The ATP-sensitive potassium channel is a key molecular complex for glucose-stimulated insulin secretion in pancreatic β cells. In humans, mutations in either of the two subunits for this channel, the sulfonylurea type 1 receptor (Sur1) or Kir6.2, cause persistent hyperinsulinemic hypoglycemia of infancy. We have generated and characterized Sur1 null mice. Interestingly, these animals remain euglycemic for a large portion of their life despite constant depolarization of membrane, elevated cytoplasmic free Ca²⁺ concentrations, and intact sensitivity of the exocytotic machinery to Ca²⁺. A comparison of glucose- and meal-stimulated insulin secretion showed that, although Sur1 null mice do not secrete insulin in response to glucose, they secrete nearly normal amounts of insulin in response to feeding. Because Sur1 null mice lack an insulin secretory response to GLP-1, even though their islets exhibit a normal rise in cAMP by GLP-1, we tested their response to cholinergic stimulation. We found that perfused Sur1 null pancreatic islets respond to FYLY3 by GLP-1, we tested their response to cholinergic stimulation. We found that perfused Sur1 null pancreatic islets secrete insulin in response to the cholinergic agonist carbachol in a glucose-dependent manner. Together, these findings suggest that cholinergic stimulation is one of the mechanisms that compensate for the severely impaired response to glucose and GLP-1 brought on by the absence of Sur1, thereby allowing euglycemia to be maintained.

Glucose-stimulated insulin secretion by the pancreatic β cell requires the coupling of changes in glucose metabolism to alterations in membrane potential (1–4). In response to a rise in the intracellular ATP/ADP ratio that occurs with glucose metabolism the closure of ATP-sensitive potassium (KATP) channels causes the β cell membrane to depolarize. This, in turn, leads to the opening of voltage-gated L-type Ca²⁺ channels, a rise in the cytoplasmic free Ca²⁺ concentration ([Ca²⁺]), and the subsequent exocytosis of insulin (5). The β cell KATP channel is an octameric complex of two proteins: an inward-rectifier K⁺ channel, Kir6.2, and the sulfonylurea receptor type 1 (Sur1), which are present in a 4:4 stoichiometry (6, 7). Kir6.2, which forms the channel pore, possesses intrinsic ATP sensitivity (8, 9), whereas Sur1, a member of a superfamily of ATP-binding cassette transporter proteins, provides sites for interaction with Mg-ADP (10). Sulfonylureas, which are widely used for treatment of patients with type 2 diabetes mellitus, act by binding to KATP channels and stimulating their closure (10).

Mutations in Sur1 are a frequent cause of persistent hyperinsulinemic hypoglycemia of infancy (PHHI), an autosomal recessive disorder characterized by excess and unregulated secretion of insulin. Because initial identification of Sur1 as a candidate gene for PHHI by Aguilar-Bryan et al. (11), more than 50 different mutations in this gene, as well as 2 mutations in Kir6.2, have been identified in PHHI patients (12). Analyses of pancreatic β cells from PHHI patients, as well as functional studies of mutated KATP channels introduced into cultured cells, suggest that impaired KATP channel function and/or expression is a common mechanism underlying PHHI (13–15).

Both Kir6.2 and Sur1 knock-out mice have previously been shown to maintain euglycemia, despite the absence of functional KATP channel in their islets (16, 17). However, the mechanisms that enable mice lacking KATP channels to maintain regulated insulin secretion are not known. Because identification of these mechanisms may lead to new insights into the regulation of insulin secretion, we have also generated mice that lack Sur1. We found that Sur1 null mice secrete normal amounts of insulin upon meal ingestion, largely through intact second phase insulin secretory responses, and that an intact response to cholinergic stimulation may explain the ability of

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these mice to regulate their insulin secretion in the absence of K\textsubscript{ATP} channels. These results suggest that, although K\textsubscript{ATP} channels play an important role in glucose-stimulated insulin secretion, other insulin secretagogues act to stimulate secretion of this hormone through K\textsubscript{ATP} channel-independent mechanisms.

