Effects of c-MYC activation on glucose stimulus-secretion coupling events in mouse pancreatic islets

Séverine M. A. Pascal, Yves Guiot, Stella Pelengaris, Michael Khan, and Jean-Christophe Jonas

1Unit of Endocrinology and Metabolism and 2Service of Pathology, Faculty of Medicine, Université catholique de Louvain, Brussels, Belgium; and 3Cancer Biology Group, Biomedical Research Institute, Warwick Medical School, and 4Department of Biological Sciences, University of Warwick, Coventry, United Kingdom

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Pascal SM, Guiot Y, Pelengaris S, Khan M, Jonas J.C. Effects of c-MYC activation on glucose stimulus-secretion coupling events in mouse pancreatic islets. Am J Physiol Endocrinol Metab 295: E92–E102, 2008. First published April 15, 2008; doi:10.1152/ajpendo.90235.2008.—Alteration of pancreatic β-cell survival and Preproinsulin gene expression by prolonged hyperglycemia may result from increased c-MYC expression. However, it is unclear whether c-MYC effects on β-cell function are compatible with its proposed role in glucotoxicity. We therefore tested the effects of short-term c-MYC activation on key β-cell stimulus-secretion coupling events in islets isolated from mice expressing a tamoxifen-switchable form of c-MYC in β-cells (MycER) and their wild-type littermates. Tamoxifen treatment of wild-type islets did not affect their cell survival, Preproinsulin gene expression, and glucose stimulus-secretion coupling. In contrast, tamoxifen-mediated c-MYC activation for 2–3 days triggered cell apoptosis and decreased Preproinsulin gene expression in MycER islets. These effects were accompanied by mitochondrial membrane hyperpolarization at all glucose concentrations, a higher resting intracellular calcium concentration ([Ca2+]i), and lower glucose-induced [Ca2+]i rise and islet insulin content, leading to a strong reduction of glucose-induced insulin secretion. Compared with these effects, 1-wk culture in 30 mmol/l glucose increased the islet sensitivity to glucose stimulation without reducing the maximal glucose effectiveness or the insulin content. In contrast, overnight exposure to a low H2O2 concentration increased the islet resting [Ca2+]i, and reduced the amplitude of the maximal glucose response as in tamoxifen-treated MycER islets. In conclusion, c-MYC activation rapidly stimulates apoptosis, reduces Preproinsulin gene expression and insulin content, and triggers functional alterations of β-cells that are better mimicked by overnight exposure to a low H2O2 concentration than by prolonged culture in high glucose.

β-cell mass; apoptosis; cytosolic calcium concentration; insulin secretion; mitochondrial membrane potential

IT IS WELL ESTABLISHED that chronic hyperglycemia exerts deleterious effects on β-cell gene expression, survival, and function by a process often referred to as glucotoxicity (11). Thus, in 90% pancreaticectomized rats and other rodent models of in vivo hyperglycemia, β-cells display decreased expression of genes important for the glucose stimulation of insulin secretion (GSIS), such as Glut2, Glucokinase, and Preproinsulin (Ppi), and of some transcription factors that control their expression, like MafA, Pdx1, Beta2/NeuroD, and Nkx6.1 (9, 17, 18). They also present increased expression of genes, such as the glycolytic enzymes Hexokinase 1 and Lactate dehydrogenase A, and of transcription factors that control their expression, like c-Myc (8, 9, 17). Altogether, these changes may contribute to the higher glucose sensitivity and lower glucose responsiveness of β-cells previously exposed to high glucose concentrations (14). This reduced degree of β-cell differentiation is associated with an increase in the size of individual β-cells (hypertrophy) and, in some models, with an increase in the rate of β-cell apoptosis (9, 15, 17, 18).

