Upregulation of Prostacyclin Synthesis–Related Gene Expression by Shear Stress in Vascular Endothelial Cells

Kazuhiro Okahara, Bing Sun, Jun-ichi Kambayashi

Abstract—Prostacyclin (prostaglandin I_2, PGI_2) has a variety of functions, including inhibition of smooth muscle cell proliferation, vasodilation, and antiplatelet aggregation. PGI_2 production in endothelial cells has been reported to increase biphasically after shear loading, but the underlying mechanism is not well understood. To clarify the mechanism for the second phase of PGI_2 upregulation, we examined the gene expression of the enzymes involved in PGI_2 production in human umbilical vein endothelial cells (HUVECs) after shear-stress (24 dyne/cm^2) loading. The production of 6-keto-$PGF_{1\alpha}$, a stable metabolite of PGI_2, increased time-dependently under shear stress. The arachidonic acid liberation from membrane phospholipids in HUVECs after 12 hours of shear loading was increased significantly compared with the static condition. No change was observed for cytosolic phospholipase A_2 expression, as detected by reverse transcription–polymerase chain reaction and Western blotting. Cyclooxygenase (COX)-1 mRNA increased after 1 hour of shear loading, and the increase lasted for 12 hours, the longest time tested, whereas COX-2 mRNA increased after 1 hour of shear loading and peaked at 6 hours. An increase of COX-1 expression was detected at 12 hours of shear loading by Western blotting. No expression of COX-2 was detected in the static control, but induced expression was observed at 6 hours after shear loading. PGI_2 synthase was also found to be upregulated. These results suggest that the elevated PGI_2 production by shear stress is mediated by increased arachidonic acid release and a combination of increased expression of COXs and PGI_2 synthase. **(*Arterioscler Thromb Vasc Biol*. 1998;18:1922-1926.)**

Key Words: shear stress ■ prostacyclin ■ gene regulation ■ endothelial cells

Downloaded from http://atvb.ahajournals.org/ by guest on August 29, 2017

Vascular endothelial cells were once considered to be a passive, antithrombogenic barrier; however, they are now known to have a variety of functions, such as regulation of vascular tone, atherogenic progression, and smooth muscle cell proliferation. Endothelial functions are regulated not only by humoral factors, such as growth factors and cytokines, but also by mechanical forces, such as shear stress, pressure, and stretch. Shear stress in particular plays an important role in endothelial functions. Shear stress can mediate a change in morphology and the release of various vasoactive substances from endothelial cells.[1,2] Decreased shear stress has been suggested to be involved in the pathogenesis of atherosclerosis. It has been reported that areas of low shear stress are more likely to become sites of atherosclerosis[3] and that low shear stress promotes the formation of intimal hyperplasia.[4] Previous studies have shown that the release of NO, C-type natriuretic peptide, and prostaglandin I_2 (PGI_2) is augmented by shear stress through elevated intracellular Ca^{2+} and increased transcription of endothelial NO synthase mRNA and C-type natriuretic peptide mRNA.[5–7]

Frangos et al[7] reported that shear stress increases PGI_2 production in a biphasic manner: the first peak was seen within several minutes of shear loading at 10 dyne/cm^2, and the second peak started at 2 hours and was sustained for several hours. Recently, the enzymes related to PGI_2 biosynthesis have been reported to be regulated by cytokines such as transforming growth factor (TGF-β) and interleukin-1α at the transcriptional level.[8] Cyclooxygenase (COX)-2 mRNA expression has also been found to be induced by shear stress.[9] In the present study, we investigated the mechanism of the second phase of shear stress–induced PGI_2 production by examining the gene expression of PGI_2 synthesis–related enzymes by use of reverse transcription–polymerase chain reaction (RT-PCR) and Western blot analysis.

Methods

Cell Culture

Human umbilical vein endothelial cells (HUVECs) were obtained from the umbilical veins by use of collagenase, as described previously.[6] Cells were cultured at 37°C in medium 199 containing 10% FCS, 90 μg/mL heparin, 150 μg/mL bovine brain extract, 100 U/mL penicillin, and 100 μg/mL streptomycin under humidified 5% CO_2/95% air. The cells were subcultured with 0.02% trypsin and used within 5 passages for all experiments.

