Constitutive NOS expression in cultured endothelial cells is elevated by fluid shear stress

VIBHU RANJAN, ZESHUAI XIAO, AND SCOTT L. DIAMOND
Bioengineering Laboratory, Department of Chemical Engineering, The State University of New York, Buffalo, New York 14260

Ranjan, Vibhu, Zeshuai Xiao, and Scott L. Diamond. Constitutive NOS expression in cultured endothelial cells is elevated by fluid shear stress. *Am. J. Physiol.* 269 (*Heart Circ. Physiol.* 38): H550–H555, 1995.—The role of chronic fluid shear stress on endothelial constitutive nitric oxide synthase (cNOS) levels may have an important role in vessel diameter control. We subjected primary human umbilical vein endothelial cells (HUVEC) or bovine aortic endothelial cells (BAEC, passages 2–14) to steady laminar shear stress. In both cell types, the intracellular level of cNOS was elevated within 3 h of flow exposure at 25 dyn/cm^2 and remained elevated at 6 and 12 h of flow exposure, compared with stationary controls, as indicated by digital immunofluorescence microscopy. Shear stress exposure for 6 h caused a 2.2 ± 0.3- and 2.8 ± 0.3-fold elevation of cNOS protein levels in BAEC ($n = 3$, $P < 0.01$) and HUVEC ($n = 3$, $P < 0.01$), respectively, in the presence or absence of 1 μM dexamethasone. Dexamethasone suppresses induction of the inducible NOS gene, indicating that cNOS was elevated by fluid shear stress. Flow exposure at 4 dyn/cm^2 caused no enhancement of cNOS levels in either cell type. The flow induction of the cNOS protein levels was not blocked by preincubation of BAEC with 100–400 μM of N^G-nitro-L-arginine methyl ester, indicating that flow-induced NO (or guanosine 3′,5′-cyclic monophosphate) was not involved in the elevation of cNOS levels. Protein kinase C inhibitor H-7 (10 μM) had no effect on induction of NOS protein in BAEC exposed to 25 dyn/cm^2. The cNOS mRNA levels were found to be elevated by two- to threefold in BAEC after 6 or 12 h of flow exposure at either 4 or 25 dyn/cm^2, and this induction of NOS mRNA occurred in the presence of dexamethasone. The elevation of cNOS levels by chronic flow exposure may provide a mechanism for chronic regulation of vessel diameter by endothelial response to prevailing blood flow.

nitric oxide synthase; endothelium; hemodynamics

NITRIC OXIDE from the vascular endothelium mediates vasodilation and can be elicited by endothelium-dependent vasodilators such as acetylcholine, bradykinin, and calcium ionophores (9) as well as fluid shear stress (5, 24). NO also inhibits the adhesion of monocytes and platelets to endothelial cells and inhibits smooth muscle cell proliferation. In endothelial cells, NO synthase (NOS) activity is predominantly membrane-bound (9), and two distinct gene products are responsible for the biosynthesis of NO. The activity of constitutive NOS (cNOS, also called endothelial NOS or eNOS) is Ca^{2+}/calmodulin and NADPH dependent (3). The inducible NOS (iNOS) protein is found in macrophages and endothelial cells (29), and its level is elevated by endotoxin and some cytokines. Furthermore, the activity of iNOS is Ca^{2+} independent and requires NADPH and tetrahydrobiopterin. Glucocorticoids such as dexamethasone inhibit the induced expression of iNOS but not the expression of cNOS in vascular endothelial cells, without direct effect on the activity of either enzyme (29).

The flow-induced release of NO by endothelial cells has been demonstrated in vitro, in vivo, and in perfused vessels (5, 15, 16). Fewer studies have been conducted to study the effect of hemodynamic forces on endothelial NOS gene expression. Recently, it was shown that chronic exercise in dogs increased coronary vascular NO production and endothelial cell NOS gene expression (33). Cultured bovine endothelial cells exposed to shear stress are reported to have elevated levels of NOS mRNA levels (27), and cyclic stretching of cultured endothelial cells may also enhance NOS levels (1).

Several endothelial genes have been shown to be enhanced by shear stress either transiently, such as *fos* and *jun* (12, 30), platelet-derived growth factor (PDGF)-B (11, 21, 31), basic fibroblast growth factor (21), and monocyte chemotactic protein 1 (35), or in a sustained manner, such as tissue plasminogen activator (tPA)(7, 8) and intracellular adhesion molecule (ICAM)(26). Other endothelial genes are downregulated by arterial levels of fluid shear stress, such as endothelin (15, 20, 22, 34) or fibronectin (10). Chronic shear stress exposure appears to have little effect on plasminogen activator-inhibitor type 1 or glyceraldehyde 3-phosphate dehydrogenase expression levels (7, 8). Using an in vitro perfusion system to expose cultured endothelial cells to steady laminar flow, we have investigated the regulation of the cNOS gene in response to fluid shear stress exposures. We show that cNOS gene expression can be elevated in a sustained manner by fluid shear stress in human and bovine endothelial cells of venous and arterial origin, respectively. The induction was independent of flow-induced NO production or protein kinase C (PKC) activation.

MATERIALS AND METHODS

Cell culture. Bovine aortic endothelial cells (BAEC) were grown to confluence in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated newborn calf serum, 0.30 mg/ml glutamine, 150 U/ml penicillin, and 0.15 mg/ml streptomycin (GIBCO, Grand Island, NY) (10% C-DMEM). Primary human umbilical vein endothelial cells (HUVEC) were extracted and cultured as previously described (7) and grown in DMEM with 20% serum (20% C-DMEM).