c-MYC is a basic helix-loop-helix leucine zipper (bHLH-LZ) transcription factor that heterodimerizes with its partner Max to modulate the expression of a large number of genes regulating cell proliferation, growth, differentiation, and apoptosis (4). Thus, although c-Myc was initially characterized as a cellular oncogene involved in the pathogenesis of Burkitt’s lymphomas and lymphoid malignancies (6), it has also been shown to sensitize cells to various apoptotic stimuli such as hypoxia, serum depletion, and nutrient deprivation (10). We and others have provided some evidence that c-MYC could play a role in β-cell glucotoxicity. Thus c-Myc mRNA levels were increased in rat islets exposed to high glucose concentrations both in vitro and in vivo (8, 9), and its forced expression or acute controlled activation in pancreatic β-cells triggered cell apoptosis more than proliferation, leading to a net reduction of β-cell mass and development of diabetes (19, 24). Under these conditions, c-MYC activation also reproduced several alterations in β-cell gene expression typically induced by hyperglycemia, such as reduced expression of Ppi and other genes important for β-cell function, and increased expression of c-MYC target genes such as Ornithine decarboxylase (Odc) and Lactate dehydrogenase A (12, 19, 24). However, other studies have shown that c-MYC expression is also increased by prolonged exposure to low glucose concentrations and by short-term treatment with hydrogen peroxide (H2O2), two conditions under which β-cell survival and function were also markedly altered, although in a different manner than by high glucose (5, 13, 30). Thus, although it has been shown that forced c-MYC expression significantly reduces the maximal rate of GSIS in parallel with the islet insulin content (12), it is unclear whether its effects on β-cell function are compatible with its proposed role in β-cell glucotoxicity. We therefore tested the effects of short-term c-MYC activation on key β-cell stimulus-secretion coupling events in islets isolated from mice expressing a switchable form of c-MYC in pancreatic β-cells (24).
MATERIALS AND METHODS

Solutions and reagents. Most experiments were performed at 37°C with a bicarbonate-buffered Krebs solution containing (in mmol/l) NaCl (120), KCl (4.8), CaCl2 (2.5), MgCl2 (1.2), NaHCO3 (24), and various glucose concentrations (Gx). This solution was supplemented with 1 g/l BSA (fraction V; Roche, Basel, Switzerland) and was continuously gassed with O2/CO2 (94/6) to maintain a pH of 7.4. When the concentration of KCl was raised to 30 mmol/l, that of NaCl was reduced to 94.8 mmol/l to keep the medium osmolality unchanged. Diazoxide (Sigma, St. Louis, MO) was dissolved to 50 mmol/l in 0.1 N NaOH and used at a final concentration of 250 μmol/l.

Animals. Plns-c-MycER

TILL Photonics, Martinsried, Germany) using excitation/emission

Morphological analysis. Pancreases were removed, weighed, fixed in Bouin’s fluid, and embedded in paraffin. Four-μm-thick sections were then simultaneously processed for hematoxylin and eosin or insulin staining as described previously (14). Images from these sections were obtained with an Axiosplan microscope coupled to an Axioscam HRc digital camera (using fixed camera settings) and analyzed with Axiovision 3.1 software (Carl Zeiss, Oberkochen, Germany). The β-cell and total cell volume densities (Vv) were estimated on insulin and hematoxylin-eosin stained sections by the point-counting technique (2 sections 400 μm apart, 3,000 points/section) at ×250 magnification (26). For each mouse, the β-cell mass (mg) was computed by multiplying the mean relative proportion of β-cells (β-cell Vv/total cell Vv) by the weight of the pancreas.

RNA extraction, cDNA synthesis, and real-time PCR. Islet total RNA was extracted and reverse transcribed into cDNA using random hexamers and 200 units of M-MLV Reverse Transcriptase Rnase H− Point Mutant (Promega, Madison, WI). Real-time PCR was performed with an iCycler IQ Real Time PCR Detection System (Bio-Rad, Hercules, CA). Primer sequences and reaction conditions are detailed in Supplemental Table S1 (supplemental data are available at the online version of this article). Gene-to-βTbp mRNA ratios were expressed relative to the mRNA ratio in WT islets.

