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Cyclic stretch induces cyclooxygenase-2 gene expression in vascular endothelial cells via activation of nuclear factor kappa- β

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Abstract

Vascular endothelial cells respond to biomechanical forces, such as cyclic stretch and shear stress, by altering gene expression. Since endothelial-derived prostanoids, such as prostacyclin and thromboxane A₂, are key mediators of endothelial function, we investigated the effects of cyclic stretch on the expression of genes in human umbilical vein endothelial cells controlling prostanoid synthesis: cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), prostacyclin synthase (PGIS) and thromboxane A₂ synthase (TXAS). COX-2 and TXAS mRNAs were upregulated by cyclic stretch for 24 hours. In contrast, PGIS mRNA was decreased and stretch had no effect on COX-1 mRNA expression. We further show that stretch-induced upregulation of COX-2 is mediated by activation of the NF- κ B signaling pathway.

Keywords

cyclic stretch; COX-1; COX-2; prostacyclin synthase; thromboxane synthase; endothelial cells; nuclear factor kappa B

Introduction

Prostacyclin (PGI₂) and thromboxane A₂ (TXA₂) are the two most important prostanoids modulating vascular thromboresistance [5]. PGI₂ is predominantly produced by endothelial cells and has potent anti-inflammatory, antiplatelet, anti-proliferative and vasodilatory effects [2]. TXA₂ is mainly produced by activated platelets where it promotes aggregation during thrombus formation. Recent evidence reveals that TXA₂ is also produced in endothelial cells where it promotes vasoconstriction as well as potentiating local platelet activation [12]. The balance between endothelial PGI₂ and TXA₂ generation is thought to regulate a wide array of vascular processes including vasomotor tone, inflammation, atherogenesis as well as thromboresistance [3;15].

The rate limiting step in PGI₂ and TXA₂ generation is metabolism of arachidonic acid to the prostaglandin intermediary H₂ (PGH₂) by the actions of the cyclooxygenase enzymes COX-1 and COX-2 [5]. PGH₂ is subsequently converted to PGI₂ and TXA₂ by the actions of prostacyclin synthase (PGIS) and thromboxane synthase (TXAS), respectively. The COX-1

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isoform is constitutively expressed in endothelial cells while the COX-2 isoform is induced by several number of physiologic stimuli, the best characterized being inflammation [5;21].

Vascular endothelial cells are exposed to a range of hemodynamic forces which can be markedly altered as consequence of cardiovascular disease. It is known that laminar shear stress is able to stimulate COX-1, COX-2 and PGIS expression in endothelial cells [6;13]. In contrast, the effects of cyclic stretch on endothelial prostenoid generation are largely unknown. To better understand how stretch regulates the balance in prostenoid production in endothelial cells, we explored the differential effects of cyclic stretch on the expression of genes involved in PGI₂ and TXA₂ generation. We further investigated the mechanism by which stretch regulates COX-2 induction.

Materials and Methods

Cell culture and materials

Human umbilical endothelial cells (HUVECs; American Type Culture Collection CRL-1730, Manassas, VA) were maintained in EGM-2 media (Lonza Inc., Allendale, NJ) under 5% CO₂ at 37 °C. Cells of passages 2 to 5 were used for all experiments. All other chemicals were purchased from Sigma-Aldrich (St. Luis, MO) unless otherwise indicated.

In vitro stretch experiments

HUVECs were cultured onto type I collagen-coated 6-well Bioflex plates (Flexcell International, Hillsborough, NC). When nearly confluent, cells were subjected to 0–10% biaxial cyclic strain delivered at 1 Hz for the indicated times at 37 °C and 5% CO₂ using an FX-4000T Tension Plus System (Flexcell International) as previously [7]. Pharmacological agents were added into cell media two hours before initiation of cyclic strain as indicated.

