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Hypoxia/reoxygenation stimulates Ca^{2+}-dependent ICAM-1 mRNA expression in human aortic endothelial cells

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Abstract

Increased endothelial ICAM-1 expression is found in normal aging and in atherosclerosis and is related to the chronic effects of oxidative stress. We examined the Ca^{2+}-dependence of ICAM-1 mRNA expression in human aortic endothelial cells (HAEC) exposed to hypoxia/reoxygenation (H/R) as a model of oxidative stress. HAEC were exposed to glucose-free hypoxia (95% N_2/5% CO_2) for 60 min and were then reoxygenated (21% O_2/5% CO_2) and observed for up to 6 h. Reactive oxygen species (ROS) generation was measured by dichlorofluorescein fluorescence and ICAM-1 mRNA was assessed by Northern blot. Upon reoxygenation after hypoxia, ROS production occurred in HAEC and was inhibited by diphenyleneiodonium and by polyethylene glycol-catalase, suggesting the involvement of NADPH oxidase-derived hydrogen peroxide. Hypoxia alone did not increase either ROS production or ICAM-1 mRNA levels, but a 2.5-fold increase in ICAM-1 mRNA was noted by 30 min of reoxygenation. This was not observed in Ca^{2+}-free buffer or in cells treated with diphenyleneiodonium. Thus, H/R upregulates ICAM-1 mRNA in HAEC by a Ca^{2+}- and ROS-dependent mechanism. Characterizing the signaling pathways involved in H/R-induced adhesion molecule expression may result in a better understanding of the vascular biology of normal aging and the pathobiology of atherosclerosis.

Keywords: ICAM-1; Aging; Calcium (cellular); Redox signaling; Hypoxia/anoxia; Reoxygenation; Atherosclerosis

The vascular endothelium in atherosclerotic lesions and the vascular endothelium in normal vessels of older individuals have many features in common. Endothelial cells with senescence-associated phenotypes have been found in human atherosclerotic lesions [1]. These cells are typically large [2] and exhibit high levels of senescence-associated β-galactosidase (β-gal) activity [1]. Among the other changes common to atherosclerosis and normal aging are an increase in the expression of matrix metalloproteinase-2 (MMP-2), transforming growth factor-β (TGF-β), and ICAM-1 [3] in vascular endothelial cells when compared to the endothelium of healthy young individuals.

The changes in the vascular endothelium in normal vessels of older individuals and in atherosclerotic lesions are believed to result, in part, from the chronic effects of increased oxidative stress and from higher levels of reactive oxygen species (ROS). Models of oxidative stress are, therefore, often studied to characterize signaling pathways that may be activated in older individuals. One of the most frequently studied models of oxidative stress in the vascular endothelium is hypoxia/reoxygenation (H/R). H/R results in the generation of ROS by the vascular endothelium and leads to specific ROS-dependent changes in cell signaling. We have previously shown that H/R stimulates the generation of repetitive spikes of intracellular calcium concentration ($[Ca^{2+}]_i$) in human aortic endothelial cells (HAEC) [4]. After a 60-min period of glucose-free hypoxia, reoxygenation in glucose-containing buffer resulted in $[Ca^{2+}]_i$ oscillations

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with an average amplitude of 570.6 ± 59.3 nmol/L at a frequency of 0.28 ± 0.04 min^{-1}. In Ca^{2+}-containing buffer, these oscillations persisted for at least 60 min of observation. In the absence of buffer Ca^{2+}, oscillations were inconsistently observed and even when present, they consisted of only a few spikes lasting approximately 20 minutes. Inhibition of $[Ca^{2+}]_i$ oscillations by polyethylene glycol (PEG)-catalase, which catalyzes the decomposition of hydrogen peroxide into water and oxygen, and by the NADPH oxidase inhibitor diphenyleneiodonium (DPI) suggested that the oscillations are initiated by NADPH oxidase-derived hydrogen peroxide [4].

