Shear stress-induced up-regulation of the intermediate-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} channel in human endothelium

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Received 8 April 2003; received in revised form 27 August 2003; accepted 9 September 2003

Time for primary review 29 days

Abstract

Objective: Wall shear stress associated with blood flow is a major stimuli for generation of endothelial vasodilating and antithrombotic factors and it also regulates endothelial gene expression. Activation of endothelial intermediate-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels (IK\textsubscript{Ca}) is important for the control of endothelial function by inducing cell hyperpolarization and thus generation of the endothelium-derived hyperpolarizing factor. In the present study we tested whether the IK\textsubscript{Ca} encoding IK\textsubscript{Ca}1 gene is regulated by laminar shear stress (LSS).

Methods: Human umbilical vein endothelial cells (HUVEC) were subjected to LSS with a magnitude of 0.5–15 dyn/cm\textsuperscript{2} and time intervals of 2–24 h in a flow cone apparatus. Expression of the IK\textsubscript{Ca}1 gene and IK\textsubscript{Ca}-functions were determined by using real time RT-PCR and patch-clamp techniques.

Results: A short 2–4 h—or long 24 h—exposure to a LSS with a low (venous) magnitude of 0.5 dyn/cm\textsuperscript{2} had no effect on IK\textsubscript{Ca}1 expression levels. An exposure for 2 and 4 h to LSS with an intermediate magnitude of 5 dyn/cm\textsuperscript{2} was also ineffective, whereas an exposure for 24 h induced a significant threefold up-regulation of IK\textsubscript{Ca}1 expression levels. An exposure to LSS with a higher (arterial) magnitude of 15 dyn/cm\textsuperscript{2}, resulted in an eightfold up-regulation of IK\textsubscript{Ca}1 expression levels after a 4 h—exposure and a fourfold increase of IK\textsubscript{Ca}1 expression levels at 24 h. The increased IK\textsubscript{Ca}1 expression levels following exposure to high levels of LSS resulted in enhanced IK\textsubscript{Ca} whole-cell currents and in an increased hyperpolarization of the endothelium in response to ATP and the IK\textsubscript{Ca} opener 1-EBIO. Inhibition of the mitogen-activated protein kinase/extracellular-signal-regulated kinase (ERK) kinase 1/2 (MEK/ERK) pathway by PD98059 prevented the LSS-induced up-regulation of IK\textsubscript{Ca}1 expression levels and IK\textsubscript{Ca} whole-cell currents indicating that augmentation of IK\textsubscript{Ca}1 expression levels is mediated by the LSS-induced activation of the MEK/ERK pathway.

Conclusion: Long term exposure to LSS up-regulates expression and function of endothelial IK\textsubscript{Ca}. This increase might represent a new important mechanism in endothelial adaptation to altered hemodynamics.

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Keywords: Blood flow; Endothelial function; Signal transduction; Gene expression; IK\textsubscript{Ca}1; K-channel

This article is referred to in the Editorial by E. van Bavel (pages 457–459) in this issue.

1. Introduction

In vivo, the vascular endothelium is constantly exposed to wall shear stress generated by the streaming blood.

Alterations in shear stress levels lead to complex changes in endothelial functions [1,2]. Early responses to elevated shear stress include activation of ion channels, cell hyperpolarization, elevation of the intracellular Ca\textsuperscript{2+} concentration, and protein phosphorylation stimulating the synthesis of vasodilatory factors such as NO, EDHF, and prostacyclin [2,3]. Later responses include endothelial remodeling and modulation of gene expression levels such as of endothelial nitric oxide synthase (eNOS), prostacyclin, cyclooxygenase, connexins, and adhesion molecules [1,4–6].

This shear stress-dependent modulation of gene expression was related to long term vessel adaptation and vaso-
regulation especially in disease states like hypertension [1]. Moreover, shear stress induced modulation of gene expression levels is regarded as an atheroprotective mechanism by increasing the generation of antithrombotic and antiinflammatory factors [8].

With respect to mechanisms by which the endothelium senses alterations in shear stress levels, opening of mechanosensitive cation channels and Ca\(^{2+}\)-activated or inwardly rectifying K\(^+\)-channels have been proposed to play an important role by inducing mechanosensitive Ca\(^{2+}\)-entry and membrane hyperpolarization [9–13], respectively, as part of the very rapid responses to elevated shear stress.

