PDGF induces migration of synthetic vascular smooth muscle cells through c-Src-dependent activation of full-length $\text{Ca}_v1.2$ L-type Ca^{2+} channels

Toshihide Kashihara, Xiaoguang Guo, Tsutomu Nakada, Mitsuhiko Yamada

Department of Molecular Pharmacology, Shinshu University School of Medicine, Japan

In atherosclerosis, vascular smooth muscle cells (VSMC) migrate from the media toward the intima of the arteries in response to platelet-derived growth factor (PDGF). However, molecular mechanism underlying the PDGF-induced migration of VSMCs remains unclear. Here we found that the migration of rat aorta-derived synthetic VSMCs, A7r5, in response to PDGF was potently inhibited by a $\text{Ca}_v1.2$ L-type Ca^{2+} ($\text{Ca}_v1.2$) channel inhibitor, nifedipine, and a Src-family tyrosine kinase (Src)/Abl inhibitor, bosutinib, in a less-than-additive manner. PDGF significantly increased $\text{Ca}_v1.2$ channel currents without altering $\text{Ca}_v1.2$ proteins expression levels in A7r5 cells. This reaction was almost completely inhibited by overexpression of C-terminal Src kinase, a selective inhibitor of Src. In contractile VSMCs, such as the aorta and cerebral artery, the C-terminus of $\text{Ca}_v1.2$ is proteolytically cleaved into the proximal and distal C-termini (PCT and DCT, respectively). Clipped DCT is noncovalently reassociated with PCT to autoinhibit the channel activity. Conversely, in synthetic A7r5 cells, we found that full-length $\text{Ca}_v1.2$ ($\text{Ca}_v1.2\text{FL}$) was expressed much more abundantly than truncated $\text{Ca}_v1.2$. In a heterologous expression system, overexpression of c-Src activated $\text{Ca}_v1.2$ channels composed of $\text{Ca}_v1.2\text{FL}$ but not truncated $\text{Ca}_v1.2$ ($\text{Ca}_v1.2\Delta1763$, $\text{Ca}_v1.2$ lacking DCT₁₇₆₄₋₂₁₄₀) or $\text{Ca}_v1.2\Delta1763$ plus clipped DCT. Further, c-Src enhanced the coupling efficiency between the voltage-sensing domain and activation gate of $\text{Ca}_v1.2\text{FL}$ channels by phosphorylating Tyr1709 and Tyr1758 in PCT. Interaction assays revealed that c-Src could more efficiently bind to and phosphorylate $\text{Ca}_v1.2\text{FL}$ compared with $\text{Ca}_v1.2\Delta1763$ irrespective of the presence or absence of clipped DCT. These results indicate that PDGF induces migration of synthetic VSMCs through c-Src-dependent activation of $\text{Ca}_v1.2\text{FL}$ channels. Therefore, in atherosclerotic lesions, phenotypic switching of VSMCs may facilitate pro-migratory effects of PDGF on VSMCs by suppressing posttranslational modifications of $\text{Ca}_v1.2$.