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Expression of yeast hexokinase in pancreatic β cells of transgenic mice reduces blood glucose, enhances insulin secretion, and decreases diabetes

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ABSTRACT **It has been proposed that endogenous hexokinases of the pancreatic β cell control the rate of glucose-stimulated insulin secretion and that genetic defects that reduce β-cell hexokinase activity may lead to diabetes. To test these hypotheses, we have produced transgenic mice that have a 2-fold increase in hexokinase activity specific to the pancreatic β cell. This increase was sufficient to significantly augment glucose-stimulated insulin secretion of isolated pancreatic islets, increase serum insulin levels *in vivo*, and lower the blood glucose levels of transgenic mice by 20–50% below control levels. Elevation of hexokinase activity also significantly reduced blood glucose levels of diabetic mice. These results confirm the role of β-cell hexokinase activity in the regulation of insulin secretion and glucose homeostasis. They also provide strong support for the proposal that reductions in β-cell hexokinase activity can produce diabetes.**

Glucose is the primary regulator of insulin secretion. It is well-established that glucose must be metabolized for insulin secretion to ensue (for review, see ref. 1): The rate of insulin secretion closely parallels its metabolic rate (2) and inhibitors of glucose metabolism effectively block secretion (3). In all cells, glucose metabolism is initiated by hexokinase-catalyzed phosphorylation. Unique to the pancreatic β cell and the hepatocyte of the liver, this reaction is catalyzed by an unusual hexokinase isoform, hexokinase IV (4, 5) (commonly referred to as glucokinase due to its relative specificity for glucose as substrate). Hexokinase IV has a K_m for glucose in the physiological range of glucose levels, ≈10 mM. This is almost two orders of magnitude higher than any other mammalian hexokinase (1). Therefore, *in vivo* hexokinase IV-catalyzed phosphorylation is proportional to blood glucose, whereas phosphorylation by other hexokinases is saturated at physiological glucose levels. Hexokinase IV is also distinct from other hexokinases in that it is not allosterically inhibited by its product glucose 6-phosphate (G6P). Due to the high K_m of hexokinase IV and the absence of end-product inhibition, glucose phosphorylation in the β cell closely parallels circulating glucose concentrations. It has therefore been proposed (6) that hexokinase IV functions as the glucose receptor or glucose sensor of the pancreatic β cell. However, until the advent of techniques allowing manipulation of gene expression, direct experimental verification of this hypothesis has not been possible and several other plausible mechanisms for glucose sensing have been proposed (7, 8).

In non-insulin-dependent diabetes mellitus (NIDDM), there is a failure to secrete adequate amounts of insulin despite the fact that pancreatic islets retain significant levels of insulin (9). This secretory deficit appears to be specific for glucose-induced secretion as other secretagogues retain normal or near normal efficacy (10, 11). The hypothesis that hexokinase IV may serve as the β-cell glucose sensor has taken on great clinical significance recently since it has been demonstrated that a nonsense mutation in only one allele of the hexokinase IV gene predisposes to some types of NIDDM (12, 13). As hexokinase IV is a monomeric enzyme, these results suggest that a simple 50% reduction in hexokinase IV activity due to mutational inactivation of one allele may lead to diabetes.

In this report we describe a direct test of the hypothesis that hexokinase IV functions as the β-cell glucose sensor by increasing hexokinase activity in pancreatic β cells of transgenic mice. Hexokinase activity was elevated by introducing the yeast hexokinase B gene under the control of the insulin promoter. Yeast hexokinase B, like hexokinase IV, is not allosterically inhibited by intracellular levels of G6P. Our results clearly demonstrate that the rate of glucose phosphorylation in the pancreatic β cell not only controls the rate of insulin secretion but also plays a major role in determining the glucose set point of the whole animal. In addition, when the yeast hexokinase transgenic mice were bred with mice having a diabetic genotype, a significant amelioration of diabetes was achieved. These results support the importance of hexokinase IV gene mutations in the etiology of NIDDM, in that moderate changes in hexokinase activity are sufficient to greatly alter glucose homeostasis and suggest that the critical cell type involved in induction of diabetes is the pancreatic β cell rather than the hepatocyte. These results in transgenic mice also demonstrate that a transgene can enhance insulin secretion and, therefore, suggest that genetic manipulation of the β cell may ultimately provide a valuable approach to enhancing insulin secretion in diabetes.