RESULTS

Characteristics of plns-c-MycER

Islets were isolated from 108 MycER and 149 WT mice between 3 and 20 mo of age (226 ± 11 days for MycER mice and 257 ± 13 days for WT mice). The body weight and fed blood glucose levels on the day of isolation were significantly lower in MycER than WT mice (25 ± 0.4 vs. 27 ± 0.4 g body wt and 4.2 ± 0.1 vs. 6.6 ± 0.1 mmol/l glucose; P < 0.0001 by Student’s t-test for both parameters). Of note, blood glucose levels decreased with age, irrespective of the genotype.
MycER transgenic mice

Metabolic characteristics of WT and heterozygous MycER transgenic mice

<table>
<thead>
<tr>
<th></th>
<th>WT (n)</th>
<th>MycER (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>27 ± 1 (10)</td>
<td>23 ± 1 (10)*</td>
</tr>
<tr>
<td>Food intake, mg·day⁻¹·g body wt⁻¹</td>
<td>179 ± 17 (10)</td>
<td>205 ± 23 (10)</td>
</tr>
<tr>
<td>Fed blood glucose, mmol/l</td>
<td>6.0 ± 0.5 (6)</td>
<td>5.8 ± 0.2 (6)</td>
</tr>
<tr>
<td>Fed plasma insulin, pmol/l</td>
<td>69 ± 7 (6)</td>
<td>61 ± 11 (6)</td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/l</td>
<td>4.6 ± 0.6 (10)</td>
<td>1.3 ± 0.1 (10)†</td>
</tr>
<tr>
<td>Fasting plasma insulin, pmol/l</td>
<td>22 ± 6 (10)</td>
<td>48 ± 7 (10)*</td>
</tr>
</tbody>
</table>

Wild-type (WT) and heterozygous pIns-c-MycERTAM (MycER) mice from the same litters were studied at 5-7 mo of age. Food intake was measured over a 3-day period. Data are means ± SE for 6 females and 4 males of each genotype (n = 10) or 3 mice of each gender and each genotype (n = 6). *P < 0.05 and †P < 0.01 vs. WT by Student’s t-test.

These results are compatible with the recent suggestion that the MycERTAM protein construct may not be fully repressed in the absence of TAM (3). To evaluate that hypothesis, the experiments were repeated in the presence of FBS, a well-characterized inhibitor of c-MYC-induced cell apoptosis (10). Under these conditions, the caspase activity in DMSO-treated MycER islets was no longer different from that in WT islets and was much less increased by TAM treatment than in the absence of FBS (Fig. 1F). Despite this inhibition of islet cell apoptosis, changes in islet DNA content were not affected by the presence of FBS (data not shown). In contrast, the Ppi mRNA levels were slightly less reduced in DMSO-treated MycER vs. WT islets than in the absence of FBS, and their reduction by TAM treatment was proportionately enhanced (Fig. 1C).

**Effects of c-MYC activation on glucose stimulus-secretion coupling events.** After 1-wk culture in serum-free RPMI medium containing 10 mmol/l glucose and 5 g/l BSA, stepwise stimulation of WT islets from 0.5 to 7, 15, and 30 mmol/l glucose (G0.5 to G7, G15, and G30, respectively) induced a concentration-dependent decrease in rhodamine-123 fluorescence, demonstrating mitochondrial membrane hyperpolarization (Fig. 2A). As expected, addition of 5 mmol/l Na⁺-azide to

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Table 1. Metabolic characteristics of WT and heterozygous MycER transgenic mice

