Journal of Cellular Biochemistry Supplement 36:99–106 (2001)

Cyclic Strain Induces Reactive Oxygen Species Production Via an Endothelial NAD(P)H Oxidase

Hidetsugu Matsushita, Kuen-ho Lee, and Philip S. Tsao*

Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, California

Abstract Vascular endothelial cells are constantly subjected to pressure-induced cyclic strain. Reactive oxygen species (ROS) have been implicated in atherosclerosis and vascular remodeling. Recent evidence indicates that a vascular NAD(P)H oxidase may be an important source of ROS in both physiologic and pathophysiologic situations. The aim of this study was to investigate cyclic strain-induced NAD(P)H oxidase activity in endothelial cells. ROS production was examined by electron paramagnetic resonance and lucigenin chemiluminescence. Cyclic strain-induced NAD(P)H oxidase activity was quantified by activity assay while the expression of p22phox was monitored by Northern blotting. Endothelial cells produce basal amounts of ROS that were enhanced by cyclic strain. Moreover subsequent stimulation with TNF-α resulted in significantly greater ROS production in cells previously exposed to cyclic strain as compared to static conditions. Cyclic strain resulted in a significant increase in message for the p22phox subunit as well as activity of the NAD(P)H oxidase. The induced oxidative stress was accompanied by increased mobilization of the transcription factor NFκB, an effect that was blocked by a pharmacological inhibitor of NAD(P)H. These results demonstrate a pivotal role for NAD(P)H oxidase in cyclic strain-induced endothelial ROS production and may provide insight into the modulation of vascular disease by biomechanical forces. J. Cell. Biochem. Suppl. 36:99–106, 2001.

Key words: superoxide anion; protein kinase C; endothelium; hemodynamic; cyclic stretch

Epidemiological studies have established an association between hypertension and a greater prevalence of atherosclerosis and occlusive vascular disease [Perloff et al., 1983; Levy et al., 1990]. The importance of hypertension was emphasized by the findings of the Veterans Administration Cooperative Study Group on Antihypertensive Agents [1967] indicating that pharmacological treatment of hypertension resulted in reduced cardiovascular morbidity and mortality. The Framingham Heart Study determined that the incidence of heart disease rises progressively with increasing levels of either systolic or diastolic blood pressure [Levy et al., 1990].

One of the most fundamental forces influencing cardiovascular structure and function in hypertensive subjects is the increase in pressure within the vasculature [Folkow et al., 1958; Chien et al., 1998]. Intravascular pressure is transmitted to the vessel wall by Laplace's law and results in mechanical stretch to vascular cells. The increase in vascular wall stress (cyclic strain) imposed by hypertension has been strongly implicated in the pathogenesis of cardiovascular disease. In hypertension, the mechanical strain of the arterial wall has been reported to increase by as much as 15% [Safar et al., 1981]. It is now recognized that the mechanical force of cyclic strain is transduced into a panoply of biochemical signals within endothelial cells, which can then modulate the expression and activity of humoral mediators affecting vascular function.

Several intracellular signaling molecules have been proposed to be affected by biomechanical forces. As an example, exposure of endothelial cells to cyclic strain induces production of reactive oxygen species (ROS) [Howard et al., 1997; Cheng et al., 1998]. Although larger concentrations of ROS have been implicated in DNA and cell damage, lower levels of these

Grant sponsor: NIH; Grant number: HL62889; Grant sponsor: Scientist Development Grant from the American Heart Association National Center to Dr. Tsao.

*Correspondence to: Philip S. Tsao, Ph.D., Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA 94305-5406. E-mail: ptsao@stanford.edu

Received 3 August 2000; Accepted 5 January 2001

This article published online in Wiley InterScience, March 19, 2001.

molecules may act as proinflammatory second-messengers controlling important cell functions such as vasomotion, release of paracrine factors, and regulation of gene expression [Satriano et al., 1993; Li and Jaiswal, 1994; Roebuck et al., 1995]. Several redox-sensitive genes, including vascular cell-adhesion molecule-1 (VCAM-1) and monocyte chemotactic protein-1 (MCP-1), are thought to play an important role in atherosclerosis and restenosis.

