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Nitric oxide regulates angiotensin-I converting enzyme under static conditions but not under shear stress

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Abstract

Mechanical forces including pressure and shear stress play an important role in vascular homeostasis via the control of the production and release of a variety of vasoactive factors. An increase in vascular shear stress is accompanied by nitric oxide (NO) release and NO synthase activation. Previously, we have demonstrated that shear stress induces angiotensin-I converting enzyme (ACE) down-regulation *in vivo* and *in vitro*. In the present study, we determined whether NO participates in the shear stress-induced ACE suppression response. Rabbit aortic endothelial cells were evaluated using the NO synthase inhibitor L-NAME, and two NO donors, diethylamine NONOate (DEA/NO) and sodium nitroprusside (SNP). Under static conditions, incubation of endothelial cells with 1 mM L-NAME for 18 h increased ACE activity by 27% (from 1.000 ± 0.090 to 1.272 ± 0.182) while DEA/NO and SNP (0.1, 0.5 and 1 mM) caused no change in ACE activity. Interestingly, ACE activity was down-regulated similarly in the presence or absence of L-NAME ($\Delta_{(0\ mM)}$ = 0.26 ± 0.055, $\Delta_{(0.1\ mM)}$ = 0.21 ± 0.22, $\Delta_{(1\ mM)}$ = 0.36 ± 0.13) upon 18 h shear stress activation (from static to 15 dyn/cm^2). Taken together, these results indicate that NO can participate in the maintenance of basal ACE levels in the static condition but NO is not associated with the shear stress-induced inactivation of ACE.

Key words
- Shear stress
- Angiotensin-I converting enzyme
- Nitric oxide
- Endothelial cells

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Shear stress is a tangential force acting on endothelial cells resulting from the contact between blood and the vascular wall. Endothelial cells respond to shear stress by altering the production of a variety of vasoactive factors, releasing nitric oxide (NO) (1) and prostacyclin (2), and inhibiting endothelin-1 (3). Shear forces also modulate the production of growth factors such as platelet-derived growth factor (PDGF) (4) and fibroblast growth factor (FGF) (5).

Laminar flow induces a biphasic NO production response with a burst of NO release at the onset of the stimulus, which is reduced after 30 min and then tonically maintained for the duration of the stimulus (6,7). NO has been reported to modulate the control of expression of several genes such as monocyte chemotactic protein-1 (MCP-1) (8), vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 (9). In addition, it has been demonstrated that NO regulates

shear stress-induced changes in PDGF-A and MCP-1 gene expression (10).

We have demonstrated that shear stress decreases angiotensin-I converting enzyme (ACE) activity and expression *in vitro* and *in vivo* (11). There is evidence that NO and NO-releasing compounds inhibit ACE activity in a concentration-dependent and competitive way, and that NO physiologically released from the endothelium reduces the conversion of angiotensin I to angiotensin II (12). *In vivo*, the ACE inhibitor can prevent the coronary vascular and myocardial remodeling induced by long-term inhibition of NO synthesis (13,14). Thus, in the present study we assessed the possible influence of NO on shear stress-induced down-regulation of ACE.

To test this hypothesis, we used a model previously characterized in our laboratory which consists of a rabbit aortic endothelial cell line transfected with 1.3 kb of rat ACE promoter controlling the luciferase reporter gene (WLuc cells). This approach allowed the simultaneous determination of ACE promoter activity by measuring luciferase activity and ACE activity as the product of the endogenous gene. After each experiment, cells were harvested in borate buffer (0.5 M boric acid, 1.125 M NaCl, 0.1% Triton X-100, pH 8.3) and luciferase and ACE activity were measured in the same sample. The luciferase activity was quantified using the Promega (Madison, WI, USA) luciferase kit according to manufacturer instructions. Briefly, luciferin was added to cellular extracts and production of light was monitored by a luminometer. ACE activity was measured by a modified fluorometric technique in which the lysed cells were incubated with substrate (Hippuryl-His-Leu 8 mM; Sigma, St. Louis, MO, USA) for 3 h at 37ºC. The amount of His-Leu formed was measured by adding 100 µl of 74.5 mM o-phthaldialdehyde and fluorescence was read at 365-nm excitation and 485-nm emission (15). All results were normalized by the amount of DNA present in the sample.

Table 1. Angiotensin-I converting enzyme (ACE) and luciferase activity of WLuc cells treated with L-NAME, DEA/NO and SNP for 18 h.

	ACE activity (U His-Leu h^{-1} µg DNA^{-1})	Luciferase activity (U light/µg DNA)
L-NAME		
0 mM	1.000 ± 0.090	1.000 ± 0.065
0.1 mM	0.993 ± 0.145	0.907 ± 0.026
0.5 mM	0.925 ± 0.037	1.363 ± 0.042*
1 mM	1.272 ± 0.182*	1.189 ± 0.037*
DEA/NO		
0 mM	1.002 ± 0.074	1.000 ± 0.039
0.1 mM	0.957 ± 0.093	1.048 ± 0.082
0.5 mM	1.048 ± 0.080	1.091 ± 0.062
1 mM	0.947 ± 0.099	1.223 ± 0.062*
SNP		
0 mM	1.006 ± 0.048	1.000 ± 0.033
0.1 mM	0.884 ± 0.0405	1.012 ± 0.041
0.5 mM	0.979 ± 0.060	1.005 ± 0.039
1 mM	0.986 ± 0.045	0.929 ± 0.043

Data are reported as the mean ± SEM of 3 experiments carried out in triplicate. DEA/NO = diethylamine NONOate; L-NAME = N^G-nitro-L-arginine methyl ester; SNP = sodium nitroprusside.
*P < 0.05 compared to no treatment (ANOVA).

