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Prolonged culture in low glucose induces apoptosis of rat pancreatic β-cells through induction of c-myc

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Abstract

Prolonged culture in low-glucose concentrations (⩽5 mM) induces apoptosis in pancreatic β-cells by a poorly defined mechanism. We now show that, in both purified rat β-cells and isolated rat islets, culture in the presence of 3 or 5 mM (G3–G5) instead of 10 mM glucose (G10) induces a large increase in c-myc expression before onset of a caspase-dependent apoptosis. These effects were prevented by addition of leucine and glutamine to G3 and G5, and were mimicked by addition of the mitochondrial poison azide to G10. In contrast, inhibition of Ca^{2+} influx and insulin secretion with diazoxide under control conditions did not stimulate islet c-myc expression nor β-cell apoptosis. In rat β-cells, adenovirus-mediated c-myc overexpression increased their rate of apoptosis, whereas antisense-c-myc expression reduced low-glucose-induced apoptosis by ∼50%. In the insulin producing MIN6 cell line, apoptosis induction by either low glucose or an activator of AMP-activated protein kinase (AMPK) was associated with c-myc mRNA and protein upregulation. In conclusion, stimulation of β-cell apoptosis by prolonged culture at low glucose partly results from early and sustained induction of β-cell c-myc expression. These effects may be due to sustained restriction in nutrient-derived metabolic signals.

Keywords: Pancreatic islet; Gene expression; MIN6; Metabolism; Cell death; c-Myc antisense

Stimulation of pancreatic β-cells with glucose induces an initial rise in β-cell energetic metabolism, which leads to the closure of ATP-sensitive K^+ channels, plasma membrane depolarization, Ca^{2+} influx, and a rise in the cytosolic Ca^{2+} concentration that triggers insulin secretion [1]. Besides these acute effects on β-cell function, glucose also chronically exerts pleiotropic effects in pancreatic β-cells. Previous studies have indicated that the viability of cultured β-cells depends on the prevailing glucose concentration, with an optimal glucose concentration for rodent β-cell survival of ∼10 mM [2,3]. Culture of β-cells at suboptimal glucose concentrations (<10 mM) triggers their apoptosis, suggesting that glucose suppresses an apoptosis program in these cells [4]. However, the mechanism leading to apoptosis induction under glucose limitation remains undefined. Glucose deprivation has been associated with increased c-myc expression in other cell systems [5,6].

c-Myc is a transcription factor of the basic helix-loop-helix-leucine zipper (bHLH-LZ) family that can induce both proliferation and apoptosis in a variety of cell types [7]. In pancreatic β-cells, chronic hyperglycemia or culture in the presence of 20–30 mM glucose induces c-myc expression in a Ca^{2+}-dependent manner [8,9]. Furthermore, β-cell-targeted c-myc overexpression decreases insulin gene expression and stimulates β-cell apoptosis more than proliferation, leading to net β-cell loss and development of diabetes [10–12].

The aim of this study was to investigate whether deregulated expression of c-myc could contribute to β-cell apoptosis induced by culture at low glucose concentrations.

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Materials and methods

Cell and islet isolation and culture. Rat pancreatic islets were isolated and cultured as previously described [9]. In brief, batches of 45–60 islets were kept in 2 ml of RPMI 1640 medium (Invitrogen) containing 5 or 10 mM glucose (G5–G10) and 5 g/L BSA, but no fetal calf serum. The culture medium was renewed after 1 day, then every other day, at which time a portion of the medium was withdrawn for insulin secretion measurement. In some experiments, the islets were precultured for 1 week in G10 and then cultured 18 h in G5 or G10 plus various test substances.

Single β-cells were obtained from rat pancreatic islets by isolation, dissociation, and purification as previously described [13,14]. The purified β-cells were then reaggregated and cultured in suspension using a HAM's serum-free medium containing 5 g/L BSA, at G3, G5 or G10, or were cultured as single cells in the same medium [15]. The general caspase-inhibitor z-Val–Ala–Asp–fluoromethylketone (z-VAD-fmk, Bachem, Bubendorf, Switzerland) was added to reaggregated β-cell cultures at a concentration of 50 μM, 1 h before transferring the cells to apoptogenic culture conditions.

Insulin-secreting MIN6 cells derived from mouse pancreatic β-cells [16] were cultured in DMEM with 15% fetal bovine serum (FBS) containing G25 unless specified otherwise. They were used at passages 20–30.

