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Original Research

Impairment of Proinsulin Processing in β-Cells Exposed to Saturated Free Fatty Acid Is Dependent on Uncoupling Protein-2 Expression

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ABSTRACT

Objective: Uncoupling protein-2 (UCP2) overexpression impairs proinsulin processing to mature insulin. Here we tested the hypothesis that induction of endogenous UCP2 by saturated free fatty acid (FFA) would also decrease proinsulin processing.
Methods: Insulinoma cells (INS-1) cells or rat islets were cultured with or without palmitic acid. Proinsulin processing was assessed by immunoblotting or ELISA for UCP2 along with changes in UCP2 expression, mitochondrial uncoupling, Adenosine-5′-triphosphate (ATP) and insulin secretion.
Results: Palmitate increased UCP2 expression and mitochondrial uncoupling and reduced ATP content. Palmitate glucose-dependently increased the proinsulin:insulin ratio up to ~3-fold in INS-1 cells and rat islets, an effect reversed by knockdown of UCP2 in INS-1 cells. Palmitate increased basal insulin secretion, insulin content and mRNA in INS-1 cells.
Conclusions: One effect of palmitate on β-cells is reduced proinsulin processing, which is associated with mitochondrial uncoupling and reduced cellular ATP. Knockdown of UCP2 can prevent the effects of palmitate on proinsulin processing.

Mots clés:
insuline
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protéine découplante 2

RÉSUMÉ

Objectifs : La surexpression de la protéine UCP2 (UCP2) altère la maturation de la proinsuline en insuline. Ici, nous avons testé l'hypothèse voulant que l'induction de l'UCP2 endogène par la saturation de l'acide gras libre (AGL) diminue aussi la maturation de la proinsuline.
Méthodes : Les cellules INS-1 ou les îlots de rat ont été cultivés avec ou sans acide palmitique. La maturation de la proinsuline a été évaluée par l'immunobuvardage ou la méthode ELISA pour l'UCP2, de même que les changements dans l'expression de l'UCP2, la protéine découplante, l'ATP et l'insulinosécrétion.
Résultats : Le palmitate a augmenté l'expression de l'UCP2 et de la protéine découplante, et a réduit la teneur en ATP. Le palmitate a augmenté d'une manière glucose-dépendante la proinsuline : le ratio de l'insuline des cellules INS-1 et des îlots de rat s'est élevé jusqu'à ~3 fois, un effet inversé par le knock-down de l'UCP2 des cellules INS-1. Le palmitate a augmenté l'insulinosécrétion basale, la teneur en insuline et l'ARNm des cellules INS-1.
Conclusions : L'un des effets du palmitate sur les cellules β est la réduction de la maturation de la proinsuline, qui est associée à la protéine découplante et à la réduction cellulaire de l'ATP. Le knock-down de l'UCP2 peut prévenir les effets du palmitate sur la maturation de la proinsuline.

Introduction

Secretion of proinsulin with severely reduced biological activity (1) is a hallmark of type 2 diabetes mellitus patients and contributes to glucose intolerance (2). Recently, diabetes susceptibility genes variants have been associated with elevated circulating

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proinsulin (3). Elevated plasma proinsulin may represent faster transit through the secretory pathway but is also caused by impaired processing in the endoplasmic reticulum (ER)-Golgi or secretory granule (4).

Maturation of insulin involves sequential cleavage at 2 sites of the proinsulin molecule by the subtilisin-like prohormone convertase enzymes PC1/3 and PC2, resulting in intermediate forms des-31,32-proinsulin and des-64-65-proinsulin, followed by release of C-peptide. Carboxypeptidase E (CPE) removes basic amino acid residues exposed by PC1/3 and PC2 action to complete the processing steps (5). The PCs also exist as proenzymes and require CPE to attain full activity (6). Adenosine-5′-triphosphate (ATP)-dependent factors including granule Ca^{2+} or pH are also important for efficient conversion of proinsulin to insulin (7). Therefore, changes in β-cell metabolism that impair ATP generation are predicted to negatively affect conversion of proinsulin to insulin.

Defects in insulin secretion have been attributed in part to the chronic hyperlipidemia seen in both human patients and rodent models of diabetes. Thus, in vitro chronic exposure of islets to elevated free fatty acids (FFA) caused impaired β-cell function, with saturated FFA more damaging than mono- or polyunsaturated FFA (8). One effect of FFA is on insulin biosynthesis and processing. Palmitate inhibits both transcriptional and posttranscriptional phases of proinsulin biosynthesis (9,10). A mixture of palmitate (saturated) and oleate (monounsaturated) increased the proinsulin/insulin ratio by 2-fold in MIN6 β-cells, at least partly due to impaired maturation of PC1/3 and PC2 (11). Recently, palmitate (1.0 to 1.5 mM) was shown to reduce CPE content but not PCs in MIN6 cells, an effect associated with an increase in the proinsulin/insulin ratio and apoptosis (12). Palmitate also has many other effects on β-cells, including induction of ER stress, altered lipid metabolism and mitochondrial function (13).

