Mechanisms of intermittent hypoxia induced hypertension

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Abstract

Exposing rodents to brief episodes of hypoxia mimics the hypoxemia and the cardiovascular and metabolic effects observed in patients with obstructive sleep apnoea (OSA), a condition that affects between 5% and 20% of the population. Apart from daytime sleepiness, OSA is associated with a high incidence of systemic and pulmonary hypertension, peripheral vascular disease, stroke and sudden cardiac death. The development of animal models to study sleep apnoea has provided convincing evidence that recurrent exposure to intermittent hypoxia (IH) has significant vascular and haemodynamic impact that explain much of the cardiovascular morbidity and mortality observed in patients with sleep apnoea. However, the molecular and cellular mechanisms of how IH causes these changes is unclear and under investigation. This review focuses on the most recent findings addressing these mechanisms. It includes a discussion of the contribution of the nervous system, circulating and vascular factors, inflammatory mediators and transcription factors to IH-induced cardiovascular disease. It also highlights the importance of reactive oxygen species as a primary mediator of the systemic and pulmonary hypertension that develops in response to exposure to IH.

Keywords: systemic hypertension ● pulmonary hypertension ● ROS ● inflammation ● NFAT ● NF-κB ● HIF ●
nervous system ● endothelin ● sleep apnoea

Introduction

Exposing rodents to brief episodes of hypoxia mimics the hypoxemia and the cardiovascular and metabolic effects observed in patients with sleep-disordered breathing (SDB) [1–11]. SDB includes different respiratory disorders – the most frequent being obstructive sleep apnoea (OSA) and hypoapnoea. OSA corresponds to recurrent episodes of complete or partial pharyngeal collapse during sleep resulting in intermittent hypoxia (IH) and sleep fragmentation [1, 12–18].

OSA affects between 5% and 20% of the population and apart from daytime sleepiness, is associated with high incidence of hypertension, peripheral vascular disease, stroke and sudden cardiac death [7, 19]. However, the mechanisms by which OSA and cardiovascular disease are linked remain obscure since patient studies are complicated by the confounding effects of disease duration and the many comorbidities present in OSA patients.

The first animal study showing that IH induces hypertension was reported by Fletcher, *et al.* [20]. They exposed rats to IH (3–5% nadir ambient oxygen) every 30 sec., 7 hrs per day for up to 35 days and demonstrated a significant increase in mean arterial blood pressure (MAP) over baseline of 13.7 ± 1.8 mmHg [20]. Follow-up studies have confirmed these observations despite multiple differences in the exposure paradigms, pCO_2 levels, species and strains of animals [1, 5, 21–29]. In addition, some of these elegant studies have demonstrated elevations of arterial blood pressure that persist after termination of the intermittent hypoxic exposure. Therefore, it is now established that systemic

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hypertension [20], dyslipidemia [30] and early atherosclerosis (14) as well as vascular remodelling [5, 20] occur after only a few weeks of IH exposure.

In summary, in animals that are not prone to hypertension, IH induces a rapid but moderate elevation in blood pressure. In contrast, in animals with a genotype that is prone to hypertension (spontaneous hypertensive rats, SHR), IH accelerates age-induced hypertension [22, 31].

The development of SDB animal models has provided convincing evidence that IH has significant vascular and haemodynamic impact that can cause the cardiovascular morbidity and mortality observed in patients with sleep apnoea. However, some investigators suggest that species differences make the applicability of animal studies to human disease states questionable. Interestingly, Tamisier, *et al.* recently described a human model of IH [32]. In their study, healthy volunteer human beings were exposed to IH to induce a 10% desaturation–resaturation difference (95% to 85%) every 120 sec., thus allowing 30 cyclic desaturation–resaturation sequences per hour during sleep for 14 and 28 days. CO_2 accumulation was insignificant. The persons experienced spontaneous central hypopnoea during the re-oxygenation phase. These respiratory events induced arousals with significant sleep fragmentation showing adaptation after 4 weeks of exposure. Persons exposed to this model for 2 weeks displayed changes in chemosensitivity. More importantly, IH exposure induced a small but significant increase in arterial blood pressure from baseline. This study suggests that results obtained in SDB animal models can translate to human pathophysiology and confirm the clinical relevance of the experimental models that are indispensable for SDB studies. However, the mechanisms of IH-induced hypertension are still not clear and under investigation. This review focuses on the most recent findings addressing these mechanisms.

Mechanisms of IH-induced systemic hypertension

Contribution of the nervous system

It has been well established that IH-induced elevation in blood pressure involves enhanced sympathetic nerve activity [33–38], increased adrenal catecholamine secretion [39–42], decreased baroreflex [28, 43] and enhanced chemoreflex sensitivity [5, 19, 21, 36, 44, 45]. These reflexes are under the control of the nucleus of the solitary tract in the brainstem. IH has been shown to induce activation of catecholaminergic structures and neuronal cell death – including hypoglossal motoneurons, pyramidal, Purkinje, catecholaminergic wake neurons and upper airway dilator motoneurons [9, 46–49]. The role each of these plays in the blood pressure response and in the decreased baroreflex sensitivity, however, is not clear.

