β-Cell-targeted Overexpression of Phosphodiesterase 3B in Mice Causes Impaired Insulin Secretion, Glucose Intolerance, and Deranged Islet Morphology*

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The second messenger cAMP mediates potentiation of glucose-stimulated insulin release. Use of inhibitors of cAMP-hydrolyzing phosphodiesterase (PDE) 3 and overexpression of PDE3B in vitro have demonstrated a regulatory role for this enzyme in insulin secretion. In this work, the physiological significance of PDE3B-mediated degradation of cAMP for the regulation of insulin secretion in vivo and glucose homeostasis was investigated in transgenic mice overexpressing PDE3B in pancreatic β-cells. A 2-fold overexpression of PDE3B protein and activity blunted the insulin response to intravenous glucose, resulting in reduced glucose disposal. The effects were “dose”-dependent because mice overexpressing PDE3B 7-fold failed to increase insulin in response to glucose and hence exhibited pronounced glucose intolerance. Also, the insulin secretory response to intravenous glucagon-like peptide 1 was reduced in vivo. Similarly, islets stimulated in vitro exhibited reduced insulin secretory capacity in response to glucose and glucagon-like peptide 1. Perfusion experiments revealed that the reduction specifically affected the first phase of glucose-stimulated insulin secretion. Furthermore, morphological examinations demonstrated deranged islet cytoarchitecture. In conclusion, these results are consistent with an essential role for PDE3B in cAMP-mediated regulation of insulin release and glucose homeostasis.

A vital part of the signaling network responsible for stimulus-secretion coupling in pancreatic β-cells involves cAMP as second messenger. In particular, several physiological enhancers of glucose-stimulated insulin release, such as pituitary adenylate cyclase-activating polypeptide and glucagon-like peptide (GLP)1, act mainly through increased formation of cAMP (1–3) and, at least in part, its subsequent activation of protein kinase A (PKA). However, the exact mechanisms by which cAMP mediates the potentiating effect on insulin secretion remain to be established. Also, glucose itself has been reported to stimulate cAMP formation in β-cells. The significance, however, of glucose-induced increase in cAMP has been debated (4, 5) and full understanding of its contribution to insulin release has not been reached. Utilization of inhibitors of PKA has been shown not to impair glucose-stimulated release of insulin (6, 7). It has therefore been suggested that cAMP plays a minor role in nutrient-stimulated insulin secretion. Recently, newly described targets for cAMP, e.g. the family of exchange proteins directly activated by cAMP (Epacs), were shown to participate in PKA-independent β-cell signaling and may prove to mediate previously unknown secretion stimulatory effects of cAMP (8–10).

The significance of accurate regulation of cAMP levels is illustrated by the large number of gene products known to generate, target, and degrade the cyclic nucleotide (reviewed in Ref. 11). Degradation of cAMP is achieved through phosphodiesterase (PDE)-catalyzed hydrolysis, which balances the activity of adenyl cyclase, and thereby contributes to the control of appropriate levels of cAMP within the cell. Inhibitors of PDE, some of which are in clinical use, are well known cAMP elevating tools. One effect of PDE inhibition is augmented insulin secretion (12–14).

PDEs comprise a superfamily of enzymes with 11 known members (PDE1–11), each containing multiple isofoms and splice variants (15, 16). The isozymes are structurally related but differ both functionally, e.g. with regard to regulation and substrate specificity, and in tissue distribution. PDE3B, which is one of two enzymes forming the PDE3 family, has been shown to reside particularly in tissues of importance for energy homeostasis (17). A critical role of PDE3B in insulin-induced inhibition of adipose tissue lipolysis is well established. Also, a role in inhibition of hepatic glycogenolysis has been described (17). Besides these tissues, β-cells of the endocrine pancreas express this particular isoform. In β-cells, PDE1 and PDE4 enzymes are expressed in addition to PDE3B (18–20), but the differential roles of these enzymes remain to be clarified. Although all three have been implicated in insulin release, the firmest evidence of an important role has been obtained by manipulating the activity of PDE3B (20, 21); when inhibited, rat islet insulin secretion was shown to be enhanced and when increased by overexpression, insulin secretion was reduced (21).
β-Cell-targeted Overexpression of PDE3B in Mice

