A B S T R A C T

Vascular smooth muscle cells (VSMCs) that function as synthetic units play important roles in cardiovascular diseases. Extracellular nucleotides, such as ATP, have been shown to act via activation of P_2 purinoceptors implicated in various inflammatory diseases, we hypothesized that extracellular nucleotides contribute to vascular diseases via up-regulation of inflammatory proteins, including cyclooxygenase-2 (COX-2) and cytosolic phospholipase A_2 ($cPLA_2$) in VSMCs. However, the mechanisms of ATP-induced $cPLA_2$ and COX-2 expression and PGE_2 synthesis remain largely unclear. We showed that pretreatment with the inhibitors of STAT3 (CBE), NADPH oxidase [diphenyleneiodonium chloride (DPI) or apocynin (APO)], ROS [N-acetyl-L-cysteine (NAC)], and PKC (Ro-318220, Gö6983, or Rottlerin) or transfection with siRNAs of STAT3 and $p47^{phox}$ markedly inhibited ATPγS-induced $cPLA_2$ and COX-2 mRNA/protein expression and promoter activity and PGE_2 secretion. ATPγS further stimulated PKC, $p47^{phox}$, and STAT3 translocation. Moreover, ATPγS-induced STAT3 phosphorylation and translocation was inhibited by pretreatment with the inhibitors of PKC, NADPH oxidase, and ROS. ATPγS enhanced NADPH oxidase activity and ROS generation in VSMCs, which were reduced by pretreatment with Ro-318220, Gö6983, or Rottlerin. Finally, we found that ATPγS significantly induced cyclin D1 expression and VSMCs proliferation, which were inhibited by pretreatment with NAC, APO, DPI, Ro-318220, Gö6983, Rottlerin, or CBE or transfection with siRNAs of COX-2 and cyclin D1. We also demonstrated that ATPγS induced cyclin D1 expression via a PGE_2-dependent pathway. These results suggested that ATPγS-induced $cPLA_2$/COX-2 expression and PGE_2 secretion is mediated through a PKC/NADPH oxidase/ROS/STAT3-dependent pathway in VSMCs.