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# Differential effects of pulsatile versus steady flow on coronary endothelial membrane potential

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**Qiu, Wei-Ping, Qinghua Hu, Nazareno Paolocci, Roy C. Ziegelstein, and David A. Kass.** Differential effects of pulsatile versus steady flow on coronary endothelial membrane potential. *Am J Physiol Heart Circ Physiol* 285: H341–H346, 2003; 10.1152/ajpheart.01072.2002.—Steady shear stress stimulates transient hyperpolarization coupled to calcium-sensitive potassium ($K_{Ca}$) channels and sustained depolarization linked to chloride-selective channels. Physiological flow is pulsatile not static, and whereas in vivo data suggest phasic shear stress may preferentially activate $K_{Ca}$ channels, its differential effects on both currents remain largely unknown. To determine this interaction, coronary endothelial cells were cultured in glass capillary flow tubes, loaded with the voltage-sensitive dye bis-(1,3-dibutylbarbituric acid)trimethine oxonol, and exposed to constant or pulsatile shear stress. The latter was generated by a custom servoperfusion system employing physiological pressure and flow waveforms. Steady shear induced a sustained depolarization inhibited by the $Cl^-$ channel blocker DIDS. Even after exposure to steady flow, subsequent transition to pulsatile shear stress further stimulated DIDS-sensitive depolarization. DIDS pretreatment "unmasked" a pulsatile flow-induced hyperpolarization of which magnitude was further enhanced by nifedipine, which augments epoxygenase synthesis. Pulse-shear hyperpolarization was fully blocked by $K_{Ca}$ channel inhibition (charybdotoxin + apamin), although these agents had no influence on membrane potential altered by steady flow. Thus $K_{Ca}$-dependent hyperpolarization is preferentially stimulated by pulsatile over steady flow, whereas both can stimulate $Cl^-$-dependent depolarization. This supports studies showing greater potency of pulsatile flow for triggering $K_{Ca}$-dependent vasorelaxation.

calcium-sensitive potassium channels; steady shear stress; vasorelaxation; endothelium-derived hyperpolarizing factor

THE ENDOTHELIUM is the primary sensor for changes in shear stress. This signaling has been recently reviewed (8, 11, 17, 29) and involves activation of specific integrin-focal adhesion kinase cascades, G proteins, intracellular $Ca^{2+}$, pH, and tyrosine and serine-threonine kinases. Steady shear also induces intracellular hyperpolarization by stimulating apamin-charybdotoxin (AP-CbTX)-sensitive potassium channels (3, 6) and voltage-sensitive $K^+$ inward rectifying current (14). In addition, lower levels of steady shear activates a sustained depolarizing $Cl^-$-selective current (1) that can override membrane hyperpolarization.

Whereas the majority of studies have examined responses to steady shear, in vivo flow is generally pulsatile, and phasic versus constant shear stress can trigger very different and even opposite responses in endothelial signaling. This has been demonstrated for nitric oxide synthase and Akt activation (4, 24), endothelial intracellular pH ($pH_i$), mitogen-activated protein (MAP) kinase, cSRC tyrosine kinase activation (32), and G protein signaling (7). There are virtually no data to date assessing whether different voltage changes are also triggered in response to pulsatile versus static shear. Such differential signaling seems plausible, however, given that $pH_i$ can affect membrane potential change induced by carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (23).

The present study tested the hypothesis that pulsatile shear stress differentially modulates membrane potential. Effects of steady versus pulsatile flow on endothelial membrane potential were examined using a novel servoperfusion system that exposed endothelial cells cultured in microcapillary tubes to physiological pulsatile pressure and flow while simultaneously monitoring potential by the fluorescent dye bis-(1,3-dibutylbarbituric acid)trimethine oxonol ($DiBAC_4$). The results provide the first evidence of differential effects of pulsatile and steady flow on endothelial membrane potential, with preferential stimulation of $K_{Ca}$-dependent hyperpolarization by pulsatile versus steady shear stress.

## MATERIALS AND METHODS

*Cell culture.* Bovine coronary endothelial cells (BCECs, Clonetics; San Diego, CA) were grown to passage 4–8 in culture medium supplemented with 5% FBS, 10 μg/l human recombinant epidermal growth factor, 1 mg/l hydrocortisone, 50 mg/l gentamicin, 50 μg/l amphotericin-B, and 12 μg/l bovine brain extract (Clonetics) in 37°C humidified atmosphere of 95% air-5% $CO_2$. After exposure to 0.25% trypsin and 0.5 mM EDTA (Clonetics), cells were plated either in 1% gelatin (Sigma Chemical; St. Louis, MO)-coated 1 $mm^2 \times 5$ cm borosilicate glass capillary tubes (VitroCom; Mountain Lakes, NJ) or on 25-mm diameter circular glass coverslips. Cells were grown to confluence for ~48 h before the study.

