Transgenic overexpression of intraislet ghrelin does not affect insulin secretion or glucose metabolism in vivo

Mika Bando,1,3 Hiroshi Iwakura,1 Hiroyuki Ariyasu,1 Hiroshi Hosoda,4 Go Yamada,2 Kiminori Hosoda,2,3 Souichi Adachi,3 Kazuwa Nakao,2 Kenji Kangawa,4 and Takashi Akamizu1,5

1Ghrelin Research Project, Translational Research Center, 2Department of Medicine and Clinical Science, Endocrinology, and Metabolism, and 3Department of Human Health Sciences, Kyoto University Hospital, Kyoto University Graduate School of Medicine, Kyoto; 4National Cerebral and Cardiovascular Center Research Institute, Osaka; and 5The First Department of Medicine, Wakayama Medical University, Wakayama, Japan

Submitted 7 July 2011; accepted in final form 17 November 2011

Bando M, Iwakura H, Ariyasu H, Hosoda H, Yamada G, Hosoda K, Adachi S, Nakao K, Kangawa K, Akamizu T. Transgenic overexpression of intraislet ghrelin does not affect insulin secretion or glucose metabolism in vivo. Am J Physiol Endocrinol Metab 302: E403–E408, 2012. First published November 22, 2011; doi:10.1152/ajpendo.00341.2011.—Whereas ghrelin is produced primarily in the stomach, a small amount of it is produced in pancreatic islets. Although exogenous administration of ghrelin suppresses insulin secretion in vitro or in vivo, the role of intraislet ghrelin in the regulation of insulin secretion in vivo remains unclear. To understand the physiological role of intraislet ghrelin in insulin secretion and glucose metabolism, we developed a transgenic (Tg) mouse model, rat insulin II promoter ghrelin-internal ribosomal entry site-ghrelin O-acyl transferase (RIP-GG) Tg mice, in which mouse ghrelin cDNA and ghrelin O-acyltransferase are overexpressed under the control of the rat insulin II promoter. Although pancreatic acetic acid ghrelin levels were elevated in RIP-GG Tg mice, pancreatic ghrelin levels were not altered in animals on a standard diet. However, when Tg mice were fed a medium-chain triglyceride-rich diet (MCTD), pancreatic ghrelin levels were elevated to ~16 times that seen in control animals. It seems likely that the gastric ghrelin cells possess specific machinery to provide the octanoyl acid necessary for ghrelin acylation but that this machinery is absent from pancreatic β-cells. Despite the overexpression of ghrelin, plasma ghrelin levels in the portal veins of RIP-GG Tg mice were unchanged from control levels. Glucose tolerance, insulin secretion, and islet architecture in RIP-GG Tg mice were not significantly different even when the mice were fed a MCTD. These results indicate that intraislet ghrelin does not play a major role in the regulation of insulin secretion in vivo.

pancreas; ghrelin; D-acyltransferase

GHRELIN IS A 28-AMINO ACID PEPTIDE HORMONE, with a unique modification of acylation at the third serine residue, first described by Kojima et al. (17) in 1999. The acyl modification of ghrelin is mediated by the recently discovered enzyme ghrelin O-acyl transferase (29), and the modification is essential for ghrelin binding to its cognate receptor (12). Ghrelin is produced primarily in the stomach, but small amounts of ghrelin are also produced in pancreatic islets (1, 5, 8, 10, 12, 26, 27). Controversy remains about which type of islet cell produces ghrelin (5, 20, 26, 27). Date et al. (5) reported that ghrelin is present in α-cells in humans and rats, whereas Volante et al. (26) reported that ghrelin is produced by β-cells in humans. In contrast, Wierup and colleagues (27, 28) and Prado et al. (20) reported that ghrelin-expressing cells comprise a new islet cell type distinct from α-, β-, and δ-cells and PP cells in human, rat, and mouse islets.

