Insulin secretory deficiency and glucose intolerance in Rab3A null mice

Received for publication, November 6, 2002, and in revised form, December 20, 2002
Published, JBC Papers in Press, January 1, 2003, DOI 10.1074/jbc.M211352200

Kazuro Yaekura‡, Richard Julyan‡, Barton L. Wicksteed‡, Lori B. Hays‡, Cristina Alarcon‡, Scott Sommers‡, Vincent Poitout‡§, Denis G. Baskin§, Yong Wang¶, Louis H. Philipson¶ and Christopher J. Rhodes†‡**

From the ‡Pacific Northwest Research Institute and Departments of Pharmacology and §Medicine, University of Washington, Seattle, Washington 98122 and the ¶Department of Medicine, University of Chicago, Chicago, Illinois 60637

Insulin secretory dysfunction of the pancreatic β-cell in type-2 diabetes is thought to be due to defective nutrient sensing and/or deficiencies in the mechanism of insulin exocytosis. Previous studies have indicated that the GTP-binding protein, Rab3A, plays a mechanistic role in insulin exocytosis. Here, we report that Rab3A−/− mice develop fasting hyperglycemia and upon a glucose challenge show significant glucose intolerance coupled to ablated first-phase insulin release and consequential insufficient insulin secretion in vivo, without insulin resistance. The in vivo insulin secretory response to arginine was similar in Rab3A−/− mice as Rab3A+/+ control animals, indicating a phenotype reminiscent of insulin secretory dysfunction found in type-2 diabetes. However, when a second arginine dose was given 10 min after, there was a negligible insulin secretory response, consistent with that in Rab3A−/− animals, that was markedly increased above that of the first arginine stimulus. There was no difference in β-cell mass or insulin production between Rab3A−/− and Rab3A+/+ mice. However, in isolated islets, secretagogue-induced insulin release (by glucose, GLP-1, glyburide, or fatty acid) was ~60–70% lower in Rab3A−/− islets compared with Rab3A+/+ controls. Nonetheless, there was a similar rate of glucose oxidation and glucose-induced rise in cytosolic [Ca2+]i, flux between Rab3A−/− and Rab3A+/+ islet β-cells, indicating the mechanistic role of Rab3A lies downstream of generating secondary signals that trigger insulin release, at the level of secretory granule transport and/or exocytosis. Thus, Rab3A plays an important in vivo role facilitating the efficiency of insulin exocytosis, most likely at the level of replenishing the ready releasable pool of β-granules. This study indicates, for the first time, that the in vivo insulin secretory dysfunction found in type-2 diabetes can lie solely at the level of defective insulin exocytosis.

Insulin is the major anabolic hormone controlling metabolic homeostasis, and without an effective supply of insulin, diabetes mellitus ensues. Type-1 diabetes occurs as a result of auto-immune destruction of pancreatic β-cells that produce insulin, and type-2 diabetes develops as a result of insulin secretory dysfunction, as well as insufficient β-cell mass, that no longer compensates for peripheral insulin resistance (1, 2). The insulin secretory dysfunction in type-2 diabetes is derived from β-cell secretory abnormalities, proposed to be either at the level of abnormal glucose metabolism required for generating secondary signals necessary to trigger insulin exocytosis and/or deficiencies in the exocytotic mechanism itself (1, 3). Insulin secretion from the β-cell is highly regulated and only occurs in response to certain nutrients, hormones, neurotransmitters, and pharmacological reagents (4). Of these, glucose is the most physiologically relevant. The secondary signals that emanate from increased glucose metabolism to stimulate insulin release in β-cells have been relatively well defined and of these, a rise in cytosolic [Ca2+]i, is a prerequisite (4, 5). Indeed, increased [Ca2+]i, is the necessary signal to trigger regulated exocytosis in most neuroendocrine cells (6). In comparison, the mechanism of insulin exocytosis is less well defined. Several proteins required for insulin exocytosis in β-cells have been indicated, including SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) proteins, analogous to the mechanism of regulated exocytosis in neurons (5). However, how such “exocytotic proteins” interact in a regulated manner to control insulin exocytosis is currently unclear (5).