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Fig. 1. Effects of c-MYC activation on Ornithine decarboxylase (Odc; A) and Preproinsulin I + II (Ppi; B and C) mRNA levels, DNA content (D), and caspase activity (E and F) in cultured mouse islets. After 4–5 days of preculture in standard medium containing 10 mmol/l glucose and 5 g/l BSA or 10% (vol/vol) FBS as indicated at top, islets from wild-type (WT) and heterozygous transgenic pIns-c-MycER TAMB⁻⁺ (MycER) mice were treated for 18 h (A–C), 48 h (E and F), or 72 h (D) with 100 mmol/l 4-OH-tamoxifen (TAM; solid columns) or vehicle alone (DMSO, 1/1,000; open columns). Gene-to-Tbp mRNA ratios were normalized to the ratio in WT islets treated with DMSO. Tbp, TATA-box binding protein; nd, not determined. Data are means ± SE for at least 3 experiments. *P < 0.05 or less by 1-way ANOVA + test of Newman-Keuls or §P < 0.05 by Student’s t-test for the effect of TAM. *P < 0.05 or less by 1-way ANOVA + test of Newman-Keuls or &P < 0.05 by Student’s t-test for the effect of strain (MycER vs. WT).
G30 rapidly depolarized the mitochondrial membrane to a level that was used as 100% reference for data normalization. The stimulation with glucose also triggered the usual changes in islet $[\text{Ca}^{2+}]_i$ and insulin secretion, with a slight decrease of $[\text{Ca}^{2+}]_i$ without changes in insulin secretion in G7, and concentration-dependent rises in $[\text{Ca}^{2+}]_i$ and secretion above G7 that were fully abrogated by addition of the K$^+$-ATP channel opener diazoxide (Dz) (Fig. 2, B and C). Subsequent depolarization with 30 mmol/l extracellular K$^+$ (K30) in the continued presence of G30 and Dz rapidly increased both $[\text{Ca}^{2+}]_i$ and insulin secretion.

These glucose stimulus-secretion coupling events and the islet insulin-to-DNA content ratio were totally unaffected by TAM treatment of WT islets (Fig. 2, A–C). In contrast, they were clearly altered in DMSO-treated MycER islets and further impaired by TAM-mediated c-MYC activation (Fig. 2, D–F). Thus, in DMSO-treated MycER vs. WT islets, the decrease in rhodamine-123 fluorescence triggered by glucose was of similar amplitude but of lower sensitivity to glucose, with only 35% of the effect occurring between G0.5 and G7 compared with 65% in WT islets (Fig. 2D). In addition, the glucose-induced rise in $[\text{Ca}^{2+}]_i$ was of lower amplitude and sensitivity to glucose, with only 25% of the maximal glucose effect occurring between G7 and G15 compared with 60% in WT islets (Fig. 2E; Table 2). This defect, together with a 60% lower insulin-to-DNA content ratio, led to a 85% reduction of the maximal glucose-induced insulin secretion (Fig. 2F; Table 3).
was markedly reduced by islets. Consequently, the stimulation of insulin secretion by glucose-induced changes in \([Ca^{2+}]_i\) was acutely increased from G0.5 to G30, TAM- and significantly reduced the amplitude of the \([Ca^{2+}]_i\) response to K30 (Fig. 2; Table 2). In addition, c-MYC response to G30 stimulation without significantly reducing the stimulus-secretion coupling events induced by c-MYC activation in the presence of BSA on subsequent glucose-induced changes in insulin secretion.

Table 2. Effects of c-MYC activation in the presence of BSA on subsequent glucose-induced \([Ca^{2+}]_i\) changes in mouse islets

<table>
<thead>
<tr>
<th>Islet Strain and Culture Conditions (BSA)</th>
<th>(n)</th>
<th>G0.5</th>
<th>(\Delta (G7-G15))</th>
<th>(\Delta (G7-G30))</th>
<th>(\Delta (G30Dz-G30K30Dz))</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT DMSO</td>
<td>22</td>
<td>95±7</td>
<td>132±16</td>
<td>214±24</td>
<td>376±22</td>
</tr>
<tr>
<td>WT TAM</td>
<td>22</td>
<td>95±6</td>
<td>154±13</td>
<td>251±18</td>
<td>396±18</td>
</tr>
<tr>
<td>MycER DMSO</td>
<td>37</td>
<td>66±4</td>
<td>18±2†</td>
<td>73±7†</td>
<td>184±10†</td>
</tr>
<tr>
<td>MycER TAM</td>
<td>35</td>
<td>114±9*</td>
<td>19±3*</td>
<td>42±6†</td>
<td>154±12†</td>
</tr>
</tbody>
</table>

