OBJECTIVE—Retinoid X receptors (RXRs) are members of the nuclear hormone receptor superfamily and are thought to be key regulators in differentiation, cellular growth, and gene expression. Although several experiments using pancreatic β-cell lines have shown that the ligands of nuclear hormone receptors modulate insulin secretion, it is not clear whether RXRs have any role in insulin secretion.

RESEARCH DESIGN AND METHODS—To elucidate the function of RXRs in pancreatic β-cells, we generated a double-transgenic mouse in which a dominant-negative form of RXRβ was inducibly expressed in pancreatic β-cells using the Tet-On system. We also established a pancreatic β-cell line from an insulinoma caused by the β-cell–specific expression of simian virus 40 T antigen in the above transgenic mouse.

RESULTS—In the transgenic mouse, expression of the dominant-negative RXR enhanced the insulin secretion with high glucose stimulation. In the pancreatic β-cell line, the suppression of RXRs also enhanced glucose-stimulated insulin secretion at a high glucose concentration, while 9-cis-retinoic acid, an RXR agonist, repressed it. High-density oligonucleotide microarray analysis showed that expression of the dominant-negative RXR affected the expression levels of a number of genes, some of which have been implicated in the function and/or differentiation of β-cells.

CONCLUSIONS—These results suggest that endogenous RXR negatively regulates the glucose-stimulated insulin secretion. Given these findings, we propose that the modulation of endogenous RXR in β-cells may be a new therapeutic approach for improving impaired insulin secretion in type 2 diabetes. *Diabetes* 59:2854–2861, 2010

The nuclear hormone receptor superfamily plays essential roles in various aspects of biological regulation, such as differentiation, cellular growth, and metabolism. The retinoid X receptors (RXRs) play a unique and central role in the activity of many members of this superfamily. They function as an obligate heterodimeric partner for retinoic acid receptors (RARs), thyroid hormone receptor, vitamin D receptor, peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs), farnesoid X receptor, and others (1). Because of this unique position within the superfamily, the modulation of the activity of RXRs is considered to cause a broad spectrum of effects (2,3).

In pancreatic β-cells, there are several lines of evidence from in vitro studies that nuclear receptors are involved in the insulin secretory mechanism. Retinoic acid affects the expression of the glucokinase and preproinsulin genes and promotes insulin secretion in RIN-m5F cells (4,5) and in isolated islets (6). In INS-1 cells, both 9-cis-retinoic acid (9cRA), a ligand of RXRs, and all-trans-retinoic acid (ATRA), a ligand of RARs, increase insulin secretion, and ATRA raises GLUT2 mRNA (7). In contrast, 9cRA and a high concentration of rosiglitazone, a ligand of PPAR-γ, are reported to inhibit glucose-stimulated insulin secretion (GSIS) in INS-1 cells (7), suggesting that the PPAR/RXR heterodimer is inhibitory to insulin secretion in β-cells. Recent reports showed that the co-overexpression of PPAR-α and RXR-α in INS-1 cells potentiated glucose-induced insulin secretion, whereas the co-overexpression of PPAR-γ and RXR-α in INS-1 cells attenuated it (8). Although these results suggest that RXR plays a role in regulating the insulin secretory machinery of β-cells, it is still controversial whether RXR has a direct effect on insulin secretion.

RXR is composed of three family members—RXR-α, -β, and -γ—that have redundant roles (8–10). Therefore, it is necessary to disrupt the genes for all three RXR members to inactivate RXR function. Considering the practical difficulty of a β-cell–specific knockout of all three genes, we took another approach using a dominant-negative form of RXR. RXRβΔC2, which lacks the ligand-binding domain, is known to act as a dominant-negative receptor and to inhibit the ligand-dependent transcriptional activation of target genes by homodimerized and heterodimerized RXRs (11). To investigate the in vivo function of RXR in β-cells, we generated a double-transgenic (Tg) mouse: one transgene expresses reverse tetracycline-regulated transactivator (rtTA) under the insulin promoter, and the other expresses RXRβΔC2 under the tetracycline-responsive TetO promoter. In this Tg mouse line, the addition of
tetracycline to the drinking water led to the expression of RXRβΔC2 in the β-cells. The tetracycline-treated Tg mice showed elevated glucose tolerance, and islets isolated from these mice also showed enhanced GSIS.