Endoplasmic reticulum (ER) plays a central role in both adaptive and pro-apoptotic response in neurons (reviewed in [50]). The unfolded protein response (UPR) in the ER represents an adaptive response to minimize accumulation of misfolded proteins that would be toxic to the cell. This response includes reduced overall protein translation, increased production of chaperones, up-regulated clearance of improperly folded proteins and increased antioxidant capacity. Several components of this protective response are mediated by phosphorylation of eIF-2a. However, when this stress is insurmountable, the ER may lead to apoptosis by activating CAAT/enhancer-binding protein homologous protein (CHOP)/ growth arrest and DNA damage inducible protein (GADD153) and caspase-7. Zhu, *et al.* have recently demonstrated that IH exposure induces ER injury contributing to death of a selective population of brainstem motoneurons [51]. In adult mice, IH selectively activates the PKR-like ER kinase (PERK ER) sensor of cell fate. Hypoglossal motoneurons, which innervate the upper airway, have high basal levels of CHOP/GADD153 and dephosphorylated p-eIF-2a and are not protected from apoptosis when they are exposed to IH. However, motor trigeminal neurons and occulomotor neurons do not have significant levels of CHOP/GADD153 at baseline, and can mount a protective eIF-2a response. More importantly, protection of eIF-2 dephosphorylation with systemically administered salubrinal (protein phosphatase inhibitor) throughout IH exposure prevented apoptosis in susceptible motoneurons [51]. Other areas, including phrenic and other spinal motoneurons, should now be examined to determine the contribution of neural apoptosis to the development of hypertension.

As mentioned above, substantial evidence demonstrates that hypoxia affects the synthesis of various neurotransmitters in the central and peripheral nervous systems. Synthesis of bioamines is affected in the brainstem. IH exposure through increased reactive oxygen species (ROS) produces a robust increase in tyrosine hydroxylase activity, dopamine level and tyrosine hydroxylase phosphorylation. Tyrosine hydroxylase is the rate-limiting enzyme in the biosynthesis of catecholamines [52]. In addition to biomines, neuropeptides play critical roles in neurotransmission, acting as transmitters or modulators in the brainstem; a region that has been implicated in the central nervous system control of cardiovascular responses. IH activates O_2-sensitive peptidylglycine α-hydroxylating monooxygenase in rat brainstem, which is the rate-limiting enzyme for the generation of carboxy-terminal α-amidated neuropeptides. ROS increases post-translational proteolytic processing leading to elevated levels of α-amidated forms of substance P and neuropeptide Y, which contribute to IH-mediated peptidergic neurotransmission in rat brainstem [53].

Studies in rodents exposed to IH suggest enhanced carotid body (CB) responsiveness to hypoxia is involved in the autonomic alterations induced by OSA and in the development of the hypertension [5, 19, 21, 36, 44, 45]. This is supported by recordings of CB chemosensory neural discharges *in situ* and *in vitro* showing that exposure to IH increases basal CB chemosensory discharges and enhances the chemosensory response to hypoxia [44, 54, 55]. Studies with anti-oxidants suggest the involvement of ROS-mediated signalling in eliciting long-term facilitation of sensory nerve discharge (sLTF) of the CB in rodents exposed to IH [56]. Peng and colleagues have recently demonstrated

increases in NADPH oxidase (NOX) activity in IH but not in control carotid bodies. This effect was associated with up-regulation of NOX2 mRNA and protein in glomus cells of the CB. sLTF was prevented by NOX inhibitors and was absent in mice deficient in NOX2. H_2O_2 generated from $O_2^{\cdot-}$ contributes to sensory plasticity of the CB [57]. These ROS-dependent functional changes may contribute to persistent reflex activation of sympathetic nerve activity in IH and sleep apnoea. In addition, recent evidence support a role for endothelin-1 (ET-1) [58–60] and increased N-methyl-D-aspartate receptors [61] as contributors to the increased CB chemoreflex sensitivity.

Contribution of circulating and vascular factors

In addition to the contribution of the sympathetic and central nervous system, other factors including circulating and tissue ROS [62, 63], angiotensin II (Ang II) [64–66] and ET-1 [23, 29, 67–69] have been implicated in the development of hypertension during IH. Indeed, ROS could be the main and common contributor to hypertension in IH and sleep apnoea. Consistent with this hypothesis is evidence that ROS generation is increased by IH in rodents [45, 62, 63, 70] and in leucocytes from patients with OSA [71].

ROS are produced as intermediates in reduction–oxidation (redox) reactions of O_2 to H_2O and comprise two major groups: free radicals – superoxide ($^{\cdot}O_2^-$), hydroxyl (OH^-) and nitric oxide –, which are highly reactive and unstable; and non-radical derivatives of O_2, for example, H_2O_2, which is less reactive and with a longer half-life than free radicals (reviewed in [72]). ROS are produced in the brain, heart, kidneys and vasculature. It is now recognized that specific enzymes, the NOX family of NADPH oxidases, may have as their sole function generation of ROS in a highly regulated fashion under physiological conditions. However, the physiological production of ROS can also occur as a by-product of other biological pathways such as peroxisomes, cytochrome P450, xanthine oxidase, uncoupled NOS, cyclooxygenase, lipoxygenase, heme oxygenases and especially mitochondrial enzymes [72]. Besides their key role in bioenergetics and ATP synthesis, it appears that mitochondria are one of the main sites of ROS generation within the cell. 80% of intracellular superoxide anion is generated by leakiness of the electron transport chain [73]. In quiescent cells, individual mitochondria undergo spontaneous bursts of superoxide generation, termed 'superoxide flashes' [74]. Mitochondrial ROS production is similar during normoxia and hypoxia but markedly increases during reoxygenation in cardiac myocytes [74, 75]. Similar results were obtained in cortical mouse neurons exposed to IH [76] suggesting the repeated hypoxia/reoxygenation of IH leads to significant increases in ROS generation.