In particular, intermediate-conductance Ca\(^{2+}\)-activated K\(^+\) channels (IK\(_{Ca}\)) encoded by the IK\(_{Ca1}\) gene [14–19] have been reported to induce endothelial hyperpolarization within seconds following shear stress stimulation [11,12]. However, whether also a modulation of IK\(_{Ca1}\) expression occurs, maybe as part of the adaptive response of the endothelium to altered hemodynamics, is still elusive.

Therefore, we tested whether shear stress regulates endothelial IK\(_{Ca1}\) gene expression in human endothelium. The results show that shear stress stimulation leads to an up-regulation of IK\(_{Ca1}\) expression levels and an increase of IK\(_{Ca}\) functions in human umbilical vein endothelium.

2. Materials and methods

2.1. Cell culture and shear stress experiments

Human umbilical vein endothelial cells (HUVEC) were isolated and cultured as described previously [20]. The investigation conformed with the principles outlined in the Declaration of Helsinki (Cardiovasc. Res. 1997;35:2–3). For shear stress (LSS) experiments, HUVEC of second passage were used to avoid senescence. After reaching confluence in fibronectin-coated petri dishes, HUVEC were passaged for 2, 4 and 24 h in a cone-plate viscosimeter as described previously [20]. For controls, each LSS experiment was performed at room temperature. Data analysis was performed as described previously [17]. Series resistance (4–10 M\(\Omega\)) was not compensated and symmetric background leak currents were subtracted manually.

2.2. Patch-clamp experiments

Patch-clamp experiments were carried out as described previously [14]. Patch pipettes were pulled from borosilicate glass capillaries with 0.3 mm wall thickness and had a tip resistance of 4 M\(\Omega\) in symmetric KCl solution. To induce complete electrical uncoupling, cells were pretreated for 5 min with 1 mM heptanol, a gap-junction inhibitor. Membrane currents in the electrically uncoupled single cells (22 ± 2 pF, \(n = 29\)) were recorded with an EPC-9 patch-clamp amplifier (HEKA) using voltage ramps (duration 1000 ms) from –120 to 100 mV and were low-pass filtered (– 3 dB, 1000 Hz) at a sampling time of 0.5 ms [17]. In another set of current-clamp experiments, cells were not electrically uncoupled and membrane hyperpolarization in response to ATP (1 \(\mu\)M), and 1-ethyl-2-benzimidazolinone (1-EBIO, 100 \(\mu\)M) were measured.

For activation of IK\(_{Ca}\) currents in whole-cell patch-clamp experiments, cells were dialyzed with a pipette solution containing (mM): KCl 135; MgCl\(_2\) 1, ETGA 1, CaCl\(_2\) 0.955 ([Ca\(^{2+}\)]\(_{\text{free}}\) = 3 \(\mu\)M), and HEPES 5 (pH 7.2). The NaCl bath solution contained (mM): NaCl 137, Na\(_2\)HPO\(_4\) 4.5, KCl 3, KH\(_2\)PO\(_4\) 1.5, MgCl\(_2\) 0.4, and CaCl\(_2\) 0.7 (pH 7.4). For current-clamp experiments, the pipette solution was prepared without CaCl\(_2\) to avoid activation of IK\(_{Ca}\) by Ca\(^{2+}\) dialysis in the patch-clamped cell. All experiments were performed at room temperature.

2.3. RNA isolation and quantitative realtime RT-PCR

Cells were harvested by cell scraping and RNA was isolated and purified using TRIZol (Life Technologies, Eggenstein, Germany), following the manufacturer’s instructions. RNA (2 \(\mu\)g) was reverse transcribed using random hexamers (Boehringer, Mannheim, Germany) and M-MLV reverse transcriptase (Life Technologies) in a 50-\(\mu\)l reaction. Expression was quantified with an ABI Prism 7700 Sequence detection system (Perkin-Elmer Applied Biosystems). Primer pairs and internal oligos for IK\(_{Ca1}\), eNOS, intercellular adhesion molecule 1 (ICAM1), von Willebrand factor (vWF), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Table 1) were positioned in the coding region and primer pairs spanned intronic sequences. Internal oligonucleotides (Biotez, Berlin, Germany) were labeled with 6-carboxy-fluorescein (FAM) on the 3’ end and 6-carboxytetramethylrhodamine (TAMRA) on the 5’ end. Identity of PCR products was verified by sequencing and linearity of each PCR assay were confirmed by serial dilutions of cDNA.