To examine the role of RXR in β-cells, we established β-cell lines (dnRXR-MIN6 cells) from an insulinoma that developed in a triple-Tg mouse harboring the SV40 T antigen gene under the insulin promoter in addition to the two transgenes for RXRβΔC2 expression. Using this cell line, we confirmed that the suppression of RXR enhanced the glucose-stimulated insulin secretion at a high glucose concentration in vitro. High-density oligonucleotide microarray analysis showed that the expression of a number of genes was affected by the suppression of RXR. Our findings suggest that modulating the activity of the endogenous RXRs in β-cells may provide a novel therapeutic approach for improving impaired insulin secretion in type 2 diabetes.

RESEARCH DESIGN AND METHODS

Generation of double-Tg mice. To generate Tg mice in which the expression of a dominant-negative form of RXRβ could be induced in β-cells, we designed two transgenes to enable the Tet-On system to work: the transgene termed Ins-rtTA (A) carried the reverse tetracycline-regulated transactivator (rtTA) gene under the human insulin promoter, and the other transgene, termed TetO-RXRβΔC2 (B), carried the RXRβΔC2 cDNA, which encodes a dominant-negative form of mouse RXRβ lacking 20 conserved COOH-terminal amino residues of the ligand-binding and dimerization domain, following the TetO promoter. DBD, DNA-binding domain; LBD, ligand-binding domain. (A high-quality color representation of this figure is available in the online issue.)

FIG. 1. DNA constructs used to generate transgenic mice. Two transgenes were constructed to enable the Tet-On system to work: the transgene termed Ins-rtTA (A) carried the reverse tetracycline-regulated transactivator (rtTA) gene under the human insulin promoter, and the other transgene, termed TetO-RXRβΔC2 (B), carried the RXRβΔC2 cDNA, which encodes a dominant-negative form of mouse RXRβ lacking 20 conserved COOH-terminal amino residues of the ligand-binding and dimerization domain, following the TetO promoter. DBD, DNA-binding domain; LBD, ligand-binding domain. (A high-quality color representation of this figure is available in the online issue.)

Oral glucose tolerance test. An oral glucose tolerance test (OGTT) was performed twice on the same groups of the double-Tg mice: after 2 weeks of Dox treatment (Dox On) and 2 weeks after the withdrawal of Dox treatment (Dox Off). The Tg mice were fasted overnight for 16 h followed by oral administration of glucose (2 g/kg body wt). Blood samples were collected by retro-orbital sinus puncture before (0 min) and 10, 30, 60, and 120 min after glucose administration. Plasma samples were obtained by centrifugation at 4°C and were stored at −80°C until analysis. The plasma glucose and insulin concentrations were measured using a blood glucose test kit (Glucose CII-test; Wako Pure Chemicals, Osaka, Japan) and an enzyme-linked immunosorbent assay (ELISA) kit (Morinaga, Yokohama, Japan), respectively.

Measurement of insulin secretion and insulin content. Double-Tg mice with or without 2 weeks of Dox treatment were killed, and their islets were isolated according to a previously described method (15). The isolated islets were cultured in RPMI medium with 11 mmol/l glucose, 10% FBS, and antibiotics. For the islets from Dox-treated Tg mice, 5 μg/ml Dox was added to the medium. After cultivation for 24 h, 10 μmol/l 9cRA was added to the culture media of half of the islets from Dox-treated Tg mice. Islets from Dox-untreated Tg mice. After further cultivation for 24 h, the islets of a similar size were washed with Krebs-Ringer bicarbonate buffer (KRBB) containing 0.2% BSA and transferred to microcentrifuge tubes (10 islets per tube; n = 5–6 for each group). They were then cultured in KRBB containing 0.2% BSA with 3, 9, or 27 mmol/l glucose for 60 min, and the supernatant was collected and assayed for insulin using an ELISA kit (Mercodia, Uppsala, Sweden). For measurement of insulin content, double-Tg mice with or without 2 weeks of Dox treatment were killed and their pancreas were isolated. Insulin was extracted from them with acid ethanol and measured by immunoassay as described above.