The following study was performed to determine the effect of H/R on ROS generation and Ca^{2+}-dependent ICAM-1 mRNA level in HAEC. We found that H/R stimulates ROS generation that is sensitive to the NADPH oxidase inhibitor DPI. H/R leads to upregulation of ICAM-1 mRNA levels in HAEC that is dependent on Ca^{2+} and is inhibited by DPI.

Materials and methods

Culture of HAEC. HAEC were obtained as proliferating quaternary cultures (Clonetics, San Diego, CA) and grown as previously described [5] to confluence to passage 5–9 in endothelial cell growth medium supplemented with 2% fetal bovine serum, 10 μg/L human-recombinant epidermal growth factor, 1 mg/L hydrocortisone, 50 μg/mL gentamicin, 50 ng/mL amphotericin-B, and 12 μg/mL bovine brain extract (Clonetics) in a 37 °C humidified atmosphere of 95% air–5% CO_2. Cells were used for experiments after reaching ~70% confluence. HAEC were perfused with a bicarbonate-buffered saline (BBS) containing (in mmol/L) NaCl 116.4, KCl 5.4, $MgSO_4$ 1.6, $CaCl_2$ 1.5, NaH_2PO_4 1.0, D-glucose 5.6, and $NaHCO_3$ 26.2 continuously gassed with 21% O_2/5% CO_2. HAEC were exposed to hypoxia by switching the perfusion solution from BBS to glucose-free BBS continuously gassed with 95% N_2/5% CO_2 for 60 min. After this period, cells were reoxygenated in glucose-containing BBS balanced with 21% O_2/5% CO_2. The timing and extent of hypoxia was measured with an oxygen electrode (Microelectrodes, Londonderry, NH).

Measurement of ROS generation. To detect the generation of intracellular reactive oxygen species (ROS), the ROS-sensitive fluorescent indicator 2′,7′-dihydrodichlorofluorescin diacetate (DCF-DH) was used in HAEC as described previously [6]. HAEC were incubated with 5 μg/mL DCF-DH (Molecular Probes, Eugene, OR) for 5 min at 37 °C. The diacetylated derivative of 2′7′-dihydrodichlorofluorescin allows the indicator to diffuse into the cell, where intracellular esterases cleave the acetyl groups, trapping DCF-DH inside the cell [7]. DCF-DH is oxidized by ROS to the highly fluorescent compound 2′,7′-dichlorofluorescein (DCF); this fluorescence is used as an index of ROS generation. After loading, HAEC on coverslips were then gently washed for 30 minutes with indicator-free Hepes-buffered saline (HBS) at room temperature to allow deesterification of the indicator. DCF fluorescence was recorded a field of two or three connected cells of a HAEC monolayer on a coverslip in a perfusion chamber mounted on the stage of a modified Nikon Diaphot inverted epifluorescence microscope. DCF fluorescence was excited at 480 ± 20 nm using a xenon short arc lamp (UXL-75 XE; Ushio) and bandpass interference filters (Omega Optical) with selected wavelength bands of emitted fluorescence at 535 ± 25 nm. Emitted DCF fluorescence was collected and measured using a spectrofluorimeter (PTI; Deltascan). Since DCF fluorescence intensity increased linearly by time during the measurement period (about 10 s), the slope of DCF fluorescence intensity increase was used to quantify intracellular ROS generation.

