EXPERIMENTAL STUDY

Gastric inhibitory polypeptide is the major insulinotropic factor in K_{ATP} null mice

Katsushi Tsukiyama^{1,3}, Yuichiro Yamada¹, Kazumasa Miyawaki¹, Akihiro Hamasaki¹, Kazuaki Nagashima¹, Masaya Hosokawa¹, Shimpei Fujimoto¹, Akira Takahashi¹, Kentaro Toyoda¹, Shinya Toyokuni², Yutaka Oiso³ and Yutaka Seino¹

Departments of ¹Diabetes and Clinical Nutrition and ²Pathology and Biology of Diseases, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan and ³Department of Endocrinology and Diabetology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan

(Correspondence should be addressed to K Tsukiyama, Department of Diabetes and Clinical Nutrition, Kyoto University Graduate School of Medicine, 54 Shogoin-Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan; Email: tsukiyama@metab.kuhp.kyoto-u.ac.jp)

Abstract

Objective: ATP-sensitive K^+ (K_{ATP}) channels in pancreatic β -cells are crucial in the regulation of glucose-induced insulin secretion. Recently, K_{ATP} channel-deficient mice were generated by genetic disruption of Kir6.2, the pore-forming component of K_{ATP} channels, but the mice still showed a significant insulin response after oral glucose loading *in vivo*. Gastric inhibitory polypeptide (GIP) is a physiological incretin that stimulates insulin release upon ingestion of nutrients. To determine if GIP is the insulinotropic factor in insulin secretion in K_{ATP} channel-deficient mice, we generated double-knockout Kir6.2 and GIP receptor null mice and compared them with Kir6.2 knockout mice.

Methods: Double-knockout mice were generated by intercrossing Kir6.2-knockout mice with GIP receptor-knockout mice. An oral glucose tolerance test, insulin tolerance test and batch incubation study of pancreatic islets were performed on double-knockout mice and Kir6.2-knockout mice.

Results: Fasting glucose and insulin levels were similar in both groups. After oral glucose loading, blood glucose levels of double-knockout mice became elevated compared with Kir6.2-knockout mice, especially at 15 min (345 ± 10 mg/dl vs 294 ± 20 mg/dl, $P < 0.05$) and 30 min (453 ± 20 mg/dl vs 381 ± 26 mg/dl, $P < 0.05$). The insulin response was almost completely lost in double-knockout mice, although insulin secretion from isolated islets was stimulated by another incretin, glucagon-like peptide-1 in the double-knockout mice. Double-knockout mice and Kir6.2-knockout mice were similarly insulin sensitive as assessed by the insulin tolerance test.

Conclusion: GIP is the major insulinotropic factor in the secretion of insulin in response to glucose load in K_{ATP} channel-deficient mice.

European Journal of Endocrinology 151 407–412

Introduction

The regulation of glucose-induced insulin secretion depends critically on glucose metabolism in pancreatic β -cells, and electrical activity controlled by plasma membrane ion channels is especially important (1). The ATP-sensitive K^+ (K_{ATP}) channel links glucose metabolism to membrane potentials (2–4). ATP closes the K_{ATP} channels, which activates voltage-dependent calcium channels, initiating insulin release.

Recently, K_{ATP} channel-deficient mice were generated by genetic disruption of Kir6.2, which forms the K^+ ion-selective pore of the channel (5). While there is no increment in insulin secretion in response to high glucose concentration *in vitro* in Kir6.2-knockout (Kir6.2^{-/-}) mice, as assessed by perfusion and batch

incubation of isolated pancreatic islets, the mice still show the insulin response after oral glucose loading *in vivo*.

Insulinotropic hormones secreted from the gut upon nutritional ingestion play an important role in glucose-induced insulin secretion (6, 7). Gastric inhibitory polypeptide (GIP) is released from duodenal and upper small intestinal endocrine K-cells upon absorption of glucose or fat (8), and potentiates insulin secretion from pancreatic β -cells by binding to its specific receptor, the GIP receptor (GIPR). This incretin stimulates adenylyl cyclase, raises cyclic AMP (cAMP), and activates protein kinase A or type-II isoform of cAMP-regulated guanine nucleotide exchange factor (cAMP-GEF II) (9). We previously generated mice with a targeted disruption of the GIPR gene (10). Islets from GIPR-knockout

(GIPR^{-/-}) mice respond to glucose as well as wild-type mice, but GIPR^{-/-} mice exhibit higher blood glucose levels and an impaired initial insulin response after oral glucose loading.