Real-time quantitative PCR

Total RNA was extracted from HUVECs using RNeasy Mini kit (Qiagen, Valencia, CA). After the treatment with DNase (Qiagen), RNA samples were subjected to reverse transcription (37 °C for 2 hours) using High Capacity cDNA reverse transcription kits (Applied Biosystems, Foster City, CA). Real-time PCR was performed with a 7900HT Sequence Detection System (Applied Biosystems) using TaqMan® Universal PCR Master Mix with specific probe and primers sets for each target gene purchased from Applied Biosystems (Human COX1: Hs00924803, COX2: Hs00153133_m1, TXAS: Hs0023423_m1, PGIS: Hs00168766_m1). mRNA expression of target gene was normalized to 18S ribosomal RNA (rRNA, Endogenous control (#4308329; Applied Biosystems)).

Western blot analysis

Western blotting was performed using primary antibodies to human COX2 (#438933; Abcam, Cambridge, MA), and beta-actin (#A5441; Sigma-Aldrich) as previously described [7;17]. Protein expression level was normalized to beta-actin.

Quantification of nuclear factor kappa B (NF- κ B) Activation. NF- κ B activation was measured by an ELISA-based method (Trans-AM NF- κ B p65; Active Motif) as previously described [17].

Statistic analysis—All data are presented as the mean $\pm$ SEM. Comparison between 2 groups is by 2-tailed t tests and between multiple groups is by one factor ANOVA followed by a Tukey's multiple comparison test for intergroup comparisons. Only P values <0.05, considered to be statistically significant, are shown.

Results

Effect of cyclic stretch on COX gene expression

We first investigated the effects of cyclic stretch (0–10%, delivered at 1 Hz for 24 hours) on the expression of COX-1 and COX-2 mRNA expression in HUVECs. While COX-1 expression was not altered by cyclic stretch, levels of COX-2 mRNA proportionally increased in response to increasing cyclic stretch (Figure 1A). In similar fashion, levels of COX-2 protein similarly increased in response to stretch (Figure 1B). Time course studies revealed that cyclic stretch caused a marked induction in COX-2 mRNA (>2.5 that of control) within 3 hours of initiation of cyclic stretch stimuli that persisted for at least 24 hours, the duration of the experiment (Figure 1C).

COX-2 induction by cyclic stretch is mediated by activation of NF- κ B

We next investigated the molecular mechanism by which cyclic stretch upregulates COX2 mRNA expression. Cyclic stretch did not alter the decay in levels of mRNA following treatment with actinomycin D (Figure 2A), indicating no change in mRNA stability. Pretreatment of HUVECs with cycloheximide also did not inhibit stretch-induced COX-2 mRNA upregulation (Figure 2B), indicating that new protein synthesis is not required. Given that nuclear factor- κ B (NF- κ B) signaling has recently been recognized as an important modulator of COX-2 gene expression [10;11;20], we investigated whether NF- κ B signaling mediates COX-2 mRNA up-regulation by cyclic stretch. Treatment of HUVECs with parthenolide, a soluble sesquiterpene lactone inhibitor of NF- κ B activation, prevented both stretch-induced NF- κ B activation (Figure 2C left panel) and COX-2 mRNA upregulation (Figure 2C right panel), indicating that cyclic stretch stimulates COX-2 mRNA expression via a mechanism that is dependent on NF- κ B signaling.

Effects of cyclic stretch on PGIS and TXAS expression

Finally, we investigated the effects of cyclic stretch on mRNA expression of key downstream enzymes that mediate generation of prostacyclin and thromboxane A₂. Cyclic stretch decreased the expression of prostacyclin synthase mRNA but increased the expression of thromboxane synthase mRNA (Figure 3), suggesting that it is capable of altering the balance of prostanoid production in human vascular endothelial cells.

Discussion

The major findings of this study are: (1) cyclic stretch stimulates COX-2 and TXAS mRNA expression, does not alter COX-1 mRNA expression and inhibits PGIS mRNA expression in human umbilical vein endothelial cells, and; 2) COX-2 upregulation by stretch is mediated via an NF- κ B-dependent signaling pathway.