Exposure of endothelial cells to shear stress. Confluent monolayers of BAEC (passages 2–14) and primary, confluent monolayers of HUVEC were treated with 1 μM dexamethasone for 24 h to inhibit the expression of iNOS during the experiment (29). The cells were then exposed to a steady laminar shear stress of 4 or 25 dyn/cm^2 in individual, sterile,

parallel-plate flow chamber systems with recirculating medium (20 ml of 10% C-DMEM with 1 μM dexamethasone for BAEC and 20% C-DMEM with 1 μM dexamethasone for HUVEC) as previously described (7, 8) or maintained in a CO_2 incubator. The pH and aeration of the perfusion media was controlled with a sterile 5% CO_2-air mixture. Immunofluorescent staining or RNA extraction were carried out on the monolayers after various shear exposure times.

Immunfluoroscence staining and digital epifluorescence microscopy. Individual glass slides with endothelial cells were fixed by incubation of the glass slides in a solution containing 2% paraformaldehyde, 0.5% Triton X-100 in phosphate-buffered saline (PBS) at 4°C for 15 min. The slides were then washed with 3% bovine serum albumin (BSA) and 0.5% Triton X-100 in PBS, and incubated with 50 μl of mouse monoclonal antibody against ecNOS (Transduction Laboratories, Lexington, KY) at a dilution of 1:100 (3% BSA and 0.5% Triton X-100 in PBS) for 1 h. The slides were washed three times with 5 ml of 3% BSA-0.5% Triton X-100 in PBS solution. The slides were then incubated with 50 μl of fluorescein goat anti-mouse IgG (H+L) conjugate (Molecular Probes, Eugene, OR) (1:100 dilution) for 30 min. The slides were washed three times with PBS and viewed by video microscopy. For epifluorescence visualization, a Leitz Aristoplan microscope (×100 objective) with image-intensified charge-coupled device camera assembly (GenIIsys-CCD72, Dage-MTI, Michigan City, IN) was used to detect the fluorescence emission. Noise reduction of the video signal was achieved with the use of a Hamamatsu Argus-10 Image Processor, but no gray scale stretching or contrast enhancement was carried out. For digital image analysis, the video signal was recorded on 0.5 in. videotape and analyzed with a Quickcapture framegrabber (Data Translation, Marlboro, MA) and a Macintosh IIci (NIH Image 1.47).

RNA isolation and Northern blot analysis. Total RNA was extracted from control and shear stress-stimulated endothelial cells by methods described previously (8). Briefly, endothelial cell monolayers (15 cm^2) were rinsed with sterile PBS, lysed in 1.0 ml of denaturing buffer (4.0 M guanidium isothiocyanate, 0.5% *N*-lauroylsarcosine, 25 mM sodium citrate, and 0.1 M mercaptoethanol), followed by addition of 0.1 ml of 2.0 M sodium acetate (pH 4.0). The mixture was extracted twice with redistilled buffered phenol and chloroform/isoamyl alcohol (49:1). RNA was precipitated by addition of equal volume of isopropyl alcohol and overnight incubation at −20°C, followed by centrifugation at 12,000 *g* for 30 min. The RNA pellet was rinsed with ethanol, dried, and redissolved in diethylpyrocarbonate-treated water.

A 296-bp segment of bovine cNOS cDNA (corresponding to positions 2730–3025 of Ref. 32) was amplified with a coupled reverse transcription-polymerase chain reaction with *Taq* DNA polymerase [30 cycles; each cycle was 94°C for 2 min, 55°C for 0.75 min, and 72°C for 2 min (15-min final cycle for A nucleotide overhang)] as previously described (8). The sense primer was 5′-GCTTGAGACCCTCAGTCAGG-3′, and the antisense primer was 5′-GGTCTCCAGTCTTGAGCTGG-3′. These primers produced a unique band of correct target size that was purified and subcloned into T-tailed pBluescript II SK(+) (Stratagene). The insert was verified by dideoxy DNA sequencing. The cDNA probe was synthesized by the random priming hexanucleotide method with [α-^{32}P]dATP. Five micrograms of each total RNA extract was run on each lane of a 1.0% agarose-formaldehyde gel. Ethidium bromide staining indicated complete integrity of the rRNA bands as well as even lane loading. The gel was blotted overnight to Millipore Immobilon N membranes, and then the membranes were probed with 10^5 cpm/ml of probe in hybridization buffer, followed by high-astringency washes. Autoradiography was performed at −75°C for 16 h. Northern blotting of BAEC total RNA produced a single band at ~4.1 kb as expected for bovine cNOS mRNA (32).

RESULTS

To inhibit the induction of iNOS, endothelial monolayers were incubated with 1 μM dexamethasone for 24 h before the experiment. The perfusion medium during the experiment also contained dexamethasone. Immunostaining for cNOS in unstimulated cells showed a very low level of diffuse staining that was elevated after 6 h of flow exposure (25 dyn/cm^2) and remained elevated after 12 h of flow exposure (Fig. 1). Dexamethasone (1 μM) had no effect on resting levels of cNOS immunofluorescence in BAEC or HUVEC after a 24-h incubation.