Establishment of the dnRXR-MIN6 β-cell lines. To establish β-cell lines with inducible RXRβΔC2 expression, Ins-rtTA/TetO-RXRβΔC2 double-Tg mice were mated with β-cell mice. Tg-6 Tg mice bear the SV40 T antigen gene under the insulin promoter and were originally MIN6 β-cells. However, we established a β-cell line that retains GSIS (16). The resulting triple-Tg mice were identified by the PCR analysis of genomic DNA obtained from the tail tips. Pancreatic β-cell lines were generated from 22 insulinomas isolated from the triple-Tg mice at the age of 9 weeks. Finally, two clones with good GSIS and stable induction of RXRβΔC2 by Dox were selected (named dnRXR-MIN6) and used for experiments.

Western blotting and immunocytochemistry. The total protein was extracted from dnRXR-MIN6 cells after cultivation with or without Dox for 4 days and subjected to Western blotting using a mouse anti-mouse RXRβ monoclonal antibody (MA3-812 [clone MOK13.17]; Affinity Bioreagents, Golden, CO) that detects RXRβΔC2 (11) and a homeradish peroxidase–conjugated second antibody. Detection was done by enhanced chemiluminescence (ECL kit; Amersham, Arlington Heights, IL). Immunocytochemistry was performed with dnRXR-MIN6 cells. The cultured cells were washed with PBS and fixed in 4% paraformaldehyde for 10 min. After fixation, the cells were rinsed with PBS, incubated for 5 min in 1% Triton X-100, and, after a second wash, incubated in a blocking reagent. The samples were incubated with the first antibody for 60 min at room temperature, washed with PBS, and then incubated with the secondary antibody for 60 min at room temperature. The primary antibody was a mouse anti-mouse RXRβ antibody (MOK13.17); the secondary antibody was Alexa Fluor 488–conjugated anti-mouse IgG1 (Molecular Probes, Eugene, OR).

Immunohistochemical analyses. Immunohistochemistry was performed with frozen sections or paraffin sections of pancreatic tissue. The 8-μm-thick frozen sections were placed on slides and fixed in cold acetone for 10 min. Pancreatic tissue was also fixed in 4% paraformaldehyde overnight and processed for paraffin embedding. Sections of paraffin-embedded pancreatic tissue (3–5 μm thick) were deparaffinized and dehydrated. The frozen and paraffin sections were incubated with 3% normal goat serum in PBS containing 10% blocking One (Nacalai Tesque, Kyoto, Japan) for 60 min at room temperature. The sections were then incubated with the first antibody at 4°C overnight and with a fluorescein-conjugated secondary antibody for 60 min at room temperature. After each antibody incubation, the sections were washed in PBS for 5 min with three changes. The first antibodies were guinea pig anti-insulin antibody (Dako, Carpentaria, CA), rabbit anti-VIP antibody for the detection of rtTA, and mouse anti-RXRβ antibody. The secondary antibodies were Alexa Fluor 568–conjugated goat anti-mouse IgG1, Alexa Fluor 488–conjugated rabbit IgG, and Alexa Fluor 488–conjugated anti-mouse IgG1 (Molecular Probes). The sections were observed by fluorescence microscopy (Olympus, Tokyo, Japan).

RT-PCR and real-time PCR analyses. The total RNA was extracted from isolated islets and dnRXR-MIN6 cells after they were cultivated with or without Dox for 4 days by the acid guanidinium-phenol-chloroform method and was subjected to cDNA synthesis using ReverTra Ace (Toyobo, Tokyo, Japan) (17). The cDNA was subjected to PCR with the following primers: rtTA forward primer, 5′-GCGTATAGGCTGCTTAAT-3′ and backward primer, 5′-TACATGTGAAGCACCATCT-3′; RXRβ forward primer, 5′-GCCGTTCTACCTGTCCTG-3′ and backward primer 5′-AGGTTGTTCACAAAAGCTGG-3′; and to the sequence within the second exon of the human growth hormone gene in the TetO-RXRβΔC2 transgene and HGPT forward primer, 5′-CTCGAAGGGTGGGTGATAACGG-3′ and backward primer, 5′-GGCTGTAGAACCTGATAGTG-3′. These
primes were designed to encompass intronic sequences to distinguish the appropriate PCR products from products amplified from contaminating genomic DNA. Real-time PCR was performed on an ABI Prism 7300 Sequence Detection System (Applied Biosystems, Foster City, CA), using the SYBR Green PCR core reagents detection system (Applied Biosystems). The primer sequences are shown in supplementary Table I. PCR was performed with an initial step of 10 s at 95°C followed by 40 cycles of 5 s at 95°C and 31 s at 60°C. The levels of targeted gene expression were normalized to that of β-actin. Statistical analysis was performed by a Student t test.

