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Activation of activin type IB receptor signals in pancreatic β cells leads to defective insulin secretion through the attenuation of ATP-sensitive K^+ channel activity

Masatoshi Nomura [a], Hidetaka Morinaga [a], Hai-Lei Zhu [b], Lixiang Wang [a], Nao Hasuzawa [a], Ryoichi Takayanagi [a], Noriyoshi Teramoto [b,c,*]

[a] Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi Ward, Fukuoka 812-8582, Japan
[b] Department of Pharmacology, Faculty of Medicine, Saga 849-8501, Japan
[c] Laboratory of Biomedical Engineering, Graduate School of Biomedical Engineering, Tohoku University, 4-1 Seiryo, Aoba Ward, Sendai 980-8575, Japan

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ABSTRACT

In studies of gene-ablated mice, activin signaling through activin type IIB receptors (ActRIIB) and Smad2 has been shown to regulate not only pancreatic β cell mass but also insulin secretion. However, it still remains unclear whether gain of function of activin signaling is involved in the modulation of pancreatic β cell mass and insulin secretion. To identify distinct roles of activin signaling in pancreatic β cells, the Cre-loxP system was used to activate signaling through activin type IB receptor (ActRIB) in pancreatic β cells. The resultant mice (pancreatic β cell-specific ActRIB transgenic (Tg) mice; ActRIBCAβTg) exhibited a defect in glucose-stimulated insulin secretion (GSIS) and a progressive impairment of glucose tolerance. Patch-clamp techniques revealed that the activity of ATP-sensitive K^+ channels (K_{ATP} channels) was decreased in mutant β cells. These results indicate that an appropriate level of activin signaling may be required for GSIS in pancreatic β cells, and that activin signaling involves modulation of K_{ATP} channel activity.

1. Introduction

Activin, a member of the transforming growth factor-β (TGF-β) superfamily, has been shown to regulate several biological functions, such as the specification, differentiation and proliferation of multiple organs, including the pancreas [1,2]. The effects of activin are mediated by a complex of the activin type I receptor (ActRI) and activin type II receptor (ActRII). Type I receptors consist of ActRIA and ActRIB, and type II receptors comprise ActRIIA and ActRIIB [3]. The binding of activin to ActRIIA or ActRIIB induces the recruitment of ActRIB [4]. Receptor heterodimerization activates ActRII kinase to phosphorylate ActRI, which recruits and phosphorylates both Smad2 and Smad3. The phosphorylated Smads are released, forming a complex with Co-Smad and Smad4. The Smad and Co-Smad complex then translocates to the nucleus to regulate the expression of target genes [5].

We reported that compound heterozygotic ActRIIB$^{+/-}$ Smad2$^{+/-}$ knockout (KO) mice have a defect in early pancreatic formation, with a reduction of adult islet mass and an impairment of glucose tolerance occurring in a gene dosage-sensitive manner [6]. Smad2 heterozygotic (+/−) mutant embryos also show decreased β cell mass and disrupted islet development, although to a lesser degree than the compound heterozygotic embryos [7]. These results suggest that activin signaling through ActRIIB and Smad2 plays a significant role in islet formation and insulin secretion. Pancreatic β cell-specific Smad2 disruption produced islet hyperplasia and impaired glucose tolerance, with decreased activity of K_{ATP} channels [8]. It remains unknown whether gain of function of activin signaling is involved in the regulation of pancreatic β cell mass and insulin secretion.

In this study, we created GFP-floxed mice carrying a transgene that directs the expression of the constitutively active form of ActRIB (ActRIBCA) upon Cre-mediated excision of the loxP-flanked GFP gene. To obtain β cell-specific ActRIBCA Tg mice (i.e. ActRIBCAβTg), GFP-floxed mice were crossed with RIP-Cre mice that express the Cre recombinase gene under the control of the rat insulin gene promoter. The resultant mice exhibited a defect in glucose-stimulated insulin secretion (GSIS) and a progressive impairment of glucose tolerance, although there was no difference in β cell mass between mutant and control mice. Patch-clamp techniques

* Corresponding author at: Department of Pharmacology, Faculty of Medicine, Saga 849-8501, Japan. Fax: +81 952 34 2013.
E-mail address: noritera@cc.saga-u.ac.jp (N. Teramoto).

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revealed that constitutive activation of ActRIB signaling alters K_{ATP} channel activity. The findings suggest that an appropriate level of activin signaling is required for normal K_{ATP} channel activity and thus GSIS.

2. Materials and methods

2.1. Ethics statement

Experiments on mice were performed according to the guidelines of the animal ethics committee of Kyushu University, Graduate School of Medicine (Fukuoka, Japan; Permit Number: A25-178-0).

