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Adult pancreatic islets require differential pax6 gene dosage

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Abstract

Pax6, a paired homeodomain transcription factor, plays crucial roles in morphogenesis of eye, central nervous system, and pancreatic islets. Recently, heterozygosity for pax6 mutation has been reported in some individuals with glucose intolerance and aniridia. To investigate the role of pax6 for pancreatic islet function, we examined the pancreatic phenotype of small eye rat strain (rSey^2) with a point mutation in the pax6 locus resulting in truncated PAX6 proteins. Analyses of the insulin secretory profile of heterozygous rSey^2/+ revealed that insulin secretion is significantly increased in response to membrane-depolarizing stimuli such as arginine, tolbutamide, and KCl. The processes of insulin granule exocytosis were suggested to be enhanced in rSey^2/+. On the other hand, pancreatic insulin and glucagon content and islet architecture in rSey^2/+ showed no significant differences compared to wild-type. These findings indicate differential requirements for pax6 gene dosage in displaying function and maintaining architecture of adult pancreatic islets.

Keywords: Pax6; Pancreatic islets; Insulin secretion; Arginine; Small eye; Pancreas

Transcription factors playing a role in pancreatic development have been shown to orchestrate the process of cell differentiation and transition by regulating the expression of numbers of genes [1,2]. To form the pancreas and organize pancreatic islets, multiple transcription factors play roles at precise steps in the developmental program. Various models in which these transcription factors are inactivated have revealed defects of pancreatic development or pancreatic islet morphogenesis.

The paired homeobox (Pax) family of transcription factors is involved in embryonic development of many organs including eyes, brain, kidney, thyroid gland, immune system, and the pancreas [3,4]. Two of its members, Pax4 and Pax6, play important roles in pancreatic endocrine cell differentiation [5,6]. In addition, Pax6 is essential for the development of eye and central nervous system and regulates the expression of various functional molecules in these tissues [7]. During the mouse embryogenesis, PAX6 protein can be detected already around E9.0 in the pancreatic endoderm, and its expression is maintained throughout pancreas development in all endocrine cells [8]. Analyses of pax6 mutant animals (Fig. 1) have revealed that differentiation of endocrine cells and the forming of proper islet architecture are severely affected in the fetal pancreas in the homozygous state. Pax6 knockout mice lack glucagon-producing α-cells and do not form proper islet structure [6]. In Sey^{NEU} mice, in which the PAX6 protein is

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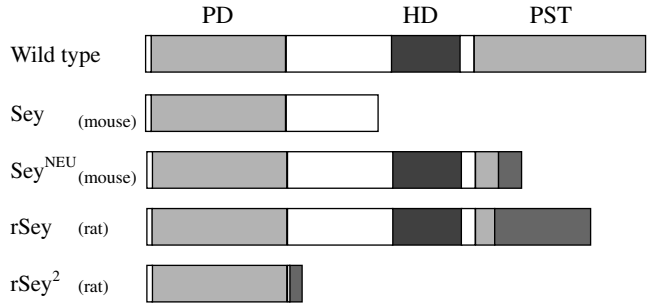

Fig. 1. Schematic diagram of coding region of pax6 gene and mutants. Wild-type PAX6 has a paired domain (PD), homeodomain (HD), and proline/serine/threonine rich transactivation domain (PST). Sey^{NEU} and rSey have a mutation, which results in a PAX6 protein that has a PD and HD but lacks the functional PST domain. Sey and $rSey^2$ (this study) have a mutation resulting in a PAX6 that has a PD but lacks the HD and PST domain completely.

truncated directly after the homeodomain, all four endocrine cell types are decreased in number [9]. Thus, Pax6 is involved in pancreatic development, particularly in endocrine cell differentiation and pancreatic islet organogenesis.

Transcription factors are involved not only in regulating pancreas development but also in pancreatic endocrine cell function. Many mutation models of transcription factors have shown that these mutations influence β-cell molecular events and the insulin secretory profile by altering the gene expression.

However, the role of PAX6 in adult islet function is little known except for the observations *in vitro* that PAX6 increases insulin, somatostatin, and glucagon gene transcription by binding with their promoters [9,10], and is involved in the regulation of enzymes and transcription factors [11,12]. The homozygous mice of pax6 knockout, Sey^{NEU} lack eyes and even model mice with conditional inactivation of pax6 in the endocrine pancreas [13] die shortly after birth, limiting the analyses of PAX6 function in the postnatal pancreatic islet function.