Glucose-stimulated insulin secretion in vitro. dnRXR-MIN6 cells were cultured in 24-well plates with Dulbecco’s modified Eagle’s medium in the presence or absence of 2 μg/ml Dox for 4 days. In some experiments, 5 μmol/l 9cRA was added for the last 2 days of culture. Cells were starved in KRB containing 0.1% BSA and 3 mmol/l glucose for 1 h, and wells were replenished with the same buffer twice. Then, cells were stimulated with 3, 10, or 25 μmol/l glucose in 500 μl KRB containing 0.1% BSA for 1 h. The supernatants were collected and assayed for insulin using an ELISA kit. The values were normalized to the protein amount of each well. Statistical analysis was performed by a Student t test.

Microarray analysis. Total RNA was extracted by the acid guanidinium-phenol-chloroform method from dnRXR-MIN6 cells, after they were cultivated with or without Dox for 4 days. The quality of the purified total RNA was examined with an Agilent 2100 Bioanalyzer. We used 500 ng verified RNA to perform by a Student t test.

RESULTS

Islet-specific overexpression of RXRβΔC2 in InsrtTA/TetO-RXRβΔC2 double-Tg mice. To achieve tetracycline-inducible expression of the RXRβΔC2 gene in vivo, two types of Tg mouse were generated. The first Tg mouse, InsrtTA, expressed rtTA under the human insulin promoter (16). A total of 22 RT-PCR analysis showed that RXRβΔC2 was expressed only in the islets of the Dox-treated double-Tg mouse (Fig. 2C). Immunohistochemical analysis showed that the nuclei of the islet β-cells of Dox-treated dnRXR Tg mouse [Dox(++)] were more strongly stained with an anti-RXRβ antibody than those of the Dox-untreated mice [Dox(−)] (Fig. 2D). The weak staining of the latter sample was probably due to the expression of endogenous RXRβ, because the pancreas samples from non-Tg mice were similarly stained. These findings indicated that islet-specific expression of RXRβΔC2 was inducible by Dox in the dnRXR Tg mice.

Suppression of RXRs increases the GSIS in vivo. To investigate the effect of dnRXR expression in β-cells on glucose homeostasis, OGTTs were performed after 2 weeks of Dox treatment (Dox On) and 2 weeks after the withdrawal of Dox treatment (Dox Off) (Fig. 3A). Interestingly, the dnRXR Tg mice showed significantly improved glucose tolerance under the expression of the dnRXRβ (Dox On) compared with after the withdrawal of the Dox treatment (Dox Off) (Fig. 3B). Plasma insulin levels at 10 min after glucose administration were significantly higher under the Dox treatment (Dox On) compared with after the withdrawal of the Dox treatment (Dox Off) (Fig. 3C). The non-Tg control mice showed no significant change in the glucose tolerance with Dox treatment (data not shown). In a separate experiment, we examined whether the Dox treatment affected the body weight of the dnRXR Tg mice. Eight-week-old dnRXR Tg mice were maintained with or without Dox treatment (n = 5 each) for 2 weeks, and their body weights were measured. The result showed that there were no significant changes in the body weight between Dox-treated and Dox-untreated mice. Therefore, the improved glucose tolerance seen under the expression of the dnRXRβ was mostly due to the improved response of β-cells to glucose.

We then examined the GSIS of isolated islets. In this analysis, the effect of 9cRA, an RXR agonist, was also examined. At 9 and 27 mmol/l glucose, the insulin secretion of the Dox(++) islets was significantly higher (~1.3-fold) than that of the Dox(−) islets (Fig. 4A). Interestingly, 9cRA treatment significantly reduced the insulin secretion at the high glucose concentration both from the Dox(+) and Dox(−) islets. This effect of 9cRA was not observed at 3 or 9 mmol/l glucose.

