Inhibition of pancreatic β-cell glucokinase by antisense RNA expression in transgenic mice: mouse strain-dependent alteration of glucose tolerance

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Abstract **We have generated transgenic mice, in either C57BL/6 or C3H background, expressing antisense glucokinase mRNA in β-cells. The glucose phosphorylating activity at 60 mM glucose in transgenic islets was significantly lower than that in controls, and the insulin secretory response to glucose was lower in transgenic islets than in those of controls in both strains. Following i.p. glucose challenge, higher blood glucose levels were observed in transgenic mice than in controls in the C57BL/6 but not the C3H background. These data suggest that a β-cell secretory defect, in combination with other undefined genetic factors, causes impaired glucose homeostasis in mice.**

Key words: Glucokinase; Antisense mRNA; Transgenic mouse; Glucose homeostasis

1. Introduction

Non-insulin-dependent diabetes mellitus (NIDDM) is considered to be a heterogeneous and polygenic disorder. It is generally accepted that this disease is caused by a combination of insulin secretory defects and insulin resistance in insulin responsive organs. Several genetic defects have been identified in patients with NIDDM. One such defect is in glucokinase, an enzyme playing an important role in glucose metabolism in the liver and pancreatic β-cells [1–3]. Recently, β-cell secretory defects were demonstrated in glucokinase-deficient diabetic patients [4]. Therefore, this type of diabetes provides us with an important opportunity to study the effects of β-cell secretory defects on glucose homeostasis. To further study these effects, we have generated a murine model with reduced pancreatic β-cell glucokinase activity by expressing antisense glucokinase mRNA in β-cells.

C57BL/6 mice were reported to be less glucose-tolerant than C3H mice [5]. In addition, it has been reported that a high-calorie diet in animals with the C57BL/6 genetic background resulted in characteristics similar to those of human NIDDM [6]. We hypothesized that reduced glucokinase activity in β-cells has different effects depending on the genetic background of the mice strains. Therefore, we backcrossed founder mice to both C57BL/6 and C3H strains. Our data indicate that a β-cell secretory defect causes impaired whole-body glucose homeostasis when combined with other, as yet to be identified, genetic factors in mice.

2. Materials and methods

2.1 Animals

(C57BL/6 × DBA/2) F1, C57BL/6 and C3H mice were purchased from Charles River Japan (Yokohama). A 268-bp mouse β-cell glucokinase cDNA fragment (−68 to 200, A of the start codon is denoted by +1) was amplified from polyA$^+$ RNA of the murine β-cell line MIN6 [7], and ligated under human insulin gene promoter as described previously [8], in the antisense orientation and micro-injected into fertilized eggs of (C57BL/6 × DBA/2) F2 mice. We used only male mice for the analysis described herein. Control mice were non-transgenic littermates.

2.2. Northern blot analysis and RNase protection assay

Transgenic and non-transgenic islets were isolated from 12- to 15-week-old littermates by collagenase infusion through the bile duct. Total RNA was isolated from murine tissues with an Isogen RNA extraction kit (Nippon Gene, Tokyo). For RNase protection assay, 268-bp β-cell glucokinase cDNA fragment described above was digested with *Sau*3A1 and a resulting 121-bp fragment was inserted into the *Eco*RI-*Bam*HI site of pGEM4Z. The plasmid was linearized at the *Eco*RI site and RNA probes were synthesized using the T7 RNA polymerase in the presence of [α-^{32}P]UTP. 3 μg of islets total RNA was assayed using an RPA II kit (Ambion, TX).

2.3. Glucose phosphorylation activity

Glucose phosphorylating activities in islet homogenates were determined by fluorometry as described previously [7]. A 12,000 × *g* supernatant of homogenate, derived from ~200 islets from one mouse, was used.

2.4. Insulin secretion from isolated islets

Islets were isolated from 14- to 18-week-old mice as described above. There was no apparent difference in islet size between control and transgenic mice. Batches of 10 islets were incubated for 60 min at 37°C in 300 μl of HEPES (10 mM)-balanced bicarbonate buffer supplemented with 0.5% BSA and varying concentrations of glucose in an atmosphere of 95% O_2 and 5% CO_2. Immunoreactive insulin was measured by RIA.

