

Distinct Effects of Glucose-Dependent Insulinotropic Polypeptide and Glucagon-Like Peptide-1 on Insulin Secretion and Gut Motility

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Glucose-induced insulin secretion from pancreatic β -cells depends critically on ATP-sensitive K^+ channel (K_{ATP} channel) activity, but it is not known whether K_{ATP} channels are involved in the potentiation of insulin secretion by glucose-dependent insulinotropic polypeptide (GIP). In mice lacking K_{ATP} channels (Kir6.2^{-/-} mice), we found that pretreatment with GIP in vivo failed to blunt the rise in blood glucose levels after oral glucose load. In Kir6.2^{-/-} mice, potentiation of insulin secretion by GIP in vivo was markedly attenuated, indicating that K_{ATP} channels are essential in the insulinotropic effect of GIP. In contrast, pretreatment with glucagon-like peptide-1 (GLP-1) in Kir6.2^{-/-} mice potentiated insulin secretion and blunted the rise in blood glucose levels. We also found that GLP-1 inhibited gut motility whereas GIP did not. Perfusion experiments of Kir6.2^{-/-} mice revealed severely impaired potentiation of insulin secretion by 1 nmol/l GIP and substantial potentiation by 1 nmol/l GLP-1. Although both GIP and GLP-1 increase the intracellular cAMP concentration and potentiate insulin secretion, these results demonstrate that the GLP-1 and GIP signaling pathways involve the K_{ATP} channel differently. *Diabetes* 54: 1056–1063, 2005

Oral glucose load elicits larger insulin secretion and less increase in blood glucose levels than intravenous administration of the equivalent amount of glucose (1,2). This phenomenon is mostly due to incretins, gut-derived factors including

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AUC, area under the curve; GEF, guanine nucleotide exchange factor; GIP, glucose-dependent insulinotropic polypeptide; GIPR^{-/-}, GIP-receptor knockout; GLP-1, glucagon-like peptide-1; K_{ATP} channel, ATP-sensitive K^+ channel; KRBH, Krebs-Ringer bicarbonate HEPES; OGTT, oral glucose tolerance test; PKA, protein kinase A; PreTx, GIP pretreatment.

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glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) (3,4). GIP and GLP-1 are released from gastrointestinal endocrine K-cells and L-cells, respectively, into the blood stream in response to the ingestion of nutrients (5), which potentiates insulin secretion from pancreatic β -cells (6–9). GIP and GLP-1 exert their insulinotropic effects by binding to GIP receptors (10) and GLP-1 receptors on the β -cell surface (11), respectively, activating adenylyl cyclase (12,13), which leads to the rise in intracellular cAMP concentration that potentiates insulin secretion by activating protein kinase A- and/or cAMP-guanine nucleotide exchange factor (GEF)-2-mediated signaling in normal pancreatic β -cells (14,15). Thus, GIP and GLP-1 share in part a common pathway of insulin secretion enhancement. However, many clinical findings suggest different mechanisms of GIP and GLP-1 action. In patients with type 2 diabetes, for example, the insulinotropic action of GLP-1 is well preserved whereas that of GIP is markedly reduced (16). The mechanism of the differing effects GLP-1 and GIP remains unknown.

Recent studies of GIP-receptor knockout (GIPR^{-/-}) mice have shown that potentiation of insulin secretion by GIP plays an important role in glucose metabolism (17). GIPR^{-/-} mice have higher glucose levels in response to oral glucose load than in response to intraperitoneal load, showing that endogenous GIP plays an important role in preventing a rise in blood glucose levels after oral load. Unlike other secretagogues that stimulate insulin secretion, GIP exerts a potentiating effect on insulin secretion only in the presence of glucose (7,18,19). The glucose dependency of the insulinotropic action of GIP has been confirmed using stepwise glucose clamp in normal human subjects (9,20,21).

ATP-sensitive K^+ channel (K_{ATP} channel) null (Kir6.2^{-/-} and SUR1^{-/-}) mice do not exhibit significant insulin secretion in response to oral glucose load (22–24). This raises the possibility that Kir6.2^{-/-} mice have either a defect in glucose-induced GIP secretion from K-cells or a defect in potentiation by GIP of insulin secretion from β -cells. Because glucose-induced GIP secretion from K-cells has been shown to occur in a K_{ATP} channel-independent manner, we investigated the potentiating effect of GIP on insulin secretion from β -cells in Kir6.2^{-/-} mice. We also examined the effects of GLP-1, the other important incretin hormone, on the potentiation of insulin secretion and

blood glucose levels after an oral glucose load in Kir6.2^{-/-} mice.

RESEARCH DESIGN AND METHODS

Kir6.2^{-/-} mice were generated as previously described (22). Because the Kir6.2^{-/-} mice had been backcrossed to the C57BL/6 mouse strain over five generations, C57BL/6 mice were used as wild-type (Kir6.2^{+/+}) mice. All animal experiments were performed in accordance with the guidelines of the Animal Care Committee of Chiba and Kobe University.

GIP secretion assay in vivo. The secretion of GIP in response to oral glucose was examined in conscious male mice (18–20 weeks old, weighing 20–25 g) in vivo. After an overnight fast (16 h), Kir6.2^{+/+} and Kir6.2^{-/-} mice were administered D-glucose (150 mg/mouse in 0.5 ml) via gavage. A blood sample (~500 μ l of whole blood) was taken 15 min after glucose load and separated by centrifugation at 12,000g for 15 min at 4°C and stored at -80°C until hormone radioimmunoassay. Blood samples for basal GIP and glucose level was taken independently 1 week before ($n = 6$ for both genotypes) and after ($n = 6$ for both genotypes) the glucose loading test. GIP concentrations and glucose levels were determined as previously described (25–27).