The levels of cNOS were 2.2 ± 0.3 times (n = 3 expts, $P < 0.01$) and 2.8 ± 0.3 times (n = 3, $P < 0.01$) higher in BAEC and HUVEC, respectively, that had been exposed to arterial shear stress (25 dyn/cm^2) for 6 h than in the matched stationary controls (Figs. 2 and 3). We have found that low shear stress of 4 dyn/cm^2 did not cause a significant induction of cNOS protein in either BAEC or HUVEC after 6 h of flow exposure.

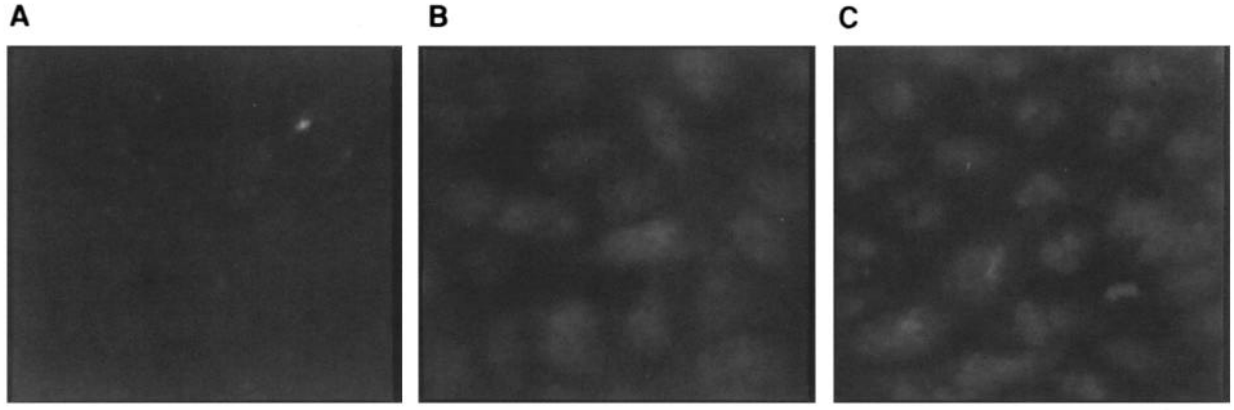

Fig. 1. Fluid shear stress induces elevation of endothelial constitutive nitric oxide synthase (cNOS) enzyme levels. Immunofluorescence staining of bovine aortic endothelial cells (BAEC) maintained under stationary conditions (*A*) or exposed to laminar shear stress at 25 dynes/cm^2 for 6 (*B*) or 12 h (*C*). Endothelial cells were preincubated with 1 μM dexamethasone before shear stress exposures with dexamethasone in perfusion media.

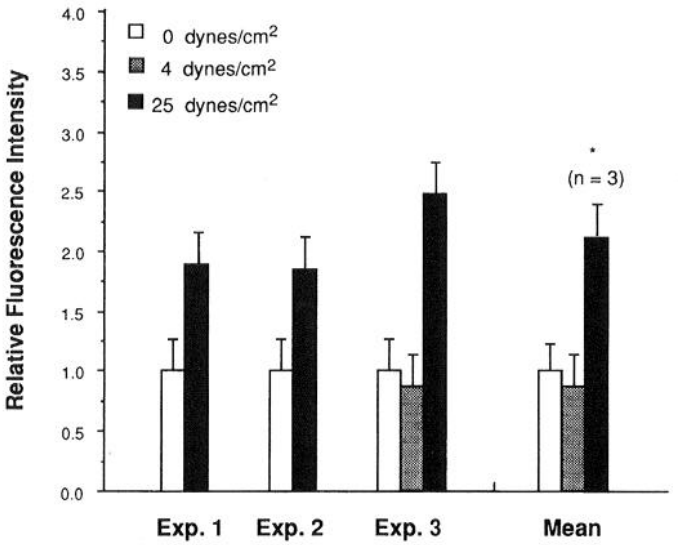

Fig. 2. cNOS immunofluorescence intensity in bovine aortic endothelial cells (BAEC) maintained in stationary culture (open bars) or exposed for 6 h to shear stress at 4 (stippled bars) or 25 dyn/cm^2 (filled bars). Average intensity (± SD) was determined in each experiment (Exp. 1–3) by digital image analysis for >30 cells in each monolayer. Average intensity values were then normalized to the average intensity of quiescent control endothelium maintained in stationary culture to give relative fluorescence intensity. Each experiment was conducted with an independent passage of BAEC. All monolayers were visualized under identical illumination and detection conditions. *$P < 0.01$; n, no. of expts.

In light of the known activation of signaling pathways such as PKC or NO (via cGMP) in shear-stressed endothelium (11, 15), we tested the effect of pharmacological inhibitors on the shear induction of cNOS protein. BAEC were pretreated for 30 min with N^G-nitro-L-arginine methyl ester (L-NAME), a potent inhibitor of NO production. These cells were then exposed to an arterial level of shear stress with the same concentration of L-NAME in the perfusion medium. The presence of L-NAME (100 μM) did not significantly inhibit the shear stress induction of the cNOS after 6 or 12 h of flow exposure (Fig. 4). We have found also that 400 μM L-NAME has no effect on shear stress induction. This would indicate that the shear stress induction of cNOS does not depend on flow-induced NO production (or cGMP).