In the present study, we have generated double-knockout Kir6.2 and GIPR null (Kir6.2^{-/-}GIPR^{-/-}) mice, and have found that Kir6.2^{-/-}GIPR^{-/-} mice lack the insulin response to oral glucose load seen in Kir6.2^{-/-} mice, clearly indicating that GIP is the major insulinotropic factor in the insulin secretory activity in these mice.

Design and methods

Generation of animal models

Kir6.2^{-/-} mice were provided by T Miki and S Seino, Chiba University Graduate School of Medicine, Japan. GIPR^{-/-} mice were generated as described previously (10). Kir6.2^{+/-}GIPR^{+/-} mice were generated by intercrossing Kir6.2^{-/-} mice with GIPR^{-/-} mice. Crossbreeding of Kir6.2^{+/-}GIPR^{+/-} mice yielded Kir6.2^{-/-}GIPR^{-/-} mice. All animals received care in accordance with the principles of laboratory animal care adopted by Kyoto University and the Declaration of Helsinki.

Batch incubation study

Pancreatic islets were isolated from 18- to 19-week-old male mice by collagenase digestion, and batch incubation was performed as described previously (11), with slight modification. Briefly, pancreatic islets (10 in each tube) were preincubated at 37°C for 60 min in Hepes-Krebs buffer containing 118.4 mmol/l NaCl, 4.7 mmol/l KCl, 1.2 mmol/l KH₂PO₄, 2.4 mmol/l CaCl₂, 1.2 mmol/l MgSO₄, 20 mmol/l NaHCO₃, 2.8 mmol/l glucose, and 10 mmol/l Hepes, supplemented with 0.2% (wt/vol) BSA. The islets were incubated for 30 min in 400 µl buffer containing various stimuli of insulin secretion. GIP and glucagon-like peptide-1 (GLP-1) were obtained from Peptide Institute, Inc., Osaka, Japan. Tolbutamide was from Nacalai Tesque Inc., Kyoto, Japan.

Immunohistochemical analysis

The pancreata of 18-week-old male mice were removed 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 was used as previously described (12), with a slight modification. After deparaffinization, normal goat serum (diluted to 1:75) (DAKO, Kyoto, Japan) for inhibition of nonspecific binding of primary antibody, rabbit anti-insulin antibody (diluted to 1:350) (DAKO) or rabbit anti-glucagon antibody (OAL-123, kindly provided by Otsuka Assay Laboratory, Tokushima, Japan)

(13) in a final dilution of 1:500, the biotin-labeled goat anti-rabbit IgG antibody (diluted to 1:300) (DAKO), and avidin-biotin-alkaline phosphatase complex (diluted to 1:100) (Vector Laboratories, Burlingame, CA, USA) were sequentially applied on sections, followed by hematoxylin nuclear counterstaining. Staining was visualized in red and black by alkaline phosphatase substrate (Vector Laboratories) for insulin and glucagon respectively. The sections were analyzed using the NIH Image software (<http://rsb.info.nih.gov/nih-image/>), and the area containing insulin-positive β-cells was calculated.

Measurements of blood glucose and insulin levels

An oral glucose tolerance test (OGTT) was performed on age-matched male mice at 10 to 11 weeks of age. After a 16-h fast, plasma insulin and blood glucose levels were measured and glucose (2 g/kg body weight) was loaded orally. In the insulin tolerance test, 0.1 unit/kg body weight of human insulin (Eli Lilly, Indianapolis, IN, USA) was injected subcutaneously in male mice (17 weeks of age) after a 16-h fast. Blood samples were taken from the tail vein at the times indicated. Blood glucose levels were measured by an enzyme-electrode method in whole blood. Plasma insulin levels were determined by a high-sensitive ELISA kit (Shibayagi, Gunma, Japan).

