Review

Endothelial cell responses to hypoxia: initiation of a cascade of cellular interactions

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Abstract

The origin of several vascular pathologies involves sudden or recurrent oxygen deficiency. In this review, we examine what the biochemical and molecular responses of the endothelial cells to the lack of oxygen are and how these responses may account for the features observed in pathological situations, mainly by modifications of cell–cell interactions. Two major responses of the endothelial cells have been observed depending on the degree and duration of the oxygen deficiency. Firstly, acute hypoxia rapidly activates the endothelial cells to release inflammatory mediators and growth factors. These inflammatory mediators are able to recruit and promote the adherence of neutrophils to the endothelium where they become activated. The synthesis of platelet-activating factor plays a key role in this adherence process. Secondly, longer periods of hypoxia increase the expression of specific genes such as those encoding some cytokines as well as for the growth factors platelet-derived growth factor and vascular endothelial growth factor. The transcriptional induction of these genes is mediated through the activation of several transcription factors, the most important one being hypoxia inducible factor-1. The link between our knowledge of the signalling cascade of the cellular and molecular events initiated by hypoxia and their involvement in several vascular pathological situations, varicose veins, tumor angiogenesis and pulmonary hypertension is discussed briefly. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

By virtue of its location and ability to synthesize various vasoactive mediators, the vascular endothelium plays a key role in the maintenance of vascular homeostasis. Indeed, numerous endothelial-derived products have been shown to regulate platelet, leukocyte or smooth muscle cell functions and disturbance of the endothelial metabolism profoundly affects the vascular functions.

Being the first cell layer in contact with the blood, endothelial cells have to cope with all changes occurring within the blood. One of these changes is the variation in the oxygen tension. The metabolic and molecular changes occurring during hypoxia/reoxygenation have been abundantly investigated and reviewed and they greatly contributed to the understanding of the pathophysiological modifications observed in ischemic/reperfused organs. However, recently, it has been found that hypoxic conditions by themselves have profound effects on the endothelial functions: hypoxia is able to activate the endothelial cells and thereby initiates a cascade of reactions involving neutrophils and smooth muscle cells. In ad-
dition, hypoxia directly regulates the expression of several genes.

In this paper, we review some of the mechanisms leading to endothelial cell activation which occurs under hypoxic conditions. We also examine the relevance of such a cascade of reactions induced by hypoxia for the appearance of some physiopathological modifications observed in vessel walls under ischemic conditions.

2. Endothelial cell–neutrophil interactions

One of the most spectacular effects of hypoxia on endothelial cells is to increase their adhesiveness for neutrophils [1–3]. This adherence is observed both in vitro and in vivo [4] under hypoxia and does not require the reoxygenation step.

In general, leukocyte emigration from the bloodstream occurs through a sequential three step process mediated by several adhesion molecules as well as chemoattractant/activator molecules [5,6]. The initial rolling phase is mediated by selectins and consists in a slowing down of the leukocyte movement at the surface of the endothelium. Subsequently, firm adherence occurs via the interaction of the leukocyte β2-integrins with ligands such as ICAM-1 (intercellular adhesion molecule-1). This binding requires activation of the integrins by exposure of the neutrophils to chemoattractant/activator molecules like interleukin-8 or platelet-activating factor (PAF). Finally, transmigration through the endothelium occurs, triggered by a gradient of chemotactic factors.

Some molecular basis for hypoxia-induced adherence of neutrophils to endothelial cells has been untangled and the data obtained on HUVEC (human umbilical vein endothelial cells) are summarized in Fig. 1. It follows the general pattern mentioned above and is the consequence of endothelial cell activation by hypoxia. Activation as used here means a direct process which does not need gene induction.