Measurement of changes in gene mRNA levels. β-Cell and islet total RNA were extracted, quantitated, and reverse transcribed into cDNA as described [9], using Ultraspec (Biotecx Laboratories, Houston, Texas) or Tripure (Roche Diagnostics, Mannheim, Germany) and the kit Superscript II (Invitrogen). Control reactions lacking the reverse transcriptase were run in parallel. Changes in gene mRNA expression were determined by semiquantitative radioactive duplex PCR in which c-myc cDNA was amplified simultaneously with the TATA-box binding protein (TBP) as internal control gene. The primers and cycling conditions for amplification of c-myc and TBP cDNAs have previously been described [9]. The amplimers were then separated by 6% PAGE in Tris–borate–EDTA buffer. The gel was dried and the amount of [α-^{32}P]dCTP incorporated in each amplimer was quantitated with a Cyclone Storage Phosphor System (Packard, Meriden, CT). The ratio "Myc/TBP" was then calculated for each sample.

Insulin determinations. Insulin concentrations in the culture media of rat islets were determined by radioimmunoassay using rat insulin as a standard and ethanol to precipitate bound insulin [17].

Detection and quantification of apoptosis. Living, apoptotic, and necrotic β-cells were detected and quantified by fluorescence microscopy using propidium iodide and Hoechst 3342 (Sigma) [4]. The majority of apoptotic cells that accumulate during culture are microscopically recognized through their fragmented nuclei that fluoresce for both compounds. Apoptotic or necrotic index is defined as: [(% necrotic or apoptotic cells in experimental condition − % necrotic or apoptotic cells in control)/(% living cells in control)] × 100.

Cells containing sub-G1 (apoptotic) nuclei were also identified and counted by FACS analysis [18,19]. This technique was applied on both primary cells and the cell line. In brief, cells ($\pm 5 \times 10^4$) were collected in 300 μl culture medium to which 1 ml cold propidium iodide (PI) lysis buffer [50 μg/ml PI, 0.1% (wt/vol) trisodium citrate, and 0.1% (vol/vol) Triton X-100] was added. The aliquots were briefly vortexed and stored overnight at 4 °C. The percentage of sub-G1 nuclei was quantified by FACS on the basis of the PI fluorescence intensity (FL-2H) histograms.

Northern blotting. Total RNA was extracted from MIN6 cells using the Rneasy mini kit (Qiagen). RNA concentration was determined spectrophotometrically. The c-myc probe was derived from a PCR-II based plasmid containing a 1.1 kbp fragment of rat c-myc cDNA as verified by restriction mapping and DNA sequencing. The actin probe was kindly provided by E. Quartier (Department of Biochemistry, Faculty of Medicine, Brussels Free University, Brussels, Belgium). c-Myc and actin probes were labeled with [α^{32}P]dCTP (Amersham) using the Megaprime labeling kit (Amersham Pharmacia Biotech) as instructed by the manufacturer.

Ten micrograms total RNA of each sample was loaded on formaldehyde gels and run at 80 V until the bromophenol blue dye front had migrated approximately 10 cm. Gels were photographed in ethidium bromide/UV. The RNA was transferred to nylon membranes using capillary transfer. After pre-hybridization in solution containing 50% formamide, 5× SSC, 2× Denhardt's reagent, and 0.1% SDS at 42 °C for 2 h, the blots were hybridized with radiolabeled probes at 60 °C for at least 6 h. After a stringent wash in 0.2× SSC + 2.5% SDS for 30 min, autoradiographs were established by exposing the filters for 9 h to X-ray film at −70 °C with intensifying screens.

Western blotting. MIN6 cells were detached from culture flasks by brief incubation at 37 °C in PBS containing 1 mM EDTA and 1% BSA. After two washes, the harvest was lysed by 30 s sonication in RIPA buffer containing protease and phosphatase inhibitors [19], and then cleared by centrifugation (12,000*g*, 5 min). Samples were boiled under reducing conditions, resolved by standard 12–15% SDS–PAGE, and transferred to nitrocellulose membranes (Schleicher and Schuell). Membranes were blocked and incubated in 5% non-fat milk (w/v) in TBS/Tween (10 mM Tris–HCl, pH 7.5, 150 mM NaCl, and 0.05% (v/v) Tween 20). Monoclonal IgG1 antibody directed against human c-Myc (clone C-33, Biosource), and a polyclonal antibody directed against β actin (Santa Cruz Biotechnology) were added at 1:200–1:1000 dilution, and the membranes were incubated overnight with shaking at 4 °C.