To investigate the mechanisms by which alterations in PAX6 affect islet function, we examined pancreatic islet function and architecture in small eye rat strain ($rSey^2$) with point mutation in the pax6 locus resulting in truncated PAX6 proteins [14] and found that in contrast to showing normal insulin secretion in response to glucose, $rSey^2/+$ showed surprisingly increased insulin release in response to membrane depolarizing stimuli and that $rSey^2/+$ had normal pancreatic islet architecture, indicating different requirement for pax6 gene dosage in the function and the morphology of the pancreatic islets.

Materials and methods

Animals. Mutant rats with small eyes ($rSey^2$) [14] were used in this study. Studies for the adult rats were performed in heterozygous ($rSey^2/+$) and their age-matched wild-type littermates. Homozygous rat embryos were obtained by inter-crossing male and female heterozygotes. Animal care and procedures were approved by the Animal Care Committee of Kyoto University.

Measurement of blood glucose, insulin, and glucagon levels. Blood glucose levels were measured by enzyme-electrode method. Plasma insulin levels were measured using ELISA kit (Shibayagi, Gunma, Japan). Plasma glucagon levels were measured using ELISA kit (Yanaihara Institute Inc., Shizuoka, Japan). Different groups of age-matched 20- to 24-week-old male rats were used for intraperitoneal glucose tolerance test. After an overnight fast, plasma insulin, glucagon, and glucose levels were measured and D-glucose (2 g/kg body weight) was loaded. In the insulin tolerance test, human insulin (1 U/kg) was injected subcutaneously in the fed condition. Blood samples were taken from the tail vein at indicated times.

Quantification of pancreatic peptide content. Protein was extracted from the dissected pancreas using acid extraction. Protein content was measured by Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA). The amount of immunoreactive insulin was determined by RIA, using rat insulin as described [15]. The amount of immunoreactive glucagon was determined by using RIA kit (Linco Research, St. Charles, MO).

Immunohistochemistry. The pancreata of rats were removed under pentobarbital anesthesia (40 mg/kg body weight) and fixed in Bouin's solution. Pancreatic specimens were embedded in paraffin and sectioned at 3.5 μm. The avidin–biotin complex method with alkaline phosphatase or with peroxidase was used as previously described [16] with a slight modification. After deparaffinization, the following were sequentially applied: normal goat or rabbit serum (diluted to 1:75, Dako, Kyoto, Japan), primary antibodies, biotin-labeled goat anti-rabbit or rabbit anti-goat IgG serum (diluted to 1:300, Dako), and avidin–biotin–alkaline phosphatase complex or avidin–biotin–peroxidase complex (diluted to 1:100, Vector Laboratories, Burlingame, CA), followed by hematoxylin nuclear counterstaining. Staining was visualized in black and red by alkaline phosphatase substrate (Vector Laboratories) and in brown by peroxidase substrate. For PAX6 analysis, paraffin sections of pancreata were deparaffinized and autoclaved for 10 min at 121 °C in 10 mM citrate buffer (pH 6.0). The following primary antibodies were used: rabbit anti-insulin polyclonal antibody (diluted to 1:350, Dako), rabbit anti-glucagon serum (diluted to 1:500, OAL-123, Otsuka Assay Laboratory, Tokushima, Japan), rabbit anti-somatostatin polyclonal antibody (diluted to 1:200, Dako), rabbit anti-pancreatic polypeptide polyclonal antibody (diluted to 1:200, Dako), goat anti-GLUT2 polyclonal antibody (diluted to 1:50, C-19, Santa Cruz Biotechnology, Santa Cruz, CA) or rabbit anti-PAX6 polyclonal antibody (diluted to 1:20, H-295, Santa Cruz Biotechnology).