Cyclic stretch is a known modulator of prostenoid generation in a number of non-endothelial cell types. For example, stretch stimulates PGI₂ production in fetal lung epithelial cells and both PGI₂ and TXA₂ production in uterine myometrial cells [4;9]. Stretch also upregulates the expression of the COX-2 gene in lung fibroblasts, patellar tendon fibroblasts and both bladder epithelial and smooth muscle cells [1;8;14;18;22]. Relevant to the current study, cyclic stretch has been previously been reported to upregulate both COX-2 and PGIs gene expression in bovine endothelial cells [16;19] While our data confirms the ability of stretch to upregulate COX-2 in human umbilical vein endothelial cells, we found that it downregulated PGIS mRNA expression. This raises the possibility that there may be differences in stretch responsiveness between endothelial cell subtypes. Our data also extends previous findings by demonstrating that cyclic stretch stimulates TXAS mRNA expression. This raises the possibility that stretch can alter the balance between endogenous PGI₂ and TXA₂ in vascular endothelial cells.

The most significant of our findings was that stretch-induced upregulation of COX-2 mRNA expression was mediated by activation of the NF- κ B signaling pathway. NF- κ B is known to mediate the upregulation of the COX-2 gene in response to stimulation with the inflammatory cytokines tumor necrosis factor- α and interleukin-1 β as well as by thrombin [10;20]. Stretch-induced COX-2 upregulation in amnion cells has also been recently reported to be mediated by activation of NF- κ B [11]. Our results reinforce the concept that NF- κ B is a major regulator of COX-2 induction in multiple cell types by a variety of physiologic stimuli. Further studies will determine if the upregulation of TXAS by stretch is also mediated by NF- κ B and whether this leads to alterations in the relative amounts of PGI₂ and TXA₂ by endothelial cells.

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Figure 1.

Effects of cyclic stretch on COX mRNA expression. HUVECs were subjected to the indicated degree of cyclic stretch at 1 Hz for 24 hours. A) The mRNA levels of COX-1 and COX-2 were measured by quantitative PCR (n=6) * $p < 0.05$, ** $p < 0.001$ vs 0% stretch control. B) Changes in COX-2 protein expression, normalized to beta-actin, were measured by Western blotting (n=3). * $p < 0.05$ vs 0% stretch control. C) The time course in COX-2 mRNA upregulation by cyclic stretch (n=3). * $p < 0.01$ vs control at time 0.

Figure 2.

Mechanism of COX-2 mRNA upregulation. A) Stretch does not alter COX-2 mRNA stability. HUVECs were subjected to cyclic stretch (10%, 1 Hz) for 2 hours and then treated with actinomycin D (AcD, 10 g/ml) with cyclic stretch (0 or 10%, 1 Hz) for additional 5 hours (n=3). B) COX-2 upregulation does not require new protein synthesis. HUVECs were subjected to cyclic stretch (0 or 10%, 1 Hz) for 24 hours after treated with cycloheximide (Chx, 5 ug/ml) for two hours (n=6). * $p < 0.001$ vs 0% stretch control in each group. C) COX-2 upregulation by stretch is mediated by NF- κ B activation. Right panel: Stretch activates NF- κ B in HUVECs. Left panel: Inhibition of NF- κ B activation prevents stretch-induced COX-2 upregulation. HUVECs were subjected to cyclic stretch (10%, 1 Hz) for 3 hours (for NF- κ B activation) or 24 hours (for COX-2 mRNA) after treated with parthenolide (Parth, 8 uM) for two hours (n=6). * $p < 0.05$, ** $p < 0.01$ vs 0% stretch control in each group.

Figure 3.

Effects of cyclic stretch on PGIS and TXAS expression. HUVECs were subjected to cyclic stretch at 1 Hz for 24 hours and changes in mRNA levels of PGIS and TXAS measured by quantitative PCR (n=6). ** p<0.001 vs 0% stretch control.