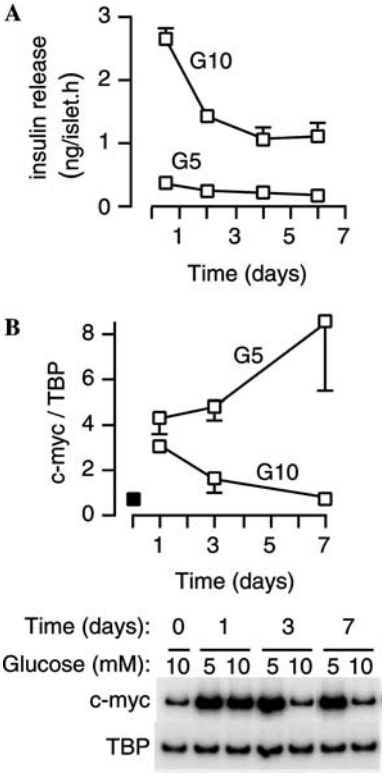

Fig. 1. Effects of prolonged culture in low glucose on islet c-myc mRNA levels and insulin secretion. Batches of 40–50 rat islets were cultured in RPMI medium in the presence of 5 mM (G5) or 10 mM (G10) glucose and various test substances. (A) The medium was renewed after 1 day, then every other day, and saved for insulin secretion measurement. (B) Total RNA was extracted from fresh islets directly after isolation (filled square) or after 1, 3, and 7 days of culture in G5 or G10 as indicated. The islet c-myc/TBP mRNA ratios were determined by duplex RT-PCR as described in Materials and methods.

