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Endoplasmic reticulum stress as a novel inducer of hypoxia inducible factor-1 activity: its role in the susceptibility to myocardial ischemia-reperfusion induced by chronic intermittent hypoxia☆,☆☆,☆☆☆

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ABSTRACT

Background: Obstructive sleep apnea (OSA) is a highly prevalent disease and a risk factor for myocardial infarction expansion in humans. Intermittent hypoxia (IH) is known to be the most important OSA feature in terms of cardiovascular morbi-mortality.
Since ER stress and HIF-1 are known to be involved in cardiomyocyte life or death, this study investigates the role of ER stress on HIF-1 activation in myocardial susceptibility to ischemia-reperfusion (I/R) induced by IH.
Methods: C57Bl6J, HIF-1$\alpha^{+/-}$ and their respective control mice were exposed to 14 days of IH (21–5% FiO_2, 60 s cycle, 8 h/day). Myocardial inter-organelle calcium exchanges, ER stress and HIF-1 activity were investigated and *in vivo* I/R was performed to measure infarct size. In additional groups, tauroursodeoxycholic acid (TUDCA, 75 mg·kg^{-1}), an ER stress inhibitor, was administered daily during exposure.
Results: In C57Bl6J mice, chronic IH induced an increase in ER-Ca^{2+} content, ER stress markers and HIF-1 activity, associated with an enhanced infarct size (33.7 ± 9.4 *vs.* 61.0 ± 5.6% in N and IH, respectively, $p < 0.05$). IH failed to increase infarct size in HIF-1α deficient mice (42.4 ± 2.7 and 24.7 ± 3.4% N and IH, respectively). Finally, TUDCA totally abolished the IH-induced increase in HIF-1 activity (1.3 ± 0.04 *vs.* 0.14 ± 0.02 fold increase in IH *vs.* IH-TUDCA respectively, $p < 0.0001$) and in infarct size (55.5 ± 7.6 *vs.* 49.9 ± 3.0 in N-TUDCA and IH-TUDCA, respectively).
Conclusion: This novel regulatory mechanism of HIF-1 activity by ER stress should be considered as a potential diagnostic tool for cardiovascular complications in OSA patients as well as a therapeutic target to limit myocardial ischemic damage.

1. Introduction

Obstructive sleep apnea (OSA) is a highly prevalent disease characterized by repetitive upper airway collapses during sleep leading to chronic intermittent hypoxia (IH) [1]. In 2008, the American Heart Association and American College of Cardiology jointly reported that OSA is independently associated with increased risk of hypertension, coronary heart disease, arrhythmias, heart failure and death [2,3]. Buchner et al., demonstrated recently that co-existing OSA promotes myocardial infarct size expansion and less myocardial salvage after acute myocardial infarction [4].

Among the different consequences of OSA, nocturnal hypoxemia seems to strongly predict the risk of sudden cardiac death [5]. Since mechanistic studies are difficult to perform in OSA patients with data interpretation limited by numerous confounders, animal models of OSA have been developed and have shown that, among the different stimuli induced by OSA, chronic IH appears to be the most detrimental for the cardiovascular system [6]. In particular, in the context of deleterious hypoxemia mimicking, we and others have shown that chronic IH induces atherosclerosis [7,8], arterial hypertension [9], an increased susceptibility to myocardial infarction [9,10], and early heart failure [11] in rodents, likely through oxidative stress [12,13] and activation of the hypoxia inducible factor-1 (HIF-1) [9,14]. HIF-1 is a transcription factor

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composed of 2 subunits, the constitutive nuclear HIF-1β subunit and the O_2-regulated cytosolic HIF-1α subunit. In normoxic conditions, HIF-1α hydroxylation by prolyl hydroxylase enzymes leads to its proteasomal degradation. In hypoxic conditions, HIF-1α is stabilized and translocated to the nucleus where it dimerizes with HIF-1β to activate the transcription of various genes [15]. Depending on the intensity and duration of the hypoxic stimulus, HIF-1 triggers the transcription of genes that are involved in either adaptive (*i.e.* inducible nitric oxide synthase) or detrimental (*i.e.* ET-1) responses. Although HIF-1 is involved in myocardial pre- and post-conditioning [16–18] it also triggers cardiovascular deleterious response to chronic IH [14]. For example, we have previously demonstrated that HIF-1 binding on the ET-1 gene increases the susceptibility of the myocardium to ischemia–reperfusion (I/R) injury [9]. Although hypoxia is a potent determinant of HIF-1 activity, recent data suggest that HIF-1 could also be activated by other stress. In particular, Werno et al. have demonstrated an increase in HIF-1α mRNA content following an endoplasmic reticulum (ER) stress [19] and Lopez-Hernandez et al. have recently suggested that HIF-1α level is regulated by ER stress in the context of chemical hypoxic neuronal death [20].