Each 25 \(\mu\)l PCR reaction consisted of 500 nM forward primer, 500 nM reverse primer, 150 nM probe, 3 \(\mu\)l cDNA, and 1 × (final concentration) TaqMan Universal Master Mix (Perkin-Elmer Applied Biosystems). PCR parameters were 50 °C × 2 min, 95 °C × 10 min, and 50 cycles at 95 °C × 15 s, 60 °C × 1 min.

The TaqMan® software was employed to calculate a threshold cycle (CT) which is defined as the cycle at which the reporter fluorescence is distinguishable from the background in the extension phase of the PCR reaction (ABI User Bulletin #2). Real-time RT-PCR signals for
IKCa1, eNOS, ICAM1, and vWF were standardized to GAPDH by using the equation: 
\[ C_tX - C_{GAPDH} = \Delta C_t \]
where \( C_tX \) is the value for the IKCa1, eNOS, ICAM1, or vWF probe, and \( C_{GAPDH} \) is the value calculated for GAPDH. The equation,
\[ \Delta C_{static} - \Delta C_{shear stress} = \Delta \Delta C_t \]
was used to determine changes in expression levels of the respective gene following LSS stimulation. Fold increase in expression was calculated by the equation,
\[ 2^{\Delta \Delta C_t} = \text{fold change in expression (ABI User Bulletin #2)} \]

2.4. Reagents
PD98059 and SB203580 were obtained from TOCRIS (Ballwin, MO). TRAM-34 (1-[(2-chlorophenyl)diphenylmethyl]-1H-pyrazole) was a kind gift of Dr. H. Wulff (UCI, CA). All other chemicals and toxins were obtained from Sigma (Deisenhofen, Germany).

2.5. Statistical analysis
Data are given as mean ± SEM. Student’s t-test was used to assess differences between groups. \( P \)-values of < 0.05 were considered significant.

### Table 1
Primer pairs and internal oligos

<table>
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<th>Gene</th>
<th>Accession no.</th>
<th>Primer pairs</th>
<th>TaqMan probe</th>
</tr>
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<tbody>
<tr>
<td>IKCa1</td>
<td>AF022797</td>
<td>F 5'-CATCACATTTCTGACCATCG-3' R 5'-ACGTGGTTCTGCTTTGTT-3'</td>
<td>5'-FAM-TGG TGA CGT GGT GCC GG C-TAMRA-3'</td>
</tr>
<tr>
<td>eNOS</td>
<td>L26914</td>
<td>F 5'-CGGATCACCTAGGGAAGAAAGA-3' R 5'-CATAGGCCCCGAGAGAT-3'</td>
<td>5'-FAM-TTTAAAGAAGTGGCCAACCC-3'</td>
</tr>
<tr>
<td>vWF</td>
<td>K03028</td>
<td>F 5'-TGAGAGGAGGAGCTACAGG-3' R 5'-CATAATTTTACACTCACCAGGCGA-3'</td>
<td>5'-FAM-TGCCCACCTTTTGAAGACACAAAGTG-TAMRA-3'</td>
</tr>
<tr>
<td>GAPDH</td>
<td>BC 013310</td>
<td>F 5'-ACCGTCAAGGCGAGACG-3' R 5'-GCCCCACTTATTTGGGAGG-3'</td>
<td>5'-FAM-CCCACATCATCTCAGGGCGA-TAMRA-3'</td>
</tr>
<tr>
<td>ICAM1</td>
<td>XM049516</td>
<td>F 5'-AAAGACCAAGACCCCGGAG-3' R 5'-TTCCGGGACAATCCCTTCT-3'</td>
<td>5'-FAM-CGTGCTCTGTATGGCCGCGACT-TAMRA-3'</td>
</tr>
</tbody>
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3. Results
3.1. Up-regulation of endothelial IKCa1 expression levels by LSS
To evaluate the regulation of endothelial IKCa1 expression by LSS, we quantified IKCa1 expression in HUVECs after different time intervals and magnitude of LSS exposure and in an equal number of static controls accompanying each LSS experiment. A LSS exposure for 2, 4 or 24 h with a magnitude of 0.5 dyn/cm² (\( n = 3 \)) as reported.