Oral glucose tolerance test and measurement of blood glucose and serum insulin levels. One-hundred micrograms of human GIP (in 0.1 ml), human GLP-1 (in 0.1 ml), or saline (0.1 ml) was given subcutaneously to overnight (16 h)-fasted male mice. Glucose (1.5 g/kg) was administered 5 min after GIP or GLP-1 pretreatment as a 15% solution via gavage. Blood glucose levels at 0, 10, 30, 60, 90, 120, and 180 min and serum insulin levels at 0, 10, and 30 min after the glucose load were measured as previously described (27). The areas under the curve (AUCs) were assessed for blood glucose levels (AUC_{glucose}) with the trapezoidal rule of suprabasal values.

Measurement of gastrointestinal transit. To evaluate gastrointestinal motility, male mice were fasted with free access to drinking water for 48 h. On the day of the experiment, the mice received an intragastric injection of 20 μ l/g test solution (25% wt/vol barium sulfate suspended in water or 50% wt/vol D-glucose solution). The mice were killed 15 min later by cervical dislocation. After dissection, the length from the pylorus to the most distal point of migration of the barium (A) and from the pylorus to terminal ileum (B) was measured. Gastrointestinal transit was expressed as percentage of A to B. To determine the effects of GIP and GLP-1 on gastrointestinal motility, mice were pretreated 5 min before test solution ingestion with 100 μ g human GIP or GLP-1.

Perfusion experiments of mouse pancreata. Overnight (16 h)-fasted male mice at 16–20 weeks of age were used in perfusion experiments as previously reported (28) with slight modifications. Briefly, after anesthesia with 80 mg/kg sodium pentobarbital, the superior mesenteric and renal arteries were ligated, and the aorta was tied off just below the diaphragm. The perfusate was infused from a catheter placed in the aorta and collected from the portal vein. The perfusate was Krebs-Ringer bicarbonate HEPES (KRBH) buffer supplemented with 4.6% dextran and 0.25% BSA and gassed with 95% O₂/5% CO₂. The flow rate of the perfusate was 1 ml/min. In experiments involving GIP and GLP-1, mouse pancreata were perfused with KRBH buffer containing 2.8 or 16.7 mmol/l glucose in the presence or absence of 1 nmol/l GIP or 1 nmol/l GLP-1. In experiments involving arginine and carbachol, pancreata were perfused with KRBH buffer containing 5.5 mmol/l glucose in the presence or absence of 20 mmol/l arginine or 50 μ mol/l carbachol. The perfusion protocols began with a 10-min equilibration period with the same buffer used in the initial step (i.e., from 1 to 5 min) shown in the figures. The insulin levels in the perfusate were measured by an ELISA kit (Mesacup Insulin Test) from BML (Nagoya, Japan).

Measurement of insulin secretion in response to arginine and carbachol in vivo. To analyze arginine- and carbachol-induced insulin secretion, overnight (16 h)-fasted male mice were administered 250 mg/kg L(+)-arginine intravenously or 750 μ g/kg carbachol intraperitoneally as previously described by Guenifi et al. (29) and Havel et al. (30). Blood samples were taken before and 2 min after load, and blood glucose and serum insulin levels were measured.

Meal ingestion test. Glucose tolerance and insulin secretory response to mixed meal was evaluated using the enteral feeding formula Twinline, which is used clinically and which consists mainly of casein from milk protein, amino acids, maltodextrin, fat from safflower oil, and tricaprillin and contains 4.05 g/dl protein, 2.78 g/dl carbohydrate, and 2.78 g/dl fat (1 kcal/ml calorie in total). After overnight fasting (16 h), male mice were administered 20 μ l/g Twinline (20 kcal/g energy and 3 g/kg carbohydrate), and blood glucose levels at 0, 30, 60, 120, and 180 min and serum insulin levels at 0, 30, and 60 min after the glucose load were measured.

Reagents. Synthetic human GIP and GLP-1 were purchased from Peptide Institute (Osaka, Japan). Arginine [L(+)-arginine monohydrochloride] was

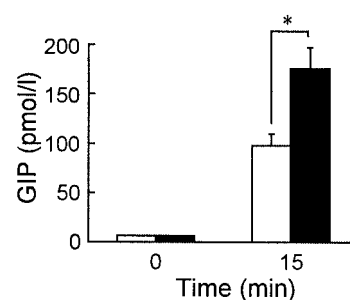


FIG. 1. GIP secretion in response to oral glucose. Plasma GIP levels in Kir6.2^{+/+} (□; $n = 12$) and Kir6.2^{-/-} (■; $n = 12$) mice. * $P < 0.0001$.

from Nacalai (033-23), and carbachol (carbarylcholine chloride, C-4382) was from Sigma. Twinline enteral formula was from Otsuka Pharmaceuticals (Tokushima, Japan).

Statistical calculations. All values are shown as means \pm SE. P values were calculated with unpaired Student's t test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Glucose-induced GIP secretion. Oral glucose load elicited a significant increase in GIP secretion after 15 min in Kir6.2^{+/+} mice (113.3 \pm 2.6 pmol/l, $n = 12$) (Fig. 1). GIP secretion was significantly increased also in Kir6.2^{-/-} mice (207.3 \pm 14.7 pmol/l, $n = 12$), suggesting that glucose-induced GIP secretion is K_{ATP} channel independent. Interestingly, the increment in plasma GIP in Kir6.2^{-/-} mice was enhanced in Kir6.2^{-/-} mice ($P < 0.0001$).