2.2. Generation of mice with pancreatic β cell-specific expression of ActRIB

A constitutively active form of ActRIB, designated ActRIBCA, was generated by amino acid substitution of the threonine at position 206 with glutamine in human ActRIB cDNA [3]. The ActRIBCA expression vector was constructed by inserting a 1.6 kb cDNA fragment, which containing the entire human ActRIBCA coding sequence, into the pCAGGS vector [9]. The ActRIBCA gene was tagged at the carboxyl terminus with an influenza virus triple hemagglutinin (HA) epitope, and its expression controlled by the CAG promoter. A 5 kb *Sal*I–*Sal*I fragment containing an internal ribosome-entry site (IRES)-bgeo cassette [10] was inserted next to the HA epitope [11]. This transgene directs the expression of ActRIBCA upon Cre-mediated excision of the loxP-flanked GFP gene. Transfected ES cells were cultured for 7 days in ES medium containing 100 μg/ml of hygromycin (Life Technologies, USA) for 7 days. Twenty colonies with GFP fluorescence were selected using a laser confocal microscope and expanded. The degree of ActRIBCA expression was confirmed with β-galactosidase staining after excision of the stuffer GFP cassette by infection with an adenovirus vector containing the Cre recombinase gene [12]. The cloned ES cells were microinjected into C57BL/6 blastocysts, and the chimeric mice were crossed with C57BL/6 mice to obtain GFP-floxed mice. Female GFP-floxed mice were crossed with male Tg mice expressing Cre recombinase under the control of rat insulin gene regulatory elements (TgN(ins2-cre)25Mgn, hereafter referred to as Rip) to obtain ActRIBCAβTg mice [13]. Mice were genotyped for the ActRIBCATg allele with PCR, using the following primers: primer F: 5′-TCT TTT TCC TAC AGC TCC TGG GCA-3′ and primer R: 5′-CCC ATC TGT CTC ACA CGT GTA GTT-3′ (Fig. 1A).

2.3. Glucose and insulin tolerance tests

Blood glucose levels of mice, fasted overnight, were measured using a Glutest Sensor (Sanwa Kagaku, Nagoya, Japan). Glucose was injected intraperitoneally (2 g dextrose/kg body weight in sterile saline), and the subsequent blood levels of glucose determined. Human regular insulin (0.75 U/kg body weight) was injected intraperitoneally to random-fed mice, and blood glucose levels measured before and after the injection. The levels of insulin in plasma were determined using a standard rat/mouse insulin ELISA kit (Morinaga Institute of Biological Science, Tokyo, Japan).

2.4. Histological analysis

The intact pancreas was removed and bisected into the head (duodenal) and tail (spleen) segments. After weighing, tissues were fixed in 4% paraformaldehyde overnight at 4 °C, dehydrated in 100% ethanol, and embedded in paraffin. The anatomy of the pancreas was assessed by hematoxylin and eosin staining [6]. For immunohistochemical detection of ActRIB, Cre recombinase, insulin, glucagon and pancreatic polypeptide, rabbit anti-HA antibody (1:100, Upstate, Charlottesville, VA, USA), rabbit anti-Cre antibody (1:2000; Merck Millipore, Billerica, MA, USA), guinea-pig anti-insulin antibody (1:200), rabbit anti-glucagon antibody (1:200) and rabbit anti-pancreatic polypeptide (PP) antibody (1:600; DAKO, Glostrup, Denmark) were used as primary antibodies. Goat anti-rabbit IgG conjugated with horseradish peroxidase (Cell Signaling Technology, Danvers, MA, USA) was used as the secondary antibody for HA, Cre recombinase and PP. 3′3′-diaminobenzidine was used as a chromogenic substrate for horseradish peroxidase. The secondary antibodies used to detect insulin and glucagon were Alexa Fluor® 594-conjugated goat anti-guinea-pig IgG and Alexa Fluor® 488-conjugated donkey anti-rabbit IgG (Life Technologies, Carlsbad, USA) respectively. The stained sections were observed and photographed using a BioZero fluorescence microscope (Keyence, Osaka, Japan).

2.5. Morphometric analyses

Computer-assisted measurements of pancreatic islet area (BioZero microscope; Keyence) were analyzed using Microsoft Excel software (Mac 2011 version 14.3.5: Microsoft Japan, Tokyo, Japan).

2.6. Analysis of pancreatic islets

Pancreatic islets from fasted adult ActRIBCAβTg and Rip control mice were isolated as described previously [8]. The islets were placed in Krebs–Ringer HEPES buffer solution comprised of (mM): NaCl 140, KCl 3.6, NaH_2PO_4 0.5, $MgSO_4$ 0.5, $CaCl_2$ 1.5, $NaHCO_3$ 2, HEPES 10 and 0.1% bovine serum albumin (BSA). Depending on the experiment, glucose 2.8, glucose 22.4, tolbutamide 0.5 or KCl 60 was added to the buffer solution. The extent of insulin secretion was determined using the rat/mouse insulin ELISA kit (Morinaga Institute of Biological Science). Western blot analysis of islets was performed with rabbit anti-Smad2 primary antibody (1:250; Cell Signaling Technology, Danvers, MA, USA) or rabbit anti-phospho-Smad2 primary antibody (1:150; Cell Signaling Technology) [8].