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*Measurements of membrane potential.* Membrane potential was assessed using the fluorescent dye $DiBAC_4$ (Molecular Probes; Eugene, OR). Cells were washed and incubated in HEPES buffer (see *Reagents*) containing 0.5 μM of $DiBAC_4$ at 37°C 15–30 min. Preliminary studies were also performed in physiological buffer containing bicarbonate as previously described (32), although prior investigation found no difference in the $Cl^-$-mediated response between HEPES and $HCO_3^-$-containing buffer (1). Furthermore, whereas acidification with steady flow is buffer dependent, pulsatile shear-induced alkalinization is similar with either (32). Both buffers were gassed with 5% $CO_2$-95% $O_2$ to maintain physiological pH. Monolayers were mounted on the stage of a modified Nikon Diaphot inverted epifluorescence microscope. Fluorescence was excited with a 75-W xenon short-arc lamp (UXL-75, Ushio, Japan) at 480 ± 20 nm, and fluorescence was collected in real time at 535 ± 25 nm.

*Pulse pressure servopump system.* Pulsatile perfusion was generated by a custom servopump system as previously described (25, 32). Mean flow was generated by a nonpulsatile flow pump, and realistic pulse pressure and flow waveforms were superimposed by means of an electromagnetic linear motor (Applied Engineering) controlled by real-time digital feedback. The servosignal was a previously recorded aortic pressure wave that could be modified to yield desired pulse amplitude and mean. An inline flowmeter (1N, Transsonic) placed just upstream of the capillary tube recorded phasic flow. A downstream hydraulic resistor placed in the outflow line was used to set mean pressure for a given mean flow.

*Reagents.* DIDS, CbTX, AP, and epoxyeicosatrienoic acids (EETs) were obtained from Sigma Chemical. Nifedipine and bradykinin were obtained from Calbiochem. 1,3-Dihydro-1-[2-hydroxy-5-(trifluroromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one (NS-1619) was obtained from Sigma RBI. For most studies, the perfusion buffer contained (in mM) 150 NaCl, 4 KCl, 1 $CaCl_2$, 2 $MgCl_2$, and 10 HEPES (pH adjusted to 7.4 with NaOH). $Cl^-$-free HEPES contained (in mM) sodium aspartate 137, potassium aspartate 4.9, $MgSO_4$ 1.2, $NaH_2PO_4$ 1.2, D-glucose 15, HEPES 20, and calcium gluconate 1.5 (pH adjusted to 7.4 with NaOH). The perfusion buffer contained the same concentration of bisoxonol dye as that in which the cells were incubated during experiments to prevent concentration gradient-driven dye washout from the cells.

*Experimental protocols.* BCECs were first perfused using nonpulsatile steady low-level flow (< 0.1 ml/min) at a mean pressure of 90 mmHg with perfusion buffer. After several minutes to allow for stabilization of the fluorescence signal, flow was increased and maintained at 0.7 ml/min (1 dyn/cm$^2$) at the same pressure for at least 10 min or until fluorescence intensity stabilized. Perfusion was then switched (typically 500 s after onset of steady flow) to pulsatile flow (90 mmHg pulse pressure at the identical mean flow and mean pressure), and this condition was maintained for 15 min or more. In additional studies, the effect of transitioning from minimal flow directly to pressurized pulsatile perfusion was also examined to assess for any accommodation response to preexposure to steady flow.

*Statistical analysis.* Data are reported as means ± SE. Statistics comparisons were made using the Student's *t*-test for paired and unpaired groups.