To study the physiological role of PDE3B in cAMP-mediated insulin release, we have generated transgenic mice with β-cell-targeted overexpression of the enzyme. Here we show that a modest degree of PDE3B overexpression impairs the insulin response to intravenous loads of glucose and cGMP-1 and that mainly the first phase of insulin secretion is impaired. Furthermore, the islet dysfunction in these mice is associated with glucose intolerance and histological examination reveals deranged islet cytarchitecture similar to the model of type 2 diabetes. The effect appeared to be mo “dose”-dependent, because glucagon hormone secretion was further impaired in a second transgenic line exhibiting a more pronounced overexpression of PDE3B. Our findings highlight the important physiological role of cAMP in the control of insulin secretion.

MATERIALS AND METHODS

Generation of PDE3B Transgenic (RIP-PDE3B) Mice—A transgene construct for β-cell-specific expression was achieved by placing a FLAG epitope-equipped cDNA for mouse PDE3B (described in Ref. 22) under control of the rat insulin promoter (RIP). To this end the pcDNA3/mPDE3B-Flag vector (22) was modified by subcloning a 689-bp fragment of RIP2 (nucleotides 241–933) into the KpnI/BamHI restriction sites upstream of mPDE3B cDNA (sitting in the Xhol site). A 958-bp genomic fragment of the human growth hormone gene (nucleotides 1193–2151) was then subcloned into the XbaI/ApaI restriction sites downstream of PDE3B cDNA in the same vector. After excision of the whole construct (6.3 kb) by HindIII/AvrII cleavage, pronuclear microinjection into C57Bl/6xCBA mouse (performed at the Transgenic Core Facility of Karolinska Institute, Stockholm, Sweden) generating the C57Bl/6xCBA-FLAG-RIP-PDE3B-Lah mice (nomenclature according to Ref 45). Transgenic founder mice were identified by PCR analysis of tail biopsies using a 5′- and 3′-primer one strand cDNA synthesis from 1 μg of total RNA using the Advantage RT-for-PCR kit (Clontech). The mPDE3B-Flag cDNA was amplified using the same primer pair as described above.

Western Blot Analysis—Tissues were homogenized in buffer consisting of 50 mM TRIS-HCl, 250 mM sucrose, 1 mM EDTA, and 0.1 mM dithiotheitol, protease inhibitors: 10 μg/ml leupeptin, 10 μg/ml antipain, and 1 μg/ml pepstatin (Peptide Institute Inc., Osaka, Japan). Homogenates were subjected to SDS-PAGE. Proteins were electrotransferred to polyvinylidene membranes (Millipore) and membranes were blocked with 0.5% (v/w) gelatin in a buffer consisting of 20 mM Tris-HCl, pH 7.6, 157 mM NaCl, and 0.1% (v/v) Tween 20 for 1 h. Membranes were probed with polyclonal PDE3B antibodies (21) for 16 h. Proteins were detected using chemiluminescence (Pierce) and a LAS 1000 Plus system (Fuji, Tokyo, Japan).

PDE Activity Measurements—Tissues were homogenized as above and PDE activity was measured in duplicates as described (23) in the presence of 3 μM OCP3911 (Otsuka Pharmaceutical Co., Tokyo, Japan). To increase the sensitivity for PDE3 in the assay, the isoform-specific PDE4 inhibitor Ro-20-1724 (10 μM) (Biologic Research Labs, Plymouth Meeting, PA) was included when analyzing muscle samples. Assays were performed at 30 °C for 20–50 min.

