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Hyperglycemia induces apoptosis and p53 mobilization to mitochondria in RINm5F cells*

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Abstract

The mechanisms related to hyperglycemia-induced pancreatic β-cell apoptosis are poorly defined. Rat insulin-producing cells (RINm5F) cultured in high glucose concentrations (30 mM) showed increased apoptosis and protein p53 translocation to mitochondria. In addition, hyperglycemia induced both the disruption of mitochondrial membrane potential ($\Delta\psi_m$), and an increase in reactive oxygen species (ROS), as shown by fluorescence changes of JC-1 and dichlorodihydrofluorescein-diacetate (DCDHF-DA), respectively. The increased intracellular ROS by high glucose exposure was blunted by mitochondrial-function and NADPH-oxidase inhibitors. We postulate that the concomitant mobilization of p53 protein to the mitochondria and the subsequent changes on the $\Delta\psi_m$, lead to an important pancreatic β-cell apoptosis mechanism induced by oxidative stress caused by hyperglycemia. (Mol Cell Biochem **281:** 163–171, 2006)

Key words: apoptosis, p53, pancreatic β-cell, hyperglycemia, mitochondria, oxidative stress

Introduction

Cell proliferation and cell death by apoptosis are very complex and involve several pathways. In both processes, the p53 tumor suppressor gene plays an important role. Under stress, the activation of p53 protein works as a transcription factor, activating a series of genes such as p21 and Bax, and their concerted action results in cell cycle inhibition [1] or in apoptosis induction, respectively [2]. However, there is growing evidence about the induction of apoptosis by independent mechanisms of the transcriptional function of p53 [3]. These studies support the idea that p53 can contribute to apoptosis in malignant cells by directly signaling the mitochondria [4, 5] and inducing cytochrome c release [6, 7].

It is likely that p53 regulates the function of the organelles within the cell. This function was confirmed with γ radiation that showed the mobilization of p53 heading towards the external mitochondrial membrane during apoptosis as a response to stress. Such mobilization is not seen when apoptosis is p53 independent and/or during cell cycle delay [4, 6]. Before p53 reaches the mitochondria and leads to changes during the induction of apoptosis, transcriptional activation

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of several oxide-reductases and production of reactive oxygen species (ROS) takes place. [8]. Also, p53 induces a transient increase in mitochondrial membrane potential followed by a decrease [9]. Previous studies in thymocytes and leukemia cells treated with γ radiation [7, 10] and oxidative stress-induced apoptosis of glia cells [11], suggest that changes occur in the mitochondrial membrane potential and in the production of ROS by this organelle as an important factor for p53-induced apoptosis [9].

Enough evidence is available about the role of apoptosis in the loss of pancreatic β cells during chronic hyperglycemic states [12–14]. During the initial hyperglycemia the number of pancreatic β cells decreases. The remaining cells increase their glucose sensitivity and the production and secretion of insulin is also increased to prevent a hyperglycemic state while in starvation [15]. In addition, in a chronic state of hyperglycemia, the pancreatic β cells become exhausted, and after a while, death occurs by apoptotic mechanisms [12, 16]. In other instances, hyperglycemia induces ROS as well as an oxidative stress condition in several tissues. This is considered as a main feature in the development of several pathophysiologic changes related to certain chronic complications of diabetes [17, 18]. Oxidative stress mainly affects pancreatic β cells [15], where catalase and peroxidase action is 20 times lower than in the liver [19]. According to these studies, it seems that the increase in ROS production is one of the earliest events in cases of glucose intolerance and it may be the cause of pancreatic β cell dysfunction, as well as the cause of changes in insulin synthesis and glucose-induced insulin secretion in type 2 diabetes [18]. Thus, we hypothesize that the p53 protein participates in changes at the mitochondrial level in pancreatic β cell death. The objective in this study was to investigate whether hyperglycemia induces apoptosis in RINm5F cells through an increase in ROS production, and if this phenomenon is associated with changes in mitochondrial potential and with the mobilization of p53 protein to the mitochondria.

Materials and methods

Reagents

The chemicals were obtained from the following sources: RPMI-1640 medium, Hank's balanced salt solution, trypsin, Na-EDTA, carbonylcyanide-m-clorophenyl-hydrazone (CCCP), rotenone, and apocinin from Sigma (Sigma Chemical Co., St. Louis, MO); fetal bovine serum and L-glutamine from GIBCO (Carlsbad, CA). DePsiper^{TM} kits (JC-1 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl benzimidazol carbocyanine iodide) from R&D systems (Minneapolis, MN); dichlorodihydrofluorescein-diacetate (DCDHF-DA) from Molecular Probes Europe (Leiden, The Netherlands); Anexin-V Fluos staining kit from Roche Antibodies Applied Science (Mexico, D.F.), anti-p53 (pab 240) and anti-cytochrome c (H-104) antibodies from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); chemiluminescence reagent from Perking Elmer Life Sciences (Boston, MA), sequi-blot-PVDF membrane and prestained and biotinylate standards from Bio-Rad (Hercules, CA). All other analytical grade chemicals were obtained from Merck (Darmstadt, Germany).