The hypoxia signalling cascade involves different

![Diagram of endothelial cell-neutrophil interactions](image-url)

Fig. 1. Metabolic and biochemical responses of endothelial cells to hypoxia leading to their activation and to the adherence and activation of neutrophils. ICAM-1, intercellular adhesion molecule-1; LTB₄, leukotriene B₄; PAF, platelet-activating factor; PGF₂α, prostaglandin F₂α; PSGL-1, P-selectin glycoligand-1; vWF, von Willebrand factor.
pathways necessary for a rapid cell activation or for the regulation of gene expression; it seems to be dependent on the cell type and varies according to the degree of hypoxia. In differentiated HUVEC exposed to oxygen deficiency, the initiating event of the endothelial cell activation is a decrease in the mitochondrial respiratory chain activity [7]. A decrease in mitochondrial potential has been observed in liver sinusoidal endothelial cells exposed to hypoxia [8]. Moreover, direct inhibition of the respiratory chain in HUVEC also leads to enhanced neutrophil adherence as hypoxia [9]. It must, however, be noted that the inhibition of mitochondrial oxidative phosphorylation does not always recapitulate the effects of hypoxia. This is particularly true for gene expression induced by hypoxia inducible factor-1 (HIF-1) (see below).

Different second messengers may be responsible for the hypoxia-induced activation of endothelial cells. Firstly, a fall in cAMP concentration is observed in hypoxic endothelial cells, which may be responsible for an increased vascular leakage [10]. Secondly, an increase in the cytosolic calcium concentration is observed in differentiated HUVEC [11,12]. Calcium ion regulates the activity of various enzymes and is a mediator of signal transduction for thrombin, histamine or bradykinin stimulation in endothelial cells. An elevated calcium concentration activates phospholipase A2 in hypoxic endothelial cells [13], leading to the synthesis of high amounts of prostaglandins. Increased PGI2 release from bovine pulmonary artery endothelial cells [14] and from HUVEC [15] and increased PGI2 (prostaglandin I2), PGF2α, PGD2 and PGE2 release from HUVEC were indeed observed under hypoxia [13], while in some experimental models, a decrease is observed [16]. PGF2α seems to be responsible for the chemotactic activity for neutrophils present in hypoxic HUVEC-conditioned medium (Arnould, unpublished data). It was recently suggested that this hypoxia-induced synthesis of prostanoids also inhibits NO production via an autocrine negative feedback mechanism [17] which could partially explain the often observed decrease of EDRF release by endothelial cells in hypoxia. Recently, Schmedtje et al. [18] have demonstrated that hypoxia induces cyclooxygenase-2 expression via the activation of the NF-κB p65 transcription factor in human endothelial cells. These data indicate that, under hypoxia, both the level of cyclooxygenase and the phospholipase A2 activity increases, which could account for the increase in prostaglandin synthesis in endothelial cells exposed to hypoxia.

Activation of phospholipase A2 in hypoxic endothelial cells not only allows a large release of prostaglandins but also the synthesis of PAF [3,19]. In vivo and in vitro studies showed that PAF is a neutrophil-activating agent promoting this adherence to the endothelium after hypoxia or ischemia [2,20]. Different structurally unrelated PAF antagonists as well as the inhibition of PAF synthesis by oleic acid indeed block neutrophil adherence to hypoxic endothelial cells [3]. Concomitantly, Pinsky et al. [21] elegantly demonstrated that hypoxia alone induces a calcium-dependent exocytosis of the Weibel-Palade bodies. These organelles, typical of endothelial cells, store the von Willebrand factor (vWF). This exocytosis leads to the release of vWF [22] and to the overexpression of P-selectin which then sustains neutrophil binding. This explains why hypoxia-induced neutrophil adherence can be blocked by antibodies against P-selectin [3,23]. In addition to PAF and P-selectin, the hypoxia-induced neutrophil adherence also requires the neutrophil β2 integrin (CD18) [1,3,23] as well as the endothelial ICAM-1. Neither ICAM-1 nor E-selectin nor VCAM-1 are upregulated by hypoxia [24]. Hence, it was suggested that the adherence process was due to its constitutive expression on endothelial cells [3]. The role of another still uncharacterized adhesion molecule only expressed on hypoxic endothelial cells but not on normoxic cells has been reported [25].

Neutrophils not only adhere but are also activated when in contact with hypoxic HUVEC. This activation is characterized by an increased cytosolic calcium concentration, by the release of high amounts of superoxide anion and by the synthesis of leukotriene B4 [26]. They are also sensitive to hypoxic conditions. Several authors have reported that hypoxia per se is able to increase CD18/CD11b expression at the surface of neutrophils [27–29] resulting in an enhanced adhesion to endothelial cells. On the other hand, hypoxia has been shown to decrease free radical production and cytokine synthesis by neutrophils in response to various stimuli [30,31]. It must be noted that the contact of neutrophils with activated endo-
thelial cells not only results in adherence and activation but also in delayed apoptosis, thereby prolonging their useful life [32].