**EXPERIMENTAL PROCEDURES**

**Cloning and Targeted Disruption of the Sur1 Gene**—Both BAC (129/SvJ) and P1 (129/Ola) clones containing the Sur1/Kir6.2 gene locus were isolated by PCR screening (Genome Systems, Inc., St. Louis, MO). The gene targeting strategy involved the placement of tandem repeats and loxP sites around a 1.01-kbp DNA fragment that contains the proximal promoter and exon 1 of Sur1, and its subsequent removal with cre recombinase (Fig. 1B). Positive selection was achieved with a phosphoglycerol kinase-neomycin resistance gene (pgk-neo) cassette, and negative selection with a phosphoglycerol kinase-herpes simplex virus type I thymidine kinase gene (pgk- tk) cassette placed outside the 3’ arm of the targeting vector. After electroporation of RW4 ES cells (Genome Systems, Inc., St. Louis, MO), 21 of 321 clones (6.5%) were found to be resistant to both G418 and gancyclovir and to have the desired 10.1-kb deletion of the targeting vector. After electroporation of RW4 ES cells (Genome Systems, Inc., St. Louis, MO), 21 of 321 clones (6.5%) were found to be resistant to both G418 and gancyclovir and to have the desired 10.1-kb deletion of the targeting vector. After electroporation of RW4 ES cells (Genome Systems, Inc., St. Louis, MO), 21 of 321 clones (6.5%) were found to be resistant to both G418 and gancyclovir and to have the desired 10.1-kb deletion of the targeting vector.

**Husbandry and Genotyping**—Mice were fed a standard rodent chow diet, maintained on 12-h light/dark cycle, and were specific pathogen-free. The Sur1\textsuperscript{lox+/H11032}, Sur1\textsuperscript{lox−/H11001}, and Sur1\textsuperscript{lox+/H11001} alleles were distinguished by PCR using two different sets of primers. The primers SUR.1 (5’-CAATTCTCCAACTGAGGCTCTTAA) and SUR.2 (5’-TGGCAGAGTACCATCAGGCTGT) amplify a 530-bp DNA fragment from the Sur1\textsuperscript{lox+} allele and a 412-bp fragment from the Sur1\textsuperscript{lox−} allele. The primers SUR.1 and Neo-5’ (5’-AGGCTGTCCTCAACATCCTA) generate a 414-bp fragment from the Sur1\textsuperscript{lox−} allele. The predicted band of 1566 bp from Sur1\textsuperscript{lox−/H11001} allele and 1323 bp from Sur1\textsuperscript{lox−/H11032} allele was obtained with ES cell clone 2G4.

**Preparation of Islets and Single β Cells**—Islets of Langerhans were isolated from 8- to 10-week-old mice by a collagenase technique (18) and dispersed into single cells in Ca\textsuperscript{2+}–free. The glucose turnover rate divided by the plasma glucose concentration (mg/ml).

**Electrophysiology**—Whole-cell K\textsubscript{ATP} currents were recorded using the perforated-patch configuration of the patch-clamp technique as described previously (20). The rate of exocytosis was determined as the rate of tracer infusion (dpm/mg) divided by the corrected plasma glucose specific activity (dpm/mg) per kilogram body weight of the mouse. The glucose clearance (mJg\textsuperscript{−1}mm\textsuperscript{−1}) was calculated as the rate of glucose turnover divided by the plasma glucose concentration (mg/ml). Blood samples were taken from the cannula implanted into the left carotid artery at indicated time.