Isolated pancreatic perfusion. The pancreas was isolated as previously described [17]. All perfusions were accomplished with Krebs–Ringer Bicarbonate Buffer (KRBB) containing 0.25% bovine serum albumin (BSA, Fraction V, Sigma, St. Louis, MO) and 4.6% dextran (mean molecular weight 70,000; Pharmacia, Uppsala, Sweden). The perfusate was gassed with 95% O_2–5% CO_2 to maintain pH 7.4 at 37 °C. The flow rate was kept constant at 1.9 ml/min. After 20 min of equilibration, the perfusate was collected at 1-min intervals by cannula inserted into the portal vein. The collected effluent was frozen immediately with 1000 U aprotinin (Bayer, Leverkusen, German). The amount of immunoreactive insulin and immunoreactive glucagon was determined by RIA as described above.

Measurement of insulin release from isolated rat pancreatic islets. Isolated islets were cultured for 18 h in RPMI 1640 medium containing 10% fetal calf serum (FCS), 100 U/ml penicillin, and 100 μg/ml streptomycin. Insulin release from intact islets was monitored using batch incubation system described previously [18] with slight modifications. Cultured islets were preincubated at 37 °C for 30 min in KRBB supplemented with 2.8 mM glucose, 0.2% BSA, and 10 mM Hepes, adjusted to pH 7.4. Groups of 10 islets were then batch-incubated for 30 min in 0.4 ml of KRBB with test materials. The amount of immunoreactive insulin was determined by RIA as described above.

Measurement of intracellular Ca^{2+}. For intracellular Ca^{2+} ($[Ca^{2+}]_i$) measurement, cultured islets were loaded with fura-PE3 during 2 h of preincubation in the presence of 2 μM fura-PE3AM (Calbiochem, La Jolla, LA) as previously described [18]. Islets were placed at 36 ± 1 °C, superfused with KRBB containing 2.8 mM glucose and 10 mM Hepes adjusted to pH 7.4 for 30 min, and subsequently exposed to the medium containing a high concentration of K^+. The islets were excited successively

at 340 and 380 nm, and fluorescence emitted at 510 nm was captured by a charge-coupled device (CCD) camera (Micro Max 5-MHz System; Roper Industries, Trenton, NJ). Fluorescence signals at 340-nm (F340) and 380-nm (F380) were detected every 20 s. Results are expressed as the ratios (F340/F380).

Measurement of insulin release from electrically permeabilized islets. Cultured islets were preincubated with KRBB with 2.8 mM glucose and 0.2% BSA for 30 min. The islets were washed twice in cold potassium aspartate buffer (KA buffer) containing 140 mM KA, 7 mM $MgSO_4$, 2.5 mM EGTA, 30 mM Hepes (pH 7.0), and 0.5% BSA, with $CaCl_2$ added to a Ca^{2+} concentration of 30 nM. The islets were then permeabilized by high voltage discharge (four exposures, each of 450 μs duration, to an electrical field of 4.0 kV/cm) in KA buffer and washed once with the same buffer. Groups of electrically permeabilized islets were then batch-incubated for 30 min at 37 °C in 0.4 ml KA buffer with various concentrations of Ca^{2+} and ATP. At the end of the incubation period, permeabilized islets were pelleted by centrifugation (15000*g*, 180 s), and aliquots of the buffer were sampled. The amount of immunoreactive insulin was determined by RIA as described above.

Statistical analysis. Results are expressed as means ± SE. Statistical significance was evaluated by unpaired Student's *t*-test. $P < 0.05$ was considered significant.

Results and discussion

One intact allele in the pax6 gene is sufficient for maintenance of adult pancreatic islet architecture