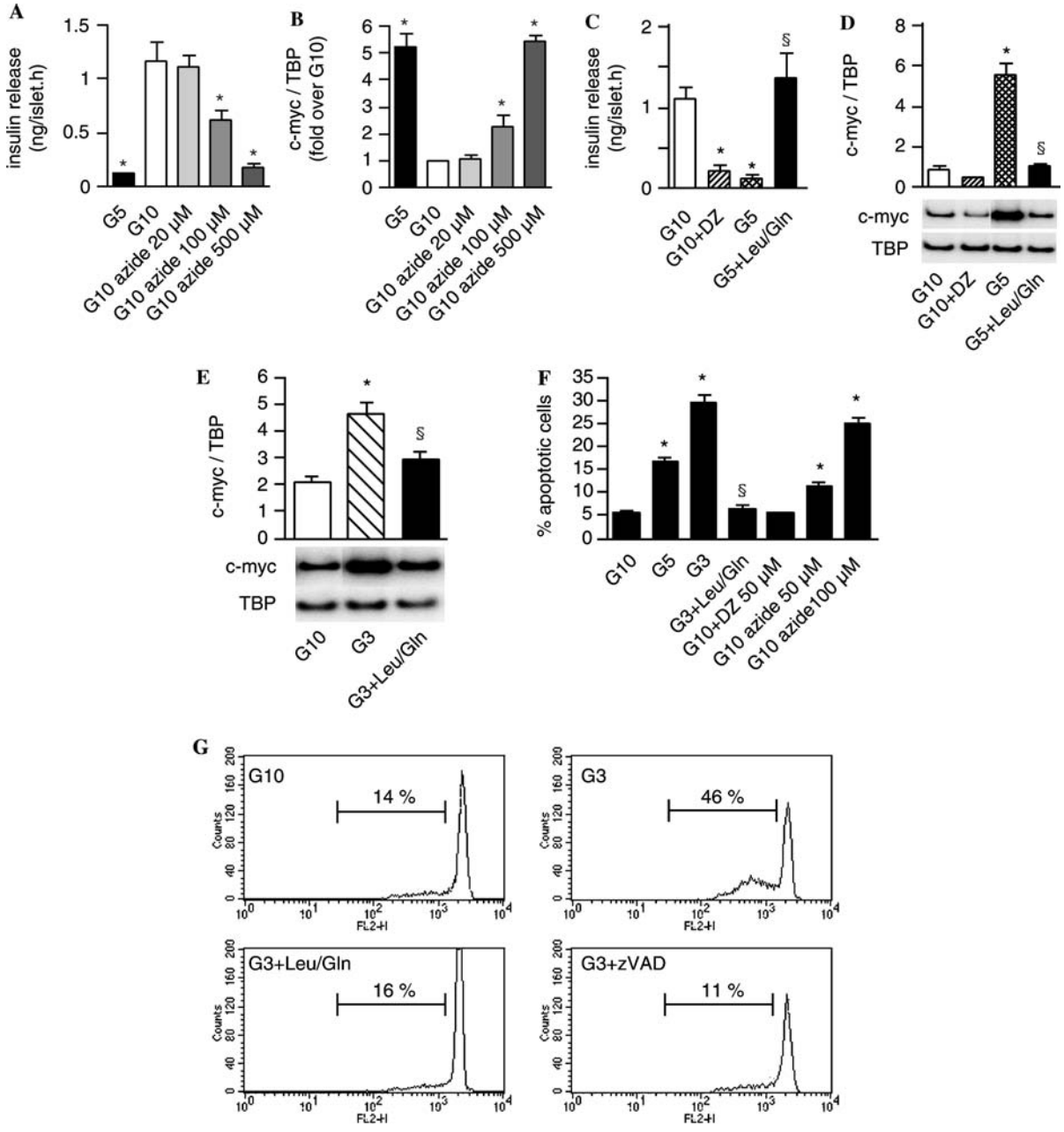

Fig. 2. Correlation between low-glucose-induced c-myc mRNA expression and apoptosis of β-cells. (A,B) After 1 week preculture in G10, the islets were cultured 18 h in G5, or in G10 plus increasing concentrations of azide, and their insulin secretion and c-myc/TBP mRNA ratio were determined. (C) Insulin secretion during the last 2 days of a 5 days culture and (D) islet c-myc/TBP mRNA ratios at the end of culture (5 days) in the presence of G5 or G10 and various test substances: 50 µM diazoxide (DZ) or 5 mM leucine + 5 mM glutamine (Leu/Gln). (E) Purified reaggregated rat β-cells were cultured for 2 days in 10 or 3 mM glucose (G10–G3) without or with 5 mM leucine + 5 mM glutamine (Leu/Gln). Total RNA was extracted and the β-cell c-myc/TBP mRNA ratio was determined by duplex RT-PCR. (F) Single β-cells were cultured for 4 days in G10, G5 or G3 alone, or G3 containing 5 mM leucine + 5 mM glutamine (Leu/Gln), or in G10 supplemented with the substances diazoxide (DZ) or azide, as indicated. The percentage of apoptotic and necrotic cells was then determined by fluorescence microscopy using the nuclear dyes propidium iodide and Hoechst 3342. Necrotic index was <5% in all conditions, except for 100 µM azide where 15.7 ± 0.8% necrosis was induced. (G) β-Cells were cultured for 3 days as described in (E), in the absence or presence of the pan-caspase-inhibitor z-VAD-fmk, after which their DNA content was quantified by FACS. The percentage of cells that displayed a sub-G1 DNA content is indicated above the horizontal bars corresponding to the gate setting. The results are means ± SE for 3–4 experiments. * Significantly different from G10 ($p < 0.05$ or less); § significant effect of test substance vs. G3 or G5 alone ($p < 0.05$ or less). FACS-histograms similar to those shown were obtained in 2–3 experiments.

They were then washed three times for 15 min in TBS/Tween and incubated with appropriate horseradish-peroxidase-linked whole secondary antibodies (Amersham). The membranes were washed as described above and revealed using the ECL reaction (Amersham).

Adenoviral infection of β-cells. Single rat β-cells were infected as previously described [20], with an adenovirus containing both the GFP- and the human c-myc gene (Ad.GFP/c-myc) or with a control adenovirus (Ad.GFP) containing only the GFP gene. In other

experiments, cells were infected with an empty adenovirus (Ad.null) or with an adenovirus encoding a rat c-myc antisense under the control of the CMV promoter (Ad.AS-c-myc), which was shown by one of us (D.K.S.) to reduce c-Myc protein expression in hepatoma cells [21]. Recombinant adenovirus Ad.GFP/c-myc expressing c-Myc was previously described [22] and kindly provided by Dr. H. Hermeking (Max-Planck-Institute for Biochemistry, Martinsried, Munich, Germany). Adenovirus-mediated expression of c-myc and AS-myc in β-cells was verified by RT-PCR using specific primers; c-myc: forward 5′-TTC TGT GGA AAA GAG GCA GGC-3′ and reverse 5′-GCT CCG TTT TAG CTC GTT CCT-3′; AS-myc: forward 5′-ACT CGC TGT AGT AAT TCC AGCG-3′ and reverse 5′-CGG AAT TCT TCT CTT CCT CGTC-3′. RT-PCR in the absence of reverse transcriptase, performed on cells infected with adenovirus expressing the c-myc gene, gave no PCR-bands.

Statistical methods. Results are shown as means ± SEM, or representative traces and pictures, for the indicated number of independent experiments. Statistical significance of differences between groups was determined by one-way ANOVA followed by a test of Newman–Keuls or by two-way ANOVA followed by a test of Bonferroni.

Results

Induction of c-myc mRNA expression and apoptosis in rat islets and β-cells cultured in low-glucose concentrations

Rat pancreatic islets were cultured for 1 week in the presence of 5 or 10 mM glucose (G5 or G10). As expected, insulin secretion was low during culture in G5 and significantly stimulated by G10 at all time points ($p < 0.01$ by two-way ANOVA followed by a test of Bonferroni) (Fig. 1A). The c-myc/TBP mRNA ratio was low in freshly isolated islets. It increased to a similar extent after overnight culture in G5 or G10, and eventually returned to its initial value within 1 week of culture in G10 (Fig. 1B) [9]. In contrast, the increase in c-myc/TBP mRNA ratio was sustained during prolonged culture in G5 (Fig. 1B). As a result, the islet c-myc/TBP mRNA ratio was significantly higher after 5 and 7 days of culture in G5 instead of G10 ($p < 0.01$) (Fig. 1B). In islets precultured for 1 week in G10, further overnight culture in G5 instead of G10 induced a ∼90% decrease in insulin secretion and a ∼5-fold increase in c-Myc/TBP mRNA ratio (Figs. 2A and B). The stimulation of islet c-myc mRNA expression by G5 was also observed during culture in a medium containing 10% FCS instead of BSA (not shown).