One protein implicated to play a role in control of insulin exocytosis is the small GTP-binding protein, Rab3A (7). In β-cells, Rab3A is located on the cytosolic face of β-granules where it is probably involved in control of β-granule transport and/or exocytosis (8, 9). Members of the Rab protein family are key to directing vesicular transport in eukaryotic cells (10). In Rab3A−/− mice, there is a defect in recruiting synaptic vesicles for exocytosis in hippocampal neurons (11), and it has been indicated that Rab3A plays a role in the later stages of synaptic vesicle exocytosis controlling the efficiency of neurotransmitter release (12). However, although there are similarities, the mechanism of synaptic vesicle exocytosis is distinct from that for large dense-core granules (13). Nonetheless, given the proposed key role that Rab3A plays in controlling insulin exocytosis, we examined whether there was a deficient insulin secretory phenotype of Rab3A−/− mice that would not only better characterize the mechanism of exocytosis in β-cells, but also reveal novel insight into insulin secretory dysfunction found in type-2 diabetes.

EXPERIMENTAL PROCEDURES

Materials—The EasyTag™ Expre35S35S protein labeling mix from PerkinElmer Life Sciences, containing 73% of L-[35S]methionine, was used for islet protein synthesis radiolabeling. Uridine 5′-[3H]triphosphate (3000 Ci/mmol) was purchased from Amersham Biosciences. [3-3H](U-14C)glucose (250–360 mCi/mmol) was purchased from PerkinElmer Life Sciences. GLP-11–30, was purchased from Bachem Inc.
Confirmation of Rab3A gene knockout in Rab3A"−/−" mouse islets and brain. Equivalent total protein (25 μg) containing lysates of isolated islets and brain from Rab3A"−/−" and Rab3A"+/−" mice were analyzed for Rab3A, and VAMP-2 as a control, protein expression by immunoblot analysis as described previously (15).

Animals—The Rab3A"−/−" on a B6 background (B6129SF2/J) and Rab3A"−/−" mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were housed on a 12-h light/dark cycle and were allowed free access to standard mouse food and water. Mice were used at 12–20 weeks of age.

Glucose, Arginine, and Insulin Tolerance Tests—Glucose (1 mg/g), arginine (1 mg/g), and insulin (0.75 milliunits/g) tolerance tests were performed on 15–17-week-old Rab3A"−/−" and Rab3A"+/−" mice after an overnight fast by intraperitoneal injection dose relative to body weight as described (14). Blood samples were obtained from the tail vein at the times indicated after the glucose injection. Blood glucose concentrations were measured with a HemoCue blood glucose analyzer (HemoCue AB, Angelholm, Sweden), and plasma insulin levels measured by enzyme-linked immunosorbent assay (Crystal Chem, Chicago, IL).

Islet Isolation and In Vitro Insulin Secretion Analysis—Pancreatic mouse islets were isolated by collagenase digestion, and insulin secretory activity examined in static or perfusion incubation studies of isolated islets as described previously (15), in response to various concentrations glucose, 1 mM GLP-1, 5 μM glyburide, or 125 μM oleate complexed to 1% (w/v) BSA.1

Histological Analyses—Pancreata from 16-week Rab3A"−/−" and Rab3A"−/−" mice were removed and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, rinsed in ethanol, and embedded in paraffin. Serial sections (10 μm) were stained with hematoxylin and eosin or immunostained with either anti-insulin or a combination of anti-glucagon/ anti-somatostatin antibodies for visualization of islet β-cells and α/β-cells using a Leica confocal microscope as described previously (16). For each section the number and cross-sectional islet area, and the number and size of β-cells and α/β-cells per islet were assessed (2).