Cell culture

RINm5F cells were grown in RPMI-1640 medium (11 mM glucose) supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 23.8 mM sodium bicarbonate, 20 μg/ml gentamycin, and 2 mM L-glutamine, in a 95% air, 5% CO_2 environment at 37 °C. Cells were harvested and sub-cultured for an additional 2 days in RPMI-1640 containing 30 mM glucose.

Apoptosis

Flow cytometry

Cells were harvested with 2 mM PBS-EDTA, washed in PBS-BSA 0.1%, and centrifuged at 200 × g for 5 min. The pellets were resuspended in 100 μl of staining-solution: 20 μl fluorescein isothiocyanate-labeled annexin-V (10 μg/ml) and 20 μl propidium iodide (PI) (50 μg/ml) in 1 ml Hepes buffer (10 mm Hepes, pH 7.4, 140 mM NaCl, 5 mM $CaCl_2$), and incubated in the dark for 15 min at room temperature. About 400 μl Hepes-buffer was added for flow cytometry analysis using a FACSCalibur instrument (Becton Dickinson System, San Jose, CA) with 488 nm excitation. Emitted light was split and collected at 530 nm band pass filter for fluorescein and 585 nm to PI. For each flow cytometer run, 10 000 cells were required. Data were analyzed using CELLQUEST software (Becton Dickinson).

DNA fragmentation

Cells were washed twice with PBS, resuspended in lysis buffer (25 mM Tris-HCl, pH 8.0 and 10 mM EDTA, 0.25% triton X-100) and stored at 4 °C for 15 min. Cell lysates were then centrifuged at 13,000 × g at 4 °C for 30 min. The supernatants were collected and incubated in the presence of 100 μg/ml RNAsa A and 240 μg/ml proteinase K at 37 °C for 1 h. Crude DNA preparations were extracted with phenol:chloroform:isoamylalcohol (25:24:1), precipitated with 0.1 volume of 3 M sodium acetate and 2.5 volumes of 100% ethanol at −20 °C overnight and centrifuged at 14,000 × g for 30 min. The DNA pellets were air-dried and resuspended

in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) [20]. The concentration of nucleic acid was determined by UV absorbance at 260 nm. The same amount of nucleic acids from each sample was found by electrophoresis on a 1.2% agarose gel and visualized by UV fluorescence after staining with ethidium bromide. Gel was visualized and photographed on an image analyzer Gel Doc 2000 using Quantity One 4.0.1 software (Bio-Rad, Hercules, CA).

Subcellular fractioning

Cells (2×10^7) were resuspended in 400 μl of buffer A (10 mM Tris-HCl, pH 7.4; 0.5 mM Na-EDTA, 250 mM sucrose, 0.1 mM PMSF, 10 μg/ml leupeptin, 5 μg/ml pepstatin, 2 μg/ml aprotinin, 10 μg/ml digitonin) and incubated for 5 min at room temperature and later homogenized in a glass Teflon homogenizer using 10 up-and-down strokes at 500 rpm. The homogenate was centrifuged at $750 \times g$ for 10 min at 4 °C. The supernatant was collected and subjected to centrifugation at $750 \times g$ for 10 min at 4 °C. The supernatant was transferred and centrifuged at $10{,}000 \times g$ for 15 min at 4 °C [21]. The resulting mitochondrial pellet was resuspended in 50 μl buffer A without digitonin. The supernatant was stored as the cytosolic fraction. Both fractions were stored frozen at −70 °C until analyzed. Protein concentrations from both fractions were determined using the Bradford assay [22]. Cytosolic fractions were analyzed for glutamate dehydrogenase (GDH) enzymatic activity. Less than 5% of the total cellular GDH activity (mitochondrial marker) was detected in cytosolic fraction [23].

Western blot analysis of cytochrome c release and p53 location

The mitochondrial and cytosolic fractions or crude cell lysates were used for cytochrome c and p53 detection. These fractions (40 μg) were separated on 10% SDS-PAGE. Biotinylated standards were used to determine molecular weights. The gels were electroblotted onto PVDF membranes (0.8 mA/cm^2, 2 h). p53 was detected with a mouse monoclonal antibody at a 1:1000 dilution and secondary goat antimouse horseradish peroxidase-labeled antibody (1:10,000). Cytochrome c was detected using rabbit polyclonal antibody at a 1:2500 dilution and secondary goat antirabbit horseradish peroxidase-labeled antibodies (1:10,000) [6, 24]. Bound antibodies were detected using an enhanced chemiluminescence kit (Perking Elmer Life Sciences, Boston USA) following the manufacture's instructions.