Exogenous ghrelin suppresses insulin secretion from pancreatic β-cells in vitro (4, 9, 22) or in vivo (3, 22, 25). Although several studies have demonstrated contradictory results (1, 5, 11, 18, 24), data from genetically engineered mice are consistent with this concept. Chronic elevation of plasma ghrelin levels suppresses insulin secretion, inducing glucose intolerance in transgenic mice (2, 13, 21), whereas ablation of ghrelin improves glucose tolerance by enhancing insulin secretion in diet-induced obesity (7) or ob/ob mouse models (23). Although in vitro studies demonstrate that intraislet ghrelin can suppress insulin secretion from isolated islets (6), the physiological role of intraislet ghrelin on the regulation of insulin secretion in vivo is unclear. Because only minimal amounts of ghrelin are produced by the pancreas compared with that made by the stomach (15), the effect of stomach-derived ghrelin may overpower the effects of intraislet ghrelin in vivo.

In this study, we developed a transgenic mouse model in which the ghrelin and ghrelin O-acyltransferase (GOAT) genes are overexpressed by pancreatic β-cells under the control of the rat insulin II promoter (RIP) to ascertain the physiological role of intraislet ghrelin on insulin secretion and glucose metabolism in vivo.

MATERIALS AND METHODS

Generation of RIP-ghrelin-GOAT transgenic mice. We designed a fusion gene comprised of RIP, mouse ghrelin cDNA, internal ribosomal entry site (IRES), and mouse GOAT cDNA coding sequences. The purified fragment (10 μg/ml) was microinjected into the pronuclei of fertilized C57B6J mouse eggs (SLC, Shizuoka, Japan). Viable eggs were transferred into the oviducts of pseudopregnant female ICR mice (SLC) by using standard techniques. Transgenic founder mice were identified by Southern blot analyses of tail DNA, using a mouse ghrelin cDNA fragment as a probe. For experimentation, we utilized heterozygous transgenic mice. Animals were maintained on a 12:12-h light-dark cycle and fed a standard diet (SD; CE-2, 352 kcal/100 g; Japan CLEA, Tokyo, Japan) or a MCTD containing 45% Dermol M5 (C8:60%, C10:40%; Research Diets, New Brunswick, NJ) as indicated. All experimental procedures were approved by the Kyoto University Graduate School of Medicine Committee on Animal Research.

http://www.ajpendo.org
0193-1849/12 Copyright © 2012 the American Physiological Society
Measurement of plasma and tissue ghrelin concentrations. Blood was drawn from the proximal end of the portal vein under ether anesthesia, transferred immediately to chilled siliconized glass tubes containing Na₂-EDTA (1 mg/ml) and aprotinin (1,000 KIU/ml), and centrifuged at 4°C. Hydrogen chloride was added to the samples at a final concentration of 0.1 N immediately after separation of plasma. Plasma was immediately frozen and stored at −80°C until assay. Plasma ghrelin concentration was determined by AIA-600 II ( Tosoh, Tokyo, Japan).

To measure tissue ghrelin concentrations, pancreata or stomachs were isolated from mice and then boiled for 5 min in the 10-fold vol/wt of water. Acetic acid was added to each solution to adjust the final concentration to 1 M before tissue homogenization. We determine the tissue ghrelin concentration in supernatants obtained after centrifugation by radioimmunoassay (RIA) using anti-ghrelin [13–28] (C-RIA) and anti-ghrelin [1–11] (N-RIA) antisera, as described previously (12, 15).

Real-time quantitative RT-PCR. Total RNA was extracted from pancreata using an RNaseasy Protect mini kit (Qiagen, Hilden, Germany). Reverse transcription (RT) was performed using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). Real-time quantitative PCR was performed on an ABI PRISM 7500 Sequence Detection System (Applied Biosystems), using the following primers and TaqMan probes: mouse ghrelin (sense, 5′-GCATGTCGGATGGA-CATG-3′; antisense, 5′-TGGTGGCTTCTGATTCCT-3′; TaqMan probe, 5′-AGCCTACACAGCCAAAGC-3′); mouse insulin (sense, 5′-CAGCTATAATCAGAGACCATC-3′; antisense, 5′-GGTGAAGAATTACCCAC-3′; TaqMan probe, 5′-CAGGTGCTGCACCAACAC-3′); GOAT (sense, 5′-AGGGACTCTAGGAAG-3′; antisense, 5′-CCCTGTGAAGGAGAAGT-3′, with Power SybrGreen). Data were normalized to the content of 18S rRNA in each sample.

