Insulin Secretion to Glucose as Well as Nonglucose Stimuli Is Impaired in Spontaneously Diabetic Nagoya-Shibata-Yasuda Mice

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To clarify the mechanisms of impaired insulin secretion in Nagoya-Shibata-Yasuda (NSY) mice, an inbred strain of mice with spontaneous development of type 2 (non–insulin-dependent) diabetes mellitus, the insulin response to glucose (5.5 to 27.8 mmol/L) and nonglucose stimuli (glibenclamide, arginine, and BayK8644, a Ca-channel opener) was studied in vitro using isolated islets from male NSY and control C3H/He mice at 36 weeks of age by the batch incubation method. Insulin response to 5.5 mmol/L glucose was not significantly different between NSY and C3H/He mice, but insulin response to a high concentration of glucose (≥11.1 mmol/L) was significantly smaller in NSY mice than in control C3H/He mice. The dose-response curve of insulin secretion showed a markedly reduced maximum response, but almost normal glucose sensitivity in NSY islets. Insulin responses to glibenclamide (1 mmol/L), arginine (20 mmol/L), and BayK8644 (0.1 mmol/L) were also significantly smaller in NSY mice than in C3H/He mice. Insulin content of islets, in contrast, was significantly higher in NSY mice than in C3H/He mice. The impaired insulin response to glucose and nonglucose stimuli together with higher insulin content in islets in the NSY mouse suggest that a defect in voltage-dependent Ca^{2+}-channel or thereafter in the cascade of insulin secretion may be responsible for impaired insulin secretion in NSY mice. NSY mice, therefore, could be a novel animal model of type 2 diabetes with a defect in insulin secretion at a different site from that in previously known animal models.

TYPE 2 (non–insulin-dependent) diabetes mellitus is a heterogeneous disorder caused by an interaction of genetic and environmental factors.[1] This heterogeneity makes it difficult to clarify the pathogenesis of the disease. Inbred animal models are helpful and necessary to understand the etiology of heterogeneous diseases, such as diabetes. Although several animal models of type 2 diabetes have been described, most of them are characterized by obesity and insulin resistance[2] rather than impaired insulin secretion, with the exception of Goto-Kakigazi (GK) rats.[3]

The NSY (Nagoya-Shibata-Yasuda) mouse is an inbred animal model of type 2 diabetes. The NSY mouse was established by selective breeding for glucose intolerance from an outbred Jcl:ICR mouse colony.[4] Unlike severely obese animal models, such as ob/ob and db/db mice, NSY mice are only mildly obese. NSY mice spontaneously develop diabetes mellitus in an age-dependent manner, as in the case of human type 2 diabetes. The cumulative incidence of diabetes is almost 100% in male NSY mice at 48 weeks of age.[5] Previous in vivo studies suggested that both impaired insulin secretion in response to glucose and insulin resistance contribute to the development of diabetes in NSY mice.[5] Elucidation of the underlying mechanisms of impaired insulin secretion and action in the NSY mouse, therefore, may increase our understanding of the etiology of human type 2 diabetes.

As a first step to clarify the mechanisms of impaired insulin secretion in NSY mice, we investigated the pancreatic β-cell function of the NSY mouse in vitro by studying insulin response to glucose and nonglucose stimuli using isolated islets.

MATERIALS AND METHODS

Animals

Male NSY and control C3H/He mice at 36 weeks of age were used in this study. By this age, most NSY mice develop diabetes as defined by blood glucose level at 120 minutes during intraperitoneal glucose tolerance test (2 g/kg glucose).[5,6] Mean (±SD) body weight in NSY (n = 5) and C3H (n = 5) mice at 36 weeks of age was 51.2 ± 3.1 and 37.3 ± 2.7 g ($P < .001$), mean fasting plasma glucose level was 5.0 ± 0.4 and 5.4 ± 0.7 mmol/L, and mean fasting insulin level was 545 ± 163 and 305 ± 89 pmol/L ($P < .05$), respectively.[5] C3H/He mice (Charles River Japan, Kanagawa, Japan) were used as the nondiabetic control strain, because this strain is one of the most common strains used as experimental mice and was used in our previous in vivo studies on glucose tolerance and insulin secretion.[5,6] All mice were given a laboratory diet, MF (Oriental Yeast, Tokyo, Japan), containing 24.6% protein, 5.6% fat, 3.1% fiber, 6.3% ash, 52.8% complex carbohydrate, and tap water ad libitum in an air-conditioned room (22° to 25°C) with a 12-hour light/dark cycle.