Confocal fluorescent imaging

After the above-mentioned treatments, the cells were washed with PBS, fixed for 3 min in 1:1 ice-cold acetone/methanol, washed with PBS, and labeled by adding the MitoFluor 589 probe (200 nM in PBS, Molecular Probes). After incubating for 20 min at room temperature, the cells were washed once with PBS and incubated with primary antibodies against p53 (diluted 1:25 in 1% BSA-PBS) for 3 h at room temperature. After washing, the cells were exposed to secondary antibodies (antimouse IgG conjugated-FITC, diluted 1:100) in the same buffer for 3 h at room temperature in the dark. Fluorescent-labeled preparations were viewed under a Zeiss Axiovert 100 M confocal scanning microscope supplemented with an argon/helium/neon laser. The excitation/emission wavelengths used were 488/510 nm for FITC, and 568/590 nm for MitoFluor dye. The acquisitions were recorded using pseudocolor representation, with LSM 5 Image Browser Program for confocal microscopy.

Measurement of ROS by DCDFH-DA fluorescence

ROS production was measured by fluorescence using the oxidation-sensitive fluorescent probe 2′,7′-dichlorodihydrofluorescein diacetate (DCDHF-DA). DCDHF-DA is a cell-permeant dye that, once inside the cell, is cleaved by intracellular esterase into its nonfluorescent form DCDHF. This form, which is no longer membrane permeable, may be further oxidized by H_2O_2 or the hydroxyl radical, to its fluorescent form, DCF. Following exposure to hyperglycemia, RINm5F cells were washed with Hank's balanced salt solution (lacking phenol red) and then incubated in the dark with the fluorescent probe DCDHF-DA (10 μM in Krebs-Ringer bicarbonate buffer) for 30 min at 37 °C and analyzed with a Fluoroskan Ascent FL (Labsystems). Fluorescence emission from DCF (green) was detected at a wavelength of 530 nm [25, 26], in the presence or absence of 5 μM rotenone, 0.1 μM CCCP or 10 μM apocinin [27].

Measurement of the mitochondrial membrane potential ($\Delta\Psi_m$) by JC-1 fluorescence

The mitochondrial membrane potential was semiquantitatively determined using a DePsiper^{TM} commercial kit, following the procedure recommended by the manufacturer for staining monolayer cells. The fluorescent probe JC-1 is a lipophilic cation that selectively accumulates within the mitochondrial matrix either as a green (527 nm) fluorescent monomer at depolarized membrane potentials or as J-aggregates with orange-red (590 nm) fluorescence at hyperpolarized membrane potentials [28]. The samples were immediately analyzed under the confocal microscopy Zeiss Axiovert 100 M microscope, using 488 nm excitation and 530 and over 580 nm emission wavelengths.