2.5 Glucose tolerance test

I.p. glucose tolerance tests (2 mg/g wt glucose administered) were performed, after a 14–16-h fast, in 8-week-old mice.

3. Results

We obtained 15 transgenic founder mice which were then bred to the C3H strain. Northern blot analysis of the islets from F1 mice showed higher expression of antisense mRNA in two lines, termed GKr15 and GKr38, than in other lines (data not shown). These lines were subsequently analysed. Blood glucose levels were determined at 2 h after cessation of free access to

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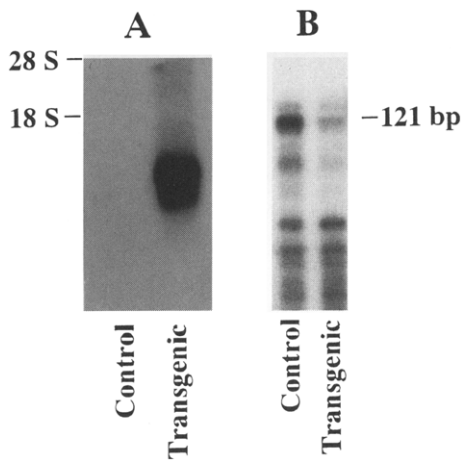

Fig. 1. Expression of antisense glucokinase mRNA and reduced glucokinase mRNA in isolated islets. (A) 5 μg of total RNA from the isolated islets of a single mouse in C57BL/6 background were electrophoresed in 2.0% formaldehyde-agarose gel, transferred onto a nylon membrane, and probed with a murine β-cell glucokinase cDNA fragment. Experiments were performed 3× with similar results for both backgrounds. (B) RNase protection assay was used to estimate the levels of glucokinase mRNA in isolated islets from C57BL/6 background mice. The 121-bp portion of T7-transcribed RNA was protected. Experiments were performed 2× with similar results for both backgrounds.

the chow in the F1 generation mice. Blood glucose levels were significantly elevated in transgenics as compared with those in non-transgenic littermates in lines GKr15 (153 ± 26 vs. 206 ± 15 mg/dl, mean ± S.D., $n = 9$) and GKr38 (167 ± 12 vs. 202 ± 11 mg/dl, $n = 4$). The F1 mice were then bred to the C3H strain. The differences in blood glucose levels between transgenic and non-transgenic littermates were minimal in this generation (data not shown). We speculated that the effects of antisense glucokinase mRNA expression were less evident in the C3H background. Therefore, the GKr15 and 38 founder mice were bred to C57BL/6 mice. Ultimately, we obtained line GKr15 and GKr38 animals which had been crossed with C3H or C57BL/6 strains for at least five generations. In the experiments described bellow, we used the GKr15 line which expressed more antisense GK mRNA than the GKr38 line. The GKr38 line yielded similar results (data not shown).

Fig. 1A shows Northern blot analysis of total RNA from pancreatic islets of GKr15 transgenic and non-transgenic littermates. An ~600-bp band was hybridized with glucokinase cDNA probes. This size coincides with the expected size of the antisense glucokinase mRNA. Islets total RNA was also subjected to RNase protection assay. An RNA fragment of 121 bp should be protected for the glucokinase mRNA. As shown in Fig. 1B, the protected RNA amount was reduced in transgenic islets than in control islets, indicating a diminished expression of glucokinase mRNA in transgenic islets.

Glucose phosphorylating activity at 0.6 or 60 mM glucose concentration was compared between islets from transgenic and non-transgenic mice (Fig. 2). Glucose phosphorylating activity in transgenic islets was lower, by ~30%, than that of control islets at 60 mM glucose in both genetic backgrounds. However, the activity at 0.6 mM glucose did not differ between control and transgenic islets. The difference in glucose phosphorylating activities at these two glucose concentrations is considered to be attributable to glucokinase activity. Therefore, we estimated the glucokinase activity in transgenic islets to be ~50% lower than that in control islets.