We and others have found that the PKC inhibitor H-7 can cause significant attenuation of shear stress induction of various genes, such as *fos* (12, 30), PDGF-B (11), or tPA (Diamond, unpublished observations). To test whether shear induction of cNOS was PKC-dependent, BAEC were preincubated with H-7 (10 μM) for 30 min and then exposed to arterial levels of shear stress for 6 h with H-7 (10 μM) in the perfusion media. The presence of H-7 did not cause any significant change in the level of induction of cNOS in H-7-treated BAEC compared with untreated BAEC exposed to arterial levels of shear stress. Although H-7 can cause nonspecific inhibition of other protein kinases, as well as disruption of the endothelial actin cytoskeleton, we conclude that the induction of cNOS is PKC-independent. Consistent with this lack of effect of H-7, we observed that 6-h incubation of BAEC with phorbol myristrate acetate (PMA), a potent PKC activator, did not cause induction of cNOS. It is interesting to note that other endothelial cell stimulants such as thrombin (0.5 U/ml) or lipopolysaccharide (1 μg/ml) had no effect on BAEC cNOS levels after 6-h incubation in the agonist compared with

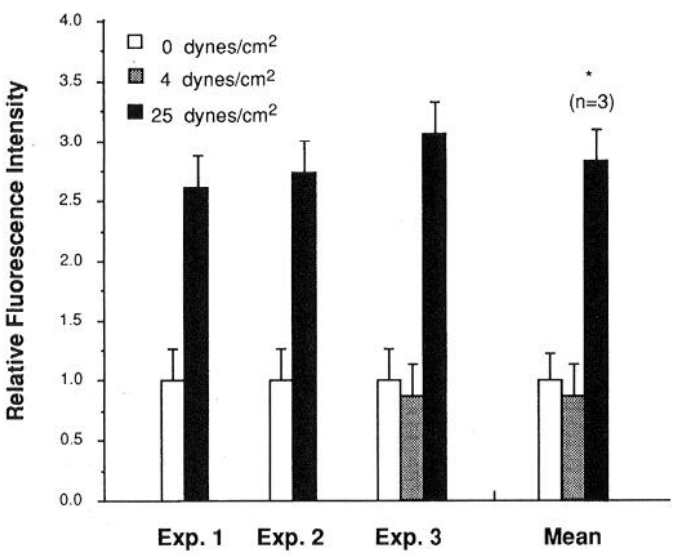

Fig. 3. cNOS immunofluorescence intensity in human umbilical vein endothelial cells (HUVEC) maintained in stationary culture (open bars) or exposed for 6 h to shear stress at 4 (stippled bars) or 25 dyn/cm^2 (filled bars). Average intensity (± SD) was determined in each experiment (Exps. 1–3) by digital image analysis for >30 cells in each monolayer. Average intensity values were then normalized to the average intensity of quiescent control endothelium maintained in stationary culture to give relative fluorescence intensity. Each experiment was conducted with an independent harvest of primary HUVEC. All monolayers were visualized under identical illumination and detection conditions. *$P < 0.01$; n, no. of expts.

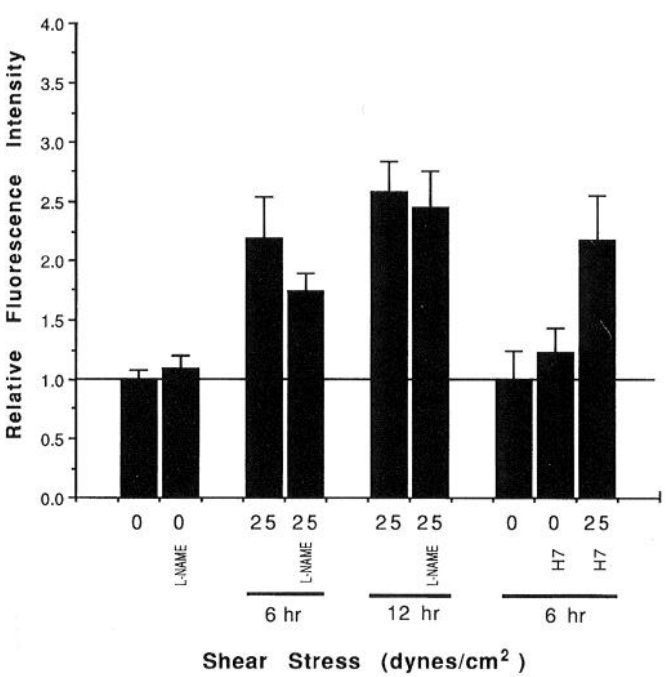

Fig. 4. cNOS immunofluorescence intensity (± SD) in BAEC maintained in stationary culture (0) or exposed for 6 or 12 h to shear stress at 25 dynes/cm^2 in presence or absence of 100 μM N^G-nitro-L-arginine methyl ester (L-NAME) or 10 μM H-7. Relative fluorescence intensity was calculated as in Figs. 2 and 3.

unstimulated controls. This suggests that cNOS was not induced by a large calcium mobilization (or elevated arachidonic acid metabolism), which would be expected with thrombin or lipopolysaccharide stimulation.