Glucose uptake in jejunum in vitro

Incorporation of D-glucose into everted jejunal rings was determined as described previously (14), with slight modifications. Briefly, mice were anesthetized with pentobarbital sodium (60 mg/kg s.c.) and a segment of jejunum was quickly excised, rinsed with saline solution (140 mmol/l NaCl, 10 mmol/l KHCO₃, 0.4 mmol/l KH₂PO₄, 2.4 mmol/l K₂HPO₄, 1.2 mmol/l CaCl₂ and 1.2 mmol/l MgCl₂, pH 7.4), everted, and cut into ~30 mg pieces. Groups of 4 intestinal rings were incubated for 15 min at 37°C and continuously gassed with O₂ in buffer containing 1.0 mmol/l D-glucose and 0.1 µCi/10 ml D-[¹⁴C]glucose (specific activity 50–60 mCi/mmol; Amersham Radiochemical Center, London, UK) in the absence and presence of 1 mmol/l phlorizin (phloretin 2'-glucoside), a potent inhibitor of Na⁺/glucose cotransporter (SGLT). At the end of the incubation period, the tissues were washed in ice-cold saline solution twice, blotted carefully to remove excess moisture, weighed, and extracted by shaking for 24 h in 1 ml 100 mmol/l HNO₃ (at 4°C). An aliquot was taken and radioactivity was determined by liquid scintillation counting on a Liquid Scintillation Analyzer (model TRI-CARB 1900CA, Packard Instrument Company Inc., Downers Grove, IL, USA). SGLT-dependent glucose uptake was determined as the glucose uptake

in the absence of phlorizin minus the glucose uptake in the presence of phlorizin.

Results

Insulin secretion in isolated islets

Insulin secretory responses of $Kir6.2^{-/-}$ $GIPR^{-/-}$ mice were examined *in vitro* by batch incubation study. Total

Figure 1 Insulin secretion from islets of $Kir6.2^{-/-}$ $GIPR^{-/-}$ mice. Insulin secretion in batch-incubated pancreatic islets of $Kir6.2^{-/-}$ $GIPR^{-/-}$ mice in response to the indicated concentrations of glucose (G), 100 μ M tolbutamide (Tolb), 100 nM GIP and 100 nM GLP-1 are shown. Values are means $\pm$ S.E.M., relative to the insulin content in averaged single islets of $Kir6.2^{-/-}$ $GIPR^{-/-}$ mice ($n = 40$). * $P < 0.05$ compared with 2.8 mmol/l glucose.

insulin content per single islet is decreased in $Kir6.2^{-/-}$ $GIPR^{-/-}$ mice compared with wild-type ($Kir6.2^{+/+}$ $GIPR^{+/+}$) mice ($47.3 \pm 3.5\%$, data not shown). In comparison with the basal level of insulin secretion at 2.8 mmol/l glucose from batch-incubated pancreatic islets, the insulin secretion in $Kir6.2^{-/-}$ $GIPR^{-/-}$ mice in response to 8.3 mmol/l glucose, 16.7 mmol/l glucose, 25.0 mmol/l glucose, 100 μ M tolbutamide in the presence of 16.7 mmol/l glucose or 100 nM GIP in the presence of 8.3 mmol/l glucose is similar. In contrast, 100 nM GLP-1 in the presence of 8.3 mmol/l glucose elicits an increased response (Fig. 1).

Immunohistochemistry of pancreatic islets

Immunohistochemical examination shows that the number of insulin-positive β -cells in islets of $Kir6.2^{-/-}$ $GIPR^{-/-}$ is increased, and that the area containing insulin-positive cells in the islets of $Kir6.2^{-/-}$ $GIPR^{-/-}$ mice is increased by 32.6% compared with $Kir6.2^{-/-}$ $GIPR^{+/+}$ mice. Glucagon-positive α -cells, which are present primarily in the periphery in islets of $Kir6.2^{+/+}$ $GIPR^{+/+}$ mice, also appear in the central region in islets of $Kir6.2^{-/-}$ $GIPR^{-/-}$ mice, as in $Kir6.2^{-/-}$ $GIPR^{+/+}$ mice (Fig. 2).