ROS, *via* oxidation of reactive cysteine residues, can activate ion channels (Ca^{2+} and K^+ channels), redox-sensitive kinases (Src, Akt, protein kinase C [PKC], MAP kinases and ρ kinase), protein tyrosine phosphatases such as SH2-domain containing phosphatase and MAP kinase (MAPK) phosphatase, and transcription factors (nuclear factor-κ-light chain enhancer of acti-
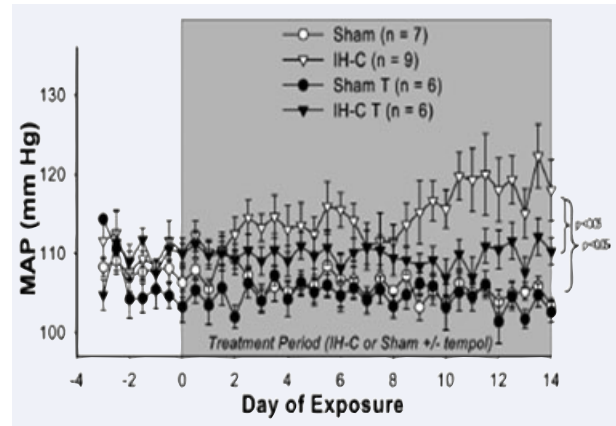

Fig. 1 MAP was recorded daily for 3 baseline days and 14 treatment days in eucapnic intermittent hypoxia (IH-C) $\pm$ Tempol and Sham $\pm$ Tempol (T). MAP increased in IH-C-vehicle rats over the 14 days of treatment. MAP did not increase in IH-C rats treated with Tempol (1 mM). Tempol did not affect MAP in sham-operated rats. *$P < 0.05$, significant difference from Sham/vehicle. Reprinted with permission from Carmen M. Troncoso Brindeiro et al. [63].

vated B cells [NF-κB], activator protein-1 [AP-1], nuclear factor of activated T cells [NFAT] [77–82], p53, Ets and hypoxia-inducible factor-1 [HIF-1]) [72].

It has been shown that IH increases in ROS production scavenge nitric oxide reducing its bioavailability leading to impaired dilation in skeletal and cerebral blood vessels [24, 26]. Impaired vasodilation contributes to hypertension. However, other studies have shown that IH leads to increased vasodilatory ROS causing a reduction in myogenic tone in skeletal muscle arterioles [83]. On the contrary, we have observed that IH augments myogenic tone in mesenteric arteries that is endothelium and ROS dependent (unpublished observations).

In spite of these disparate results the literature clearly supports a role for ROS in IH-induced hypertension. Administration of a superoxide dismutase mimetic (tempol) prevents IH-induced increases in plasma ET-1 levels and blood pressure (Fig. 1) [63].

Hypoxia has been shown to induce ET-1 transcription in several cell types including cardiomyocytes and endothelial cells [84–87]. In addition, ROS increases ET-1 mRNA and peptide levels in endothelial, smooth muscle and CB (glomus) cells [88, 89]. Consistent with a role of ET-1 as a contributor to IH-induced hypertension, chronic administration of an ET$_A$R blocker (BQ123) prevents IH-induced increases in MAP in rats [68].

Our group has also demonstrated that small resistant arteries from IH-exposed rats have selective increased constrictor reactivity to ET-1 [67]. The intracellular signalling pathways that mediates ET-1 vasoconstriction are altered in IH exposed compared with air exposed rats whereas the mechanisms that mediates phenylephrine constriction are unaltered. The

mechanism involves activation of PKC delta-mediated Ca^{2+} sensitization [90].

ROS can also increase angiotensinogen synthesis contributing to the activation of the renin-angiotensin system (RAS) [91, 92]. Interestingly, Ang II by increasing ROS production leads to up-regulation of ET-1 [93, 94]. Therefore, ROS has not only been implicated as activator of these systems but also as part of the signalling downstream of both ET-1 [95] and Ang II [93, 96–98] in the vasculature. In summary, oxidative stress and ET-1 are main contributors to the aetiology of IH-induced hypertension.

The role of Ang II in the increased blood pressure observed in animals exposed to IH and in OSA patients is still controversial. Some studies clearly demonstrate activation of RAS in animals [64] and OSA patients [99, 100]. However other studies have shown no changes in the components of RAS in patients [101–103] and that administration of an Ang II receptor inhibitor (losartan) does not prevent IH-induced hypertension in rats [37].

Although it may not be acting as a pressor agent, elevated plasma levels of Ang II in OSA patients appears to contribute to increases in plasma levels of vascular endothelial growth factor (VEGF) [100, 104]. VEGF is a potent angiogenic cytokine that also contributes to atherogenesis. Therefore, it is possible that Ang II through VEGF contributes to angiogenesis, endothelium repair and atherosclerosis more than to hypertension in OSA.

Role of transcription factors in the inflammatory and cardiovascular consequences of IH

Increases in inflammatory factors have been associated with induction of vascular damage and hypertension. Three transcription factors – NF-κB, NFAT and HIF-1– have been implicated in the initiation of the inflammatory response and the associated vascular damage induced by IH.