Uncoupling protein-2 (UCP2) is a mitochondrial inner membrane protein that decreases ATP production in β-cells (14) and is implicated as a mediator of lipotoxicity (15). FFA induced UCP2 expression (16–18) and overexpression of UCP2 inhibited glucose-stimulated insulin secretion (GSIS) (16,19) whereas its

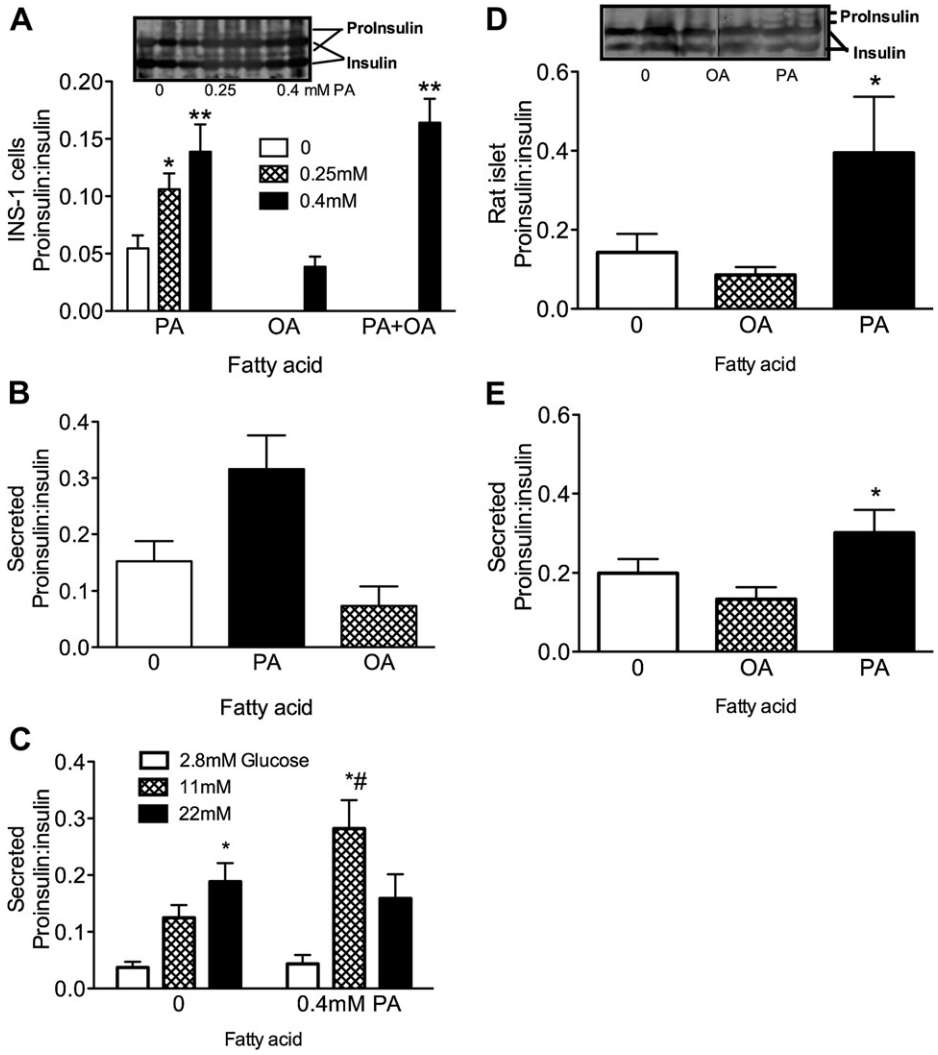

Figure 1. Quantification of proinsulin to insulin ratio in INS-1 cells and rats islets by immunoblotting and ELISA. (A) Proinsulin/insulin in INS-1 cells visualized by immunoblotting. Insulin and proinsulin were identified as described in the Methods section. The ratio of proinsulin/insulin bands was computed by first summing the densities of the proinsulin bands from blots as shown, then summing the densities of the insulin bands, then dividing total proinsulin by total insulin (n=9–10). □ control medium (11 mM glucose + 1% BSA); ⊠ 0.25 mM and ■ 0.4 mM palmitate (PA), oleate (OA), or 0.2 mM PA + 0.2 mM OA; *p<0.05, **p<0.01. (B) The proinsulin/insulin ratio in control, PA- and OA-treated INS-1 preparations, using ELISA (n=6–10) after culture in medium with 11.0 mM glucose; p<0.05 comparing PA with OA. (C) An interaction between glucose and 0.4 mM PA on the proinsulin/insulin ratio was also detected (*p<0.01 comparing effect of glucose; #p<0.05 comparing PA to 0). (D) Conversion of proinsulin to insulin visualized by immunoblotting lysates of isolated rat islets; *p<0.05, n=5–8. (E) The proinsulin/insulin ratio in control, OA-treated and PA-treated rat islets (n=5–7) measured by ELISA after culture in medium containing 8.3 mM glucose; *p<0.05 comparing PA to OA.