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Control/Tracking Number: A-18-1387-EASD
Activity: Abstract
Current Date/Time: 4/4/2018 1:48:27 PM

Metallothionein 1 inhibits glucose-stimulated insulin secretion and is differentially regulated in conditions of beta cell compensation and failure

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Abstract:

Background and aims: The mechanisms responsible for β cell compensation in obesity and for β cell failure in type 2 diabetes (T2D) are poorly defined. Metallothioneins play a role in both Zn^{2+} homeostasis and the regulation of cellular redox state. The mRNA levels of several metallothionein genes are upregulated in islets from subjects with T2D, but their role in β cells is not clear. Here we examined: 1) the temporal changes of islet *Mt1* and *Mt2* gene expression in models of β cell compensation and failure, and 2) the role of *Mt1* and *Mt2* in β cell function and glucose homeostasis.

Materials and methods: *Mt1* and *Mt2* expression was assessed in islets from control lean (chow diet) and diet-induced obese (DIO) mice (8 weeks high fat diet), and prediabetic (6-week-old) and diabetic (16-week-old) *db/db* mice and age-matched *db/+* (control) mice. *Mt1-Mt2* double knockout (KO) mice, *Mt1* overexpressing transgenic mice (Tg-*Mt1*) and corresponding control mice were studied. *Mt1* and *Mt2* were inhibited in MIN6 cells by small interfering RNAs. mRNA levels were assessed by real-time RT-PCR, plasma insulin and islet metallothionein levels by ELISA, glucose tolerance by *i.p.* glucose tolerance tests (*ip*GTT) and fasting-1h refeeding tests, insulin secretion by RIA, cytosolic free Ca^{2+} with Fura-2 LR, NAD(P)H by autofluorescence and cytosolic thiol redox state using roGFP1 ratiometric thiol redox probe.

Results: Increased plasma insulin levels (β cell compensation) correlated with marked downregulation of *Mt1* and *Mt2* mRNA levels in islets of DIO mice (*Mt1*: ~4-fold, $p<0.01$ and *Mt2*: ~4.5-fold, $p<0.05$), and prediabetic *db/db* mice (both by ~2-fold, $p<0.01$). These findings were confirmed in β cells of DIO mice (β cell-specific translating ribosome affinity purification model). In contrast, β cell failure in islets from diabetic *db/db* mice correlated with a tendency for increased *Mt1* (~1.3-fold) and significantly upregulated *Mt2* (~1.6-fold, $p<0.05$) mRNA levels. *Ex vivo* treatment of islets for 18-48h

in high glucose (10-30 mM *vs.* 2-5 mM) strongly downregulated *Mt1* and *Mt2* mRNA and protein levels in parallel with increased insulin secretion. Interestingly, KO mice displayed markedly improved glucose tolerance during *ip*GTT ($p<0.01$) and fasting-refeeding tests ($p<0.01$), in association with increased plasma insulin levels (30 min following *ip*GTT, $p<0.05$). Glucose-stimulated insulin secretion (GSIS) was potentiated in islets isolated from KO mice *vs.* control islets while insulin content was unchanged. In MIN6 cells, knockdown of *Mt1*, but not *Mt2*, potentiated GSIS by ~1.8-fold ($p<0.01$). The potentiation of GSIS in KO islets occurred despite similar rises in intracellular Ca^{2+} and NAD(P)H levels, and the mRNA levels of β cell enriched genes preproinsulin, *Pdx1*, *Glut2* and *Pc* and stress response genes *Hmox1*, *Hspa5* and *Ddit3* were unchanged. Nevertheless, basal and acute H_2O_2-induced cytosolic roGFP1 oxidation was slightly lower in KO islets. On the other hand, overexpression of *Mt1* in islets from Tg-*Mt1* inhibited GSIS by ~1.5-fold ($p<0.01$). Moreover, treatment of control islets with $ZnCl_2$, a potent inducer of metallothioneins, reduced GSIS. This effect was absent in KO islets. **Conclusion:** We identified *Mt1* as a novel negative regulator of GSIS in mouse β cells. Our studies suggest a role for *Mt1* downregulation in β cell compensation in obesity, and for *Mt1* upregulation in β cell failure in T2D.

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Keyword (Complete): 14 Insulin secretion in vitro and exocytosis
Study information (Complete):
***Human studies**: No
***Animal Studies**: Yes

Grant Acknowledgement (Complete):
Please select Yes or No: Yes
Support: : MOVE-in Louvain fellowship (MB), ARC, NHMRC (DRL), SFD-2016 (JCJ)

Clinical Trial Registration Number (Complete):
: No
: N/A

Status: Complete