**Biochemical Analyses**—Blood glucose levels were determined by a glucose oxidase method using a blood glucose analyzer (Hemocue, Mission Viejo, CA). Plasma glucose levels were determined by the UV method (29). Plasma insulin concentrations were measured using either RIA kit (Linco Research, St. Louis, MO) or ELISA kit (Crystal Chem, Chicago, IL). Insulin concentrations in the pancreas perfusate were determined by RIA using anti-insulin coated tubes (ICN, Orangeburg, NY) and radiolabeled insulin (Diagnostic Products, Los Angeles, CA). Plasma GLP-1 (active form) concentrations were determined using ELISA kit (Linco Research, St. Louis, MO).

**Statistical Analysis**—Results are expressed as mean ± S.E. Differences between groups were evaluated using Student’s t test. p < 0.05 was considered to be significant.

**RESULTS**

**Sur1 and Kir6.2 Gene Structure**—Overlapping BAC and P1 clones containing the mouse Sur1/Kir6.2 gene locus were obtained and analyzed by DNA sequencing to determine intron/exon structure. The mouse Sur1 gene was found to consist of 39 exons ranging in size from 33 to 243 nucleotides and to span ~80 kbp (see Fig. 1A). In contrast, the Kir6.2 gene is intronless, spans only 1.5 kbp, and lies ~5 kbp downstream of the 3’ end of the Sur1 gene.

**Sur1 Knock-out Mice**—The gene targeting strategy shown in Fig. 1 (B–D) was used to generate mice containing the Sur1\textsuperscript{lox−/lox} allele. To create a null allele for Sur1 (Sur1\textsuperscript{lox−}), a ~1-kbp gene segment containing both promoter and exon 1 sequences was removed by cre-mediated recombinase. Analysis of 199 offspring from the intercrossing of mice that were heterozygous for the Sur1\textsuperscript{lox−} allele (e.g. Sur1\textsuperscript{lox+/lox}), where w indicates the wild type allele) showed a Mendelian distribution of genotypes (50 Sur1\textsuperscript{lox+/lox}, 107 Sur1\textsuperscript{lox−/lox}, and 42 Sur1\textsuperscript{lox+/lox}). Mice lacking Sur1 (e.g. Sur1\textsuperscript{lox−/lox}) were fertile and indistinguishable from their wild-type littermates in terms of normal growth (data not shown).

**Elevated [Ca\textsuperscript{2+}], in Sur1-deficient Mouse β Cells**—Pancreatic β cells from mice lacking Sur1 exhibited a continuous train of action potentials that was blocked by an inhibitor of the L-type voltage-dependent Ca\textsuperscript{2+} channel, similar to that reported by Seghini et al. (17). This is consistent with the lack of functional K\textsubscript{ATP} channels in Sur1\textsuperscript{lox−/lox} β cells as assessed by measurements of whole-cell K\textsubscript{ATP} currents. By using the standard whole-cell configuration of the patch-clamp technique, cells were depolarized to potentials between ~60 and +50 mV in 10-mV steps from a holding potential of ~70 mV. This protocol was...
there was no difference in plasma insulin level between 
Sur1\textsuperscript{neo} and Sur1\textsuperscript{lox/lox} mice (Fig. 3B). To test the possibility that the exocytotic machinery in Sur1\textsuperscript{neo} β cells is impaired by the chronically elevated [Ca\textsuperscript{2+}] by measuring an increment of cell capacitance at different Ca\textsuperscript{2+} concentrations. Either 0.9 or 10 μM free Ca\textsuperscript{2+} produced a more pronounced increase in cell capacitance in Sur1\textsuperscript{neo} β cells compared with Sur1\textsuperscript{lox/lox} β cells, indicating a higher sensitivity of the exocytotic machinery to Ca\textsuperscript{2+} in Sur1\textsuperscript{neo} β cells than in Sur1\textsuperscript{lox/lox} β cells (Fig. 4A).

Approximating the Hill equation to the average rates of exocytosis in response to various concentrations of free Ca\textsuperscript{2+} (Fig. 4B) yielded EC\textsubscript{50} values of 0.47 μM for Sur1\textsuperscript{lox/lox} β cells and 0.43 μM for Sur1\textsuperscript{neo} β cells. The results demonstrate that sensitivity of the exocytotic machinery to Ca\textsuperscript{2+} is not impaired in Sur1\textsuperscript{neo} β cells.

