# Linoleic acid stimulates $Ca^{2+}$ release from $InsP_3$-sensitive $Ca^{2+}$ storage sites, leading to a reduction in voltage-gated $Ca^{2+}$ currents in primary cultured rat pancreatic β-cells

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It has been well-established that free fatty acids (FFAs) stimulates immediate insulin secretion from pancreatic β-cells, although the underlying mechanism is unclear. An orphan G-protein-coupled receptor, GPR40, has been demonstrated as a specific receptor for FFAs. This receptor is abundantly expressed in pancreatic islet cells. Linoleic acid, an unsaturated FFA, is a high affinity ligand to GPR40. We have shown that linoleic acid significantly decreased the amplitude of voltage-gated $K^+$ currents in primary cultured rat pancreatic β-cells through GPR40 and intracellular cAMP-PKA system. This reduction in $K^+$ currents may indirectly increase $Ca^{2+}$ influx into and insulin secretion from β-cells. The aim of this study was to clarify the direct effect of linoleic acid on voltage-gated $Ca^{2+}$ currents in the same cell preparation obtained from rats killed using a $CO_2$ chamber. Nystatin-perforated whole-cell recording configuration was employed to record voltage-gated $Ca^{2+}$ currents. Using a bath solution containing TEA-Cl (40 mM, $K^+$ channel blocker) and TTX (1μM, $Na^+$ channel blocker) and pipette solution containing $Cs^+$ (to replace $K^+$), two types of $Ca^{2+}$ currents were isolated by different holding potentials (-80mV and -40mV) and the employment of specific L-type $Ca^{2+}$ channel blocker (nifedipine) as the L- and T-type $Ca^{2+}$ currents. The major component of the total $Ca^{2+}$ currents was the L-type current. Local application of linoleic acid onto recorded cells significantly, and reversibly, decreased the amplitude of both L- and T-type currents, while methyl-linoleate, which has a similar structure to linoleic acid but no binding affinity to GPR40, showed no effect on currents. The linoleic acid-induced reduction in $Ca^{2+}$ currents was sustained when cells were pre-incubated with a specific protein kinase A inhibitor H89 (1 μM) but was completely abolished by 30 min pre-incubation of cells with a specific intracellular inositol 1,4,5-triphosphate ($InsP_3$) $Ca^{2+}$ store depleting reagent thapsigargin (1 μM) or 10 min pre-incubation with an inhibitor of the $InsP_3$-gated $Ca^{2+}$ channel 2-APB (5 μM). We conclude that linoleic acid acts on GPR40 to evoke $Ca^{2+}$ release from $InsP_3$ sensitive $Ca^{2+}$ pools, which in turn increases $[Ca^{2+}]i$ levels leading to a reduction of the voltage-gated $Ca^{2+}$ currents in rat pancreatic β-cells. Supported by NHMRC and Eli Lilly Australia.