Glucose tolerance and insulin secretion in vivo

Fasting glucose levels are similar in $Kir6.2^{-/-}$ $GIPR^{-/-}$, $Kir6.2^{-/-}$ $GIPR^{+/+}$, and $Kir6.2^{+/+}$ $GIPR^{+/+}$ mice. However, after oral glucose loading, blood glucose

Figure 2 Immunohistochemical analyses of pancreatic islets. Immunohistochemical analyses of pancreatic islets of $Kir6.2^{+/+}$ $GIPR^{+/+}$ mice (A, D), $Kir6.2^{-/-}$ $GIPR^{+/+}$ mice (B, E), and $Kir6.2^{-/-}$ $GIPR^{-/-}$ mice (C, F) are shown. Pancreatic β -cells and α -cells were stained using rabbit anti-insulin antibodies (A, B and C) or anti-glucagon antibodies (D, E and F). Bar = 100 μ m.

levels of Kir6.2^{-/-} GIPR^{-/-} mice become elevated compared with Kir6.2^{-/-} GIPR^{+/+} mice, especially at 15 min (345 ± 10 mg/dl vs 294 ± 20 mg/dl, $P < 0.05$) and 30 min (453 ± 20 mg/dl vs 381 ± 26 mg/dl, $P < 0.05$) (Fig. 3A). The time of peak glucose response is at 30 min both in Kir6.2^{-/-} GIPR^{-/-} mice and Kir6.2^{-/-} GIPR^{+/+} mice. Plasma insulin levels were measured to determine the cause of the glucose intolerance in Kir6.2^{-/-} GIPR^{-/-} mice. Fasting insulin levels in Kir6.2^{-/-} GIPR^{-/-} mice and Kir6.2^{-/-} GIPR^{+/+} mice are similar. The insulin response after oral glucose loading is almost completely lost in Kir6.2^{-/-} GIPR^{-/-} mice, and the insulin levels at 15 min and 30 min in Kir6.2^{-/-} GIPR^{-/-} mice are lower than in Kir6.2^{-/-} GIPR^{+/+} mice (Fig. 3B), indicating that the glucose intolerance in Kir6.2^{-/-} GIPR^{-/-} mice is due to insufficient insulin secretion.

Glucose uptake by the jejunum *in vitro*

We investigated glucose uptake by the jejunum *in vitro* using everted jejunal rings, and found no difference among Kir6.2^{+/+} GIPR^{+/+}, Kir6.2^{-/-} GIPR^{+/+} and Kir6.2^{-/-} GIPR^{-/-} mice (Fig. 4).

Figure 3 Glucose tolerance test. (A) Blood glucose levels after oral glucose loading are shown: Kir6.2^{+/+} GIPR^{+/+} mice, open circles; Kir6.2^{-/-} GIPR^{+/+} mice, hatched circles; Kir6.2^{-/-} GIPR^{-/-} mice, solid circles ($n = 5$). (B) Plasma insulin levels after oral glucose tolerance test are shown: Kir6.2^{+/+} GIPR^{+/+} mice, open bars; Kir6.2^{-/-} GIPR^{+/+} mice, hatched bars; Kir6.2^{-/-} GIPR^{-/-} mice, solid bars ($n = 5$). Values are means $\pm$ S.E.M. # $P < 0.05$ vs Kir6.2^{+/+} GIPR^{+/+} mice; * $P < 0.05$ vs Kir6.2^{-/-} GIPR^{+/+} mice.

Insulin sensitivity *in vivo*

The glucose-lowering effect of insulin was assessed by an insulin tolerance test. Kir6.2^{-/-} GIPR^{-/-} mice and Kir6.2^{-/-} GIPR^{+/+} mice are similarly insulin sensitive, and more so than Kir6.2^{+/+} GIPR^{+/+} mice (Fig. 5).

Discussion

GIP and GLP-1 are physiological incretins (7, 15) that stimulate insulin secretion from pancreatic β -cells upon ingestion of nutrients. GIP and GLP-1 bind to specific receptors, the GIPR and the GLP-1 receptor (GLP-1R), and increase the intracellular cAMP concentration, which produces insulin secretion. However, functional differences have become clear in recent studies of knockout mice (10, 16–18). GIPR^{-/-} mice exhibit normal glucose tolerance after intraperitoneal glucose loading but impaired glucose tolerance after oral glucose loading, while GLP-1R-knockout (GLP-1^{-/-}) mice exhibit impaired glucose tolerance

Figure 4 Glucose uptake in the jejunum. Glucose uptake in the jejunum in Kir6.2^{+/+} GIPR^{+/+} mice, open bar; Kir6.2^{-/-} GIPR^{+/+} mice, hatched bar; Kir6.2^{-/-} GIPR^{-/-} mice, solid bar is shown ($n = 4$). Na⁺/glucose cotransporter (SGLT)-dependent glucose uptake was determined as the glucose uptake in the absence of phlorizin minus the glucose uptake in the presence of 1 mmol/l phlorizin. Values are means $\pm$ S.E.M.

