The circadian clock nuclear receptor Rev-erbα is implicated in autophagy alteration and beta-cell deficit under diabetogenic conditions

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Background and aims:
Type 2 diabetes (T2D) is characterized by hyperglycemia secondary to pancreatic beta-cell deficit. Circadian disruption is considered as a risk factor for T2D. At the molecular level, circadian rhythms are controlled by Clock-Bmal1 with nuclear receptor Rev-erbα as a repressor. In contrast to Clock and Bmal1, Rev-erbα has received little attention in beta-cells. Importantly, in addition to its circadian function, Rev-erbα is a repressor of the autophagy degradation pathway, the latter being crucial for beta-cell health. Nevertheless, little is known about the clock genes/autophagy interplay that may contribute to beta-cell failure in T2D. Therefore, in the present study, we set out to address whether Rev-erbα-mediated inhibition of autophagy caused by diabetogenic stress is involved in beta-cell deficit. The objectives are: 1) To evaluate the impact of Rev-erbα overexpression on beta-cell integrity and autophagy. 2) To investigate whether the negative modulation of Rev-erbα could protect beta-cells from diabetogenic stressors.

Materials and methods: Experiments were performed with pancreatic beta-cell lines (rat beta-cell line INS-1E, human beta-cell line EndoC-βH1) and human islets. Rev-erbα protein levels were evaluated by western blot analysis. Levels of LC3-II (marker of autophagosome number) and p62 (also known as sequestosome-1) were used to monitor autophagic degradation and evaluated by western blot. Since p62 aggregated forms/inclusions are an additional marker for defective autophagy, p62 was also detected by immunofluorescence. Apoptosis was evidenced by cleaved caspase-3 emergence. Glucose-induced insulin secretion was assessed by Homogeneous Time Resolved Fluorescence (HTRF) technology.

Results:
Exposure of INS-1E cells to either glucotoxicity (30 mM glucose for 48h) or cytokines (cytomix of IL-1β, TNFα and IFNγ for 24h) resulted in robust induction of Rev-erbα expression (1.5-2 fold, $p<0.05$) and corresponded with impaired autophagy flux characterized by increased protein levels of p62 (1.5-2 fold, $p<0.05$). Consistent with these data, exposure of beta-cells and human islets to a Rev-erbα agonist (SR9009) was characterized by impaired autophagy/lysosomal degradation as shown by increased LC3-II and p62 levels ($p<0.05$). Importantly, p62-positive inclusions were almost exclusively detected in SR9009-treated dispersed human beta-cells. As a consequence, defective glucose-stimulated insulin secretion (70 % decrease, $p<0.05$) and increased beta-cell apoptosis (increased cleaved caspase-3, $p<0.01$ *vs* vehicle) were detected in SR9009-treated INS-1E cells and human islets. In contrast, pharmacological inhibition of Rev-erbα (antagonist SR8278) or its knock-down by siRNA protected beta-cells from deleterious effects of glucotoxicity (INS-1E and EndoC-βH1) or cytokines-induced inflammation (INS-1E and human islets) by attenuating beta-cell apoptosis (~30%, $p<0.05$).

Conclusion:
Taken together, these data reveal for the first time an underexplored link between the core circadian clock nuclear receptor Rev-erbα, autophagy and beta-cell failure under diabetogenic

conditions. These data also suggest a therapeutic potential of elaborating new Rev-erbα-based strategies to preserve a functional beta-cell mass in T2D.