Most studies to date have implicated a role of ROS in cyclic strain utilizing various pharmacological scavengers of different free radical species. [Satriano et al., 1993; Chien et al., 1998; Brar et al., 1999]. We, therefore, designed this study to directly monitor the production of ROS by endothelial cells using electron spin resonance and investigate the enzymatic source of ROS production induced by cyclic strain. We demonstrate that, (1) endothelial cells in culture elaborate ROS under basal conditions and that production is enhanced by cyclic strain, (2) the majority of the cyclic strain-induced ROS is due to increased activity of an NAD(P)H sensitive oxidase, and (3) pharmacologic inhibition of NAD(P)H oxidase abrogates the ROS production and activation of NFκB resulting from cyclic strain.

MATERIALS AND METHODS

Cell Culture

Human umbilical vein endothelial cells (HUVEC) were obtained from Clonetics Corp. (San Diego, CA), and cultured in MEDIUM 199 supplemented with endothelial cell growth supplement (50 μg/ml; Collaborative Research), heparin (50 μg/ml, porcine intestinal; Sigma), antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin), and 10% fetal bovine serum. Cells were incubated at 37°C in a humidified atmosphere of 95% air/5% CO_2 with medium changes every two days. All the cells were used within passage 5–6. For transfection studies, transformed HUVEC (ECV304; ATCC) were grown in M199 + 10% FBS.

In Vitro Cyclic Stretch

HUVEC were seeded on sheet of flexible silicone rubber with a hydrophilic surface coated with collagen I (Sigma) that can be horizontally stretched. After the cells reached confluence, cells were exposed to cyclic strain as previously described [Hirata et al., 2000]. Briefly, the sheet was fixed on one side of the base plate while the opposite side is attached to a movable piston driven by the camshaft of a variable speed monitor. The piston then pulls the sheet of endothelial cells to a predetermined length to approximate physiological cyclic strain (6%) at a frequency of 60Hz for 6 h.

Measurement of ROS

Nitroxides are spin traps that have been shown to terminate or decrease the radical process in rapid radical–radical reactions resulting in stable non-toxic diamagnetic species. One of the typical nitroxides, Tempamine (TA; Aldrich) reacts directly with superoxide anion. Thus, quantification of superoxide anion is accomplished by monitoring the reduction in the electron paramagnetic signal of TA. EPR spectra were obtained using a Bruker ESP300 spectrometer equipped with a low-noise microwave amplifier and a loop gap resonator in a Suprasil microcapillary tube. The EPR spectrometer operated at X-band (9.6 GHz) with 50 kHz modulation frequency at room temperature. The spectra were taken at a constant microwave power without saturation (10 mW) during data acquisition time of 1 min with 0.5 sec time constant. The modulation amplitude was set carefully at no greater than 1/8th of the line width. Data acquisition was digitized to 1024 points and was collected on IBM PC interfaced to a HP-3457A multimeter. The quantitation of the EPR signal intensity was determined by comparing the double integration of recorded first derivative EPR peaks of each sample with a standard TA spin solution. In some cases, additional experiments were performed with lucigenin chemiluminescence. Cells exposed to static or cyclic strain conditions were isolated from the silicone membranes and incubated in PBS containing lucigenin (25 μM, Sigma). Cell superoxide production was monitored as single photon counts in a scintillation counter (Beckman) every minute for a total of 10 min. Experimental groups were then compared to static control cells done in parallel and data was finally expressed relative to control. HUVEC viability was assessed by trypan blue exclusion and all measurements were normalized to viable cell number.

Northern Analysis

Total RNA was isolated using TRIzol reagent (Life Technologies). RNA (20 μg as quantified by

UV spectroscopy) was separated on 1.3% agarose gels containing 2.2 M formaldehyde and transferred to nylon membrane (Osmonics Inc). Specific cDNA probes for p22phox (kindly provided by Kathy Griendling) or cyclophilin were labeled with ^{32}P-dCTP by random primer kit (Amersham) and hybridized at 42°C overnight in the presence of 48% formamide and 10% dextran sulfate. After hybridization, the membranes were washed twice at 65°C with 1XSSC and 0.5% SDS. The blots were autoradiographed using Kodak Biomax MR film (Eastman Kodak Co., Rochester, NY) at −80°C and the resultant bands were quantified by densitometry using NIH Image software.