MATERIALS AND METHODS

Construction of the Inshex Gene and Production of Transgenic Mice. The fragment of the rat insulin II promoter utilized to direct β-cell-specific expression has been described (14). This promoter fragment contains 692 base pairs (bp) of sequence 5′ to the insulin transcription initiation site. A *Hin*dIII linker was inserted 3′ to the initiation site as described (15). The yeast hexokinase B gene (provided by W. Konigsberg, Yale University, New Haven, CT), contained in a 1681-bp *Ssp* I fragment that included the entire coding sequence, was inserted behind the insulin promoter by blunt-end ligation using the *Hin*dIII linker mentioned above. An 870-bp *Sal* I–*Bam*HI fragment of simian virus 40 (derived from the plasmid PMSG; Pharmacia) containing a simian virus 40 early region splice and polyadenylylation site was inserted behind the hexokinase B gene. For production of

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Abbreviations: G6P, glucose 6-phosphate; NIDDM, non-insulin-dependent diabetes mellitus; KIC, ketoisocaproate.
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transgenic mice, plasmid sequences were removed from the gene by *Bam*HI/*Nde* I digestion, and the resultant 3275-bp fragment was purified as described (15).

Transgenic mice were generated by microinjection of DNA into single-cell embryos by standard techniques (16, 17). Embryos were isolated from superovulated 4- to 6-week-old FVB/N females. Microinjections of purified DNA at 2 μg/ml in 10 mM Tris·HCl/0.1 mM EDTA, pH 7.6, were into one pronucleus for each embryo, 12–14 h after fertilization. Embryos that survived microinjection were implanted into pseudopregnant females and allowed to develop to term. Founder mice were crossed with ICR mates and transgenic lines were maintained on an ICR background.

DNA and RNA Analyses. Founder mice were identified on Southern blots prepared with 10 μg of tail DNA digested with *Eco*RI. Blots were hybridized to a 1200-bp *Hin*dIII–*Xho* I fragment of the Inshex gene radiolabeled with ^{32}P by random priming. Routine identification of transgenic mice was made by PCR analysis with oligonucleotides identical to the rat insulin II promoter between positions −600 and −575 (GCTCTGAAGCAAGCACCTCTTATG) and complementary to the yeast hexokinase coding sequence 125 bp 3′ to the translation start site (GGAAATGAAGTGCTTGGTAACG).

Expression of yeast hexokinase RNA was determined by S1 nuclease protection analysis of poly(A)$^+$ pancreatic RNA. Poly(A)$^+$ RNA was prepared on oligo(dT) columns from pancreatic RNA isolated by the guanidinium thiocyanate method. An RNA probe was prepared from an 1125-bp fragment of the Inshex gene, from position −620 of the insulin promoter to a *Hin*dIII site in the hexokinase coding sequence, 505 bp 3′ of the expected transcription initiation site of the insulin promoter. This fragment was inserted into plasmid pSP64, cut with *Eco*RI in the insulin promoter and in the pSP64 polylinker and radiolabeled with SP6 polymerase and [^{32}P]CTP. The radiolabeled RNA probe was hybridized to pancreatic poly(A)$^+$ RNA in 80% (vol/vol) formamide at 50°C and digested with 1000 units of S1 nuclease at 37°C. Protected products were identified by PAGE and autoradiography. ^{32}P-end-labeled *Hae* III digests of plasmid ϕ-174 served as molecular size standards.

Islet Isolation. Transgenic and normal islets were isolated in parallel from 1- to 2-week-old litters. Genotype was determined by PCR analysis as described above. Islets were released from pancreatic acinar tissue by digestion with collagenase P (Boehringer). After two washes in Hanks' balanced salt solution, islets were purified by centrifugation on Percoll (Pharmacia) gradients (18). Islets were then further purified by picking under a dissection microscope. In most instances, islets were cultured overnight in RPMI 1640 medium (GIBCO) containing 10% (vol/vol) fetal calf serum, 5 mM glucose, and 10 mM Hepes (pH 7.4). In these experiments overnight cultured and freshly isolated islets yielded equivalent results.