In order to determine whether static basal NO levels interfere with ACE, WLuc cells were treated with the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME; Sigma) (Table 1). A time-course response using 0.1, 0.5 and 1 mM L-NAME did not modify ACE or luciferase activity up to 12 h of treatment (data not shown). After 18 h of incubation with 0.5 or 1 mM L-NAME, there was an increase in luciferase activity, whereas the 1-mM concentration also led to increased ACE activity. To further assess whether NO influences ACE under static conditions, two spontaneous NO donors were administered (Table 1). Treatment of WLuc cells with 0.1, 0.5 and 1 mM diethylamine NONOate (DEA/NO; OXIS International, Inc., Portland, OR, USA) and sodium nitroprusside (Sigma) did not change ACE activity. The 1-mM concentration of DEA/NO for 18 h led to an increase in luciferase activity. The effect of NO donors on the cell culture media was confirmed by measuring by chemilumines-

cence the amount of nitrite and nitrate, which increased by 8-fold (data not shown).

These results indicated that NO may have an inhibitory influence on basal ACE activity levels and expression in endothelial cells. Furthermore, this influence appears to be mediated at least in part at the transcriptional level and the 1.3-kb ACE promoter fragment studied contains some of the regulatory elements involved.

As reported previosly (11), shear stress of 15 dyn/cm^2 for 18 h decreases ACE activity and expression and increases NO production. To determine if the decrease in ACE activity induced by shear stress is mediated by NO, WLuc cells were treated with L-NAME 1 h before starting the shear stress stimulus and for the remaining period of the experiment. Pretreatment with L-NAME was performed to guarantee that NO synthase was inhibited when the shear stress stimulus was started. Shear stress was produced using a cone plate system as described by Malek and Izumo (3). This system consists of a cone with a 0.5º angle rotating on the cell culture medium. Under these conditions, shear stress is proportional to the angular velocity and can be calculated using the following formula: $\tau = \eta\omega/\alpha$, where τ is shear stress, η is medium viscosity, ω is angular velocity, and α is the cone angle. As shown in Table 1, 0.1 mM L-NAME did not modify basal ACE or luciferase activity, whereas 1 mM L-NAME led to their increase by 27%. When cells were subjected to shear stress in the presence of L-NAME (0.1 and 1 mM), the decrease in ACE activity was of the same magnitude as in the absence of the NO synthase inhibitor (Figure 1). The amount of decrease between the static and shear condition was the same among all groups studied ($\Delta_{(0\ mM)} = 0.26 \pm 0.055$, $\Delta_{(0.1\ mM)} = 0.21 \pm 0.22$, $\Delta_{(1\ mM)} = 0.36 \pm 0.13$; $P = 0.7862$). These results are consistent with the idea that NO does not participate in shear stress-induced ACE down-regulation.

The mechanotransduction of shear stress has not been well characterized. There is evidence that shear forces can activate potassium ion channels and the pathways of G proteins and mitogen-activated protein kinase (MAPK) (16). It was also demonstrated that signaling of the extracellular signal-regulated kinase (17) and c-Jun N-terminal kinase (18,19) pathways are activated by shear stress. The interplay and/or synergism among different pathways remains elusive. These transduction cascades can be activated by growth factor receptors or mechanical stimuli (for example, stretch and shear stress). When endothelial cells are subjected to shear stress, the cytoskeletal network is rearranged and the intracellular tension is redistributed. During this process, the integrins are involved and the kinases that are present in the focal adhesion can be activated and trigger the MAPK pathway. It is known that shear stress also regulates the production of growth factors such as PDGF and FGF that also activate MAPK signaling. The autocrine/paracrine release of these hormones can also be responsible for the activa-

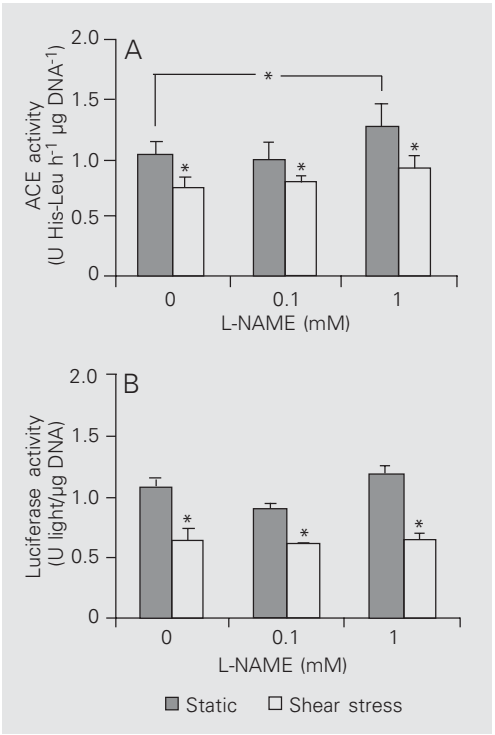

Figure 1. Angiotensin-I converting enzyme (ACE) (A) and luciferase (B) activity in WLuc cells under static conditions and subjected to shear stress of 15 dyn/cm^2 for 18 h. The cells were treated with N^G-nitro-L-arginine methyl ester (L-NAME) 1 h before the beginning of shear stress and for the remaining period of the experiment. Each bar indicates the mean ± SEM of 8 experiments. The results were normalized to static cells with no treatment. *P < 0.05 compared to static control (ANOVA).

tion of MAPK by shear stress. The potential sensor for the shear stress stimulus remains unknown, with a variety of reports demonstrating the activation of different transduction cascades (ion channels, G protein, and MAPK). While NO is a likely mediator interacting with such signaling pathways under several conditions, our results provide evidence that NO can influence basal ACE levels but may not influence the shear stress-induced ACE down-regulation.

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