Figure 5 Insulin tolerance test. Changes in blood glucose levels upon insulin injection are shown: Kir6.2^{+/+} GIPR^{+/+} mice, open circles; Kir6.2^{-/-} GIPR^{+/+} mice, hatched circles; Kir6.2^{-/-} GIPR^{-/-} mice, solid circles ($n = 5$). Values are means $\pm$ S.E.M., relative to basal blood glucose levels. # $P < 0.05$ compared with Kir6.2^{+/+} GIPR^{+/+} mice.

both orally and intraperitoneally. $GIPR^{-/-}$ mice also exhibit an increased number of pancreatic β -cells while $GLP-1R^{-/-}$ mice show a decreased number. In addition, GLP-1 causes a decreased gastric emptying rate by intravenous infusion in humans (19), and produces a marked reduction in food and water intake in rats through a central mechanism (20). Apparently, GIP contributes to glucose homeostasis by raising insulin secretion in response to an oral glucose load while GLP-1 contributes by pancreatic β -cell proliferation and other factors in addition to insulin secretion. In the present study using double-knockout mice, we establish that GIP is the insulinotropic factor in the insulin response to oral glucose loading in K_{ATP} channel-deficient mice. $Kir6.2^{-/-}GIPR^{-/-}$ mice show no increase in plasma insulin levels in response to an oral glucose load, demonstrating that the insulin secretory response in $Kir6.2^{-/-}GIPR^{+/+}$ mice to an oral glucose load is abolished in the absence of the GIP signal.

Immunohistochemical examination of pancreatic islets of $Kir6.2^{-/-}GIPR^{-/-}$ mice shows that the area containing insulin-positive β -cells is larger than in $Kir6.2^{-/-}GIPR^{+/+}$ islets at 18 weeks of age. It has been reported that the number of β -cells in $Kir6.2^{-/-}$ mice is similar to wild-type ($Kir6.2^{+/+}$) mice before 16 weeks of age, but that a decrease becomes evident in more aged $Kir6.2^{-/-}$ mice (21). The result is consistent with the previous study (17) that showed that the total pancreatic insulin content of $GIPR^{-/-}$ mice is lower than that of wild-type ($GIPR^{+/+}$) mice, although the pancreatic β -cell area is increased.

We have shown that the plasma GLP-1 levels are similar in $GIPR^{+/+}$ mice and $GIPR^{-/-}$ mice, and that $GIPR^{-/-}$ mice exhibit higher insulin release in response to GLP-1 (17). These results suggest that the action of GLP-1 is increased in the absence of GIP signaling. Since GLP-1 signaling is still intact in $Kir6.2^{-/-}GIPR^{-/-}$ mice, the increased number of pancreatic β -cells in $Kir6.2^{-/-}GIPR^{-/-}$ mice may be due to the action of GLP-1. These results indicate both that GIP is the major insulinotropic factor in insulin secretion in K_{ATP} channel-deficient mice and that GLP-1 contributes to glucose homeostasis in these mice by stimulating pancreatic β -cell proliferation.

Despite the nearly complete loss of the insulin secretory response to an oral glucose load, $Kir6.2^{-/-}GIPR^{-/-}$ mice still show only mild glucose intolerance. Since GIP receptors are also expressed in the small intestine and in insulin-target tissues such as adipocytes (22), we investigated glucose uptake by the small intestine using everted jejunal rings *in vitro*. Glucose uptake in $Kir6.2^{-/-}GIPR^{-/-}$, $Kir6.2^{-/-}GIPR^{+/+}$, and $Kir6.2^{+/+}GIPR^{+/+}$ mice was similar in this study, suggesting that other factors may contribute to glucose homeostasis in this case, such as increased glucose uptake to adipocytes and skeletal muscle or extrapancreatic glucose-lowering effects of GLP-1 (23).

