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Research Article

Activation of vascular p38MAPK by mechanical stretch is independent of c-Src and NADPH oxidase: influence of hypertension and angiotensin II

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Abstract

Little is known about vascular MAPK regulation in response to mechanical strain. Whether mechanically-sensitive pathways are altered in hypertension is unclear. We examined effects of stretch and Ang II on activation of p38MAPK in vascular smooth muscle cells (VSMC) from WKY and SHR. The role of c-Src and redox-sensitive pathways in stretch-induced effects were examined. VSMC from mesenteric arteries were plated onto flexible silastic plates and exposed to acute or chronic cyclic stretch (10%, 1 Hz) with or without Ang II (0.1 uM). Acute stretch stimulated p38MAPK activation in WKY and SHR, independently of c-Src and reactive oxygen species (ROS), since PP2 (c-Src inhibitor) and apocynin (NADPH oxidase inhibitor), failed to alter stretch-mediated p38MAPK. Chronic stretch blunted p38MAPK phosphorylation in WKY and increased phosphorylation in SHR. Stretch, in the presence of Ang II, induced an increase in procollagen-1 expression. This was blocked by SB203580 (p38MAPK inhibitor). Accordingly, vascular p38MAPK is a mechano-sensitive MAPK, differentially regulated by acute and chronic stretch in WKY and SHR. Functionally, stretch and Ang II, amplify profibrotic responses in a p38MAPK-dependent manner, responses that are perturbed in SHR. Such molecular process may influence vascular fibrosis in hypertension and appear to be independent of c-Src and ROS. J Am Soc Hypertens 2012;6(3):169–178.

Keywords: Mechanical strain; vascular remodeling; MAP kinases; SHR; signal transduction.

Introduction

Blood vessels in vivo are continually exposed to a wide array of both humoral and mechanical stimuli. Humoral factors include vasoactive agents (angiotensin II [Ang II], endothelin-1, aldosterone, vasopressin), growth factors (epidermal growth factor, plate-derived growth factor, insulinlike growth factor 1), and cytokines, chemokines, and adipokines that regulate vascular tone and structure through complex signaling pathways.[1–3] Mechanical stimuli include both shear stress (generated at the vascular wall by the flow of blood) and radial stretch (generated by the pulsatile pressure within the vessels).[4] These dynamic mechanical forces can both activate and modulate signaling cascades in vascular cells, probably through highly sensitive mechanotransducers.[5,6]

Of the myriad signaling pathways important in vascular cell regulation is the mitogen-activated protein kinase (MAPK) family that is responsive to both humoral and mechanical stimulation.[7,8] MAPKs are a family of serine/threonine kinases, including ERK1/2, p38MAP kinase, and JNK, and are capable of inducing diverse actions in vascular smooth muscle cells (VSMCs), including contraction, migration, adhesion, cell growth, apoptosis,

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inflammation, and differentiation.[7–11] Aberrant MAPK signaling is associated with endothelial dysfunction, vascular injury, and inflammation, important in vascular remodeling in hypertension.[10–14] Critical in these processes is the prohypertensive, proinflammatory vasoactive peptide Ang II.[15,16] Studies from our group and others have demonstrated that activation of ERK1/2 and p38MAPK is augmented in hypertension, leading to an increase in remodeling processes, such as VSMC growth, inflammation, and collagen deposition.[17,18] These processes involve activation of the nonreceptor tyrosine kinase, c-Src, and NADPH oxidase-derived reactive oxygen species (ROS).[19]