NAD(P)H Oxidase Assay

NAD(P)H oxidase activity was measured with a lucigenin-based assay [Ushio-Fukai et al., 1996]. HUVEC exposed to either control or cyclic strain conditions were washed and lysed in buffer containing protease inhibitors (20 mM monobasic potassium phosphate (pH 7.0), 1 mM EGTA, 10 μg/ml aprotinin, 0.5 μg/ml leupeptin, 0.7 μg/ml pepstatin, 0.5 mM PMSF). The cell suspension was then subjected to Dounce homogenization (100 times, on ice), and the homogenate was stored on ice until use. Protein content was measured in an aliquot of the homogenate by the Lowry method. Oxidase activity was measured by a luminescence assay in a 50 mM phosphate buffer, pH 7.0, containing 1 mM EGTA, 150 mM sucrose, 500 μM lucigenin as the detector and NADH or NADPH as the substrate (final volume 0.9 ml). NADH or NADPH were used at a final concentration of 100 μM. The reaction was started by the addition of 100 μl of homogenate (100 μg of protein) and single photon emission was measured every 15 sec for 15 min in a Beckman scintillation counter in out-of-coincidence mode. In some experiments, diphenyl iodinium was added to samples 15 min before readings.

Transfection and NFκB Reporter Assay

For transient transfection, cells were seeded on the collagen-coated silicone membrane at 50–70% confluence. NFκB-sensitive luciferase or SV40-β-galactosidase vectors were transfected by conventional cationic liposome transfection methods (Effectene, Qiagen). Following 12 h of cyclic stretch, luciferase and β-galactosidase activity were measured by Dual-Light Kit (Tropix, Bedford, MA) according to manufacturer's instructions. Results are reported as luciferase activity normalized to cotransfected β-galactosidase activity.

Statistical Analysis

All values are expressed as mean ± SEM. Analysis of variance (ANOVA) with subsequent Bonferroni's test was used to determine significant differences in multiple comparisons. $P < 0.05$ was considered statistically significant.

RESULTS

Cyclic Strain Increases ROS Production

Recent observations suggest that ROS play an important signaling role in vascular physiology. To document the participation in the endothelial response to cyclic strain, oxygen-derived free radical production was analyzed by EPR using the spin trap, tempamine. In solution, TA demonstrates a paramagnetic signature whereby the area under the peaks is directly proportional to the concentration. In a series of studies we have demonstrated the stability of this signal in solution over several months (data not shown). However, when TA reacts with other radical species such as superoxide anions, it loses its paramagnetic triplet signal. The reduction in peak height (and thus area under the peaks) is directly proportional to the amount of superoxide produced. Incubation of static endothelial cells with TA reduces the EPR signal, indicating basal production of ROS. As shown in Figure 1A, cyclic strain induced a marked exaggeration of the effect upon TA peak height. Normalization of the reduced signal to cell number indicates that 6 h of cyclic strain results in twice as much ROS production compared to static cells. To confirm the participation of superoxide anion in the reduction of TA signal due to cyclic strain, HUVEC were incubated with PEG-SOD 15 min prior and throughout the period of cyclic strain. PEG-SOD effectively scavenged the ROS induced by cyclic strain, thereby protecting the triplet signature of TA. As summarized in Figure 1B, PEG-SOD scavenged ROS to below basal levels (Static 76.2 ± 7.5; Cyclic Strain 161.9 ± 16.3; Cyclic Strain + PEG-SOD 35.6 ± 21.4 fmols/cell/6h, $P < 0.05$). This implicates a role for superoxide anion not only in the oxidative stress induced by cyclic strain, but also in at least a portion of the ROS produced under basal conditions.

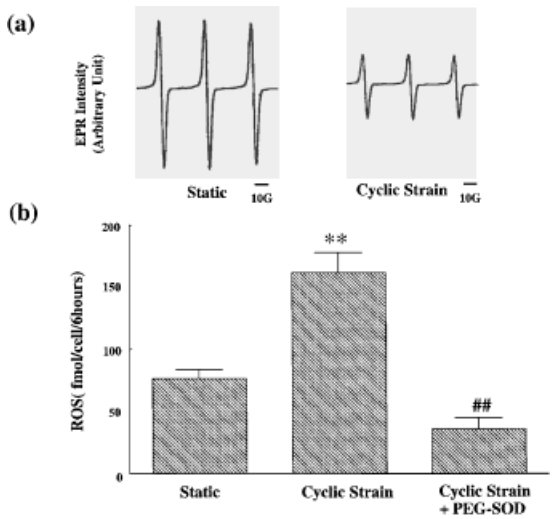

Fig. 1. **a:** Typical ESR spectra of endothelial cells exposed to 6 h of static (left) or cyclic strain (right) conditions. **b:** Effect of cyclic strain on ROS production. Values are expressed as mean ± SEM of eight independent experiments. $^{**}P < 0.01$ vs. Static control. $^{\#\#}P < 0.01$ vs. Cyclic strain.