Next, we studied glucose-stimulated insulin secretion from freshly isolated islets of control and transgenic mice. As shown in Fig. 3, insulin secretion from both C3H and C57BL/6 transgenic islets tended to be lower than that from control mice at 10 and 25 mM glucose ($P = \sim 0.07$) and significantly lower at 15 mM glucose ($P < 0.05$).

To examine the effects of this diminution in glucose-stimulated insulin secretion from β-cells on whole-body glucose homeostasis, we performed an i.p. glucose challenge test in these mice. As shown in Fig. 4, transgenic mice in the C57BL/6 background had elevated blood glucose levels as compared with non-transgenic siblings at 45 and 90 min. In contrast, blood glucose levels did not differ between transgenic and non-transgenic mice in the C3H background.

To elucidate the mechanism underlying the different effects of impaired glucose-stimulated insulin secretion on glucose tolerance test in mice with the different genetic backgrounds, we assessed the expression of GLUT4 in skeletal muscle and that of hepatic glucokinase in these mice. The amount of GLUT4 glucose transporter protein in skeletal muscle did not differ between these strains (data not shown). In contrast, as shown in Fig. 5, hepatic glucokinase mRNA levels in C57BL/6 mice were lower by ~50% than those in C3H mice, although there was no difference between control and transgenic tissues from mice with the same genetic background.

4. Discussion

Antisense glucokinase mRNA expression was used to make a β-cell glucokinase-deficient mice. Glucose phosphorylating activity and glucose-stimulated insulin secretion from transgenic islets were decreased as compared with those from control

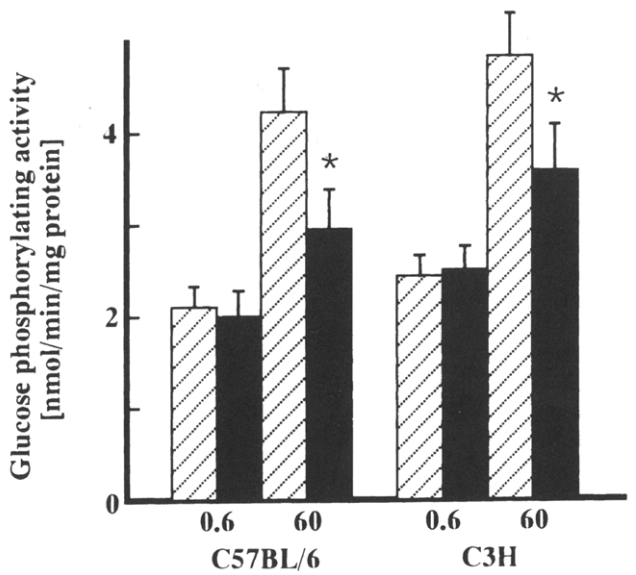

Fig. 2. Glucose phosphorylating activity in islets from control (hatched column) and transgenic (shaded columns) mice in both the C57BL/6 and the C3H strain. Glucose phosphorylating activity was measured at 30°C at 0.6 or 60 mM glucose concentration. Values are mean ± S.D. of eight experiments, each performed in triplicates. *Difference from control at $P < 0.05$.