Glucose-stimulated Insulin Secretion in Sur1\textsuperscript{neo} Mice Is Markedly Attenuated—We next examined glucose-stimulated insulin secretion in Sur1\textsuperscript{neo} mice during a hyperglycemic clamp. The plasma glucose concentration was held at ~300 mg/dl in conscious animals for 2 h by infusion of glucose solution through an implanted cannula. In response to this prolonged hyperglycemic stimulus Sur1\textsuperscript{loox} mice showed an increase in their plasma insulin concentrations of 5-fold (0.55 ± 0.13 to 2.72 ± 0.70 ng/ml, p = 0.025). In contrast, the Sur1\textsuperscript{neo} mice showed only a 1.5-fold increase in their plasma insulin concentrations (0.55 ± 0.13 to 0.81 ± 0.26 ng/ml, p = 0.115) (Fig. 5). The glucose infusion rate necessary to maintain hyperglycemia, as well as the glucose turnover and glucose clearance rates in Sur1\textsuperscript{neo} mice, were all lower than in Sur1\textsuperscript{lox/lox} mice (37, 58, and 58%, respectively; see Fig. 5 and Table 1). The reduction of all of these parameters indicates
diminished glucose tolerance in the Sur1\textsubscript{neo} mice because of impaired glucose-stimulated insulin secretion.

Secretagogue-specific Impairment of Insulin Secretion in Sur1\textsubscript{neo} Mice—The finding that mice without Sur1 are eu-
glycemic for much of their life was in apparent conflict with their markedly impaired glucose-stimulated insulin secretion. 
Therefore, to test the possibility that non-glucose secretogogues were acting to stimulate insulin secretion in the absence of 
functional K\textsubscript{ATP} channels, we measured blood glucose and plasma insulin levels in both Sur1\textsubscript{w} and Sur1\textsubscript{neo} mice during the postprandial period. After a 16-h fast, the mice were given free access to food and both blood glucose and insulin levels were determined. Both sets of mice were observed to begin refeeding within a few min and to eat frequently for at least 30 min. The blood glucose levels in both the Sur1\textsubscript{w} and Sur1\textsubscript{neo} mice increased rapidly until about 60 min, then gradually decreased (Fig. 6A, upper graph). Although the difference in mean values between the two groups was not significant, 2 of 4 Sur1\textsubscript{neo} mice studied showed abnormally high levels of postprandial glucose concentration (> 250 mg/dl at 60 min), whereas none of the Sur1\textsubscript{w} animals showed any values over 200 mg/dl through the experimental period (Fig. 6A, inset). Sur1\textsubscript{w} mice exhibited a 9-fold increase in their plasma insulin

TABLE I

| Summary of metabolic parameters determined during hyperglycemic clamp experiments |
|---------------------------------|-------------------------------|
|                                  | Sur1\textsubscript{w}         | Sur1\textsubscript{neo}         |
|                                  | (n = 6)                       | (n = 6)                        |
| Plasma glucose (mg/dl)           | 301 ± 9                      | 298 ± 6                       |
| Plasma insulin (mg/ml)           | 2.72 ± 0.7                   | 0.81 ± 0.3                    |
| Glucose infusion rate (mgkg\textsuperscript{-1}min\textsuperscript{-1}) | 51.9 ± 9.3                   | 19.5 ± 2.9b                   |
| Glucose turnover (mgkg\textsuperscript{-1}min\textsuperscript{-1}) | 53.7 ± 5.9                   | 31.1 ± 1.3b                   |
| Glucose clearance (mkg\textsuperscript{-1}min\textsuperscript{-1}) | 18.0 ± 2.4                   | 10.5 ± 0.5b                   |

\textsuperscript{a} p < 0.05 vs. Sur1\textsubscript{w} mice.