Measurement of Glucose Oxidation—Glucose oxidation assays were performed as described previously (17). Briefly, groups of 40–50 islets were suspended in 200 μL of Krebs-Ringer bicarbonate buffer, 16 mM HEPES, and 0.1% (w/v) BSA containing 2.8 mM glucose or 16.7 mM glucose in rounded bottom cups sealed with a rubber-sleeved stopper. Then, −2 × 10^7 cpm of [U-14C]glucose in samples containing glucose as a substrate was added to the islet suspensions. The cups were then sealed within a 20-ml borosilicate glass scintillation vial using a rubber-sleeved stopper and incubated for 2 h at 37 °C. Then islet-cell oxidation was halted by the addition of 20 μL of 100 mM sodium phosphate buffer (pH 6.0) containing 100 μM rotenone to the islets in the cups via the sleeves stoppers. Afterward, 100 μL of HClO4 was added to the islets in the cup, and 300 μL of 1 mM benzethonium was added to the bottom of the scintillation vials via the appropriate sleeved stoppers. The samples then were incubated for an additional 2 h at 37 °C in a shaking water bath. Then the seals were removed, the cups were discarded, and 10 μl of scintillation mixture was added to the vials. The vials then were kept at 25 °C overnight before scintillation counting.

Proinsulin Biosynthesis and Preproinsulin mRNA Analysis—Freshly isolated islets from 16-week Rab3A"−/−" and Rab3A"+/−" mice were incubated for 1 h at 37 °C in 200 μL of Krebs-Ringer buffer (pH 7.4) containing a basal 2.8 mM or stimulatory 16.7 mM glucose and 0.1% (w/v) BSA. Messenger RNA levels were analyzed by the RNase protection assay, as described previously (18). Immunoprecipitation analysis of proinsulin biosynthesis in isolated islets pulse-radiolabeled with [35S]methionine was as described previously (18).

Standard Wide-field Epifluorescence Imaging—Dual-wavelength excitation microspectrophotometry was used to measure [Ca2+]i, as described previously (19). Isolated islets from 18-week Rab3A"−/−" and Rab3A"−/−" mice were loaded with Fura-2 by a 25-min incubation at 37 °C in Krebs-Ringer buffer containing basal 2.8 glucose and 5 μM Fura-2/AM (Molecular Probes Inc., Eugene, OR) and then placed into a temperature-controlled perfusion chamber (Medical Systems Inc.)

---

1 The abbreviations used are: BSA, bovine serum albumin; IPGTT, intraperitoneal glucose tolerance test(s); IPITT, intraperitoneal insulin tolerance test(s); GLP-1, glucagon-like peptide-1.
mounted on an inverted epifluorescence microscope (Diaphot, Nikon, Inc.) and perfused by a continuous flow (rate: 2.5 ml/min) of 5% CO₂-bubbled Krebs-Ringer buffer at 37 °C. Groups of islets were visualized with a ×20 quartz objective. Fura-2 dual wavelength excitation at 340 and 380 nm, and detection of single wavelength emission at 510 nm was accomplished using the Metafluor/Metamorph system (Universal Imaging Corp.); images were collected with an intensified CCD camera.

Statistical Analysis—Where appropriate, results are expressed as a mean ± S.E. Statistical analysis was performed by unpaired Student’s t test or repeated measure analysis of variance, where p < 0.05 was considered significant.