In the insulin tolerance test, $Kir6.2^{-/-}GIPR^{-/-}$ mice and $Kir6.2^{-/-}GIPR^{+/+}$ mice are similarly insulin sensitive, and more so than $Kir6.2^{+/+}GIPR^{+/+}$ mice. Previous studies have shown that insulin sensitivity is increased by disruption not only of $Kir6.2$ but also of SUR2, the other component of skeletal muscle K_{ATP} channels. The glucose-lowering effect of insulin, as assessed by the insulin tolerance test, is increased in $Kir6.2^{-/-}$ mice (5), and a 2-deoxy-[3H]glucose uptake experiment *in vivo* has shown that basal and insulin-stimulated glucose uptake in skeletal muscles and adipose tissues of $Kir6.2^{-/-}$ mice is enhanced compared with $Kir6.2^{+/+}$ mice (24). In SUR2-knockout ($SUR2^{-/-}$) mice, enhanced insulin action in skeletal muscle has been reported, and *in vitro* insulin-stimulated glucose transport is 1.5-fold greater than in wild-type ($SUR2^{+/+}$) mice (25). It is thought that K_{ATP} channels consisting of $Kir6.2$ and SUR2 participate in glucose uptake in skeletal muscles directly while the $Kir6.2$ -containing channels in adipose tissues are involved in glucose uptake only indirectly.

Acknowledgements

We thank Dr T Miki and Prof. S Seino, Chiba University Graduate School of Medicine, Department of Cellular and Molecular Medicine, who provided the $Kir6.2^{-/-}$ mice. This study was supported, in part, by Grants-in-Aids for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by Health and Labor Sciences Research Grants for Research on Human Genome, Tissue Engineering and Food Biotechnology from the Ministry of Health, Labor and Welfare of Japan, and by Health and Labor Sciences Research Grants for Comprehensive Research on Aging and Health from the Ministry of Health, Labor and Welfare of Japan.

References

- 1 Ashcroft FM & Rorsman P. Electrophysiology of the pancreatic beta-cell. *Progress in Biophysics and Molecular Biology* 1989 **54** 87–143.
- 2 Meglasson MD & Matschinsky FM. Pancreatic islet glucose metabolism and regulation of insulin secretion. *Diabetes and Metabolism Reviews* 1986 **2** 163–214.
- 3 Cook DL, Satin LS, Ashford ML & Hales CN. ATP-sensitive K^+ channels in pancreatic beta-cells. Spare-channel hypothesis. *Diabetes* 1988 **37** 495–498.
- 4 Misler S, Barnett DW, Gillis KD & Pressel DM. Electrophysiology of stimulus-secretion coupling in human beta-cells. *Diabetes* 1992 **41** 1221–1228.
- 5 Miki T, Nagashima K, Tashiro F, Kotake K, Yoshitomi H, Tamamoto A, Gono T, Iwanaga T, Miyazaki J & Seino S. Defective insulin secretion and enhanced insulin action in K_{ATP} channel-deficient mice. *PNAS* 1998 **95** 10402–10406.
- 6 Unger RH & Eisentraut AM. Entero-insular axis. *Archives of Internal Medicine* 1969 **123** 261–266.
- 7 Creutzfeldt W. The incretin concept today. *Diabetologia* 1979 **16** 75–85.