## RESULTS

*Steady versus pulsatile shear stress effects on endothelial voltage.* Figure 1, *A–E*, displays example tracings for protocols in which endothelial cells were first exposed to steady shear stress and then abruptly switched to pulsatile shear at the identical mean pressure and flow rate. Summary results are provided in Fig. 1*E*. With the acute onset of steady shear, intracellular voltage ($V_i$) rose (i.e., a depolarization) reaching a steady state within several minutes (Fig. 1*A*, control HEPES buffer on *left*, physiological $HCO_3^-$ buffer on *right*). Once stabilized, this depolarization was maintained until shear stress was discontinued (data not shown), as previously reported (1). However, when steady shear stress was changed to pulsatile shear, there was subsequent further depolarization, which also quickly reached a plateau. The magnitude of depolarization triggered by initial exposure to steady shear stress was more than twice that observed following the transition to pulsatile shear stress (Fig. 1*E*). In separate studies, we initiated shear stress in the pulsatile mode, and the response was similar to that observed when pulsatile shear followed steady shear (data not shown). Results using physiological $HCO_3^-$-containing buffer were qualitatively similar to those with HEPES buffer, although the relative magnitude of steady versus pulsatile shear stress-induced depolarization was slightly larger with the $HCO_3^-$ buffer ($P < 0.05$).

Because a shear-activated $Cl^-$-selective depolarizing current was previously documented in endothelial cells subjected to flow, experiments were performed using the $Cl^-$ channel blocker DIDS (1 mM × 1 h; and same concentration added to perfusate during study). DIDS reduced the magnitude of the steady shear stress-induced depolarization by slightly >50% ($P < 0.001$) and converted the depolarization previously observed when changing to pulsatile shear stress to a hyperpolarization ($P < 0.05$; Fig. 1, *B* and *E*). These results suggest that pulsatile shear stress activates both a depolarizing $Cl^-$ current and a hyperpolarizing current, the net response being depolarization under control conditions. $Cl^-$ channel inhibition "unmasked" the pulsatile shear-induced hyperpolarizing membrane current.

Shear stress has been reported to stimulate endothelial-derived hyperpolarizing factors (EDHFs) (2), which may include cytochrome *P*-450-derived EETs (3, 6, 10). Studying EET signaling in vitro can be difficult because these signaling pathways are downregulated in cultured endothelial cells. To study the role of EET in pulsatile flow-induced hyperpolarization, endothelial cells were first preincubated for 24 h with 10 μM nifedipine because this treatment enhances endothelial cytochrome *P*-450-derived EET production in endothelial cells(9). Nifedipine pretreatment had no influence on the depolarizing response to steady shear stress, but the subsequent pulsatile shear-induced depolarization was blocked, converting to a hyperpolarization ($P = 0.058$; Fig. 1, *C* and *E*). Finally, exposure to both nifedipine pretreatment and DIDS (Fig. 1*D*) yielded steady shear depolarization similar to DIDS alone but more consistent hyperpolarization with pulsatile shear stress ($P = 0.0001$, Fig. 1*E*). Similar results were obtained by adding nifedipine to $Cl^-$-free