**RESULTS**

Pancreatic β-cells are located in the islets of Langerhans of the endocrine pancreas. By immunoblot analysis it was first confirmed that Rab3A protein was indeed absent in isolated Rab3A−/− mice versus Rab3A+/+ control mice compared with other β-granule proteins involved in insulin exocytosis, such as VAMP-2 (Fig. 1). At >3 months old, a glucose-intol erant phenotype emerged in Rab3A−/− mice. Male Rab3A−/− mice at 4 months old exhibited significant (p < 0.05) fasting hyperglycemia (127.4 ± 3.0 mg/dl; n = 25) compared with male Rab3A+/+ animals (102.2 ± 4.1 mg/dl; n = 15). Fasting blood glucose levels did not significantly differ in female Rab3A−/− mice compared with Rab3A+/+ controls (108.0 ± 4.2 mg/dl; n = 22 versus 102.9 ± 3.2 mg/dl; n = 11, respectively). It is not uncommon for a diabetic glucose-intolerant phenotype to be much more severe in male versus female transgenic mouse models of this age (20). Intraperitoneal glucose tolerance tests (IPGTT) indicated that male Rab3A−/− mice were significantly glucose-intolerant showing an increased excursion in blood glucose in response to a glucose load compared with control Rab3A+/+ animals (Fig. 2A). Fasting insulin levels in male Rab3A−/− mice were lower (349 ± 36 pg/ml; n = 16) but not statistically significantly different from male Rab3A+/+ mice (428 ± 56 pg/ml; n = 12). However, in response to a glucose challenge of an IPGTT, Rab3A−/− mice showed a severe decrease in first phase plasma insulin secretory response accompanied by a marked ~75% decrease in plasma insulin levels compared with Rab3A+/+ mice (Fig. 2B). An intraperitoneal insulin tolerance tests (IPITT) indicated that insulin sensitivity was unaltered in either male Rab3A−/− mice (Fig. 2C), indicative of minimal insulin resistance in these animals.

In contrast to the IPGTT, an intraperitoneal arginine stimulus (1 mg/g) showed no apparent deficiency in the in vivo insulin secretory response in Rab3A−/− versus Rab3A+/+ control mice (Fig. 3A). However, when a second intraperitoneal dose (1 mg/g) was given 10 min after the first, the subsequent insulin secretory response to arginine was negated (p < 0.05) in Rab3A−/− mice compared with an enhanced response in Rab3A+/+ control animals (Fig. 3B). Blood glucose levels in Rab3A−/− or Rab3A+/+ mice did not appreciably alter during the in vivo arginine stimulus (Fig. 3C).

The insulin secretory-deficient phenotype of the Rab3A−/− mice did not appear to be based at the level of defective (pro)insulin production. Isolated pancreatic islets from Rab3A−/− mice had similar insulin content stores to that of Rab3A+/+ mouse islets (Fig. 4A). Likewise, preproinsulin mRNA levels were equivalent in islets from Rab3A−/− versus Rab3A+/+ mice (Fig. 4B), and translational control of glucose-induced prosu lin biosynthesis was unaffected in isolated Rab3A−/− mouse islets (Fig. 4C). Immunohistochemical examination of pancreata from Rab3A−/− and Rab3A+/+ mice indicated no discernable difference in islet architecture, with insulin-expressing β-cells in the central core of an islet and glucagon expressing α-cells and somatostatin expressing δ-cells around the periphery (Fig. 4). Further analysis of pancreatic serial sections showed no obvious change between Rab3A−/− and Rab3A+/+ mice in islet number per pancreas, islet size, number of β-cells, and non-β-cells per islet or size of β-cells and non-β-cells per islet (data not shown). The weight and size of pancreata from Rab3A−/− and Rab3A+/+ mice were equivalent, and as such it is reasonable to deduce that there was no change in pancreatic β-cell mass between Rab3A−/− and Rab3A+/+ mice. It follows...
Experimental Procedures. Results are shown as a mean ± S.E. of three experiments performed in duplicate.

An increase in β-cell glucose metabolism is required to generate secondary signals for glucose-induced insulin secretion (4, 21) and proinsulin biosynthesis (17, 21). We compared the rate of glucose oxidation in isolated islets from Rab3A−/− and Rab3A+/+ mice and found that there was no significant difference in glucose oxidation at either a basal 2.8 mM or stimulatory 16.7 mM glucose concentration (Fig. 5). As such, the insulin secretory deficiency in Rab3A−/− mouse β-cells does not lie at the level of defective glucose metabolism.