2.7. RNA isolation and quantitative real time-PCR analysis

Total RNAs (500 ng) from islets were reverse transcribed (QuantiTect Reverse Transcription Kit; Qiagen, Hilden, Germany), and quantitative real-time PCR undertaken using 2× Power SYBR Green PCR Master Mix and an ABI Prism 7000 sequence detection system (Life Technologies, CA, USA). The PCR conditions were: 95 °C for 10 min, then 40 cycles at 95 °C for 15 s and 60 °C for 45 s. The primer sequences used were as follows:

Kir6.2: 5′-CTGGCCATCCTCATTCTCAT-3′/5′-CTCTTTCGGAGGTCCCCTAC-3′,
SUR1: 5′-CCCTCTACCAGCACACCAAT-3′/5′-CAGTCTGCATGAGGCAGGTA-3′,
GAPDH: 5′-ATGCCCCCATGTTTGTGATG-3′/5′-CCATCACTGCCACCCAGAAG-3′.

Relative gene expression *vs.* control was normalized to the expression of GAPDH genes.

2.8. Electrophysiological recordings and data analysis

Pancreatic islets from fasted adult control Rip and ActRIBCAβTg mice were isolated and the patch-clamp and data recording systems were as described previously [8,14]. Experiments were carried out at 21–23 °C. Single ion channel data was low-pass filtered at 2 kHz and sampled at a 80 μs intervals using 'PAT' software (Dr Dempster, Strathclyde University, UK). The pipette and