To examine whether expression of the dnRXRβ affected the transcription of insulin genes, the mRNA for the two insulin genes was quantified. The total RNA of islets isolated from Dox-treated and Dox-untreated mice was subjected to semi-quantitative RT-PCR analyses for the insulin 1 and insulin 2 genes. The results showed no significant differences in the expression level of these genes between the Dox(+) and Dox(−) islets (data not shown). We further examined whether expression of dnRXRβ affected the insulin content of the pancreas. Pancreases were isolated from Dox-treated and Dox-untreated mice, and insulin content was measured. No significant differences were observed in the insulin content between the Dox(+) and Dox(−) pancreases (Fig. 4B). These results suggested that the expression of the dnRXRβ affected insulin secretion through a mechanism occurring after insulin production.

dnRXRβ increases the GSIS in a β-cell line. To examine the molecular mechanism underlying the elevated GSIS of islets cells by the expression of the dnRXRβ, we sought to establish β-cell lines with Tet-inducible RXRβΔC2 expression using a triple-Tg mouse strategy involving the IT-6 Tg mouse expressing the SV40 T antigen gene under the human insulin promoter (16). A total of 22 β-cell lines were established from each insulinoma developed in the pancreases of the triple-Tg mice and were tested for GSIS. Most lines exhibited appropriate GSIS. Two lines (19 and 21) were ultimately selected, termed dnRXR-MIN6 cells, and subjected to further analyses. Both clones showed similar results, and the data with clone 19 were presented. In dnRXR-MIN6 cells, the stable expres-
sion of rtTA and the Dox-mediated induction of RXRβΔC2 were confirmed by RT-PCR analysis, Western blotting, and immunocytochemistry (Fig. 5A–C). We also confirmed that Dox treatment affected neither the proliferation nor apoptosis of dnRXR-MIN6 cells (supplementary Figs. 1 and 2).

After incubation with or without Dox for 4 days, insulin secretion was measured at different glucose concentrations. In this analysis, the effect of 9cRA was also examined. At 10 and 25 mmol/l glucose, the insulin secretion of the Dox-treated cells was significantly higher (1.5-fold) than that of the Dox-untreated cells (Fig. 5D). Interestingly, 9cRA treatment significantly reduced the insulin secretion at the high glucose concentration from both Dox-treated and untreated dnRXR-
NEGATIVE REGULATION OF GSIS BY RXR

MIN6 cells. This effect of 9cRA was not observed at 3 or 10 mmol/l glucose.

cDNA microarray analysis. RXRs function as transcription factors in a homodimeric or heterodimeric form. Therefore, the induced expression of the dnRXRβ must influence the transcriptional levels of various target genes, some of which are assumed to be related to the regulation of insulin secretion in β-cells. We used high-density (Affymetrix) oligonucleotide microarrays to identify genes that were differentially expressed by the Dox-treated and untreated dnRXR-MIN6 cells. To correct for dye bias and exclude false-positive genes, dye swapping and subsequent normalization were performed. Supplementary Tables 2 and 3 list the genes for which a mean twofold or greater change in transcript level was demonstrated in the dye-swap experiment. We noted that the list included genes that might be related to the regulation of insulin secretion. We selected some of these genes to analyze by real-time PCR (Fig. 6). The results showed that these genes were clearly down- or upregulated by the Dox treatment, consistent with the microarray analysis.

DISCUSSION

Several studies have shown that RXR agonists have a beneficial effect on glucose tolerance in type 2 diabetes. The RXR agonist LG100268 was reported to protect the insulin secretory capacity in the db/db mouse, a model for this disease (19,20). A recent report also showed that LG101506, a heterodimer-selective RXR stimulator, also has insulin-sensitizing activity in the fa/fa rat (21). Although these data suggest that RXR agonists are beneficial for glucose tolerance, there has been no direct evidence for an effect of RXR on the insulin secretory mechanism in β-cells. Thus, to elucidate the function of RXRs in β-cells, we produced a Tg mouse and established

![Fig. 3. Experimental protocols for the Dox treatment (A) and OGTT in the dnRXR Tg mice (B and C). The Dox-containing water was given for 2 weeks and renewed every 4 days. The OGTTs were performed after 2 weeks of the Dox treatment (Dox On) and 2 weeks after the withdrawal of the Dox treatment (Dox Off). Compared with the Dox On state, the withdrawal of Dox (Dox Off) deteriorated the glucose tolerance. Plasma insulin levels were also measured at each time point (C). Values are expressed as the mean ± SE. Statistical analyses were carried out by the Student t test. *P < 0.05, **P < 0.01. n = 6.