NF-κB

The NF-κB is a key mediator of the inflammatory response under many conditions. NF-κB is a member of the Rel family of proteins and was first described as a nuclear factor necessary for immunoglobulin k light chain transcription in B-cells [105]. NF-κB exists in the cytosol in an inactive form bound to the inhibitor, IκB. An appropriate endogenous or exogenous inflammatory stimulus triggers IκB ubiquitin-mediated degradation releasing NF-κB to translocate to the nucleus. In the nucleus, NF-κB up-regulates the transcription of several pro-inflammatory genes responsible for encoding inflammatory cytokines (tumour necrosis factor [TNF]-α, interleukin [IL]-6 and IL-8), chemokines, surface adhesion molecules (intercellular adhesion molecule-1) and enzymes (cyclo-oxygenase-2) (reviewed in [106]).

There is evidence suggesting IH increases NF-κB activity and its downstream products, particularly TNF-α in cultured cell, animal models and OSA patients. TNF-α has been shown to play a major role in inflammation-mediated vascular damage in OSA patients and animal models (reviewed in [107]).

Cultured HeLa cells (an epithelial cell line) and bovine aortic endothelial cells exposed to IH show activation of NF-κB [108]. IH activation of NF-κB is dependent on IκB kinase and p38 MAPK activity [109].

The same group also demonstrated increases in TNF-α and the NF-κB-dependent cytokine, IL-8 in plasma of OSA patients [108, 110]. In mice, IH increased the expression of NF-κB -dependent gene product, iNOS, in cardiovascular tissues (heart, aorta and lung) of mice [111]. Finally, OSA patients have significantly elevated monocyte and neutrophil NF-κB activity, which was normalized following elimination of apnoeas with continues positive airway pressure (CPAP) therapy [111, 112].

Interestingly, NF-κB is a redox-sensitive transcription factor [113, 114] that can initiate inflammatory and immune responses by promoting activation of endothelial cells, leucocytes and platelets. These activated cells express adhesion molecules and pro-inflammatory cytokines that may contribute to endothelial injury and dysfunction and consequently to the development of cardio- and cerebro-vascular morbidities in IH.

NFAT

Another member of the Rel family of transcription factors that has recently been implicated in the development of hypertension in IH exposed mice is the nuclear factor of activated T cells (NFAT) (Fig. 2) [29]. First identified in activated T cells, the calcium (Ca^{2+})-dependent transcription factor, NFAT, has since been shown to play a role in non-immune cells, including cells of the cardiovascular system (reviewed in [115]). The NFAT transcription factor family is comprised four well-characterized members, designated NFATc1 (NFAT2/c), NFATc2 (NFAT1/p), NFATc3 (NFAT4/x) and NFATc4 (NFAT3). The signature of this family is a shared NFAT homology region in the N-terminal portion of the protein. This region mediates binding of the Ca^{2+}-activated phosphatase calcineurin and other regulatory functions necessary for nuclear translocation and contains nuclear localization and nuclear export sequences along with phosphorylation sites for a number of serine/threonine kinases. The C-terminal region of NFAT proteins contains a DNA-binding domain. NFAT activation is regulated primarily through control of its subcellular localization. In unstimulated cells, NFAT is a hyperphosphorylated cytosolic protein. An elevation in intracellular Ca^{2+}, induced by a variety of mechanisms, increases the activity of the Ca^{2+}-calmodulin-dependent phosphatase, calcineurin. Activated calcineurin dephosphorylates the regulatory region of NFAT, inducing a conformational change that exposes nuclear localization signals and allows import of NFAT into the nucleus (reviewed in [115, 116]). NFAT binds DNA with very low affinity in the absence of a cofactor so that formation of an NFAT-cofactor-DNA ternary complex is required for
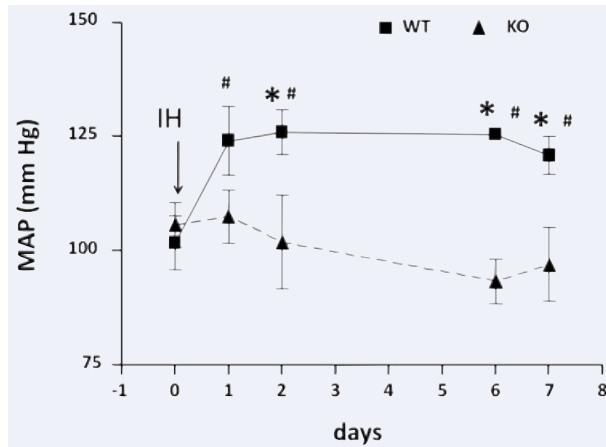
Fig. 2 MAP values, including those for the day before IH exposure (day 0 = air exposure), for NFATc3 wild-type and knockout mice. *$P < 0.05$ *versus* knockout; #$P < 0.05$ *versus* day 0 (two-way repeated-measures anova and Holm–Sidak test). Reprinted with permission from Sergio de Frutos *et al.* [29].

significant NFAT-mediated transcriptional activity. Egr-1 [117, 118], activator protein 1 (AP-1) [119, 120], YY1 [121], the MADS family member – MEF2 [122–124] and members of the GATA family [125, 126] (reviewed in [127]) have been shown to be NFAT cofactors.

NFAT activation is generally associated with an increase in gene expression [116, 127]. However, several reports have shown that NFAT can also function as a repressor of gene expression [128–131].