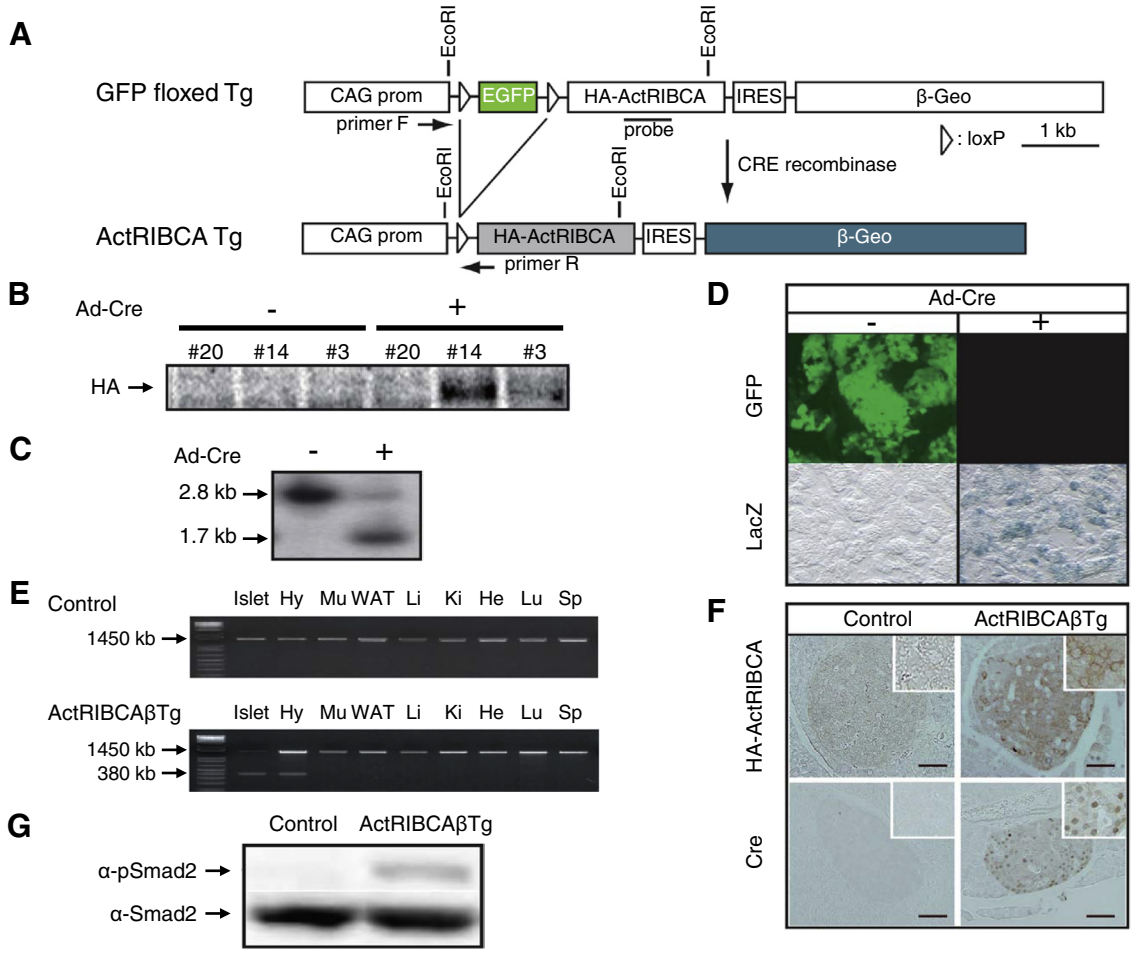

Fig. 1. Generation of ActRIBCAβTg mice. (A) Schematic of transgene construction. The HA-tagged ActRIBCA gene is expressed after excision of the EGFP gene by Cre recombinase. (B) Western blot analysis of HA-tagged ActRIBCA expression. (C) Southern blot analysis of the #14 ES cell line. The genomic DNA purified from the #14 ES cell line was digested with *Eco*RI and hybridized with the genomic probe described in (A). (D) Validation of the Cre-LoxP system in the cloned ES cells. Green fluorescence disappeared after Cre recombination, whereas β-galactosidase staining was clearly present. (E) PCR analysis of Cre-mediated recombination in various tissues from control Rip and ActRIBCAβTg mice. Primers F and R indicated in (A) were used. (F) Expression of ActRIBCA and Cre recombinase in the islets. (G) Western blot analysis of the expressions of Smad2 protein and phospho-Smad2 protein in pancreatic islets (control Rip and ActRIBCAβTg mice). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

bath solutions used were high K^+ solutions, producing symmetrical 140 mM K^+ conditions. The compositions of the pipette and bath solutions, respectively, were (in mM): 140 KCl, 1 $CaCl_2$, 1.5 $MgCl_2$, 5.5 glucose, 10 HEPES/Tris (pH 7.35–7.40); and 140 KCl, 4.6 $MgCl_2$, 1 EGTA, 10 glucose, 10 HEPES/Tris (pH 7.35–7.40).

2.9. Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). Differences between sample means were assessed by one-way ANOVA test. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Generation of ActRIBCAβTg mice

The transgene directs the expression of ActRIBCA upon Cre-mediated excision of the loxP-flanked GFP gene (Fig. 1A). Twenty colonies with positive GFP fluorescence signals were selected under a laser microscope. The expression of HA-tagged ActRIBCA was analyzed in each cell line after excision of the stuffer GFP cassette by infection with an adenovirus vector containing a Cre recombinase gene. As shown in Fig. 1B, the expression level varied between cell lines. The cell line with the highest expression level (i.e. clone #14) was used for further investigation. Southern blot analysis revealed that the transgene was properly integrated into the genome and that the loxP-flanked GFP gene was excised by Cre recombinase (Fig. 1C). After excision of the GFP stuffer gene by Cre recombinase, ActRIBCA expression from the transgene was confirmed by β-galactosidase staining (Fig. 1D). The cell line of clone #14 was used to generate ActRIBCAβTg mice as described in Section 2.

To confirm specific expression of ActRIBCA genes in ActRIBCAβTg mice, genomic DNAs were analyzed by PCR. As shown in Fig. 1E, the GFP stuffer cassette gene was completely deleted in the islets of ActRIBCAβTg mice, but was detected in other tissues. Note that the HA-tagged ActRIBCA gene was detected in the hypothalamus of ActRIBCAβTg mice (Fig. 1E). The expression of ActRIBCA in islets was further investigated using immunohistochemical techniques. As shown in Fig. 1F (upper panels), immunostaining of the pancreas with anti-HA antibody revealed that HA-tagged ActRIBCA was predominantly expressed on the membranes of pancreatic β cells. The anti-Cre antibody produced nuclear staining in ActRIBCAβTg pancreatic β cells, but no staining in control mice (Fig. 1F, lower panels). Western blot analysis of Smad2 phosphorylation revealed that Smad2 was substantially activated in ActRIBCAβTg pancreatic β cells (Fig. 1G).

3.2. Analysis of β cell mass and the histological structure of the islets

Histological and immunohistological analyses of pancreatic islets from ActRIBCAβTg and control Rip mice were performed at 12 and 40 weeks of ages. Pancreatic sections were immunostained for insulin, glucagon and PP. A typical islet morphology of a core of insulin-positive cells with a mantle of glucagon- and PP-positive cells was observed in ActRIBCAβTg mice (Fig. 2A), suggesting that cell lineage specification of β cells in the islet seems to be independent of the ActRIB-Smad2 signaling pathway. Morphometric analysis revealed that there was no significant difference in β cell mass between wildtype and mutant mice at both 12 and 40 weeks of ages. The average islet size at 12 weeks was 3570 ± 800 μm^2 in control Rip and 2750 ± 700 μm^2 in ActRIBCAβTg mice. At 40 weeks, the average islet size was 11,300 ± 2600 μm^2 in control Rip and 12,000 ± 3100 μm^2 in ActRIBCAβTg mice. The distribution of islet areas is shown in Fig. 2B as a percentage of the islets measured.