Isolation of Pancreatic Islets and Assessment of Insulin Secretory Capacity

Pancreatic islets were isolated from 5 to 6 male NSY or C3H/He mice in each experiment by collagenase digestion.[7] The isolated islets were cultured overnight in RPMI 1640 medium (Sigma, St Louis, MO) containing 11.1 mmol/L glucose, 10% fetal calf serum, and 1% glutamic acid. The isolated islets were preincubated for 60 minutes at 37°C in Hank's Balanced Salt Solution (HBSS) medium with 5.5 mmol/L glucose and 0.1% bovine serum albumin. Each batch of 10 islets was incubated for 60 minutes at 37°C in 1.0 mL HBSS medium with 0.1% bovine serum albumin and different glucose concentrations (5.5, 8.3, 11.1, 16.7, or 27.8 mmol/L), L-arginine (5.5 mmol/L glucose + 20 mmol/L L-arginine), glibenclamide (5.5 mmol/L glucose +

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1 mmol/L glibenclamide) or BayK8644, a Ca-channel opener (5.5 mmol/L glucose + 0.1 mmol/L BayK8644).

Insulin Concentration and Insulin Content

Following incubation, the islets were collected by centrifugation. The insulin concentration of the incubation medium was measured by radioimmunoassay (ShionoRIA insulin; Shionogi, Osaka, Japan) with rat insulin (Novo, Copenhagen, Denmark) as a standard. DNA content was determined by fluorometric assay,[8] and insulin secretion was normalized by DNA content of the islets. Insulin was extracted from the islets by the acid ethanol method,[9] and insulin content of islets was measured by radioimmunoassay.

Statistical Analysis

All results are expressed as mean ±SEM. Statistical analysis was performed by Student's *t* test.

RESULTS

Insulin Response to Glucose

Insulin response to glucose increased in a dose-dependent manner in both NSY and C3H mice (Fig 1). Insulin response to 5.5 mmol/L glucose in NSY mice was comparable to that in C3H/He mice (NSY *v* C3H/He: 30.3 ± 2.0 *v* 31.2 ± 2.5 pg/ng islet DNA · 60 min). Insulin response to 8.3 mmol/L glucose was slightly, but not significantly, lower in NSY mice than C3H/He mice. Insulin responses to 11.1 mmol/L (NSY *v* C3H/He: 41.3 ± 1.6 *v* 57.3 ± 5.6 pg/ng islet DNA · 60 min; $P < .01$), 16.7 mmol/L (44.8 ± 2.9 *v* 60.8 ± 6.1 pg/ng islet DNA · 60 min; $P < .05$), and 27.8 mmol/L (44.7 ± 3.2 *v* 58.8 ± 5.8 pg/ng islet DNA · 60 min; $P < .05$) glucose were significantly lower in NSY mice than in C3H/He mice. The dose-response curve of glucose-stimulated insulin secretion showed a markedly reduced maximum response (44.8 ± 2.9 *v* 60.8 ± 6.1 pg/ng islet DNA · 60 min; $P < .05$), but almost normal glucose sensitivity in NSY islets (EC50, NSY *v* C3H/He: 8.2 *v* 8.7 mmol/L) (Fig 1).