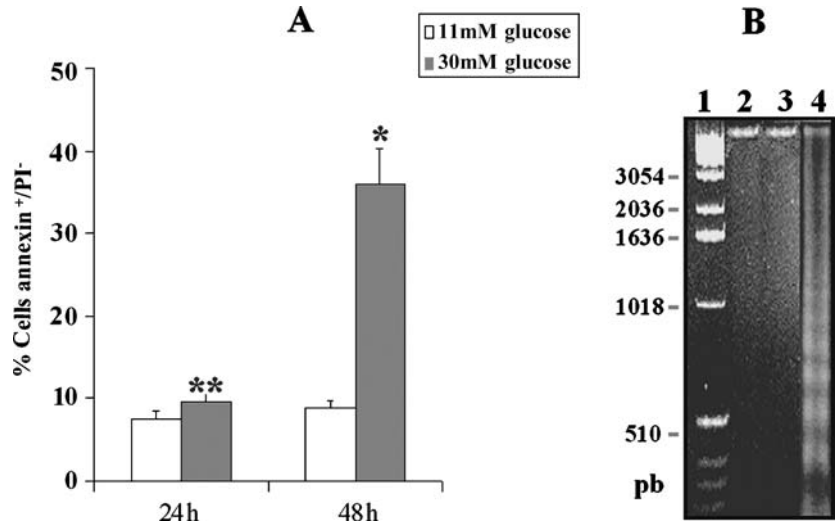

Fig. 1. Apoptosis of cultured RINm5F cells in the presence of glucose (11 or 30 mM) for 24 and 48 h, followed by staining with annexin-V-FITC (10 μg/ml) and PI (50 μg/ml) and analyzed by flow cytometry. (A) Rate of apoptotic cells or positive cells to annexin-V-FITC and negative to PI at 24 and 48 h. Results are a mean $\pm$ S.E.M. in five separate experiments. (*) indicates $p < 0.001$ and ** indicates $p < 0.005$ when compared to controls using one-way ANOVA and the Bonferroni test. (B) Agarose gel electrophoresis of DNA fragmentation in the RINm5F cells. *Lane 1*: molecular DNA weight markers. *Lane 2*: DNA obtained from RINm5F cells cultured in the presence of glucose (11 mM) for 48 h. *Lanes 3 and 4*: DNA obtained from RINm5F cells in the presence of hyperglycemia (30 mM) for 24 and 48 h, respectively. This figure is representative of five independent experiments.

Data analysis

Data were evaluated using variance analysis for multiple comparisons with the Sigma Stat v2.0 package (Sigma-Aldrich Co., San Luis Missouri, USA). A p value <0.05 was considered significantly different.

Results

Effects of hyperglycemia on the survival of the RINm5F cells

The RINm5F cells were cultured in a medium containing 11 or 30 mM glucose. Apoptosis was quantified measuring exposure of phosphatidylserine and by oligonucleosomal DNA fragmentation. Annexin V-FITC binds to phosphatidylserine in the presence of Ca^{+2} [29], which is exposed on the outer leaflet of the (intact) plasma membrane as an early marker of apoptosis. To exclude a possible contribution as a result of necrosis, we also treated cells with PI, that labels the nuclei of nonintact (necrotic) cells. Increased staining with annexin V (or FL1 fluorescence) alone is indicative of apoptosis, whereas staining with both annexin V and PI is indicative of necrosis. Our results proved that a 48 h hyperglycemia induces an increase in the positive cell rate to Annexin-V but negative to PI, with a statistically significant difference ($p < 0.001$) compared to controls (11 mM glucose) ($36 \pm 11.3\%$ and $5.08 \pm 0.56\%$, respectively) (Fig. 1A). Gel electrophoresis of DNA extracted from RINm5F cells (Fig. 1B) confirmed that endonucleolytic DNA breakdown, a late event in apoptosis, was present with 48 h hyperglycemia.

Since the RINm5F cells cultured during 24 h under hyperglycemia showed no significant difference ($p < 0.05$) as compared to controls concerning early apoptosis in annexin V assays, in subsequent experiments we show only the results after 48 h incubation.

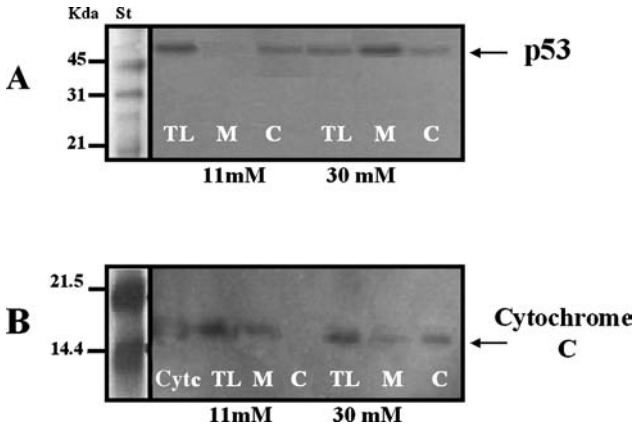

Fig. 2. Immunoblott detection of (A) p53 and (B) cytochrome c in mitochondrial, cytosolic fraction and total cell lysate of RINm5F cells cultured in the presence of glucose (11 or 30 mM) for 48 h. Subcellular fractioning was performed as described in the "Materials and methods" section. 40 μg protein were resolved on 10% SDS-PAGE, blotted, and detected using p53, and cytochrome c antibodies. St: molecular weight standard proteins; TL: total lysate; M: mitochondrial fraction; C: cytosolic fraction; Cyt. c: cytochrome c standard. The immunoblots are representative of five independent experiments.

Subcellular location of p53 and cytochrome c release

Recent studies have shown that the mitochondria are targets for p53 during induction of apoptosis, p53 is translocated to the mitochondria and is involved in the regulation of mitochondrial permeability of tumor cells [7]. In this study, we show evidence that hyperglycemia induces the mobilization of p53 protein to the mitochondria. The analysis by WB detected the presence of this protein in the mitochondrial and not in the cytosolic fraction of cells cultured during 48 h under hyperglycemic conditions (Fig. 2A). Additionally, hyperglycemia-induced mitochondrial permeability (Fig. 5) and led to cytochrome c release to the cytosol in the same cells (Fig. 2B).