In Vivo Experiments—Mice, either fed or fasted for 3 h, were anesthetized with an intraperitoneal injection of midazolam (0.15 mg/ml) injection containing 0.2 mg/mouse; Dormicum® (Hoffmann-La Roche, Basel, Switzerland) and a combination of 0.4 mg/mouse flunisolide and 0.02 mg/mouse fentanyl (Hypnorm® Janssen, Beere, Belgium). After induction of anesthesia, a blood sample was taken from the retrobulbar, intraorbital capillary plexus, and then 1 g of t-glucose (British Drug House) was kg body weight was injected rapidly into the tail vein. The volume load was 10 μl/kg body weight. New blood samples were taken after 1, 5, 10, 20, 50, and 75 min. The samples were taken in heparinized tubes and stored on ice. After centrifugation, plasma was separated and stored at −20 °C until analysis. For arginine tests 0.25 g of L-arginine (Sigma) per kg was adminis-
tered intravenously as described above; in experiments assessing GLP-1 effects, glucose (1 g/kg) in combination with either saline or GLP-1 (7–36 amide) (Peninsula Labs, Merseyside, United Kingdom) was used. In insulin tolerance tests, mice, fasted for 24 h, were injected intraperitoneally with insulin (1 unit/kg) and blood samples were collected at 10, 15, 20, 25, 30, 35, 40, 45, and 60 min after the injection.

Immunohistochemistry and Morphometry—Pancreases were dissected, fixed with 4% paraformaldehyde and 0.2% picric acid in 0.1 M PBS, pH 7.2, rinsed thoroughly in Tyrode solution containing 10% sucrose, and frozen on dry ice. Sections (10 μm thickness) were cut and thaw-mounted on slides. Alternatively, specimens were fixed in 4% paraformaldehyde and embedded in paraffin. Sections (6 μm thickness) were mounted on coated slides, deparaffinized, and hydrated before further handling. Antibodies were diluted in PBS, pH 7.2, containing 0.25% bovine serum albumin and 0.25% Triton X-100. Sections were incubated overnight at 4 °C with the following primary antibodies: insulin, dilution 1:2560 (code 9003, Euro Diagnostica, Malmö, Sweden); glucagon, dilution 1:5120 (code 5708, Euro Diagnostica); GLUT2, dilution 1:6400 (code AB 1342, Chemicon, Temecula, CA). After rinsing in PBS with Triton X-100, secondary antibodies with specificity for rabbit- or guinea pig-IgG, and coupled to fluorescein isothiocyanate (DAKO, Copenhagen, Denmark), were applied on the sections and incubated for 1 h at room temperature. Sections were again rinsed in Triton X-100-enriched PBS and then mounted in PBS-glycerol, 1:1. Sections from paraffin-embedded specimens were immersed in 3% BSA, to block endogenous peroxidase activity and incubated with primary antibodies as above. Unlabeled secondary antibody (dilution 1:160) was then applied to the sections and incubated for 30 min, followed by incubation with peroxidase anti-peroxidase complex, dilution 1:100 (Dako) for 30 min. Peroxidase was visualized with diaminobenzidine tetrahydrochloride. The specificity of immunostaining was tested using primary antisera preadsorbed with homologous antigen (100 μg of peptide/ml of antiserum at working dilution), or by omission of primary antibodies. Immunofluorescence was examined in epifluorescence microscope (Olympus, BX60). Diaminobenzidine tetrahydrochloride was examined in bright field. Images were captured with a digital camera (Olympus).

To measure islet size, islets were analyzed using Image Pro Plus. The pancreatic specimens (n = 5 in each group) were analyzed at three levels with a distance of 800 μm between each level. Three sections with 100 μm distance were taken at each level. Images of all islets immunostained for insulin within each section were captured and the islet areas were measured. Image Pro Plus was used for calculation of the mean islet area.

Insulin Secretion and Insulin Content—Pancreatic islets were isolated using a collagenase digestion technique (21) modified after Lacy and Kostianovsky (24). After preincubation (1 h) in Krebs-Ringer bicarbonate buffer (KRB: 120 mM NaCl, 5 mM NaHCO3, 5 mM KCl, 1.2 mM KH2PO4, 2.5 mM CaCl2, 1.2 mM MgSO4, 0.2% bovine serum albumin, 10 mM Hepes, pH 7.2–7.4) at 37 °C in 5% CO2 the islets were distributed to 96-well plates and incubated in 200 μl of KRB containing glucose at the indicated concentrations, with or without addition of 100 mM GLP-1 or 10 μM OCP3911, for 1 h at 37 °C. Buffer was then withdrawn for determination of the amount of insulin secreted.

Insulin content of isolated islets was determined by acid-ethanol extraction. Groups of 3 islets were homogenized in 100 μl of 0.5% cold ethanol (0.18% HCl in 95% ethanol) and frozen on dry ice. This step was repeated once, and after centrifugation supernatants were stored at −20 °C until analysis.

