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Review

Intermittent hypoxia-induced cardiorespiratory long-term facilitation: A new role for microglia

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ABSTRACT

Intermittent hypoxia induces plasticity in neural networks controlling breathing and cardiovascular function. Studies demonstrate that mechanisms causing cardiorespiratory plasticity rely on intracellular signalling pathways that are activated by specific neurotransmitters. Peptides such as serotonin, PACAP and orexin are well-known for their physiological significance in regulating the cardiorespiratory system. Their receptor counterparts are present in cardiorespiratory centres of the brainstem medulla and spinal cord. Microglial cells are also important players in inducing plasticity. The phenotype and function of microglial cells can change based on the physiological state of the central nervous system. Here, we propose that in the autonomic nuclei of the ventral brainstem the relationship between neurotransmitters and neurokines, neurons and microglia determines the overall neural function of the central cardiorespiratory system.

1. Introduction

The cardiorespiratory network of the brainstem displays an anatomically distinct neural circuitry. Peripheral sensors (neural, oxygen and pH) detect changes in the external milieu relay information via afferent pathways to brainstem centres. These sensory inputs are integrated within brainstem regulatory centres resulting in appropriate changes in the activity of efferent neurons in order to restore homeostasis (Pilowsky and Goodchild, 2002). This neural reflex loop is not hard-wired; rather, there exists a capacity for considerable plasticity. Acute intermittent hypoxia (AIH) causes long-term changes to the way neurons fire in the brainstem nuclei (Xing and Pilowsky, 2010). A well-established concept of this plasticity in the cardiorespiratory regulating pathways of the brain, following intermittent exposures of hypoxia, is termed long-term facilitation (LTF; pLTF in the phrenic motor pathway for breathing, and sLTF for the sympathetic output to the cardiovascular system). Two types of intermittent hypoxia protocols are used for experimental models to study complications arising from the cardiorespiratory system. These include: AIH (Dick et al., 2014, 2004, 2007; Xing et al., 2013, 2014; Xing and Pilowsky, 2010), and chronic intermittent hypoxia (CIH) (Braga et al., 2006; Leuenberger et al., 2005; Prabhakar et al., 2005; Zoccal et al., 2007, 2008). The physiological importance of LTF in the respiratory and sympathetic pathway remains inconclusive.

Communication of signals between neurons is achieved by neurotransmitters. Certain experiences prompt various combinations of endogenous neurotransmitters to be released, and this leads to a change in system behaviour. Additionally, the integrity of neural connections is supported and regulated by microglial cells. Microglia actively interact with neurons to shape the network, and the structure of the central nervous system (CNS) (Kettenmann et al., 2013). Microglia are extremely plastic in nature; the state of the CNS determines the morphological and functional phenotype of the cells. An interplay between neurons and microglia is mediated by various chemicals, such as neurotransmitters (Pocock and Kettenmann, 2007), cytokines, brain-derived neurotrophic factor (BDNF), and transforming growth factor (TGF)-β (Biber et al., 2007). Therefore, we hypothesise that neurotransmitters and microglia in the cardiorespiratory system may play a key role in generating neuronal plasticity.

The mechanisms underlying the generation of cardiorespiratory plasticity following acute intermittent hypoxia are well-studied, but remain unclear (Dale-Nagle et al., 2010; Devinney et al., 2013; Mitchell et al., 2001; Xing et al., 2013, 2014). Here we review

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the known molecular pathways involved in LTF. We suggest that other neurotransmitters are involved in the development of LTF. Furthermore, the role of microglia in supporting the central cardiorespiratory network and the role microglial cells may play in generating intermittent hypoxia-induced LTF will also be discussed.

2. Mechanisms in the generation of pLTF after intermittent hypoxia

Long term facilitation in neural activity following intermittent hypoxia conventionally refers to facilitation in the activity recorded from the phrenic nerve. Facilitation in phrenic nerve activity is a form of serotonin- and protein synthesis-dependent plasticity that takes place within the phrenic motor nucleus in the spinal cord (Bach and Mitchell, 1996; Baker-Herman et al., 2004; Fuller et al., 2001). Induction of pLTF is a state of enhanced respiratory neural output, that is independent of increased phrenic nerve activity (Baker and Mitchell, 2000; Xing et al., 2013). Furthermore, the plasticity is mediated by the action of neuromodulators (Dale-Nagle et al., 2010; Dick et al., 2007; Toyama et al., 2009). Although the current understandings of mechanisms giving rise to pLTF remain incomplete, substantial progress has been made, and the key elements will be discussed below.