In fetal pancreas in the homozygous state ($rSey^2/rSey^2$), insulin-positive cells are remarkably reduced and the alignment of the endocrine cells is not preserved (Fig. 2A). Especially, few or no glucagon-positive cells were found in the pancreas, and glucagon content could not be detected in RIA study of pancreas extract (data not shown). Recently, it has been reported that PAX6 is important especially for the endocrine cells to obtain final differentiation, rather than to proliferate [13]. Pax6 knockout mice have been shown to completely lack glucagon-producing cells in fetal pancreas [6]. In contrast, in the homozygous (Sey^{NEU}/Sey^{NEU}) mice, in which the PAX6 protein has a paired domain and homeodomain but lacks the transactivation domain (Fig. 1), the number of α-cells is reduced but is still present in the late fetal stage [9]. In the homozygous Sey mice [19] and $rSey^2$ rats (this study), in which the PAX6 protein has a paired domain but lacks a homeodomain and transactivation domain (Fig. 1), few or no glucagon-positive cells were detected in the later fetal stage in the homozygous state. These findings suggest that homeodomain is especially important in the formation of the pancreatic α-cells. In contrast to the homozygous fetal pancreatic islets, immunohistochemical evaluation of pancreata from adult heterozygote rats ($rSey^2/+$) revealed normal islet morphology with insulin-positive β-cells located in the center of the islet (Fig. 2B and C), and glucagon-positive α-cells (Fig. 2B and C), somatostatin-positive δ-cells (data not shown), and pancreatic polypeptide-positive PP-cells (data not shown) located in the periphery of the islets. Pancreatic insulin and glucagon contents in $rSey^2/+$ were the same as in wild-type (data not shown). Because of a recent report showing that expression of the glucose transporter GLUT2 was down-regulated in the pancreas of conditional inactivation of pax6 model mice [13], we examined GLUT2 expression in $rSey^2/+$ pancreatic islets. Adult $rSey^2/+$

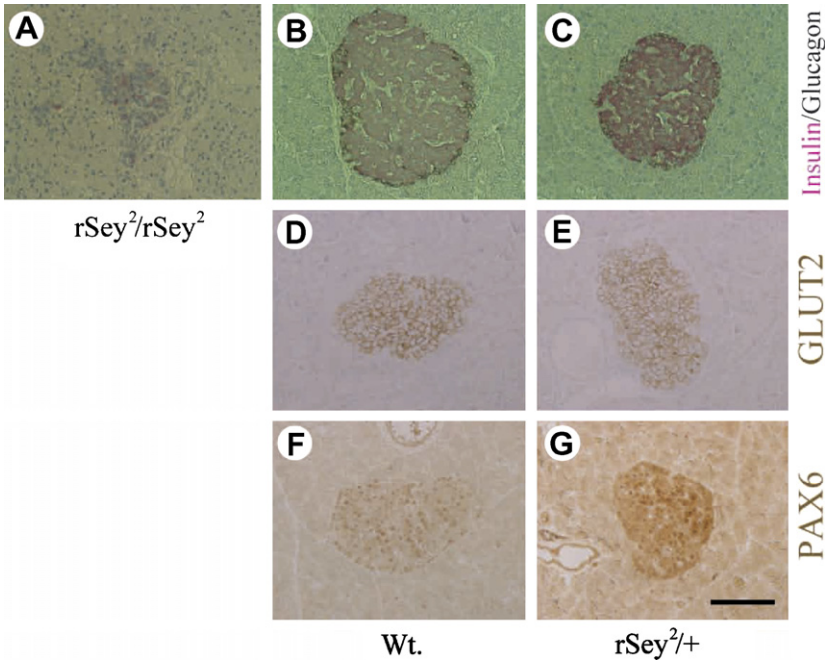

Fig. 2. One intact allele in the pax6 gene is sufficient for maintenance of adult pancreatic islet architecture. Immunohistochemical staining for islet protein was performed on paraffin-embedded sections of the pancreas. The sections were double-labeled for insulin (red) and glucagon (black) in the fetal (20.5E) homozygous state ($rSey^2/rSey^2$) (A) and adult wild-type (B), $rSey^2/+$ (C) rat pancreas or labeled for GLUT2 (D,E) and PAX6 (F,G) in the adult wild-type (D,F) and $rSey^2/+$ (E,G) rat pancreas. Bar 100 μm.

showed normal GLUT2 expression in the pancreatic endocrine cells (Fig. 2D and E). Thus, one allele of the wild-type pax6 gene is essential and sufficient to maintain morphologically normal pancreatic islets in adult. Anti-PAX6 antibody, which recognizes the C-terminus of PAX6 protein, was used to identify wild-type PAX6 protein derived from the wild-type pax6 allele, and rSey^2/+ was found to have similar PAX6 protein expression pattern in the nuclei of pancreatic islets (Fig. 2F and G).