ICAM-1 mRNA expression. ICAM-1 mRNA levels were determined by Northern blot and expressed relative to the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH); mRNA levels after reoxygenation were normalized to normoxic control HAEC. After the experimental protocol, HAEC were lysed by exposure to 700 μL lysis/binding solution. The lysate was collected with a rubber spatula, vortexed vigorously, and mixed with an equal volume of 64% ethanol. The lysate/ethanol mixture was then applied to an RNAqueous filter cartridge (Ambion, Austin, TX) which was washed twice by wash solutions and brief centrifuging. Preheated elution solution was applied to the center of the filter to collect total RNA samples. Total RNA (6 μg for each sample) was subjected to 1% agarose gel electrophoresis and Northern Blotting using NorthernMax-Gly (Ambion). Briefly, each sample of RNA was mixed with an equal volume of Glyoxal Load Dye, incubated 30 min at 50 °C, and loaded into the gel. After electrophoresis, RNA was transferred to the positively charged BrightStar-Plus nylon membrane (Ambion) using the transfer buffers supplied by the manufacturer. The RNA-transferred, damp membrane was immediately treated in UV Stratalinker 2400 (Stratagene, La Jolla, CA) to crosslink RNA. Prehybridization was performed at 42 °C for 60 min in pre-warmed ULTRAhyb buffer and then 10 ng biotinylated ICAM-1 cDNA probe was added and continued to hybridize for overnight. The membrane was then washed by low and high stringency wash solution.

BrightStar BioDetect from Ambion was used to detect ICAM-1 mRNA signal. The hybridized membrane was washed twice, each for 5 min in wash buffer and was then incubated twice, each for 5 min, in blocking buffer and continued to incubate for an additional 30 min. Then the membrane was incubated for 30 min in diluted Strep-Alkaline Phosphatase (Strep-AP, 1 μL/10 mL Blocking Buffer). The membrane was then incubated for 10 min in blocking buffer and washed three times each for 5 min in wash buffer, incubated twice, each for 2 min in assay buffer and then incubated for 5 min in CDP-Star and finally shaken off excess CDP-Star and exposed to film. After exposure, the membrane was boiled to remove ICAM-1 probe and re-probed with biotinylated GAPDH cDNA fragment to evaluate sample loading. The quantitative analysis of each band intensity was performed using the UN-SCAN-IT gel program (Silk Scientific, Orem, Utah). ICAM-1 mRNA levels were normalized to GAPDH and then expressed as percentage of normoxia control. A 0.96 kB, ICAM-1 cDNA fragment (digested by *SalI*/*PstI* from ICAM-1 cDNA construct, a kind gift from Dr. Timothy A. Springer, Harvard Medical School) was labeled with biotin using nonisotopic labeling kit-BrightStar Psoralen-Biotin (Ambion) according to manufacturer's manual. Briefly, 10 ng/μL of the ICAM-1 cDNA fragment was denatured at 100 °C for 10 min and immediately quick-chilled in an ice-water bath. This was then mixed with BrightStar Psoralen-Biotin (1/10 μL DNA), irradiated at 365 nm for 45 min, and finally extracted twice with 200 μL water-saturated *n*-butanol. The biotinylated probe was stored at −80 °C.

Data analysis and statistics. Data are reported as means ± SEM. Statistical comparisons were made by use of Student's *t* test for the paired and the unpaired groups. ANOVA was used when multiple group comparisons were performed. A difference was considered significant at a value of $p < 0.05$.

Results

Timing and degree of hypoxia

There have been many systems used to create hypoxia in the laboratory. Variation in these systems and in the timing (onset and duration) and extent of hypoxia may

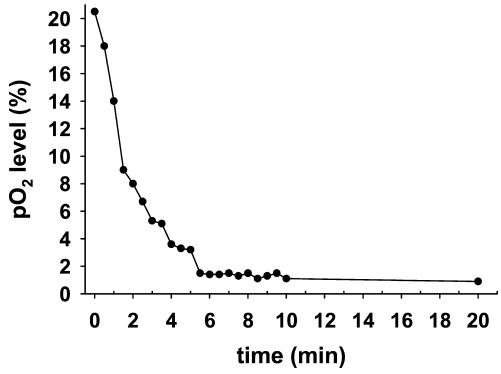

Fig. 1. Degree of hypoxia. The pO_2 level, measured with an oxygen electrode, declined rapidly upon transition to buffer gassed with 95% N_2/5% CO_2. The pO_2 remained low throughout the 60-min period of hypoxia (only 20 min shown).