Insulin Response to Nonglucose Stimuli

To investigate the mechanism of the impaired insulin response to glucose in NSY mice, we examined insulin response to nonglucose stimuli, which stimulate insulin secretion at a different step of the cascade in glucose-stimulated insulin secretion: glibenclamide, arginine, and BayK8644, a Ca-channel opener, in the presence of 5.5 mmol/L glucose. Insulin response to both 1 mmol/L glibenclamide (NSY *v* C3H/He: 151% ± 9% *v* 203% ± 22% of basal; $P < .01$) and 20 mmol/L arginine (NSY *v* C3H/He: 154% ± 11% v 197% ± 14% of basal; $P < .05$) was impaired in NSY mice as compared with control C3H/He mice, to almost the same degree as that to 16.7 mmol/L glucose (Fig 2). Insulin response to 0.1 mmol/L BayK8644 was also impaired in NSY mice (NSY *v* C3H/He: 162% ± 19% *v* 225% ± 20% of basal; $P < .05$). To investigate the insulin response to BayK8644 in detail, insulin response to different concentrations of BayK8644 was studied (Fig 3). Insulin responses to more than 0.1 mmol/L BayK8644 were significantly lower in NSY mice than in C3H/He mice.

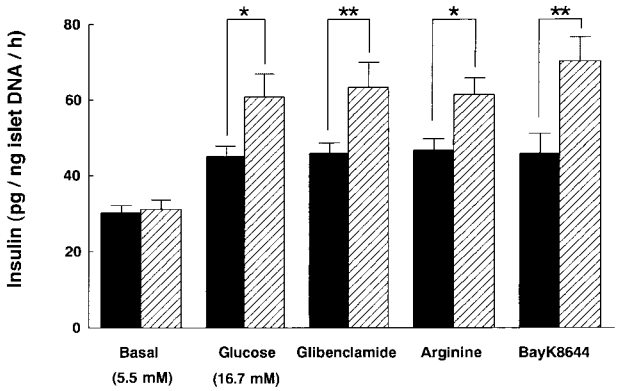

Fig 2. Insulin response to glucose (16.7 mmol/L), glibenclamide, arginine, and BayK8644 in isolated islets from male NSY (closed bars) and control C3H/He (hatched bars) mice. Data are mean ± SEM. *$P < .05$, **$P < .01$.

Islet Insulin Content in NSY and C3H/He Mice

Islet insulin content was significantly higher in NSY mice than in C3H/He mice (NSY *v* C3H/He: 775 ± 56 *v* 441 ± 42 ng/10 islets). The relative insulin content of islets in these mice

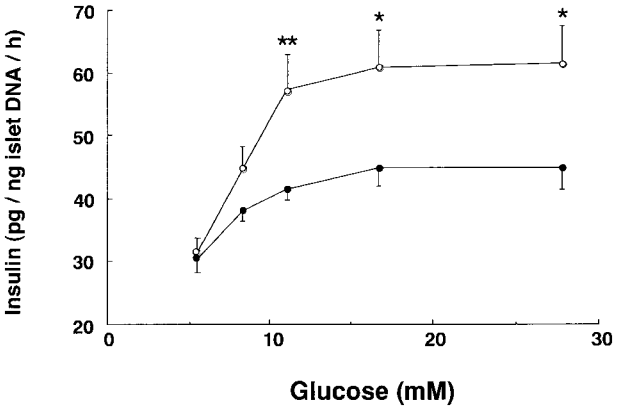

Fig 1. Insulin response to glucose in isolated islets from male NSY (●) and control C3H/He (○) mice. Data are mean ± SEM. *$P < 0.05$, **$P < .01$.