Effects of TNF-α on ROS Production in Cells Exposed to Cyclic Strain

Cytokines such as tumor necrosis factor-α (TNF-α) can induce ROS production in endothelial cells [Matsubara and Ziff, 1986; Mohazzab et al., 1994]. To investigate the effects of cyclic strain upon TNF-α stimulated ROS production, HUVEC were exposed to static or cyclic strain conditions for 6 h. Subsequently, cells were then incubated with TNF-α for an additional 6 h under static conditions. As previously demonstrated, TNF-α stimulation of static HUVEC produced significantly greater amounts of ROS compared to unstimulated cells. Interestingly, this effect was greater in cells previously exposed to cyclic strain compared to static conditions (Fig. 2; $P < 0.01$).

Cyclic Strain Activates an NAD(P)H Oxidase in Endothelial Cells

There are several possible sources of O_2^- found in vascular cells. To begin to investigate which of these systems is activated by cyclic strain, pharmacological antagonists of xanthine oxidase (allopurinol 100 μM), lipoxygenase (eicosatriynoic acid 100 μM), cyclooxygenase (indomethacin, 100 μM), and NAD(P)H oxygenase (dipheylene iodinium 10 μM) were incubated with HUVEC 15 min prior to and throughout the measurement of superoxide production by lucigenin chemiluminescence.

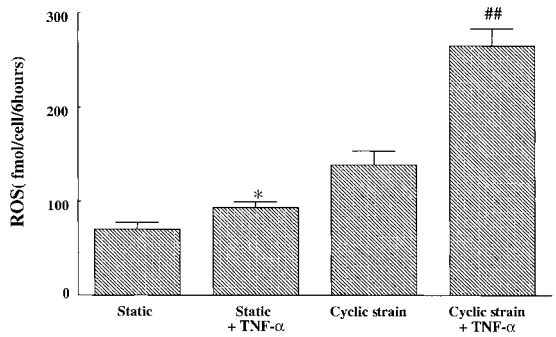

Fig. 2. Previous exposure to cyclic strain results in enhanced TNF-α induced ROS production. HUVEC were first exposed to static or cyclic strain conditions. Subsequently cells were exposed to TNF-α or vehicle for an additional 6 h under static conditions. Values are expressed as mean ± SEM of six independent experiments. $^{*}P < 0.05$ vs. static control, ##P < 0.01vs. Static control.

Cyclic strain-induced superoxide production was significantly reduced by the NAD(P)H oxidase inhibitor diphenyl iodonium (Fig. 3; $P < 0.01$). In contrast, inhibitors of xanthine oxidase, lipoxygenase, and cyclooxygenase had little effect. To further investigate the role of NAD(P)H oxidase, we performed an NAD(P)H-dependent activity assay on endothelial cell homogenates. Cyclic strain induced an increase of NAD(P)H activity, (NADH: Static 100%; Cyclic Strain 160.7 ± 17.4%; $P < 0.01$, NADPH: Static 100%; Cyclic Strain 181.0 ± 25.4%; $P < 0.01$) and this increased activity was inhibited by DPI as shown in Figure 4. Moreover, both NADH and NADPH could be used as substrates in cyclic strain-induced oxidase activity.

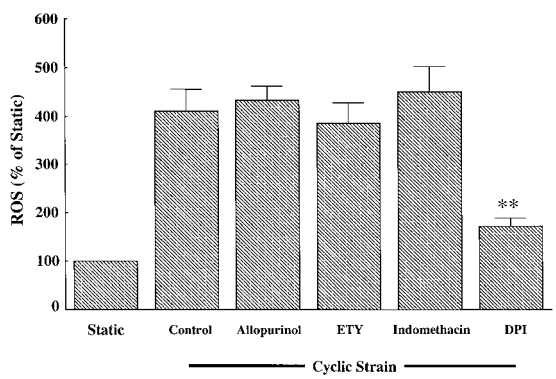

Fig. 3. Effect of pharmacological antagonists to endothelial oxidases on cyclic strain-induced ROS production. ETY = eicosatriynoic acid; DPI = diphenyl iodonium. Values are expressed as mean ± SEM of five independent experiments. ##$P < 0.01$vs. Cyclic strain control.