Islet Assays. Measurement of hexokinase activity was performed by the radiometric procedure of Bedoya *et al.* (19). In brief, batches of 25–50 islets were homogenized in 100 μl of extraction buffer [30 mM Hepes, pH 7.6/130 mM KCl/4 mM $MgCl_2$/14 mM 2-mercaptoethanol/0.2% (wt/vol) bovine serum albumin] and then centrifuged at 60,000 × *g* for 30 min at 4°C. Pellets were used for measurement of DNA content. Aliquots (10 μl) of the extract were assayed for hexokinase activity in incubation buffer [50 mM Hepes, pH 7.6/120 mM KCl/8 mM $MgCl_2$/14 mM 2-mercaptoethanol/5 mM ATP/0.1% bovine serum albumin/3 μCi of D-[2-^{3}H(N)]glucose/3 mM G6P]. Assays were performed for 60 min at 30°C. After the initial incubation, the reaction products were quantitated by measuring the release of ^3H_2O (19) from [^{3}H]G6P by the action of phosphoglucose isomerase (Boehringer). Determinations of hexokinase activity in other tissues were performed by the same procedure.

Insulin secretion was assayed by consecutive static assays of isolated islets in increasing concentrations of glucose (3–15 mM) or ketoisocaproic acid (KIC, 1–9 mM). Assays were performed in modified Krebs-Ringer bicarbonate buffer (KRB = 133 mM NaCl/5 mM $NaHCO_3$/5 mM KCl/1.2 mM $MgSO_4$/1.2 mM KH_2PO_4/2.5 mM $CaCl_2$/15 mM Hepes, pH 7.4/0.1% bovine serum albumin. Briefly, batches of 10–20 islets were picked into tubes containing KRB for a 30-min preincubation at 37°C and then the medium was replaced with 0.4 ml of KRB containing the lowest concentration of glucose or KIC to be tested. After 30 min at 37°C, the medium was removed for radioimmunoassay of insulin and the incubation medium was replaced with the next higher concentration of secretagogue. After the last incubation, islets were extracted with acidifed ethanol for measurement of islet insulin content. Perifusion assays were performed as described (20) on freshly microdissected islets from 3- to 4-month-old transgenic and control mice.

Measurement of Glucose and Insulin. Determinations of insulin levels in serum samples and secretion assays were made by double-antibody RIA using a coated-tube insulin RIA kit (Diagnostic Products, Los Angeles) and rat insulin standards (Novo Biolabs, Danbury, CT). Blood samples sufficient for RIA were obtained by decapitation. Serum samples were prepared by centrifugation after incubating blood samples overnight at 4°C. Glucose assays were performed on whole blood with the One Touch blood glucose monitor (Lifescan, Mountain View, CA).

RESULTS

Expression of Yeast Hexokinase in Transgenic Mice. A transgene, designated Inshex, was constructed consisting of 700 bp of the rat insulin II promoter linked to the intronless yeast hexokinase B gene (21) and polyadenylylation sequences derived from the simian virus 40 early region. The yeast hexokinase gene was chosen because yeast hexokinase B, like hexokinase IV, is free from allosteric inhibition by G6P (22) and catalyzes the same reaction as hexokinase IV. Yeast hexokinase B has a higher affinity for glucose than hexokinase IV and, therefore, should produce a more obvious effect on β-cell function. Four founder lines were produced initially that carried one or more copies of the Inshex gene. To determine whether the transgene was expressed, pancreatic RNA was isolated from members of each line and subjected to an S1 nuclease protection assay. As shown in Fig. 1 only mice of transgenic line 5032 produced significant

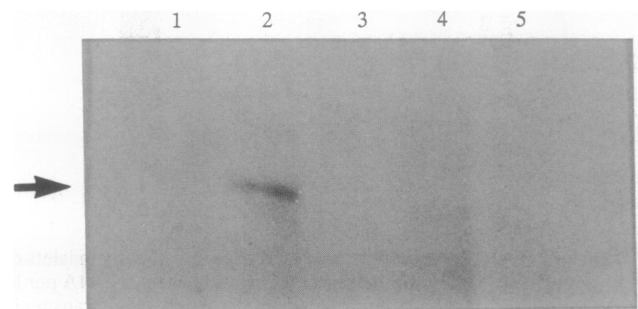

FIG. 1. S1 nuclease protection assays of pancreatic RNA indicate that the Inshex gene is transcribed in line 5032. S1 protection assays were performed on pancreatic RNA by using a ^{32}P-labeled RNA probe derived from the Inshex gene from nucleotide −620 of the insulin promoter to nucleotide 505 of the hexokinase gene. Arrow, predicted position for a 505-nucleotide RNA probe fragment protected by a properly initiated transcript from the Inshex transgene. Sources of pancreatic RNA are as follows: Lanes, 1, control mice; 2, line 5032 mice; 3, line 5039 mice; 4, line 5040 mice; 5, line 5041 mice.