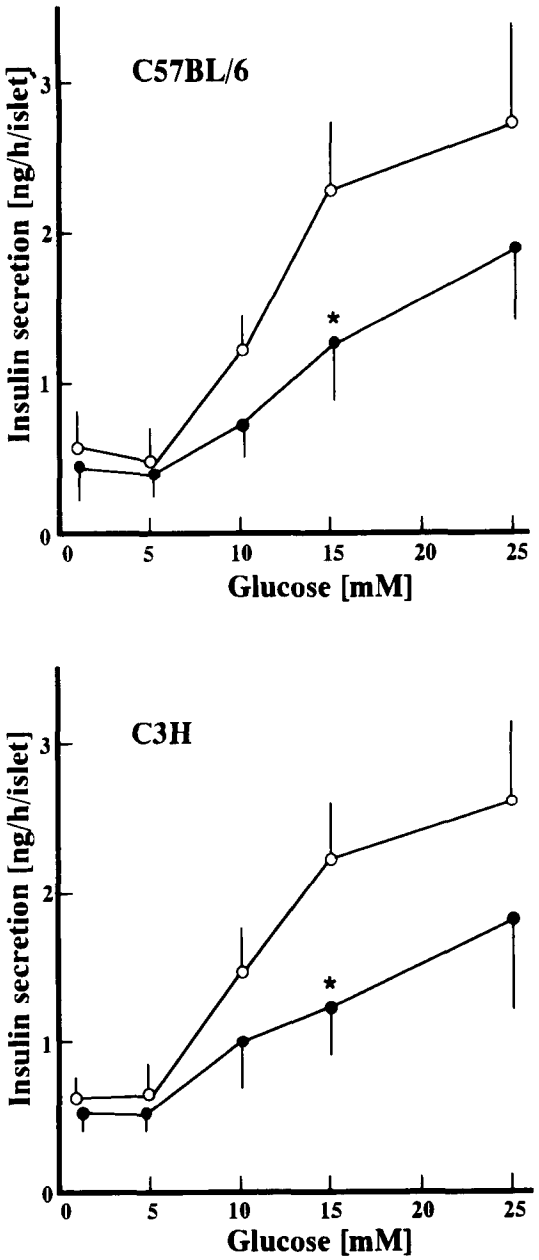

Fig 3. Glucose-stimulated insulin secretion from isolated islets from control and transgenic mice in the C57BL/6 or the C3H background. Control (open circles) and transgenic (closed circles) islets were isolated in parallel. Batches of 10 islets were incubated for 60 min at 37°C with varying concentrations of glucose. Values are mean ± S.D. of five or four experiments using mice in the C57BL/6 or the C3H background, respectively, each performed in triplicate. *Difference from control at $P < 0.05$.

islets. These observations are consistent with glucokinase being the glucose-sensor molecule for glucose-stimulated insulin secretion, as recently demonstrated by several investigators [9–11].

Another β-cell glucokinase-deficient mice were recently reported, in which β-cell glucokinase activities were suppressed by expression of glucokinase-targeted ribozyme [12]. The ribozyme-targeted mice had the C3H background and were reported to exhibit no significant changes in whole-body glucose homeostasis, which is consistent with the results obtained with our transgenic C3H background mice. The findings in both types of transgenic mice in the C3H background are different from those of human glucokinase-deficient patients, in whom glucose homeostasis impairment is mild but clinically significant. The reasons for the differences are currently unclear. However, one possible explanation is that a glucokinase deficiency outside of β-cells contributes significantly to the development of the impaired whole-body glucose homeostasis. Glucokinase is also expressed in hepatocytes, and glucokinase expression in pancreatic α-cells and certain neuroendocrine cell types has recently been reported [13]. The glucokinase expression outside of β-cells is presumably impaired in patients with a defect in the glucokinase gene, while that in our transgenic mice is likely to have remained normal. In this context, impaired glucagon secretion was recently documented in glucokinase-deficient diabetic patients [14]. Thus, suppression of glucokinase alone in β-cells may not be sufficient for the development of impaired glucose homeostasis in mice in the C3H background.