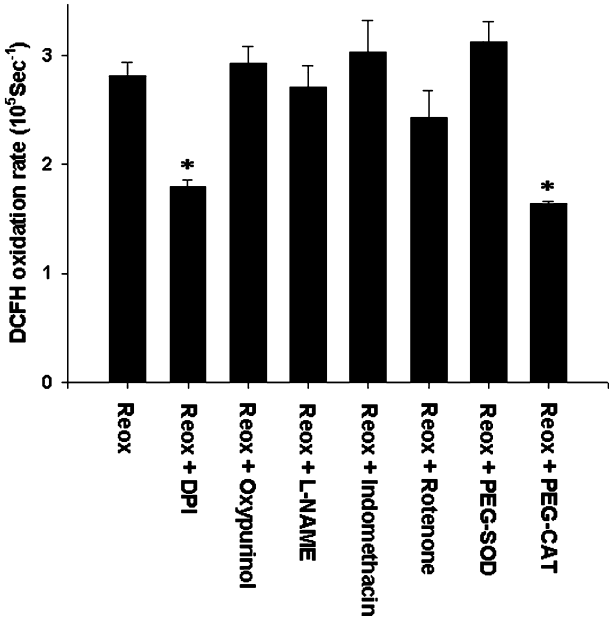

Fig. 2. ROS generation in HAEC reoxygenated after a 60-min period of hypoxia. Averaged data showing that upon reoxygenation after a period of glucose-free hypoxia, HAEC generated ROS as assessed by the rate of oxidation of DCF-DH ("Reox"). This was inhibited by diphenyleneiodonium ("Reox + DPI") and by polyethylene glycol-catalase ("Reox + PEG-CAT") but not by any other inhibitor of ROS-generating systems or free radical scavenger (data represent means ± SEM of 3 experiments, $*p < 0.05$).

contribute to discrepancies in findings from one laboratory to another. In our laboratory, we employed a system in which hypoxia was created by perfusing HAEC monolayers in 100 mm^2 tissue culture plates with a solution gassed continuously with 95% N_2/5% CO_2. After a period of hypoxia, cells were reoxygenated with a solution gassed continuously with 21% O_2/5% CO_2. The culture plate was also gassed continuously with either 95% N_2/5% CO_2 (for hypoxia) or 21% O_2/5% CO_2 (during the reoxygenation period). The timing and extent of hypoxia was assessed using an oxygen electrode and is shown in Fig. 1. The pO_2 of the solution bathing the cells began to decline quite rapidly from a baseline of 20.5% (152.1 Torr), reaching a nadir after 5–6 min of 1.4% (10.3 Torr). This degree of hypoxia was maintained throughout the total duration of 60 min (only the first 20 min of hypoxia shown in the figure).

Post-hypoxic reoxygenation stimulates ROS generation in HAEC

We previously showed that H/R initiates $[Ca^{2+}]_i$ oscillations in HAEC that were blocked by the NADPH oxidase inhibitor DPI (10 μmol/L) and by PEG-catalase (5000 U/mL), suggesting the involvement of NADPH oxidase-derived hydrogen peroxide. To determine whether H/R stimulates ROS generation in HAEC, cells were loaded with DCF-DH and the rate of oxidation of DCF-DH was assessed during H/R in the presence of various known or potential inhibitors of the enzymatic sources of ROS generation, including DPI (10 μmol/L), the xanthine oxidase inhibitor oxypurinol (100 μmol/L), the nitric oxide synthase inhibitor L-NAME (1 mmol/L), the cyclooxygenase inhibitor indomethacin (10 μmol/L), or the mitochondrial complex I inhibitor rotenone (10 μmol/L). We also examined the rate of oxidation of DCF-DH in the presence of the superoxide anion scavenger polyethylene glycol-superoxide dismutase (PEG-SOD, 1000 U/mL, Sigma) or in the presence of PEG-catalase (5000 U/mL, Sigma). Whereas no significant ROS generation was noted during hypoxia alone (DCF-DH oxidation rate = $1.56 \pm 0.11 \times 10^5$ per second, $n = 3$, p = NS vs. normoxia), ROS generation was observed during reoxygenation as assessed by the rate of oxidation of DCF-DH (Fig. 2). This was significantly inhibited by DPI or PEG-CAT, but was not affected by any other inhibitor or scavenger examined.