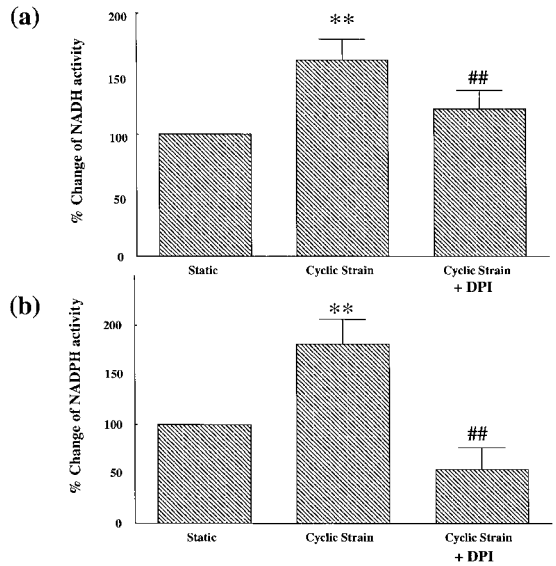

Fig. 4. **a:** NADH- and NADPH- dependent oxidase activity induced by cyclic strain and its inhibition by DPI. Values are expressed as mean ± SEM of five independent experiments. $^{**}P < 0.01$ vs. Static control. $^{\#\#}P < 0.05$ vs. Cyclic strain.

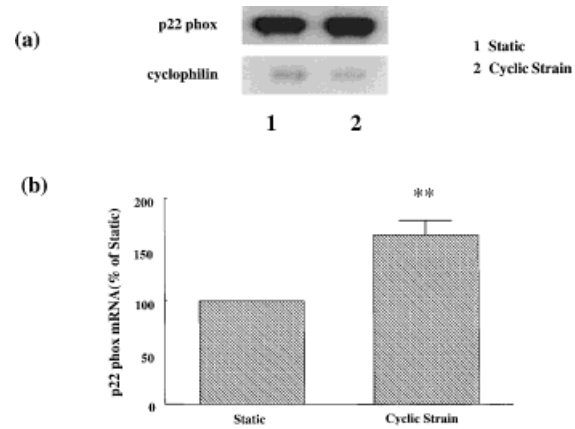

Fig. 5. **a:** Northern blot analysis of p22phox mRNA in HUVEC exposed to static control or cyclic strain for 12 h. **b:** Percent changes in p22phox mRNA expression in endothelial cells exposed to static (control) or cyclic strain as determined by densitometry. Values are expressed as mean ± SEM of six independent experiments. $^{**}P < 0.05$ vs. Static control.

p22phox is Induced by Cyclic Strain

p22phox is a component of the membrane-bound cytochrome b-558 complex, which transfers an electron from NADPH to oxygen in the phagocyte NADPH oxidase system [Thelen et al., 1993]. Recent studies have shown that p22phox may be a common component of phagocytic and vascular oxidases. The importance of p22phox in ROS production in non-phagocytic cells is supported by several investigations [Ushio-Fukai et al., 1996; Griendling and Ushio-Fukai, 1998]. To check the expression of p22phox mRNA induced by cyclic strain, we performed Northern blotting. As shown in Figure 5, significant increase of p22phox mRNA expression was observed in cells exposed to cyclic strain as compared to static conditions ($P < 0.05$).

Effects of Cyclic Strain on NFκB Activity

Transcription factor, NFκB, controls the expression of a multitude of genes involved in inflammation and proliferation. To investigate the effect of cyclic strain-induced ROS upon activation of the NFκB, cells were transfected with a luciferase reporter gene construct driven by multiple repeats of a putative NFκB binding site. Increase of NFκB activity was observed in cells exposed to cyclic strain as compared to static conditions ($P < 0.01$) and this increase was inhibited by DPI, as shown in Figure 6. Results are reported as luciferase activity normalized to cotransfected β-galactosidase activity.

