Title: Down-regulation of voltage-gated Ca2+ channels and depletion in Ca2+ store are Involved in the pathogenesis of diabetes mediated cytotoxicity in beta RINm5F cell

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Abstract

Aims:. Glucose-stimulated insulin secretion in pancreatic islet-cells is initiated by ATP-induced closure of ATP-sensitive potassium channels (K-ATP channels), subsequent depolarization and opening of voltage-gated Ca^{2+} channels (VGCC), eventually leading to insulin exocytosis. Endoplasmic reticulum (ER) stress has been implicated in both type 1 and 2 diabetes mellitus (DM), but its impact on beta-cell plasmalemmal ion channels is unknown. We investigated whether the ionic channels instrumental in insulin secretion were affected in ER-stressed RINm5F cells.

Methods: The rat insulinoma RINm5F cells viability was measured using the MTT method. Total RNA was extracted from cells using a TRIzol kit (MDBio, Inc., Taiwan). Electrophysiological experiments were performed as in a previous report; INm5F cells were voltage-clamped using the whole-cell configuration. (Endocrinology 2005, 146; 4766). The difference was significant if $p < 0.05$. (ANOVA).

Results: Glucose- and KCl-stimulted Ca^{2+} signals were substantially attenuated after a 24-hr cyclopiazonic acid (CPA, a Ca2+ store depletor) treatment in microfluorimetric Ca^{2+} imaging. The VGCC currents were much reduced after a 24-h CPA treatment in the patch clamp experiments. Quantitative RT-PCR experiments showed that gene expression of alpha-1A and alpha-1C was reduced, suggesting that expression of P/Q- and L-type VGCC was down-regulated. Voltage-gated K^+ channels, KATP channels and store-operated Ca^{2+} entry were unaffected in ER-stressed cells. Pharmacological inhibition of extracellular signal-regulated kinases (ERK) and c-Jun N-terminal kinases (JNK) could not rescue the KCl-stimulted Ca^{2+} signal attenuated by ER stress.

Conclusions: It is the first time to shown that ER stress in beta-cells leads to selective down-regulation of VGCC; this process is possibly via pathways other than ERK or JNK. The deficiency in voltage-gated Ca^{2+} channels activities in ER-stressed beta cells may in part account for the pathogenesis in DM. **(*Acknowledgement: This study was funded by: National Science Council of Taiwan no. NSC-101-2320-B-039-030-MY2 to KL Wong, NSC97-2320- B-039-029-MY3 to YM Leung)**.