Results are means ± SE for the indicated no. of islets (n). In each experiment illustrated in Fig. 2, B and E (see Fig. 2 legend for experimental details), the delta intracellular calcium concentration ([Ca^{2+}]_i) between G7 and G15, G7 and G30, and G30Dz and G30K30Dz was computed from the average [Ca^{2+}]_i during the last 3 min in G0.5, the last 10 min in G7, G15, G30 and G30Dz, and the last 7 min in G30K30Dz. G0.5, 0.5 mmol/l glucose; G7, 7 mmol/l glucose; and so forth. K30, 30 mmol/l extracellular K+; Dz, diazoxide. *P < 0.001 or less for the effect of 4-OH-tamoxifen (TAM) vs. DMSO (Student’s t-test). †P < 0.001 for the effect strain (MycER vs. WT) (2-way ANOVA).

stimulation were much less affected in DMSO-treated MycER vs. WT islets.

In MycER islets treated for 3 days with 100 nmol/l TAM, the normalized rhodamine-123 fluorescence levels during perfusion with G0.5 were lower than in DMSO-treated MycER islets, suggesting that their mitochondria were more hyperpolarized. Nevertheless, stepwise glucose stimulation reduced the rhodamine-123 fluorescence as in DMSO-treated islets, indicating that glucose was still able to hyperpolarize their mitochondria (Fig. 2D). c-MYC activation also increased islet resting [Ca^{2+}]_i, measured in G0.5 or G30+Dz by ~50 mmol/l, and significantly reduced the amplitude of the [Ca^{2+}]_i rise in response to G30 stimulation without significantly reducing the response to K30 (Fig. 2E; Table 2). In addition, c-MYC activation decreased the islet insulin-to-DNA content ratio by ~55%, down to a level that was <20% of the ratio in WT islets. Consequently, the stimulation of insulin secretion by glucose was completely suppressed, whereas the effect of K30 was markedly reduced by ~95% (Fig. 2F; Table 3). When glucose was acutely increased from G0.5 to G30, TAM-mediated activation of c-MYC similarly affected the basal and glucose-induced changes in [Ca^{2+}]_i, with a clear reduction of both the initial peak and the subsequent oscillations of [Ca^{2+}]_i, (Fig. 3, A and D).

Because culture in the presence of FBS improved cell survival in both DMSO- and TAM-treated MycER islets (Fig. 1, E and F), we also tested whether the alterations of glucose stimulus-secretion coupling events by c-MYC activation would be reduced by FBS. As expected, acute stimulation of WT islets from G0.5 to G30 induced a transient decrease in [Ca^{2+}]_i, followed by a rapid rise in [Ca^{2+}]_i, and insulin secretion that was only slightly further increased by addition of K30 with Dz (Fig. 3, B and C). These responses were almost identical in TAM-treated WT islets. As observed after culture in the absence of FBS, MycER islets displayed a 30–50% lower glucose-induced rise in [Ca^{2+}]_i than WT islets (Fig. 3E, Supplemental Table S2) and a 60% reduction of their insulin-to-DNA content ratio (P < 0.01 by 2-way ANOVA + test of Bonferroni), leading to a 95% lower glucose-induced insulin secretion (Fig. 3F, Supplemental Table S3). Under these culture conditions, TAM-mediated c-MYC activation increased islet resting [Ca^{2+}]_i, by ~40 nmol/l and further reduced the amplitude of the first and second phases of glucose-induced rise in [Ca^{2+}]_i, by ~50% (Fig. 3E, Supplemental Table S2). In addition, c-MYC activation reduced the insulin-to-DNA content ratio by 77% (P < 0.05 by 2-way ANOVA + test of Bonferroni) and tended to decrease by 44% and delay by 4 min the first peak of glucose-induced insulin secretion without affecting its second phase (Fig. 3E, Supplemental Table S3). c-MYC also completely suppressed the stimulation of insulin secretion by K30 despite the persisting elevation of [Ca^{2+}]_i, (Fig. 3, E and F).