3.3. Impaired glucose-induced insulin secretion

Glucose tolerance tests were performed to analyze the effects of ActRIBCA on the regulation of glucose metabolism by β cells. In mice fed a normal chow diet, a statistical difference in glucose profile between control Rip and ActRIBCAβTg mice was not found at 8 and 22 weeks of age, although ActRIBCAβTg mice showed a tendency toward a higher glucose profile (Fig. 3A and B). When the mice were fed a high-fat chow diet from 8 weeks of age onward, glucose tolerance in ActRIBCAβTg mice became impaired at 12 (Fig. 3C) and 20 (Fig. 3D) weeks of age. At 20 weeks of age, the insulin levels increased as expected in control Rip mice after a glucose challenge, whereas little or no change was observed in ActRIBCAβTg mice (Fig. 3E). In contrast, the glucose and insulin levels after overnight fasting were not significantly different between control Rip and ActRIBCAβTg mice (Fig. 3F, 3G). There was no significant difference in body weight (Fig. 3H). When insulin tolerance tests were performed, no difference in insulin sensitivity was observed between control Rip and ActRIBCAβTg mice (Fig. 3I). These results imply that the observed impairment in glucose tolerance in ActRIBCAβTg mice may be due to an impairment of GSIS.

To characterize further the effects of ActRIBCA on GSIS, islets were isolated from control Rip and ActRIBCAβTg mice and any potential changes in insulin secretion measured. Consistent with the glucose tolerance test, the insulin secretary response to glucose (22.4 mM) was significantly lower in ActRIBCAβTg islets than that in control Rip islets (Fig. 4A). When islets were incubated with 0.5 mM tolbutamide, a selective K_{ATP} channel inhibitor, insulin secretion was also significantly lower in ActRIBCAβTg islets compared to control mice. However, when islets were incubated in the presence of 60 mM KCl, there was no significant difference in insulin secretion between control Rip and ActRIBCAβTg islets. Since an increase in the ATP/ADP ratio is known to be a trigger for the closure of K_{ATP} channels, the ratio of ATP/ADP was measured in isolated islets. It was found that the ratio of ATP/ADP in ActRIBCAβTg islets was comparable to that in control Rip islets at both 2.8 and 16.7 mM glucose (Fig. 4B). Real-time PCR analysis of the expression of components of K_{ATP} channel subunits, namely Kir6.2 and SUR1,

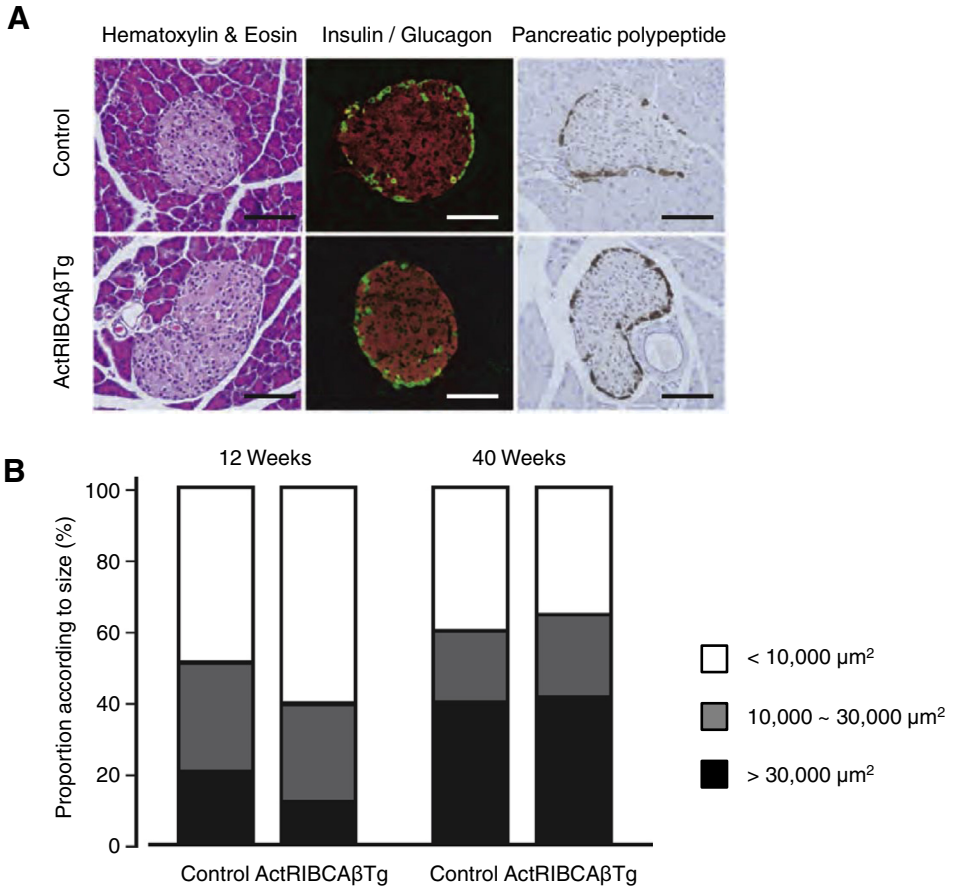

Fig. 2. Histological analysis of pancreatic islets in ActRIBCAβTg mice. (A) Hematoxylin and eosin staining, and immunofluorescence staining for insulin (red), glucagon (green) and PP. Scale bars = 100 μm. (B) Morphometric analysis of islet area in pancreases from ActRIBCAβTg and control Rip mice. For controls, n = 198; for the mutants, n = 208. The distribution of islet areas <10,000 (white), 10,000–30,000 (grey) or >30,000 μm^2 (black) is shown as a percentage of the islets measured. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