Activators of Gq-couple receptors (*i.e.* ET-1, Ang II, catecholamines, uridine 5′-triphosphate), elevated plasma glucose levels and increased intravascular pressure are potent activators of NFAT [115, 132–136]. NFAT activation not only plays an important role in modulating the immune response and inflammation [116], it is also important in vascular development [137, 138], maintenance of the contractile phenotype of vascular smooth muscle cells (VSMC) [126, 139, 140] and regulation of VSMC contractility through the down-regulation of voltage-dependent (K_V 2.1) channel and large conductance (BK) potassium channels [128, 131, 141].

Smooth muscle-specific expression of NFAT isoforms has been described. NFATc1 and NFATc2 were the first reported in thoracic aortic smooth muscle cells from rat [142]. More recently human aortic smooth muscle was shown to express NFATc1 and c3, but not NFATc2 or c4 [126]. Extensive studies of the cerebral vasculature reveal NFATc3 and c4, but not NFATc1 or c2 [132–135]. A recent study in the rat pulmonary circulation shows that NFATc4 is the only isoform expressed in PASMC, whereas we showed that NFATc3 is expressed in murine pulmonary arteries [143] and mesenteric arteries [29].

We have recently shown that systemic hypertension caused by IH in mice is associated with NFATc3 activation in aorta and mesenteric arteries [29]. In the same study, we demonstrated that

genetic ablation or pharmacological inhibition of NFATc3 prevents IH-induced hypertension (Fig. 2), demonstrating the importance of NFAT in IH-induced cardiovascular pathology. We have also found that NFATc3 is required for IH-induced mesenteric arterial remodelling with increased expression of the hypertrophic/differentiation marker smooth muscle α-actin (unpublished observations). Smooth muscle α-actin has been identified as a gene regulated by NFATc3 in cooperation with serum response factor and AP-1 [140, 143].

NFAT is also an inflammatory modulator that regulates the expression of TNF-α [144], IL-6 [145, 146] and osteopontin (OPN) [147]. TNF-α, described above, and IL-6 are clearly elevated in patients with SDB [148], suggesting they may be mediators of SDB-associated cardiovascular disease. In particular, IL-6 appears to contribute to atherogenesis [149] and is associated with higher mortality in patients with coronary arterial disease and a higher risk for myocardial infarction in otherwise healthy men [150, 151].

OPN is a multifunctional protein important in proliferation, migration and remodelling in the vasculature. OPN is also linked to multiple human diseases involving systemic inflammation and autoimmunity [152]. OPN plasma levels are increased in hypertension [153, 154], coronary artery disease [155] and by hypoxia [156]. OPN expression is induced by a variety of growth factors and cytokines including IL-1, TNF-α and Ang II [157–159]. In models of systemic inflammatory response, OPN levels have been found to directly correlate with secretion of IL-6 [160]. Interestingly, ROS also enhance expression of OPN in VSMC [161, 162].

ROS also activate NFAT in many different cell types including neuronal-like cells, immune cells, osteoclasts and cardiac myocytes [77–82]. The mechanism by which ROS activates NFAT has not been completely established but, in cardiomyocytes, H_2O_2 induces a time- and dose-dependent activation of NFAT [81]. This activation appears to be mediated by ERK-dependent activation of AP-1, a cofactor of NFAT. This evidence further supports a role for ROS as an upstream activator of a complex signalling pathway initiated by IH that leads to changes in inflammatory and vascular-specific gene expression.

HIF-1

Hypoxia-inducible factor (HIF)-1 is a heterodimeric transcription factor formed by a constitutively expressed β-subunit and an α-subunit that contains an oxygen-dependent ubiquitin- proteasomal degradation domain [163, 164]. Therefore, HIF-1 is suppressed in normoxia.

However, in hypoxia, HIF-1 is stabilized by inhibition of its degradation allowing binding to the regulatory regions of its target genes and regulation of their expression [165]. HIF-1 is the major regulator of oxygen homeostasis within cells by mediating the shift to increased glycolysis and anaerobic metabolism at low oxygen tensions through down-regulation of mitochondrial

oxygen consumption [166]. One of the first genes shown to be regulated by HIF-1 was erythropoietin (EPO) whose gene promoter contains hypoxia response elements. Up-regulation of EPO leads to increased red blood cell production and enhanced blood oxygen-carrying capacity [167]. Studies from our group have shown that IH without CO_2 supplementation (hypocapnia) increases haematocrit in rats [168]. However, IH with CO_2 supplementation (eucapnia) does not increase haematocrit in either rats [168] or mice [29] similar to OSA patients that have no or modest elevation in haematocrit [169]. These results suggest that HIF-1-mediated EPO up-regulation is blunted under hypoxic-eucapnic conditions.

HIF-1 also contributes to inflammation by increasing the survival of pro-inflammatory cells [170].

Another HIF-1 gene product is VEGF, which it has been demonstrated to be elevated in OSA patients with severe nocturnal hypoxemia [171]. VEGF induces migration of mature endothelial cells towards hypoxic areas of tissue, thereby promoting angiogenesis [172]. IH increases HIF-1 in endothelial cells in culture [173, 174] to mediate cell migration and promote an angiogenic phenotype [174]. Therefore, HIF-1-mediated VEGF up-regulation could be involved in IH-induced angiogenesis.