\textsuperscript{b} p < 0.01 vs. Sur1\textsubscript{w} mice.
levels 30 min after initiation of refeeding and maintained plasma insulin concentrations that were within 80% of this peak level during rest of the experimental period (Fig. 6A, lower graph). Interestingly, although the plasma insulin level did not rise as rapidly during the first 30 min in Sur1neo mice, similar or higher levels were observed thereafter until the end of the 150-min test period.

The oral intake of glucose is known to stimulate the secretion of incretin hormones such as glucagon-like peptide 1 (GLP-1), which augment the effect of insulin on the islet (26, 27). Thus, to determine whether ingested glucose might have contributed to the increase of insulin secretion after refeeding of Sur1neo mice, we performed a gastric glucose tolerance test. After a 16-h overnight fast, glucose (2 g/kg body weight) was administered via gavage. This caused the blood glucose concentrations to increase rapidly in both the Sur1w/w and Sur1neo/neo mice during the first 20-min period (Fig. 6B, upper graph). In the Sur1w/w mice the blood glucose concentration reached a peak of 374 ± 10 mg/dl at 20 min before declining, whereas in the Sur1neo/neo mice the blood glucose peaked at 433 ± 26 mg/dl at 30 min. However, whereas the plasma insulin concentration increased rapidly in the Sur1w/w mice, it did not change in Sur1neo/neo mice (Fig. 6B, lower graph). The peak plasma insulin levels achieved after meal ingestion in the Sur1w/w mice was over 3-fold of that observed in response to the glucose gavage. For the Sur1neo/neo mice this difference was even more striking, with the peak plasma insulin level being ~13-fold higher for the meal compared with the glucose gavage.

The lack of any significant increase in plasma insulin concentrations in Sur1neo mice after gastric glucose gavage led us to test whether incretins were being secreted, whether they were able to cause a normal rise in cAMP levels, and whether they were able to augment insulin secretion in Sur1neo mice. To explore these possibilities, we first measured the plasma concentration of GLP-1-(7–36) amide during refeeding after an overnight fast but found no differences between the Sur1w/w and Sur1neo/neo animals (6.6 ± 2.2 pmol/l versus 7.5 ± 1.5 pmol/l, respectively). The lack of any difference suggests that GLP-1 is secreted normally in the Sur1neo mice. We next examined the effect of GLP-1-(7–36) on insulin secretion in these animals. In Sur1w/w mice a transient but drastic increase in plasma insulin level was observed 5 min after intravenous administration of GLP-1-(7–36) (10 nmol/kg body weight) with glucose (1g/kg body weight), whereas only a moderate increase was observed with glucose alone (Fig. 6C). In marked contrast, GLP-1-(7–36) failed to stimulate any rise in the plasma insulin concentration in Sur1neo mice at 5 min after administration. Only a slight increase in the insulin concentration, which resulted in a modest improvement of glucose tolerance, was observed 20 min after administration of GLP-1-(7–36) in these mice (Fig. 6C). Changes in blood glucose concentration during this test showed that glucose tolerance was significantly improved in Sur1w/w mice but only slightly in Sur1neo mice by GLP-1-(7–36) administration (Fig. 6C). The inability of GLP-1 to stimulate insulin secretion in Sur1neo mice does not reflect a lack of cAMP production. As shown in Table II, we measured cAMP levels in isolated islets and observed Sur1neo islets produced a similar amount of cAMP to
**Sulfonylurea Receptor Type 1 Knock-out Mice**

*Sulfonylurea Receptor Type 1* islets in response to GLP-1-1(7–36).