Glucose-induced insulin secretion is preserved in heterozygous small eye rat strain (rSey^2/+)

Both male and female rSey^2/+ had similar body weight as wild-type rats (Fig. S1(A)). Pancreatic weight measured in males was similar in rSey^2/+ and wild-type (data not shown). Male rSey^2/+ showed normal fasting blood glucose levels, but had significantly higher fasting plasma insulin levels (Table 1). In the fed state, rSey^2/+ showed significantly lower blood glucose levels, and the plasma insulin level was similar in the two groups (Table 1). As these findings indicate relative hyperinsulinemia in rSey^2/+, we assessed the glucose-lowering effect of insulin by insulin tolerance test (ITT). rSey^2/+ and wild-type showed similar insulin sensitivity (Fig. S1(B)), indicating that the hyperinsulinemia is not derived from insulin resistance. In intraperitoneal glucose tolerance test (IPGTT), plasma glucose elevation elicited by glucose load in rSey^2/+ was similar to that of wild-type rats (Fig. S1(C)). Insulin secretion during IPGTT was also similar (Fig. S1(D)). Thus, it is possible that insulin secretion in response to secretagogues other than glucose is enhanced, resulting in hyperinsulinemia in rSey^2/+ rats. Glucagon is not a candidate as plasma glucagon levels were similar in the fasted and fed state (Table 1).

Insulin secretion induced by arginine is augmented in rSey^2/+ perfused pancreas

To determine which secretagogues contribute to the enhanced insulin release of rSey^2/+ in vivo, pancreatic perfusion was performed. rSey^2/+ rats showed the same biphasic insulin release from isolated perfused pancreas in response to stepwise increases in glucose concentration from 5.5 to 16.7 mM. However, insulin release in response to 10 mM arginine at the basal glucose level was significantly increased in rSey^2/+ rats (Fig. 3A). The integrated response to 10 mM arginine in the presence of 5.5 mM glucose was 1062 ± 285 ng of insulin in wild-type ($n = 5$) versus 2068 ± 131 ng of insulin in rSey^2/+ rats ($n = 5$) ($P < 0.05$) (Fig. 3B). On the other hand, rSey^2/+ rats showed similar glucagon release from the perfused pancreas in response to 10 mM arginine in the presence of 5.5 mM glucose (data not shown). This finding demonstrates that increased insulin response to 10 mM arginine in rSey^2/+ is not due to simultaneous enhancement of glucagon release.

Insulin secretory response to membrane-depolarizing stimuli in rSey^2/+ pancreatic islets

As one of the mechanisms by which arginine potentiates insulin release is direct depolarization of the pancreatic β-cell membrane, we examined insulin secretion evoked by membrane-depolarizing insulin secretagogues other than arginine. In batch incubation experiments, insulin release from isolated pancreatic islets in response to glucose stimulation was similar in rSey^2/+ (16.7 mM glucose: rSey^2/+ 0.82 ± 0.065 ng/islet/30 min ($n = 4$)) and wild-type rats (0.97 ± 0.087 ng/islet/30 min ($n = 4$)) ($P = 0.22$). However, insulin secretory responses to 10 mM arginine, 30 mM K^+, or 100 μM tolbutamide were significantly increased in rSey^2/+ pancreatic islets (10 mM arginine in the presence of 5.5 mM glucose: rSey^2/+ 0.55 ± 0.028 ng/islet/30 min ($n = 5$) vs. wild-type 0.21 ± 0.017 ng/islet/30 min ($n = 4$), $P < 0.001$; 30 mM K^+ in the presence of 2.8 mM glucose: rSey^2/+ 0.58 ± 0.035 ng/islet/30 min ($n = 5$) vs. wild-type 0.39 ± 0.040 ng/islet/30 min ($n = 4$), $P < 0.01$; 100 μM tolubutamide in the presence of 2.8 mM glucose: rSey^2/+ 0.45 ± 0.034 ng/islet/30 min ($n = 6$) vs. wild-type 0.31 ± 0.022 ng/islet/30 min ($n = 6$), $P < 0.01$) (Fig. 3C).