Hypoxia/reoxygenation upregulates ICAM-1 mRNA

When HAEC were reoxygenated after a 60-min period of glucose-free hypoxia, a 2.5-fold increase in ICAM-1 mRNA was noted by about 30 min, as shown in Fig. 3A. This increase was maintained, but demonstrated no additional increase, over a 6-h period of observation (Fig. 3B). Of note, hypoxia alone did not significantly affect ICAM-1 mRNA levels. Under Ca^{2+}-free conditions, no increase in ICAM-1 mRNA was observed during H/R (Fig. 3B). DPI (10 μmol/L) significantly inhibited the increase in ICAM-1 mRNA during reoxygenation. In DPI-treated cells ($n = 3$), the normalized ICAM-1/GAPDH mRNA ratio at 60 min of reoxygenation was 0.84 ± 0.22 vs. 2.51 ± 0.28 for control cells subjected to H/R, ($n = 5$, $p < 0.01$). Of note, DPI itself

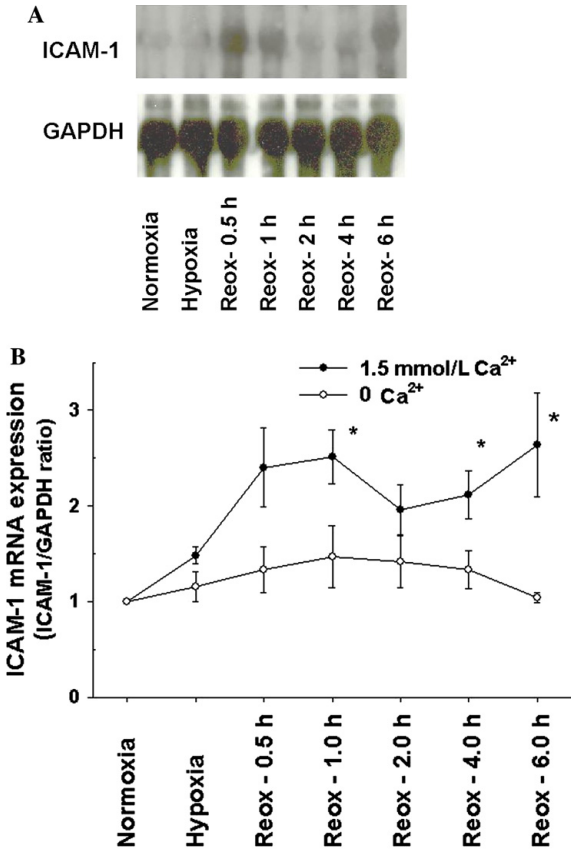

Fig. 3. Effect of H/R on ICAM-1 mRNA expression in HAEC. (A) Representative Northern blot showing the effect of hypoxia/reoxygenation (Reox) on ICAM-1 mRNA expression. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. (B) Averaged data showing the time course of ICAM-1 mRNA expression in HAEC exposed to hypoxia and reoxygenation in buffer with 1.5 mmol/L Ca^{2+} (closed circles) and in buffer without added Ca^{2+} and with 1 mmol/L EGTA (open circles). An increase in ICAM-1 mRNA was observed within 1 h of reoxygenation, but only in the presence of buffer Ca^{2+} (data represent means ± SEM of 5 experiments for 1.5 mmol/L Ca^{2+} and 4 experiments for Ca^{2+}-free conditions; data are expressed as the ratio of ICAM-1 to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) normalized to normoxic control, $*p < 0.05$).

did not affect ICAM-1 levels (normalized ICAM-1/GAPDH mRNA ratio of 0.86 ± 0.07, $n = 3$, p = NS vs. untreated control HAEC not exposed to H/R).