Perfusion Experiments—The kinetics of insulin release was studied in vitro using the perfusion technique as described by Skoglund et al. (25). Islets were perfused at a rate of 0.9 ml/min using UB buffer containing 3.3 mM glucose. After 10 min the buffer was switched to UB buffer containing 16.7 mM glucose, and after 30 min to buffer containing 16.7 mM glucose and 35 mM KCl (the concentration of NaCl was changed from 125 to 96 mM). Samples were collected every minute and analyzed for insulin using enzyme-linked immunomassay (Mercodia, Uppsala, Sweden). Insulin and Glucose Measurements—Glucose concentrations were determined by radioimmunoassays (if not otherwise stated) using reagents from Linco Research Inc. (St. Charles, MI). Glucose concentra-
tions were determined by either the glucose (Trinder) kit from Sigma or glucose (oxidase) reagent from Thermo Trace (Melbourne, Australia).
**RESULTS**

**PDE3B Expression in RIP-PDE3B Mice**—A RIP2-controlled cDNA construct was used to generate mice with a β-cell-specific overexpression of PDE3B. By PCR analysis, 11 transgene-positive founder mice were identified and subsequently analyzed for islet PDE3B expression. Western blot analysis revealed variable degrees of overexpression, ranging between ~2 and 10-fold, in all founders (data not shown). Based on these results and analyses of islet PDE3 activity we established two transgenic lines. One line, here designated RIP-PDE3B/2, exhibits a 2–3-fold increase in islet PDE3B expression and activity compared with wild-type mice, whereas in islets of the other line (RIP-PDE3B/7), the increase is 7–10-fold (Fig. 1). None or very weak signs of transgene expression were observed in other tissues, as studied both by reverse transcriptase-PCR and Western blot analysis (data not shown), and PDE3 activity determination (Table I). Mice of the two RIP-PDE3B lines appear normal with regard to behavior, as inspected from birth to adult age, and they reproduce normally with a Mendelian distribution of offspring in heterozygous mating. Body weight monitored throughout the life span of the mice did not differ significantly between transgenic and wild-type mice (Table II).

**RIP-PDE3B Mice Exhibit Reduced Insulin Secretion and Impaired Glucose Tolerance in Response to Glucose and GLP-1**—To assess the impact of β-cell-targeted overexpression of PDE3B on insulin release, intravenous glucose tolerance tests were conducted. Initial experiments revealed insulin secretion response and glucose tolerance different from wild-type in male but not female transgenic mice. Therefore, for further experiments, groups of male mice only were used. Experimental series included mice between 6 and 34 weeks of age. In 12-week-old RIP-PDE3B/2 mice, glucose concentrations in plasma collected immediately before glucose was administered did not differ from control mice values (13.8 ± 1.0 mM versus 13.3 ± 0.4 mM (n = 8)) (Table II). In contrast, fasting (3 h) plasma glucose was increased in RIP-PDE3B/7 mice (10-week-old) (15.4 ± 0.9 mM versus 10.7 ± 0.6 mM for wild-type (p < 0.001; n = 8)) (Table II). Plasma insulin concentrations before injection of glucose varied and showed no significant differences in any of the transgenic animals compared with their wild-type counterparts.