Confocal microscopy analysis of p53 intracellular distribution

To further document the intracellular distribution of p53, we used confocal microscopy analysis of p53 after MitoFluor 589 labeling of the mitochondria (Fig. 3). The RINm5F cells

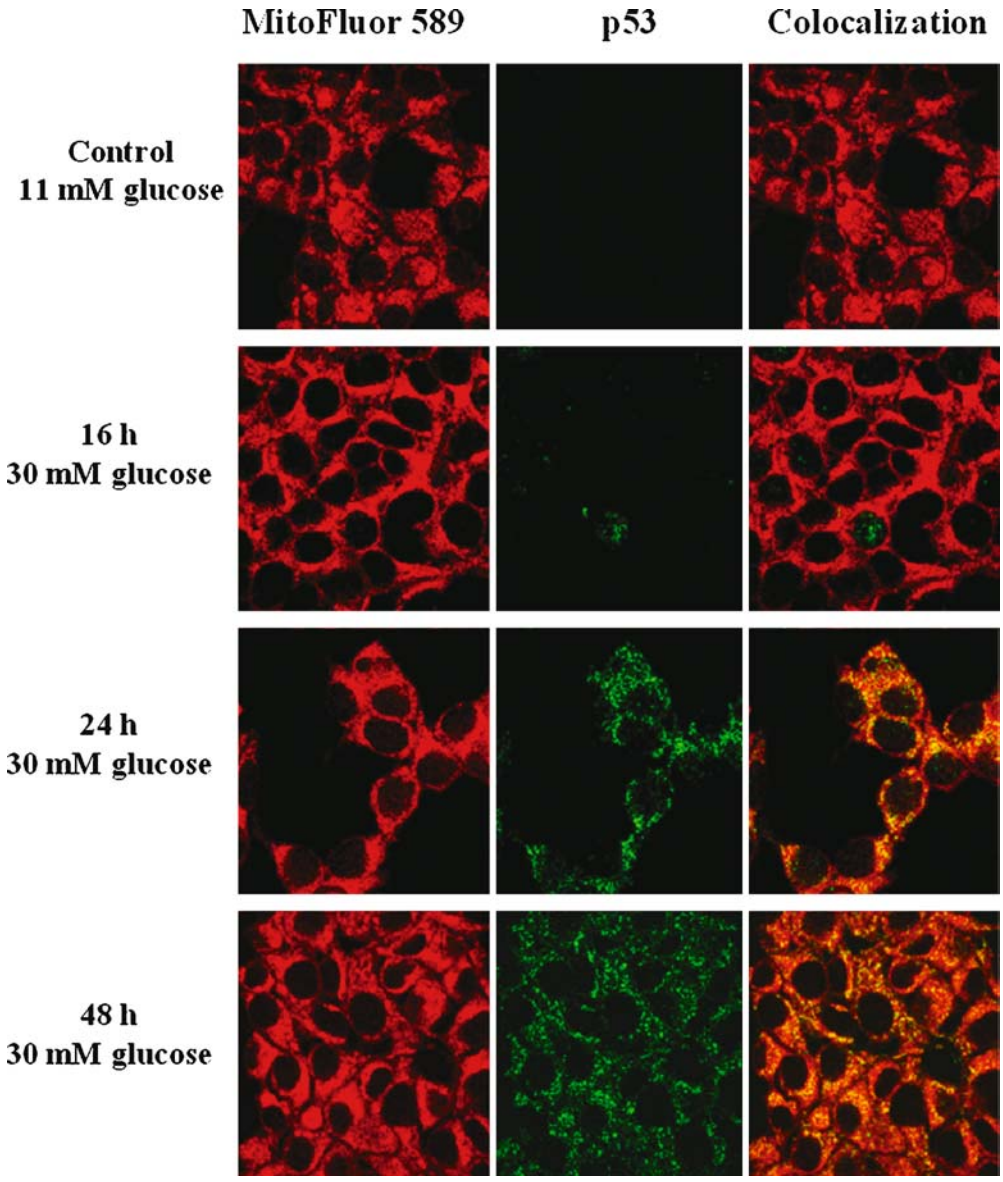

Fig. 3. Confocal microscopy analysis of p53 redistribution and mitochondrial staining of cultured RINm5F cells in the presence of glucose (11 or 30 mM) for 16, 24, and 48 h. In controls only the 48 h picture is shown since no changes were found at other times. MitoFluor 589 stained mitochondria are shown in the first column (*red*). The second column shows p53 staining (*green*) and the third column indicates when both are present (*yellow*). The RINm5F cells showed p53 protein and mitochondria located together at 24 h and increasing in number after 48 h of hyperglycemia.

were cultured with 11 or 30 mM glucose and analyzed at 16, 24, and 48 h. Confocal microscopy analysis showed a clear mitochondrial location of p53 after hyperglycemia exposure for 24 h compared to control cells (11 mM glucose), with an evident increase at 48 h.