Interestingly, HIF-1α partially deficient mice do not display the typical cardio-respiratory response to IH. Specifically, IH did not: elicit sLTF of the chemoreceptor activity, augment hypoxic ventilatory response, cause LTF of breathing, elevate blood pressure, elevate brain ROS levels, up-regulate brain HIF-1α or increase plasma noradrenaline [45]. This study suggests that IH involves complex positive interactions between HIF-1 and ROS.

The contribution of HIF-1α to IH-induced hypertension may involve up-regulation of ET-1. The pre-pro ET-1 promoter contains hypoxia response elements and it has been demonstrated that pre-pro ET-1 transcription is increased by hypoxia *via* recruitment of HIF-1, AP-1, GATA-2, CAAT-binding factor (NF-1) and CREB binding protein (p300/CBP) to the transcripsome [175–177]. In addition, IH-induced ET-1 up-regulation in the CB requires HIF-1 [178]. Consistent with a role for HIF-1 and ET-1 in IH, Belaidi, *et al*. recently demonstrated a major role for HIF-1 and the endothelin system in promoting myocardial infarction and hypertension in an animal model of OSA [69]. They found that IH accelerates the development of hypertension and enhances infarct size in SHR compared with that seen in control rats. This was accompanied by an increase in myocardial pre-pro ET-1 levels, ET-1 protein and ET-A receptor expression and by enhanced coronary vascular reactivity to ET-1 in SHR only. Myocardial HIF-1 activity was also increased and *in vivo* chromatin immunoprecipation assay on myocardial extracts provided evidence that HIF-1α targets the ET-1 gene in SHR after IH. Moreover, administration of bosentan, a mixed ET receptor antagonist, during IH prevented both the increase in blood pressure and in infarct size. This study demonstrates that activation of the ET system, mediated by HIF-1 activity, is responsible for the enhanced susceptibility to IH and for its associated cardiovascular consequences leading to hypertension and ischemic injury.

Yuan G, *et al*. have investigated the molecular mechanism of HIF-1α up-regulation during IH [179]. The mechanism includes both increased mTOR-dependent HIF-1α synthesis during re-oxygenation and decreased hydroxylase-dependent HIF-1α degradation during hypoxia. HIF-1α up-regulation also appears to require increased generation of ROS by NOX. Anti-oxidants prevented IH decreased hydroxylation of HIF-1α protein. Ca^{2+}-dependent activation of phospholipase Cγ and PKC are also required for decreased HIF-1α degradation.

There is also an active NF-κB binding site in the proximal promoter site of the HIF-1 gene and NF-κB appears to regulate basal levels of HIF-1 gene expression [180]. Indeed, hypoxia up-regulation of HIF-1 transcription appears to be through a NF-κB-dependent mechanism and vice versa [180–182]. Interestingly these studies further established a role for ROS as a factor that contributes to the significant crosstalk between NF-κB and HIF-1, suggesting mutual dependence between the pathways. The O_2 regulated α subunit of HIF-2α, also known as endothelial PAS domain protein-1, is another member of the HIF family [183]. HIF-2α shares 80% sequence homology to HIF-1α and also interacts with HIF-1β [184]. Similar to HIF-1, continuous hypoxia leads to HIF-2α accumulation and the resulting transcriptional activation of VEGF [185]. In contrast to the up-regulation of HIF-1α, it has been shown that IH down-regulates HIF-2α protein levels *via* activation of calpain proteases [186]. IH-evoked HIF-2α degradation led to inhibition of SOD2 transcription, resulting in greater oxidative stress. Systemic administration of calpain inhibitors restored HIF-2α levels in IH-exposed rats and prevented IH-induced oxidative stress and cardiovascular abnormalities.

Together these studies suggest that IH activates HIF-2α leading to increases in ROS, which act in concert with the low O_2 levels to up-regulate HIF-1 and contribute to IH-induced hypertension.

Intermittent hypoxia induced pulmonary hypertension

Pulmonary hypertension (PH) is an additional cardiovascular complication of OSA. Patients with OSA demonstrate cyclical increases in pulmonary arterial pressure coinciding with apnoeic episodes, which has been largely attributed to acute hypoxic pulmonary vasoconstriction (HPV) [187, 188]. OSA additionally exacerbates pulmonary hypertension in patients with COPD (a condition termed 'overlap syndrome'), and CPAP is effective in reducing the severity of pulmonary hypertension in these individuals [189, 190]. However, whether OSA directly causes sustained daytime pulmonary hypertension in the absence of co-existing chronic lung disease or obesity-related hypoventilation is debated. Interestingly, recent studies suggest that OSA is an independent risk factor in PH [187, 191–193], with an incidence of approximately 20–40% in patients without clinically identifiable cardiopulmonary disease [187, 190–197]. Supporting this finding is evidence that CPAP reduces daytime pulmonary arterial pressure in pulmonary
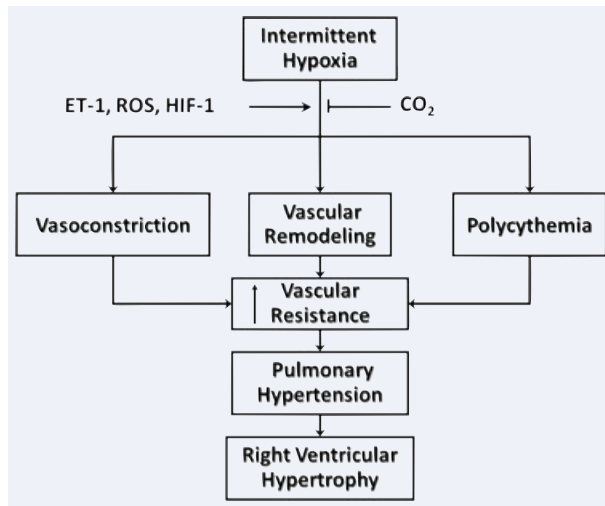
Fig. 3 Proposed mechanisms of IH-induced pulmonary hypertension. ET-1, ROS and HIF-1 may play important roles in the vasoconstrictor, remodelling and polycythemic components of pulmonary hypertension. Supplemental CO_2 blunts IH-induced right ventricular hypertrophy, arterial remodelling and polycythemia.