Discussion

The present study shows that reoxygenation after a 60-min period of glucose-free hypoxia stimulates Ca^{2+}- and ROS-dependent upregulation of ICAM-1 mRNA in human aortic endothelial cells. H/R-stimulated ROS generation was inhibited by DPI and by PEG-CAT, suggesting that H/R resulted in the production of H_2O_2 via activation of an NADPH oxidase in HAEC. After 30 min of reoxygenation following 60 min of glucose-free hypoxia, a 2.5-fold increase in ICAM-1 mRNA was noted that was not observed in Ca^{2+}-free buffer or when cells were exposed to DPI. These results, together with our previous observation that H/R stimulates $[Ca^{2+}]_i$ oscillations in HAEC that are dependent on buffer Ca^{2+} and are inhibited by DPI [6], suggest that intracellular Ca^{2+} and ROS are critical second messengers in the signaling pathways leading to ICAM-1 upregulation in human endothelial cells exposed to H/R.

The dependence on extracellular Ca^{2+} suggests that Ca^{2+} influx is critical to ICAM-1 expression during H/R, as has been shown when ICAM-1 expression has been measured after other stimuli [8,9]. This is likely because of the effect of Ca^{2+} influx on $[Ca^{2+}]_i$, since chelation of intracellular Ca^{2+} has been previously shown to decrease ICAM-1 expression in human endothelial cells in several other models [10–12]. To the best of our knowledge, no previous study has documented Ca^{2+} dependence of ICAM-1 upregulation during H/R alone in human endothelial cells. Hattori et al. [9] found that the increase in ICAM-1 mRNA levels during reoxygenation of rat coronary microvascular endothelial cells that had been subjected to 30 min of glucose-free acidic hypoxia was diminished when experiments were performed in Ca^{2+}-free buffer. The experimental model used in our study was very different from the one employed by Hattori et al. In the present study, HAEC were exposed to 60 min of hypoxic, glucose-free buffer at physiologic pH in an anaerobic atmosphere continuously gassed with 95% N_2/5%CO_2. The pO_2 of the solution bathing the cells was carefully monitored with an O_2 electrode, confirming the extent of hypoxia. In the study by Hattori et al., the pH of the hypoxic solution was adjusted to 6.8 with 5 N HCl and the degree of hypoxia achieved was not reported. As opposed to our model in which a small, but significant, increase in $[Ca^{2+}]_i$ of approximately 50 nmol/L was observed during 60 min of hypoxia followed by the appearance of $[Ca^{2+}]_i$ oscillations during reoxygenation [4], Hattori, et al. [9] reported a large increase in $[Ca^{2+}]_i$ of more than 500 nmol/L during 30 min of acidic hypoxia without a subsequent rise during reoxygenation.

Despite these differences, in both models the increase in ICAM-1 mRNA during reoxygenation was inhibited in Ca^{2+}-free buffer. This is not surprising, since an increase in $[Ca^{2+}]_i$ has been shown to be necessary for the activation and subsequent binding of both NF-κB and NFAT to the promoter region of the ICAM-1 gene [13]. Ca^{2+} and ROS may function synergistically as second messengers in the activation of the transcription of NF-κB- or NFAT-dependent genes like ICAM-1. The fact that H/R did not affect ICAM-1 mRNA levels in Ca^{2+}-free buffer or in the presence of DPI suggests that H/R-stimulated Ca^{2+} signaling and ROS generation are each necessary, but that neither alone is sufficient, to lead to ICAM-1 transcription. True et al. [14] found that

H_2O_2 initiated the binding of NF-κB to the ICAM-1 promoter but this bound NF-κB was transcriptionally "silent." Since H_2O_2 alone was found not to affect phosphorylation of the NF-κB p65 subunit, these authors speculated that phosphorylation of this subunit is required for the transcriptional activity of NF-κB [14]. Phosphorylation of NF-κB p65 is mediated by IκB-kinase [15], an enzyme whose activity is Ca^{2+}-dependent [16]. In this way, ROS and Ca^{2+} may function synergistically in the regulation of ICAM-1 expression during H/R.