levels of RNA complementary to an antisense yeast hexokinase RNA probe. Two additional transgenic lines were made but these also failed to express the transgene. The lack of expression in five of six transgenic lines may have been due to the absence of introns in the yeast hexokinase gene (17).

To determine whether production of yeast hexokinase mRNA resulted in elevated enzyme levels, pancreatic islets were isolated from transgenic and nontransgenic siblings of line 5032 and assayed for hexokinase activity (19). The results of these assays are shown in Fig. 2*A*. Transgenic high-affinity hexokinase activity was increased 5-fold over normal when measured at 0.5 mM glucose. At 5 mM glucose, selected because it is in the physiological range of glucose concentrations, hexokinase activity was increased ≈2-fold. This moderate increase could be sufficient to affect β-cell function *in vivo*, if secretion is directly regulated by the rate of glucose phosphorylation. These assays were performed in the presence of 3 mM G6P since G6P is a potent endogenous inhibitor of all mammalian hexokinases except hexokinase IV: In the β cell, intracellular G6P inhibits the endogenous high-affinity hexokinases by >90% (23). To confirm tissue specificity of expression, hexokinase assays were also performed on brain, liver, kidney, and heart from transgenic and nontransgenic mice (Fig. 2*B*). Despite the fact that these assays were performed under conditions of maximal sensitivity for yeast hexokinase (0.5 mM glucose/3 mM G6P), no significant increase in enzyme activity was apparent in these tissues.

Yeast Hexokinase Increases Insulin Secretion in Isolated Islets. The most direct measurements of β-cell glucose sensitivity can be made in isolated pancreatic islets. Islets were isolated from transgenic mice of line 5032 and their nontransgenic littermates. Insulin secretion was measured by incubating islets successively in increasing concentrations of glucose (Fig. 3*A*). Since yeast hexokinase B has a high affinity for glucose, the greatest effect of this transgene would be at relatively low concentrations of glucose, as was found. At the lower concentrations of glucose tested, 3–9 mM, transgenic secretion rates were ≈2-fold higher than normal ($P < 0.025$). At 15 mM glucose, transgenic and normal secretion rates were essentially the same. If this enhanced secretion to glucose was a direct effect of the enzymatic activity of yeast hexokinase then the secretory response to secretagogues that are not hexokinase substrates should not be affected. We chose KIC for this test since KIC, like glucose, is a fuel secretagogue dependent on metabolism to induce secretion. However, unlike glucose, KIC is not a substrate for hexokinase (1); therefore, the transgenic hexokinase should not augment KIC-induced secretion. As shown in Fig. 3*B*, at no concentration of KIC did the transgenic islets demonstrate increased secretion relative to normal islets. These results demonstrate that the enhanced secretion response of transgenic islets was specific to substrates of the transgene and a direct effect of elevated hexokinase activity.

Yeast Hexokinase Lowers Blood Glucose and Elevates Insulin Secretion *in Vivo*. The 2-fold elevation of islet hexokinase activity measured at physiological glucose concentrations and the enhancement of glucose-stimulated insulin secretion in isolated islets led us to anticipate alterations in β-cell function that might be reflected in altered glucose homeostasis of the whole animal. Random blood glucose assays