$[Ca^{2+}]_i$ elevation in pancreatic islets induced by 30 mM K^+-induced membrane depolarization in rSey^2/+ rats

To determine if the increase in K^+-induced insulin release in rSey^2/+ isolated islets is associated with increased intracellular Ca^{2+}, fura-PE3 was used to measure changes in $[Ca^{2+}]_i$. Five minutes before and 15 min after exposure to 30 mM K^+ in the presence of 2.8 mM glucose, $[Ca^{2+}]_i$ of rSey^2/+ islets was somewhat lower than that of wild-type islets (Fig. 4A and B). However, there was no difference between the depolarization-stimulated increment ratio in

Table 1
Blood glucose and plasma pancreatic hormone levels

	Blood glucose (mg/dl)		Plasma insulin (ng/ml)		Plasma glucagon (ng/ml)	
	Fed	Fasted	Fed	Fasted	Fed	Fasted
Wt	91 ± 2.1 ($n = 14$)	76 ± 1.0 ($n = 17$)	2.4 ± 0.36 ($n = 12$)	0.30 ± 0.09 ($n = 8$)	0.80 ± 0.03 ($n = 9$)	1.2 ± 0.44 ($n = 9$)
rSey^2/+	$80 \pm 1.8^{**}$ ($n = 11$)	72 ± 1.3 ($n = 15$)	1.9 ± 0.23 ($n = 11$)	$0.91 \pm 0.15^{*}$ ($n = 6$)	0.73 ± 0.05 ($n = 9$)	1.1 ± 0.22 ($n = 9$)

Values indicated as means ± SE.
* $P < 0.01$.
** $P < 0.001$ for rSey^2/+ vs. Wt.