Given the rapid induction of cNOS protein levels in cells exposed to shear stress, we tested whether NOS mRNA levels were elevated in shear-stressed endothelium. Northern blot analysis revealed the induction of NOS at arterial levels of shear stress (25 dyn/cm^2) as well as at low levels of shear stress (4 dyn/cm^2) after 6 h of flow exposure (Fig. 5). The mRNA level of the cNOS band was elevated by threefold over stationary controls at 6 h and remained elevated at 12 h of exposure at 25 dyn/cm^2 (Fig. 6). In ten independent monolayers of BAEC exposed to shear stress (25 dyn/cm^2 up to 12 h), we observed a two- to threefold elevation of cNOS mRNA compared with matched stationary control cultures. We evaluated whether other stimulants elevated cNOS mRNA levels in BAEC. A 16-h stimulation of BAEC with lipopolysaccharide (1 μg/ml), phorbol ester

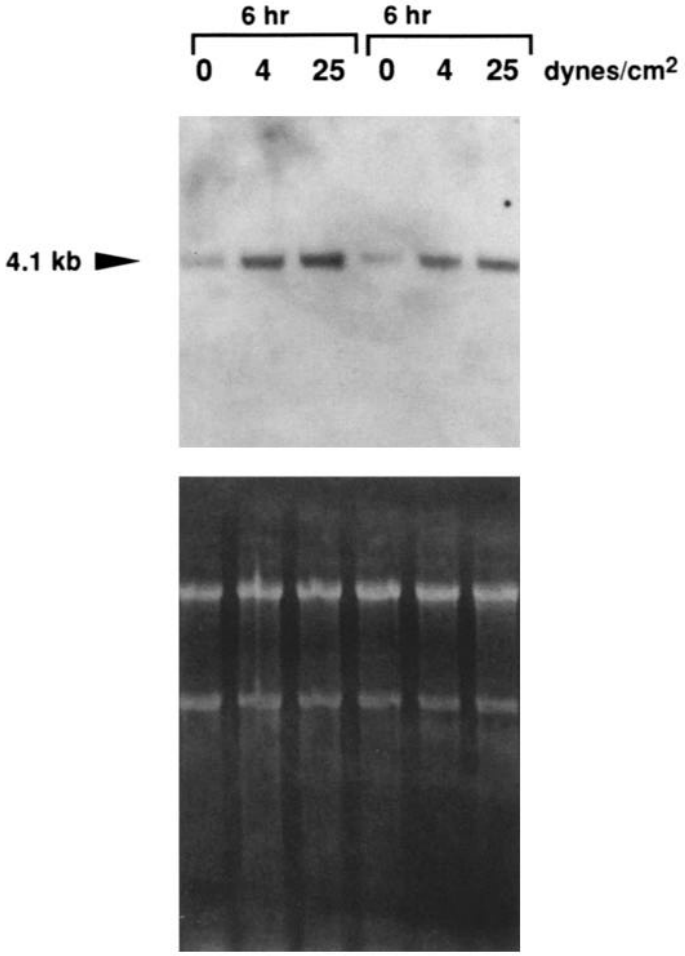

Fig. 5. Fluid shear stress causes elevation of cNOS mRNA levels in BAEC exposed to shear stress of 4 or 25 dyn/cm^2 for 6 h compared with stationary controls (0 dyn/cm^2). Two experiments were conducted, each with an independent passage of BAEC (first experiment, *lanes 1–3*; second experiment, *lanes 4–6*) (*top*). By scanning densitometry, elevated cNOS mRNA levels were 2.1 ± 0.2 (n = 2, P < 0.01) and 2.4 ± 0.5 (n = 2, P < 0.01) times higher at 4 and 25 dyn/cm^2, respectively, than levels found in stationary controls. Five micrograms of total RNA were subjected to electrophoresis in each lane, and ethidium bromide staining indicated uniform lane loading (*bottom*).

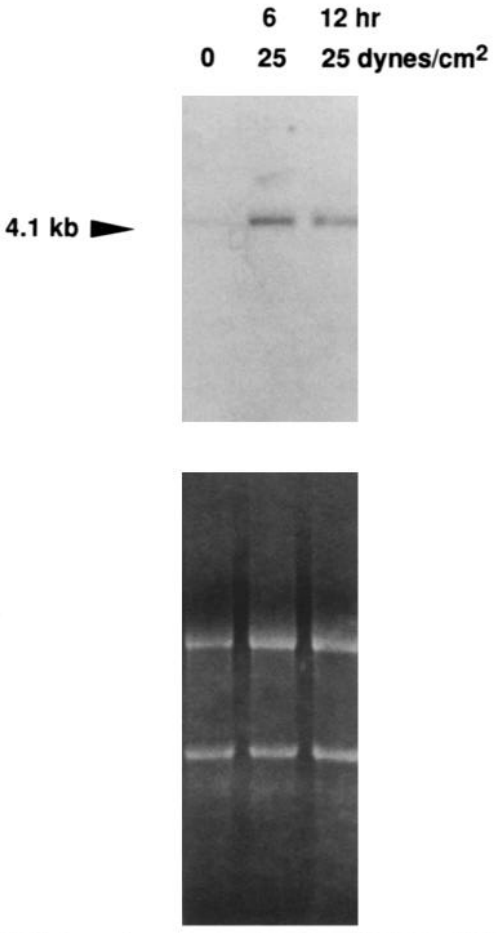

Fig. 6. Fluid shear stress causes elevation of cNOS mRNA levels in BAEC exposed to 25 dyn/cm^2 for 6 and 12 h compared with stationary controls (0 dyn/cm^2) (*top*). Five micrograms of total RNA were subjected to electrophoresis in each lane, and ethidium bromide staining indicated uniform lane loading (*bottom*).

(500 ng/ml), L-NAME (200 μM) ± dexamethasone (1 μM), or hyperosmotic shrinkage (45 mM mannitol) had no stimulatory effect on cNOS mRNA levels (data not shown). This indicated that the shear stress induction of cNOS in BAEC was somewhat unique, and similar elevations in cNOS mRNA levels could not be achieved with agonists known to greatly elevate other genes, such as ICAM (28) or tPA (18, 19) that are inducible by shear stress.