DISCUSSION

The current findings indicate that, (1) cultured endothelial cells produce ROS under basal conditions, (2) cyclic strain enhances both basal and TNF-α stimulated superoxide anion production, (3) an NAD(P)H sensitive oxidase is responsible for the majority of cyclic strain-

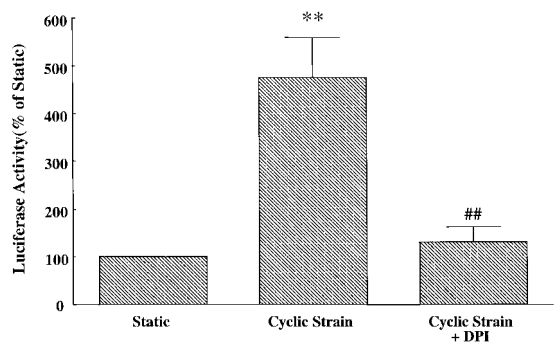

Fig. 6. Effect of cyclic strain on the expression of NFκB activity assessed by Luciferase assay. Values are expressed as mean ± SEM of six independent experiments. $^{**}P < 0.01$ vs. Static control. $^{\#\#}P < 0.01$ vs. Cyclic strain. Results are reported as luciferase activity normalized to cotransfected β-galactosidase activity.

induced ROS production, and (4) inhibition of NAD(P)H activity reduces the activation of NFκB by cyclic strain.

Endothelial cells are constantly exposed to several biomechanical forces including cyclic strain. In pathophysiological situations such as hypertension, cyclic strain is enhanced and is thought to be a central mechanism in the accelerated development of atherosclerosis. Our results indicate that a potential atherogenic signal induced by cyclic strain is the production of ROS. Recent studies have shown that the pathogenesis of atherosclerosis may be viewed as an inflammatory disease with an underlying abnormality in redox-mediated signals in the vasculature [Offermann and Medford, 1994; Medford, 1995]. ROS play a key role in mediating the pathologic manifestations of endothelial dysfunction associated with atherosclerosis. Regions of high cyclic strain are predisposed to lesion formation, whereas regions of high shear stress and low cyclic strain are resistant to atherosclerosis [Nerem, 1992]. Therefore, elucidation of the mechanism whereby cyclic strain activates local oxidative stress may have important implications for the site-specific nature of atherogenesis.

By nature, ROS are extremely unstable and thus, their activity is difficult to monitor. Previous studies indicating that cyclic strain increased intracellular oxidative stress have relied either on pharmacological inhibitors or cell free assays. In this study, we used three separate assays to measure endothelial oxidative stress. To our knowledge, this is the first report quantifying ROS production induced by cyclic strain by EPR. In addition, our studies using the luminescent indicator, lucigenin, support the observed increase in superoxide anion production.

There are several possible enzymatic sources of ROS production in endothelial cells. Our findings indicate that an NAD(P)H oxidase may be preferentially stimulated by cyclic strain and the main source of ROS in this setting. Moreover, subsequent stimulation with TNF-α resulted in significantly greater ROS production in cells previously exposed to cyclic strain as compared to static conditions. Previous studies have also indicated that cytokines such as TNF-α can activate a membrane-bound NAD(P)H oxidase to produce ROS in vascular endothelial cells [Matsubara and Ziff, 1986; Mohazzab et al., 1994].

The current observations indicate that cyclic strain-induced NAD(P)H oxidase activity may be important in the inflammatory response of endothelial cells. We demonstrate that activation of the transcription factor NFκB induced by cyclic strain is inhibited by a pharmacological inhibitor of NAD(P)H oxidase, diphenyl iodonium. The transcription factor, NFκB, controls the expression of a multitude of genes involved in inflammation and proliferation, including VCAM-1 [Tsao et al., 1997], ICAM-1 [Cheng et al., 1998], and MCP-1 [Wang et al., 1995]. It is interesting to note that another redox-sensitive transcription factor, Egr-1, is also induced by cyclic strain via a mechanisms involving intracellular oxidative signaling [Wung et al., 1999].

In addition to their role as signaling molecules, ROS are normal byproducts of various metabolic reactions. Thus, cells possess several antioxidant enzymes to inhibit the accumulation of these ROS. For example, superoxide anion is efficiently converted to the less reactive radical, hydrogen peroxide (H_2O_2) by several isoforms of superoxide dismutase within the cell. However, H_2O_2 has also been demonstrated to have significant effects upon autocrine as well as paracrine cell function. Recent evidence indicates that H_2O_2 may induce growth of vascular smooth muscle cells. Rao and Berk [1992] reported that exogenous superoxide anion could induce proliferation of rat aortic VSMC. Furthermore, generation of ROS by ligand-receptor interactions occurs in response to several growth factors including angiotensin II [Griendling and Ushio-Fukai, 1998], PDGF [Brar et al., 1999], and TGF-β [Thannickal and Fanburg, 1995]. Thus, cyclic strain-induced free radical production from endothelial cells may contribute to the vascular hypertrophy noted in various models of hypertension and intimal hyperplasia.