Effects of high glucose and H2O2 on glucose stimulus-secretion coupling events. The alterations of glucose stimulus-secretion coupling events induced by c-MYC activation in mouse islets were clearly different from those reported in rat islets cultured for 1 wk in 30 instead of 10 mmol/l glucose (14). They were, in contrast, similar to those reported in rat islets exposed overnight to a low concentration of H2O2 (13). To allow formal comparison of the effects of c-MYC activation with those induced by high glucose or H2O2 in the mouse, we measured glucose-induced changes in mitochondrial membrane potential, [Ca^{2+}]_i, and insulin secretion in WT islets cultured for 1 wk in 30 instead of 10 mmol/l glucose (G30 vs.

Table 3. Effects of c-MYC activation in the presence of BSA on subsequent glucose-induced changes in insulin secretion by mouse islets

<table>
<thead>
<tr>
<th>Islet Strain and Culture Conditions (BSA)</th>
<th>(n)</th>
<th>G0.5</th>
<th>(\Delta (G7-G15))</th>
<th>(\Delta (G7-G30))</th>
<th>(\Delta (G30Dz-G30K30Dz))</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT DMSO</td>
<td>3</td>
<td>0.02±0.01</td>
<td>1.18±0.18</td>
<td>6.84±1.51</td>
<td>8.41±2.27</td>
</tr>
<tr>
<td>WT TAM</td>
<td>3</td>
<td>0.02±0.01</td>
<td>1.14±0.21</td>
<td>6.48±1.43</td>
<td>7.04±2.03</td>
</tr>
<tr>
<td>MycER DMSO</td>
<td>3</td>
<td>0.31±0.13</td>
<td>0.08±0.08*</td>
<td>1.08±0.80*</td>
<td>8.86±5.58</td>
</tr>
<tr>
<td>MycER TAM</td>
<td>3</td>
<td>1.05±0.83</td>
<td>-0.09±0.09*</td>
<td>-0.09±0.12*</td>
<td>0.34±0.17†</td>
</tr>
</tbody>
</table>

Results are means ± SE for the indicated no. of islets (n). In each experiment illustrated in Fig. 2, C and F (see legend for experimental details), the delta insulin secretion between G7 and G15, G7 and G30, and G30Dz and G30K30Dz was computed from the mean insulin secretion during the last 8 min in G0.5, the last 3 min in G30Dz, and the last 10 min in G7, G15, G30, and G30K30Dz. *P < 0.05 or less for the effect strain (MycER vs. WT) (2-way ANOVA). †The relative reduction in secretion rate induced by TAM treatment was significantly larger in MycER vs. WT islets (94 ± 5 vs. 13 ± 14%), P < 0.01 by Student’s t-test.)
and, at least for changes in \([Ca^{2+}]_i\), in islets exposed overnight to 58 \(\mumol/l\) \(H_2O_2\) in G10. After culture in G30, the normalized rhodamine-123 fluorescence level during perfusion with G0.5 was lower than in control islets cultured in G10 and was only slightly further decreased upon stepwise glucose stimulation (Fig. 4A), suggesting that, as in the rat, these islets were more sensitive to glucose. One week of culture in G30 also induced a small elevation of islet resting \([Ca^{2+}]_i\) (in both G0.5 and G30) and a significant increase in their glucose sensitivity, with half-maximal and maximal glucose effects observed at 7 and 15 mmol/l instead of 15 and 30 mmol/l in control islets (Fig. 4C). Consequently, these islets, which had a normal insulin-to-DNA content ratio, displayed a higher sensitivity to glucose for the stimulation of insulin secretion without changes in their maximal rate of secretion (Fig. 4B). One week of culture in G30 similarly affected the glucose-induced changes in \([Ca^{2+}]_i\), in MycER islets (Fig. 4D). Compared with these effects of high glucose, 18-h culture in the presence of 58 \(\mumol/l\) \(H_2O_2\) also significantly increased the islet resting \([Ca^{2+}]_i\) by ~50 mmol/l but reduced the amplitude of their maximal glucose response by ~30%, as observed after c-MYC activation in MycER islets (Fig. 5 and Table 4). These results therefore suggest that the alterations of glucose stimulus-secretion coupling events by c-MYC activation are better mimicked by low concentrations of \(H_2O_2\) than by prolonged exposure to high glucose.