Glucose tolerance tests. For glucose tolerance testing, the ad libitum-fed mice were injected intraperitoneally with 1.5 g/kg glucose. Blood was sampled from the tail veins before and 30, 60, 90, and 120 min after the injection. Blood glucose levels were determined by the glucose oxidase method using a Glutest sensor (Sanwa Kagaku, Kyoto, Japan).

Insulin release. Ad libitum-fed mice were injected with 3.0 g/kg glucose intravenously. Plasma was sampled from a retroorbital vein by 10.220.33.3 on October 21, 2017 http://ajpendo.physiology.org/ Downloaded from AJP-Endocrinol Metab • doi:10.1152/ajpendo.00341.2011 • www.ajpendo.org

RESULTS

Generation of RIP-ghrelin-IRES-GOAT transgenic mice. After the RIP-ghrelin-IRES-GOAT transgene was injected into 286 eggs, we obtained three lines (3–4, 9–3, and 11–5) confirmed to be rat insulin II promoter-ghrelin-IRES-GOAT transgenic (RIP-GG Tg) mice. For further analyses, we selected the 9–3 line, which had the highest expression of ghrelin and GOAT mRNA in the pancreas (data not shown). The expression levels of pancreatic ghrelin mRNA in the 9–3 line of RIP-GG Tg mice were ~20-fold higher than those seen in controls (Fig. 1B), whereas GOAT mRNA levels were ~80-fold higher than those in controls (Fig. 1C). There was also an increment in ghrelin and GOAT mRNA levels in the hypothalamus of RIP-GG Tg mice (non-Tg vs. Tg: ghrelin, 1.0 ± 0.28 vs. 25.6 ± 5.6; GOAT, 1.0 ± 0.26 vs. 5,735.5 ± 1,189.1, arbitrary unit; n = 8, P < 0.01).

Pancreatic and plasma ghrelin levels in RIP-GG Tg mice. Total ghrelin levels measured by C-RIA were significantly elevated in the pancreata of RIP-GG Tg mice on a SD or MCTD (Fig. 2A). However, the ghrelin levels measured by N-RIA were elevated only when RIP-GG Tg mice were fed a MCTD (Fig. 2B). Although ghrelin levels 16-fold higher than those seen in control littermates were observed in the pancreata of RIP-GG Tg mice fed a MCTD, these absolute levels were low compared with those isolated from stomach (Fig. 2, D and E). Furthermore, the ratio of ghrelin to total ghrelin in the pancreata of RIP-GG Tg mice was significantly low on SD, which was elevated on a MCTD (Fig. 2C). Still, the level was significantly lower compared with that of the stomach (Fig. 1F).

Immunohistochemistry showed that the ghrelin-like immunoreactivities were increased in the core of the islets of RIP-GG Tg mice on a MCTD (Fig. 3), indicating that increased tissue levels of pancreatic ghrelin were originated from β-cells.
We measured plasma ghrelin levels in the portal veins of RIP-GG Tg mice fed a MCTD to determine whether this level of ghrelin overexpression in islets could affect plasma ghrelin levels. No significant changes were observed in either ghrelin or desacyl ghrelin levels in the portal veins of RIP-GG Tg mice (Fig. 4, A and B), indicating that ghrelin overexpression from the transgene in islets produces minimal effect on plasma ghrelin levels.