Shear Loading

The system we used was modified from the cone-plate viscometer device first described and characterized by Bussolari et al,[10] which allowed us to expose cultured HUVECs to a well-defined laminar

Received May 19, 1998; revision accepted May 29, 1998.

From the Maryland Research Laboratories, Otsuka America Pharmaceutical, Inc, Rockville, Md.

Correspondence to Jun-ichi Kambayashi, MD, PhD, Maryland Research Laboratories, Otsuka America Pharmaceutical, Inc, 9900 Medical Center Dr, Rockville, MD 20850. E-mail junichik@mrl.oapi.com

© 1998 American Heart Association, Inc.

***Arterioscler Thromb Vasc Biol.* is available at http://www.atvbaha.org**

fluid flow. The shear-stress device consists of a cone that rotates above a stationary base plate containing the cultured HUVECs. The base plate is made from a 35-mm-diameter gelatin-coated polystyrene dish (Corning). The cone makes an angle of 0.5° with the culture plate and is coupled to a variable-speed motor. The distance between the cone and the culture dish was adjusted to 50 μm. The shear-stress device was operated in a CO_2 incubator.

Confluent HUVECs in a 35-mm dish coated with 0.2% gelatin (Sigma Chemical Co) were washed once with PBS, and 1 mL of culture medium was added. The cells were then exposed to shear stress (24 dyne/cm^2) for 1, 3, 6, and 12 hours after 30 minutes of preincubation in the incubator. Control samples were obtained by the same procedure but without exposure to shear stress.

6-Keto-prostaglandin $F_{1\alpha}$ Measurement by Enzyme Immunoassay

After shear loading, the cells were washed 3 times with PBS, and 1 mL of medium 199 medium without phenol red was added. The cells were incubated in a CO_2 incubator for 30 minutes, and then the medium was collected. After centrifugation at 1500*g* for 5 minutes, supernatant was collected and the 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) concentration was measured with a 6-keto-$PGF_{1\alpha}$ enzyme immunoassay kit (Amersham) according to the manufacturer's protocol.

Arachidonic Acid Liberation Assay

HUVECs were preincubated with 0.5 mCi of ^{3}H-labeled arachidonic acid (AA; 0.1 mCi/mL, DuPont NEN) in 1 mL of culture medium for 24 hours and then washed 3 times with PBS. After the addition of 1 mL of culture medium, the control cells were placed in the CO_2 incubator, and the cells for shear loading were exposed to shear stress (24 dyne/cm^2) for 12 hours. Then the cells were washed with PBS and fed with culture medium. After incubation for 30 minutes at 37°C, the medium was collected and the cells were lysed in 2% SDS; the medium and cell lysate were counted in a liquid scintillation counter (Wallac). The liberation rate was calculated by dividing the count from the medium by the total count from both medium and lysate.

RT-PCR

Total RNA from HUVECs was extracted with RNA Stat-60 (Teltest).[11] The purity and degradation of RNA were checked by electrophoresis in 0.8% agarose gel containing ethidium bromide and by spectrophotometric measurements at 260 and 280 nm. Total RNA (2 μg) from HUVECs was transcribed into cDNA with a Superscriptase II kit (Gibco BRL) and random hexamers. Aliquots of 1/50 RT reaction were used for each PCR amplification with cytosolic phospholipase A_2 (cPLA_2), COX-1, COX-2, PGI_2 synthase (PGIS), and GAPDH specific primers and a thermal cycler (model 480, Perkin-Elmer). The sequences of the sense and antisense primers used were cPLA_2 sense, 5′-CAGTATTCCCACAAGTTTACG-3′; cPLA_2 antisense, 5′-ACCATTTCAGTGACTTGGTTG-3′; COX-1 sense, 5′-AGAAGCAGTTGCCAGATGC-3′; COX-1 antisense, 5′-AGCCGCAGTTGATACTGAC-3′; COX-2 sense, 5′-CAGTGCACTACATACTTACC-3′; COX-2 antisense, 5′-TCTAGCCAGAGTTTCACCG-3′; PGIS sense, 5′-CAGCTTCCTCACGAGGATGA-3′; PGIS antisense, 5′-CAGCCACTGCCTGCTTCTG-3′; GAPDH sense, 5′-GTGCCAAAAGGGTCATCATCTC-3′; and GAPDH antisense, 5′-GATGGCATGGACTGTGGT CATG-3′. The expected sizes of these products were cPLA_2, 360 bp; COX-1, 235 bp; COX-2, 461 bp; PGIS, 324 bp; and GAPDH, 200 bp. PCR was performed for 25 cycles for GAPDH and 30 cycles for all others. The amplification products were quantified by separation on the 1.5% agarose gel and transferred to nylon membranes (Hybond N$^+$, Amersham). Specific oligonucleotide probes were labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase reaction and hybridized to the membrane at 37°C overnight. The membranes were washed 3 times with 2× SSC for 10 minutes and autoradiographed. Autoradiographs were scanned, and the bands were quantified by use of SigmaScan software. All values were normalized to GAPDH.