After intravenous administration of glucose (1 g/kg body weight), the transient peak of plasma insulin detected after 1 min was significantly lower in both groups of transgenic mice compared with wild-type. In RIP-PDE3B/2 mice (Fig. 2A), the acute insulin response was 30% of that seen in wild-type animals (increase from 0.35 ± 0.06 to 0.75 ± 0.10 nM in RIP-PDE3B/2 versus from 0.32 ± 0.07 to 1.67 ± 0.18 nM in wild-type (n = 8)), whereas insulin levels at 5–50 min did not differ. A complete failure of insulin secretory response at 1 min after glucose administration was observed in RIP-PDE3B/7 mice (Fig. 3A) as opposed to the 2.6-fold increase in insulin in their wild-type littermates (0.88 ± 0.18 to 0.69 ± 0.08 nM in RIP-PDE3B/7 versus 0.77 ± 0.20 to 2.02 ± 0.15 nM in wild-type (n = 8)). As a consequence of the reduced insulin response, glucose disposal was impaired in both lines (Figs. 2B and 3B). Moderately increased glucose levels were observed in plasma of RIP-PDE3B/2 mice at 20 and 50 min after the glucose injection (30.0 ± 1.0 and 21.1 ± 0.6 mM in RIP-PDE3B/2 versus 25.2 ± 0.8 and 18.7 ± 0.8 mM in wild-type (p < 0.001 and p < 0.05; n = 8)). In RIP-PDE3B/7 mice, the rise in glucose concentration at 5 min post-injection was significantly higher compared with wild-type (35.7 ± 0.8 mM versus 30.9 ± 1.2 mM (p < 0.01; n = 8)) and the elimination from plasma was remarkably reduced with end point (75 min) glucose levels remaining significantly higher than in wild-type mice (20.7 ± 0.9 mM versus 13.7 ± 0.8 mM, p < 0.001; n = 8) (Fig. 3B). Repeated glucose tolerance tests during the age span of 6–25 weeks of both animal groups produced similar results and we did not observe significant age-related differences. Intravenous administration of arginine (0.25 g/kg) did not produce significant differences between
wild-type and transgenic mice with regard to insulin secretory response or glucose elimination (Figs. 2C and 3C), indicating that the above described effects are specific for glucose stimulation.

To assess the physiological insulin secretory response of RIP-PDE3B/7 mice to GLP-1 stimulation, GLP-1 (1 nmol/kg) was administered intravenously together with glucose (1 g/kg). While unable to elevate plasma insulin in response to glucose only, these mice did respond to glucose in combination with GLP-1 (Fig. 4). However, the 1-min peak insulin value in RIP-PDE3B7 mice was only 48% of that of wild-type (4.1 ± 0.6 nM in RIP-PDE3B/7 mice versus 8.6 ± 0.6 nM in wild-type mice.

### Table II

Baseline value characteristics of RIP-PDE3B mice at the age of 10–12 and 23–25 weeks

| Mice          | Body weight | Insulin | Glucose |
|---------------|-------------|---------|---------|
|               | 10–12 w     | 23–25 w | 12 w    |
| Wild-type     | 28.1 ± 0.9  | 35.5 ± 1.7 | 0.32 ± 0.07 | 1.67 ± 0.18 | 13.3 ± 0.4 |
| RIP-PDE3B/7   | 29.1 ± 1.0  | 41.7 ± 1.6 | 0.88 ± 0.15 | 0.69 ± 0.08 | 15.4 ± 0.9 |
| Wild-type     | 29.3 ± 1.0  | 37.1 ± 2.2 | 0.77 ± 0.20 | 2.02 ± 0.15 | 10.7 ± 0.6 |

**Fig. 2.** Intravenous glucose tolerance test performed in RIP-PDE3B/2 (TG) and wild-type (WT) mice. Mice at the age of 12 weeks were used. Blood samples were drawn immediately before and after an intravenous injection of glucose (1 g/kg). Plasma insulin (A) and glucose (B) levels at the indicated time points were determined. In control experiments, mice at 24 weeks of age were injected intravenously with L-arginine (0.25g/kg) (C). Values are presented as the mean ± S.E. (n = 6–8 mice in each group). *, p < 0.05; ***, p < 0.001.
As evident from Fig. 4B, this amount of insulin secreted did not suffice to significantly increase glucose disposal. At 75 min post-injection plasma glucose concentration was 21.2 ± 1.3 mM in RIP-PDE3B/7 and 10.9 ± 0.9 mM in wild-type (p < 0.001; n = 4).

PDE3 enzyme activity assayed in hypothalamic homogenates did not reach detectable levels (Table I). To check the possibility that, still, a low expression of the transgene specifically in hypothalamic neurons could have altered their signaling and created an insulin-resistant condition, we performed insulin tolerance experiments in the RIP-PDE3B/2 mice. Insulin doses at 1 unit/kg, given intraperitoneally, induced equal elimination rates of plasma glucose in the transgenic mice and wild-type control animals (Fig. 5), indicating that insulin sensitivity is not reduced in the RIP-PDE3B/2 mice.

