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Research Letters

Mutation of the Pax6 gene causes impaired glucose-stimulated insulin secretion

To the Editor: The paired-box gene that encodes Pax6 is a member of the *Pax* gene family and is expressed in eye, nose, pancreas and the central nervous system. It is well known that aniridia, a panocular human eye malformation, is caused by mutations in *Pax6* [1]. Mutant rats with small eyes (rSey) were identified during the course of breeding Sprague–Dawley rats [2], and it was shown that about 600 base pairs of Pax6 mRNA are deleted in these rats. At the genomic level, a single base (G) insertion in the exon produces the truncated mRNA [3]. Heterozygotes (rSey/+) have small eyes, while homozygotes (rSey/rSey) lack eyes and a nose, resulting in perinatal death. We previously evaluated glucose tolerance in six patients with aniridia and found that five of the patients possessed a *Pax6* mutation and had impaired insulin secretion and glucose intolerance [4]. Based on this finding, we hypothesised that a reduction in Pax6 expression or activity leads to the deterioration of beta cell function.

In the present study we evaluated pancreatic beta cell function in rats with a heterozygous mutation in *Pax6* (rSey/+ rats) to investigate whether insufficient insulin secretion is attributable to Pax6 inactivation. It should be noted that the rats used in this study were inbred.

To evaluate pancreatic beta cell function we performed an IVGTT. Glucose-stimulated first-phase insulin secretion (at 2 min) was significantly lower in rSey/+ rats than in +/+ rats (Fig. 1a), although the two groups had similar plasma glucose levels (Fig. 1b). In contrast, arginine-stimulated insulin secretion in rSey/+ rats was almost the same as that in +/+ rats (Fig. 1c). To further evaluate glucose-stimulated insulin secretion, we performed a perifusion study using islets isolated from rSey/+ and +/+ rats. As shown in Figure 1d, glucose-stimulated insulin secretion was significantly lower in islets isolated from rSey/+ rats than in those from +/+ rats. It should be noted that a marked difference in glucose-stimulated first-phase insulin secretion was observed at 1 min. As in the in vivo study, arginine-stimulated insulin secretion in islets from rSey/+ rats was almost the same as that in islets from +/+ rats (Fig. 1e). KCl-stimulated insulin secretion in rSey/+ islets was also comparable to that in +/+ islets (data not shown). Taken together, these results indicate that the heterozygous mutation in *Pax6* specifically impaired glucose-stimulated insulin secretion without influencing arginine- or KCl-stimulated insulin secretion.

To examine islet morphology and insulin biosynthesis, we performed immunostaining for insulin and examined insulin content in the pancreas. There were no differences in islet morphology (data not shown) or insulin content between pancreata from rSey/+ and +/+ rats. To elucidate the mechanism by which mutation of *Pax6* causes impairment of glucose-stimulated insulin secretion, we used RT-PCR to examine the expression of various beta-cell-related factors in the islets of the rats. We assumed that there are some defects in the pathway between glucose entry and membrane depolarisation in the beta cells of the rSey/+ rats. Glucose is the primary physiological stimulus for insulin secretion, and this process requires glucose sensing [5]. GLUT2 facilitates the rapid equilibration of glucose across the plasma membrane, and glucokinase is the rate-limiting step in glycolytic flux for insulin secretion [6]. Both of these proteins are crucial for glucose-stimulated insulin secretion. The K_{ATP} channel, which contains Kir 6.2 and sulphonylurea receptor 1 (SUR1), is also important for glucose-induced insulin secretion. However, we observed no differences in the levels of expression of glucokinase, GLUT2, Kir 6.2 or SUR-1 between the two groups of rats (data not shown). These results indicate that the heterozygous mutation in *Pax6* causes impaired glucose-stimulated insulin secretion without influencing islet morphology, insulin content in the pancreas or the expression of various beta-cell-related factors. Thus, it remains unknown how mutation of *Pax6* causes impairment of glucose-stimulated insulin secretion. Although not examined in this study, it was recently reported that the transcription of the gene encoding the glucose-6-phosphatase catalytic subunit-related protein is regulated by Pax6 [7], and it is possible that this is affected by mutation of *Pax6*.

In conclusion, the results of the present study indicate that in rSey/+ rats, glucose-stimulated insulin secretion is specifically impaired, while arginine-stimulated insulin secretion, islet morphology and expression of various beta-cell-related factors are almost the same as in +/+ rats. Since it is well known that a deficiency in glucose-stimulated early-phase insulin secretion is a strong predictor for the development of type 2 diabetes [8], we postulate that decreased Pax6 expression or activity could lead to type 2 diabetes.