An important role for NAD(P)H oxidase-derived free radicals has also been established in angiotensin II-induced hypertension [Rajagopalan et al., 1996]. Studies from Griendling and colleagues [Ushio-Fukai et al., 1996; Fukui et al., 1997]; indicate that angiotensin II increases vascular smooth muscle expression of the 22 kDa subunit of NAD(P)H oxidase (p22phox) and superoxide anion production antisense to p22phox reduces angiotensin II-induced superoxide production. Furthermore, the expression of p22phox was increased in atherosclerotic lesions [Azumi et al., 1999;

Cahilly et al., 2000]. As shown in Figure 5, exposure of endothelial cells to cyclic strain also enhances p22phox expression. It is plausible that cyclic strain may induce the production of angiotensin II by endothelial cells, which in turn is responsible for the enhanced p22phox expression and ROS production. Indeed, the increase in expression of p22phox by cyclic strain is comparable to that seen after exposure to angiotensin II or TNF-α. A similar mechanism is known to exist in vascular smooth muscle cells whereby cyclic strain was demonstrated to increase vascular smooth muscle proliferation via autocrine production of angiotensin II and PDGF [Li et al., 1997].

In conclusion, HUVEC exposed to cyclic strain produce significant amounts of ROS from an NAD(P)H sensitive oxidase. This effect is in part, due to enhanced expression of the important regulatory subunit, p22phox, by cyclic strain. The subsequent oxidative stress may have deleterious consequences to enhance redox-sensitive gene transcription via transcription factors such as NFκB. These observations further implicate a role for biomechanical forces in basal cell signaling as well as responses to various cytokines and growth factors.

REFERENCES

Azumi H, Inoue N, Takeshita S, Rikitake Y, Kawashima S, Hayashi Y, Itoh H, Yokoyama M. 1999. Expression of NADH/NADPH oxidase p22phox in human coronary arteries. Circulation 100:1494–1498.

Brar SS, Kennedy TP, Whorton AR, Murphy TM, Chitano P, Hoidal JR. 1999. Requirement for reactive oxygen species in serum-induced and platelet-derived growth factor-induced growth of airway smooth muscle. J Biol Chem 274:20017–20026.

Cahilly C, Ballantyne CM, Lim DS, Gotto A, Marian AJ. 2000. A variant of p22(phox), involved in generation of reactive oxygen species in the vessel wall, is associated with progression of coronary atherosclerosis [see comments]. Circ Res 86:391–395.

Cheng JJ, Wung BS, Chao YJ, Wang DL. 1998. Cyclic strain-induced reactive oxygen species involved in ICAM-1 gene induction in endothelial cells. Hypertension 31:125–130.

Chien S, Li S, Shyy YJ. 1998. Effects of mechanical forces on signal transduction and gene expression in endothelial cells. Hypertension 31:162–169.

Folkow B, Gimby G, Thulesius O. 1958. Adaptive structural changes of the vascular wall in hypertension and their relationship to control of peripheral resistance. Act Physiol Scand 44:225–272.

Fukui T, Ishizaka N, Rajagopalan S, Laursen JB, Capers Qt, Taylor WR, Harrison DG, de Leon H, Wilcox JN, Griendling KK. 1997. p22phox mRNA expression and NADPH oxidase activity are increased in aortas from hypertensive rats. Circ Res 80:45–51.

Griendling KK, Ushio-Fukai M. 1998. Redox control of vascular smooth muscle proliferation. J Lab Clin Med 132:9–15.

Hirata K, Ishida T, Matsushita H, Tsao PS. 2000. Quertermous T. Regulated expression of endothelial cell-derived lipase. Biochem Biophys Res Commun 272:90–93.

Howard AB, Alexander RW, Nerem RM, Griendling KK, Taylor WR. 1997. Cyclic strain induces an oxidative stress in endothelial cells. Am J Physiol 272:C421–C427.

Levy D, Wilson PW, Anderson KM, Castelli WP. 1990. Stratifying the patient at risk from coronary disease: new insights from the Framingham Heart Study. Am Heart J (discussion 717) 119:712–717.