ER stress occurs in both physiological and pathological conditions (*i.e.* oxidative stress, ischemia, hypoxia, Ca^{2+} disturbances…). Especially, it is involved in various OSA-associated pathologies such as atherosclerosis, diabetes, and ischemic heart disease [21,22]. Calcium homeostasis disturbances and/or unfolded proteins accumulation in the ER triggers the unfolded protein response (UPR). UPR is initiated by the release of glucose-regulated protein 78 kDa (Grp78) from transmembrane ER sensors to resolve ER stress. Briefly, UPR leads to activation of 3 pathways characterized by (i) double-stranded RNA-activated protein kinase-like ER kinase (PERK), (ii) activating transcription factor 6 (ATF6), (iii) inositol required enzyme 1 (IRE-1), resulting in ER homeostasis recovery. However, when ER stress is excessive and prolonged, the UPR promotes cell death especially through activation of the pro-apoptotic transcription factor C/EBP homologous protein (CHOP). The role of ER stress in cardiovascular diseases is well recognized [23]. In particular, intense ER stress plays a pivotal role in the cell death induced by myocardial ischemia–reperfusion [21]. Recent studies have evidenced an ER stress in both brain [24–26] and heart [27–29] of rodents exposed to chronic IH. Furthermore, the IH-induced ER stress in the brain appears to be related to HIF-1 since CHOP deletion abolished the IH-dependent increase in HIF-1α mRNA content [25]. While ER stress and HIF-1 pathways have been viewed as two major factors in triggering damage to the heart under the condition of IH, the interrelationship between them in this pathophysiological condition has not been established. Activation of the ER stress/HIF-1 pathway by chronic IH could represent a major contributing factor in the enhanced response to myocardial ischemia–reperfusion and possibly, a potential therapeutic target against OSA-associated cardiovascular pathologies.

The aims of the present study were: first, to determine whether chronic IH exposure *per se* induces a myocardial ER stress that predisposes the myocardium to an increased susceptibility to I/R *in vivo*; and second, to investigate the relationship between IH-induced ER stress, HIF-1 activation and consequently myocardial sensibility to ischemia-reperfusion injury.

2. Methods

2.1. Animals

Male C57BL/6J mice, HIF-1α +/- or their wild-type (WT, Swiss x S129) littermates (8 week old) were randomized to 14 days of IH or air (normoxia, N). The IH stimulus was applied automatically during daytime, as rodents preferentially sleep during this period. IH consisted of repetitive cycles of hypoxia and reoxygenation. Briefly, cages were flushed for 30 s with nitrogen to achieve a hypoxia plateau (5% FiO_2) followed by 30 s of air to restore N (21% FiO_2).

A set of mice were intraperitonealy (i.p.) treated 5 days per week of exposure, during the exposure, with the tauroursodeoxycholic acid (TUDCA) an ER stress inhibitor (75 $mg \cdot kg^{-1}$) The experiments were conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Council of Europe, European Treaties ETS 123, Strasbourg, 18 March 1986) and with the Guide for Care and Use of Laboratory Animals (NIH Publication No. 85–23, revised 1996) and were approved by an Institutional Animal Care and Use Committee (agreement number B 38 516 10 006, n° 273_UHTA-U1042-EBC-02).

2.2. Ischemia-reperfusion protocol

At the end of the IH exposure, HIF-1α$^{+/-}$ and their WT control mice, as well as C57Bl6J mice, treated or not with TUDCA, (Fig. 1) were anesthetized by an i.p. injection of sodium pentobarbital (70 $mg \cdot kg^{-1}$). Animals were intubated with a tidal volume of 0.2 ml and a breathing rate of 160 per minute. Body temperature was maintained at 37 °C. A left thoracotomy was performed and the pericardium was removed. A 7-0 silk suture was passed around the left interventricular artery. Successful left interventricular coronary artery occlusion was confirmed by an ST segment shift on the electrocardiogram (Powerlab) and whitening of the ischemic area. After 45 min of ischemia, the ligature was removed and the myocardium was reperfused for 90 min, according to a previous study showing that 90 min is enough to induce a stable comparable necrosis area [30].

2.3. Infarct size measurement

At the end of reperfusion, the coronary artery was briefly re-occluded, and 1 ml Unisperse blue pigment was injected intravenously to delineate the area at risk (AAR). Then, hearts were excised and cut into five 1 mm-thick transverse slices. Each slice was incubated for 20 min in a 1% triphenyltetrazolium chloride solution at 37 °C to differentiate infarcted from viable myocardial areas [31]. Extent of AAR and area of necrosis (AN) was quantified by planimetric analysis (Image J software) and corrected by the weight of each slice [32].

2.4. Murine adult cardiomyocytes isolation and calcium measurements

Cardiomyocytes were isolated with enzymatic digestion according to a previously described procedure [33]. To measure cytosolic, endoplasmic reticulum or mitochondrial Ca^{2+}, cardiomyocytes were loaded for 20 min at 37 °C with Fluo4-AM (3 μM; Invitrogen), Fluo-5N (3 μM; Invitrogen), or Rhod2-AM (6 μM; Invitrogen), respectively. After being loaded, probes were washed out with Tyrode solution and calcium organite content was recorded by confocal microscopy (Dynascope-LSM710 equipped with a 63× lens). Fluo4-AM, Fluo-5N and Rhod2-AM fluorescence signals were obtained by excitation at 488 nm and detected with a spectral range between 495 and 699 nm (HFT488 dichroic beamsplitter). Cells were analyzed with paired combinations of probes (Fluo4-Rhod2/Fluo5-Rhod2). For each probe, fluorescence recorded after ER leakage, induced by inositol triphosphate receptor-mediated stimulation (histamine 10 μM), was expressed relative to baseline (F1/F0). Results (mean values of 4 cells per heart) were computed by calculating the Ca^{2+} slope between 100 and 50 s ($slope_{100–50}$) after histamine.

2.5. Assessment of myocardial ER stress and HIF-1α

Myocardial ER stress was assessed in C57Bl6J mice, either at the end of the IH exposure (in left ventricle from mice treated or not with TUDCA) or after the I/R protocol (in AAR, after 15 min of reperfusion). HIF-1 α mRNA content or nuclear/cytosolic ratio were assessed at the