Fig. 2. LSS increases IKCa functions in human umbilical vein endothelial cells. (A) Representative whole-cell recordings of HUVEC exposed for 24 h to LSS with a magnitude of 15 dyn/cm² and of HUVEC kept under static conditions. IKCa currents were activated by dialysis of the cells with 3 \( \mu \)M Ca²⁺ (B) Quantitative analysis of KCa current densities (pA/pF) at a holding potential of 0 V in HUVEC subjected to LSS with a magnitude of 15 dyn/cm² for 4 h (cells, \( n = 9 \)), 6 h (cells, \( n = 12 \)), and 24 h (cells, \( n = 13 \)) compared to controls kept in stationary culture (cells, \( n = 21 \)). Data are given as mean ± SEM. *\( P < 0.01 \) vs. static controls.

Fig. 1. Shear stress-induced up-regulation of endothelial IKCa1 gene expression levels. Quantitative real time RT-PCR analysis of IKCa1 gene expression levels in human umbilical vein endothelial cells after exposure to LSS of 0.5, 5 or 15 dyn/cm² for the indicated time intervals. \( \Delta \Delta C_t = \Delta C_{static} - \Delta C_{shear stress} \) (left axis). Data are given as mean ± SEM; *\( P < 0.05 \), \( n = 3–8 \).
to be present in veins [21] had no effects on $IKCa_1$ expression levels (Fig. 1). Also an exposure for 2 and 4 h to a LSS with an intermediate magnitude of 5 dyn/cm$^2$ ($n=5$ each) did not induce considerable changes of $IKCa_1$ expression levels. However, at this higher magnitude of LSS, an exposure for 24 h ($n=6$) resulted in a threefold increase in $IKCa_1$ expression levels (Fig. 1).

At a LSS with a higher magnitude of 15 dyn/cm$^2$ as reported to be present in arteries [21], the time course of modulation of $IKCa_1$ expression levels was different (Fig. 1). A steep eightfold increase in $IKCa_1$ expression was already observed after an exposure interval of 4 h ($n=5$), but with no significant increase at 2 h ($n=7$). A longer exposure for 24 h induced a fourfold increase in $IKCa_1$ expression ($n=8$), similar to the increase in channel expression levels detected after an exposure for 24 h to a LSS with a magnitude of 5 dyn/cm$^2$.

To relate this up-regulation of $IKCa_1$ expression levels to previously reported shear stress-sensitive or -insensitive endothelial cell-specific gene regulation, we determined expression of eNOS and ICAM1 as shear stress-sensitive genes [5,7] and of vWF as a shear stress-insensitive gene [22]. Similar to earlier reports on the regulation of eNOS expression by LSS in HUVECs [7,23], a twofold up-regulation of eNOS expression levels in HUVECs was only present after a 24-h exposure to an arterial LSS of 15 dyn/cm$^2$. 