**Carbachol Stimulates Insulin Secretion in Perfused *Sur*<sup>1<sub>neo</sub></sup> Pancreata**—To understand the mechanisms by which *Sur*<sup>1<sub>neo</sub></sup> mice are able to secrete nearly normal amount of insulin in response to feeding, we tested the effect of carbachol, a cholinergic agonist, on insulin secretion from perfused pancreas. Cholinergic stimulation is known to potentiate sustained insulin secretion from normal islets in glucose-dependent manner (28). When the glucose concentration was shifted from 3 to 11 mM, a clear biphasic insulin secretion was observed in *Sur*<sup>1<sub>neo</sub></sup> pancreata, whereas only a very small amount of insulin was secreted in *Sur*<sup>1<sub>w</sub></sup> pancreata (Fig. 7). A 20-min administration of 50 μM carbachol in combination with 3 mM glucose induced a small peak of insulin secretion during the first 3 min, similarly in both *Sur*<sup>1<sub>neo</sub></sup> and *Sur*<sup>1<sub>w</sub></sup> pancreata. After this transient secretion, *Sur*<sup>1<sub>neo</sub></sup> pancreata exhibited no additional secretion during the rest of period. In marked contrast, insulin secretion was sustained in the *Sur*<sup>1<sub>neo</sub></sup> pancreata at ~50% of the initial peak (Fig. 7). In the presence of 11 mM glucose, carbachol potentiated both phases of insulin secretion in the *Sur*<sup>1<sub>neo</sub></sup> pancreata. When compared with insulin secretion stimulated by 11 mM glucose alone, carbachol caused 3.5-fold increase in the first phase of secretion and 13.7-fold increase in the second phase. Insulin secretion was also observed in the *Sur*<sup>1<sub>neo</sub></sup> pancreata; however, the total amount of insulin secreted during a 20-min period was about half that of the *Sur*<sup>1<sub>neo</sub></sup> pancreata. Moreover, the insulin secretion profile of the *Sur*<sup>1<sub>neo</sub></sup> pancreata was monophasic, compared with the biphasic response of the wild-type pancreata (Fig. 7). Insulin secretion caused by carbachol in *Sur*<sup>1<sub>neo</sub></sup> pancreata was 3.4-fold higher in the presence of 11 mM glucose compared with in the presence of 3 mM glucose.

**DISCUSSION**

The K<sub>ATP</sub> channel has been thought to play a key role in insulin secretion by the β cell. However, these studies demonstrate mice that lack *Sur*<sub>1</sub>, a key component of K<sub>ATP</sub> channel, secrete nearly a normal amount of insulin in response to a meal despite constant membrane depolarization in β cells and marked impairment of glucose-stimulated insulin secretion. Analysis of the postprandial insulin secretion profile suggests that a greater than normal second phase of insulin secretion may compensate for the lack of a first phase of secretion in *Sur*<sup>1<sub>neo</sub></sup> mice. Thus, although there are changes in the kinetics of insulin secretion after feeding, the ability of β cells in *Sur*<sub>1</sub> null mice to regulate the secretion of insulin remains remarkably intact. Our finding that carbachol was able to stimulate insulin secretion in the perfused *Sur*<sup>1<sub>neo</sub></sup> pancreas points to cholinergic stimulation of the β cell as a likely compensatory mechanism that may circumvent the markedly impaired response to glucose brought on by the lack of functional K<sub>ATP</sub> channels.

Our initial expectation was that *Sur* 1 knock-out mice would provide a model for PHHI in humans. However, although *Sur*<sup>1<sub>neo</sub></sup> β cells mimic the electrical properties described for β cells from PHHI-affected individuals (29, 30), the absence of functional K<sub>ATP</sub> channels on insulin secretion and glucose homeostasis in mice has far less impact than in humans. Insulin secretion has long been known to comprise both a first and second phase. The first phase is a transient burst of insulin secretion from granules that exist in a readily releasable form, whereas the second phase is thought to reflect secretion from a pool of granules that must first be mobilized and docked to the plasma membrane before they can be secreted (31, 32). A recent study using evanescent wave microscopy has provided additional evidence for this model (33). A first phase of insulin secretion was observed from a pool of previously docked granules, whereas the second phase of insulin secretion occurred largely from granules that were newly recruited to plasma-membrane docking sites. Indeed, although a single β cell may contain more than 10,000 secretory granules (34), only 40 or so appear to exist in a readily releasable form (31). Thus, the process of granule mobilization and docking to the plasma membrane may play an important role in the regulation of insulin secretion.