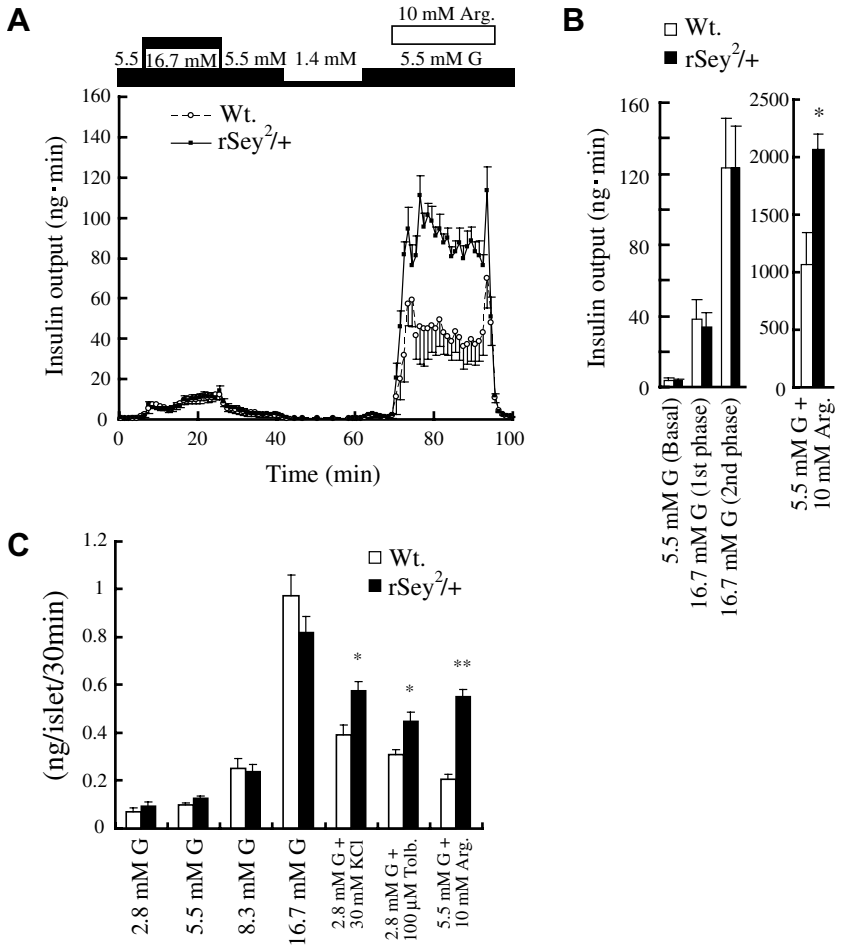

Fig. 3. (A,B) Insulin secretory responses from isolated perfused pancreas. Insulin release in response to glucose and arginine from the pancreas of rSey^2/+ (■) and wild type littermates (○). Values are expressed as mean ± SE. (B) AUC of insulin release at 5.5 mM glucose (basal level, 1–6 min), first (6–13 min) and second (13–26 min) phase insulin release at 16.7 mM glucose, and insulin release in response to 5.5 mM glucose and 10 mM arginine (70–96 min) from the perfused pancreas of wild type (open bars, $n = 5$) and rSey^2/+ (filled bars, $n = 5$). Values are expressed as mean ± SE. * $P < 0.05$, for rSey^2/+ vs. wild type. G: glucose, Arg.: arginine. (C) Depolarization-induced insulin release from isolated islets. Insulin release from batch-incubated islets of wild type (open bars) and rSey^2/+ (filled bars) was examined in response to the indicated concentrations of glucose with or without membrane depolarizing insulin secretagogues. Values are expressed as mean ± SE of 4–7 determinations from several experiments. * $P < 0.01$, ** $P < 0.001$ for rSey^2/+ vs. wild type. G: glucose, Tolb.: tolbutamide, Arg.: arginine.

rSey^2/+ islets and wild-type islets (rSey^2/+ 1.076 ± 0.0033 vs. wild-type 1.073 ± 0.0044, $P = 0.54$) (Fig. 4B).

Ca^{2+} efficacy in insulin release under low ATP condition in rSey^2/+ pancreatic islets

To determine if intracellular Ca^{2+} efficacy is altered in rSey^2/+ islets, we measured insulin release from pancreatic β-cell at $[Ca^{2+}]_i$ clamped by extracellular medium. As shown in Fig. 4C and D, raising the Ca^{2+} concentration from 30 nM to 10 μM elicited an increase in insulin release from electrically permeabilized islets. In the presence of 5 mM ATP, insulin release in rSey^2/+ was similar to wild-type at all Ca^{2+} concentrations (Fig. 4C). This result is commensurate with the findings that rSey^2/+ has a similar insulin secretory response to glucose as wild-type. However, in the presence of 1 mM ATP, insulin release in rSey^2/+ islets at Ca^{2+} concentrations from 30 to 1000 nM was greater than in wild-type islets (at 30 nM Ca^{2+}: rSey^2/+ 0.44 ± 0.031 vs. wild-type 0.30 ± 0.032 ($n = 8$), $P < 0.01$; at 100 nM Ca^{2+}: rSey^2/+ 0.44 ± 0.039 vs. wild-type 0.32 ± 0.035 ($n = 8$), $P < 0.05$; at 300 nM Ca^{2+}: rSey^2/+ 0.66 ± 0.040 vs. wild-type 0.45 ± 0.052 ($n = 7$), $P < 0.01$; at 1000 nM Ca^{2+}: rSey^2/+ 0.85 ± 0.033 vs. wild-type 0.54 ± 0.074 ng/islet/30 min ($n = 8$), $P < 0.01$) (Fig. 4D). This might well underlie the increased insulin secretion seen in rSey^2/+ β-cells in response to membrane depolarizing stimuli at the basal glucose level.

Thus, our findings show that pax6 gene mutation modifies the insulin secretory profile of adult pancreatic islets and that the disturbance in the insulin secretory mechanism in rSey^2/+ pancreatic islets is in the triggering of insulin granule exocytosis by the rise in $[Ca^{2+}]_i$, although the molecular mechanism remains to be determined.

In this study, rSey^2/+ islets showed increased insulin response to membrane-depolarizing stimuli such as