In conclusion, hypoxia per se is able to activate the endothelial cells as well as to initiate all stages of recruitment, rolling, adhesion and activation of neutrophils in ischemic organs.

In addition to this overall cascade of reactions, other endothelial cell functions are also modulated by hypoxia. Endothelial cells maintained under hypoxia for 24 h display a significant increase in procoagulant activity [33] which correlates with a marked decrease of thrombomodulin expression at the surface of endothelial cells [34]. The expression of the tissue factor [35] and of some cytokines like interleukin-6 [36,37], interleukin-1α [38], interleukin-8 [39,40] and MCP-1 (macrophage chemotactic protein-1) [39] is also enhanced under hypoxic conditions. The production of these proinflammatory cytokines in addition to the cascade described in Fig. 1 may explain why an inflammatory response develops in ischemic tissues.

3. Tissue remodeling

The vascular tone is regulated under normal conditions through the release by endothelial cells of both vasorelaxing molecules such as EDRF and prostacyclin and vasoconstricting agents like endothelin-1 (ET-1). The molecular mechanism beyond the regulation of the synthesis of these vasoactive mediators is still under investigation but the best documented hypothesis suggests the presence of ‘mechanosensors’ which would sense the shear stress undergone by the cells [41].

Hypoxia differently affects these two types of molecules creating conditions favorable to vasoconstriction [42]. Indeed, the basal and agonist-stimulated release of EDRF by endothelial cells is quickly inhibited by hypoxia [43,44] and the NO production remains very low even after long periods of hypoxia (24–48 h). This seems to be due to a decrease in the endothelial constitutive NO synthase expression [45,46]. Recent data however indicate that, unlike endothelial constitutive NO synthase, inducible NO synthase is induced by hypoxia in pulmonary artery endothelial cells [47]. The overall result of these changes, i.e. the effect of hypoxia on NO production, thus remains highly controversial.

In contrast, hypoxia generally enhances the release of ET-1 by endothelial cells in vitro [48,49] but this depends on experimental conditions since, in two cases, a decrease has been reported [50,51]. In vivo, an increase in ET-1 circulating level is also observed and this was correlated with an induction of ET-1 gene transcription in lungs [52].

The effect of hypoxia on the release of mitogenic molecules for smooth muscle cells is also well evidenced. Experimentally, it was shown that short-term hypoxic endothelial cell-conditioned medium induces the proliferation of smooth muscle cells. By the use of cyclooxygenase inhibitors and neutralizing antibodies, PGF2α and basic fibroblast growth factor (bFGF) have been identified as being the mediators of this effect [53]. bFGF seems to be released from intracellular stores by hypoxic endothelial cells since there is no induction of its synthesis by hypoxia [54]. Other mitogens, such as platelet-derived growth factor-B (PDGF-B), have also been detected in sustained hypoxia [54]. The presence of PDGF and of ET-1, which also has mitogenic properties for smooth muscle cells, contributes to the pro-proliferative activity present in conditioned media from endothelial cells undergoing a sustained hypoxia [55]. In conclusion, the increased production of these different mitogens combined with the suppression of endothelial NO synthase would be expected to accelerate smooth muscle cell growth and to induce vascular remodeling. In addition to increase the release of growth factors, hypoxia stimulates the production of some extracellular matrix proteins, such as thrombospondin-1 in human endothelial cells [56]. Thrombospondin-1 modulates smooth muscle cell proliferation and migration and may be a negative regulator of angiogenesis [57]. Chronic in vivo hypoxic exposure indeed induces medial hypertrophy of pulmonary vessels [58–62]. Cell proliferation and matrix deposition in hypoxia reflects an imbalance between pro-proliferative and anti-proliferative stimuli, most of them derived from the endothelium.