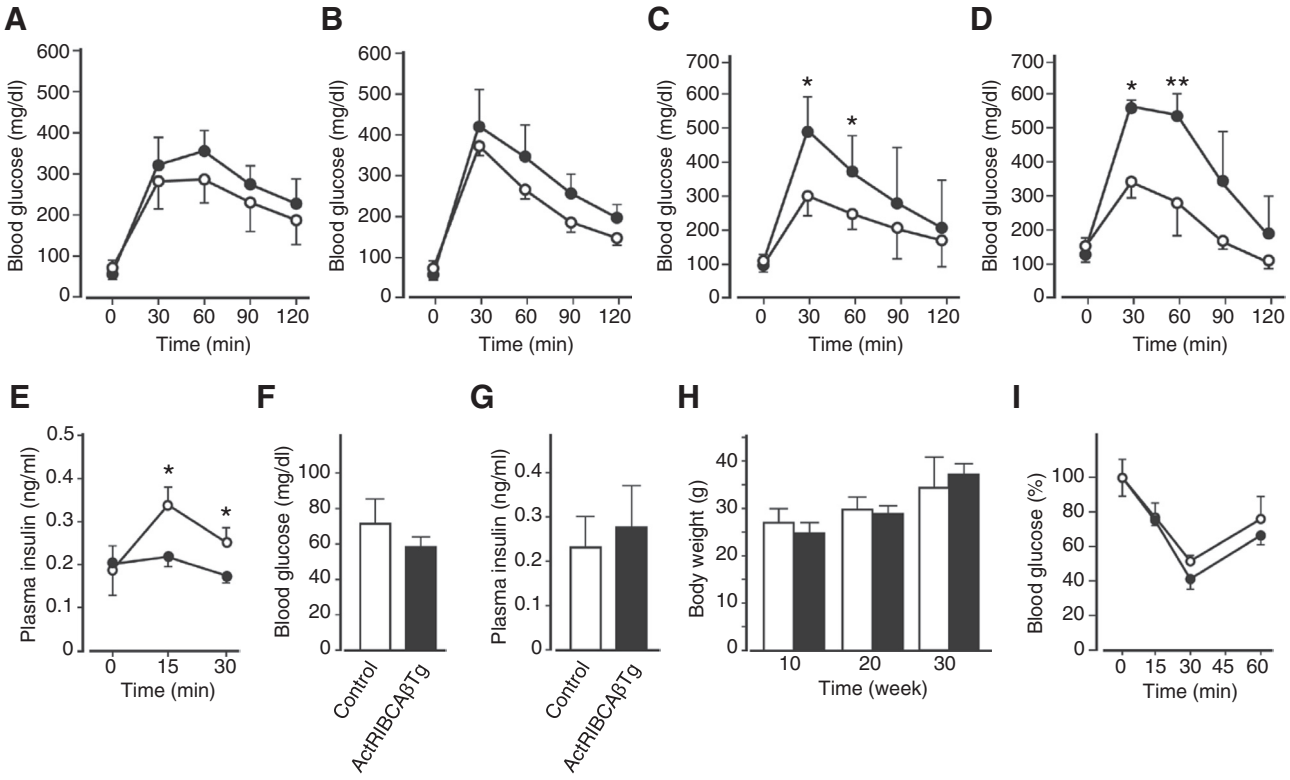

Fig. 3. Impaired glucose tolerance in ActRIBCAβTg mice. (A, B) Intraperitoneal glucose tolerance tests in control Rip (white circles) and ActRIBCAβTg male mice (black circles) with normal diets, carried out at 8 (A) and 22 (B) weeks of age. (C, D) Intraperitoneal glucose tolerance tests in control Rip (white circles) and ActRIBCAβTg male mice (black circles) fed a high-fat diet from 4 weeks of age onward ($n = 10$ per group), carried out at 12 (C) and 20 (D) weeks of age. (E) Serum insulin levels in the mice after glucose injection in control Rip (white circles) and ActRIBCAβTg male mice (black circles) as shown in (D). (F) Blood glucose and insulin levels in control Rip (white bars, $n = 8$) and ActRIBCAβTg mice (black bars, $n = 8$) at 12–14 weeks of age, measured after overnight fasting. (G) Body weights at 10, 20 and 30 weeks of age ($n = 7$) in control Rip (white bars) and ActRIBCAβTg mice (black bars). (H) Insulin tolerance test carried out at 12–14 weeks of age. Insulin (0.75 U/kg) was injected intraperitoneally and the glucose levels were measured at the times indicated in control Rip (white circles) and ActRIBCAβTg male mice (black circles). Data are expressed as the mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$.

revealed no difference between control Rip and ActRIBCAβTg islets (Fig. 4C). These results indicate that impaired GSIS in ActRIBCAβTg mice may be attributable to dysfunction of K_{ATP} channels.