In contrast to mice in the C3H background, we found that

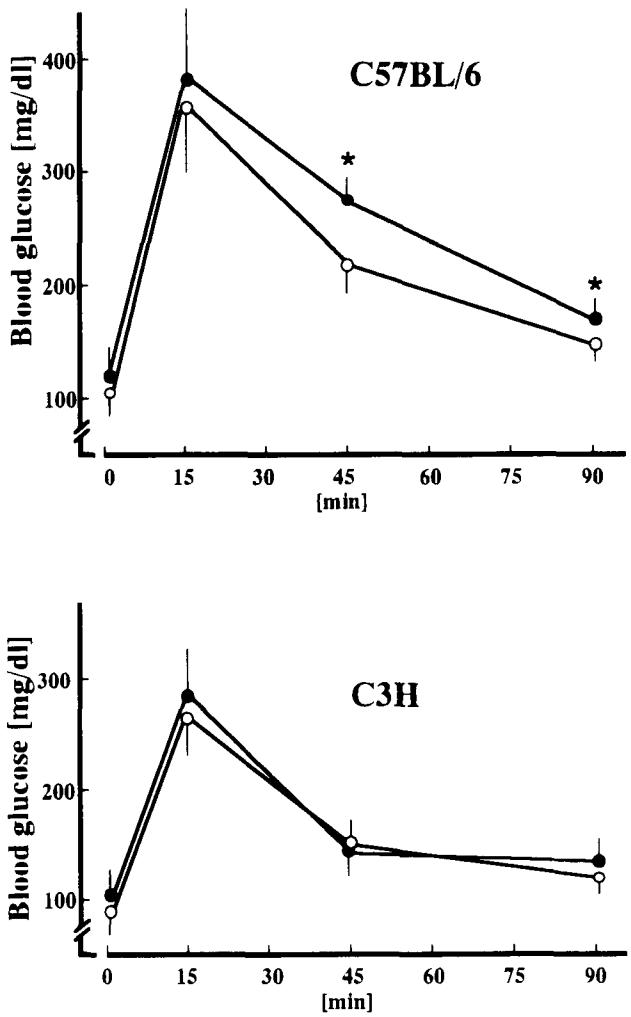

Fig. 4. I.p glucose tolerance test in control (open circles) and transgenic (closed circles) mice in the C57BL/6 or the C3H background. Mice were fasted for 14–16 h and then given an i.p injection of 2 mg of glucose/g of body weight. Blood samples were drawn at the indicated time points from a tail vein. Values are mean ± S.D. of 12 transgenic and 9 control mice in the C57BL/6 background or 8 transgenic and 6 control mice in the C3H background. *Difference from control at $P < 0.05$.

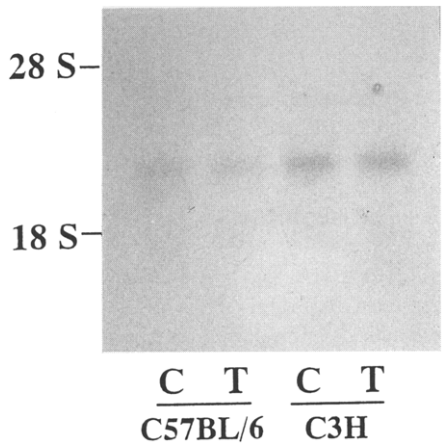

Fig. 5. Expression of hepatic glucokinase in the C57BL/6 or C3H background mice. 20 μg of total RNA from the liver of control (C) and transgenic (T) mice were electrophoresed in a 1.0% formaldehyde-agarose gel, transferred onto a nylon membrane, and probed with rat liver glucokinase cDNA fragment. Experiments were performed 3× using different animals with similar results.

transgenic C57BL/6 background mice exhibited impaired glucose homeostasis. We speculated that as yet unknown genetic factors in the C57BL/6 strain, which differ from those in the C3H strain, had different effects on glucose metabolism in vivo, even though glucose-stimulated insulin secretion was impaired in transgenic mice of both strains. Several differences have been reported between C57BL/6 and C3H strains [5,15,16]. The C3H strain has been reported to be one of the strains with the highest hepatic glucokinase activity [17]. We found that hepatic glucokinase mRNA levels were lower in C57BL/6 mice than in C3H mice. Reduced activity of glucokinase in liver would result in diminished hepatic glucose uptake. Although a decrease in insulin secretion due to reduced β-cell glucokinase activity is likely to cause an insufficient suppression of hepatic gluconeogenesis and glycogenolysis in transgenic mice of both strains, this impaired suppression would result in greater deterioration of glucose metabolism in the liver with a lower glucose uptake. This might account for transgenic mice in the C57BL/6 but not C3H background exhibiting impairment in whole-body glucose homeostasis. Sufficient expression of hepatic glucokinase could mask a reduced suppressive effect on hepatic glucose production, caused by impaired insulin secretion, in transgenic C3H background mice. Although it is not clear whether the lower hepatic glucokinase is the only factor to render C57BL/6 strain more sensitive to the inhibition of β-cell glucokinase expression, this finding suggests that hepatic glucokinase expression is one factor contributing to the regulation of whole- body glucose homeostasis. Nonetheless, our results, obtained in mice in different genetic backgrounds, demonstrate that a β-cell secretory defect leads to impairment of whole-body glucose homeostasis when combined with other unknown genetic factors.

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