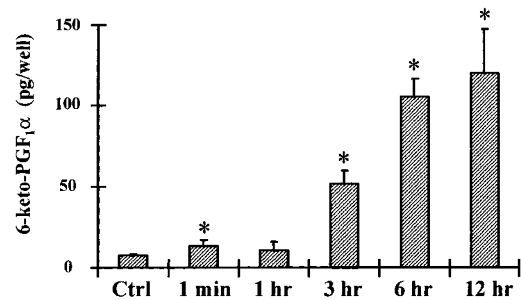

Figure 1. Amount of 6-keto-$PGF_{1\alpha}$ released from HUVECs under shear stress over time. Bar graph shows 6-keto-$PGF_{1\alpha}$ release into the medium for 30 minutes before (basal control) and after shear loading (24 dyne/cm^2), measured by enzyme immunoassay. Cells were washed after shear stress exposure and incubated with serum-free medium 199 for 30 minutes. Values are mean±SEM (n=4 each). *$P<0.05$.

Western Blotting

The cells were homogenized in lysis buffer (HEPES 50 mmol/L, NaCl 150 mmol/L, EGTA 2.5 mmol/L, EDTA 1 mmol/L, Triton X-100 1%, PMSF 200 mmol/L, aprotinin 2 mg/mL, and leupeptin 1 mg/mL), and the protein concentration was determined with a BCA protein assay kit (Pierce). Loading buffer was added to achieve a final concentration of Tris-HCl 62.5 mmol/L, SDS 2%, glycerol 10%, and 2-mercaptoethanol 5% before being heated to 95°C for 5 minutes. Twenty micrograms of each sample was used and separated by 10% SDS–polyacrylamide gel electrophoresis. Proteins were electrophoretically transferred to membranes (PVDF, Bio-Rad). After incubation for 1 hour at room temperature or overnight at 4°C in TBS/Tween-20 (Tris 25 mmol/L, NaCl 0.5 mol/L containing Tween-20 0.2%, pH 7.4) with 5% skim milk and 1% BSA to reduce nonspecific binding, the membranes were incubated with anti-cPLA_2 polyclonal antibody (Santa Cruz, 1:200), anti–COX-1 monoclonal antibody (Cayman Chemical, 0.5 μg/mL), anti–COX-2 monoclonal antibody (Cayman Chemical, 0.5 μg/mL), and anti-PGIS monoclonal antibody (Cayman Chemical, 0.4 μg/mL) for 2 hours at room temperature. The second antibodies used were anti-mouse peroxidase-conjugated IgG (1:2000) for monoclonal antibody or anti-rabbit peroxidase-conjugated IgG (1:5000) for polyclonal antibody. Finally, the membranes were washed in TBS/Tween-20 and visualized with enhanced chemiluminescence detection reagent (ECL, Amersham).

Statistical Analysis

All values are expressed as mean±SEM. Statistical analysis between 2 groups was performed with unpaired Student's *t* test. Values of $P<0.05$ were considered statistically significant.

Results

6-Keto-$PGF_{1\alpha}$ Production Under Shear Stress

PGI_2 production increased under shear stress, as measured by its stable metabolite 6-keto-$PGF_{1\alpha}$ (Figure 1). The basal release of 6-keto-$PGF_{1\alpha}$ during the 30-minute incubation at 37°C was 7.9 pg/well. After 1 minute of shear loading at 24 dyne/cm^2, 6-keto-$PGF_{1\alpha}$ generation increased to 1.7 times the basal level. After 1 hour of shear loading, 6-keto-$PGF_{1\alpha}$ production dropped slightly (1.4 times basal). The sustained phase of the 6-keto-$PGF_{1\alpha}$ increase began after 3 hours of shear loading with 6.6 times the control level and increased further to 15.3 times at 12 hours. This biphasic pattern of PGI_2 production in shear-loaded HUVECs is similar to previous findings.[7]