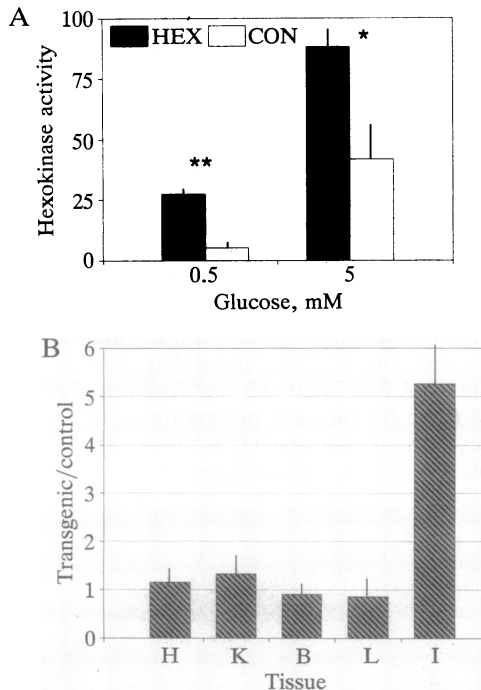

FIG. 2. Hexokinase activity was increased specifically in islets of transgenic mice. (*A*) Hexokinase activity (fmol per ng of DNA per h) was determined in homogenates of islets isolated from transgenic mice of line 5032 (HEX) and their nontransgenic siblings (CON). Assays were performed at the indicated glucose concentrations in the presence of 3 mM G6P. Assays were performed in triplicate on at least three islet preparations. Each islet preparation was isolated from three or more transgenic or control mice. **, $P < 0.025$; *, $P < 0.05$ (different from control). (*B*) The ratio of transgenic to control levels of hexokinase activity in homogenates of various tissues obtained from transgenic and control mice. Assays were done at 0.5 mM glucose in the presence of 3 mM G6P. Four or more assays were performed for each tissue from at least two preparations. The vertical lines show the SEM. H, heart; K, kidney; B, brain; L, liver; I, islets.

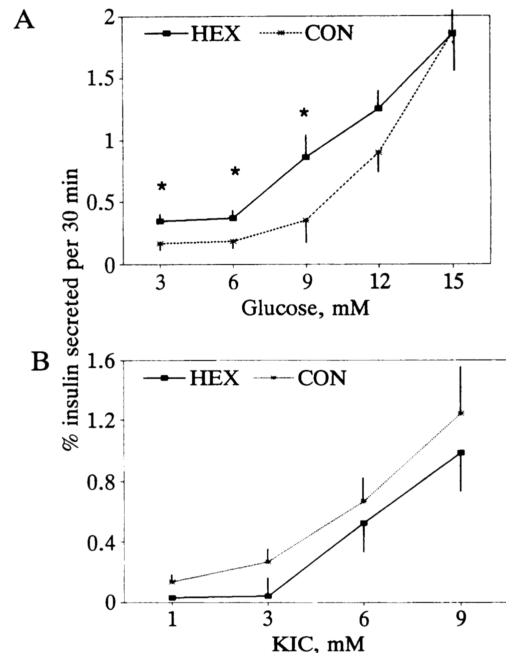

FIG. 3. Glucose- but not KIC-stimulated insulin secretion is increased in transgenic islets. Transgenic (HEX) and control (CON) islets were isolated in parallel from at least three mice of each type. Insulin secretion was assayed by consecutive 30-min incubations at the indicated concentrations of glucose (*A*) or KIC (*B*). After the last incubation islets were extracted to determine insulin content. The results shown for glucose-stimulated secretion are the averages from 16 assays performed on five preparations of transgenic and control islets. The results shown for KIC-stimulated secretion are the averages from 7 assays performed on two preparations of transgenic and control islets. Both the glucose and KIC secretion results have each been replicated in three or more perifusion assays of microdissected transgenic and control islets (data not shown). *, $P < 0.025$ (different from control).

indicated that glucose levels were reduced in line 5032, which expressed the hexokinase transgene and that blood glucose levels were normal in the five nonexpressing lines of transgenic mice. To analyze the reduction in glucose levels of line 5032 systematically, we performed intraperitoneal glucose tolerance tests on overnight-fasted transgenic mice and their nontransgenic siblings (age, 20–30 days). These results (Fig. 4) confirmed our initial impressions: Relative to their normal siblings transgenic mice of line 5032 had significantly lower fasted glucose levels, and their peak glucose levels after i.p. administration of glucose were also much lower. In addition, within 180 min of glucose administration, transgenic blood glucose values returned to near their original low fasted level. These results indicate that the level of β-cell hexokinase activity controls whole-animal glucose homeostasis. Thus we report a transgene producing a permanent reduction of blood glucose values.