Downstream of increased glucose metabolism, a rise in cytosolic [Ca2+]i in pancreatic β-cells is essential to trigger glucose-induced insulin secretion (4, 5, 7). We examined whether the insulin secretory-deficient phenotype of Rab3A−/− mice was due to a defect in generating secondary signals required to trigger insulin exocytosis by measuring glucose-induced increases in β-cell [Ca2+]i. Changes in cytosolic [Ca2+]i, was monitored by Fura-2 fluorescence imaging as described previously (19). Increasing the glucose concentration from a basal 2 mM glucose to a stimulatory 14 mM glucose induced a significant rise in cytosolic [Ca2+]i, after a short lag period, that was equivalent in isolated islet β-cells from Rab3A−/− and Rab3A+/+ mice (Fig. 6). However, there was a subtle difference in subsequent glucose-induced oscillations in [Ca2+]i. Although the amplitude of the [Ca2+]i, oscillations did not appreciably change the frequency of [Ca2+]i, oscillations were slower in Rab3A−/− compared with Rab3A+/+ islet β-cells (Fig. 6, A versus B). Nonetheless, despite this disparity in glucose-induced [Ca2+]i, oscillations the total increase in [Ca2+]i, was only 4% lower in Rab3A−/− islet β-cells. This was reaffirmed in that depolarization of islet β-cells with 30 mM KCl at 2 mM glucose

![Image](http://www.jbc.org/)

Fig. 4. Insulin production is unaffected in Rab3A−/− mice. A, total insulin content was measured in isolated Rab3A−/− (open bar) and Rab3A+/+ (closed bar) mouse islets by enzyme-linked immunosorbent assay (n ≥ 18). B, proinsulin mRNA and, as a control, glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA levels were measured by nuclease protection assay in isolated islets from Rab3A−/− and Rab3A+/+ mouse islets incubated for 1 h at basal 2.8 mM or stimulatory 16.7 mM glucose as described under “Experimental Procedures.” A representative autoradiograph analysis is shown. C, proinsulin biosynthesis was analyzed by immunoprecipitation of [35S]proinsulin from lysates of isolated islets from Rab3A−/− and Rab3A+/+ mouse islets incubated for 1 h at basal 2.8 mM or stimulatory 16.7 mM glucose, the last 20 min of which was in the presence of [35S]methionine as described under “Experimental Procedures.” A representative autoradiograph analysis is shown. D, analysis of 10-μm serial sections of pancreata from Rab3A−/− and Rab3A+/+ mice, stained with hematoxyline and eosin (H & E; left panels) or immunofluorescence for glucagons/somatostatin (center panels) and insulin (right panels). A representative image of an islet in pancreatic serial sections is shown.

Fig. 5. Glucose oxidation is unaffected in Rab3A−/− islet β-cells. Isolated islets from Rab3A+/+ and Rab3A−/− mice were analyzed for glucose oxidation rates at a basal 2.8 mM (open bars) or stimulatory 16.7 mM glucose (hatched bars) induced as described under "Experimental Procedures." Results are shown as a mean ± S.E. of four experiments performed in duplicate.

that the insulin secretory-deficient phenotype of Rab3A−/− mice does not reside at the level of insufficient insulin production.
Insulin Deficiency and Glucose Intolerance in Rab3A Null Mice