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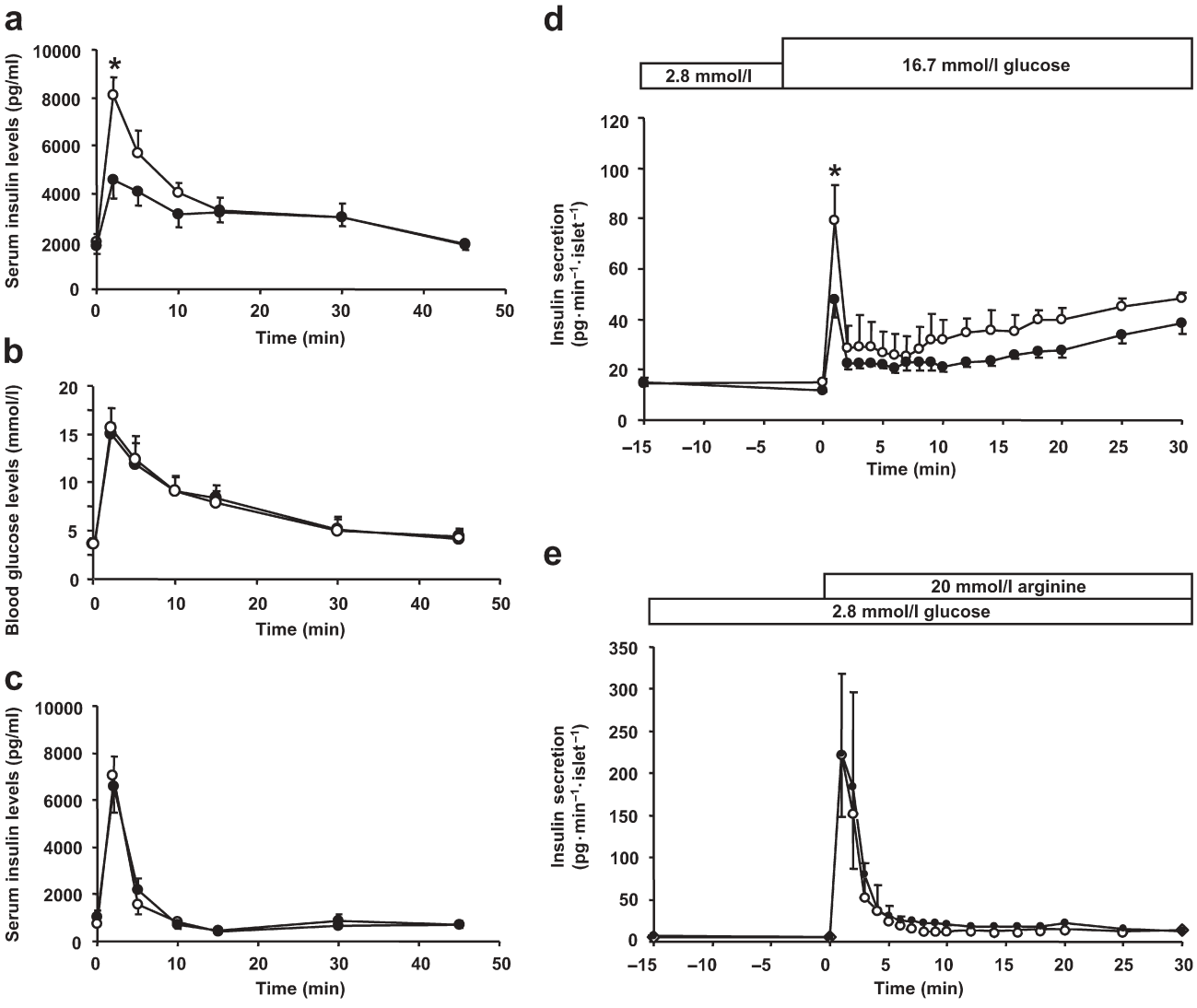

Fig. 1 a–c. An IVGTT and an intravenous arginine load test were performed in 12-week-old rSey/+ (●) and +/+ rats (○). After an overnight fast, glucose was injected intravenously at a dose of 0.5 g/kg body weight, then plasma insulin (**a**) and glucose (**b**) levels were measured. Similarly, after an overnight fast, arginine was injected intravenously at a dose of 0.1 g/kg body weight, then plasma insulin levels were measured (**c**). Data are shown as means ± SEM, (n=4–6). * p<0.05 vs rSey/+ rats. **d, e.** Glucose-stimulated insulin secretion (**d**) and arginine-stimulated insulin secretion (**e**) were evaluated using islets isolated from 12-week-old rSey/+ (●) and +/+ rats (○). Groups of 50 isolated islets were perifused in a Krebs–Ringer bicarbonate buffer containing 2.8 mmol/l glucose. Following this stabilisation period, the islets were perifused with 16.7 mmol/l glucose or 20 mmol/l l-arginine for 30 min. Data are shown as means ± SEM (n=4–6). * p<0.05 vs rSey/+ rats

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