Downloaded from http://atvb.ahajournals.org/ by guest on August 29, 2017

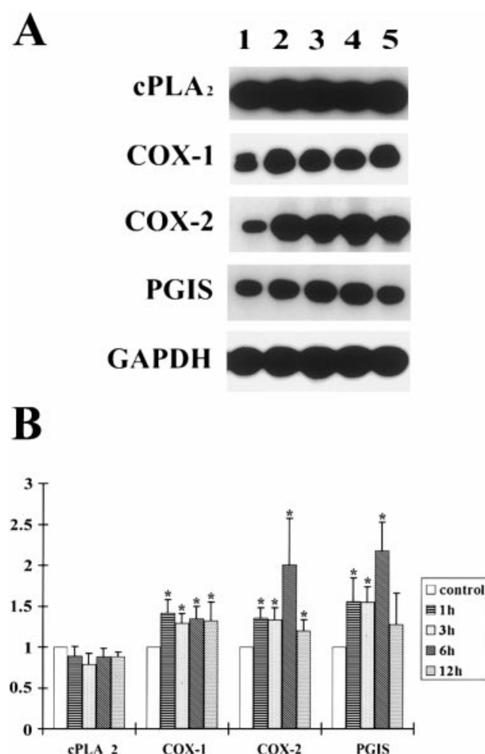

Figure 2. mRNA expression of $cPLA_2$, COX-1, COX-2, PGIS, and GAPDH (for normalization) in HUVECs determined by RT-PCR. A, Representative set from 4 individual RT-PCR/Southern blotting experiments after shear stress loading at 24 dyne/cm^2. Lane 1, static control; lane 2, 1 hour; lane 3, 3 hours; lane 4, 6 hours; lane 5, 12 hours. B, Normalized mRNA level determined by RT-PCR/Southern analysis. Values of static control are taken as 1. Data are mean±SEM (n=4 each). *$P<0.05$.

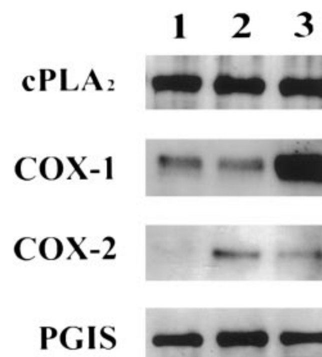

Figure 3. Western blotting of $cPLA_2$, COX-1, COX-2, and PGIS. Expression of $cPLA_2$, COX-1, COX-2, and PGIS in HUVECs before and at 6 and 12 hours after shear loading (24 dyne/cm^2). Twenty micrograms of total protein per lane was loaded for polyacrylamide gel electrophoresis. Lane 1, static control; lane 2, 6 hours after shear loading; and lane 3, 12 hours after shear loading.

Downloaded from http://atvb.ahajournals.org/ by guest on August 29, 2017

Arachidonic Acid Liberation

The release of AA from HUVECs was examined. It was found that the AA liberation rate (see Methods) was 0.60±0.05% in the static control (n=6). The rate increased significantly (1.5-fold) after 12 hours of shear loading at 24 dyne/cm^2 (0.91±0.09%, n=5, $P<0.05$).

Expression of $cPLA_2$, COX-1, COX-2, and PGIS

$cPLA_2$, COX-1, COX-2, and PGIS are the enzymes involved in PGI_2 synthesis. The present study showed that $cPLA_2$ mRNA was expressed at a low level in static conditions, as shown by RT-PCR (Figure 2). No increase was observed after 1, 3, 6, and 12 hours of shear loading at 24 dyne/cm^2. COX-1 mRNA was also detected at a low level in the static control. The expression increased significantly (1.4-fold) at 1 hour and remained at that level up to 12 hours of shear loading. COX-2 mRNA was detected as a faint band in the static control; its expression became apparent at 1 hour of shear loading and peaked at 6 hours (2.0-fold). However, the expression returned to basal levels at 12 hours. The expression of PGIS was also detected in the static control; its expression increased at 1 hour with a peak at 6 hours (2.2-fold, $P<0.05$), and then the mRNA levels decreased at 12 hours of shear loading.