Glucose-lowering effect of GIP in vivo. Oral glucose tolerance test (OGTT) was performed on mice pretreated with or without GIP, as previously reported of GLP-1 (31). GIP pretreatment (PreTx) significantly increased glucose tolerance in Kir6.2^{+/+} mice [AUC_{glucose}; PreTx(-), 2,146 \pm 18 mmol/l in 180 min; PreTx(+), 1,649 \pm 78 mmol/l in 180 min; $P < 0.005$] (Fig. 2A). However, GIP pretreatment failed to increase glucose tolerance in Kir6.2^{-/-} mice [AUC_{glucose}; PreTx(-), 1,901 \pm 75 mmol/l in 180 min; PreTx(+), 2,191 \pm 375 mmol/l in 180 min; not significant] (Fig. 2B), indicating that the glucose-lowering effect of GIP is abolished completely in Kir6.2^{-/-} mice.

Effect of GIP on gastrointestinal transit. We assessed gut motility by measuring gastrointestinal transit of orally ingested barium sulfate. GIP did not affect gastrointestinal transit [67.1 \pm 6.7% in PreTx(-) and 74.9 \pm 6.0% in PreTx(+)] in Kir6.2^{+/+} mice, which shows that GIP does not inhibit gastrointestinal transit, at least in our protocol (Fig. 2C). However, there was a significant increase in gastrointestinal transit of oral glucose load. To evaluate involvement of the K_{ATP} channels in glucose-responsive enteric neurons in regulating gut motility of glucose load, we compared transit of oral glucose load in Kir6.2^{+/+} and Kir6.2^{-/-} mice. Gastrointestinal transit was similarly increased by oral glucose in Kir6.2^{+/+} [glucose (-), 41.9 \pm 2.1%; glucose (+), 57.5 \pm 4.0%] and Kir6.2^{-/-} [glucose (-), 34.1 \pm 1.7%; glucose (+), 61.4 \pm 1.7%] mice (Fig. 2D), indicating that gut motility is not regulated by K_{ATP} channel-mediated glucose sensing in enteric neurons.

Glucose-lowering effect of GLP-1 in vivo. We then performed OGTTs with and without GLP-1 pretreatment, as was done with GIP. GLP-1 pretreatment reduced the elevation in blood glucose significantly in Kir6.2^{+/+} mice

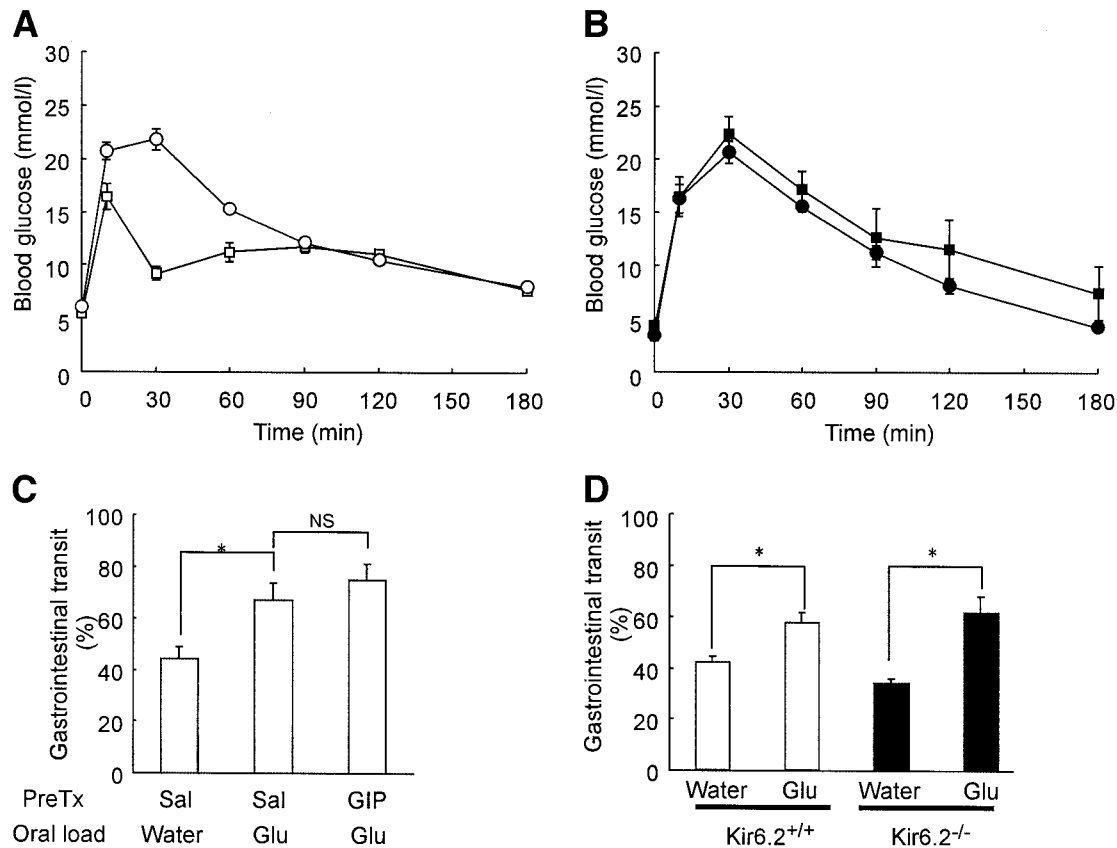


FIG. 2. Effect of GIP on blood glucose and gastrointestinal transit. **A** and **B**: Effect of GIP on blood glucose levels after oral glucose challenge in Kir6.2^{+/+} and Kir6.2^{-/-} mice. Changes in blood glucose levels of Kir6.2^{+/+} (**A**) and Kir6.2^{-/-} (**B**) mice ($n = 5-9$, for each group) during OGTT are shown. Kir6.2^{+/+} (**A**) and Kir6.2^{-/-} (**B**) mice were pretreated subcutaneously with saline (●) or GIP (■), and glucose was administered orally. **C**: Effect of GIP on gut motility in Kir6.2^{+/+} mice. Gastrointestinal transit was measured in the three groups ($n = 4$ for each group). The mice were pretreated subcutaneously with saline (Sal) or GIP and then administered barium sulfate orally suspended in glucose (Glu) or water. * $P < 0.05$; NS, not significant. **D**: Effect of glucose on gut motility in Kir6.2^{+/+} (□) and Kir6.2^{-/-} (■) mice. Gastrointestinal transit was measured in Kir6.2^{+/+} ($n = 9$) and Kir6.2^{-/-} ($n = 9$) mice in response to orally administered barium sulfate suspended in glucose or water. * $P < 0.005$.