2.1. Q pathway

Acute intermittent hypoxia-induced pLTF is characterised by a prolonged facilitation in respiratory motor activity from the phrenic and hypoglossal (XII) nerves that are regulated by various neurotransmitters (Bach and Mitchell, 1996; Baker and Mitchell, 2000). First, respiratory neural activity is regulated by transmission from serotonin-containing neurons of the raphe nuclei to the spinal cord (Murphy et al., 1995; Pilowsky et al., 1990). Secondly, hypoxia activates the pontine catecholamine neurons in the locus coeruleus and A5 that release noradrenaline in spinal and cranial motornuclei including phrenic nucleus, the nucleus ambiguus and the hypoglossal (XII) nerve (Berkowitz et al., 2005; Gatti et al., 1999; Sun et al., 2002, 2003, 1995). Induction of pLTF by AIH occurs via mechanisms requiring the activation of spinal serotonergic ($5HT_2$) receptor subtypes, or brainstem α_1-adrenoreceptors that are coupled to the G_q protein (Dale-Nagle et al., 2010; Dick et al., 2007); thus the pathway is referred to as the '*Q pathway*'. The initiation, but not sustained elevation, of pLTF by AIH is blocked by administration of $5HT_2$ receptor (Fuller et al., 2001) and α_1-adrenoreceptor (Neverova et al., 2007) antagonists. Adrenergic α_1 receptor activation, however, is mechanistically sufficient but not necessary for pLTF (Huxtable et al., 2014). The maintenance in the persistent state of motor discharge requires contributions from intracellular proteins within the spinal phrenic motor neurons. Episodic spinal $5HT_2$ receptor activation is necessary and sufficient for new synthesis of BDNF (Baker-Herman et al., 2004) from the activated protein kinase C (PKC) θ effector protein isoform that is strongly coupled to $5HT_2$ receptors (Devinney et al., 2015), and an increased expression of the high-affinity TrkB receptor leading to ERK/MAPK signalling (Hoffman et al., 2012). Downstream of ERK, there is an increase in post-synaptic glutamate receptor phosphorylation. This leads to an enhancement in glutamatergic transmission within phrenic motor neurons, causing a robust increase in phrenic nerve activity (Fig. 1).

2.2. S pathway

The mechanism by which is pLTF generated is incomplete, however metabotropic receptors coupled to G_s protein, referred to as the *'S pathway,'* are believed to make a significant contribution. Hypoxia increases extracellular adenosine levels via active transport and ATP degradation (Gourine et al., 2002). Adenosine release leads to the activation of G_s protein-coupled A_{2A} receptor in the spinal cord. The G_s receptor pathway is mediated by exchange protein directly activated by cAMP (EPAC) effectors (Fields et al., 2015), requiring newly synthesised proteins comprised of immature TrkB isoforms via mTORC1 signalling, and downstream activation of PI3-kinase/Akt signalling (Golder et al., 2008) (Fig. 1). Q and S pathways are distinct mechanisms that interact via 'cross-talk' inhibition (Hoffman et al., 2010). 'Cross-talk' mechanisms between the intracellular G_q and G_s pathways have been frequently demonstrated in past studies (Lai et al., 1997; Zimmermann and Taussig, 1996). Activation of $5\text{-}HT_7$ receptors in the cervical spinal cord elicits pLTF (S pathway), but also constrains $5\text{-}HT_2$ receptor-induced pLTF (Q pathway); this phenomenon is likely to be mediated by divergent cAMP/PKA signalling (Fields et al., 2015). However, some model systems do require PKA activation to induce certain forms of plasticity e.g. CREB-mediated plasticity (Lonze and Ginty, 2002; Silva et al., 1998).

Interestingly, the Q and S pathways adopt differential roles that are dependent on the 'intensities' of hypoxemic challenges placed on the animals. The 'intensity' of intermittent hypoxia stimuli depends on two factors: (a) the level of oxygen in the hypoxic gas mixture (i.e. the% of oxygen, balanced in nitrogen), and (b) the number of cycles, or frequency of the hypoxia (Navarrete-Opazo and Mitchell, 2014), which leads to the overall hypoxemic state of the animal. The S pathway does not contribute to pLTF following 'moderate' hypoxia (three 5-min episodes of isocapnic inspired 11% O_2 to maintain PaO2 at 45–55 mmHg, separated by 5-min intervals of baseline 51% O_2) (Hoffman et al., 2010; Nichols et al., 2012), but dominates pLTF following severe AIH (P_aO_2 level at 25–30 mmHg) (Nichols et al., 2012). The underlying mechanism is that severe, but not moderate, AIH activates the mTOR protein that is essential for activating PI3-kinase/Akt pathway (Dougherty et al., 2015). A difference in the level of extracellular adenosine accumulation during severe hypoxia may also contribute to the transition, increasing the number of activated A_{2A} receptors. These findings demonstrate that cross-talk inhibition ensures the switch of dominance from one mechanism to the other. Furthermore, the transition between the one pathway to other follows a reference P_aO_2 threshold: in that levels above 35 mmHg elicit pLTF via the Q pathway, whereas levels of 30 mmHg or below elicit pLTF via the S pathway (Nichols et al., 2012). Indeed, central serotonin knockout ($Tph2^{-/-}$) C57BL/6-129SV mice display physical ventilatory LTF following exposure to severe intermittent hypoxia (i.e. lower oxygen concentration and greater frequency of hypoxic bursts), comprised of 4-min periods of 10% oxygen (balanced with nitrogen) interspersed with 4-min recovery intervals, 12 times for 10 days (12th hypoxia lasted for 45 min) (Hickner et al., 2014). Also, smaller animals (mice vs. rats) were used, which cause discrepancies in the relative capacity of accessory respiratory muscles to express plasticity. It is most likely that respiratory facilitation was caused by a shift in dominance from the impaired serotonin-dependent Q pathway to the S pathway.

2.3. Orexin

A hallmark of pLTF is pattern sensitivity. Phrenic LTF is elicited by intermittent, but not continuous, hypoxia. In spite of the significant advances made to understand the neuromodulator-mediated mechanisms underlying the generation of pLTF, the reasons that pattern-oriented stimuli are crucial remain unclear. Kuwaki and colleagues demonstrated that orexin-containing neurons are activated by distinct pattern-dependent intermittent hypoxic challenges (Terada et al., 2008; Toyama et al., 2009; Yamaguchi et al., 2015). In addition, functional anatomy and *in vitro* studies show that orexin neurons send dense fibre networks to