Western blot analysis revealed similar levels of $cPLA_2$, COX-1, COX-2, and PGIS protein expression in HUVECs under shear stress. It was found that $cPLA_2$ expression was not changed by shear stress. One typical set of results (n=3) is presented in Figure 3. The expression of COX-1 at the protein level was increased only at 12 hours of shear loading, which is different from mRNA expression. COX-2 was not detectable in the static control. After 6 and 12 hours, COX-2 expression was detected by Western blotting, with higher expression at 6 hours, which is consistent with the RT-PCR results. Western blotting also showed PGIS expression to be increased after shear stress. The increase was observed after 6 hours of shear loading. The expression returned to static levels at 12 hours. The pattern of increased PGIS expression at the protein level is correlated with the increased mRNA levels.

Discussion

The existence of a second phase of increased PGI_2 production in ECs under shear stress was confirmed in the present study by measurement of 6-keto-$PGF_{1\alpha}$. Several types of phospholipase have been reported to be involved in AA liberation. Among these, $cPLA_2$ is considered to play a major role.[12] Activation of $cPLA_2$ has been suggested to play a role in the early phase of shear stress–induced PGI_2 production.[13] $cPLA_2$ is a calcium-dependent phospholipase and is activated by micromolar concentrations of intracellular calcium. Because shear stress elevates the intracellular calcium of vascular ECs during the first several minutes of shear loading,[14] $cPLA_2$ is believed to be involved in the initial burst of shear-induced AA liberation and PGI_2 production. However, the mechanism of the prolonged second phase of PGI_2 production under shear stress remains unclear.

It has been reported that shear stress activates diacylglycerol lipase.[15] It was found that diacylglycerol lipase inhibitor inhibits shear stress–induced PGI_2 production during the prolonged second phase. The present results are in agreement with previous observations. Although AA liberation increased 1.5-fold after 12 hours of shear loading, no increase in either mRNA or protein levels for $cPLA_2$ expression was observed, which also suggests that

shear stress augments AA liberation through activation of other lipase(s) or increased activity of $cPLA_2$,[13] rather than through increased $cPLA_2$ expression. Nevertheless, the augmented AA liberation, as observed after 12 hours of shear loading in the present study, may contribute to the enhanced second phase of PGI_2 production.

COX has both cyclooxygenase and peroxidase catalytic activity. COX utilizes AA as its substrate and catalyzes the conversion of AA to PGG_2 and further to PGH_2.[16] Numerous reports have indicated that COX plays a major regulatory role in prostanoid biosynthesis.[17–19] Two isoforms (COX-1 and COX-2) have been molecularly cloned and characterized biochemically.[20,21] COX-1 is considered a constitutive enzyme, which is expressed in most cell types. Recently, it was found that the expression of COX-1 is regulated by several growth factors and cytokines, such as TGF-β, interleukin-1α, and phorbol-12-myristate 13-acetate in ECs.[8,22,23] By DNA sequence analysis, we found that the sequence of shear stress–responsive elements (5′-GAGACC/GGTCTC-3′) is present in the promoter region of the COX-1 gene (–261 to –256 and –492 to –487).[24] Our results also clearly demonstrated that the expression of COX-1 at the levels of both transcription and translation in HUVECs was increased by shear stress. The second cyclooxygenase, COX-2, is undetectable in most mammalian cells.[25,26] COX-2 expression was also reported to be induced by cytokines and growth factors.[27] Using a differential display technique, Topper et al[9] recently reported that shear stress induced COX-2 mRNA expression. In the present study, COX-2 was not detected in HUVECs by Western blotting in the static control. After shear loading for 1 hour at 24 dyne/cm^2, the level of COX-2 mRNA was found to be elevated. Although it is unclear whether the increase in COX-1 and COX-2 mRNA levels is due to increased transcription or decreased degradation, the elevated mRNAs induced by shear stress resulted in increased COX expression at the protein level. The total increase of COX expression may also contribute to the prolonged second phase of PGI_2 production under shear stress.

The final enzyme involved in PGI_2 biosynthesis is PGIS, which catalyzes the conversion of PGH_2 to PGI_2.[28] PGIS is also reported to have the shear stress–responsive element sequence in the promoter region.[29] Our results showed that PGIS was significantly increased by shear stress at both the mRNA and protein levels. PGH_2 is also a substrate of thromboxane (TX) synthase, which produces TXA_2. Measurement of TXB_2, a metabolite of TXA_2, in the same samples revealed that a shear stress–induced increase in PGI_2 production was not accompanied by a concomitant increase in TXA_2 (data not shown). Therefore, shear stress appears to selectively augment PGI_2 production in HUVECs.