Effect of hyperglycemia on ROS production

RINm5F cells cultured under hyperglycemic conditions showed a 2.5-fold increase in ROS versus controls as measured by DCF fluorescence (Fig. 4A). In order to determine the origin of these free radicals, the cells were treated with two mitochondrial inhibitors (Rotenone and CCCP), and with the NADPH oxidase inhibitor (apocinin), before adding the ROS fluorescent probe. The results showed that these inhibitors decrease the ROS production, even in the control group with a 40% average inhibition (data not showed), while in the treated groups a 66% inhibition was achieved. However, no inhibition differences were found among the three inhibitors (49, 44 and 40% in control cells and 62, 66 and 66% in hyperglycemia cells treated with rotenone, CCCP and apocinin, respectively) (Fig. 4B). No additional effect was found when the cells were treated with the combination of inhibitors (Rotenone + CCCP 59%, CCCP + apocinin 62%, rotenone + apocinin 61%), except for rotenone + CCCP + apocinin (73%) (Fig. 4C).

Effect of hyperglycemia on the $\Delta\psi_m$ of RINm5F cells

To assess whether hyperglycemia-induced apoptosis involves changes in mitochondrial membrane potential, the RINm5F cells were cultured at high glucose concentrations and $\Delta\psi_m$ was measured by JC-1 fluorescence (Fig. 5). In live cells, JC-1 forms aggregates and emits fluorescence at 590 nm (red), while dead cells with collapsed $\Delta\psi_m$, JC-1 emit fluorescence at 530 nm (green). Green fluorescence was greater in cells cultured with 30 mM glucose (Fig. 5B) compared to control cells (11 mM glucose) (Fig. 5A).

Discussion

The decrease in pancreatic β cells during chronic states of hyperglycemia occurs through apoptotic mechanisms [13].

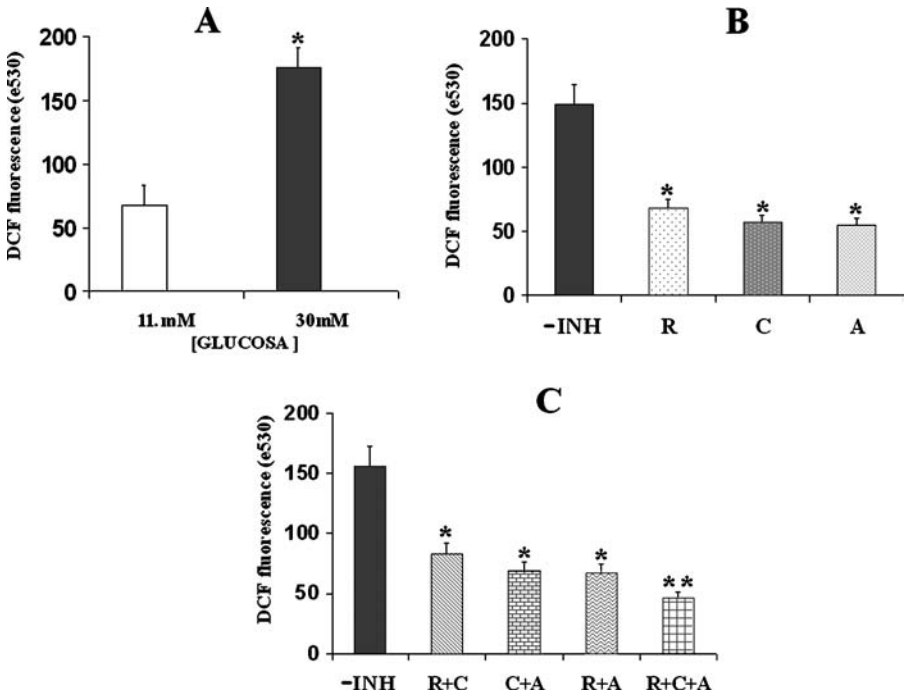

Fig. 4. Effects of glucose on intracellular ROS production in RINm5F cells. (A) The cells were incubated in RPMI containing 11 or 30 mM glucose for 48 h followed by staining with DCDHF-DA, and the fluorescence was measured at 530 nm. Data are expressed as 530 nm fluorescence units. Results are mean ± S.E.M. in five separate experiments. * indicates $p < 0.001$ when comparing 11 vs. 30 mM glucose using one-way ANOVA with the Bonferroni test. (B) RINm5F cells were incubated in RPMI containing 30 mM glucose for 48 h in the absence or presence of rotenone (R), CCCP (C), apocinin (A). (C) or with a combination of these inhibitors. Data are expressed as 530 nm fluorescence units of DCF. Results are a mean ± S.E.M. in five separate experiments. * indicate $p < 0.001$ when comparing vs. with inhibitor and without inhibitor (-INH) and ** indicate $p < 0.001$ when comparing vs. between groups using one-way ANOVA with the Bonferroni test.