**Islets Isolated from RIP-PDE3B Mice Exhibit Impaired Insulin Secretion**—Islets were isolated after completion of the in vivo experiments and the secretory capacity was examined in static incubations with groups of 3 freshly isolated islets, matched according to size, i.e. avoiding remarkably large or small islets. As seen in Fig. 6A, the secretory response to 16.7 mM glucose was significantly reduced in islets from RIP-PDE3B/2 mice in comparison with wild-type islets (1.8 ± 0.3 nM versus 2.7 ± 0.4 nM insulin per 3 islets (p < 0.05, n = 3)). Inclusion of the selective PDE3 inhibitor OPC3911 (10 μM) together with glucose at 16.7 mM potentiated glucose-stimu-
addition of 10 nM GLP-1 in the islets of transgenic mice caused reversal of the diminished insulin secretory response. The response to 100 nM GLP-1 in the islets of transgenic mice was virtually equal in islets of wild-type and the two transgenic lines compared with wild-type or RIP-PDE3B/7 mice (not shown), after normalization for total islet protein the insulin content was significantly increased in islets from the RIP-PDE3B/2 mice (Fig. 6A). Similar results were obtained in islets isolated from RIP-PDE3B/7 mice (data not shown).

The evaluation of secretory performance was supplemented with measurements of islet insulin content. For such experiments, isolated islets were randomly (i.e. without size-matching) subdivided in groups of 3 islets. As seen in Fig. 6A, there was a marked reduction in first phase insulin secretion compared with wild-type islets. Two minutes after the introduction of 16.7 mM glucose, the secretory response of the transgenic islets was 22.5 ± 3.0 pg/islet/min as compared with 42.0 ± 6.2 pg/islet/min for wild-type islets (p < 0.001, n = 6). The reduction was significant at all time points during the first phase (Fig. 5B). Suprabasal values for the area under the curve disclosed a 42% reduction in first phase insulin release (186 ± 25 versus 107 ± 17 pg of insulin/islet/min in wild-type versus RIP-PDE3B/7 (p < 0.05; n = 6 and 5)) (Fig. 6C, inset). Accordingly, static incubations of islets performed in parallel showed reduced insulin response to 16.7 mM glucose in transgenic as compared with wild-type islets (data not shown).

β-Cell-specific Overexpression of PDE3B Results in Altered Islet Morphology—To characterize islets morphologically and to estimate islet size, immunostaining for insulin, glucagon, and GLUT2 was performed. Morphometry revealed that the transgenic mice had greatly increased mean islet area compared with wild-type mice (Fig. 7). The increase was more pronounced in pancreases of RIP-PDE3B/7 than those of RIP-PDE3B/2 mice. Both transgenic lines exhibited increased frequency of islets with unevenly distributed insulin immunostaining, with some islet areas having low insulin expression, compared with control mice (Fig. 8, A–C). In pancreases of RIP-PDE3B/7 mice, some islets displayed more irregular shape and generally weaker insulin expression as well as more uneven insulin immunostaining than RIP-PDE3B/2 islets; occasionally large islet areas had barely detectable insulin immunoreactivity (Fig. 8C).

To study the location of α-cells in relation to the β-cells, double immunostaining for glucagon and insulin was performed. As expected, in islets of control mice, the α-cells were located at the peripheral rim of the islets (Fig. 8D). As a sign of altered islet cell topography, centrally located α-cells were regularly seen in RIP-PDE3B islets (Fig. 8, E–F). The frequency of islets with this anomaly was higher in RIP-PDE3B/7 than in RIP-PDE3B/2 mice. This type of islet reorganization has been observed in several models of impaired glucose tolerance (26–28).

Wild-type islets displayed intense GLUT2 immunostaining in the plasma membrane of the β-cells (Fig. 8G). Islets with markedly reduced expression were regularly observed in both RIP-PDE3B/2 and RIP-PDE3B/7 islets. In such islets there was only a weak cell membrane immunostaining and an irregular intra-islet distribution with large areas lacking detectable GLUT2 expression (Fig. 8, H–I). Furthermore, at the cellular level an abnormal distribution of GLUT2 with very little membrane staining and instead a weak to moderate cytoplasmic staining was seen in some islets. Such changes in GLUT2 are known to be indicators of β-cell dysfunction (29, 30). In RIP-PDE3B/2 pancre-
ases, islets with only a few cells with a normal pattern of GLUT2 immunostaining were seen, and in RIP-PDE3B/7 islets the vast majority of the cells were abnormal (Fig. 8I).