DISCUSSION

The endothelial regulation of basal levels of the constitutive and inducible forms of NOS under physiological conditions is not fully resolved. In contrast, the influence of fluid shear stress on NO production under chronic and acute flow conditions has been extensively studied in perfused vessels (5) and more recently under conditions of a well-defined steady laminar flow (14, 15). We present experimental evidence that cNOS mRNA and protein levels are elevated in endothelial cells exposed to arterial levels of shear stress. A lower venous level of shear stress (4 dyn/cm^2) was sufficient to elevate cNOS mRNA but caused no significant corresponding elevation of cNOS protein levels. A rigid parallel-plate flow chamber eliminated any deformation of the sub-

strate, thus preventing a situation analogous to circumferential distension of the vessel wall during increased flow. Our findings are consistent with a previous study carried out in the absence of any chemicals to inhibit iNOS induction that showed a flow induction of NOS levels at 24 h (27, 36). We have found that the induction within 3 h of cNOS occurred fairly rapidly and is somewhat more rapid than the shear stress induction of the secreted protein tPA (7).

The cNOS expressed in the vascular endothelium shares common biochemical and pharmacological properties with the neuronal NOS (9). However, cloning of NOS from human and bovine endothelial cells (23, 27, 32) has indicated the existence of a family of constitutive NO synthases. cDNA clones for human endothelial NOS predicted a protein of 1203 amino acids sharing 94% identity with the bovine endothelial protein but only 60% identity with the human or rat neuronal isoform. Sequence inspection of the 5′-flanking region of the human endothelial NOS gene reveals multiple potential *cis*-regulatory DNA sequence motifs (23). A *cis*-regulatory consensus sequence indicated to be a shear stress response element in the PDGF-B chain (31) is also present in the 5′-region of the human constitutive endothelial NOS gene. Two sets of AP-1 and AP-2 may participate in the transcriptional response to phorbol esters and/or adenosine 3′,5′-cyclic monophosphate (cAMP), respectively. However, we have found that phorbol ester did not cause induction of cNOS mRNA or protein. A nuclear factor (NF)1 binding site is present, which has been implicated in adenovirus replication and transforming growth factor-β responses. A putative cAMP-responsive element present in this region is identical to the cAMP-responsive-2 element in human proenkephalin promoter known to mediate transcriptional responses to changes in the intracellular level of cAMP. However, these elements have not been characterized in the regulation of endothelial NOS gene expression. Interestingly, the induction by arterial levels of shear stress of PDGF mRNA levels is rather transient and PKC dependent (11). Our results indicate that the elevation of cNOS mRNA and protein levels is sustained and independent of PKC activity. Although shear stress regulation of endothelin secretion appears to depend on shear-induced NO production via a cGMP pathway, the shear stress elevation of cNOS mRNA and protein levels was not inhibited by the L-arginine analogue, L-NAME. The presumable blockade (14) of the shear stress-suppression of endothelin-1 (ET-1) by L-NAME in our experiments had no effect on NOS induction by shear stress, suggesting that prevailing ET-1 secretion rates have no effect on cNOS gene regulation. Although PKC modulates receptor-independent activation of the endothelial NOS activity (6), it does not seem to play a major role in the shear stress-induced activation of the cNOS enzyme levels, because the presence of the PKC inhibitor H-7 did not significantly reduce the shear-mediated induction of the cNOS protein levels in BAEC.

While ET-1 gene expression is suppressed by sustained arterial flow exposures, the suppression is not seen in stretched endothelial cells (4, 20). The suppression by shear stress is reported to require NO production, because it can be completely blocked by 400 μM N^{ω}-nitro-L-arginine (24). Yet both shear and substrate stretching (1) appear to upregulate NOS gene expression. The suppression of ET-1 by flow-induced NO (but not stretch-induced NO) may suggest additional regulatory agents (repressors) present in shear-stressed but not stretched endothelium. From our studies it does not appear that NO has an autocrinic role in the shear-enhanced cNOS expression, although there is evidence that NO can autoregulate NOS activity (2).

It has been shown that the initial burst of NO production is Ca^{2+}/calmodulin dependent and appears to be dependent on a G protein, whereas the sustained release of NO is Ca^{2+}/calmodulin and G protein independent (15, 16). These workers found that dexamethasone did not have any effect on the sustained release of NO production, ruling out a role of the iNOS for its production. Dexamethasone is known to interfere with the binding of the nuclear factor NF-κB to its consensus DNA binding sequence and to interfere with the function of AP-1 complexes without diminishing the binding of the *fos-jun* to its cognate *cis* element (25). However, the cNOS promoter does not contain an identifiable NF-κB consensus element, and our findings indicate that the elevation of cNOS expression by shear stress does not require NF-κB or NF-κB-dependent pathways.

If shear stress causes a transient elevation in Ca^{2+}, this putative elevation in Ca^{2+} does not appear to have an important role in the elevation of cNOS gene expression in endothelial cells exposed to shear stress. Whether the shear stress elevation of NO production is a calcium-dependent process or a calcium-activated process is not clear. Studies with inhibitors of calmodulin or intracellular chelators of calcium (15) do not fully resolve the mechanisms by which NO production is elevated, and this lack of understanding is compounded by the known variability in demonstrating shear stress-induced calcium mobilization. Regardless of the mechanism, elevated production of NO by shear stress does not cause autocrinic enhancement of cNOS mRNA and protein levels.