In purified β-cells precultured overnight in G10, the c-myc/TBP mRNA ratio was significantly increased after a further 2 days culture in G3 rather than G10 (Fig. 2E). The prolonged culture in G3, or in G5, subsequently induced β-cell apoptosis (sixfold increase in G3 vs. G10, $p < 0.01$; Fig. 2F), an effect that was completely prevented by addition of the caspase-inhibitor z-VAD-fmk (Fig. 2G).

Effect of a combination of leucine and glutamine on β-cell c-myc mRNA expression and apoptosis at low-glucose concentration

When islets were cultured in G5 + 5 mM Leu and 5 mM Gln, insulin secretion was stimulated to the same extent as during culture in G10 (Fig. 2C). After 5 days of culture under these conditions, the c-myc/TBP mRNA ratio was not different from that measured after culture in G10 (Fig. 2D). In purified β-cells also, the rise in c-myc/TBP mRNA ratio caused by a 2-day-culture in G3 was prevented by addition of the combination of Leu and Gln to the medium (Fig. 2E). Furthermore, this combination also suppressed the induction of β-cell apoptosis by G3, as documented by microscopical quantitation of apoptotic β-cells (Fig. 2F) or FACS analysis of the β-cell DNA content (Fig. 2G).

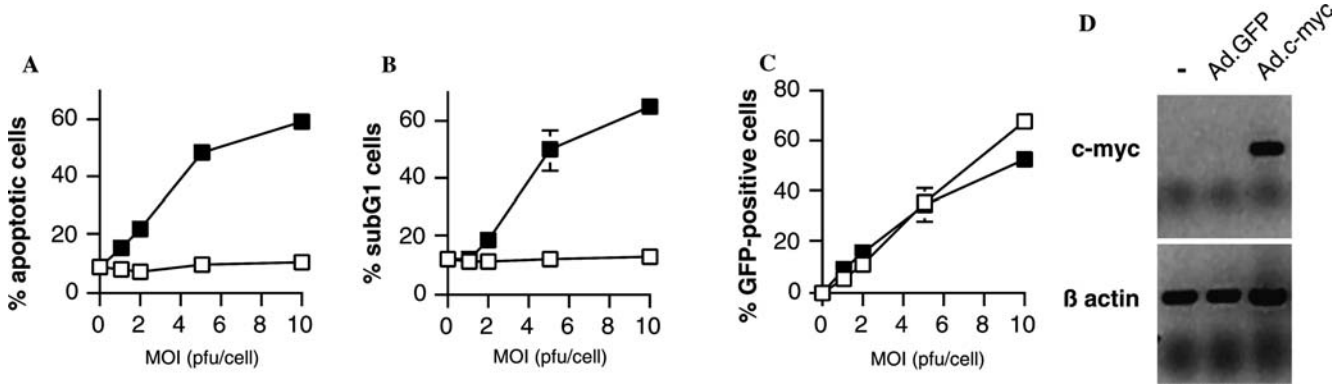

Fig. 3. Infection of rat β-cells by adenovirus expressing c-myc leads to induction of apoptosis. Single rat β-cells were infected at the indicated multiplicities (MOI) with recombinant adenovirus Ad.GFP/c-myc (closed symbols) or Ad.GFP (open symbols), which expressed c-myc and GFP or only GFP, respectively. The percentage of apoptotic cells was evaluated after 3 days of expression, either by fluorescence microscopy (A) as in Fig. 2B or by quantifying sub-G1 cells in FACS (B) as in Fig. 2C, while the percentage of transfected cells (% GFP-positive) had been evaluated by fluorescence microscopy (C) after 1 day of viral expression. Data represent means ± SE of 3–4 experiments. (D) Total RNA was extracted from uninfected β-cells or cells infected with either Ad.GFP or Ad.GFP/c-myc, and RT-PCR was performed for detecting c-myc and β actin mRNA as described in Materials and methods.

Effects of diazoxide and azide on β-cell c-myc mRNA expression and apoptosis in G10

During culture in G10, diazoxide, an opener of ATP-dependent K^+ channels, inhibited insulin secretion by ~80% to a level that was not different from that measured during culture in G5 (Fig. 2C). After 5 days of culture under these conditions, the islet c-myc/TBP mRNA ratio was not different from that measured after culture in G10 (Fig. 2D). In purified β-cells, diazoxide did not induce β-cell apoptosis in the presence of G10 (Fig. 2F).