[AUC_{glucose}; PreTx(-), $2,057 \pm 86$ mmol/l in 180 min; PreTx(+), $1,347 \pm 127$ mmol/l in 180 min; $P < 0.005$] (Fig. 3A) as well as Kir6.2^{-/-} mice [AUC_{glucose}; PreTx(-), $2,513 \pm 156$ mmol/l in 180 min; PreTx(+), $1,403 \pm 155$ mmol/l in 180 min; $P < 0.0001$] (Fig. 3B).

Effect of GLP-1 on gastrointestinal transit in vivo. We also examined the effect of GLP-1 on gut motility. Gastrointestinal transit was significantly suppressed by GLP-1 pretreatment (Fig. 3C). GLP-1 was similarly effective on gut motility in Kir6.2^{+/+} and Kir6.2^{-/-} mice, suggesting that the effect of GLP-1 is independent of K_{ATP} channel activity. In addition, GLP-1 similarly suppressed gastrointestinal transit in glucose-loaded and water-loaded mice, showing that GLP-1 and glucose regulate gut motility through independent mechanism.

Potentiation of insulin secretion by GIP and GLP-1 in vivo. We examined insulin secretion during OGTT with and without GIP or GLP-1 pretreatment (Fig. 4). Ten minutes after glucose loading, serum insulin levels were already elevated in Kir6.2^{+/+} mice (77.2 ± 11.5 pmol/l at 0 min; 275.5 ± 42.5 pmol/l at 10 min) (Fig. 4). Insulin secretion at 10 min was significantly enhanced by GIP pretreatment (440.8 ± 53.8 pmol/l, $P < 0.05$) or by GLP-1 pretreatment (474.7 ± 49.2 pmol/l, $P < 0.05$). Secretion in Kir6.2^{+/+} mice was no longer enhanced by GIP or GLP-1 pretreatment at 30 min, when the blood glucose levels are lower (Figs. 2A and 3A).

Interestingly, the insulinotropic effect of GIP was completely absent in Kir6.2^{-/-} mice at 10 min (83.1 ± 9.0 pmol/l at 0 min; 66.2 ± 17.0 pmol/l at 10 min; not significant); however, there was significant potentiation of insulin secretion at 10 min by GLP-1 pretreatment (242.9 ± 13.0 pmol/l, $P < 0.0005$) (Fig. 4). In contrast, at 30 min after glucose load, there was significant potentiation of insulin secretion in 30 min in Kir6.2^{-/-} mice both by GIP pretreatment (245.5 ± 8.51 pmol/l) and by GLP-1 pretreatment (240.4 ± 21.1 pmol/l), even though glucose-induced insulin secretion was not observed (112.8 ± 12.5 pmol/l) (Fig. 4).

Effects of GIP and GLP-1 on insulin secretion in perfused pancreas. To examine the time course of the insulin secretory response to GIP and GLP-1 in Kir6.2^{-/-} mice, perfusion experiments were performed in the absence (Fig. 5A) or presence of GIP (Fig. 5B and C) or GLP-1 (Fig. 5D). In Kir6.2^{+/+} mice, 16.7 mmol/l glucose elicited insulin secretion [the amount of secreted insulin (AUC_{insulin}) after glucose stimulation (from 5 to 25 min); 61.4 ± 5.5 ng in 20 min, $n = 3$] (Fig. 5A), which was further potentiated by 1 nmol/l [AUC_{insulin}; 217.9 ± 12.3 ng, $n = 3$, $P < 0.005$ vs. GIP(-)] or 10 nmol/l GIP [AUC_{insulin}; 278.8 ± 25.4 ng, $n = 3$, $P < 0.05$ vs. GIP(-)] (Fig. 5B and C). In contrast, in Kir6.2^{-/-} mice, 16.7 mmol/l glucose barely elicited a rise in insulin secretion (AUC_{insulin}; 23.3 ± 2.7 ng, $n = 3$) (Fig. 5A), and there was only slight potentiation in insulin secretion by 1 nmol/l (AUC_{insulin}; 37.1 ± 4.2 ng, $n =$