These studies indicate that there exists a potentially important mechanism in vessel-diameter regulation involving endothelial gene expression in response to shear stress. Rabbit carotid arteries constrict by 21% within days after flow is reduced by 70% (17). The adaptive regulation to chronic changes in flow (up or down) of canine carotid artery (13) also indicate a strong coupling between prevailing blood flow and endothelial release of vasodilators and vasoconstrictors. That arterial levels of shear stress can elevate cNOS mRNA and protein levels while suppressing ET-1 mRNA and secretion (14, 20, 22, 34) by cultured endothelium may provide some insight into these adaptive responses.

The authors thank Diane Schmidt and the nursing staff of Millard Fillmore Suburban Hospital for generous assistance. DNA sequencing services were provided by Dr. L. Hall through the Center of Advanced Molecular Biology and Immunology at SUNY-Buffalo.

This study was supported by National Heart, Lung, and Blood Institute Grant HL-47486 and National Science Foundation (NSF)

Grant BCS 9211197. S.L. Diamond is a recipient of the NSF National Young Investigator Award.

Address for reprint requests: S. L. Diamond, Bioengineering Laboratory, Dept. of Chemical Engineering, SUNY, Buffalo, NY 14260.

Received 18 November 1994; accepted in final form 15 February 1995.

REFERENCES

1. **Awolesi, N., W. C. Sessa, and B. Sumpio.** Cyclic strain upregulates the endothelial cell nitric oxide synthase gene (Abstract). *FASEB J.* 7: 4360, 1993.
2. **Buga, G. M., J. M. Griscavage, N. E. Rogers, and L. J. Ignarro.** Negative feedback regulation of endothelial cell function by nitric oxide. *Circ. Res.* 73: 808–812, 1993.
3. **Busse, R., and A. Mulsch.** Calcium dependent nitric oxide synthesis in endothelial cytosol is mediated by calmodulin. *FEBS Lett.* 265: 133–136, 1990.
4. **Carosi, J. A., S. G. Eskin, and L. V. McIntire.** Cyclical strain effects on production of vasoactive materials in cultured endothelial cells. *J. Cell Physiol.* 151: 29–36, 1992.
5. **Cooke, J. P., E. Rossitch, Jr., N. A. Andon, L. Loscalzo, and V. J. Dzau.** Flow activates an endotelial potassium channel to release an endogenous nitrovasodilator. *J. Clin. Invest.* 88: 1663–1671, 1991.
6. **Davda, R. K., L. J. Chandler, and N. J. Guzman.** Protein kinase C modulates receptor-independent activation of endothelial nitric oxide synthase. *Eur. J. Pharmacol.* 266: 237–244, 1994.
7. **Diamond, S. L., S. G. Eskin, and L. V. McIntire.** Fluid flow stimulates tissue plaminogen activator secretion in cultured human endothelial cells. *Science Wash. DC* 243: 1483–1485, 1989.
8. **Diamond, S. L., J. B. Sharefkin, C. Dieffenbach, K. F. Scott, L. V. McIntire, and S. G. Eskin.** Tissue plasminogen activator messenger RNA levels increase in cultured human endothelial cells exposed to shear stress. *J. Cell Physiol.* 143: 364–371, 1990.
9. **Forstermann, U., J. S. Pollock, H. H. H. W. Schmidt, M. Heller, and F. Murad.** Calmodulin-dependent endothelium-derived relaxing factor/nitic oxide synthase is present in particulate and cytosolic fractions of bovine aortic endothelial cells. *Proc. Natl. Acad. Sci. USA* 88: 1788–1792, 1991.
10. **Gupte, A., and J. A. Frangos.** Effects of flow on the synthesis and release of fibronectin by endothelial cells. *In Vitro Cell. Devel. Biol.* 26: 57–60, 1990.
11. **Hsieh, H. J., N. Q. Li, and J. A. Frangos.** Shear induced platelet-derived growth factor gene expression in human endothelial cells is mediated by protein kinase C. *J. Cell Physiol.* 150: 552–558, 1992.
12. **Hsieh, H. J., N. Q. Li, and J. A. Frangos.** Pulsatile and steady flow induces *c-fos* expression in human endothelial cells. *J. Cell Physiol.* 154: 143–151, 1993.
13. **Kamiya, A., and T. Togawa.** Adaptive regulation of wall shear stress to flow change in the canine carotid artery. *Am. J. Physiol.* 239 (*Heart Circ. Physiol.* 8): H14–H21, 1980.
14. **Kuchan, M. J., and J. A. Frangos.** Shear stress regulates endothelin-1 release via protein kinase C and cGMP in cultured endothelial cells. *Am. J. Physiol.* 264 (*Heart Circ. Physiol.* 33): H150–H156, 1993.
15. **Kuchan, M. J., and J. A. Frangos.** Role of calcium and calmodulin in flow-induced nitric oxide production in endothelial cells. *Am. J. Physiol.* 266 (*Cell Physiol.* 35): C628–C636, 1994.
16. **Kuchan, M. J., H. Jo, and J. A. Frangos.** Role of G proteins in shear-mediated nitric oxide production by endothelial cells. *Am. J. Physiol.* 267 (*Cell Physiol.* 36): C753–C758, 1994.