3.4. ActRIB signaling is crucial for the activity of K_{ATP} channels in pancreatic β cells

Next, single-channel recordings were performed using patch-clamp techniques. In the presence of 250 μM diazoxide, K_{ATP} channel openings were detected in isolated pancreatic β cells from control Rip mice, when the cell-attached configuration was used at the holding membrane potential of −70 mV (Fig. 4D). When the membrane patches were excised into ATP-free solution (i.e. from a cell-attached mode to an inside-out mode), K_{ATP} channel activity was initially stimulated. However, channel activity then gradually decreased and disappeared (i.e. a run-down phenomenon). Similar results were obtained in a total of 5 patches. In contrast, K_{ATP} channel openings were not observed in isolated pancreatic β cells from ActRIBCAβTg mice (cell-attached mode), even in the presence of 250 μM diazoxide (Fig. 4E). When the membrane patches were excised, an increase in the activity of K_{ATP} channels was not elicited. Similar results were obtained in a total of 5 patches.

4. Discussion

In the present study, we have investigated the roles of activin signaling in the differentiation and function of pancreatic β cells. Since ActRIB is known to specify the activin signaling pathway, conditional Tg mice were established in which the constitutively active form of ActRIB (ActRIBCA) could be transcribed upon Cre-mediated excision of the stuffer cassette. Expression of ActRIBCA protein in pancreatic β cells was successfully achieved by crossing the mice with Rip-Cre mice (ActRIBCAβTg). Genomic PCR analysis in various tissues revealed excision of the stuffer cassette in the hypothalamus as well as in pancreatic islets [8,15]. Mice lacking Smad2 in both pancreatic β cells and the hypothalamus show late-onset obesity due to an increase in food intake [8]. In contrast, the body weight of ActRIBCAβTg mice was comparable to that of control mice. In addition, the plasma leptin level and food intake were not significantly different between mutant and control mice (data not shown). These results imply that an overexpression of the ActRIBCA in the hypothalamus is unlikely to have an impact on the regulation of food intake.

It has been reported that activin signaling plays important roles in the development of pancreatic islets [6,16]. Recently, we have reported that tissue-specific disruption of Smad2 in pancreatic β cells leads to islet hyperplasia and a defect in GSIS [8]. On the other hand, overexpression of either a dominantly negative or constitutively active form of ActRIB, with multiple copies of the transgenes driven by the human insulin gene promoter, resulted in a lower survival rates, smaller islet area and lower insulin content in the whole pancreas, with impaired glucose tolerance [17]. These observations suggest that a precisely regulated level of activin signaling is necessary for normal islet development, and that activin plays a role in the postnatal functional maturation of islet β cells. However, the role of activin signaling in the adult stage, with normal islet architecture and morphology, has remained largely unknown. In the present study, the Tg mutant mice showed normal survival, fertility and pancreatic islet morphology, suggesting that