To determine whether the lower glucose levels *in vivo* were a function of enhanced insulin secretion, age-matched transgenic and nontransgenic mice were sacrificed to obtain samples adequate for measurement of serum insulin levels. Prior to sacrifice mice were either free-fed or fasted for 5 h so that insulin levels could be determined over a broad range of glucose values. Serum insulin and blood glucose levels were determined from the same sample. As shown in Fig. 5, the relationship between blood glucose and serum insulin has been markedly shifted by the transgene. Insulin levels in transgenic mice are higher at all glucose values (no control values are shown for 3 and 4 mM glucose since no control mice had glucose levels <5 mM; no transgenic mice had glucose values >8 mM). In addition, the rise in serum insulin levels of hexokinase mice was steeper with increasing blood glucose levels than was seen in control mice. The elevation of serum insulin could not have been due to increased levels of pancreatic insulin, as observed in some transgene-induced β-cell tumors (14), since several assays of total pancreatic insulin content indicated an ≈50% reduction in pancreatic insulin content of hexokinase transgenic mice in both neonates and adults (data not shown). The elevated serum insulin values of transgenic mice over a broad range of glucose values demonstrate *in vivo* that increased hexokinase activity enhances insulin secretion and confirms that altered whole animal glucose homeostasis is a function of enhanced insulin secretion rather than a nutritional or peripheral metabolic effect.

To determine whether increasing β-cell glucose phosphorylation could be beneficial in diabetes, hexokinase transgenic mice were bred to a diabetic transgenic line. The diabetic line selected was the line OVE26 (15, 24), which overexpresses

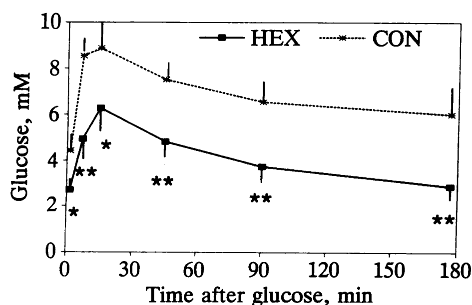

FIG. 4. Transgenic mice have lower blood glucose levels than control mice. Transgenic mice (HEX) and their nontransgenic siblings (CON) were fasted overnight and then administered an i.p. injection of 1 mg of glucose per g (body weight). Blood samples were taken at the indicated times from the tail vein. Blood glucose was determined with a Lifescan glucometer. $N = 9$ (transgenic) or 10 (control) mice per point. *, $P < 0.01$; **, $P < 0.001$ (different from control).

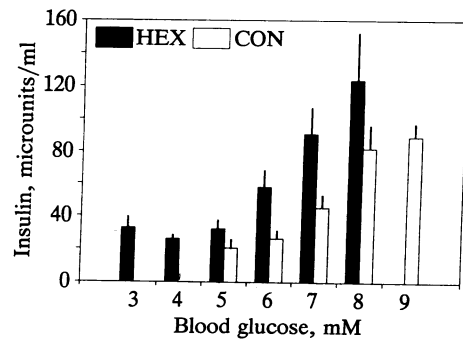

FIG. 5. Transgenic serum insulin values are higher than control values relative to blood glucose level. Forty- to 60-day-old transgenic (HEX) or normal (CON) mice were free fed or fasted for 4–6 h. One blood glucose value and one serum insulin value were obtained from each mouse by using a single blood sample obtained on sacrifice. Samples were grouped on the basis of blood glucose level. At least three determinations were obtained for each point. No control mice had blood glucose levels <5 mM and no transgenic mice had blood glucose >8 mM. Vertical bars indicate the SEM.

calmodulin in pancreatic β cells. OVE26 transgenic mice develop diabetes due to an insulin secretory defect and a depletion of pancreatic insulin. Four litters from these crosses were assayed for blood glucose at 24 days of age, which is after the onset of diabetes but before complete destruction of the β-cell population (24). The hexokinase transgene significantly reduced diabetic blood glucose levels produced by the calmodulin transgene. Blood glucose values of CaM^+/Hex^+ mice (transgenic for calmodulin and yeast hexokinase; 11.6 ± 0.7 mM glucose; $n = 10$) were 2.3 mM lower than glucose levels of CaM^+/Hex^- mice (transgenic for calmodulin only; 13.9 ± 0.7; $n = 6$) ($P < 0.025$). This hexokinase-induced reduction corresponds to ≈40% of the difference between blood glucose levels of CaM^+/Hex^- mice and normal mice (CaM^-/Hex^-; 8.0 ± 0.3; $n = 10$) ($P < 0.01$). Measurements of pancreatic insulin content from several of these mice indicated that the hexokinase transgene exacerbated the calmodulin-induced depletion of pancreatic insulin (data not shown). This implies that increased hexokinase activity does not directly reverse the defect produced by calmodulin but can enhance secretion despite this defect.