**DISCUSSION**

Evidence *in vivo* for a significant role of the cAMP-hydrolyzing enzyme PDE3B in insulin secretion has been lacking. Here...
we show that β-cell-targeted overexpression of PDE3B in mice is associated with blunted insulin release and impaired glucose homeostasis. Already a 2–3-fold overexpression of PDE3B (RIP-PDE3B/2) resulted in impaired insulin secretory response to intravenously administered glucose. The effects correlated to the amount of PDE3B present, as seen by the comparison with mice exhibiting 7–10-fold overexpression (RIP-PDE3B/7), the secretory capacity of which was further impaired. This apparently dose-dependent phenotype strongly suggests that the observed effects are because of the enzymatic activity of PDE3B and not explained by insertional mutagenesis, i.e. random incorporation of the transgene leading to inactivation of another gene. The effects as studied hitherto are most salient in male mice and it thus appears that female mice are insusceptible to the transgene-induced β-cell defects. Whereas we do not yet have an explanation for this discrepancy, we note that a gender difference in transgenic mice, which most frequently leaves the female mice unaffected, is commonly observed (31, 32). It is also worth noting that the impaired insulin secretion observed in male RIP-PDE3B mice follows altered expression of one single PDE isozyme out of at least three (PDE1, PDE3B, and PDE4) known to be present in the pancreatic β-cell. This is in agreement with the hypothesis that PDEs, which are differentially regulated and localized in cells, specifically regulate and segregate the generation, amplitude, duration, and compartmentalization of cyclic nucleotide signals and responses (11, 33–35). Initial evidence for the presence of discrete non-overlapping signaling pathways regulated by distinct PDEs came from studies using family-specific PDE inhibitors (36). More recently, the generation of PDE4B and PDE4D knock-out mice, which show distinct phenotypes, also indicates non-redundancy with regard to PDEs (37, 38).

The defective insulin release of RIP-PDE3B mice is associated with glucose intolerance. In RIP-PDE3B/2 mice the acute insulin response to glucose loads was significantly reduced (about one-half of the wild-type level). Despite this, the deterioration of glucose elimination was moderate. This is, however, expected because glucose tolerance in mice is only partially dependent on insulin secretion (39). Furthermore, a defective insulin secretory response might have been counteracted by increased insulin sensitivity. Accordingly, in a situation where the secretory response is further aggravated, as in the RIP-PDE3B/7 mice demonstrating inability to produce an immediate insulin response to glucose, a more pronounced glucose intolerance is seen. Islet mass is another parameter that may influence the secretory performance and, subsequently, glucose tolerance. It is possible that the increased mean area of islets in the RIP-PDE3B mice represents a compensatory increase in β-cell mass, serving as adaptation to an insulin-deficient condition. This is supported by the higher insulin content detected in randomly selected RIP-PDE3B/2 islets. Increased insulin content could also be explained by increased insulin gene expression. However, in this case increased β-cell mass is the most likely explanation because the insulin content was similar to wild-type levels after normalization for total islet protein. In accordance, when we chose to specifically stimulate a subset of larger RIP-PDE3B/2 islets they were found to secrete more insulin (data not shown). In contrast, both insulin content and insulin secretory performance of RIP-PDE3B/7 islets were consistently reduced and appeared not to vary with islet size. The molecular explanation for this difference is unclear, but may be

**Fig. 7.** Morphometric analysis. The histogram depicts the mean area of islets in sections of wild-type (WT) and transgenic (RIP-PDE3B) pancreases. Islet areas were measured at various depths of pancreatic specimens (n = 5 in each group) as described under “Materials and Methods.” ***p < 0.001.