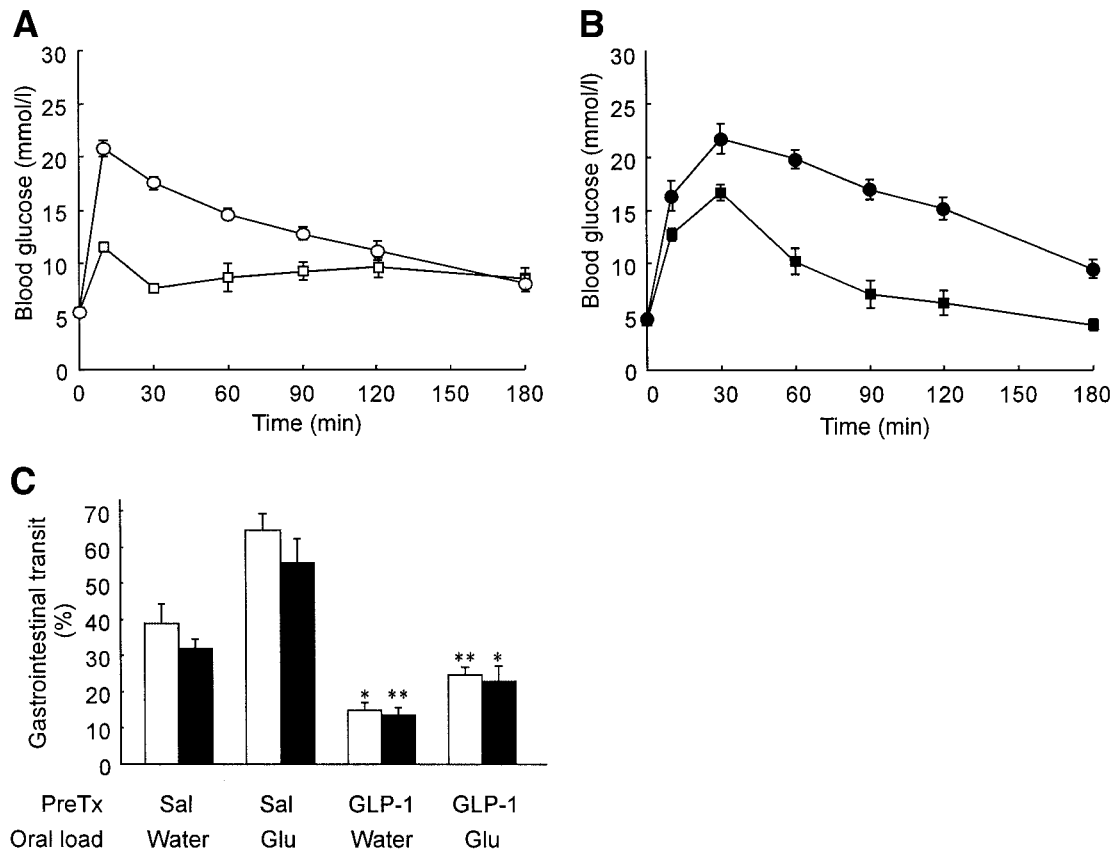


FIG. 3. Effect of GLP-1 on blood glucose after oral glucose challenge and gastrointestinal transit in Kir6.2^{+/+} and Kir6.2^{-/-} mice. **A** and **B**: Changes in blood glucose levels in Kir6.2^{+/+} (**A**) and Kir6.2^{-/-} (**B**) mice ($n = 10-14$, for each group) during OGTT are shown. Mice were pretreated subcutaneously with saline (●) or GLP-1 (■) or and were administered orally ingested glucose. **C**: Effect of glucose on gut motility in Kir6.2^{+/+} (□) and Kir6.2^{-/-} (■) mice. Gastrointestinal transit was measured ($n = 7-9$ for each group) as in Fig. 2C. The mice were pretreated subcutaneously with saline (Sal) or GLP-1 and then administered barium sulfate orally suspended in glucose (Glu) or water. * $P < 0.005$ and ** $P < 0.0001$ for comparison between GLP-1-pretreated (GLP-1) versus GLP-1-untreated (Sal) mice.

3) or 10 nmol/l GIP (AUC_{insulin} ; 55.8 ± 12.7 ng, $n = 3$) (Fig. 5B and C). The potentiation of insulin secretion by 1 nmol/l GLP-1 also was attenuated in Kir6.2^{-/-} mice (AUC_{insulin} ; 103.8 ± 40.6 ng, $n = 3$) compared with that of Kir6.2^{+/+} mice (AUC_{insulin} ; 329.1 ± 20.1 ng, $n = 3$), but the secretion was nevertheless more potent than that by 1 nmol/l GIP (Fig. 5D). When insulin secretion was assessed

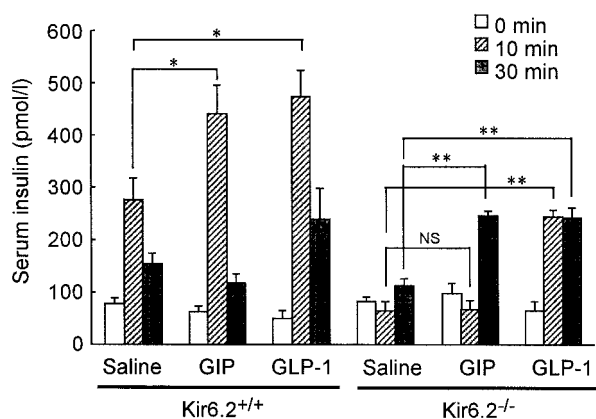


FIG. 4. Effect of GIP and GLP-1 pretreatment on insulin secretion after oral glucose challenge. Serum insulin levels in Kir6.2^{+/+} and Kir6.2^{-/-} mice before (□, $n = 5-9$) and 10 min (▨, $n = 5-9$) or 30 min (■, $n = 5-9$) after glucose load. * $P < 0.05$; ** $P < 0.0005$.

by the AUC_{insulin} , 1, 10, and 1 nmol/l GLP-1 potentiated insulin secretion in Kir6.2^{+/+} mice 3.5-, 3.5-, and 5.4-fold, respectively (Fig. 5E). In contrast, in Kir6.2^{-/-} mice, 1 and 10 nmol/l GIP increased insulin secretion only by 1.6- and 2.4-fold, whereas 1 nmol/l GLP-1 increased insulin secretion by 4.5-fold (Fig. 5E). In addition, glucose-induced insulin secretion in Kir6.2^{-/-} mice became apparent in the presence of 1 nmol/l GLP-1 [fold increase in the insulin secretory rate before and after stimulation with 16.7 mmol/l glucose; 1.52 ± 0.10 -fold in the absence of GLP-1 ($n = 3$) (Fig. 5A), 4.14 ± 0.06 -fold in 1 nmol/l GLP-1 ($n = 3$) (Fig. 5D); $P < 0.05$], indicating that Kir6.2^{-/-} mice were endowed with glucose responsiveness by stimulation with 1 nmol/l GLP-1.

