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Transcriptional responses to intermittent hypoxia

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ABSTRACT

Recurrent apneas are characterized by transient repetitive cessations of breathing (two breaths duration or longer) resulting in periodic decreases in arterial blood PO_2 or chronic intermittent hypoxia (IH). Patients with recurrent apneas and experimental animals exposed to chronic IH exhibit cardio-respiratory morbidities. The purpose of this article is to highlight the current information on the transcriptional mechanisms associated with chronic IH. Studies on rodents and cell cultures have shown that IH activates a variety of transcription factors including the hypoxia-inducible factor-1 (HIF-1), *c-fos* (immediate early gene), nuclear factor of activated T-cells (NFAT), and nuclear factor kB (NF-kB). The signaling pathways associated with transcriptional activation associated with IH differ from continuous hypoxia (CH). Compared to same duration and intensity of CH, IH is more potent in activating HIF-1 and *c-fos* and also results in long-lasting accumulation of HIF-1α and *c-fos* mRNA, a phenomenon that was not seen with CH. IH-evoked transcriptional activation by HIF-1, *c-fos* as well as the resulting activator protein-1 (AP-1) requires reactive oxygen species (ROS)-mediated signaling and involves complex feed forward interactions between HIF-1 and ROS. Chronic IH-evoked cardio-respiratory responses are absent in *Hif-1a*$^{+/-}$ mice, and hypertension elicited by chronic IH is absent in mice lacking NFAT3c. These studies indicate that cardio-respiratory responses to chronic IH depend on complex interactions between various transcription factors resulting in alterations in several down stream genes and their protein products.

1. Introduction

Recurrent apneas are characterized by transient repetitive cessations of breathing (two breaths duration or longer) resulting in cyclical decreases in arterial blood PO_2 or chronic intermittent hypoxia (IH). An estimated 4–5% of adult males, 2–4% of females after menopause, and 50–70% of premature infants experience chronic IH as a consequence of recurrent apneas (Nieto et al., 2000; Poets et al., 1994). Patients with recurrent apneas exhibit cardio-respiratory co-morbidities including pulmonary as well as systemic hypertension, myocardial infarction, stroke, ventilatory abnormalities, and sudden death (Shahar et al., 2001). Similar cardio-respiratory changes were also reported in rodents exposed to chronic IH (reviewed in Prabhakar et al., 2007). Studies on rodents and cell cultures have shown that IH activates several transcription factors. The purpose of this article is to summarize what is currently known on the effects chronic IH on activation of transcriptional factors, underlying mechanisms and the potential contribution of transcriptional activators on chronic IH-evoked cardio-respiratory responses. In contrast to IH associated with recurrent apneas, wherein each hypoxic episode lasts no more than couple of breaths, exposing individuals to few hours of hypoxia per day for a few weeks, which is also intermittent in nature, improves cardio-respiratory functions (Serebrovskaya et al., 1999). Due to constraints of space, this article focuses on transcriptional responses to IH simulating recurrent apneas only.

2. Hypoxia and transcription factors

Hypoxia activates several genes via recruiting specific transcription factors. The resulting protein products maintain homeostasis by enhancing tissue perfusion, ATP generation, glycolysis, etc.

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Transcriptional activators that are affected by continuous hypoxia (CH) include: hypoxia-inducible factors (HIF-1 and HIF-2); nuclear factor kappa B (NF-kB), cyclic AMP-response element-binding protein (CREB), activating protein-1 (AP-1), p53, early growth response-1 (Egr-1), nuclear factor for interleukin 6 (NF-IL6) (Cummins and Taylor, 2005). With the exception of HIF-1, the effects of hypoxia on other transcription factors can be cell-type and cell-state specific. The following section summarizes the effects of IH on some of these transcription factors.

3. IH and hypoxia-inducible factor-1 (HIF-1)

HIF-1 is a heterodimeric protein that is composed of a constitutively expressed HIF-1β subunit and an O_2-regulated HIF-1α subunit (Wang et al., 1995). HIF-1-mediated transcriptional activation requires increased HIF-1α expression, dimerization with HIF-1β and interaction with co-activators p300 (adenovirus EIA-associated 300-kDa protein) and CBP (cyclic AMP-responsive element-binding protein).

3.1. IH and HIF-1α protein expression

IH up-regulates HIF-1α protein in the central nervous system of mice (Peng et al., 2006), and in PC12 cell cultures (Yuan et al., 2005). Lam et al. (2008) reported an increase in the HIF-1α transcript but not the protein during IH. HIF-1α accumulation by CH requires decreased O_2-dependent proline hydroxylation, ubiquitination, and proteasomal degradation of the HIF-1α subunit (Coleman and Ratcliffe, 2007). The mechanism associated with IH-evoked HIF-1α accumulation is complex and requires not only decreased proline hydroxylation but also increased protein synthesis via activation of mammalian target of rapamycin (mTOR) as summarized in Fig. 1. Recent study by Yuan et al. (2008) reported that reactive oxygen species (ROS) generated by NADPH oxidase and the resulting changes in intracellular Ca^{2+} are the primary signaling events that trigger HIF-1α accumulation by IH.