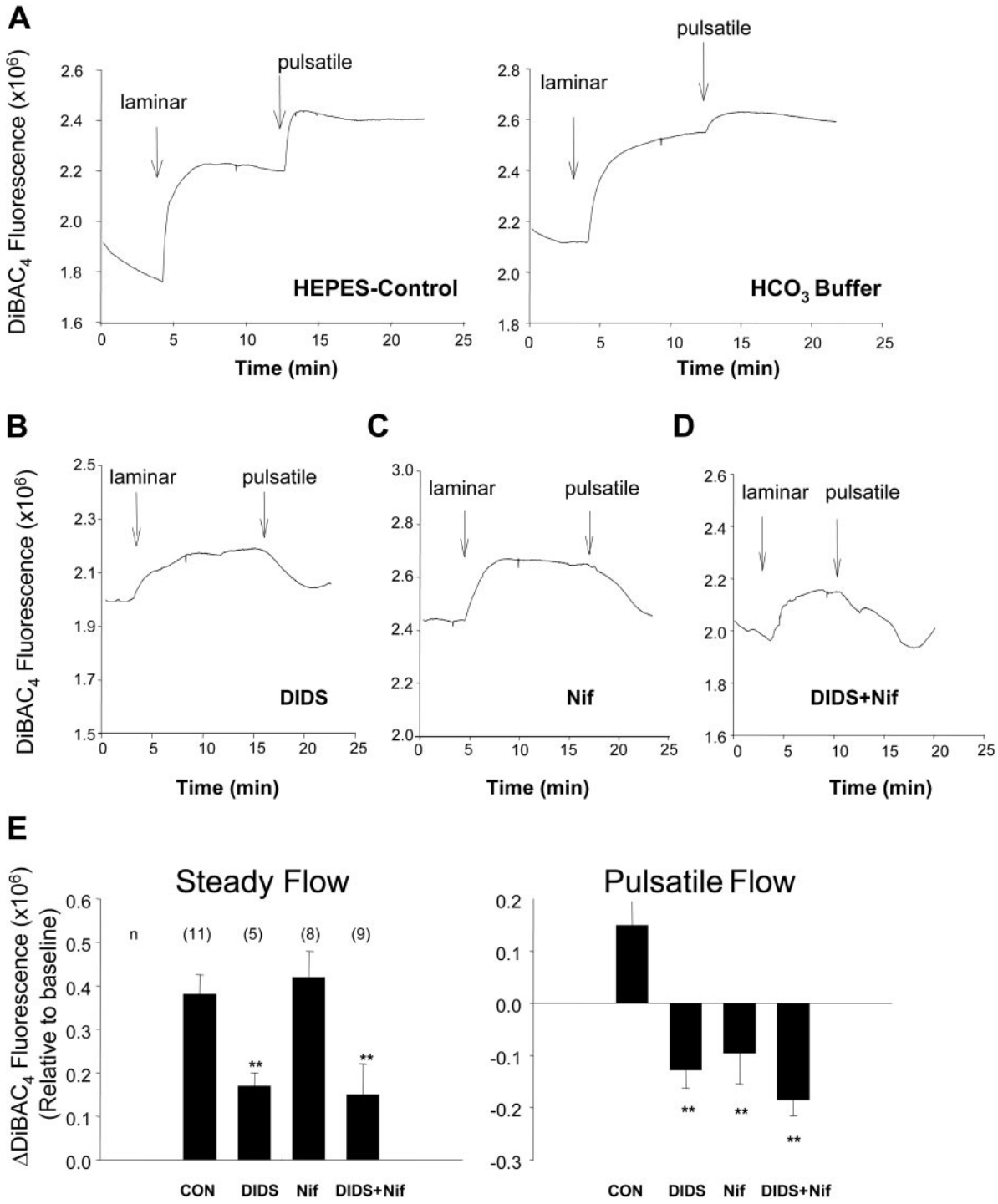


Fig. 1. Response of intracellular voltage measured by bis-(1,3-dibutylbarbituric acid)trimethine oxonol ($DiBAC_4$) fluorescence to steady versus pulsatile shear stress. *A*: response to steady flow pressure followed by a transition to pulsatile perfusion. Both stimuli induced a rapid depolarization with quick stabilization. Magnitude of the response was approximately twice as great with steady flow compared with pulsatile perfusion. *Left*, studies performed using HEPES buffer; *right*, physiological buffer with $HCO_3^-$. *B*: inhibition of steady shear-induced depolarization and reversal (hyperpolarization) with pulse shear by $Cl^-$-channel blocker DIDS. *C*: pretreatment with nifedipine (Nif) to enhance epoxyeicosatrienoic acid (EET) signaling had no effect on the initial steady shear depolarization but abolished pulse-shear depolarization, converting to a hyperpolarization. *D*: cell pretreated with Nif + DIDS showed reduced both steady shear response and hyperpolarization during pulsatile flow. *E*: summary of results, given as means ± SE, with peak (plateau) amplitude change relative to baseline shown for each condition. $^{**}P < 0.001$ vs. control response. All individual voltage changes were significant ($P < 0.01$) with the exception of Nif in pulsatile flow studies, which reached borderline significance ($P = 0.058$).

medium (data not shown). These data are consistent with a differential effect of pulsatile versus steady flow on endothelial membrane potential, with a $Cl^-$-dependent depolarizing current triggered by both stimuli but $Cl^-$-independent hyperpolarizing current more tightly linked to pulsatile shear.

*$K_{Ca}$ activation by pulsatile versus steady perfusion.* To test whether hyperpolarization with pulse-perfusion in the presence of nifedipine pretreatment and DIDS was due to $K_{Ca}$ activation, studies were performed with pharmacological inhibitors of these channels. Bradykinin (1 μM) and the $K_{Ca}$ agonist NS-1619 (30 μM) were first used to show that, in the absence of a shear stress stimulus, agonists known to hyperpolarize via $K_{Ca}$ stimulation (12) do so in this model (Fig. 2, *A* and *B*). Both agents induced hyperpolarization stabilizing over several minutes. Whereas this response was considerably slower than that reported with direct electrophyisiological recordings (5, 12), it was similar to prior data with $DiBAC_4$ (20). Hyperpolarization was also confirmed in response to $EET_{13,14}$ ($-0.2 \pm 0.04$ unit change, $P < 0.02$, data not shown). Preincubation with CbTX (100 nM) and AP (200 nM) inhibited bradykinin hyperpolarization by >50% (not shown).