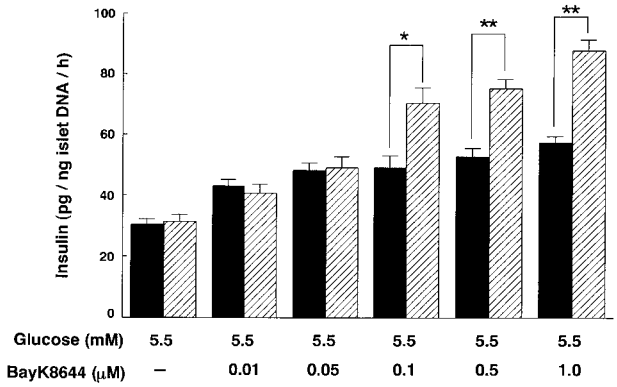

Fig 3. Insulin response to different concentrations of BayK8644 in isolated islets from male NSY (closed bars) and control C3H/He (hatched bars) mice. Data are mean ± SEM. *$P < .05$, **$P < .01$.

was comparable to pancreatic insulin content in our previous study.[5]

DISCUSSION

Type 2 diabetes is caused by a defect in insulin secretion and/or insulin action. Most naturally-occurring animal models reported thus far are characterized by massive obesity and severe insulin resistance. The GK rat is one of a few models that are not severely obese and are characterized by impaired insulin secretion rather than insulin resistance. Studies on β-cell function in GK rats have been reported by several investigators and have contributed to our understanding of the pathogenesis of impaired insulin secretion in human type 2 diabetes. GK rats, however, show hyperglycemia as early as 4 weeks of age.[3] Type 2 diabetes in NSY mice, in contrast, is late in onset, and both impaired insulin secretion and action contribute to type 2 diabetes.[5] Moreover, the phenotype, such as glucose intolerance and insulin resistance, is enhanced by a high-fat diet or sucrose supplementation, as in the case of human diabetes (manuscript in preparation). Studies on β-cell function in NSY mice will, therefore, provide further information that may be relevant to human type 2 diabetes with impaired insulin secretion.

The present study showed that glucose-induced insulin secretion in vitro was markedly impaired in NSY mice, as was previously observed in vivo during an intraperitoneal glucose tolerance test.[5] The dose-response curve of glucose-stimulated insulin secretion showed markedly reduced maximum insulin secretion, but almost normal glucose sensitivity of NSY islets. This is in clear contrast to insulin secretion in heterozygous mice with targeted disruption of the pancreatic β-cell–specific glucokinase gene,[10] which is characterized by reduced glucose sensitivity.

Impaired insulin response to glucose was observed and has been extensively studied in a genetically determined subgroup of type 2 diabetes, maturity-onset diabetes of the young (MODY). The characteristics of insulin secretion have been reported for 3 subtypes of MODY, MODY1, MODY2, and MODY3, which are caused by mutations in genes encoding hepatocyte nuclear factor (HNF)-4α,[11] glucokinase,[12] and HNF-1α,[13] respectively. Studies on insulin secretion in normoglycemic subjects with genetic markers of MODY2 indicated that MODY2 is characterized by reduced glucose sensitivity, but a normal maximum response.[14] In contrast, MODY1 and MODY3, which are caused by mutations in transcriptional factors, HNF-4α and HNF-1α, respectively, were reported to be characterized by normal insulin secretion at lower glucose concentrations, but reduced insulin secretory response at higher plasma glucose concentrations.[15,16] The dose-response curve of glucose-induced insulin secretion in NSY mice is, therefore, close to that in MODY1 and MODY3, but not to MODY2 subjects. Reduced insulin responses in MODY1 and MODY3 subjects were reported to be evident when the plasma glucose increases above a threshold of 7-8 mmol/L. Reduced insulin response in the NSY mouse is also evident when glucose concentration exceeds 8 mmol/L, although the present study was an in vitro study, whereas previous studies in MODY subjects were in vivo studies.

In this study, insulin response to arginine was impaired in NSY mice as compared with control C3H mice, to almost the same degree as that to 16.7 mmol/L glucose. Most amino acids have been shown to stimulate insulin release. Arginine and leucine are the 2 most potent stimulators.[17] Although the insulin response to nonglucose stimuli, such as arginine, has been reported to be relatively preserved in type 2 diabetic patients as compared with that to glucose stimuli,[18,19] decreased insulin secretion in response to nonglucose secretagogues, such as arginine,[20] intravenous (IV) glucagon,[21] IV terbutaline,[21] and a test meal,[21] has recently been reported in subjects with mutations in the HNF-4α gene. Moreover, marked reduction in insulin secretory response to arginine, as well as glucose, was reported in homozygous mice with a null mutation in the HNF-1α gene.[22] These characteristics are quite similar to those in NSY mice reported in this study. NSY mice, therefore, may be a unique model of type 2 diabetes with a defect in insulin secretion, whose characteristics are similar to those observed in MODY1 and MODY3, which are caused by mutations in transcription factors.