Fig. 3. Properties and pharmacology of $IKCa_1$ in human umbilical vein endothelial cells. (A) $IKCa_1$ currents after substitution of the 4 mM K$^+$ bath solution (a) by a 140 mM K$^+$ solution (b). Inhibition of $IKCa_1$ current in HUVEC by (B) TRAM-34 (100 nM), by (C) clotrimazole (CLT; 100 nM), and by (D) charybdotoxin (ChTX; 100 nM). Apamin (APA; 100 nM), a selective blocker of SKCa, had no blocking effects. (E) Concentration-dependent blockade of $IKCa_1$ currents by clotrimazole (C) $K_D 23 \pm 2$ nM, TRAM-34 (●) $K_D 13 \pm 3$ nM, or charybdotoxin (□) $K_D 5 \pm 1$ nM, $n=3-4$, for calculation of $K_D$ values, data points were fitted to the Boltzmann equation. (F) Increase of $IKCa_1$ currents by 1-EBIO (100 μM).
sure to 15 dyn/cm² expression levels completely returned after 4 h (Fig. 2A). Exposure intervals for 2, 4 and 24 h with an intermediate LSS of 5 dyn/cm² had no effect on eNOS expression (at 2 h: ΔΔCt 0.7 ± 0.4, n = 4). After 24 h of LSS exposure with a magnitude of 15 dyn/cm², an increase in IKCa current densities in individual cells and thus increased surface expression of the IKCa channel was first observed after 6 h of LSS exposure (p = 0.002 vs. controls kept under static conditions), but not after 4 h, although upregulation of IKCa/mRNA expression was already present at this time point. After 24 h of LSS exposure IKCa current densities were still up-regulated (p = 0.001, Fig. 2A,B), but did not reach the level observed at 6 h after LSS exposure. Thus, in keeping with the bell shape like up-regulation of IKCa1 expression, LSS exposure resulted in a similar bell shape like up-regulation of IKCa functions.

This IKCa current reversed near the K⁺ equilibrium potential of −89 mV at a low extracellular K⁺ concentration of 4 mM and at near 0 mV at a high extracellular K⁺ concentration of 140 mM thus demonstrating K⁺ selectivity (Fig. 3A). IKCa currents in HUVEC were blocked by the selective IKCa-blockers TRAM-34, CLT, and ChTX [25], with potencies (K_D 13 ± 3 nM for TRAM-34, K_D 23 ± 2 nM for CLT, and K_D 5 ± 1 nM for ChTX; Fig. 3B–E) similar to cloned IKCa1 channels,
native IKCa in rat arterial ECs [14] and T-lymphocytes [16]. Iberiotoxin, a selective blocker of large-conductance KCa (not shown), or apamin, a selective blocker of small-conductance KCa (SKCa), had no blocking effect on the KCa current (Fig. 3D). The SKCa/IKCa opener 1-EBIO enabled IKCa currents (Fig. 3F). This pharmacological profile indicates that KCa currents in these HUVEC were indeed mediated by IKCa.

In keeping with the observed increased IKCa-current densities in individual cells, we tested whether LSS exposure also increases the hyperpolarisation response induced by ATP-mediated intracellular Ca2+-mobilization and subsequent IKCa activation.

In confluent and electrically coupled cells (membrane capacity>500 pF) resting membrane potentials (RMP) ranged between −15 and −26 mV (Table 2), similar to values reported by others [12] and human mesenteric endothelium in situ [17]. Following LSS exposure for 24 h with a magnitude of 15 dyn/cm², RMP was not altered while ATP-induced hyperpolarisation was significantly increased (Fig. 4, Table 2). In addition, the potentiation of this hyperpolarisation response by the SKCa/IKCa opener 1-EBIO was more pronounced following LSS exposure (Fig. 4, Table 2).

3.3. Up-regulation of IKCa1 expression requires activation of the Ras/Raf/MEK/ERK signal transduction cascade

Activation of the Ras/Raf/MEK/ERK-signaling system has been shown to up-regulate functional IKCa expression in rat fibroblast in vitro [26]. The activation of this signaling cascade has also been considered important for modulation of endothelial gene expression in response to LSS stimulation [27,28]. We therefore tested whether the Ras/Raf/MEK/ERK-signaling cascade is involved in LSS-induced up-regulation of IKCa1 expression levels.

A pretreatment of the cells with the MEK1/2 inhibitor PD98059 (25 μM, n = 4) prevented the LSS-induced up-regulation of IKCa1 expression levels and also abolished the LSS-induced up-regulation of IKCa current densities (Fig. 5A,B). Moreover, the hyperpolarization responses to ATP and 1-EBIO were significantly reduced (Table 2). In contrast to inhibition of MEK/ERK-signaling, inhibition of p38 signaling cascade by SB203580 (10 μM, n = 3) was ineffective in preventing LSS-induced up-regulation of IKCa1 expression levels as well as IKCa current densities (Fig. 5A,C). Accordingly, hyperpolarization responses to ATP and 1-EBIO were similar to those observed in absence of SB203580 (Table 2).