In 1 week precultured islets, azide, an inhibitor of the respiratory chain complex IV, dose-dependently inhibited insulin secretion during further overnight culture in G10 to a level close to that measured in G5 (Fig. 2A). Under these or similar conditions, azide dose-dependently induced islet c-myc mRNA expression and β-cell apoptosis to the same extent as did culture in G3 or G5 (Figs. 2B and F).

Effect of adenovirus-mediated c-myc overexpression on β-cell apoptosis in vitro

β-Cells were infected with an adenovirus expressing the c-myc gene (Ad.GFP/c-myc) or with a control adenovirus (Ad.GFP) and then cultured for 3 days in G10. In contrast with Ad.GFP, Ad.GFP/c-myc induced a dose-dependent increase in β-cell apoptosis (Figs. 3A and B). Comparable infection efficiencies (ranging from 0% to 60% with MOI from 0 to 10 pfu/cell) were obtained with both viruses, as monitored by GFP-fluorescence of the cells (Fig. 3C). As expected, Ad.GFP/c-myc, but not Ad.GFP, strongly increased β-cell c-myc mRNA expression under these conditions (Fig. 3D).

Effect of c-myc antisense expression on β-cell apoptosis during culture in low glucose

β-Cells were infected with an adenovirus expressing part of the c-myc gene in antisense orientation (Ad.AS-c-myc) or with an empty control adenovirus (Ad.null), and then cultured for 3 days in G10, G5 or G3. Compared to Ad.null-infected or uninfected β-cells, the rate of apoptosis induced by culture in G5 and G3 instead of G10 was reduced by half in Ad.AS-c-myc infected β-cells (Fig. 4A). Only cells infected with Ad.AS-c-myc expressed RNA that could be amplified by RT-PCR using c-myc antisense-specific primers (Fig. 4B).

Effect of AMPK-activation on c-myc expression in MIN6 cells

Mouse insulinoma MIN6 cells also underwent apoptosis when exposed to low-glucose concentrations (Fig. 5A) [23]. In these cells, the c-myc mRNA (Fig. 5B)

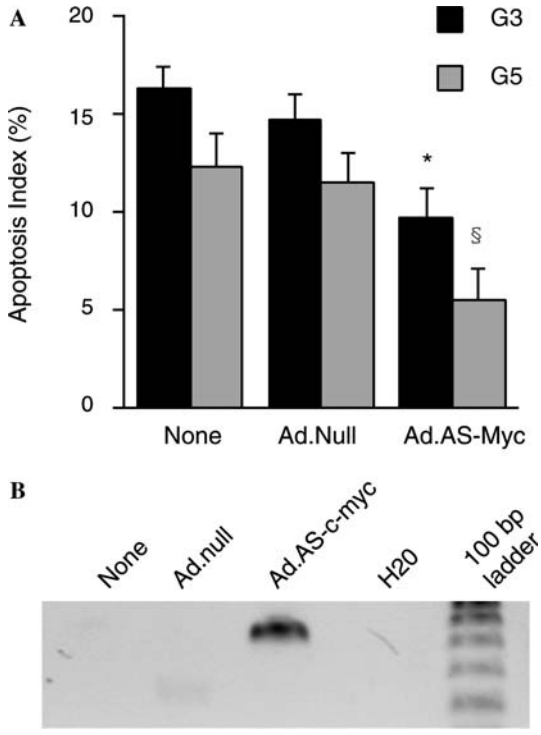

Fig. 4. Adenovirus-mediated antisense c-myc expression reduces low-glucose-induced apoptosis in β-cells. (A) Single rat β-cells were infected at MOI 20 for 2 h either with an adenovirus expressing part of the c-myc gene in antisense orientation (Ad.AS-c-myc) or with an empty adenovirus (Ad.null) (None) the cells remained uninfected. Virus was removed from the medium and the cells were further incubated for 3 days in G10, G5 or G3. Apoptosis was measured by fluorescence microscopy as in Fig. 2A. Data are expressed as apoptotic index (see Materials and methods) in G5 and G3 using G10 as control condition, and are means ± SEM for 6 independent experiments (*$p < 0.01$ vs. Ad.null or uninfected control by ANOVA). The necrotic index measured under the same conditions showed <2% variation between Ad.null and Ad.AS-c-myc infected cells. In G10, the absolute rate of apoptosis varied by <2% regardless of the virus used. (B) β-Cells either uninfected or infected at MOI 20 as in (A) were collected after 24 h culture in G10. Their RNA was extracted and probed for expression of antisense-c-myc message by RT-PCR using specific primers. Products from a 40-cycle reaction were electrophoresed on agarose gel showing specific amplification of the antisense product only in Ad.AS-c-myc infected cells. Result shown is representative for three experiments.

and protein levels (Fig. 5C) were increased after 24 h of culture in low glucose.