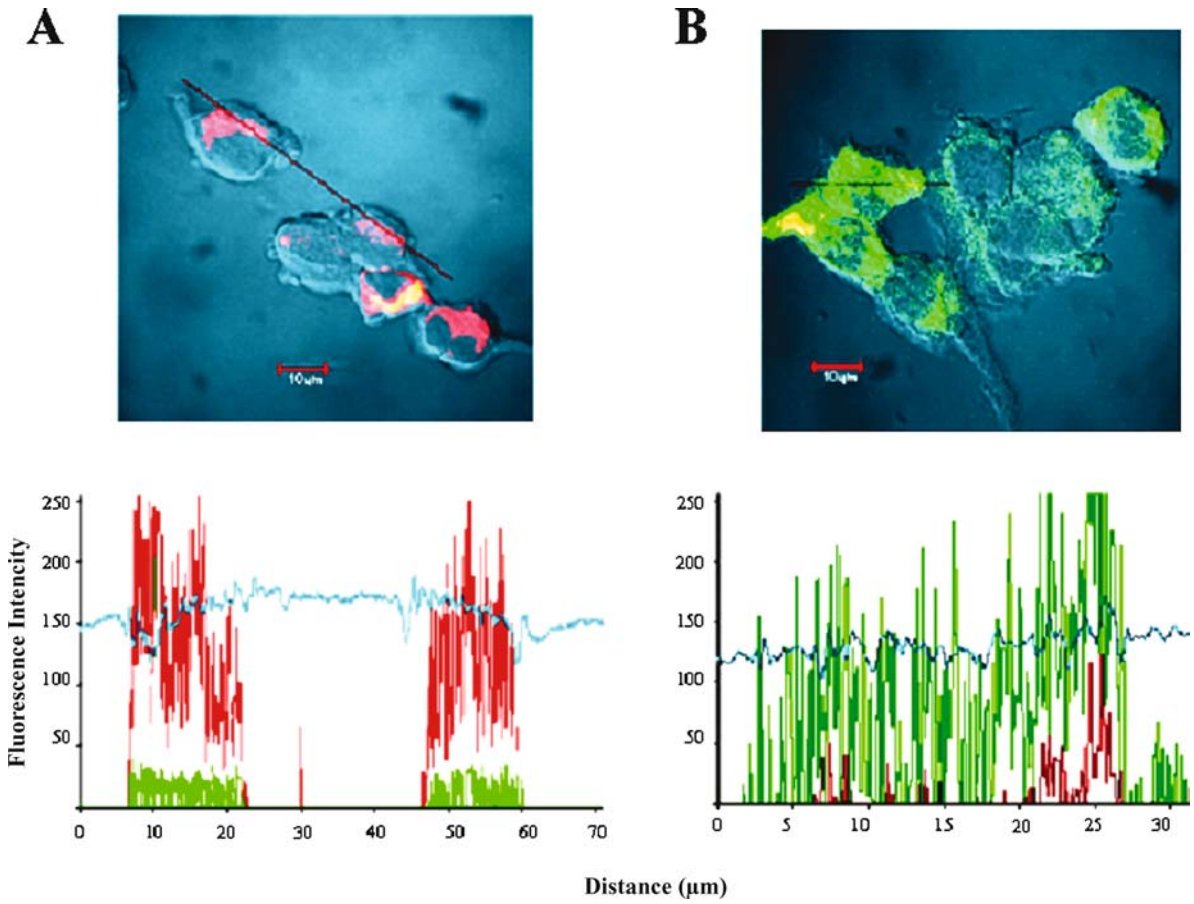

Fig. 5. Effect of hyperglycemia on $\Delta\psi_m$ in RINm5F cells. The cells were exposed for 48 h to (A) 11 or (B) 30 mM glucose, and the effect upon $\Delta\psi_m$ was determined by staining with 10 μM JC-1 and confocal microscopy scan. In living cells, JC-1 forms more J-aggregates (*red*) with increasing mitochondrial membrane potential, and in dead cells with collapsed $\Delta\psi_m$, JC-1 emits green fluorescence. The magnification is 100×. Figure is representative of five independent experiments.

However, the exact mechanism by which hyperglycemia induces β cell death is currently unknown. Recent studies on this topic have focused on mitochondrial metabolism as the main source for ROS and its role in apoptosis [30].