Li Y, Jaiswal AK. 1994. Human antioxidant-response-element-mediated regulation of type 1 NAD(P)H:quinone oxidoreductase gene expression. Effect of sulfhydryl modifying agents. Eur J Biochem 226:31–39.

Li Q, Muragaki Y, Ueno H, Ooshima A. 1997. Stretch-induced proliferation of cultured vascular smooth muscle cells and a possible involvement of local renin-angiotensin system and platelet-derived growth factor (PDGF). Hyper Res 20:217–223.

Matsubara T, Ziff M. 1986. Increased superoxide anion release from human endothelial cells in response to cytokines. J Immunol 137:3295–3298.

Medford RM. 1995. Antioxidants and endothelial expression of VCAM-1: a molecular paradigm for atherosclerosis. In: Gallo LL, editor. Cardiovascular disease 2. New York, NY: Plenum Press. p. 121–127.

Mohazzab KM, Kaminski PM, Wolin MS. 1994. NADH oxidoreductase is a major source of superoxide anion in bovine coronary artery endothelium. Am J Physiol 266: H2568–H2572.

Nerem RM. 1992. Vascular fluid mechanics, the arterial wall, and atherosclerosis. J Biomech Eng 114:274–282.

Offermann MK, Medford RM. 1994. Antioxidants and atherosclerosis: a molecular perspective. Heart Dis Stroke 3:52–57.

Perloff D, Sokolow M, Cowan R. 1983. The prognostic value of ambulatory blood pressures. JAMA 249:2792–2798.

Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK, Harrison DG. 1996. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. J Clin Invest 97:1916–1923.

Rao GN, Berk BC. 1992. Active oxygen species stimulate vascular smooth muscle cell growth and proto-oncogene expression. Circ Res 70:593–599.

Roebuck KA, Rahman A, Lakshminarayanan V, Janakidevi K, Malik AB. 1995. H2O2 and tumor necrosis factor-alpha activate intercellular adhesion molecule 1 (ICAM-1) gene transcription through distinct cis-regulatory elements within the ICAM-1 promoter. J Biol Chem 270:18966–18974.

Safar ME, Peronneau PA, Levenson JA, Toto-Moukouo JA, Simon AC. 1981. Pulsed Doppler: diameter, blood flow velocity and volumic flow of the brachial artery in sustained essential hypertension. Circulation 63:393–400.

Satriano JA, Shuldiner M, Hora K, Xing Y, Shan Z, Schlondorff D. 1993. Oxygen radicals as second messengers for expression of the monocyte chemoattractant

protein, JE/MCP-1, and the monocyte colony-stimulating factor, CSF-1, in response to tumor necrosis factor-alpha and immunoglobulin G. Evidence for involvement of reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxidase. J Clin Invest 92:1564–1571.

Thannickal VJ, Fanburg BL. 1995. Activation of an H2O2-generating NADH oxidase in human lung fibroblasts by transforming growth factor beta 1. J Biol Chem 270: 30334–30338.

Thelen M, Dewald B, Baggiolini M. 1993. Neutrophil signal transduction and activation of the respiratory burst. Physiol Rev 73:797–821.

Tsao PS, Wang B, Buitrago R, Shyy JY, Cooke JP. 1997. Nitric oxide regulates monocyte chemotactic protein-1. Circulation 96:934–940.

Ushio-Fukai M, Zafari AM, Fukui T, Ishizaka N, Griendling KK. 1996. p22phox is a critical component of the superoxide-generating NADH/NADPH oxidase system and regulates angiotensin II-induced hypertrophy in vascular smooth muscle cells. J Biol Chem 271:23317–23321.

Veterans Administration Cooperative Study Group on Antihypertensive Agents. 1967. Effects of treatment on morbidity and in hypertension. Results in patients with diastolic blood pressure averaging 115–129 mm Hg. JAMA 202:1028–1035.

Wang DL, Wung BS, Shyy YJ, Lin CF, Chao YJ, Usami S, Chien S. 1995. Mechanical strain induces monocyte chemotactic protein-1 gene expression in endothelial cells. Effects of mechanical strain on monocyte adhesion to endothelial cells. Circ Res 77:294–302.

Wung BS, Cheng JJ, Chao YJ, Hsieh HJ, Wang DL. 1999. Modulation of Ras/Raf/extracellular signal-regulated kinase pathway by reactive oxygen species is involved in cyclic strain-induced early growth response-1 gene expression in endothelial cells. Circ Res 84: 804–812.