Insulin secretory response to arginine, cholinergic stimuli, and mixed meal. Insulin secretion by arginine in vivo was significantly impaired in Kir6.2^{-/-} mice (Fig. 6A), but marked secretion was observed 2 min after administration of carbachol in vivo in both Kir6.2^{+/+} and Kir6.2^{-/-} mice (Fig. 6B). The insulin secretory response was also examined in perfusion experiments. Similar to the findings in vivo, insulin secretion from Kir6.2^{-/-} pancreata was markedly impaired in response to 20 mmol/l arginine [AUC_{insulin} during stimulation (from 5 to 10 min); 39.1 ± 4.2 ng in Kir6.2^{+/+} mice ($n = 3$), 8.4 ± 2.8 ng in Kir6.2^{-/-} mice ($n = 3$)] but remained unaffected in response to 50

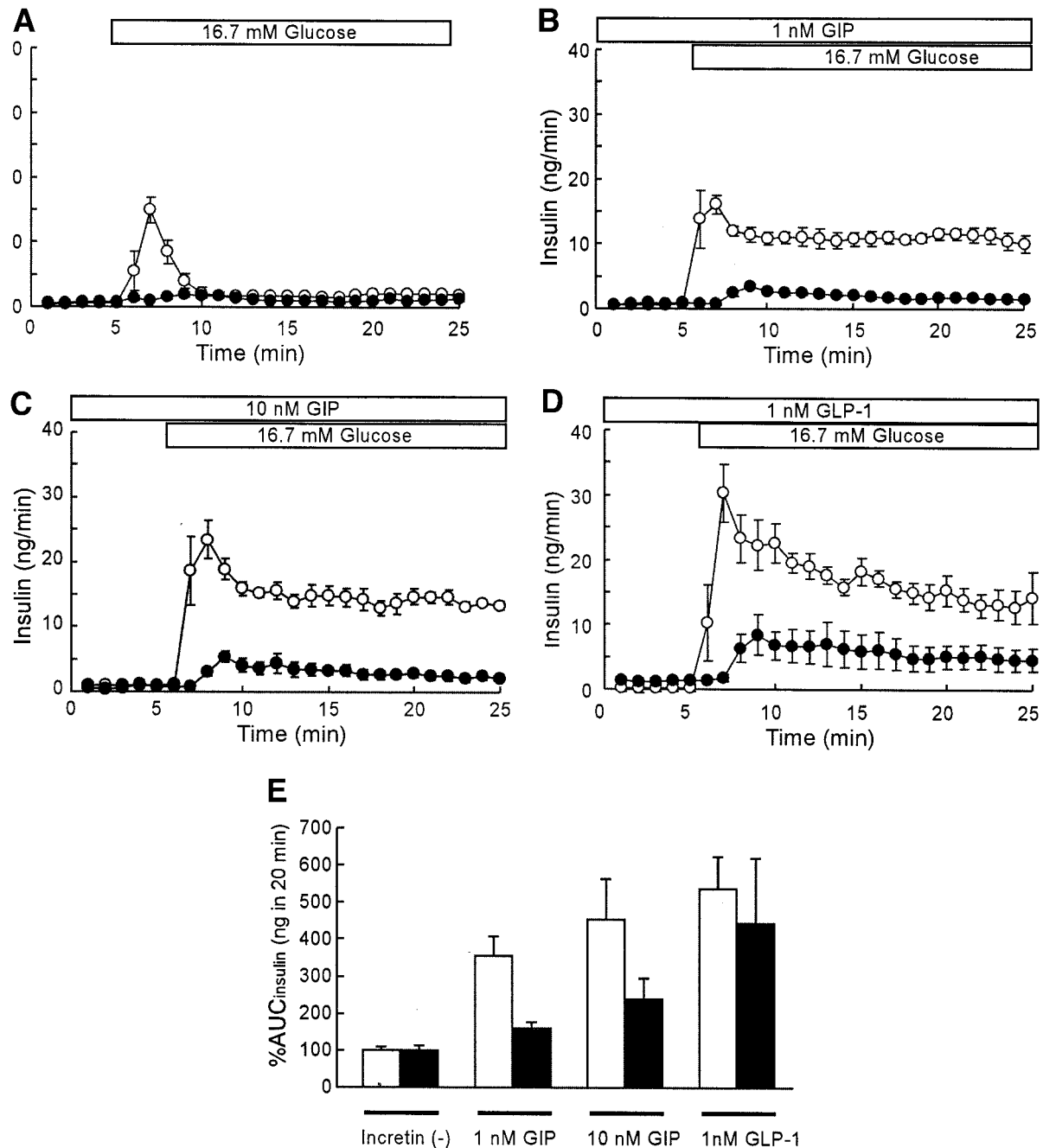


FIG. 5. Insulin secretion in perfused pancreata in response to high glucose, GIP, and GLP-1. *A–D*: Insulin secretion from perfused pancreata of Kir6.2^{+/+} (○) and Kir6.2^{-/-} (●) mice is shown. Glucose concentration was shifted from 2.8 to 16.7 mmol/l at 5 min in the absence of incretin (*A*) or in the presence of 1 nmol/l GIP (*B*), 10 nmol/l GIP (*C*), or 1 nmol/l GLP-1 (*D*). The data represent the mean \pm SE of three mice. *E*: The amounts of secreted insulin of Kir6.2^{+/+} (□) and Kir6.2^{-/-} (■) mice after glucose stimulation are expressed as the AUC_{insulin} from 5 to 25 min in *A–D*. Data are percent relative to that in the absence of incretin.