Studies were next performed to test the relevance of CbTX + AP inhibition of $K_{Ca}$ currents on altered intracellular voltage induced by steady versus pulsatile shear stress. For these studies, endothelial cells were pretreated with nifedipine and DIDS to unmask hyperpolarization. Rapid depolarization induced by steady shear stress was unaltered by $K_{Ca}$ channel blockade. However, hyperpolarization induced by pulsatile shear stress was fully blocked by CbTX + AP (Fig. 2, *C* and *D*).

## DISCUSSION

The present study is the first to demonstrate differential responses of endothelial cell voltage to steady versus pulsatile shear stress. As previously reported (1), steady flow triggered a DIDS-sensitive depolarizing current thought due to $Cl^-$, and this current was further activated by a transition to pulsatile flow. However, the latter response was smaller and appeared to reflect coactivation of an AP-CbTX-sensitive hyperpolarizing current, consistent with $K_{Ca}$ stimulation. Hyperpolarizing responses were enhanced by nifedpine pretreatment, which upregulates epoxygenase synthase. These results suggest different channel activation by steady versus pulsatile shear stress and support in vivo findings showing a greater influence of EDHF-$K_{Ca}$-dependent vasodilation by augmented pulsatile perfusion (22).

Several methodological issues related to the perfusion system and fluorescent probe used to assess voltage should be noted. As pressurization was required to generate phasic shear stress [pressure was servocontrolled (25)], patch-clamp analysis was precluded. Al-