During hypoxia, ICAM-1 mRNA levels did not change significantly in HAEC (Fig. 3), a finding also observed by Hess et al. [17] in human brain microvascular endothelial cells. Others have also found that hypoxia has little effect on ICAM-1 protein expression in human endothelial cells [18–20]. This is not surprising, since hypoxia alone did not increase ROS production as assessed by the rate of oxidation of DCF-DH in the present study and only resulted in a small increase in $[Ca^{2+}]_i$ without any $[Ca^{2+}]_i$ oscillations in our prior study [4]. As shown in Fig. 3, ICAM-1 mRNA levels increase early during reoxygenation as also reported by others [17]. ICAM-1 protein expression in endothelial cells exposed to H/R follows soon after, and has been reported to occur as early as 4 h after reoxygenation [19–21].

In summary, this study shows that H/R upregulates ICAM-1 mRNA in HAEC by a Ca^{2+} and ROS-dependent mechanism. Given our previous observations that H/R stimulates $[Ca^{2+}]_i$ oscillations in HAEC that are dependent on buffer Ca^{2+} and are inhibited by DPI [6] and given the dependence of the binding of NF-κB to the ICAM-1 gene on $[Ca^{2+}]_i$ [13] and ROS [14], it appears that increases in $[Ca^{2+}]_i$ and ROS early in reoxygenation act together to affect adhesion molecule expression in the vascular endothelium. Characterizing the signaling pathways involved in H/R-induced adhesion molecule expression may result in a better understanding of the pathobiology of atherosclerosis and of the vascular biology of normal aging. The observations that $[Ca^{2+}]_i$ [22] and ROS levels [23] are both increased in endothelium from older animals suggest that upregulation of ICAM-1 in aging [3] may result from these two signaling pathways acting in concert, as appears to occur during H/R.