Rab3A−/− mice are glucose-intolerant and exhibit loss of first-phase insulin secretory response to glucose, but the insulin secretory response to a single dose of arginine was similar in Rab3A−/− versus Rab3A+/+ control mice, which are typical characteristics of insulin secretory dysfunction in type-2 diabetes (1, 3). This insulin secretory dysfunction in the Rab3A−/− mice did not appear to be driven by peripheral insulin resistance, but rather by a primary defect in the pancreatic β-cell. This was reaffirmed in that stimulated insulin secretion from Rab3A−/− mouse isolated islets in vitro was markedly decreased compared with control Rab3A+/+ islets. However, since Rab3A−/− mice may have impaired secretory function in other neuroendocrine cells (11), it is possible that the β-cell secretory defect could be secondary, particularly when considering hypothalamic or adrenergic cells. However, when isolated islets from Rab3A−/− mice are cultured for 24 or 48 h, that would give time to recover from any in vivo influence of circulating factors, the insulin secretory response to glucose ± glyburide, GLP-1, or oleate remained 60–70% inhibited compared with similarly cultured Rab3A+/+ control islets (data not shown), as found in freshly isolated islets (Fig. 7). Moreover, if the blunted insulin secretory response in β-cells were secondary to increased α-adrenergic activity, one would also expect to see a blunted first phase insulin secretory response to arginine in vivo (22, 23), a decrease in glucose- and K+–induced rise in cytosolic [Ca2+]i (24, 25), as well as glucose-induced proinsulin biosynthesis (26). However, there were no differences found in these parameters between Rab3A−/− and Rab3A+/+ control mouse islets (Figs. 4, 5, and 6). Thus, the most likely scenario is that the insulin secretory dysfunction in the Rab3A−/− mice is due to a primary defect in the β-cell itself.

There was no difference in pancreatic β-cell mass in between Rab3A−/− versus Rab3A+/+ mice and neither was insulin production and intracellular stores of insulin affected in Rab3A−/− mouse islets. As such, the insulin secretory deficiency in Rab3A−/− mice was not due to an insufficient store or supply of insulin. Glucose metabolism is a prerequisite to generate secondary signals for glucose-induced insulin secretion and proinsulin biosynthesis, but rates of glucose oxidation were similar in Rab3A−/− and Rab3A+/+ mouse islets (Fig. 5). This indicated that insulin secretory dysfunction in Rab3A−/− mice was not due to defective β-cell glucose sensing or metabolism. Indeed, the observation that glucose-induced translational control of proinsulin biosynthesis was unaffected in Rab3A−/− indicated that stimulus-response coupling mechanisms were intact in Rab3A−/− β-cells. This was reaffirmed in that glucose- and K+–induced depolarization of Rab3A−/− mouse islets caused an increase in cytosolic [Ca2+]i, similar to that in Rab3A+/+ control β-cells. As such, the defect in insulin secretion in Rab3A−/− mouse β-cells most likely lies downstream of generating secondary signals, at the level of insulin exocytosis. A defective exocytosis mechanism in Rab3A−/− insulin secretory dysfunction would also be consistent with the observation that the insulin secretory response to all secretagogues tested in vitro was reduced by ≈50% in Rab3A−/− islets, despite these secretagogues stimulating insulin secretion via distinct signaling mechanisms (4).

In pancreatic β-cells, Rab3A is mostly located on the cytosolic face of β-granule membranes and has been implicated to play a role in control of insulin exocytosis (8, 9), as it does for Ca2+-dependent exocytosis in other neuroendocrine cell types (10). Here, we find that the absence of Rab3A in β-cells decreases secretagogue-induced insulin secretion in vivo and in vitro, reaffirming an important role of Rab3A in control of insulin exocytosis. Rab GTP-binding proteins specifically direct vesicular transport by an interaction with a particular “effector
Insulin Deficiency and Glucose Intolerance in Rab3A Null Mice

protein," characteristic of an individual Rab protein and neuroendocrine cell type (10). Rab3A has been shown to interact with several candidate effector proteins (10), but the Rab3A-calmodulin interaction appears most pertinent in control of Ca^{2+}-regulated insulin exocytosis (27). Recently, it has been found that the Rab3A-calmodulin interaction on β-granules provides a platform for local activation of the Ca^{2+}/calmodulin-dependent phosphoprotein phosphatase, calcineurin, in response to increased [Ca^{2+}]_i (15, 28). Activation of calcineurin on β-granules then leads to dephosphorylation activation of the ATP-dependent motor, kinesin, and subsequent transport of β-granules to a “readily releasable pool” docked at the β-cell plasma membrane committed to undergo exocytosis (15). Therefore, in the absence of Rab3A, calmodulin cannot be readily sequestered to the β-granule membrane for local activation of calcineurin and kinesin in response to elevated [Ca^{2+}]_i, so that β-granule transport becomes much less efficient, the number of β-granules recruited to a readily releasable pool is reduced, and consequently insulin exocytosis is impaired. A defect in replenishing the readily releasable pool of β-granules in β-cells of Rab3A^-/- mice would be consistent with the observations of a blunted first-phase glucose-induced insulin release in vitro and in vivo, as well as a severely diminished insulin secretory response to a second arginine stimulus where the prior arginine stimulus would have emptied the readily releasable pool of β-granules. Moreover, such a mechanism is also consistent with observations of a decay in synaptic transmissions in hippocampal neurons of Rab3A^-/- mice (27). Recently, it has been tied the readily releasable pool of β-granules, which are normally sustained as found in Rab3A^-/- mice (11).