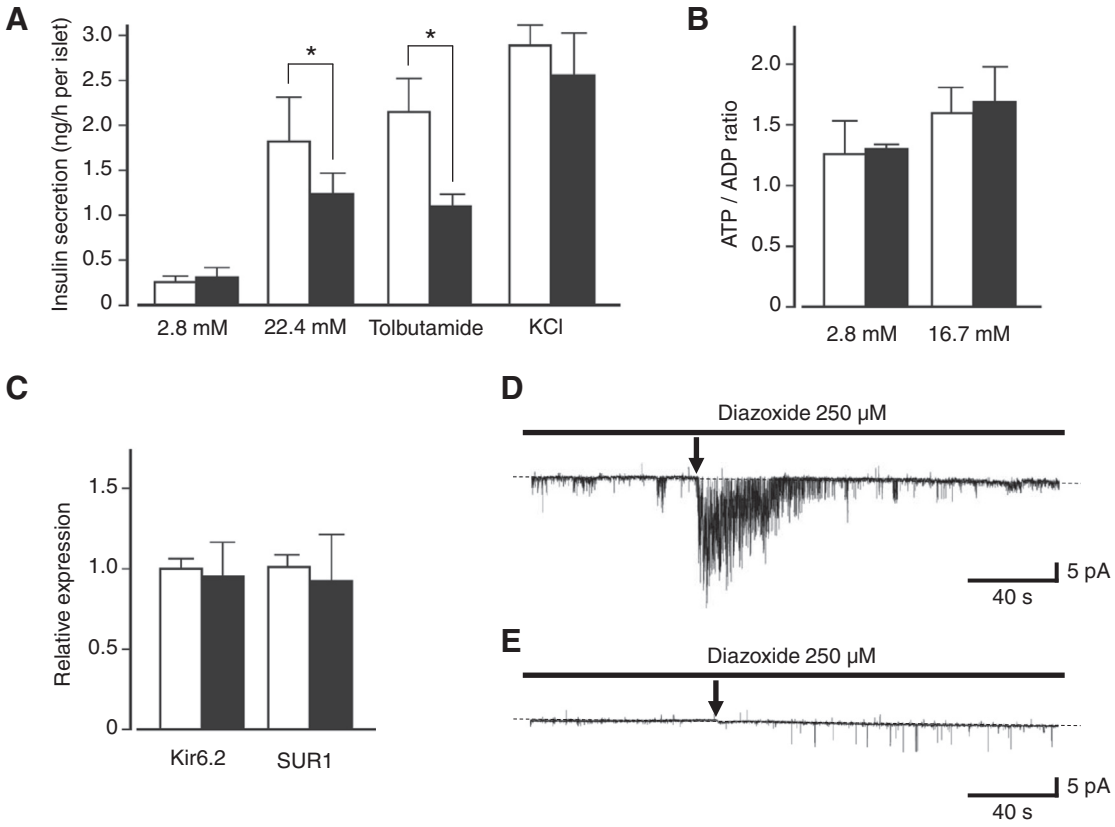

Fig. 4. Defective glucose-stimulated insulin secretion in islets from ActRIBCAβTg mice. (A) GSIS in islets isolated from control Rip (white bars) and ActRIBCAβTg (black bars) mice. Fifteen islets were used for the experiment in each well. $^{*}P < 0.05$. (B) ATP/ADP ratios in islets from control Rip (white bars) and ActRIBCAβTg (black bars) mice were measured under 2.8 mM and 16.7 mM glucose conditions. (C) Quantification of Kir6.2 and SUR1 mRNAs by real-time PCR in islets from control Rip (white bars) and ActRIBCAβTg (black bars) mice. (D) Diazoxide (250 μM)-induced K_{ATP} channel activity in control Rip pancreatic β cells. When an inside-out patch was established from the cell-attached configuration (excision of the patch membrane: the black arrow), K_{ATP} channels showed a rapid run-down even in the presence of diazoxide. The membrane potential was held at −70 mV. (E) In ActRIBCAβTg pancreatic β cells, K_{ATP} channel activity was not elicited by 250 μM diazoxide. When an inside-out patch was established from the cell-attached configuration (excision of the patch membrane: the black arrow), K_{ATP} channels showed no activity in the presence of diazoxide.

there may be a threshold of activin signaling for the determination of β cells mass. Importantly, ActRIBCAβTg mice may allow us to analyze the roles of activin signaling after maturation of the islets.

Although the morphometric analysis of the islets revealed no significant difference between control Rip and ActRIBCAβTg mice, there was a severe defect in GSIS in isolated islets from ActRIBCAβTg mice. Using electrophoretic analysis, the roles of activin signaling on GSIS in pancreatic β cells were clarified. It is well known that K_{ATP} channels play important roles in the regulation of insulin secretion, coupling glucose metabolism to the electrical activity of pancreatic β cells. K_{ATP} channels are heteromeric, consisting of at least two subunit proteins: (1) Kir6.x, which forms the K^+ conducting pore of an inwardly-rectifying K^+ channel; and (2) a modulatory sulphonylurea receptor (SUR), a member of the ATP-binding cassette (ABC) protein super-family [18,19]. It is also generally accepted that molecular combination of Kir6.2/SUR1 represents the predominant isoform present in pancreatic β cells [18,19]. In the present experiments, single-channel recordings revealed a significant difference between control Rip and ActRIBCAβTg mice in terms of the activity of pancreatic β cell K_{ATP} channels. The simplest explanation is that the K_{ATP} channel density may be reduced in pancreatic β cells of ActRIBCAβTg mice in comparison to those of control mice. However, our RT-PCR study indicated that the expressions of both Kir6.2 and SUR1 proteins were not significantly different between control Rip and ActRIBCAβTg mice at mRNA levels. These results suggest that in pancreatic β cells of ActRIBCAβTg mice, the number of functional K_{ATP} channels, but not the expression of K_{ATP} channel proteins, is reduced due to modulation of the channel kinetics, and that this modulation of K_{ATP} channel activity arises from activation of signaling through ActRIB, most likely utilizing Smad2 (such as through dephosphorylation of channel subunits). Future studies will cast further light on the relationship between Smad2 and K_{ATP} channel activity in pancreatic β cells.

In conclusion, the present findings indicate that constitutive activation of signaling by ActRIB leads to an alteration of K_{ATP} channel activity and thus insulin secretion. Precise regulation of the level of the activin signaling is mandatory for normal K_{ATP} channel activity and therefore GSIS.

Acknowledgments

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