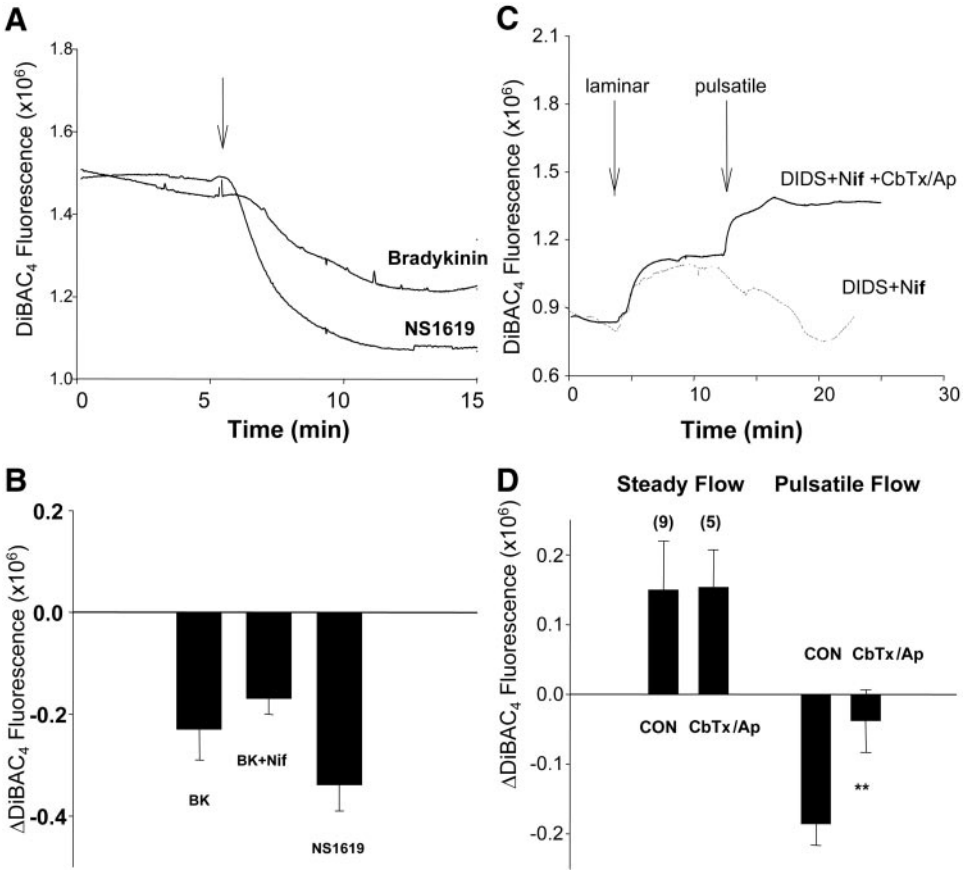


Fig. 2. Role of potassium-sensitive $Ca^{2+}$ ($K_{Ca}$) activation. *A*: rapid hyperpolarization of nonshear-stimulated cells exposed to bradykinin (BK) or to the $K_{Ca}$ agonist NS-1619. Magnitude of change as indexed by $DIBAC_4$ fluorescence was similar to that with pulsatile shear in cells cotreated with DIDS and Nif. *B*: summary results for hyperpolarization induced by BK (1 μM) and NS-1619 (30 μM). *C*: response to steady and pulsatile perfusion in cells pretreated with DIDS and Nif and then exposed to the $K_{Ca}$ blockade charybdotoxin (CbTX) + apamin (AP). Steady shear response was unaltered, whereas hyperpolarization induced by pulsatile perfusion was essentially inhibited. *D*: summary data for $K_{Ca}$ blocker studies. $^{**}P < 0.001$ vs. control.

though $DiBAC_4$ fluorescence provided an alternative, its kinetics were slower than those measured by direct electrical recordings [e.g., bradykinin response (5, 12)] reflecting both dye properties (20) and mixing in our flow system. However, the behavior of the signal in the presence of $K_{Ca}$ antagonists supported its indexing similar membrane currents.

In addition to slower kinetics, higher shear stress cannot be studied with $DiBAC_4$ because this generates an immediate artifactual signal due to nonspecific interaction of the dye with flow (1). We also confirmed this in our system using preincubated cells killed by exposure to hypoosmotic solution. Whereas there was no signal change with bradykinin in such cells, rapid shear stress-induced depolarization was observed at $>5$ dyn/cm$^2$. At the mean shear stresses used in the present study (1 dyn/cm$^2$) there was no such artifact.

The depolarizing response to steady shear stress observed in the present study is similar in time course and amplitude to that reported by Barakat et al. (1). We further provide novel evidence that cells already flow adapted to steady shear stress exhibit further depolarization by a DIDS-sensitive current. In contrast to the prior study, we did not observe an early rapid hyperpolarization on shear initiation and were unable to fully inhibit the depolarizing current with DIDS or diphenylamine carboxylate. A potential cause for this difference was the use of low-passage bovine coronary endothelial cells in the present work compared with late passage (15–25) bovine aortic endothelial cells in the earlier study.

Unlike the depolarizing $Cl^-$ current that was activated by both steady and pulsatile shear, stimulation of a $K_{Ca}$ channel-dependent hyperpolarizing current appeared to only be present with pulsatile shear. Whereas AP + CbTX blocked hyperpolarization induced by bradykinin or NS-1619 as well as pulsatile shear stress (12, 30), blockade of small and intermediate $K_{Ca}$ channels had no effect on voltage responses to steady shear stress. This means that either the magnitude of depolarizing current with steady shear was so large that $K_{Ca}$ activation could not overcome it or stimulation of $K_{Ca}$ channels was favored during pulsatile perfusion in our model system. Evidence favoring the latter interpretation stems from data showing that amplification of EDHF signaling by nifedipine did not alter voltage responses to steady shear stress, but rather only to pulsatile shear stress, where depolarization was converted to hyperpolarization. Furthermore, the effect of DIDS on the depolarizing current was similar with both steady and pulsatile shear stress, yet its net impact was more marked with the latter, supporting coexistence of a more prominent hyperpolarizing current. It is possible that at higher shear stresses, the hyperpolarizing current would be of greater magnitude, although the current data may well be relevant to conditions of lower mean shear such as bifurcation points in vivo.

Although this is the first study to examine the effects of pulsatile versus steady flow on endothelial membrane potential, several previous studies have shown that the vascular endothelium can distinguish between the types and rates of change of shear stress. Differential signaling responses during pulsatile versus steady flow have been described for intracellular $Ca^{2+}$ (13, 15, 28), $pH_i$, tyrosine kinase and MAP kinase signaling (32), and cytoskeletal organization and orientation (16). We previously showed that pulsatile shear induces intracellular alkalinization (32), whereas steady shear stimulates a decrease in $pH_i$ (31, 33). This is intriguing because in smooth muscle cells, intracellular alkalinization enhances single large $K_{Ca}$ channel activity and current amplitude, whereas acidification has the opposite effect (19, 27).