Glucose metabolism and insulin secretion in RIP-GG Tg mice. No significant changes in blood glucose levels were seen by intraperitoneal glucose tolerance tests between 10-wk-old RIP-GG Tg mice and controls on a MCTD (Fig. 5A). Plasma insulin levels before and after a glucose load were not altered significantly in 15-wk-old RIP-GG Tg mice on a MCTD (Fig. 5B). There were also no significant changes in blood glucose or plasma insulin levels after glucose load in old mice (≈84-wk old) or in female mice (Fig. 5, C–F).

Islet architecture. There were no obvious abnormalities in intraislet cytoarchitecture or in the cell numbers of insulin-, glucagon-, somatostatin-, and polypeptide-producing cells in the islets of RIP-GG Tg mice on MCTD (Fig. 6, A–D).

Fig. 2. Pancreatic and gastric ghrelin levels in RIP-GG Tg mice on standard (SD) or medium-chain triglyceride-rich diet (MCTD). A and B: pancreatic ghrelin levels in RIP-GG Tg mice (black bars) and nontransgenic controls (open bars) measured by using anti-ghrelin [13–28] (C-RIA; A) and anti-ghrelin [1–11] (N-RIA; B). Although total ghrelin levels measured by C-RIA were elevated in RIP-GG Tg mice on both SD and MCTD, ghrelin levels measured by N-RIA were elevated only when RIP-GG Tg mice were fed MCTD. D and E: gastric ghrelin levels of RIP-GG Tg mice (black bars) and nontransgenic controls (open bars) measured by C-RIA (D) or N-RIA (E) were significantly higher than pancreatic levels, regardless of diet. C and F: The ratio of N-RIA/C-RIA (N/C). **P < 0.01 and *P < 0.05 compared with controls. ##P < 0.01 compared with SD; n = 5–7.

Fig. 3. Immunohistochemical analysis of the expression of ghrelin in the islets of RIP-GG Tg mice. Ghrelin-like immunoreactivities were increased in the core of the islets of RIP-GG Tg mice on MCTD.

Fig. 4. Portal ghrelin levels of RIP-GG Tg mice. A and B: portal ghrelin (A) and desacyl ghrelin levels (B) in male Tg (black bars) and non (open bars) fed MCTD; n = 7–8.
Staining intensities for these four islet hormones within islets of RIP-GG Tg mice did not differ from those of nontransgenic littermates.

DISCUSSION

In previous studies, we developed transgenic mice in which mouse ghrelin cDNA is overexpressed in pancreatic β-cells under the control of the rat insulin II promoter to identify the effect of ghrelin on pancreatic islets (15). However, these Tg mice displayed elevated expression of desacyl ghrelin only within the pancreas. At that time, the mechanism by which ghrelin received an n-octanoyl modification was unknown. Recently, Yang et al. (29) identified GOAT as the enzyme mediating this modification. In this study, we developed a transgenic mouse in which ghrelin produced in the pancreas might be both overexpressed and modified, with the overexpression of both mouse ghrelin and GOAT cDNA in pancreatic β-cells under the control of the rat insulin II promoter.

To our surprise, whereas pancreatic desacyl ghrelin levels were elevated in RIP-GG Tg mice, pancreatic levels of (active, modified) ghrelin were unchanged on a SD. Ghrelin levels were elevated only when mice were fed a MCTD. Similar results were reported by Kirchner et al. (16), who created a transgenic mouse in which ghrelin and GOAT cDNA were overexpressed in the liver under the control of

Fig. 5. Glucose metabolism in RIP-GG Tg mice. A, C, and E: glucose tolerance tests in 10-wk-old male (A), 11-wk-old female (C), or 83-wk-old male (E) Tg on MCTD (■) and non (●); n = 7–10. B, D, and F: serum insulin levels at baseline and at 2 or 30 min after intravenous glucose injection in 15-wk-old male (B), 10-wk-old female (D), or 84-wk-old male (F) Tg fed MCTD (black bars) and in non (open bars); n = 5–10.
the apolipoprotein E promoter. These mice demonstrated elevated plasma ghrelin levels only when mice were fed a medium-chain fatty acid-rich diet. Considering that gastric ghrelin-producing cells can produce ghrelin regardless of diet, even in a fasting state, it is likely that these gastric cells possess a specific machinery to generate the octanoyl acid necessary for acylation, which is lacking from pancreatic \(\beta\)-cells or hepatocytes.