Our study of the Rab3A^-/- insulin secretory phenotype indicates that the root of β-cell secretory dysfunction in type-2 diabetes does not necessarily lie at the level of glucose-sensing and β-cell metabolism from which secondary signals emanate (e.g. a rise in [Ca^{2+}]_i), but rather at the level of a defective insulin exocytosis mechanism. This would be symptomatic of insulin secretory dysfunction in type-2 diabetes arising from an “overworked” β-cell trying hard, but not quite succeeding, to compensate for peripheral insulin resistance (3). In the face of persistent hyperglycemia in type-2 diabetes, the β-cell is chronically stimulated to produce and secrete much higher amounts of insulin than usual, and as such there is a higher rate of β-granule turnover. This, in turn, compromises β-granule transport that depletes the ready releasable pool of β-granules, so that insulin secretory insufficiency develops leading to β-cell secretory dysfunction (29). Such is the case with Rab3A^-/- mice, best illustrated by a severe reduction in the in vivo insulin secretory response to a second consecutive arginine spike.
stimulus that is contrastingly augmented in normal Rab3A−/− animals. However, there are no reports in the literature of an insulin secretory response to such a double consecutive arginine stimulus test in human type-2 diabetics, but if pursued could well reveal a similar insulin secretory insufficiency found in Rab3A−/− mice that would be supportive of insulin secretory dysfunction in human type-2 diabetes arising from deficiencies in the insulin exocytic mechanism due to β-cell exhaustion (3, 29). In this regard, it should be noted that if the β-cell is induced to rest its secretory activity in type-2 diabetics, normal insulin secretory function can be recovered (30). Notwithstanding, the glucose-intolerant/insulin secretory deficient phenotype of Rab3A−/− mice, in the absence of insulin resistance or changes in β-cell mass, emphasizes the important contribution that insulin secretory dysfunction makes in the pathogenesis of type-2 diabetes and further suggests that protection of β-cell function is a worthy consideration for the treatment of type-2 diabetes (2).

Acknowledgment—We thank Cynthia Jacobs for the preparation of this manuscript.