In summary, our results suggest that increases in the liberation of AA and the expression of COX and PGIS may contribute to the prolonged second phase of PGI_2 production in HUVECs under shear stress. The level of shear stress used in this study is within the physiological range of the arterial system (10 to 55 dyne/cm^2).[30] Therefore, we postulate that physiological shear stress stimulates PGI_2 production without affecting TXA_2 formation. Lowered shear stress, which has been observed at sites of atherosclerosis, may result in decreased PGI_2 production and may be implicated in acceleration of atherosclerosis formation in vivo.

Acknowledgments

We thank S. Lockyer and P. Randolph for reviewing the manuscript.

References

1. Ando J, Kamiya A. Blood flow and vascular endothelial cell function. *Front Med Biol Eng*. 1993;5:245–264.
2. Helmlinger G, Geiger RV, Schreck S, Nerem RM. Effects of pulsatile flow on cultured vascular endothelial cell morphology. *J Biomech Eng*. 1991;113:123–131.
3. Caro CG, Fitz-Gerald JM, Schroter RC. Atheroma and arterial wall shear: observation, correlation and proposal of a shear-dependent mass transfer mechanism for atherogenesis. *Proc Roy Soc Lond*. 1971;B177:109–159.
4. Friedman MH, Peters OJ, Bargeron CB, Hutchins GM, Mark FF. Shear-dependent thickening of the human arterial intima. *Atherosclerosis*. 1986; 60:161–171.
5. Noris M, Morigi M, Donadelli R, Aiello S, Foppolo M, Todeschini M, Orisio S, Remuzzi G, Remuzzi A. Nitric oxide synthesis by cultured endothelial cells is modulated by flow conditions. *Circ Res*. 1995;76: 536–543.
6. Okahara K, Kambayashi J, Ohnishi T, Fujiwara Y, Kawasaki T, Monden M. Shear stress induces expression of CNP gene in human endothelial cells. *FEBS Lett*. 1995;373:108–110.
7. Frangos JA, Eskin SG, McIntire LV, Ives CL. Flow effects on prostacyclin production by cultured human endothelial cells. *Science*. 1985; 227:1477–1479.
8. Jackson BA, Goldstein RH, Roy R, Cozzani M, Taylor L, Polgar P. Effects of transforming growth factor beta and interleukin-1 beta on expression of cyclooxygenase 1 and 2 and phospholipase A2 mRNA in lung fibroblasts and endothelial cells in culture. *Biochem Biophys Res Commun*. 1993;197:1465–1474.
9. Topper JN, Cai J, Falb D, Gimbrone MA Jr. Identification of vascular endothelial genes differentially responsive to fluid mechanical stimuli: cyclooxygenase-2, manganese superoxide dismutase, and endothelial cell nitric oxide synthase are selectively up-regulated by steady laminar shear stress. *Proc Natl Acad Sci U S A*. 1996;93:10417–10422.
10. Bussolari SR, Dewey CF, Gimbrone MA Jr. Apparatus for subjecting living cells to fluid shear stress. *Rev Sci Instrum*. 1982;53:1851–1854.
11. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem*. 1987;162:156–159.
12. Clark JD, Schievella AR, Nalefski EA, Lin LL. Cytosolic phospholipase A2. *J Lipid Mediat Cell Signal*. 1995;12:83–117.
13. Pearce MJ, McIntyre TM, Prescott SM, Zimmerman GA, Whatley RE. Shear stress activates cytosolic phospholipase A_2 ($cPLA_2$) and MAP kinase in human endothelial cells. *Biochem Biophys Res Commun*. 1996; 218:500–504.
14. James NL, Harrison DG, Nerem RM. Effects of shear on endothelial cell calcium in the presence and absence of ATP. *FASEB J*. 1995;9:968–973.
15. Nollert MU, Eskin SG, McIntire LV. Shear stress increases inositol trisphosphate levels in human endothelial cells. *Biochem Biophys Res Commun*. 1990;170:281–287.
16. Otto JC, Smith WL. Prostaglandin endoperoxide synthase-1 and -2. *J Lipid Mediat Cell Signal*. 1995;12:139–156.
17. Nursing RM, Klein T, Siegle R, Ullrich V. *Regulation of Prostanoid-Synthesis in the Cardiovascular System: Mediators in the Cardiovascular System*. Basel, Switzerland: Birkhauser Verlag; 1995:1–10.
18. Sanduja SK, Tsai AL, Matijevic-Aleksic N, Wu KK. Kinetics of prostacyclin synthesis in PGHS-1-overexpressed endothelial cells. *Am J Physiol*. 1994;267:C1459–C1466.
19. Xu XM, Ohashi K, Sanduja SK, Ruan KH, Wang LH, Wu KK. Enhanced prostacyclin synthesis in endothelial cells by retrovirus-mediated transfer of prostaglandin H synthase cDNA. *J Clin Invest*. 1993;91:1843–1849.
20. Hla T, Neilson K. Human cyclooxygenase-2 cDNA. *Proc Natl Acad Sci U S A*. 1992;89:7384–7388.