In our recent whole genome screening, we have mapped a gene, *Niddln*, which affects insulin secretion and glucose tolerance, to an interval on mouse chromosome 11.[23] A candidate gene in the interval is a transcription factor, HNF-1β, whose mutation has recently been reported in patients with MODY (MODY5).[24] A nucleotide substitution leading to an amino acid change at the DNA binding domain was detected in the NSY mouse as compared with the control laboratory strain.[23] These data, together with the abnormality in insulin secretion in the NSY mouse as observed in the present study, make this variant in HNF-1β an attractive candidate for *Niddln*. The region where *Niddln* has been mapped is, however, still very large, with many genes contained in the region. It is therefore premature to conclude that a mutation in HNF-1β is responsible for impaired insulin secretion in the NSY mouse. Fine mapping of the gene and further characterization of the molecular defects in insulin secretion in the NSY mouse are necessary to clarify whether or not HNF-1β is responsible for impaired insulin secretion in the NSY mouse.

Glucose is a major secretagogue of insulin. According to the present model of glucose-induced insulin secretion, glucose is transported into β cells through glucose transporter (GLUT2), metabolized, and the subsequent increase in adenosine triphosphate/adenosine diphosphate (ATP/ADP) ratio closes the ATP-sensitive K^+-channels. The subsequent plasma membrane depolarization opens voltage-dependent calcium-channels and increases intracellular Ca^{2+} concentration, which is thought to be the primary trigger for insulin secretion. Insulin secretagogues, such as glucose, glibenclamide, arginine, and BayK8644, stimulate insulin secretion at different sites in this cascade. Glibenclamide is a second generation sulfonylurea and stimulates insulin release through the sulfonylurea receptor, which is a subunit of the ATP-sensitive K^+-channel. Arginine was reported to cause membrane depolarization as a cationic amino acid and increase intracellular Ca^{2+} concentration, which leads to insulin secretion, although metabolism of the amino acid itself may also partially contribute to the response.[25] An agonist for the L-type calcium channel, BayK8644, directly acts on the L-type calcium channel, a major subtype of voltage-dependent calcium channel in β cells involved in insulin se-

cretion.[26,27] In this study, insulin response to not only glucose, but also nonglucose stimuli, was impaired in NSY mice. Since islet insulin content in NSY mice was higher than that in C3H/He mice, a defect in insulin synthesis does not appear to be a primary cause of impaired insulin secretion in NSY mice. Considering the mechanism of insulin secretion in response to glucose via the ATP-sensitive K^+-channel, the site responsible for impaired insulin secretion in NSY mice is thought to be located at the voltage-dependent calcium channel or thereafter in the cascade of insulin secretion, such as transfer and fusion of the insulin vesicle to the membrane.

In conclusion, insulin secretion in response to glucose and nonglucose stimuli, glibenclamide, arginine, and BayK8644 was impaired in NSY mice as compared with control C3H/He mice. In contrast, insulin content of islets was significantly higher in NSY mice than in C3H/He mice. These data indicate that NSY mice have a defect in the insulin secretory response to glucose, as well as nonglucose stimuli, and suggest that the site responsible for impaired insulin secretion in NSY mice is located at the voltage-dependent calcium channel or thereafter in the cascade of insulin secretion of the ATP-sensitive K^+-channel-dependent pathway. The NSY mouse, therefore, could be a unique animal model whose insulin secretion is impaired at a different site from that in previously known animal models of diabetes.

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