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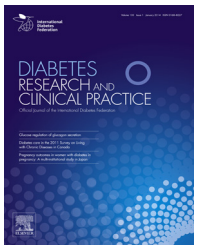

Prevention by metformin of alterations induced by chronic exposure to high glucose in human islet beta cells is associated with preserved ATP/ADP ratio

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ABSTRACT

Aim: We have explored whether the insulin secretory defects induced by glucotoxicity in human pancreatic islets could be prevented by metformin and investigated some of the possible mechanisms involved.
Methods: Human pancreatic islets and INS-1E cells were cultured for 24 h with or without high glucose (16.7 mM) concentration in the presence or absence of therapeutical concentration of metformin and then glucose-stimulated insulin release, adenine nucleotide levels and mitochondrial complex I and II activities were measured. Islet ultrastructure was analyzed by electron microscopy.
Results: Compared to control islets, human islets cultured with high glucose showed a reduced glucose-stimulated insulin secretion that was associated with lower ATP levels and a lower ATP/ADP ratio. These functional and biochemical defects were significantly prevented by the presence of metformin in the culture medium, that was also able to significantly inhibit the activity of mitochondrial complex I especially in beta cells exposed to high glucose. Ultrastructural observations showed that mitochondrial volume density was significantly increased in high glucose cultured islets. The critical involvement of mitochondria was further supported by the observation of remarkably swollen organelles with dispersed matrix and fragmented cristae. Metformin was able to efficiently prevent the appearance of all these ultrastructural alterations in human islets exposed to high glucose.
Conclusions: Our results show that the functional, biochemical and ultrastructural abnormalities observed in human islet cells exposed to glucotoxic condition can be significantly prevented by metformin, further highlighting a direct beneficial effect of this drug on the insulin secreting human pancreatic beta cells.

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1. Introduction

The concept of glucotoxicity, indicating the possibility that chronic exposure to high glucose concentrations could contribute to beta-cell dysfunction during the development of type 2 diabetes mellitus, was first introduced in 1985 by Unger and colleagues [1]. Since then, a large body of evidence has been accumulated showing that substained or repeated exposure of beta cells to supraphysiological glucose levels can cause deleterious effects on beta-cell gene expression, function, survival and growth [2]. With particular reference to the well known abnormalities in insulin secretion observed in type 2 diabetes, it has been demonstrated that in vitro chronic exposure of human and rat pancreatic islets to high glucose concentrations impairs their glucose-stimulated secretory function and decreases the processing of insulin [3,4]. Normal beta cells function as glucose sensors, finely adjusting insulin release to blood glucose levels, and mitochondria play a crucial role in the stimulus-secretion coupling. Glucose mitochondrial metabolism regulates insulin release by generating ATP and increasing the ATP/ADP ratio that is tightly associated to glucose-induced insulin release [5].

In a previous research, we showed that human pancreatic islets isolated from subjects with diabetes are characterized by a reduced insulin secretion in response to glucose when compared to control islets, and that this defect was associated with lower ATP levels, a lower ATP/ADP ratio and impaired hyperpolarization of the mitochondrial membrane [6].

The first aim of the present study was to investigate whether these functional abnormalities could be experimentally reproduced by the chronic exposure of human islets to high glucose levels. We explored also the ability of metformin (dimethylbiguanide) to prevent the high glucose-induced dysfunction in human islets, including impaired insulin secretion, ATP production and beta cell ultrastructure. Metformin is widely recognized as the first-line drug in the treatment of type 2 diabetes mellitus. It reduces hyperglycaemia primarily by its effect on peripheral tissues that makes them more sensitive to insulin action [7]. However, recently direct beneficial effects of metformin on beta cells have been demonstrated. In particular, metformin was shown to improve glucose-stimulated insulin secretion [8,9] and to ameliorate the deleterious effects of chronic high glucose and NEFA levels in isolated islets [10,11].

2. Materials and methods

2.1. Human islet preparation

Pancreatic islets were prepared by collagenase digestion and density gradient purification, as previously reported [12]. All protocols were approved by the local Ethics Committee. For this study, islets were obtained from 9 non-diabetic human multiorgan donors (age 64 ± 7 years, BMI 25.3 ± 1.6 kg/m^2, mean $\pm$ SEM). Isolated islets were cultured overnight in M199 culture medium (containing 5.5 mmol/l glucose), supplemented with 10% adult bovine serum, antibiotics (penicillin, 100 U/ml; streptomycin, 100 μg/ml; gentamicin, 50 μg/ml; and amphotericin B, 0.25 μg/ml) at 37 °C in 5% CO_2, and then for 24 h in the same medium containing 5.5 or 16.7 mmol/l glucose, in the presence or absence of 2.4 μg/ml metformin (a concentration within the therapeutical range).

2.2. Insulin secretion

At the end of the culture, insulin secretion studies were performed by the batch incubation technique as previously described [13]. Groups of approximately 30 islets of comparable size were incubated at 37 °C for 45 min in Krebs–Ringer bicarbonate solution (KRB), 0.5% albumin, pH 7.4, containing 3.3 mmol/l glucose. Then, medium was completely removed, assayed to measure "basal" insulin secretion, and replaced with KRB containing 16.7 mmol/l glucose. After additional 45-min incubation, medium was removed, and insulin levels were measured to assess "stimulated" insulin release. Insulin secretion was expressed as stimulation index (SI), i.e. the ratio of stimulated over basal insulin secretion [14]. Insulin concentrations were measured by a commercially available immunoradiometric assay (Pantec Forniture Biomediche, Turin, Italy).

2.3. Adenine nucleotide measurement

At the end of the culture, adenine nucleotides were measured as previously reported [15]. After 1 h incubation with 3.3 or 16.7 mmol/l glucose, the experiments were stopped by the addition of 0.125 ml of trichloracetic acid (TCA) (Sigma, St. Louis, MO, USA). ATP and ADP were assayed in triplicate by a luminometric method [16]. To measure total ATP + ADP, ADP was first converted into ATP. Samples, with known concentrations of ADP, without ATP, were run in parallel to check that the transformation was complete. ATP was measured by the addition of a reagent containing luciferase and luciferin (Sigma, St. Louis, MO, USA). The emitted light was measured in a luminometer (Junior LB 9509-Berthold Technologies, Germany). To measure only ATP, the same previously described procedure was followed, except that in the first incubation step pyruvate kinase was lacking. ADP levels were then calculated by subtracting ATP from the total ATP + ADP. Blanks and ATP standards were run through the entire procedure, including the extraction steps.

2.4. Electron microscopy evaluation

Electron microscopy studies were performed as previously described [17]. Islets were fixed with 2.5% glutaraldehyde in 0.1 mol/l cacodylate buffer, pH 7.4 for 1 h at 4 °C. After rinsing in cacodylate buffer, the tissue was postfixed in 1% cacodylate buffered osmium tetroxide for 2 h at room temperature, then dehydrated in a graded series of ethanol, briefly transferred to propylene oxide and embedded in Epon-Araldite. Ultrathin sections (60–80 nm thick) were cut with a diamond knife, placed on formvar-carbon coated copper grids (200 mesh), and stained with uranyl acetate and lead citrate. Morphometric analyses were performed as previously described [17]. Micrographs, obtained at 10,000× were analyzed by overlay with a graticule (11 cm 11 cm) composed of 169 points. Volume density was calculated according to the formula: volume