Acknowledgments

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References

[1] T. Minamino, H. Miyauchi, T. Yoshida, Y. Ishida, H. Yoshida, I. Komuro, Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction, Circulation 105 (2002) 1541–1544.
[2] K.F. Bürrig, The endothelium of advanced arteriosclerotic plaques in humans, Arterioscler. Thromb. 11 (1991) 1678–1689.
[3] Z. Li, J. Froehlich, Z.S. Galis, E.G. Lakatta, Increased expression of matrix metalloproteinase-2 in the thickened intima of aged rats, Hypertension 33 (1999) 116–123.
[4] Q. Hu, R.C. Ziegelstein, Hypoxia/reoxygenation stimulates intracellular calcium oscillations in human aortic endothelial cells, Circulation 102 (2000) 2541–2547.
[5] Q. Hu, S. Corda, J.L. Zweier, M.C. Capogrossi, R.C. Ziegelstein, Hydrogen peroxide induces intracellular calcium oscillations in human aortic endothelial cells, Circulation 97 (1998) 268–275.
[6] Q. Hu, Z.X. Yu, V.J. Ferrans, K. Takeda, K. Irani, R.C. Ziegelstein, Critical role of NADPH oxidase-derived reactive oxygen species in generating Ca^{2+} oscillations in human aortic endothelial cells stimulated by histamine, J. Biol. Chem. 277 (2002) 32546–32551.
[7] D.A. Bass, J.W. Parce, L.R. Dechatelet, P. Szejda, M.C. Seeds, M. Thomas, Flow cytometric studies of oxidative product formation by neutrophils: a graded response to membrane stimulation, J. Immunol. 130 (1983) 1910–1917.
[8] Y. Yamaguchi, F. Matsumura, F.S. Wang, E. Akizuki, J. Liang, T. Matsuda, K. Okabe, H. Ohshiro, T. Horiuchi, S. Yamada, K. Mori, M. Ogawa, Neutrophil elastase enhances intercellular adhesion molecule-1 expression, Transplantation 65 (1998) 1622–1628.
[9] R. Hattori, H. Otani, Y. Moriguchi, H. Matsubara, T. Yamamura, Y. Nakao, H. Omiya, M. Osako, H. Imamura, NHE and ICAM-1 expression in hypoxic/reoxygenated coronary microvascular endothelial cells, Am. J. Physiol. 280 (2001) H2796–H2803.
[10] R. Blaber, E. Stylianou, A. Clayton, R. Steadman, Selective regulation of ICAM-1 and RANTES gene expression after ICAM-1 ligation on human renal fibroblasts, J. Am. Soc. Nephrol. 14 (2003) 116–127.
[11] A. Clayton, R.A. Evans, E. Pettit, M. Hallett, J.D. Williams, R. Steadman, Cellular activation through the ligation of intercellular adhesion molecule-1, J. Cell Sci. 111 (1998) 443–453.
[12] C.C. Barnett Jr., E.E. Moore, C.C. Silliman, E.K. Abdalla, D.A. Partrick, S.A. Curley, Cytosolic phospholipase A_2-mediated ICAM-1 expression is calcium dependent, J. Surg. Res. 99 (2001) 307–310.
[13] K.L. Quinlan, S.M. Naik, G. Cannon, C.A. Armstrong, N.W. Bunnett, J.C. Ansel, S.W. Caughman, Substance P activates coincident NF-AT- and NF-κB-dependent adhesion molecule gene expression in microvascular endothelial cells through intracellular calcium mobilization, J. Immunol. 163 (1999) 5656–5665.
[14] A.L. True, A. Rahman, A.B. Malik, Activation of NF-κB induced by H_2O_2 and TNF-α and its effects on ICAM-1 expression in endothelial cells, Am. J. Physiol. 279 (2000) L302–L311.
[15] I. Mattioli, A. Sebald, C. Bucher, R.P. Charles, H. Nakano, T. Doi, M. Kracht, M.L. Schmitz, Transient and selective NF-κB p65 serine 536 phosphorylation induced by T cell costimulation is mediated by IκB kinase beta and controls the kinetics of p65 nuclear import, J. Immunol. 172 (2004) 6336–6344.
[16] J. Petranka, G. Wright, R.A. Forbes, E. Murphy, Elevated calcium in preneoplastic cells activates NF-κB and confers resistance to apoptosis, J. Biol. Chem. 276 (2001) 37102–37108.

[17] D.C. Hess, W. Zhao, J. Carroll, M. McEachin, K. Buchanan, Increased expression of ICAM-1 during reoxygenation in brain endothelial cells, Stroke 25 (1994) 1463–1467.
[18] A. Pietersma, N. De Jong, J.F. Koster, W. Sluiter, Effect of hypoxia on adherence of granulocytes to endothelial cells in vitro, Am. J. Physiol. 267 (1994) H874–H879.
[19] J.A. Buras, G.L. Stahl, K.K. Svoboda, W.R. Reenstra, Hyperbaric oxygen downregulates ICAM-1 expression induced by hypoxia and hypoglycemia: the role of NOS, Am. J. Physiol. 278 (2000) C292–C302.
[20] H. Ichikawa, S. Flores, P.R. Kvietys, R.E. Wolf, T. Yoshikawa, D.N. Granger, T.Y. Aw, Molecular mechanisms of anoxia/reoxygenation-induced neutrophil adherence to cultured endothelial cells, Circ. Res. 81 (1997) 922–931.
[21] D.D. Koo, K.I. Welsh, N.E. West, K.M. Channon, A.J. Penington, J.A. Roake, P.J. Morris, S.V. Fuggle, Endothelial cell protection against ischemia/reperfusion injury by lecithinized superoxide dismutase, Kidney Int. 60 (2001) 786–796.
[22] G.G. Geary, J.N. Buchholz, Selected contribution: effects of aging on cerebrovascular tone and $[Ca^{2+}]_i$, J. Appl. Physiol. 95 (2003) 1746–1754.
[23] R.S. Sohal, R. Weindruch, Oxidative stress, caloric restriction, and aging, Science 273 (1996) 59–63.