors including the dopamine-norepinephrine antagonists alpha-methytyrosine, and haloperidol and the endorphin antagonist, naloxone (Millhorn et al., 1981). In anesthetized rats, systemic blockade of N-Methyl-D-aspartic acid receptors (NMDAR) with intravenous delivery of MK-801 causes a slower onset of phrenic STP during hypoxia, and also extends the time course of STP offset (Poon et al., 1999). Accordingly, the authors suggested that NMDA receptors function as a “molecular” switch involved with both the induction and recovery phases of STP (Poon et al., 1999). Nitric oxide may also be involved in STP since intraperitoneal delivery of the nitric oxide synthase 1 (NOS-1) inhibitor 7-nitroindazole prior to acute hypoxia attenuates or eliminates STP of ventilation (Kline et al., 2002).

Pamenter and Powell (2016) recently proposed a molecular model to explain how stimulation of carotid chemoafferent neurons leads to STP. Sustained glutamate release leads to activation of post-synaptic NMDA receptors on second-order nucleus tractus solitarius (NTS) neurons and intracellular Ca^{2+} accumulation. Increased levels of Ca^{2+} activate calmodulin-dependent protein kinase II (CaMKII). CaMKII modifies membrane-dissociated neuronal NOS (nNOS) and stimulates production of nitric oxide. Nitric oxide then defuses back across the synaptic cleft to stimulate guanalyl cyclase-mediated production of cyclic guanosine monophosphate (cGMP). cGMP increases presynaptic glutamate release, thereby enhancing excitatory signaling that