To rule out that PD98059 or SB203580 may exert direct effects on IKCa activity, PD98059 or SB203580 were directly applied to cells exhibiting IKCa activity. However, both substances, PD98059 or SB203580, had no detectable effects on channel activity (data not shown).

Taken together, these results suggest that LSS-induced up-regulation of IKCa1 expression levels and IKCa function was mediated through activation of the MEK/ERK signaling cascade, similar to the increased IKCa functions in mitogen-activated fibroblasts [26].

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Fig. 5. Inhibition of the MEK/ERK signaling cascade prevents LSS-induced up-regulation of IKCa1 expression levels. (A) Real-time RT-PCR analysis of IKCa1 expression levels after exposure for 24 h to a LSS with a magnitude of 15 dyn/cm² with or without pretreatment with the MEK inhibitor PD98059 (25 μM) or the p38 inhibitor SB203580 (10 μM). ΔΔCt = ΔCt shear stress + PD or SB − ΔCt shear stress (left axis). Data are given as mean ± SEM. **P < 0.01, n = 3–4 each. (B) Representative IKCa currents in HUVEC after LSS exposure following pretreatment with PD98059 or SB203580. (C) Quantitative analysis of IKCa-current densities (pA/pF) at a holding potential of 0 mV in HUVEC following LSS exposure with or without pretreatment with PD98059 or SB203580. Data are given as mean ± SEM. *P < 0.05, n = 11–20 each.
4. Discussion

Shear stress-dependent gene expression has been shown for several genes such as the eNOS, cyclooxygenases, and the adhesion molecule ICAM1 [1,4–6]. However, there are only few reports regarding shear stress-dependent modulation of endothelial ion channel expression. For instance, recent in vitro studies revealed a shear stress-induced up-regulation of stretch-activated channels in HUVEC [20] and of K\textsubscript{ATP} channels in pulmonary endothelium of the rat [29]. In the present study using the HUVEC/cone-and-plate apparatus system as a standard in vitro model system for investigating shear stress-modulated gene regulation in human EC [3], we demonstrated that LSS up-regulates IKCa1 expression levels and IKCa\textsubscript{1} functions in human umbilical vein endothelium in vitro. This up-regulation arose in a time- and shear stress magnitude-dependent manner. Since IKCa\textsubscript{1} activation plays a major role in endothelium-dependent vasodilation [12,30], it is likely that the shear stress-dependent up-regulation of IKCa1 expression levels and subsequently increased IKCa functions might represent a novel mechanism to enhance endothelial vasodilatory and atheroprotective functions.

In HUVECs, IKCa1 expression and IKCa\textsubscript{1} functions were not altered after exposure to venous levels of shear stress, while both, intermediate and higher arterial levels resulted in comparable increases in IKCa1 expression after a 24-h exposure. However, the time course of up-regulation of IKCa1 expression was markedly different at these two different LSS levels. While up-regulation of IKCa1 expression was first detectable after a 24-h exposure to a LSS of intermediate magnitude, up-regulation of IKCa1 expression by the higher LSS of arterial magnitude occurred in a more bell shape like fashion with the highest increase of IKCa1 expression already present after 4 hrs and of IKCa functions after 6 h, which was followed by a less strong increase of IKCa1 expression and IKCa\textsubscript{1} functions after 24 h. This may indicate that an exposure to higher LSS levels activates more potently the signal transduction pathway or even additional signal transduction pathways resulting in stimulation of IKCa1 expression at earlier time points. Time and magnitude dependent effects of LSS on gene expression in endothelial cells have also been described for, i.e. the monocyte chemotactic protein 1 expression [31] and for ICAM1 as shown in the present study and by others [5]. However, the up-regulation of expression of these genes was transient and expression levels returned to basal levels within 4 h. In contrast, IKCa1 expression was still up-regulated after a 24-h exposure to intermediate and arterial LSS levels. Such persisting alterations in gene expression have also been shown for, e.g. eNOS expression as shown here and previously by others [7,23] and have been interpreted as an adaptive response to altered hemodynamics [1].