In our study, we found that RINm5F cells cultured in a high glucose medium (30 mM) for 16 h, had no biochemical nor morphological changes consistent with apoptosis. The externalization of phosphatidylserine and DNA oligonucleosomal fragmentation, characteristic events of apoptotic cell death, were seen at 48 h of hyperglycemia.

During the process of apoptosis, the mitochondria play a central role in the execution phase storing cell death-activators and effectors, which are released when membrane permeability changes, such as cytochrome c. During this process there is a translocation of signaling proteins and effector molecules between the nucleus, cytoplasm, and mitochondria. The mobilization of proapoptic proteins towards the mitochondria promotes the dysfunction of this organelle [31]. It was surprising to see that during the apoptosis of RINm5F cells cultured under hyperglycemic conditions for 24 and 48 h, the p53 protein moved towards the mitochondria. Endogenous p53 present in the mitochondria was identified by *in situ* immunofluorescence of whole cells after 24 and 48 h of hyperglycemia. Confocal analysis showed that a significant amount of p53 is found in the mitochondria at 24 h, with an important increase at 48 h. The mobilization of p53 to the mitochondria at 24 h of hyperglycemia in the RINm5F cells was an early event related to phosphatydylserine translocation a well accepted early event of the apoptotic process. In addition, the p53 translocation at 48 h coincided with the decrease in $\Delta\psi_m$, cytochrome c release and nuclear DNA fragmentation.

Our results suggest that mitochondrial p53 is part of a death signaling pathway in RINm5F cells under hyperglycemic conditions. Previous studies conducted by Mihara *et al.* [7], in tumor cells have shown that the release of cytochrome c is a result of the p53 translocation towards the external mitochondrial membrane. They used rat liver isolated

mitochondria incubated in the presence of mouse p53, and found that 90% of cytochrome c was released within the first 30 min. On the other hand, the collapse of $\Delta\psi_m$ is a key element for the release of proapoptotic mitochondrial factors [30]. Our results show that hyperglycemia induces apoptosis of pancreatic β cells, possibly through the mobilization of the p53 protein to the mitochondrial membrane and $\Delta\psi_m$ decrease.

The exact nature of the changes induced by p53 at the mitochondrial level is unknown, although two possibilities have been hypothesized. One possibility is that the p53 protein produces aggregates with members of Bcl-2 family [7, 32]. On the other hand, the apoptosis process in response to p53 depends on an increase in ROS production which alters mitochondrial permeability and allows the release of apoptotic factors. It has been previously shown that oxidative stress induces p53 expression [33] and that this protein stimulates ROS production [34] through the activation of genes associated with the metabolism of these radicals [5, 35]. Under these circumstances, p53 induces cell aging and the apoptotic response is seen when there is an overproduction of ROS from an additional stimulus, which in the case of pancreatic β cells could be a chronic state of hyperglycemia. The biochemical mechanism behind glycotoxicity involves a state of chronic oxidative stress. Pancreatic β cells are particularly sensitive to its effects [15, 36]. It has been previously shown that in several cell lines, hyperglycemia increases the production of ROS, even within short periods such as 15 min [17, 18, 37]. Our results show that the incubation of RINm5F cells under hyperglycemic conditions (30 mM glucose) for a 48 h period produces an important increase in ROS production, as much as 2.5 fold compared to controls. The inhibition of ROS production in the presence of rotenone and CCCP revealed that the oxidative stress generated by hyperglycemia is due, at least partly, to an increase in the production of the superoxide anion through the mitochondrial electron-transport chain [26, 38]. It is also known that apocinin decreases about 89% superoxide anion production in endothelial cell cultures under hyperglycemic conditions [27]. In our study, we also found that apocinin decreases ROS production, although this effect was not statistically different from mitochondrial inhibitors. A similar effect was seen when ROS was quantified in the presence of a combination of inhibitors. This suggests that the activation of the NADPH oxidase system significantly contributes along with the mitochondria to oxidative stress in RINm5F cells cultured with 30 mM glucose. These results are consistent with those published by Oliviera *et al.* [39]. They showed the presence of NADPH oxidase system in rat pancreatic β and HIT-T15 cells, and described its role in H_2O_2 production. The role of NADPH oxidase in the pancreatic β cells is still undefined, although given the importance of the free radicals in several signaling pathways under physiological conditions, such as the mobilization of Ca^{+2}, some authors have suggested it is involved in the insulin secretion mechanism [39].

Conclusion

Our results suggest, that the mobilization of p53 from the cytoplasm to the mitochondria and the changes in mitochondrial membrane potential are involved in the apoptosis induction mechanism of the β cell during oxidative stress produced by hyperglycemia.

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