In previous studies, we demonstrated that the chronic elevation of plasma ghrelin levels at ~10-fold higher than the normal range suppresses insulin secretion and induces glucose intolerance in mice (13). In this study, RIP-GG Tg mice, which produce 16-fold higher ghrelin levels from the pancreas as normal mice, exhibited normal glucose tolerance and insulin secretion. The pancreatic ghrelin levels in RIP-GG Tg mice, although elevated, were still considerably lower than the gastric ghrelin level. We tried to compare the ghrelin levels in pancreatic vein with those in artery, as Dezaki et al. (7) did using rats, but it was difficult to determine the ghrelin levels in pancreatic veins of mice due to the small body size. We measured ghrelin levels in the portal vein instead, which were not elevated in RIP-GG Tg mice. We cannot determine the exact concentration of ghrelin in the microenvironment surrounding \(\beta\)-cells, but these levels still seem to be overpowered by the circulating ghrelin produced by the stomach. Although it is possible that additional overproducing of ghrelin in islets could eventually suppress insulin secretion, further enhancement of ghrelin expression by islets would not be in the realm of physiological relevance. In vitro, intraslelet ghrelin may suppress insulin secretion in a paracrine (or autocrine) manner where the effect of circulating ghrelin is eliminated (6). However, this study indicates that intraslelet ghrelin does not play a major role in controlling insulin secretion in vivo, where high levels of circulating ghrelin are generated by the stomach.

One drawback of this study is that elevated pancreatic ghrelin levels in RIP-GG Tg mice could not be obtained without feeding mice a MCTD. The MCTD consists of medium-chain fatty acids (C6–C10) that can enter mitochondria without the carnitine shuttle. Medium-chain triglycerides generally have favorable effects on obesity or diabetes (19), suppressing fat accumulation and improving insulin sensitivity. We cannot exclude the possibility that a MCTD may have interfered with the effects of ghrelin within islets. In addition, ghrelin and GOAT mRNA levels were increased not only in the islet but also in the hypothalamus of RIP-GG Tg mice. There is a possibility that the overexpressed ghrelin in the hypothalamus may have influenced the effects of overexpressed ghrelin in the islet.

In summary, we have developed RIP-GG Tg mice, in which intraslelet ghrelin levels were elevated to ~16 times the control levels when mice were fed a MCTD. The glucose tolerance and insulin secretion of RIP-GG Tg mice were unchanged, indicating that intraslelet ghrelin does not play a major role in regulating insulin secretion in vivo.

ACKNOWLEDGMENTS

We thank Chieko Ishimoto and Chinami Shiraiwa for their excellent technical assistance.

GRANTS

This study was supported by funds from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and the Ministry of Health, Labour, and Welfare of Japan.

DISCLOSURES

The authors have nothing to declare.

AUTHOR CONTRIBUTIONS

M.B., H.I., and H.H. performed the experiments; M.B. and H.I. analyzed the data; M.B., H.I., H.A., H.H., G.Y., K.H., S.A., K.N., K.K., and T.A. interpreted the results of the experiments; M.B. and H.I. prepared the figures; M.B., H.I., and T.A. drafted the manuscript; M.B., H.I., H.A., S.A., K.N., K.K., and T.A. edited and revised the manuscript; M.B., H.I., K.N., K.K., and T.A. approved the final version of the manuscript; H.I., K.H., K.N., K.K., and T.A. did the conception and design of the research.

Fig. 6. Islet morphology in RIP-GG Tg mice. The pancreatic sections from RIP-GG Tg mice and non were stained with anti-insulin, anti-glucagon, anti-somatostatin, or anti-pancreatic polypeptide antibodies. Representative images are presented.

# Table of Contents

- **EAP-Endocrinol Metab** • doi:10.1152/ajpendo.00341.2011 • www.ajpendo.org
REFERENCES