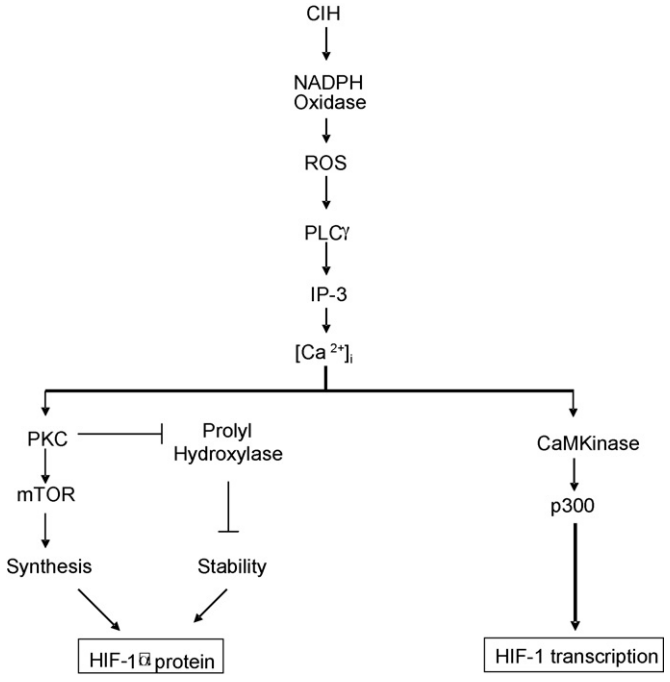

Fig. 1. NADPH oxidase signaling in intermittent hypoxia-induced HIF-1α protein expression and HIF-1 transcription. *Key*: CIH, chronic intermittent hypoxia; ROS, reactive oxygen species; PLC_γ, phospholipase C gamma; IP-3, inositol triphosphate; PKC, protein kinase C; mTOR, mammalian target of rapamycin; HIF-1, hypoxia-inducible factor-1.

The effects of IH and CH on HIF-1α accumulation differ in the following aspects: (a) for a given intensity and duration, IH is more potent in increasing HIF-1α protein than CH (Yuan et al., 2005) and (b) following IH, HIF-1α levels remain elevated during re-oxygenation, whereas they return to control levels within 10 min of re-oxygenation following CH (Yuan et al., 2008). The persistent accumulation of HIF-1α protein during re-oxygenation following IH requires increased protein synthesis via activation of mTOR signaling (Yuan et al., 2008).

3.2. IH and HIF-1-mediated transcriptional activation

The effects of IH on HIF-1-mediated transcriptional activation were examined in PC12 cells (Yuan et al., 2005). IH-activated HIF-1-dependent transcriptional activity in a stimulus-dependent manner. Like HIF-1α protein expression, CH of comparable, cumulative duration of IH was ineffective in activating HIF-1-dependent transcription. Previous studies showed that mitogen-activated protein kinases (MAPKs) and phospho-ionositol-3 (PI-3) kinases are critical for continuous hypoxia-evoked activation of HIF-1-mediated transcription (Sang et al., 2003; Seta et al., 2003). Although MAPKs (ERK-1 &2; Jun Kinase) are activated by IH, inhibitors of MAPKs and PI-3 kinase were ineffective in blocking IH-elicited activation of HIF-1-mediated transcriptional activation (Yuan et al., 2005).

HIF-1 activation by IH was inhibited by BAPTA-AM, an intracellular Ca^{2+} chelator (Yuan et al., 2005), suggesting the involvement of Ca^{2+} signaling pathways. Calcium-calmodulin-dependent kinases (CaM kinases) are one of the important Ca^{2+} signaling molecules. PC12 cells express CaMK-II and CH causes a transient and modest increase in CaM kinase activity (Premkumar et al., 2000). In striking contrast, IH resulted in robust and persistent activation of CaM kinase (~5-fold activation) and more importantly, CaM kinase inhibitor KN93 prevented HIF-1 transcriptional activation, but not HIF-1α accumulation by IH (Yuan et al., 2005).

Transcriptional activation by HIF-1 requires N- and C- terminal transactivation domains (N-TAD and C-TAD), which are separated by intervening inhibitory domain. FIH-1 (factor inhibiting HIF-1) binds to the inhibitory domain (Mahon et al., 2001) and mediates the O_2-dependent hydroxylation of asparagine (Asn-803), which prevents binding of the co-activators. CaMK II stimulates C-TAD domain function of HIF-1 via a mechanism that is independent of asparaginyl hydroxylation (Yuan et al., 2005). Several lines of evidence suggest that phosphoproteins p300 and CBP (Yaciuk and Moran, 1991) are the major co-activators for HIF-1 activation (Sang et al., 2003; Ruas et al., 2002; Dames et al., 2002). Hypoxia leads to hyperphosphorylation of p300 in PC12 cells via Ca^{2+} signaling by IP-3 receptors (Zakrzewska et al., 2005). CaMK II phosphorylated p300 *in vitro* (Yuan et al., 2005) and CaM kinase inhibitor, KN-93 prevented activation of p300 by IH, These observations suggest that IH-induced HIF-1 transcriptional activity is mediated by a novel signaling pathway involving phosphorylation of p300 by CaM kinase (Fig. 1).

3.3. Physiological significance of IH-induced HIF-1 activation

3.3.1. Cardio-respiratory responses to chronic IH

Chronic IH has profound effects on cardio-respiratory physiology. Rodents exposed to chronic IH exhibit elevated blood pressures (Fletcher, 2001; Kumar et al., 2006; Peng et al., 2006; Kanagy et al., 2001), increased plasma catecholamine (Bao et al., 1997; Kumar et al., 2006; Peng et al., 2006), and endothelin (a peptide vasoconstrictor) levels (Kanagy et al., 2001). Basal sympathetic nerve activity