Although human and rat β cells respond to glucose with a progressively increasing second phase insulin release, mouse β cells respond with a smaller and flatter second phase of insulin release (35). This difference has been suggested to be caused, at least in part, by the failure of high glucose to activate phospholipase C protein kinase C signal pathway (36), and by lower production of cAMP by glucose stimulation in mouse β cells (35). Thus, species-specific differences may explain why humans with defective K<sub>ATP</sub> channel function develop hypoglycemia from increased and unregulated insulin secretion, whereas mice lacking *Sur*<sub>1</sub> appear to have compensatory mechanisms that prevent the hypersecretion of insulin.

Our data indicate that *Sur*<sup>1<sub>neo</sub></sup> β cells have elevated basal

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**TABLE II**

Cyclic AMP production by GLP-1 in isolated islets

| GLP-1 | (−) | (+) |
|-------|-----|-----|
| *Sur*<sup>1<sub>neo</sub></sup> | 96 ± 9 | 827 ± 67 |
| *Sur*<sup>1<sub>neo</sub></sup> | 88 ± 19 | 767 ± 88 |

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**Fig. 7.** Insulin secretion in perfused *Sur*<sup>1<sub>neo</sub></sup> pancreata in response to carbachol. Pancreas was perfused with Krebs-Ringer bicarbonate buffer containing glucose and carbachol at concentrations indicated. The buffer and pancreas were maintained at 37 °C through the experimental period. Data for males and females were not significantly different and were combined. The data represent the mean ± S.E. of 6 *Sur*<sup>1<sub>neo</sub></sup> and 5 *Sur*<sup>1<sub>neo</sub></sup> mice. Insulin secretion in *Sur*<sup>1<sub>neo</sub></sup> pancreata is significantly different from that in *Sur*<sup>1<sub>neo</sub></sup> pancreata at time 8–9 min (p < 0.005), 44–61 min (p < 0.001), 62–65 min (p < 0.05), 76 min (p < 0.001), 77–79 min (p < 0.05), 87–88 min (p < 0.05), and 90 min (p < 0.05).
sensitivity to cholinergic stimulation is increased in basal glucose concentration. Although it is possible that the second phase of insulin secretion following the first phase secretion does not occur in \textit{Sur1/\text{neo} \textit{neo}} pancreas compared with that in the \textit{Sur1/\text{null} \textit{null}} pancreas (data not shown). Thus, we suggest that the lack of refilling of the readily releasable pool with granules from the reserve pool at the basal glucose level may help prevent the unregulated secretion of these insulin granules.

Cholinergic muscarinic agonists, including the endogenous neurotransmitter acetylcholine and the synthetic non-hydrolyzable analogue carbachol, are known to enhance glucose-stimulated insulin secretion (28). Acetylcholine is released by intrapancreatic vagal nerve endings and stimulates insulin secretion in \textit{β} cells mainly by activating phospholipase C/ protein kinase C signal pathways (37). Vagal stimulus of the endocrine pancreas is thought to persist during the preabsorptive and absorptive phases of feeding, although there is no direct evidence because of quick degradation of acetylcholine (37). For this reason, we studied the effect of carbachol on the insulin secretion using perfused pancreas and found that carbachol stimulates insulin secretion in \textit{Sur1/\text{neo} \textit{neo}} pancreas in a glucose-dependent manner. This finding suggests that the cholinergic stimulation may be one of the mechanisms whereby nearly normal insulin secretion in response to feeding is maintained in the \textit{Sur1/\text{neo} \textit{neo}} mice.

