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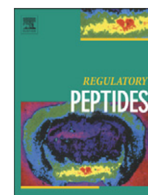

Beta-cell selective K_{ATP}-channel activation protects beta-cells and human islets from human islet amyloid polypeptide induced toxicity

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ABSTRACT

Background and aims: In type 2 diabetes mellitus (T2DM) chronic beta-cell stimulation and oligomers of aggregating human islet amyloid polypeptide (h-IAPP) cause beta-cell dysfunction and induce beta-cell apoptosis. Therefore we asked whether beta-cell rest prevents h-IAPP induced beta-cell apoptosis.
Materials and methods: We induced beta-cell rest with a beta-cell selective K_{ATP}-channel opener ($K_{ATP}CO$) in RIN cells and human islets exposed to h-IAPP versus r-IAPP. Apoptosis was quantified by time-lapse video microscopy (TLVM) in RIN cells and TUNEL staining in human islets. Whole islets were also studied with TLVM over 48 h to examine islet architecture.
Results: In RIN cells and human islets h-IAPP induced apoptosis ($p<0.001$ h-IAPP versus r-IAPP). Concomitant incubation with $K_{ATP}CO$ inhibited apoptosis ($p<0.001$). $K_{ATP}CO$ also reduced h-IAPP induced expansion of whole islets (disintegration of islet architecture) by ~70% ($p<0.05$). Thioflavin-binding assays show that $K_{ATP}CO$ does not directly inhibit amyloid formation.
Conclusions: Opening of K_{ATP}-channels reduces beta-cell vulnerability to apoptosis induced by h-IAPP oligomers. This effect is not due to a direct interaction of $K_{ATP}CO$ with h-IAPP, but might be mediated through hyperpolarization of the beta-cell membrane induced by opening of K_{ATP}-channels. Induction of beta-cell rest with beta-cell selective K_{ATP}-channel openers may provide a strategy to protect beta-cells from h-IAPP induced apoptosis and to prevent beta-cell deficiency in T2DM.

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1. Introduction

Chronic stimulation of beta-cells imposed by insulin resistance is a risk factor for the development of beta-cell dysfunction and type 2 diabetes (T2DM) since it causes depletion of islet insulin stores and beta-cell loss [1–3]. Chronic elevations of glycemia are considered to be a central mechanism for this effect since in subjects with T2DM protection of beta-cells from the stimulatory effect of glucose by overnight inhibition of insulin secretion (induction of beta-cell rest) causes quantitative normalization of insulin secretion upon subsequent stimulation [4]. Similarly, depletion of islet insulin stores and impairments of biphasic and pulsatile insulin secretion induced by prolonged exposure of human islets to elevated glucose concentrations can be prevented by intermittent induction of beta-cell rest using a beta-cell selective K_{ATP}-channel opener ($K_{ATP}CO$) [2]. Besides these effects on beta-cell function chronic hyperglycemia also influences beta-cell survival by creating a proapoptotic cellular environment [5–7]. This is partially due to chronically increased shuttling of client proteins through the endoplasmic reticulum (ER). In consequence ER-associated stress pathways and proapoptotic genes are upregulated leading to an increased vulnerability of the beta-cell to additional toxic factors [8]. One of these factors may be islet amyloid polypeptide (IAPP). In T2DM the islet is characterized by islet amyloid deposits derived from IAPP and a ~65% deficit in beta-cell mass [9–11]. IAPP is a beta-cell hormone and in humans but not in rodents it has the propensity to aggregate into fibrils and form islet amyloid deposits [9,11–15]. Oligomers or intermediates of this aggregation process have been shown to be cytotoxic and induce beta-cell apoptosis [13–18]. Moreover IAPP oligomers damage cell-to-cell adherence in human islets leading to disruption of islet architecture and a decrease in coordinate islet function (increased entropy of insulin secretion and diminished coordinate insulin secretory pulses) [17]. Thus human IAPP (h-IAPP) induced islet damages contribute to islet dysfunction and beta-cell deficiency in T2DM. Since induction of beta-cell rest by opening of beta-cell K_{ATP}-channels provided protection from chronic beta-cell stimulation and glucotoxicity and improved beta-cell survival [2,19,20] we

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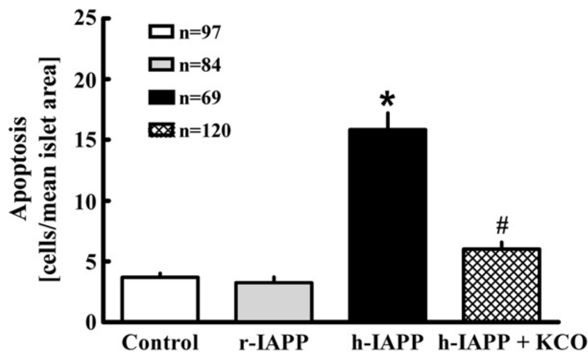

Fig. 1. Mean number of apoptotic cells in human islets (n = 5 donors) after incubation for 48 h with vehicle (control), rat IAPP (40 μM), h-IAPP (40 μM) or h-IAPP (40 μM) + K_{ATP}CO (3 μM). Numbers indicate islet numbers studied per group. Data ± SEM. P-value derived by ANOVA. Group comparisons by Tukey's multiple comparison tests; *$p < 0.05$ versus control. #$p < 0.05$ versus h-IAPP.

hypothesize that beta-cell rest reduces the vulnerability of beta-cells and human islets towards h-IAPP induced toxicity. More specifically we were asking the question whether the induction of beta-cell rest using a beta-cell selective K_{ATP}-channel opener prevents h-IAPP induced beta-cell apoptosis and disruption of human islet architecture.