Downloaded from http://atvb.ahajournals.org/ by guest on August 29, 2017

21. Yokoyama C, Tanabe T. Cloning of human gene encoding prostaglandin endoperoxide synthase and primary structure of the enzyme. *Biochem Biophys Res Commun*. 1989;165:888–894.
22. Maier JA, Hla T, Maciag T. Cyclooxygenase is an immediate-early gene induced by interleukin-1 in human endothelial cells. *J Biol Chem*. 1990; 265:10805–10808.
23. Xu XM, Tang JL, Hajibeigi A, Loose-Mitchell DS, Wu KK. Transcriptional regulation of endothelial constitutive PGHS-1 expression by phorbol ester. *Am J Physiol*. 1996;270:C259–C264.
24. Wang LH, Hajibeigi A, Xu XM, Loose-Mitchell D, Wu KK. Characterization of the promoter of human prostaglandin H synthase-1 gene. *Biochem Biophys Res Commun*. 1993;190:406–411.
25. Sirois J, Simmons DL, Richards JS. Hormonal regulation of messenger ribonucleic acid encoding a novel isoform of prostaglandin endoperoxide H synthase in rat preovulatory follicles: induction in vivo and in vitro. *J Biol Chem*. 1992;267:11586–11592.
26. Evett GE, Xie W, Chipman JG, Robertson DL, Simmons DL. Prostaglandin G/H synthase isoenzyme 2 expression in fibroblasts: regulation by dexamethasone, mitogens, and oncogenes. *Arch Biochem Biophys*. 1993;306:169–177.
27. Belvisi MG, Saunders MA, Haddad el-B, Hirst SJ, Yacoub MH, Barnes PJ, Mitchell JA. Induction of cyclo-oxygenase-2 by cytokines in human cultured airway smooth muscle cells: novel inflammatory role of this cell type. *Br J Pharmacol*. 1997;120:910–916.
28. Needleman P, Turk J, Jakshik BA, Morrison AR, Lefkowith JB. Arachidonic acid metabolism. *Annu Rev Biochem*. 1986;55:69–102.
29. Yokohama C, Yabuki T, Inoue H, Tone Y, Hara S, Hatae T, Nagata M, Takahashi E, Tanabe T. Human gene encoding prostacyclin synthase (PTGIS): genomic organization, chromosomal localization, and promoter activity. *Genomics*. 1996;36:296–304.
30. Lipowsky HH. Shear stress in the circulation. In: Bevan JA, Kaley G, Rubanyi GM, eds. *Flow-Dependent Regulation of Vascular Function*. Oxford, UK: Oxford University Press; 1995:28–45.

Downloaded from http://atvb.ahajournals.org/ by guest on August 29, 2017

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

Downloaded from http://atvb.ahajournals.org/ by guest on August 29, 2017

Upregulation of Prostacyclin Synthesis–Related Gene Expression by Shear Stress in Vascular Endothelial Cells

Kazuhiro Okahara, Bing Sun and Jun-ichi Kambayashi

Arterioscler Thromb Vasc Biol. 1998;18:1922-1926
doi: 10.1161/01.ATV.18.12.1922
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/18/12/1922

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Arteriosclerosis, Thrombosis, and Vascular Biology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in thePermissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Arteriosclerosis, Thrombosis, and Vascular Biology* is online at:
http://atvb.ahajournals.org//subscriptions/