Recent findings from Kourembanas’ laboratory suggest that this proliferative response is regulated by a negative feed back loop mediated through smooth muscle cell-derived CO. They observed an increased CO production in smooth muscle cells
under hypoxia which is the result of the transient induction by hypoxia of heme oxygenase-1 expression [63]. CO released by hypoxic smooth muscle cells was shown to be able to decrease the hypoxia-induced transcription of PDGF-B and ET-1 in endothelial cells by increasing their cGMP content. This cascade eventually inhibits smooth muscle cell proliferation initiated by hypoxia [64]. In addition, the increased CO release by smooth muscle cells under hypoxic conditions was shown to be directly responsible for smooth muscle cell growth inhibition [65]. Fig. 2 summarizes some of the interplay between endothelial and smooth muscle cell mediators under hypoxia.

Endothelial cell proliferation itself is also affected by hypoxia. The most potent and specific mitogen for the endothelial cells is vascular endothelial growth factor (VEGF). It is known to initiate angiogenesis in vivo [66]. Its expression is upregulated by hypoxia in numerous cell types including endothelial cells [67,68]. In addition, an increased expression of the VEGF receptor Flt-1 [69] and a functional upregulation of the VEGF receptor KDR or Flk-1 by hypoxia [70] have been reported in response to hypoxia, which can be responsible for the stronger VEGF-induced mitogenic response of hypoxic endothelial cells compared to normoxia.

The release of all these cytokines and growth factors by cells like the endothelial cells is generally the result of a complex cascade of processes which ends with the activation of transcription factors. Some of them have been found to be activated under hypoxic conditions but not by the inhibition of mitochondrial respiration: this is the case for AP-1 [71], nuclear
factor-interleukin-6 (NF-IL-6) [36], NF-κB-like [37,39,72] and Egr-1 [73] (see Faller et al. for a review [74]). However, the prototype of the transcription factors regulated by hypoxia is HIF-1. HIF-1 is a ubiquitous basic helix-loop-helix PAS heterodimer which is activated by hypoxia [75–77]. It is composed of the two subunits, HIF-1α and HIF-1β or ARNT (aryl/hydrocarbon receptor nuclear translocator), the first being upregulated by hypoxia. Very little is known about the mechanisms of oxygen sensing, the signalling pathway and the subsequent activation of HIF-1. Possible mechanisms include protein stability and degradation by proteasome, phosphorylation, redox processes and Hsp90 binding regulating intracellular localization and more than one could be involved [78]. Moreover, a major line of research is the identification of the cellular oxygen sensor. Redox processes, for example involving H2O2, initiated by a postulated heme oxygen sensor have been proposed [79–81]. The mitochondrion, which generates reactive oxygen species in higher amounts under hypoxic conditions, is another possible candidate [82]. Further investigations are still needed to elucidate the mechanism leading to the hypoxic activation of HIF-1.

Recent findings identified the presence of HIF-1 binding sites in the 5’ or 3’ flanking regions of the genes encoding erythropoietin [83], VEGF [67], heme oxygenase [84], VEGF receptor Flt-1 [69], glucose transporter Glut-1 [85] and several glycolytic enzymes [86] as well as for the lactate dehydrogenase A [87]. However, the hypoxia-dependent increase in VEGF expression seems to be controlled both at the transcriptional level and at the level of mRNA stability [88,89]. It must be noted that recently, a novel bHLH-PAS factor, termed EPAS-1 [90] or HIF-2α has been described which is very similar to HIF-1α and forms heterodimers with ARNT and is activated by hypoxia. Interestingly, its expression is abundant in endothelial cells. The endothelial-specific tyrosine kinase tie-2 [90] and VEGF [91] were identified as target genes of HIF-2. The essential role of HIF-1 during development was demonstrated by major vascularization defects and abnormal neural fold formation in HIF−/− embryos [92,93]. On the other hand, EPAS-1 deficient mice display normal morphological development of the circulatory system but die at midgestation due to a profound defect in catecholamine production [94]. All together, these results indicate that both transcription factors play an essential but
completely distinct role in embryo development. Fig.
3 schematically illustrates the effect of hypoxia on
gene transcription in endothelial cells.

Taken together, these results indicate that hypoxia
strongly affects the regulatory pathways of endothe-
lial cells as well as of smooth muscle cells, leading to
the activation of several transcription factors and to
the release of cytokines and growth factors. The end
result is the formation of conditions favorable to
vasoconstriction and proliferative activity, participat-
ing in the remodeling of the vascular wall.