DISCUSSION

By producing transgenic mice with increased β-cell hexokinase activity, we have established the 24-year-old hypothesis (6) that the rate of glucose phosphorylation is the principle regulator of insulin secretion. Although other β-cell proteins may significantly contribute to glucose-regulated secretion, it is apparent that changes in glucose phosphorylation are sufficient to alter secretion. Since we used yeast hexokinase B rather than hexokinase IV or any other vertebrate hexokinase, it is evident that it is the rate of phosphorylation rather than the identity of the active enzyme that is critical to β-cell function. In normal islets, the total glucose phosphorylation rate is the sum of hexokinase IV activity plus a low level of mammalian high-affinity hexokinase activity. In the transgenic islet, yeast hexokinase activity is added to this sum. Since yeast hexokinase B has a very high affinity for glucose (K_m, <0.25 mM; ref. 25), it would be expected that basal phosphorylation rates would be increased. This is consistent with the elevated plateau of secretion that we observed both in isolated islets (Fig. 3A) and *in vivo* (Fig. 5) at low glucose levels. As glucose levels rise, the enhancement of transgenic secretion was probably due to the addition of constant yeast hexokinase activity to ever increasing hexokinase IV phosphorylation rates. Higher levels of yeast hexokinase expres-

sion in transgenic mice would probably produce a greater and potentially lethal shift in the secretory response. The sensitized β-cell response to glucose was not limited to insulin secretion, since we have also found that the electrophysiological bursting response of the β cell to glucose is enhanced in transgenic islets (unpublished results). The enhanced response could not have been due to a nonspecific sensitization of the β cell since KIC-stimulated insulin secretion was not enhanced by the transgene (Fig. 3*B*).

The primary *in vivo* effect of increased insulin secretion was a reduction in transgenic blood glucose values by 20–50% (Figs. 4 and 5). This result emphasizes the preeminant role of β-cell phosphorylation kinetics in regulating whole-animal glucose homeostasis. The reduction in blood glucose did not appear to be deleterious to the health of the transgenic mice: fertility and body weight were not notably different from normal. In addition, the reduction in blood glucose appeared to be very stable with increasing age; blood glucose measurements of 1-year-old transgenic mice revealed low glucose values similar to those found in 20-day-old transgenic mice. Because the transgenic mice maintained low blood glucose levels, the mice did not become markedly hyperinsulinemic. Hyperinsulinemia, which is seen primarily with severe insulin resistance, produces a number of untoward effects including obesity. Our results are in striking contrast to previous reports on β-cell transgenes that have produced either no effect on blood glucose levels (26) or more commonly the induction of diabetes (27–29). The phenotype produced by the yeast hexokinase transgene is an indication that genetic manipulation can enhance β-cell function and, thereby, reduce blood glucose levels.

A mutation in the hexokinase IV gene has recently been shown (12) to produce maturity onset diabetes of the young (MODY). MODY is a significant subset of all patients with NIDDM and is very similar to NIDDM except for a typically earlier age of onset. The mutation is dominant, meaning that a loss of only 50% of hexokinase IV activity in the β cell and/or the liver is sufficient to induce diabetes. Our results support the clinical significance of moderate changes in hexokinase activity, since at physiological glucose levels hexokinase activity in our transgenic mice was increased only 2-fold (Fig. 2*A*). The fact that liver hexokinase activity was not increased in our transgenic mice (Fig. 2*B*) would suggest that the β cell may be the critical site for hexokinase IV gene mutations to induce diabetes. We have also demonstrated that, by increasing β-cell hexokinase activity, we can ameliorate diabetes in at least one murine model of diabetes. Genetic modification of the β cell *in situ* is not presently feasible in humans. However, genetic manipulation of isolated islets is feasible. Since islet-cell transplantation is being pursued as a treatment of diabetes, genetic upregulation of hexokinase activity in isolated islets may provide a means to improve the efficacy of this procedure.

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