$\mu\text{mol/l}$ carbachol [AUC_{insulin} during stimulation (from 20 to 25 min); 8.7 ± 1.5 ng in Kir6.2^{+/+} mice ($n = 3$), 10.6 ± 1.2 ng in Kir6.2^{-/-} mice ($n = 3$)] (Fig. 6C). We also compared glucose tolerance and insulin secretion in response to orally ingested meal in Kir6.2^{+/+} and Kir6.2^{-/-} mice (Fig. 6D and E). In Kir6.2^{-/-} mice, glucose tolerance was significantly impaired in response to mixed meal (Fig. 6D), and early-phase insulin secretion was significantly diminished (serum insulin levels 30 min after meal ingestion; Kir6.2^{+/+}, 322.2 ± 47.5 pmol/l; Kir6.2^{-/-}, 134.0 ± 14.9 pmol/l, $P < 0.005$) (Fig. 6E).

DISCUSSION

GIP is released from gastrointestinal endocrine K-cells (32) in a glucose-dependent manner (7,33). Although glucose-induced insulin secretion from pancreatic β -cells is critically dependent on K_{ATP} channel function (22–24), our present study indicated that glucose-induced GIP secretion occurs independently of K_{ATP} channel function (Fig. 1).

Because GIP pretreatment did not reduce the elevation of blood glucose in Kir6.2^{-/-} mice after oral glucose load

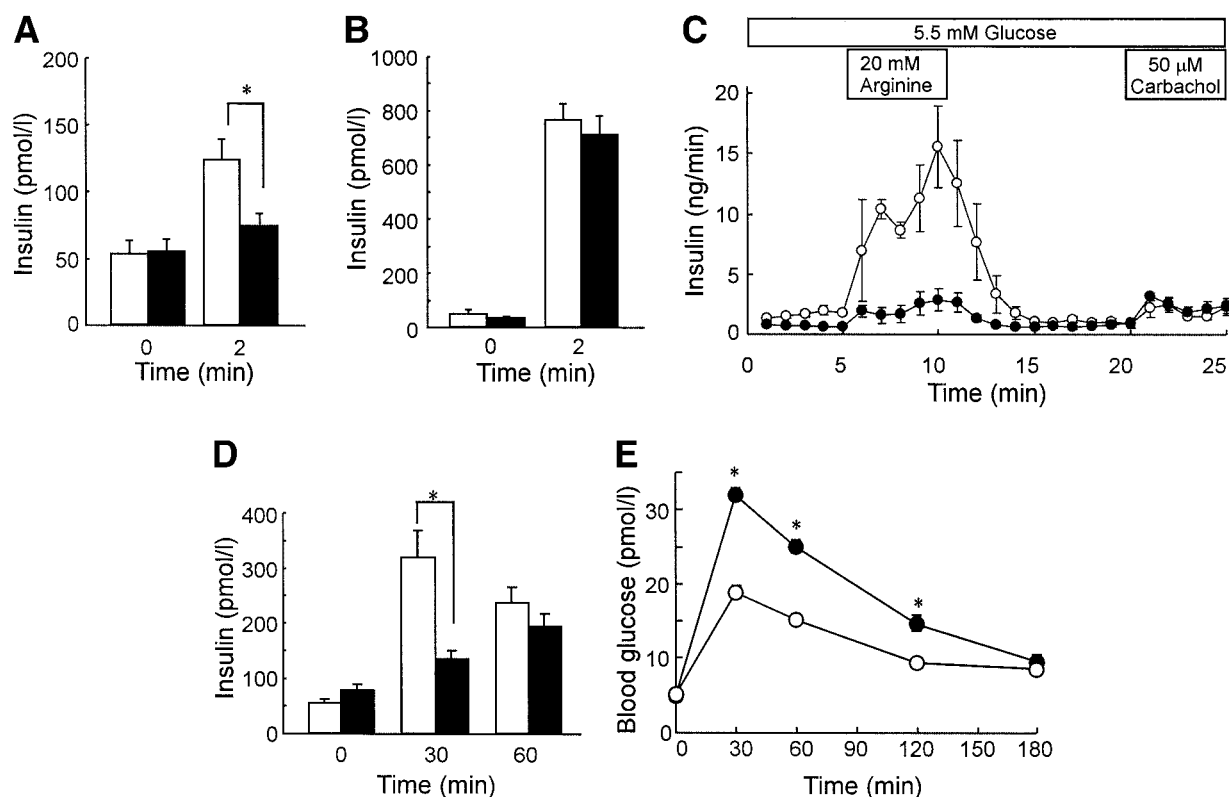


FIG. 6. Insulin secretion and glucose tolerance in response to arginine, carbachol, and a mixed meal. **A:** Early phase of insulin secretion in response to arginine is shown. Serum insulin levels in Kir6.2^{+/+} (□, *n* = 8) and Kir6.2^{-/-} (■, *n* = 9) mice 2 min after intravenous arginine administration. **P* < 0.005. **B:** The insulin secretion in response to carbachol is shown. Serum insulin levels in Kir6.2^{+/+} (□, *n* = 9) and Kir6.2^{-/-} (■, *n* = 10) mice 2 min after intraperitoneal carbachol administration. There is no significant difference in serum insulin levels between Kir6.2^{+/+} and Kir6.2^{-/-} mice. **C:** Insulin secretion from perfused pancreata of Kir6.2^{+/+} (○) and Kir6.2^{-/-} (●) mice in response to 20 mmol/l arginine and 50 μmol/l carbachol is shown. **D:** Changes in serum insulin levels in response to mixed meal ingestion. Serum insulin levels in Kir6.2^{+/+} (□, *n* = 15) and Kir6.2^{-/-} (■, *n* = 15) mice at indicated time points are shown. **P* < 0.005. **E:** Changes in blood glucose levels in response to mixed meal ingestion. Blood glucose levels in Kir6.2^{+/+} (○, *n* = 15) and Kir6.2^{-/-} (●, *n* = 15) mice are shown. **P* < 0.0001.