hypertensive OSA patients without significant heart disease, lung disease [191–193] or daytime hypoxemia [191, 192]. This relationship between OSA and PH has been reviewed extensively elsewhere [187, 189, 190, 198]. Despite the growing recognition of the prevalence of this disorder, the cardiovascular sequelae leading to the progression of OSA-induced PH in human beings remain poorly characterized. However, animal models of OSA that involve chronic exposure to IH have begun to address these mechanisms of PH.

Many of the rodent models of OSA reproduce not only the systemic hypertension characteristic of OSA [23, 63, 67, 68] as detailed earlier in this review, but provide evidence for PH as well [27, 168, 199–202]. Mechanisms of IH-induced PH are generally considered to be parallel to those associated with chronic sustained hypoxia (CH), and include pulmonary vasoconstriction, vascular remodelling (characterized by smooth muscle hypertrophy and hyperplasia in small pulmonary arteries) and polycythemia (Fig. 3). The degree of pulmonary hypertension closely correlates with right ventricular hypertrophy [203], an adaptive response to the increase in afterload. Although clinical manifestations of right heart failure and peripheral oedema resulting from severe PH can develop in patients with overlap syndrome, such complications are uncommon in individuals with OSA uncomplicated by concomitant lung disease [187, 190–197].

Whether CH and IH produce PH through similar mechanisms is not clear, but studies comparing the two models suggest that the PH resulting from IH is less severe. For example, Fagan [200] found that mice exposed to CH (barometric pressure = 410 mmHg, 4 weeks) demonstrate greater increases in right ventricular systolic pressure compared to IH mice (2 min. cycles of 10%/21% O_2, 8 hrs/day, 4 weeks). Consistent with these findings, right ventricular hypertrophy was greater in CH *versus* IH animals, although no significant differences were observed between groups with respect to the degree of polycythemia or neomuscularization of small pulmonary arteries. A recent study from our group demonstrates a similar effect of IH (3 min. cycles of 5%/21% O_2, 7 hrs/day, 2 weeks) to increase pulmonary arterial wall thickness, haematocrit, and right ventricular weight in rats [168]. However, the severity of these responses again tended to be reduced compared to effects of sustained CH (barometric pressure = 380 mmHg, 2 weeks).

Vasoconstrictor responses associated with IH have not been well studied, although HPV likely contributes to increases in pulmonary arterial pressure during hypoxic episodes, and possibly to sustained hypertension in OSA patients that exhibit daytime hypoxemia. However, it is possible that HPV-independent vasoconstrictor mechanisms additionally contribute to PH following IH. Indeed, vasoconstriction appears to play a central role in mediating PH associated with CH, evident by effects of vasodilators to dramatically lower pulmonary vascular resistance in CH rats that have been acutely returned to a normoxic environment [204, 205]. Recent studies from our group and others suggest that the vasoconstrictor response to sustained CH may be multifaceted, with contributions of both spontaneous pulmonary arterial tone [206] and enhanced agonist-dependent vasoconstriction [168, 207, 208]. Whereas IH also enhances pulmonary vasoreactivity to the thromboxane mimetic, U-46619, the magnitude of this response is markedly blunted compared to that observed with CH [168]. Additional research is needed to define the contribution of vasoconstriction to IH-induced pulmonary hypertension, and whether increased vasoreactivity in this setting is the result of altered VSM signal transduction or endothelial dysfunction, both of which occur following CH [205–207, 209, 210].

Previous studies from our group have identified ET-1 as a critical mediator of IH-induced systemic hypertension in rats [23, 68] (discussed above). Given the well established contribution of ET-1 to CH-induced PH [211–213], that circulating ET-1 levels are elevated in IH rats [23], and pre-pro ET-1 mRNA is up-regulated in lungs from IH mice [29] a distinct possibility is that ET-1 similarly contributes to IH-induced PH. Future studies are needed to assess the potential role of ET-1 in this response, and potential influences of ET-1 on the remodelling and vasoconstrictor responses to IH. In agreement with evidence that IH augments pulmonary vasoconstrictor sensitivity to U-46619 [168], preliminary studies from our group indicate that IH similarly augments VSM reactivity to ET-1 in pressurized small pulmonary arteries from rats through a PKC signalling pathway [214]. Interestingly, this mechanism of increased PKC-dependent vasoconstriction following IH stands in marked contrast to our findings of enhanced ρ kinase-mediated myofilament Ca^{2+} sensitivity in the pulmonary circulation of CH rats [207]. Whether such increases in pulmonary vasoconstrictor sensitivity to ET-1 contribute to IH-dependent PH remains to be established.