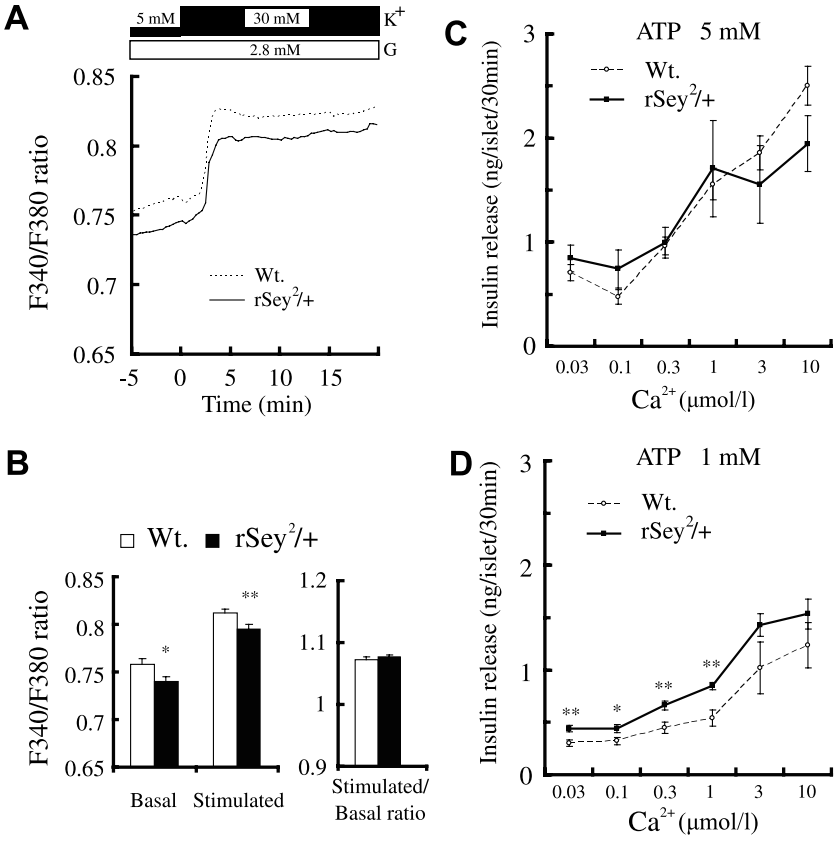

Fig. 4. (A,B) Fluorescence measurement of $[Ca^{2+}]_i$ elevation induced by 30 mM K^+-induced depolarization in the presence of 2.8 mM glucose. (A) Time course of $[Ca^{2+}]_i$ in perfused islets. Values represent mean of 83 (wild-type) and 74 (rSey^2/+) determinations from the several experiments. (B) Left, average values calculated from the data from (A). Basal, average values from −5 to 0 min in the presence of 5 mM K^+ with 2.8 mM glucose. Stimulated, average value from 0 to 15 min in the presence of 30 mM K^+ with 2.8 mM glucose. Right, values represent means ± SE of the ratio of stimulated value to basal value. $*P < 0.05$, $**P < 0.01$ for rSey^2/+ vs. wild-type. G, glucose. (C,D) Ca^{2+} dose–response of insulin release from electrically permeabilized islets. After preincubation with 2.8 mM glucose for 50 min, islets were electrically permeabilized and incubated with medium containing Ca^{2+} and ATP at the concentration indicated in the figure. Ca^{2+} dose-dependent insulin release from electrically permeabilized islets of wild-type (○) and rSey^2/+ (■) in the presence of 5 mM (C) or 1 mM (D) ATP. Values represent means ± SE of 7–8 determinations in the same experiment for each. $*P < 0.05$, $**P < 0.01$ for rSey^2/+ vs. wild-type.

arginine. This findings may underlie the relative hyperinsulinemia of rSey^2/+ rats in vivo. In contrast to rSey^2/+, it recently has been reported that rSey/+, another small eye rat strain (Fig. 1), in which the pax6 mutation is located in the transactivation domain [20], has impaired insulin response to glucose but show normal insulin secretory response to arginine [21]. These differences in pancreatic islet function suggest that pax6 plays a key role in regulating the insulin secretory response to various nutrients in pancreatic islets.

Morphological and functional analyses of rSey^2/+ pancreatic islets have important implications regarding gene dosage on pancreatic islet architecture and function. The maintenance of islet morphology in adult pancreas showed low sensitivity to quantity of pax6 gene. In contrast, islet function is necessarily more sensitive to alterations in pax6 gene. While the pax6 gene mutation in rSey^2/+ altered the insulin secretory profile, glucagon secretion was unaffected by the same mutation. These findings indicate that for displaying normal function, insulin-secreting β-cells among the pancreatic islet hormone-secreting cells require higher quantity of pax6 gene than glucagon-secreting α-cells. Outside of the pancreas, it is well known that pax6 is a key regulator of eye formation and that heterozygous pax6 mutations result in eye size reduction due to sensitivity to pax6 gene dosage for eye formation [22,23]. rSey^2/+ eye size also is reduced [14], indicating that the pancreas and the eye of same individual has differing sensitivity to the quantity of pax6 gene. Thus, there are differential dosage requirements for the pax6 gene between organs as well as between morphological and functional characteristics.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2006.11.105.

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