As with primary cells [4], glucose dose-dependently suppressed apoptosis in the MIN6 cells (Fig. 5D), and the combination of Leu and Gln reduced apoptosis at the lowest glucose concentration tested by ~50% (Fig. 5D). In these cells, low-glucose-induced apoptosis was also prevented by addition of a combination of lactate and pyruvate (Fig. 5D).

AMP-activated protein kinase (AMPK), a cellular sensor for energy depletion, is known to be activated in MIN6 cells cultured in low glucose [23,24]. Interestingly, the AMPK-activator 5-aminoimidazole-4-carboxamide-

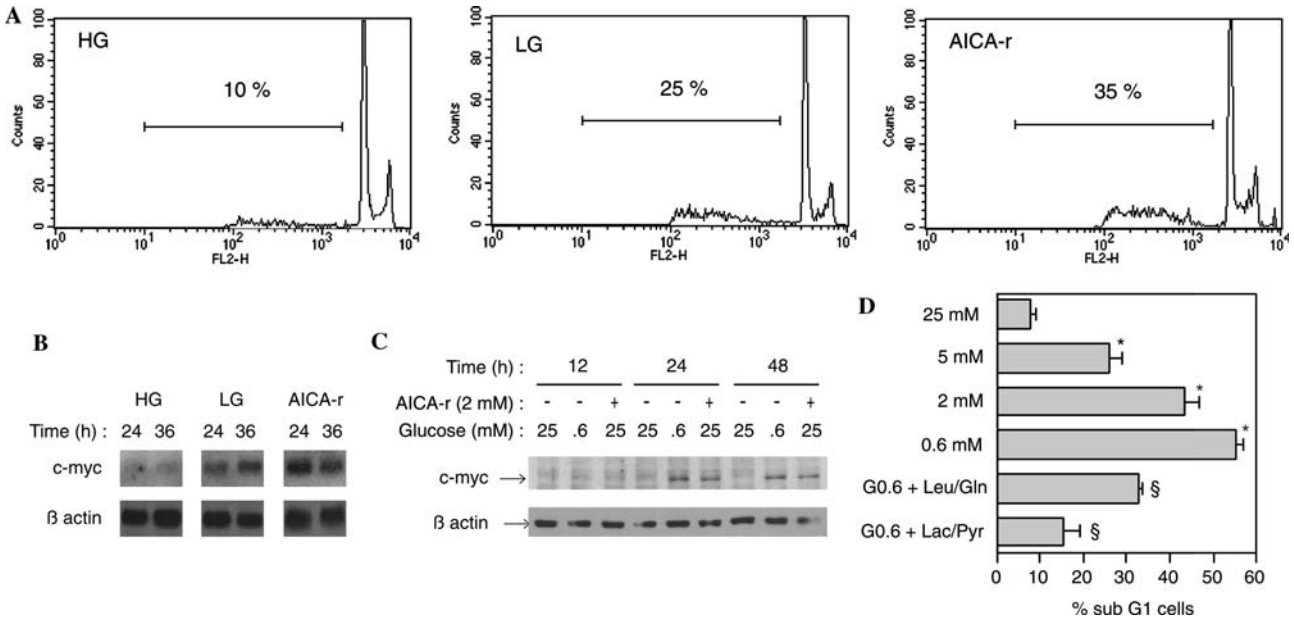

Fig. 5. Low-glucose- and AMPK-mediated apoptosis of MIN6 cells is associated with increased c-myc expression. MIN6 cells were exposed for various periods of time to medium containing either 25 or 0.6 mM glucose (HG and LG, respectively), or to medium containing 25 mM glucose supplemented with 2 mM AICA-riboside (AICA-r). (A) Representative FACS histograms for propidium iodide fluorescence of MIN6 cells that were exposed for 24 h. The sub-G1 range is indicated by a horizontal bar above which the percentage of nuclei containing a sub-G1 amount of DNA is indicated. (B) Northern blotting with 10 μg of total RNA extracted from the MIN6 cells using rat c-myc cDNA as a probe. Signals obtained with a β actin cDNA probe indicate relative RNA loading. (C) Western blotting with 25 μg of total protein from the MIN6 cells using a monoclonal anti-c-Myc antibody (c-33). Actin was detected to evaluate protein loading. Results shown are representative of three experiments. (D) MIN6 cells were cultured for 36 h in the indicated glucose concentrations, without additions, or with 5 mM leucine + 5 mM glutamine (Leu/Gln) or 10 mM lactate + 1 mM pyruvate (Lac/Pyr). Apoptotic cells containing sub-G1 DNA were detected as described in (A). Their percentage of total cells is expressed as mean ± SE of four experiments (*$p < 0.01$ vs G25; § $p < 0.01$ significant effect vs G0.6).

riboside (AICA-riboside) upregulated c-myc mRNA and protein levels in MIN6 cells cultured in the presence of 25 mM glucose to a similar extent as culture in 0.6 mM glucose (Figs. 5B and C).