17. **Langille, L., and F. O'Donnell.** Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. *Science Wash. DC* 231: 405–407, 1986.
18. **Levin, E. G., and L. Santell.** Cyclic AMP potentiates phorbol ester stimulation of tissue plasminogen activator release and inhibits release of plasminogen activator inhibitor-1 from human endothelial cells. *J. Biol. Chem.* 263: 16802–16808, 1988.
19. **Levin, E. G., L. Santell, and F. Saljooque.** Hyperosmotic stress stimulates tissue plasminogen activator expression by a PKC-independent pathway. *Am. J. Physiol.* 265 (*Cell Physiol.* 34): C387–C396, 1993.
20. **Malek, A., and S. Izumo.** Physiological fluid shear stress causes downregulation of endothelin-1 mRNA in bovine aortic endothelium. *Am. J. Physiol.* 263 (*Cell Physiol.* 32): C389-C396, 1992.
21. **Malek, A. M., G. H. Gibbons, V. J. Dzau, and S. Izumo.** Fluid shear stress differentially modulates expression of genes encoding basic fibroblast growth factor and platelet-derived growth factor B chain in vascular endothelium. *J. Clin. Invest.* 92: 2013–2021, 1993.
22. **Malek, A. M., A. L. Greene, and S. Izumo.** Regulation of endothelin 1 gene by fluid shear stress is transcriptionally mediated and independent of protein kinase C and cAMP. *Proc. Natl. Acad. Sci. USA* 90: 5999–6003, 1993.
23. **Marsden, P. A., H. H. Q. Heng, S. W. Scherer, R. J. Stewart, A. V. Hall, X. M. Shi, L. C. Tsui, and K. T. Schappert.** Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. *J. Biol. Chem.* 268: 17478–17488, 1993.
24. **Miller, V., and J. C. Burnett, Jr.** Modulation of NO and endothelin by chronic increases in blood flow in canine femoral arteries. *Am. J. Physiol.* 263 (*Heart Circ. Physiol.* 32): H103–H108, 1992.
25. **Mukaida, N., M. Morita, Y. Ishikawa, N. Rice, S. I. Okomoto, T. Kashanara, and K. Matsushima.** Novel mechanism of glucocorticoid-mediated gene repression. *J. Biol. Chem.* 269: 13289–13295, 1994.
26. **Nagel, T., N. Resnick, W. J. Atkinson, C. F. Dewey, Jr., and M. A. Gimbrone.** Shear stress selectively upregulates intracellular adhesion molecule-1 expression in cultured human vascular endothelial cells. *J. Clin. Invest.* 94: 885–891, 1994.
27. **Nishida, K., D. G. Harrison, J. P. Navas, A. A. Fisher, S. P. Dockery, M. Uematsu, R. M. Nerem, R. W. Alexander, and T. J. Murphy.** Molecular cloning and characterization of the constitutive bovine aortic endothelial cell nitric oxide synthase. *J. Clin. Invest.* 90: 2092–2096, 1992.
28. **Pohlman, T. H., K. A. Stanness, P. G. Beatty, H. D. Ochs, and J. M. Harlan.** An endothelial cell surface factor(s) induced in vitro by lipopolysaccharide, interleukin 1, and tumor necrosis factor increases neutrophil adherence by a CDw18-dependent mechanism. *J. Immunol.* 136: 4548–4556, 1986.
29. **Radomski, M. W., R. M. J. Palmer, and S. Moncada.** Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. *Proc. Natl. Acad. Sci. USA* 87: 10043–10047, 1990.
30. **Ranjan, V., and S. L. Diamond.** Fluid shear stress induces synthesis and nuclear localization of c-fos in cultured human endothelial cells. *Biochem. Biophys. Res. Commun.* 196: 79–84, 1993.
31. **Resnick, N., T. Collins, W. Atkinson, D. T. Bonthron, C. Forbes Dewey, Jr., and M. A. Gimbrone, Jr.** Platelet derived growth factor B chain promoter contains a *cis*-acting fluid shear stress response element. *Proc. Natl. Acad. Sci. USA* 90: 4591–4595, 1993.
32. **Sessa, W. C., J. K. Harrison, C. M. Barber, D. Zeng, M. E. Durieux, D. D. D'Angelo, K. R. Lynch, and M. J. Peach.** Molecular cloning and expression of a cDNA encoding endothelial cell nitric oxide synthase. *J. Biol. Chem.* 267: 15274–15276, 1992.
33. **Sessa, W. C., K. Pritchard, N. Seyedi, J. Wang, and T. H. Hintze.** Chronic exercise in dogs increases coronary nitric oxide production and endothelial cell nitric oxide synthase gene expression. *Circ. Res.* 74: 349–353, 1994.
34. **Sharefkin, J. B., S. L. Diamond, S. G. Eskin, L. V. McIntire, and C. W. Dieffenbach.** Fluid flow decreases preproendothelin mRNA levels and suppresses endothelin-1 peptide release in cultured human endothelial cells. *J. Vasc. Surg.* 14: 1–9, 1991.
35. **Shyy, Y. J., H. J. Hsieh, S. Usami, and S. Chien.** Fluid shear stress induces a biphasic response of human monocyte chemotactic protein 1 gene expression in vascular endothelium. *Proc. Natl. Acad. Sci. USA* 91: 4678–4682, 1994.
36. **Uematsu, M., J. P. Navas, K. Nishida, Y. Ohara, T. J. Murphy, R. W. Alexander, R. M. Nerem, and D. G. Harrison.** Mechanisms of endothelial cell NO synthase induction by shear stress (Abstract). *Circulation* 88: I-184, 1993.