The ability of mechanical stimuli to activate MAPKs, particularly ERK1/2, has been demonstrated in numerous experimental settings, including cultured endothelial cells exposed to shear, coronary arteries exposed to stretch,[20] stretched rabbit aorta,[21] cyclic stretch of VSMCs,[22] and fluid shear stress of endothelial cells.[23] In contrast, mechanical activation of p38MAPK in the vasculature has been less well studied. Cyclic stretch of porcine VSMCs induces p38MAPK activation[24] and also in intact human saphenous vein, p38MAPK activation is increased[25]; however, little is known about the pathways leading to stretch-induced activation of p38MAPK, an important stress-activated MAPK. Nor do we understand how stretch-induced activation of vascular p38MAPK can be affected by the presence of additional factors, such as Ang II, or in cells from hypertensive phenotypes. In this study, we used isolated VSMCs to directly examine stretch-induced responses, and how these may be influenced by a hypertensive milieu. Accordingly, the specific aims of this study were (1) to determine whether stretch-induced activation of p38 MAPK is differentially regulated in VSMCs from hypertensive and normotensive rats; (2) to investigate whether activation of p38MAPK could be modulated by Ang II in a synergistic or additive manner; and (3) to examine potential upstream signaling pathways that mediate stretch-induced activation of p38 MAPK in VSMCs, focusing on NADPH oxidase and redox-sensitive c-Src.

Methods

Cell Culture

The study was approved by the Animal Ethics Committee of the University of Ottawa and performed according to the recommendations of the Canadian Council for Animal Care. VSMCs were isolated from mesenteric resistance arteries of normotensive Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR) as described previously.[26] Each cultured cell line was initially prepared from mesenteric artery beds pooled from 10 rats. Cells were cultured until passage 3 before being frozen for storage in liquid nitrogen, and once thawed did not undergo repeated freeze-thaw cycles. Cells were cultured in Dulbecco Minimal Essential Medium (DMEM), supplemented with 10% fetal bovine serum and used between passages 4 and 7.

For experiments involving mechanical stretch, cells were plated onto 6-well Flexwell plates (Flexcell International, Hillsborough, NC) that had been coated with diluted Matrigel basement membrane matrix (diluted 1:6 in DMEM, BD Biosciences, Mississauga, Canada) to improve cell adhesion to the flexible silicone base of the culture dishes. Cells were grown to confluence and serum-starved for 24 hours before experiments to render the cells quiescent.

Experimental Protocols

Mechanical stretch was applied to cells using the Flexcell FX-4000 system (Flexcell International). This system uses a vacuum manifold to apply equibiaxial stretch of defined magnitude and frequency to cells. For experiments in this study, cyclic sinusoidal stretch of 10% elongation and 1 Hz was used.

In some experiments, cells were also stimulated with Ang II (0.1 μmol/L). Both cyclic stretch and Ang II were applied to cells in the absence and presence of various pharmacological inhibitors, including apocynin (30 μmol/L, NADPH oxidase inhibitor), PP2 (10 μmol/L, c-Src inhibitor), irbesartan (10 μmol/L, angiotensin type 1 receptor [AT_1R] antagonist), and SB203580 (10 μmol/L, p38MAPK inhibitor). These concentrations were used based on previous studies that demonstrated selectivity and sensitivity.[17–19] For all inhibitor studies, cells were treated with the inhibitors 30 minutes before stimulation with Ang II or stretch. For static controls, cells were plated onto Flexwell plates and maintained under identical culture conditions without exposure to cyclic stretch.

Western Blotting

Following stimulation, proteins (30 μg) were extracted from VSMCs, separated by polyacrylamide gel electrophoresis, and transferred to a nitrocellulose membrane. Incubation for 1 hour in 5% skim milk in Tris-buffered saline with Tween (TBS-T) was used to block nonspecific binding sites. Membranes were then incubated overnight with phospho-specific or total antibodies diluted in TBS-T with 3% bovine serum albumin. Primary antibodies used were as follows: phospho-c-Src (Tyr^{418}) (Biosource International, Burlington, Canada) (1:1000), phospho–p38 MAPK (Thr^{180}/Tyr^{182}) (1:1000), c-Src and p38 MAPK (Cell Signaling Technology Inc, Beverly, MA) (1:1000), procollagen I (Santa Cruz Biotechnology, Inc, Santa Cruz, CA) (1:500), fibronectin (Sigma-Aldrich) (1:5000), and proliferating cell nuclear antigen (PCNA) (Santa Cruz Biotechnology, Inc) (1:3000). After incubation with specific secondary antibodies, signals were revealed using chemiluminescence (PicoWest Super Signal, Pearce, Rockford, IL), visualized