The mechanisms by which endothelial cells differentiate between phasic versus static shear stress remain to be elucidated. Whereas much work has evolved regarding specific integrins and kinase activation cascades stimulated by static shear stress (18, 21, 26), little is known about how these same processes are modulated by phasic shear stress. The question is clearly relevant as most in vivo vascular biology occurs in the setting of phasic stress that can have potent effects on cell signaling and cytoprotection (24). Experimental systems such as that employed in this study and others to provide realistic pulsatile stimuli under highly controlled conditions should help provide these needed insights.

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## REFERENCES


1. **Barakat AI, Leaver EV, Pappone PA, and Davies PF.** A flow-activated chloride-selective membrane current in vascular endothelial cells. *Circ Res* 85: 820–828, 1999.
2. **Brandes RP, Schmitz-Winnenthal FH, Feletou M, Godecke A, Huang PL, Vanhoutte PM, Fleming I, and Busse R.** An endothelium-derived hyperpolarizing factor distinct from NO and prostacyclin is a major endothelium-dependent vasodilator in resistance vessels of wild-type and endothelial NO synthase knockout mice. *Proc Natl Acad Sci USA* 97: 9747–9752, 2000.
3. **Busse R, Edwards G, Feletou M, Fleming I, Vanhoutte PM, and Weston AH.** EDHF: bringing the concepts together. *Trends Pharmacol Sci* 23: 374–380, 2002.
4. **Busse R and Fleming I.** Pulsatile stretch and shear stress: physical stimuli determining the production of endothelium-derived relaxing factors. *J Vasc Res* 35: 73–84, 1998.
5. **Bychkov R, Burnham MP, Richards GR, Edwards G, Weston AH, Feletou M, and Vanhoutte PM.** Characterization of a charybdotoxin-sensitive intermediate conductance $Ca^{2+}$-activated K channel in porcine coronary endothelium: relevance to EDHF. *Br J Pharmacol* 137: 1346–1354, 2002.
6. **Campbell WB and Gauthier KM.** What is new in endothelium-derived hyperpolarizing factors? *Curr Opin Nephrol Hypertens* 11: 177–183, 2002.
7. **Cullen JP, Sayeed S, Sawai RS, Theodorakis NG, Cahill PA, Sitzmann JV, and Redmond EM.** Pulsatile flow-induced angiogenesis: role of G(i) subunits. *Arterioscler Thromb Vasc Biol* 22: 1610–1616, 2002.
8. **Fisher AB, Chien S, Barakat AI, and Nerem RM.** Endothelial cellular response to altered shear stress. *Am J Physiol Lung Cell Mol Physiol* 281: L529–L533, 2001.
9. **Fisslthaler B, Hinsch N, Chataigneau T, Popp R, Kiss L, Busse R, and Fleming I.** Nifedipine increases cytochrome P4502C expression and endothelium-derived hyperpolarizing factor-mediated responses in coronary arteries. *Hypertension* 36: 270–275, 2000.