In vivo evidence that the MycERTAM protein construct is slightly active in the absence of TAM treatment. Because both cell survival and function were significantly impaired in DMSO-treated MycER vs. WT islets, we searched for further support to our hypothesis that the MycERTAM protein construct may be slightly active even in the absence of TAM treatment.
On the basis of the observation that blood glucose levels were lower and plasma insulin levels higher in fasted MycER vs. WT mice (Table 1), we postulated that they may have an increased \(\beta\)-cell mass. We therefore looked at pancreatic sections from young and old MycER mice and their WT littermates. As previously reported, the islet size and \(\beta\)-cell morphology were grossly similar in 3-mo-old MycER and WT mice (Fig. 6, A and B). In contrast, in mice 1 yr old, MycER pancreases displayed normal-size islets as well as very large islets, with a clear increase in \(\beta\)-cell mass (21.5 \(\pm\) 7.7 and 2.7 \(\pm\) 0.5 mg in MycER vs. WT mice; \(n = 3\), \(P < 0.05\) for the comparison of log-transformed data by Student’s \(t\)-test) (Fig. 6, C–E). At that age, MycER islets had several morphological abnormalities, such as the presence of large heterogeneous nuclei, a high nucleocytoplasmic ratio, and a spatial organization typical of human insulinomas and MYC-induced \(\beta\)-cell tumors (epithelial cords of \(\beta\)-cells disposed along large blood vessels) (24, 28).

Table 4. Effects of overnight treatment with hydrogen peroxide on subsequent glucose-induced \([Ca^{2+}]_i\) changes in mouse islets

<table>
<thead>
<tr>
<th>Inlet Strain and Culture Conditions (BSA)</th>
<th>(n)</th>
<th>G0.5</th>
<th>(\Delta) (G0.5–G30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>8</td>
<td>74 (\pm) 3</td>
<td>187 (\pm) 8</td>
</tr>
<tr>
<td>WT H(_2)O(_2)</td>
<td>10</td>
<td>126 (\pm) 11*</td>
<td>123 (\pm) 11*</td>
</tr>
</tbody>
</table>

In each experiment illustrated in Fig. 5 (see legend for experimental details), we computed the difference between the average \([Ca^{2+}]_i\) during the last 5 min in G0.5 and the last 10 min in G30. Results are means \(\pm\) SE for the indicated no. of islets (\(n\)). *\(P < 0.001\) for the effect of H\(_2\)O\(_2\) (Student’s \(t\)-test).
were equally observed in double transgenic mice obtained by crossing MycER mice with RIP7-Bcl-(x)L mice that express the anti-apoptotic protein Bcl-(x)L under the control of the insulin promoter (23). At the age of 3 mo, these mice rapidly develop insulinomas upon TAM-mediated activation of c-MYC (24). However, even if they were not treated with TAM, these mice displayed marked fed hypoglycemia [MycER @ Bcl-(x)L vs. Bcl-(x)L: 3.0 ± 0.2 vs. 6.3 ± 0.4 mmol/l; n = 11–14, P < 0.0001 by Student’s t-test] and a high frequency of large encapsulated insulinomas visible at macroscopic examination at >1 yr of age (see Fig. 7, F and G). They also displayed the alterations of islet size, β-cell morphology, and islet vascularization already observed in old MycER mice (Fig. 7, A–E).

