SHEAR STRESS CAUSES NUCLEAR LOCALIZATION OF ENDOTHELIAL GLUCOCORTICOID RECEPTOR AND EXPRESSION FROM THE GRE PROMOTER

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INTRODUCTION

The anti-inflammatory and immunosuppressive actions of glucocorticoid receptor (GR) make glucocorticoid hormones one of the major pharmacological drugs. While unstimulated, the GR is present mainly in the cytoplasm. Upon activation by ligand binding, the receptors subsequently dimerize and translocate into the nucleus where gene transcription is regulated at specific DNA sequences called glucocorticoid response element (GRE). GR activation at its transcriptional regulatory region also involves phosphorylated on threonine and serine residues by MAPK and cyclin-dependent kinases complexes [1].

In the cardiovascular system, fluid shear stress in blood vessels stimulates the production of nitric oxide (NO), a potent vasodilator, by endothelial cells, possibly through various signal transduction pathways (NF-κB, AP-1) [2]. There is evidence for the presence of GR in both endothelial and smooth muscle cells, and vascular inflammation processes are becoming increasingly implicated in atherosclerosis pathology [3]. The anti-inflammatory effects of glucocorticoids on the endothelium suggest a possible role for the glucocorticoid receptor (GR) in mediating atheroprotective actions of shear stress in the vasculature.

Cytokine induction of inducible NOS in endothelium is inhibited by pretreatment with dexamethasone, a potent glucocorticoid [4], while induction of eNOS expression by shear stress can occur in the presence of dexamethasone [5]. Recent studies have linked corticosteroid mediated eNOS activation and NO production to its acute cardiovascular protective effects [6]. Effects of GR on the vasculature has not yet been elucidated, and this study focuses on interactions between shear stress-induced and dexamethasone-induced transcription pathways.

Specifically, the present studies the hypothesis that steady laminar shear stress activates the glucocorticoid receptor signaling pathway in endothelial cells in the context of shear activated kinases activities and NO production that may also regulate GR function. Such regulation is relevance to the atheroprotective role of unidirectional shear stress on endothelial function.

METHODS

A functional chimeric protein of the green fluorescent protein (GFP) and the glucocorticoid receptor (GR), transcribed from a pGFP-GR plasmid, is used to track translocation of the receptors in cells. The pGRE-SEAP plasmid (Clontech) utilizes a secretable human alkaline phosphatase (SEAP) driven by three tandem copies of the GRE sequence, GGTACA$(N)_3$TGTTCT, fused to a weak TATA-like (P_{TAL}) promoter.

Confluent bovine aortic endothelial cells (BAEC) on slides were transfected with pGFP-GR or pGRE-SEAP using Lipofectamine™ (Invitrogen). The 3T3 fibroblasts stably express a GFP-rat GR chimeric protein intrinsically. Cells were exposed to laminar shear stress in parallel plate flow chambers attached to flow loops for media recirculation (15ml) in a 37°C incubator.

After GFP-GR localization experiments, cells were digitally imaged at 40X using a Leica fluorescence microscope driven by OpenLab software. SEAP was quantified using a SEAP Fluorescence Detection Kit (Clontech) according to manufacturer's instructions.

Human internal mammary artery segments were fixed, sectioned, and stained with primary antibody against C-terminal of human GR and CD31 for positive control. Detection is done using biotinylated secondary antibody as part of the Vectastain Elite Kit with DAB peroxidase substrate kit (Vector Labs).

RESULTS

In both bovine aortic endothelial (BAEC) and 3T3 fibroblast cells, shear stress at 10 and 25 dynes/cm^2 activates the receptors, causing GFP-GR to nuclear localize in a manner similar to induction with 25 μM dexamethasone, a potent glucocorticoid (Figure 1). Western blots indicated translocation of endogenous GR into nucleus of sheared BAEC. However, in BAEC cells, but not in 3T3 cells, GFP-GR nuclear localization is blocked substantially by kinase inhibitors (PD098059 and LY294002).