10. **Fisslthaler B, Popp R, Kiss L, Potente M, Harder DR, Fleming I, and Busse R.** Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature* 401: 493–497, 1999.
11. **Fleming I and Busse R.** Molecular mechanisms involved in the regulation of the endothelial nitric oxide synthase. *Am J Physiol Regul Integr Comp Physiol* 284: R1–R12, 2003.
12. **Frieden M, Sollini M, and Beny J.** Substance P and bradykinin activate different types of KCa currents to hyperpolarize cultured porcine coronary artery endothelial cells. *J Physiol* 519: 361–371, 1999.
13. **Helmlinger G, Berk BC, and Nerem RM.** Calcium responses of endothelial cell monolayers subjected to pulsatile and steady laminar flow differ. *Am J Physiol Cell Physiol* 269: C367–C375, 1995.
14. **Hoger JH, Ilyin VI, Forsyth S, and Hoger A.** Shear stress regulates the endothelial Kir2.1 ion channel. *Proc Natl Acad Sci USA* 99: 7780–7785, 2002.
15. **Howard AB, Alexander RW, Nerem RM, Griendling KK, and Taylor WR.** Cyclic strain induces an oxidative stress in endothelial cells. *Am J Physiol Cell Physiol* 272: C421–C427, 1997.
16. **Hsiai TK, Cho SK, Honda HM, Hama S, Navab M, Demer LL, and Ho CM.** Endothelial cell dynamics under pulsating flows: significance of high versus low shear stress slew rates (d$\tau$/dt). *Ann Biomed Eng* 30: 646–656, 2002.
17. **Labrador V, Chen KD, Li YS, Muller S, Stoltz JF, and Chien S.** Interactions of mechanotransduction pathways. *Biorheology* 40: 47–52, 2003.
18. **Li S, Kim M, Hu YL, Jalali S, Schlaepfer DD, Hunter T, Chien S, and Shyy JY.** Fluid shear stress activation of focal adhesion kinase. Linking to mitogen-activated protein kinases. *J Biol Chem* 272: 30455–30462, 1997.
19. **Mori K, Nakaya Y, Sakamoto S, Hayabuchi Y, Matsuoka S, and Kuroda Y.** Lactate-induced vascular relaxation in porcine coronary arteries is mediated by $Ca^{2+}$-activated $K^+$ channels. *J Mol Cell Cardiol* 30: 349–356, 1998.
20. **Nakache M and Gaub HE.** Hydrodynamic hyperpolarization of endothelial cells. *Proc Natl Acad Sci USA* 85: 1841–1843, 1988.
21. **Okuda M, Takahashi M, Suero J, Murry CE, Traub O, Kawakatsu H, and Berk BC.** Shear stress stimulation of p130(cas) tyrosine phosphorylation requires calcium-dependent c-Src activation. *J Biol Chem* 274: 26803–26809, 1999.
22. **Paolocci N, Pagliaro P, Isoda T, Saavedra FW, and Kass DA.** Role of calcium-sensitive $K^+$ channels and nitric oxide in in vivo coronary vasodilation from enhanced perfusion pulsatility. *Circulation* 103: 119–124, 2001.
23. **Park KS, Jo I, Pak K, Bae SW, Rhim H, Suh SH, Park J, Zhu H, So I, and Kim KW.** FCCP depolarizes plasma membrane potential by activating proton and $Na^+$ currents in bovine aortic endothelial cells. *Pflügers Arch* 443: 344–352, 2002.
24. **Peng X, Haldar S, Deshpande S, Irani K, and Kass DA.** Wall stiffness suppresses Akt/eNOS and cytoprotection in pulse-perfused endothelium. *Hypertension* 41: 378–381, 2003.
25. **Peng X, Recchia FA, Byrne BJ, Wittstein IS, Ziegelstein RC, and Kass DA.** In vitro system to study realistic pulsatile flow and stretch signaling in cultured vascular cells. *Am J Physiol Cell Physiol* 279: C797–C805, 2000.
26. **Rizzo V, Sung A, Oh P, and Schnitzer JE.** Rapid mechanotransduction in situ at the luminal cell surface of vascular endothelium and its caveolae. *J Biol Chem* 273: 26323–26329, 1998.
27. **Schubert R, Krien U, and Gagov H.** Protons inhibit the BK(Ca) channel of rat small artery smooth muscle cells. *J Vasc Res* 38: 30–38, 2001.
28. **Shen J, Luscinskas FW, Connolly A, Dewey CF Jr, and Gimbrone MA Jr.** Fluid shear stress modulates cytosolic free calcium in vascular endothelial cells. *Am J Physiol Cell Physiol* 262: C384–C390, 1992.
29. **Shyy JY and Chien S.** Role of integrins in endothelial mechanosensing of shear stress. *Circ Res* 91: 769–775, 2002.
30. **Sollini M, Frieden M, and Beny JL.** Charybdotoxin-sensitive small conductance K(Ca) channel activated by bradykinin and substance P in endothelial cells. *Br J Pharmacol* 136: 1201–1209, 2002.
31. **Wittstein IS, Kass DA, Pak PH, Maughan WL, Fetics B, and Hare JM.** Cardiac nitric oxide production due to angiotensin-converting enzyme inhibition decreases beta-adrenergic myocardial contractility in patients with dilated cardiomyopathy. *J Am Coll Cardiol* 38: 429–435, 2001.
32. **Wittstein IS, Qiu W, Ziegelstein RC, Hu Q, and Kass DA.** Opposite effects of pressurized steady versus pulsatile perfusion on vascular endothelial cell cytosolic pH: role of tyrosine kinase and mitogen-activated protein kinase signaling. *Circ Res* 86: 1230–1236, 2000.
33. **Ziegelstein RC, Cheng L, and Capogrossi MC.** Flow-dependent cytosolic acidification of vascular endothelial cells. *Science* 258: 656–659, 1992.