(Fig. 2B), we considered the possibility that GIP inhibits gut motility in a K_{ATP} channel-dependent manner, but this apparently is not the case (Fig. 2C). This is compatible with a recent study of GIP action on gut motility in humans (34). We also found a significant increase in gastrointestinal transit by oral glucose load. We established previously that K_{ATP} channels comprising Kir6.2 and SUR1 are found in glucose-responsive neurons in the hypothalamus (35) and in the ileum (36), and we proposed that the K_{ATP} channel in gut cholinergic neurons plays a role in glucose-evoked reflexes (36). Ingestion of carbohydrate is known to stimulate gastrointestinal motility (37), but it was unclear whether the K_{ATP} channel in glucose-responsive enteric neurons is involved in regulating glucose-induced gut motility. Our present findings on gastrointestinal transit in Kir6.2^{-/-} mice clearly show that gut motility is not regulated by K_{ATP} channel-mediated glucose sensing in the enteric neurons (Fig. 2D).

Measurement of serum insulin at 10 min after oral glucose load revealed that GIP pretreatment in vivo failed to potentiate the early-phase (38) insulin secretion during OGTT in Kir6.2^{-/-} mice (Fig. 3), indicating that the K_{ATP} channel in β -cells is essential in the insulinotropic effect of GIP. It would be likely, therefore, that the glucose-dependent effects of GIP depend on the activity of the K_{ATP} channel. In contrast, there was significant potentiation of late-phase insulin secretion (2.17-fold increase) in Kir6.2^{-/-} mice by GIP pretreatment. However, the physi-

ological significance of this late-phase insulin secretion remains uncertain, because there was no significant reduction in blood glucose levels even after 30 min in GIP-pretreated Kir6.2^{-/-} mice compared with GIP-untreated Kir6.2^{-/-} mice. These results also suggest that rapid enhancement of early-phase insulin secretion by GIP is required for its glucose-lowering effect after oral glucose load.

In contrast to GIP, GLP-1 did potentiate the insulin secretion (3.7-fold increase in 10 min) and had an obvious antihyperglycemic effect in Kir6.2^{-/-} mice (Fig. 4A, B, and D). Perfusion experiments of mouse pancreata are applicable only for a short period (less than 45 min of sampling) of secretion study of insulin. Thus, it is difficult to perform multiple stimuli in the same mouse pancreas, and a number of experiments are required to compare the secretory differences among different stimuli. However, when compared with the study of isolated islets, this method has an advantage because we can neglect cellular damage during islet isolation or unexpected effects by culturing the islets.

We performed perfusion experiments in Kir6.2^{-/-} mice and found that differences in the insulinotropic effects between GIP and GLP-1 in Kir6.2^{-/-} mice were also shown in the perfusion experiments (Fig. 5). Accordingly, the mechanism of potentiation of insulin secretion differs for GIP and GLP-1: insulin secretion by GIP depends critically on the K_{ATP} channel, whereas that by GLP-1 does not. Both

GIP and GLP-1 increase the intracellular cAMP concentration and potentiate insulin secretion by activating protein kinase A (PKA)- and/or cAMP-GEF2-mediated signaling in normal pancreatic β -cells (39). We previously reported that GIP-potentiated insulin secretion is almost completely suppressed in islets treated both with PKA blocker H-89 and antisense oligodeoxynucleotides against cAMP-GEF2, whereas GLP-1-potentiated insulin secretion remains nearly normal (15). Apparently, the insulinotropic action of GLP-1 is mediated by a pathway other than that involving PKA and cAMP-GEF2. In addition, we found that whereas GIP had almost no effect on gut motility, GLP-1 significantly suppressed gastrointestinal transit (Fig. 3C). Because the effect of GLP-1 is independent of the K_{ATP} channels, GLP-1 may well delay glucose absorption and prevent a rise in blood glucose levels after glucose load in Kir6.2^{-/-} mice. Thus, GLP-1 is suggested to participate in the postprandial glycemic control in K_{ATP} channel-independent manners by potentiating insulin secretion and by delaying gastric emptying. Although the importance of the K_{ATP} channel in the potentiation of insulin secretion by cAMP has been shown in SUR1 knockout mice (24,40), we clarify here the involvement of the channel in the potentiation of insulin secretion by GIP and GLP-1.

Although arginine treatment elicited impaired insulin secretion, the insulin secretion of Kir6.2^{-/-} mice in response to carbachol was intact, indicating that the exocytotic machinery of Kir6.2^{-/-} β -cells is intact and that the cause of impaired insulin secretion differs according to the stimulus (Fig. 6A–C). Insulin secretion is stimulated by multiple signals in pancreatic β -cells, including nutrients (carbohydrate, proteins, and fat), incretins (GIP and GLP-1), and neuronal input (mainly cholinergic). Our results indicate that mice lacking the Kir6.2 pore-forming subunit of K_{ATP} channels have an impaired insulin secretory response to glucose, arginine, and GIP, whereas the insulin secretion elicited by carbachol is comparable with that in Kir6.2^{+/+} mice. Kir6.2^{-/-} mice were also shown to exhibit glucose intolerance and delayed insulin secretion in response to mixed meal (Fig. 6C and D). The K_{ATP} channel thus plays an important role in regulating blood glucose levels both after glucose load and after ingestion of a mixed meal. The present study shows that the K_{ATP} channel in pancreatic β -cells is required for the insulinotropic effects of GIP through the potentiation of glucose-induced insulin secretion. In contrast, the potentiation of insulin secretion by GLP-1 depends on K_{ATP} channel-independent and -dependent mechanisms. The differing pathways of the action of GLP-1 and GIP on both the potentiation of insulin and gut motility might well account for the differences seen in their therapeutic efficacy in type 2 diabetes.

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