REFERENCES
1. Leahy, J. L. (1990) Diabetes Care 13, 992–1010
2. Lingohr, M. K., Buettrner, R., and Rhodes, C. J. (2002) Trends Mol. Med. 8, 375–384
3. Kahn, S. E. (2001) J. Clin. Endocrinol. Metab. 86, 4947–4958
4. Prentki, M., Tornheim, K., and Corkey, B. E. (1997) Diabetologia 40, Suppl. 2, S22–S41
5. Eason, R. A. (2000) Semin. Cell Dev. Biol. 11, 253–266
6. Jahn, R., and Su dhof, T. C. (1997) Annu. Rev. Biochem. 68, 863–911
7. Wallheim, C. B., Lang, J., and Regazzi, R. (1996) Diabetes Rev. 4, 276–297
8. Regazzi, R., Ravazzola, M., Lezzi, M., Lang, J. C., Zabraou, A., Anderggen, E., Merel, P., Takai, Y., and Wallheim, C. B. (1996) J. Cell Sci. 109, 2265–2273
9. Iezzi, M., Escher, G., Meda, P., Charoilais, A., Baldini, G., Darchen, F., Wollheim, C. B., and Regazzi, R. (1999) Mol. Endocrinol. 13, 292–212
10. Zerial, M., and McBride, H. (2001) Nat. Rev. 2, 107–117
11. Geppert, M., Bolshakov, V. Y., Siegelbaum, S. A., Takei, K., De Camilli, P., Hammer, R. E., and Sudhof, T. C. (1994) Nature 369, 493–497
12. Geppert, M., Goda, Y., Stevens, C. F., and Rhodes, C. J. (2002) J. Biol. Chem. 277, 24232–24242
13. Ensimne, J. W., Baskin, D. G., Vahl, T. P., Vogel, R. E., Laschansky, E. C., Francis, B. H., Hoffman, R. C., Krakover, J. D., Stamm, M. R., Low, M. J., Rubinstein, M., Otero-Corchon, V., and D’Alessio, D. A. (2003) Endocrinology 143, 2599–2609
14. Skelly, R. H., Wicksteed, B. L., Antinozzi, P. A., and Rhodes, C. J. (2001) Diabetes 50, 1791–1798
15. Wicksteed, B., Herbert, T. P., Alarcon, C., Lingohr, M. L., Mass, L. G., and Rhodes, C. J. (2001) J. Biol. Chem. 276, 22553–22558
16. Roe, M. W., Worley, J. F., 3rd, Qian, F., Tamarina, N., Mittal, A. A., Dralyuk, F., Blair, N. T., Mertz, R. J., Philipson, L. H., and Dukes, I. D. (1998) J. Biol. Chem. 273, 10402–10410
17. Kido, Y., Burks, D. J., Withers, D., Bruning, J. C., Kahn, C. R., White, M. F., Ashcroft, S. J. H. (1980) Acta Physiol. Scand. 119, 199–205
18. Ashcroft, S. J. H. (1980) Diabetesology 18, 5–15
19. Ahren, B., and Taborsky, G. J. (1988) Acta Physiol. Scand. 132, 143–150
20. Ortiz-Alonso, F. J., Herman, W. H., Zobel, D. L., Perry, T. J., Smith, M. J., and Halter, J. B. (1991) Diabetes 40, 1194–1202
21. Drews, G., Debueyser, A., Nenquin, M., and Henquin, J. C. (1990) Endocrinology 126, 1646–1653
22. Wollheim, C. B., Pipeleers, D. G., and Levy, J. (1974) Biochim. Biophys. Acta 362, 121–125
23. Coppel, T., Perret-Menoud, V., Luthi, S., Farnsworth, C. C., Glomset, J. A., and Regazzi, R. (1999) EMBD J. 18, 5885–5891
24. Kajo, H., Olszewski, S., Rosner, P., Debuyser, A., Otero-Corchon, V., and Wollheim, C. B., and Regazzi, R. (1999) Mol. Endocrinol. 13, 292–212
25. Geppert, M., Bolshakov, V. Y., Siegelbaum, S. A., Takei, K., De Camilli, P., Hammer, R. E., and Sudhof, T. C. (1994) Nature 369, 493–497
26. Eason, R. A. (2000) Semin. Cell Dev. Biol. 11, 253–266
27. Prentki, M., Tornheim, K., and Corkey, B. E. (1997) Diabetologia 40, Suppl. 2, S22–S41
28. Regazzi, R., Ravazzola, M., Lezzi, M., Lang, J. C., Zabraou, A., Anderggen, E., Merel, P., Takai, Y., and Wallheim, C. B. (1996) J. Cell Sci. 109, 2265–2273
29. Rorsman, P., Eliasson, L., Renstrom, E., Gromada, J., Barg, S., and Gopel, S. (1999) EMBO J. 18, 5885–5891
30. Laedtke, T., Kjems, L., Parksen, N., Schmitz, O., Veldhuis, J., Kao, P. C., and Butler, P. C. (2000) Am. J. Physiol. Endocrinol. Metab. 279, E520–E528