In keeping with the magnitude dependent effects on the time course of LSS-induced IKCa1 expression, it is tempting to speculate that the more rapid and persisting up-regulation of IKCa1 gene expression levels by high LSS could represent a rapid adaptation to increased hemodynamic forces. On the other hand, the slowly occurring up-regulation in IKCa1 gene expression levels induced by prolonged exposure to intermediate LSS levels may be related to a LSS-mediated control of basal endothelial gene expression for establishing or maintaining adequate endothelial function. With respect to the mechanisms of shear stress-dependent gene regulation, alterations of gene expression have been shown to be mediated by LSS response element containing promoters [32] as found in several shear stress-regulated genes such as the ICAM1 gene [32] and as a result of activation of MAPK signaling cascades [33]. The promoter of the IKCa1 gene lacks shear stress response elements [34]. Regarding the control of IKCa1 transcription, up-regulation of IKCa1 expression levels have been reported to occur in mitogen-stimulated fibroblasts [29] and human T-lymphocytes [16]. This mitogen-induced up-regulation of IKCa1 expression levels is mediated via the MEK/ERK signaling cascade in fibroblast, and in T-lymphocytes augmentation of IKCa1 levels occurs as a result of AP1-dependent transcription. In the present study, the shear stress-induced up-regulation of IKCa1 gene expression levels in HUVECs was prevented by inhibition of the MEK/ERK signaling cascade but not of the p38 signaling cascade. This indicates that in shear stress-stimulated HUVECs similar to mitogen-stimulated fibroblasts [26], augmentation of IKCa1 expression levels and IKCa functions requires the activation of MEK/ERK signaling cascade.

With respect to the role of IKCa channels in endothelial function in general, previous studies revealed so far, that endothelial IKCa\textsubscript{1} are rapidly activated after humoral or shear stress stimulation and induce membrane hyperpolarization [10,17–19,35]. This IKCa\textsubscript{1}-mediated hyperpolarization has been proposed to be essential for initial endothelial mechanoreception and short term regulation of vascular tone [10,11,30] by stimulating synthesis of vasodilating factors either indirectly by stimulating membrane potential-driven Ca\textsuperscript{2+} influx and subsequent stimulation of Ca\textsuperscript{2+} dependent NO and prostacyclin synthesis [36] or directly, given that endothelial hyperpolarization is propagated via gap-junctions to the smooth muscle cells [34]. It has also been suggested that K\textsuperscript{+} efflux from the endothelium through IKCa channels could stimulate inwardly rectifying K\textsuperscript{+} channel and Na/K ATPases leading to vasodilation [37]. As such EDHF-mediated responses can be eliminated by blocking these endothelial IKCa [18,38] it is widely accepted that besides other presumed EDHFs such as cytochrome P450 generated metabolites of arachidonic acid [38] activation of endothelial IKCa channels is essential for the generation of the EDHF response [14,18,19,35,39].

The findings of the present study show that arterial levels of LSS induced an augmentation of endothelial IKCa1 transcription and thereby enhances IKCa\textsubscript{1} function in our in vitro model system. As a consequence of increased IKCa1 expression and function, the ability of the endothelium to...
hyperpolarize is increased as shown in the present study. This increased ability to hyperpolarize in turn enhances membrane potential-driven Ca$^{2+}$ influx and subsequently Ca$^{2+}$-dependent synthesis of endothelial vasodilators and in a more direct fashion the EDHF signaling. Such an up-regulation of the EDHF signaling may be especially important in compensating the diminished availability or loss of other endothelium-derived vasodilator such as NO as reported in salt-sensitive hypertension as well as in eNOS knock-out mice [40,41]. Furthermore, an increased capacity of the endothelium to hyperpolarize may be antithrombotic factors [8].

In conclusion, shear stress-induced modulation in endothelial IKCa1 gene expression and function might therefore be an important mechanism in long term vessel adaptation and vasoregulation in response to increased hemodynamic forces as present in hypertension [1]. In addition to other genes such as eNOS and cyclooxygenase [7,23] the endothelial IKCa1 can now be regarded as new class of shear stress-regulated gene.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (FOR 341/1/5/7, HO 1103/2-4, GRK 276/2). The authors wish to thank Heike Wulff for the gift of TRAM-34. We are grateful to Klaus Schlotter for excellent technical assistance.

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