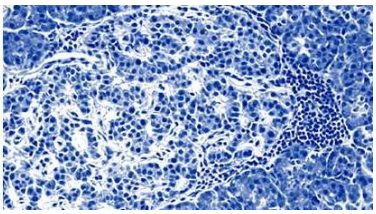
nPOD
Network for Pancreatic Organ Donors with Diabetes



Coxsackievirus Proteases Subvert Hormone Secretion by Affecting Stimulus-Secretion Coupling in Pancreatic Beta Cells

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PURPOSE

Studies of post-mortem pancreas specimens have challenged the simplified notion that beta cells are completely lost during the development of type 1 diabetes (T1D). At disease onset, a large proportion of individuals with T1D have residual insulin-positive beta cells. How these cells have escaped the immune attack and why they are dysfunctional remains to be understood. Of possible interest is the observation that many patients with residual beta cell mass also have beta cells that are positively stained by antibodies that detect the enterovirus protein VP1 after immunohistochemical analysis. Enterovirus proteins have also been identified by mass spectrometry in nPOD cases with T1D and human pancreatic islets infected with a Coxsackie B virus (CVB) infection *in vitro* show impairments in glucose-induced insulin secretion (GIIS). Collectively, these observations imply that a direct infection of the beta cell may contribute to disease development, yet information regarding the mechanism(s) by which enteroviruses negatively affect beta cell function is lacking. Such information could however be of critical importance for the design of disease intervention trials or for restoration of beta cell function in pre-diabetic and/or recent onset T1D patients. Enteroviruses including the CVBs produce so-called non-structural proteins that affect the infected host cell. In the present study, we investigated whether two of these, namely the viral proteases $2A^{pro}$ and $3C^{pro}$, play a role in affecting hormone secretion during CVB infection.

METHODS

Primary human pancreatic islets from the Nordic Network for Clinical Islet Transplantation, Uppsala, Sweden, and INS-1 832/13 cells were infected with CVB3 or CVB4. The expression of $2A^{pro}$ and $3C^{pro}$ proteases was assessed by RT-PCR and Western blot. cDNA encoding viral proteins were cloned from CVB3 (Nancy) to generate plasmids for transfections and for the production of recombinant active and catalytically inactive $2A^{pro}$ and $3C^{pro}$ proteases. ELISA was used to measure insulin and human growth hormone (hGH). Following transfection of INS-1 832/13 cells with plasmids encoding the viral proteases and reporter genes, insulin secretion was assessed by GIIS assay. Exocytosis was measured using patch-clamp and live cell total internal reflection fluorescence (TIRF) microcopy. Ca^{2+} flux was measured by patch-clamp.

SUMMARY OF RESULTS

We show that CVB infected beta cells have a reduced capacity to secrete insulin and that this is concomitant with the expression of the viral proteases $2A^{pro}$ and $3C^{pro}$. After transfecting INS-1 832/13 cells with plasmids encoding the proteases and performing subsequent GIIS assays, we identified impairments in the stimulus secretion coupling in cells expressing either of the proteases. Patch-clamp and TIRF microscopy analyses revealed that both $2A^{pro}$ and $3C^{pro}$ blocked beta cell exocytosis in response to membrane depolarization. Finally, through patch-clamp analysis we saw that the proteases caused impairments in Ca^{2+}-influx induced by membrane depolarization.

CONCLUSIONS

The present study demonstrates that the enteroviral proteases $2A^{pro}$ and $3C^{pro}$ contribute to the subversion of hormone secretion by blocking membrane depolarization-induced Ca^{2+}-influx and exocytosis. These observations provide important insights into how a CVB infection affects the function of the pancreatic beta cell. In addition, our studies suggest that antivirals targeting the viral proteases may be critical for the prevention or cure of enterovirus-mediated diseases including T1D.