**Fig. 8.** Immunofluorescence photomicrographs. A, D, and G, wild-type islets. B, E, and H, RIP-PDE3B/2 islets. C, F, and I, RIP-PDE3B/7 islets. A–C stained for insulin. Note unevenly distributed insulin staining in RIP-PDE3B/2, and generally weaker insulin staining with poorly immunostained areas in RIP-PDE3B/7. D–F, double stained for insulin (green) and glucagon (red). Note several more centrally localized α-cells in the RIP-PDE3B/2 and RIP-PDE3B/7 compared with the wild-type. G–I, stained for GLUT-2. Note also weak GLUT-2 membrane staining and more cytoplasmic localization in RIP-PDE3B/2 and RIP-PDE3B/7 compared with wild-type. Scale bars = 20 μm. Bar in A is valid for A–C and G–I. Bar in D is for D–F.
attributed to the increased occurrence of islets with a distorted structure in mice of this line. In vivo, the secretory performance of RIP-PDE3B/7 mice was markedly reduced in response to glucose, whereas they were able to respond when glucose was given in combination with GLP-1. Although the amount of insulin secreted was significantly lower than in wild-type mice, this demonstrates that at least the signaling pathways for potentiation of glucose-stimulated insulin secretion are still functional.

We also demonstrate that it is the first phase of glucose-induced insulin secretion that is reduced by PDE3B overexpression. Mechanisms governing the first phase are as yet incompletely understood but have been attributed to the release of already docked and primed granules, i.e., the readily releasable pool of secretory granules (40). We have shown that PDE3B is membrane-located, and given that it operates in close association with the exocytotic machinery, as was suggested from experiments with single β-cells flooded with cAMP (21), it is tempting to speculate that PDE3B controls pools of cAMP directly involved in granule emptying. The reported binding of cAMP to Rab3-interacting molecule 2 (8) and cAMP-guanylate nucleotide exchange factor II (10, 41), proteins that are involved in PKA-independent exocytosis and suggested to regulate vesicular fusion with the plasma membrane, makes such a speculation plausible. Further investigations have been initiated to resolve the role of PDE3B in this context.

Because of the diversity in regulatory mechanisms controlling cAMP levels, effects of dysregulated cAMP could be expected to arise at different cellular locations. The proposed function for cAMP in granule priming by binding to granule-associated proteins indicates one possible location where defective targeting of cAMP could directly impair exocytosis of insulin. In fact, such a defect was recently suggested to partly explain the lack of an early PKA-independent component in SUR 1-deficient mice (10). Formation of cAMP by receptors coupled to adenylyl cyclase is an early PKA-independent component in SUR 1-deficient mice (10). Formation of cAMP by receptors coupled to adenylyl cyclase activity represents another critical step. The known potentiation effect of, for example, pituitary adenylate cyclase-activating polypeptide on insulin secretion involves increased cAMP formation and it was recently reported that mice lacking the pituitary adenylate cyclase-activating polypeptide-selective receptor (PAC1) exhibit reduced islet size, defective insulin secretory response to glucose, and glucose intolerance (42). In the current study we show, in transgenic mice, that also increased degradation of cAMP by a phosphodiesterase may render the insulin secretory machinery dysfunctional.

In summary, we show that β-cell-specific overexpression of PDE3B results in reduced insulin release and deranged islet cytoarchitecture associated with glucose intolerance. This suggests a role for β-cell PDE3B in overall energy homeostasis. Accordingly, altered expression of β-cell PDE3B or defects in the PDE3B gene might contribute to the development of hypo- as well as hyperinsulinemia found at different stages of type 2 diabetes. In fact, decreased expression of PDE3B has been observed in adipose tissue from patients with diabetes (43) and recently, a sibling study suggests a coupling of a polymorphism in exon 4 of the human PDE3B gene to hyperinsulinemia.2 Treatment of obesity and diabetes by use of PDE3 inhibitors has been discussed (44). Based on the findings of the present study, it seems further is plausible that development of PDE3 inhibitors specifically targeting β-cell PDE3B would be beneficial.

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2 M. Klannemark, E. Degerman, and L. Groop, unpublished data.
β-Cell-targeted Overexpression of Phosphodiesterase 3B in Mice Causes Impaired Insulin Secretion, Glucose Intolerance, and Deranged Islet Morphology

Linda Härndahl, Nils Wierup, Sven Enerbäck, Hindrik Mulder, Vincent C. Manganiello, Frank Sundler, Eva Degerman, Bo Ahrén and Lena Stenson Holst

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