# Palmitate induces reactive oxygen species production and β-cell dysfunction by activating nicotinamide adenine dinucleotide phosphate oxidase through Src signaling

Yuichi Sato, Shimpei Fujimoto, Eri Mukai, Hiroki Sato, Yumiko Tahara, Kasane Ogura, Gen Yamano, Masahito Ogura, Kazuaki Nagashima, Nobuya Inagaki

Journal of Diabetes Investigation
Article first published online : 3 OCT 2013, DOI: 10.1111/jdi.12124

**ABSTRACT**

**Aims/Introduction:** Chronic hyperlipidemia impairs pancreatic β-cell function, referred to as lipotoxicity. We have reported an important role of endogenous ROS overproduction by activation of Src, a non-receptor tyrosine kinase, in impaired glucose-induced insulin secretion (GIIS) from diabetic rat islets. In this study, we investigated the role of ROS production by Src signaling in palmitate-induced dysfunction of β-cells.

**Materials and Methods:** After rat insulinoma INS-1D cells were exposed to 0.6 mmol/L palmitate for 24 h (palmitate exposure), GIIS, ROS production, and NADPH oxidase (NOX) activity were examined with or without exposure to10 μmol/L PP2, a Src inhibitior, for 30 or 60 min. Male KK-$A^y$ mice and control C57/BL6 mice (Clea Japan, Tokyo, Japan) were used. All experiments were carried out with mice aged 8-10 weeks. Pancreatic islets were isolated as previously described.

**Results:** Exposure to palmitate (C16:0) concentration-dependently decreased glucose-induced insulin secretion (GIIS) and increased reactive oxygen species (ROS) production. Oleic acid (C18:1) slightly increased ROS production but arachidonic acid (C20:4 n-6) did not increase ROS production. We have previously described an important role of endogenous ROS production that involves Src activation in impaired GIIS in diabetic islets. In this study, we investigated the effects of PP2, a specific Src inhibitor, on impaired GIIS and augmented ROS production by lipotoxicity. Exposure to 10 μmol/L PP2 for 30 min recovered impaired GIIS caused by exposure to 0.6 mmol/L palmitate for 24 h (palmitate exposure) but did not affect GIIS in the control condition without palmitate exposure. Increased ROS production by palmitate exposure was reduced by 60 min exposure to 10 μM PP2. Src down-regulation ameliorated glucose-induced insulin secretion of INS-1D cells cultured with palmitate. These results suggest that Src activation may be involved in impaired GIIS and augmented ROS production due to palmitate exposure. Src is a non-receptor tyrosine kinase that is

associated with cell membrane and plays important roles in various signal transductions. Its activity is regulated by intramolecular interactions that depend on tyrosine phosphorylation and phosphorylation of $Tyr^{418}$ at the kinase domain that results in Src activation. Palmitate exposure caused Src activation indicated by an increased protein level of $Tyr^{418}$-phosphorylated Src. NADPH oxidase activity was prominently increased by palmitate exposure. Palmitate exposure also increased mRNA level and protein level of NOX2. Exposure to 10 μmol/L PP2 for 30 min reduced augmented ROS production but did not affect protein level of NOX2. Palmitate exposure caused an increase in protein level of $p47^{phox}$ in membrane fraction that was reduced by exposure to PP2 for 30 min. Palmitate exposure caused a decrease in protein level of $p47^{phox}$ in cytosol fraction that was increased by exposure to PP2 for 30 min. Taken together, these findings indicate that palmitate exposure increases NADPH oxidase activity mainly by increasing translocation of $p47^{phox}$ to plasma membrane via Src signaling. Increased protein level of $p47^{phox}$ in membrane fraction by palmiate exposure was reduced by transfection with *$p47^{phox}$* siRNA. Down-regulation of $p47^{phox}$ level was also observed without palmitate exposure by *$p47^{phox}$* knockdown. *$p47^{phox}$* knockdown ameliorated impaired GIIS and decreased augmented ROS production by palmitate exposure. These results indicate that $p47^{phox}$ is involved in impaired GIIS and ROS overproduction by palmitate exposure. Impairment of GIIS from islets of KK-$A^y$ mice was ameliorated by exposure to PP2 for 30 min. ROS production and level of $p47^{phox}$ protein in membrane fraction in the islets of KK-$A^y$ mice was reduced by exposure to PP2. Protein level of $Tyr^{418}$-phosphorylated Src was increased in KK-$A^y$ mice islets compared to that in control islets. These results suggest that activation of NADPH oxidase via Src signaling may be involved in impairment of GIIS from islets of KK-$A^y$ mice.

**Conclusions:** Activation of NOX via Src signaling plays an important role in ROS overproduction and impaired GIIS caused by chronic exposure to palmitate, suggesting a lipotoxic mechanism of β-cell dysfunction of obese mice.