Discussion

This work shows that β-cell apoptosis induced by culture in low glucose partly results from the upregulated expression of the pro-apoptotic transcription factor c-Myc. First, in whole islets and purified β-cells, culture in G3 or G5 triggered a large, rapid and sustained increase in c-myc expression that was followed by induction of a caspase-dependent apoptosis. Our study also demonstrates that culture in low glucose for 24–48 h induces a parallel increase in c-myc mRNA and protein expression in insulin-secreting MIN6 cells. Second, adenovirus-mediated in vitro c-myc overexpression was sufficient to induce apoptosis of primary β-cells, in agreement with recent reports of an increased rate of β-cell apoptosis in transgenic mice that overexpress c-myc in vivo under the control of the insulin promoter [11,12]. It therefore appears that c-Myc is a potent effector of apoptosis in pancreatic β-cells, both in vivo and in vitro. Third, we showed that adenovirus-mediated expression of antisense-c-myc, which reduced c-Myc protein levels and transcriptional activity in a rat hepatoma cell line by ~50% [21], inhibited β-cell apoptosis induced by culture in G3 or G5 to a similar extent. The partial reversal of apoptosis may result from only a partial c-myc "knockdown," likely due to incomplete Ad.AS-c-myc transfection under our culture conditions, as observed for Ad-c-myc/GFP and AdGFP transfection experiments.

Our results suggest that the stimulation of β-cell c-myc expression and apoptosis by culture in low glucose results from a reduction in mitochondrial metabolism, rather than from a lack of Ca^{2+} influx and insulin secretion under these conditions. Thus, β-cell apoptosis was prevented by leucine and glutamine, a combination which stimulates β-cell metabolism, hence Ca^{2+} influx and insulin secretion, in a similar manner as a rise in glucose [25,26]. Conversely, c-myc expression and apoptosis were induced in G10 by inhibition of β-cell ATP production and insulin secretion with the complex IV respiratory chain inhibitor azide [27]. In contrast, c-myc expression and apoptosis were not induced by inhibition of G10-induced Ca^{2+} influx and insulin secretion with a low concentration of the ATP-sensitive K^+ channel opener diazoxide that does not reduce β-cell energetic level [28]. In addition, low-glucose-induced apoptosis

was suppressed by lactate and pyruvate in MIN6 cells that, unlike primary β-cells, seem to efficiently metabolize these exogenous substrates, probably because they express lactate dehydrogenase and the monocarboxylate transporter 4 [29,30]. Our findings indicate that both c-myc expression and apoptosis in β-cells are induced as a result of energy depletion secondary to glucose deprivation. It has been shown that culture of β-cells or MIN6 cells at low-glucose concentrations activates their AMP-activated protein kinase (AMPK), a metabolic sensor for energy depletion ([23,24,31–33] and unpublished results). Interestingly, the AMPK-activator AICA-riboside induced parallel increases in MIN6 cell c-myc expression and apoptosis [23,33]. It is therefore possible that AMPK is involved in the stimulation of β-cell c-myc expression by prolonged nutrient limitation. However, our findings do not exclude other pro-apoptotic signal transduction pathways unrelated to AMPK. Noteworthy, β-cell c-myc expression is also stimulated by pro-apoptotic cytokines [34]. How a decrease in metabolic fuel stimulates c-myc expression in β-cells therefore requires further study.

Switching on c-myc profoundly affects growth, proliferation, differentiation, and survival in various cell types [7]. In differentiated cells, c-myc expression is kept at very low, almost undetectable levels [11]. c-Myc induction may therefore be a marker of phenotypic alteration in normal cells. c-Myc expression can be induced by culture in either low glucose (the present study) or high glucose (G30 instead of G10) [9]. The V-shaped glucose concentration response curve for islet c-myc expression, with a minimum in the presence of G10, is reminiscent of a similar curve for β-cell apoptosis [35]. However, in purified rat β-cells, the rate of apoptosis was not increased by prolonged culture in G20 instead of G10 [4]. Therefore, the possible link between β-cell apoptosis and high-glucose-induced c-myc expression merits further investigation.

In conclusion, prolonged culture of β-cells in the presence of low-glucose concentrations markedly induces c-myc expression, an effect that contributes to the subsequent stimulation of β-cell apoptosis under these conditions.

Acknowledgments

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