## Receptor- and metabolite-mediated increase in $[Ca^{2+}]_i$ in rat pancreatic islet cells by free fatty acids

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Long-chain free fatty acids (FFAs) stimulate immediate insulin secretion from pancreatic islet β-cells. Constant exposure of β-cells to high level of FFAs evokes a clear dysfunction of β-cells *in vitro* and *in vivo*. Such dysfunction of β-cells occurs in type 2 diabetes. Recent work demonstrated that effects of FFAs on β-cells are achieved through two pass-ways mediated by intracellular metabolites of FFAs and membrane receptor GPR40. In order to clarify the signalling process of FFAs, role of intracellular metabolites and membrane receptor GPR40 in linoleic acid (LA)-induced increase in cytosolic free $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) was investigated in primary cultured rat pancreatic β-cells loaded with Fura-2. Levels of $[Ca^{2+}]_i$ in islet cells were reflected by ratio of 510 nm emission fluorescent intensity with excitation wavelength of 340 and 380 nm at room temperature. LA (20 μM for 10 min) induced a transient peak (the first phase) and a subsequent strong, long-lasting (the second phase) increase in $[Ca^{2+}]_i$ in β-cells. Transient application (2 min) of LA induced a weak second phase increase in $[Ca^{2+}]_i$ without changing significantly the first phase increase. GW9508, a non-metabolic agonist of GPR40, mimicked the effects of transient 2 min LA application in producing a transient, strong first phase increase in $[Ca^{2+}]_i$. Inhibition of phospholipase C (PLC) by U73122 eliminated the first transient phase without changing the second phase increase in $[Ca^{2+}]_i$ in response to 10 min LA stimulation. In contrast, blockade of intracellular LA metabolism by acyl-CoA synthetase inhibitor, Triacsin C, suppressed the second phase but not the first phase increase in $[Ca^{2+}]_i$. The first phase increase in $[Ca^{2+}]_i$ was therefore due to activation of GPR40 and PLC system to induce a $Ca^{2+}$ release from endoplasmic reticulum (ER) $Ca^{2+}$ stores. This was then confirmed by its elimination by thapsigargin pre-treatment for 60 min. The second phase increase in $[Ca^{2+}]_i$ was composed of two parts: the minor one was suppressed by extracellular $Ca^{2+}$ removal or by thapsigargin pre-treatment, suggesting a store-operated $Ca^{2+}$ entry (SOCE); and the major component was not suppressed by either extracellular $Ca^{2+}$ removal or thapsigargin pre-treatment but was eliminated after mobilization of mitochondrial $Ca^{2+}$ by inducing mitochondrial membrane permeability transition pore (PTP) using triphenyltin. This indicates a large component of $Ca^{2+}$ mobilization from mitochondrial compartment. In conclusion, LA (FFAs) mobilizes ER $InsP_3$-sensitive $Ca^{2+}$ stores and induces the subsequent SOCE through activating GPR40 receptor, PLC system. Intracellular LA metabolites induce a release of mitochondrial $Ca^{2+}$ mobilization in β-cells through an increase in mitochondrial membrane permeability. Increase in $[Ca^{2+}]_i$ contributes to the FFA-stimulated insulin secretion through $Ca^{2+}$-triggered exocytosis, whereas increase in mitochondrial membrane permeability and $Ca^{2+}$ mobilization represents a dysfunction of mitochondrial, which may play a role in FFA-induced β-cell apoptosis and desensitization to glucose stimulation.

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