2. Materials and methods

Human pancreatic islets were isolated from the pancreas retrieved from a total of five non-diabetic, heart-beating organ donors by the Diabetes Institute for Immunology and Transplantation, University of Minnesota (Bernhard J. Hering) and the Northwest Tissue Center Seattle (R. Paul Robertson). The islets were maintained in RPMI culture medium at 5 mM glucose and 37 °C in humidified air containing 5% CO_2. After the islet isolation process the islets were cultured for three to five days before experiments were performed.

Data in control and IAPP groups from static incubation of human islets, TLVM of RIN cells and human islets and thioflavin T assays have partially been published before [14,17,21].

2.1. Static incubation

For static incubation experiments aliquots of human islets were incubated for 48 h with vehicle (H_2O + 0.5% acetic acid), 40 μM r-IAPP, 40 μM h-IAPP and 40 μM h-IAPP + 3 μM K_{ATP}CO (NN414; 6-chloro-3-(1-methylcyclopropyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide [2]). After static incubation the number of apoptotic cells in human islets was detected using the TUNEL staining method (In Situ Cell Death Detection Kit, AP; Roche Diagnostics, Indianapolis, IN). Tissue samples were analyzed on an inverted microscope (Inverted System Microscope IX 70; Olympus, Melville, NY) as described previously [17].

2.2. Time-lapse video microscopy (TLVM)

TLVM is a technique that allows real-time imaging of living tissue. RIN cells (a gift from C. J. Rhodes), a rodent beta-cell line, were maintained in culture in Click's culture medium with 10 mmol/l glucose and 10% FBS at 37 °C in humidified air containing 5% CO_2. After trypsin digestion of the primary cell culture, aliquots of suspended cells were placed in a specially prepared microculture dish (2.3-cm diameter, ΔT Culture Dish; Bioptechs, Butler, PA) and kept in a conventional incubator (Model 3110; Forma Scientific, Marjetta, OH) over the following 24 h. TLVM was then performed as previously described [14]. Briefly, the microculture dish was removed from the incubator and mounted onto the motorized stage (H107, ProScan; Prior Scientific) of an inverted microscope (Inverted System Microscope IX 70; Olympus, Melville, NY). The temperature inside the dish was dynamically controlled to 37 ± 0.1 °C (ΔT Culture Dish Controller, Bioptechs). For time-lapse experiments images of selected fields were acquired with an analogue camera (3-CCD camera; Optornics) every 10 min, stored and analyzed on a personal computer.

TLVM of isolated human islets was performed over a 48-hour period as described for RIN cells. Images of islets in static incubation were acquired every 10 min. Analysis was performed as previously described [17]. To examine the impact of experimental treatments on islet morphology and integrity we measured the islet cross-sectional area of human islets in 4-hour intervals during the full experimental period. The results are expressed as changes of the islet cross-sectional area in μm^2 as a function of time. In prior experiments we established that under

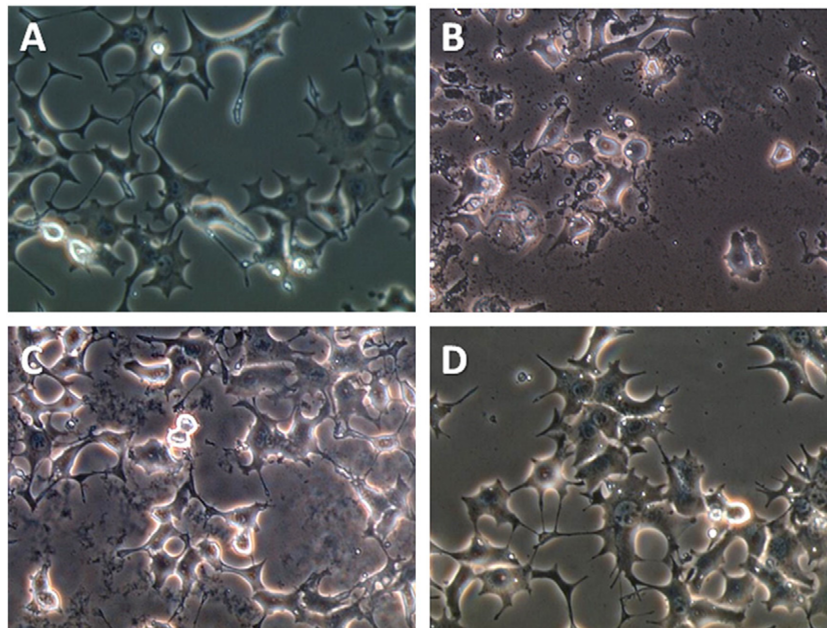

Fig. 2. Representative images of RIN cells cultured on the stage of a time-lapse microscope. (A) Vehicle (control). (B) Human IAPP. (C) Human IAPP + K_{ATP}CO (D) Rat IAPP.

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