β-Cell Adaptation to Hyperglycemia

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Recently, we showed that β-cell dysfunction in hyperglycemic 90%-pancreatectomized rats is associated with downregulation of key islet genes and transcription factors concomitant with marked upregulation of several suppressed genes, including hexokinase 1 and lactate dehydrogenase-A (1). These findings strengthened our hypothesis (2) that the loss of β-cell differentiation is a critical factor for the insulin secretory defect in type 2 diabetes. Interestingly, the adipocyte transcription factor C/EPBβ is also increased with diabetes, thus raising the possibility of lipid metabolism contributing to β-cell dysfunction induced by the diabetic milieu. In addition to functional failure, hypertrophy of β-cells develops in association with increased expression of c-*myc*, a transcription factor involved in both cell growth and apoptosis. We hypothesized that stress genes may be important for the survival of hypertrophic β-cells. In this study, we examined the expression of genes involved in lipid metabolism and cell stress response in islets after pancreatectomy (Px) to investigate their significance to β-cell function, structure, and survival under hyperglycemic conditions.

Islets were isolated and mRNA was extracted from rats 4 weeks after Px or sham-Px surgery for gene expression analysis using semiquantitative radioactive multiplex reverse transcriptase–polymerase chain reaction. For the period 2–4 weeks after surgery, Px rats showed stable hyperglycemia (average blood glucose 232 ± 14 mg/dl for Px vs. 80 ± 2 for sham-Px, $P < 0.001$), whereas neither plasma triglyceride (TG) nor nonesterified fatty acid (NEFA) levels were altered. After normalization of the specific gene to an internal control gene, mRNA levels were expressed as a percent of sham.

The peroxisome proliferator–activated receptors (PPARs) regulate the expression of target genes central to lipid homeostasis (3). Their role in islets is unknown, but in other tissues, PPAR-α and PPAR-γ appear to play key roles in the regulation of genes involved in lipid catabolism and adipocyte lipogenesis, respectively. We found reciprocal regulation of PPAR-α and PPAR-γ after Px (Table 1). Consistent with marked reduction in PPAR-α, the peroxisomal lipid oxidation enzyme acyl-CoA oxidase, a transcriptional target, was downregulated, whereas expression of the mitochondrial enzyme carnitine palmitoyltransferase-1 displayed discordant regulation. Expression levels of lipogenic genes (acetyl-CoA carboxylase and fatty acid synthase) were modestly increased after Px in association with the induction of PPAR-γ.

Hypertrophy may cause cells to be more susceptible to apoptosis (4). However, 4 weeks after Px, a stable population of hypertrophic β-cells is preserved, suggesting possible activation of protective stress genes. Normally, islets express low levels of constitutive stress genes (5). In this study, we examined the regulation of stress genes after Px (Table 2). Protective genes were upregulated with markedly

TABLE 1
Lipid gene expression

	Sham	Px
Lipid metabolism genes		
Acetyl-CoA carboxylase	100 ± 3	156 ± 19*
Carnitine palmitoyltransferase-1	100 ± 14	142 ± 7*
Fatty acid synthase	100 ± 2	143 ± 11*
Acyl-CoA oxidase	100 ± 6	78 ± 7†
Transcription factors		
PPAR-γ	100 ± 9	298 ± 43‡
PPAR-α	100 ± 10	24 ± 3‡

Data are means ± SE. *$P < 0.01$; †$P < 0.05$; ‡$P < 0.001$.

TABLE 2
Stress gene expression

	Sham	Px
Antioxidant genes		
HO-1	100 ± 5	893 ± 281*
Mn-superoxide dismutase	100 ± 2	138 ± 11†
Glutathione peroxidase	100 ± 2	519 ± 76‡
Catalase	100 ± 13	95 ± 10
Antiapoptotic genes		
A20	100 ± 1	467 ± 125†
BCL-2	100 ± 1	80 ± 6†
Heat shock protein 70	100 ± 16	134 ± 23
Transcription factors		
NF-κB (p65)	100 ± 1	116 ± 8
p53	100 ± 7	100 ± 11
Cell surface protein		
Fas	100 ± 5	209 ± 16‡

Data are means ± SE. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

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HO-1, heme oxygenase-1; NEFA, nonesterified fatty acid; NF, nuclear factor; PPAR, peroxisome proliferator–activated receptor; Px, pancreatectomy; TG, triglyceride.

increased expression of the antioxidant genes heme oxygenase-1 (HO-1) and glutathione peroxidase as well as the anti-apoptotic gene A20. Although nuclear factor (NF)-κB expression levels did not change, activation of NF-κB targets, such as A20, HO-1, and stress genes, indicates a role for this factor in regulating gene expression under diabetic conditions. In addition to the activation of potentially protective genes, we found an increased expression of the inducible isoform of nitric oxide synthase in association with a threefold induction of nitric oxide (NO) production from Px islets ($P < 0.05$). Increased NO may be deleterious to β-cell function and survival, but may also promote changes in stress gene expression.

The changes in gene expression described in this study were either partially or completely reversed by phlorizin treatment of Px rats (treatment that normalized hyperglycemia without affecting plasma TG or NEFA), indicating the specificity of hyperglycemia in eliciting the response. Hence, in islets of Px rats, changes in gene expression may be mediated directly by hyperglycemia or indirectly through the induction of oxidative stress and/or NO.

In conclusion, β-cell adaptation to hyperglycemia is accompanied by *1*) altered expression of genes involved not only in glucose metabolism, but also in lipid metabolism, and *2*) induction of stress gene expression and NO production.

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