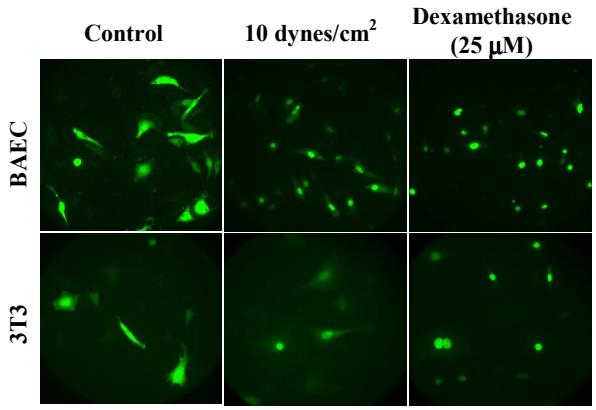

Figure 1. Nuclear translocation of GFP-GR is observed after 1 hr induction with 25 μM Dex as well as with laminar shear stress of 10 dynes/cm^2 in both BAEC and 3T3 fibroblast cells. BAEC cells on glass slides are transiently transfected with GFP-GR plasmids while 3T3 cells intrinsically express the GFP-GR chimeric protein. Laminar shear stress is induced in parallel plate flow chambers.

Promoter construct studies using endogenous GR and GRE drive expression of secreted alkaline phosphatase (SEAP) indicated that BAEC exposed to shear stress of 10 and 25 dynes/cm^2 for 8 hr produced > 9-fold more SEAP (n = 6; $P < 0.005$) than control cells, a level comparable to that observed with dexamethasone induction (Figure 2). However, shear stress enhanced SEAP expression at 6 hr was reduced 50 % (n = 5; P < 0.005) by MEK1/2 or PI 3-kinase inhibitors.

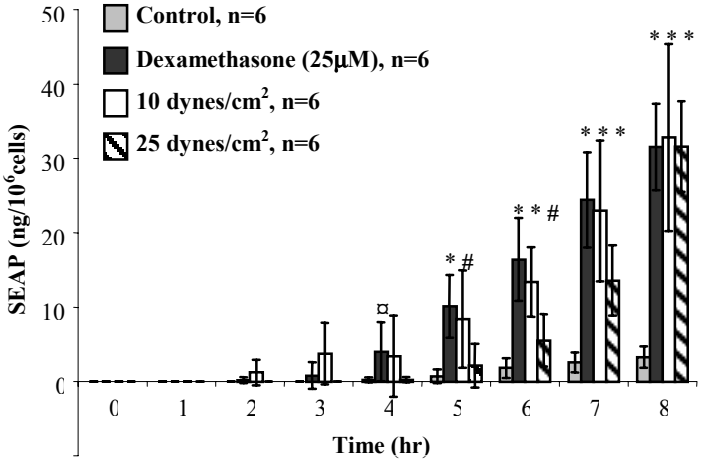

Figure 2. Effect of shear stress on GRE promoter activation in BAEC. Shear stress at both 10 and 25 dynes/cm^2 significantly activated the transcription of GRE-SEAP reporter plasmid and the production of SEAP at a level comparable with induction by dexamethasone at 25 μM. Data are mean ± SE (n = 6). *$P < 0.01$, #$P < 0.05$, ¤$P < 0.05$, all versus static control condition at the same time point. A schematic of GRE-SEAP promoter construct is included.

Finally, GR staining on sections of human internal mammary artery revealed that in contrast to CD31 images, GR staining displayed marked nuclear localization within endothelial cells.

SUMMARY

In summary, this study demonstrated that steady arterial levels of unidirectional shear stress cause nuclear localization of endothelial GR through processes that are MAPK and PI 3-kinase dependent. The finding that hemodynamic forces can be as potent as high dose glucocorticoid steroid in activating GR and GRE-regulated expression correlates with the atheroprotective responses of endothelial cells to unidirectional arterial shear stress. These results indicate the potential role glucocorticoid receptors play in shear-induced anti-inflammatory responses and transcriptional changes in endothelial cells. Future studies include: disturbed flow conditions, further kinase pathway studies on GR phosphorylation by dexamethasone and shear stress, and bioinformatic analysis of putative GRE regulated genes in sheared endothelium.

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