4. Physiological relevance

The finding that the endothelial expression of
some growth factors, cytokines as well as other genes
is influenced by variations in oxygen concentration
has obvious physiological implications. Two main
cascades of reactions have been characterized de-
pending on the duration of the oxygen deficiency.
Following acute hypoxia, endothelial cells become
activated and neutrophil adherence is observed.
One consequence of this process is the development
of a local inflammatory reaction in ischemic organs
which is then made worse if reperfusion occurs.
When chronic hypoxic conditions persist then the
expression of growth factors, cytokines and pro-co-
gulation molecules is increased. Several pathological
conditions are known to be linked to hypoxic con-
ditions. Three of them are briefly discussed.

First, blood stasis is a situation commonly associ-
ated with chronic venous insufficiency. It leads to
the development of ischemic conditions in leg veins. The
alterations observed in the varicose vein wall could be
the result of the infiltration of activated neutro-
phils after their adherence to the hypoxic endothe-
lium by the mechanism described here and from the
hypoxia-induced release by endothelial cells of
growth factors stimulating the proliferation of
smooth muscle cells. Such a process would have to
be steadily repeated over a long period of time and
superimposed on other factors in order to bring irre-
versible changes as the ones observed in varicose
veins [95,96].

Second, adaptation to hypoxia represents a key
step in tumor progression. On the one hand, these
conditions induce the activation of HIF-1 allowing
the cells to survive via an adaptive modification of
their energetic metabolism: HIF-1 increases the ex-
pression of several glycolytic enzymes and hence the
glycolysis rate [97]. HIF-1 has actually been found to
be overexpressed in various human tumor types in
comparison with the respective normal tissue [98].
On the other hand, HIF-1 is involved in the estab-
lishment of vascular supply. This neoangiogenesis is
thought to be triggered by growth factors released by
the tumor itself becoming hypoxic while enlarging. In
order for tumors to grow, they must become vascu-
larized [99]. In addition, vascularization enhances the
metastasis properties of the tumor [100]. The over-
expression of VEGF, through HIF-1 activation, is
certainly one of the main factors involved in this
process [101,102]. VEGF displays strong angiogene-
sis properties and VEGF mRNA is substantially up-
regulated in most tumors. A correlation between
VEGF expression and microvessel density is ob-
served in several malignancies [103]. Based on this
knowledge, approaches utilizing hypoxia-responsive
elements for HIF-1 are now being developed to tar-
get the expression of therapeutic genes to tumor cells
[104,105].

Third, pulmonary hypertension is a frequent he-
modynamic complication associated with respiratory
system disorders. Chronic alveolar hypoxia is the
main determinant of the increase in pulmonary vas-
cular resistance due to persistent vasoconstriction
and vascular structural remodeling [106,107]. Short
term exposure of the pulmonary circulation to a re-
duced level of oxygen tension both in vitro and in
vivo elicits an immediate vasoconstrictive response
probably through potassium channel inhibition in
smooth muscle cells. Under conditions of chronic
alveolar hypoxia, structural remodeling of the pul-
monary vasculature occurs with medial smooth
muscle cell hypertrophy and proliferation and abnor-
mal deposition of extracellular matrix components.
Growth factors such as ET-1 which can be released
by hypoxic endothelial cell or smooth muscle cells
may participate in this remodeling [52]. Moreover,
a critical role for HIF-1 in the pathogenesis of pul-
monary hypertension has recently been reported
[108].

Our knowledge of the transduction cascade initi-
ated by hypoxia leading either to direct activation or
to the regulation of gene expression is detail. In par-
allel, the involvement of hypoxic conditions and the role played by cell ‘activation’ induced by oxygen deprivation in several pathological situations becomes clearer. However, many questions are still open. What is the threshold of oxygen tension responsible for the activation of the endothelial cells in vivo? What is the duration or the number of hypoxic events needed for irreversible alterations of the tissue? One last stimulating question raised by these discoveries is whether we can influence the overall process in patients. Some of the drugs used in the treatment of the peripheral ischemia or in varicose veins have been tested and found to be protective for the hypoxia-induced activation of endothelial cells in vitro. However, the question of the potency of these drugs or of other newly designed molecules for endothelial protection against ischemic conditions and via the same mechanism in patients is still unanswered. This observation by itself would justify further investigation to understand the role of hypoxia in these pathologies.

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