**DISCUSSION**

This study clearly demonstrates that, in addition to the stimulation of β-cell apoptosis and reduction of Ppi gene expression (12, 19, 24), c-MYC activation triggers functional alterations of β-cells that are completely different from those induced by prolonged culture in high glucose concentrations.
but rather resemble those induced by overnight exposure to a low concentration of $H_2O_2$. Thus c-MYC activation markedly increased the caspase activity of islets, reduced their DNA content, and markedly decreased the $Ppi$ mRNA levels and the islet insulin-to-DNA content ratio. These changes were accompanied by mitochondrial hyperpolarization at all glucose concentrations, higher resting $[Ca^{2+}]_i$, a lower amplitude of glucose-induced rise in $[Ca^{2+}]_i$, and the abrogation of glucose- and high $K^+$-induced insulin secretion, at least when culture was carried out in the absence of FBS. When apoptosis was reduced by addition of FBS to the culture medium, c-MYC activation triggered similar alterations of β-cell function, except for the reduction of glucose-induced insulin secretion, which was less pronounced, in particular during the second phase of secretion.

Interestingly, similar functional alterations could be triggered by exposure to low $H_2O_2$ concentrations for 18 h (13) and 50 mmol/l d-ribose for 1 wk (S. Pascal, unpublished...
observations), two conditions that, like hyperglycemia in rat islets, induce an oxidative stress, increase c-Myc mRNA levels, and trigger β-cell apoptosis (27, 29). In contrast, high glucose markedly increased the islet sensitivity to glucose for the stimulation of mitochondrial membrane hyperpolarization, [Ca\textsuperscript{2+}]\textsubscript{i} rise, and insulin secretion. These results are similar to those previously reported in human and rat islets, except that the loss of glucose-induced rise in [Ca\textsuperscript{2+}]\textsubscript{i}, and the reduction of insulin content and maximal rate of insulin secretion were not observed in mouse islets (2, 14, 20, 21). The latter observation confirms a recent study showing that mouse islets (at least from C57BL6 mice) are less prone to in vitro induction of glucotoxicity than rat islets (31). Despite this species difference in islet susceptibility to glucotoxicity, we can conclude that c-MYC does not contribute to the increase in glucose sensitivity in islets previously cultured at high glucose. It remains possible, however, that c-MYC contributes to some extent to other changes induced by prolonged exposure to high glucose, such as the decrease in Ppi gene expression and insulin content, and the stimulation of β-cell apoptosis. Proving that hypothesis will require testing whether conditional inactivation of c-MYC can prevent or reduce this type of β-cell alteration in a mouse strain that is more susceptible to glucotoxicity.

Quite unexpectedly, our results suggested that the MycER\textsuperscript{TAM} protein construct is slightly active even in the absence of TAM treatment, at least when expressed in β-cells under the control of the relatively strong insulin promoter. This low level of c-MYC activation induced significant alterations of β-cell survival and function and, in old mice, morphological changes of α-cells characteristic of insulinomas and an increase in β-cell mass leading to fasting hyperinsulinaemic hypoglycaemia. The increase in β-cell mass was not reported until recently (3), likely because MycER mice had previously been used at 3 mo of age when their β-cell morphology and mass were not obviously altered. These results suggest that, at very low levels of c-MYC activation, its proliferative effect may exceed its proapoptotic effect, in contrast to what is observed upon strong c-MYC activation (24, 25).

In conclusion, conditional c-MYC activation in MycER islets rapidly stimulates apoptosis, reduces Ppi gene expression and insulin content, and triggers functional alterations of β-cells that are better mimicked by overnight exposure to a low H\textsubscript{2}O\textsubscript{2} concentration than by prolonged culture in high glucose. The precise role of c-MYC in the alterations of β-cell function and survival after exposure to H\textsubscript{2}O\textsubscript{2}, d-ribose, and high glucose concentrations merits further investigation.

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