- 8 Pederson RA, Schubert HE & Brown JC. Gastric inhibitory polypeptide. Its physiologic release and insulinotropic action in the dog. *Diabetes* 1975 **24** 1050–1056.
- 9 Kashima Y, Miki T, Shibasaki T, Ozaki N, Miyazaki M, Yano H & Seino S. Critical role of cAMP-GEFII–Rim2 complex in incretin-potentiated insulin secretion. *Journal of Biological Chemistry* 2001 **276** 46046–46053.
- 10 Miyawaki K, Yamada Y, Yano H, Niwa H, Ban N, Ihara Y, Kubota A, Fujimoto S, Kajikawa M, Kuroe A, Tsuda K, Hashimoto H, Yamashita T, Jomori T, Tashiro F, Miyazaki J & Seino Y. Glucose intolerance caused by a defect in the entero-insular axis: a study in gastric inhibitory polypeptide receptor knockout mice. *PNAS* 1999 **96** 14843–14847.
- 11 Fujimoto S, Ishida H, Kato S, Okamoto Y, Tsuji K, Mizuno N, Ueda S, Mukai E & Seino Y. The novel insulinotropic mechanism of pimobendan: direct enhancement of the exocytotic process of insulin secretory granules by increased Ca^{2+} sensitivity in beta-cells. *Endocrinology* 1998 **139** 1133–1140.
- 12 Hsu SM, Raine L & Fanger H. A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. *American Journal of Clinical Pathology* 1981 **75** 734–738.
- 13 Imagawa K, Nishino T, Shin S, Uehata S, Hashimura E, Yanaihara C & Yanaihara N. Production of anti-glucagon sera with a C-terminal fragment of pancreatic glucagon. *Endocrinologia Japonica* 1979 **26** 123–131.
- 14 Lostao MP, Urdaneta E, Martinez-Anso E, Barber A & Martinez JA. Presence of leptin receptors in rat small intestine and leptin effect on sugar absorption. *FEBS Letters* 1998 **423** 302–306.
- 15 Kreymann B, Williams G, Gbatei MA & Bloom SR. Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet* 1987 **2** 1300–1304.
- 16 Scrocchi LA, Brown TJ, MacClusky N, Brubaker PL, Auerbach AB, Joyner AL & Drucker DJ. Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nature Medicine* 1996 **2** 1254–1258.
- 17 Pamir N, Lynn FC, Buchan AM, Ehses J, Hinke SA, Pospisilik JA, Miyawaki K, Yamada Y, Seino Y, McIntosh CH & Pederson RA. Glucose-dependent insulinotropic polypeptide receptor null mice exhibit compensatory changes in the enteroinsular axis. *American Journal of Physiology. Endocrinology and Metabolism* 2003 **284** E931–E939.
- 18 Ling Z, Wu D, Zambre Y, Flamez D, Drucker DJ, Pipeleers DG & Schuit FC. Glucagon-like peptide 1 receptor signaling influences topography of islet cells in mice. *Virchows Archiv* 2001 **438** 382–387.
- 19 Wettergren A, Schjoldager B, Mortensen PE, Myhre J, Christiansen J & Holst JJ. Truncated GLP-1 (proglucagon 78–107-amide) inhibits gastric and pancreatic functions in man. *Digestive Diseases and Sciences* 1993 **38** 665–673.
- 20 Navarro M, Rodriguez de Fonseca F, Alvarez E, Chowen JA, Zueco JA, Gomez R, Eng J & Blazquez E. Colocalization of glucagon-like peptide-1 (GLP-1) receptors, glucose transporter GLUT-2, and glucokinase mRNAs in rat hypothalamic cells: evidence for a role of GLP-1 receptor agonists as an inhibitory signal for food and water intake. *Journal of Neurochemistry* 1996 **67** 1982–1991.
- 21 Miki T, Iwanaga T, Nagashima K, Ihara Y & Seino S. Roles of ATP-sensitive K^{+} channels in cell survival and differentiation in the endocrine pancreas. *Diabetes* 2001 **50** (Suppl 1) S48–S51.
- 22 Miyawaki K, Yamada Y, Ban N, Ihara Y, Tsukiyama K, Zhou H, Fujimoto S, Oku A, Tsuda K, Toyokuni S, Hiai H, Mizunoya W, Fushiki T, Holst JJ, Makino M, Tashita A, Kobara Y, Tsubamoto Y, Jinnouchi T, Jomori T & Seino Y. Inhibition of gastric inhibitory polypeptide signaling prevents obesity. *Nature Medicine* 2002 **8** 738–742.
- 23 Valverde I, Villanueva-Penacarrillo ML & Malaisse WJ. Pancreatic and extrapancreatic effects of GLP-1. *Diabetes & Metabolism* 2002 **28** 3S85–3S88; discussion 3S108–3S112.
- 24 Miki T, Minami K, Zhang L, Morita M, Gono T, Shiuchi T, Minokoshi Y, Renaud JM & Seino S. ATP-sensitive potassium channels participate in glucose uptake in skeletal muscle and adipose tissue. *American Journal of Physiology. Endocrinology and Metabolism* 2002 **283** E1178–E1184.
- 25 Chutkow WA, Samuel V, Hansen PA, Pu J, Valdivia CR, Makielski JC & Burant CF. Disruption of Sur2-containing K_{ATP} channels enhances insulin-stimulated glucose uptake in skeletal muscle. *PNAS* 2001 **98** 11760–11764.

Received 16 January 2004

Accepted 16 June 2004