Under a basal glucose concentration, \textit{Sur1/\text{null}} pancreas responds to carbachol with only a small first phase of insulin secretion. In contrast, \textit{Sur1/\text{neo} \textit{neo}} pancreas shows a sustained second phase of insulin secretion following the first phase secretion. This result suggests that carbachol stimulates granule mobilization from a reserved pool to releasable pool under a basal glucose concentration. Although it is possible that the sensitivity to cholinergic stimulation is increased in \textit{Sur1/\text{neo} \textit{neo}} pancreas, it is more likely that carbachol promotes the granule processing similarly in both \textit{Sur1/\text{null}} and \textit{Sur1/\text{neo} \textit{neo}} pancreas but that insulin secretion does not occur in \textit{Sur1/\text{null}} pancreases because of opening of K\textsubscript{ATP} channels at this glucose concentration. This is supported by the fact that the insulinotropic effect of acetylcholine under a non-stimulating glucose concentration is unmasked by sulfonylurea treatment of mouse islets (37). Thus, we speculate that the sustained insulin secretion observed from the \textit{Sur1/\text{neo} \textit{neo}} pancreas in the presence of carbachol and 3 mM glucose represents the same, unregulated mode of insulin secretion that is observed in humans with PHHI.

In this study, we also examined the effect of GLP-1 on insulin secretion in \textit{Sur1/\text{neo} \textit{neo}} mice. Binding of GLP-1 to its G protein-coupled receptor leads to the activation of adenylate cyclase and generation of cAMP. In \textit{β} cells, cAMP, via activation of protein kinase A (PKA), affects ion channel activity, [Ca\textsuperscript{2+}]\textsubscript{i}, handling, and the mobilization of granules to potentiate glucose-stimulated insulin secretion (38, 39). Both subunits of K\textsubscript{ATP} channel are also target of PKA, and the function of this channel is thought to be modulated by phosphorylation by PKA (40). Furthermore, the recent study showed that the cAMP-binding protein cAMP-GEFII, by interacting with Rim2, a target of small GTP-binding protein Rab3, mediates cAMP-dependent PKA-independent exocytosis (41), and that this PKA-independent pathway is critical in the potentiation of insulin secretion by incretins (42). Thus, the late step of granule processing, i.e., downstream of granule mobilization, is a major site for the action of incretins. A bolus injection of GLP-1 together with glucose caused a strong potentiation of the first phase of insulin secretion in the control mice, but no change in the first phase and only a slight increase in the second phase of insulin secretion in \textit{Sur1/\text{neo} \textit{neo}} mice. There was no defect in cAMP generation by GLP-1 in \textit{Sur1/\text{neo} \textit{neo}} islets. These results provide additional evidence for the lack of a readily releasable pool of insulin granules in \textit{Sur1/\text{neo} \textit{neo}} mice during the fasted condition.

Depolarization of the \textit{β} cell plasma membrane has long been thought to be a key step in the regulation of insulin secretion. Indeed, prevention of \textit{β} cell depolarization by directing the expression of a constitutively active form of Kir6.2 to pancreatic \textit{β} cells in transgenic mice markedly impairs insulin secretion, thereby causing severe diabetes and death of the animals within 5 days of birth (43). However, our studies, as well as those of others, clearly illustrate that mice lacking K\textsubscript{ATP} channels, as achieved either via \textit{Sur1} or Kir6.2 gene knock-outs, continue to regulate their secretion of insulin despite constant depolarization of the plasma membrane of \textit{β} cells in these mice. Thus, although membrane depolarization is clearly necessary for insulin secretion, the mechanisms for regulation of insulin secretion in the intact animal cannot be explained simply by the closure of K\textsubscript{ATP} channels. The precise nature of all the K\textsubscript{ATP} channel-dependent mechanisms involved in regulation of insulin secretion remain to be determined.

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