Considering the contribution of ROS to IH-induced systemic hypertension [63], and the well-established role of ROS in various models of hypoxic PH [215–217], a reasonable hypothesis is that
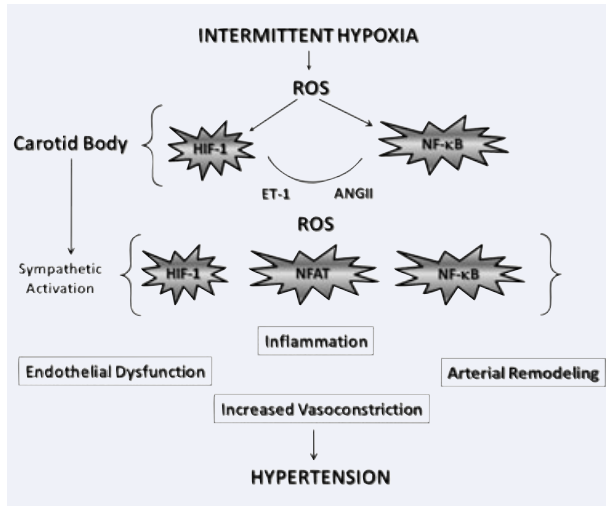
Fig. 4 Working model of the mechanisms of IH-induced hypertension. ROS = reactive oxygen species; ET-1 = endothelin-1; ANG II = angiotensin II; HIF-1 = hypoxia inducible factor-1; NF-κB = nuclear factor-κ-light chain enhancer of activated B cells; NFAT = nuclear factor of activated T cells.

ROS similarly contribute to the development of IH-induced pulmonary hypertension. NOX [215, 218–221] and xanthine oxidase [222, 223] have been the major sources of ROS implicated in the development of pulmonary hypertension. Indeed, a role for NOX in IH-dependent PH has recently been reported by Nisbet and colleagues [201], who found that the NOX subunits NOX4 and p22phox and ROS levels are increased in lungs from IH mice (1.3 min. cycles of 10%/21% O_2, 8 hrs/day, 5 days/ week, 8 weeks). Evidence from this same study that IH-induced RV hypertrophy and arterial remodelling are abolished in NOX2 (gp91phox) knockout mice further supports a major contribution of NOX2-derived ROS in this pulmonary hypertensive response.

The mechanisms by which ROS mediate IH-dependent pulmonary hypertension are not understood, but may involve both enhanced pulmonary vasoconstrictor sensitivity [207, 214] and increases in gene expression secondary to activation of HIF-1 [179]. Consistent with the latter possibility and as reviewed above, studies using HIF-1α heterozygous mice suggest that HIF-1-mediated gene expression is central to not only the development of CH-induced PH [224], but to CB-mediated ROS generation and associated cardiorespiratory responses to IH as well [45]. Whether a functional link exists between NOX, HIF-1 activity and vasoconstrictor sensitivity in the development of IH-induced pulmonary hypertension remains to be determined.

Apnoeic episodes in patients with SDB are associated not only with hypoxemia but with hypercapnia as well [225]. Therefore, studies that employ a purely IH stimulus without provision of supplemental CO_2 do not accurately reflect the blood gas changes that occur during apnoeic episodes in OSA patients. Rather, the hypoxic increases in ventilation resulting from IH alone lead to cyclical reductions in arterial Pco_2 and consequent alkalemia [168]. To more closely mimic the arterial blood gas and pH changes observed in patients with sleep apnoea, studies in our group have begun to compare effects of IH with and without supplemental CO_2 on indices of pulmonary hypertension, polycythemia and pulmonary vasoreactivity. Interestingly, we found that supplemental CO_2 significantly reduces the arterial remodelling, polycythemic and right ventricular hypertrophic responses to IH [168]. These results are consistent with earlier studies demonstrating a protective influence of CO_2 on the development of CH-induced pulmonary hypertension [226, 227]. Although the mechanisms by which CO_2 reduces the severity of PH are not understood, it is possible that supplemental CO_2 leads to suppression of oxidant stress [227] and hypoxia-inducible gene expression through an as yet unidentified mechanism.

In summary, animal models of SA involving chronic exposure to IH have provided new insight into the complex aetiology of SA-associated PH. Although these models of PH share some characteristics of those involving CH, some notable differences have been revealed with respect to the severity of PH and intracellular signalling pathways responsible for enhanced vasoconstrictor sensitivity. In addition, important new questions have been raised regarding the mechanisms that mediate both vasoconstrictor and arterial remodelling components of IH-dependent PH, including the roles of ET-1, ROS, PKC and HIF-1-induced gene expression. Neither is it understood how CO_2 blunts the pulmonary hypertensive response to IH, nor whether a similar protective influence of CO_2 exists in human beings. Animal models of PH will continue to improve our understanding of potential differences in the pathogenesis of CH *versus* IH-induced PH, which in turn is necessary to indentify effective therapeutic strategies to treat these conditions.

Conclusions

Rodent models of IH have provided a tremendous advance in our understanding of the cardiovascular alterations observed in patients with SDB.

After reviewing the literature on this field is clear that ROS plays a major role in the pathogenesis of the cardiovascular morbidities induced by IH and also present in SDB patients. We are proposing a working model where ROS is the activator of the main factors enhanced by IH but also is part of the intracellular signalling pathways initiated by these factors (Fig. 4), which contribute to inflammation, endothelial dysfunction, vascular remodelling and increased vasoconstriction leading to hypertension.

Acknowledgements

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