![Fig. 4. Analysis of insulin secretion in the dnRXR Tg mice. Islets were isolated from Dox-treated and -untreated dnRXR Tg mice and cultured in the presence and absence of Dox, respectively (A). Insulin secretion from isolated islets in response to different concentrations of glucose is shown (n = 5–6 each). The effect of 9cRA treatment on the GSIS was also examined (A). For measurement of insulin content, dnRXR Tg mice with or without 2 weeks of Dox treatment were killed, and their pancreases were isolated (n = 6 each) (B). Values are expressed as the mean ± SE. Statistical analyses were carried out by the Student t test. *P < 0.05, **P < 0.01.

![Fig. 5. Real-time PCR assays for the list of genes that were differentially expressed in the Dox-treated and -untreated dnRXR-MIN6 cells. To correct for dye bias and exclude false-positive genes, dye swapping and subsequent normalization were performed. The list included genes that might be related to the regulation of insulin secretion. We selected some of these genes to analyze by real-time PCR. The results showed that these genes were clearly down- or upregulated by the Dox treatment, consistent with the microarray analysis.](http://diabetes.diabetesjournals.org/content/59/11/2858)

![Fig. 6. Real-time PCR analysis of selected genes. The real-time PCR was performed to confirm the transcriptional changes of the selected genes (Table 2). The RNA was extracted from isolated islets of doxycycline (Dox)–treated and -untreated (Dox-) dnRXR Tg mice. The expression levels of each gene were normalized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression. The expression level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control in the Dox treatment.](http://diabetes.diabetesjournals.org/content/59/11/2858)
pression for both isolated islets and dnRXR-MIN6 cells (Figs. 4A and 5D). It was probably due to incomplete inhibition of the RXR function by the dominant-negative form of RXRβ/H9252.

In contrast, the basal insulin secretion at 3 mmol/l glucose from isolated islets or dnRXR-MIN6 cells was not significantly affected by the suppression of RXR (Fig. 4B). In addition, the suppression of insulin secretion by 9cRA treatment was seen only at high glucose for both isolated islets and dnRXR-MIN6 cells (Figs. 4A and 5D). These findings suggested that RXR might function to inhibit excessive insulin release under high-glucose conditions. These opposing results for insulin release between the high- and low-glucose responses suggest that the function of the RXR on β-cells may change, depending on the ambient glucose concentration. Thus, RXR may deeply participate in the regulatory mechanisms of GSIS.

Although the mechanism of the inhibitory effect of RXR is unknown, several reports suggest that RXR elicits diverse effects in β-cells by combining with different dimerization partners. PPAR-γ agonists are reported to prevent the impairment of the insulin secretory capacity induced by oxidative stress and lipotoxicity (22). A recent report showed that the combination of a PPAR-α agonist and an RXR agonist acted synergistically and led to protection against lipotoxicity in β-cells (23). Thus, the activation of PPAR/RXR signaling protects β-cells against oxidative stress and apoptosis. LXR is another het-

![Graph](image-url)
Thus, the function of RXR may contribute to the glucose-induced insulin secretion from MIN6 cells, but the addition of 9cRA inhibits the effect of T0901317, especially on the insulin release.
responses: new insights into coordinated regulation of the PPAR-RXR complex. FEBS Lett 2008;582:32–38
4. Fernandez-Mejia C, Davidson MB. Regulation of glucokinase and proinsulin gene expression and insulin secretion in RIN-m5F cells by dexamethasone, retinoic acid, and thyroid hormone. Endocrinology 1992;130:1660–1668
5. Chertow BS, Driscoll HK, Goking NQ, Primerano D, Cordle MB, Matthews KA. Retinoid-X receptors and the effects of 9-cis-retinoic acid on insulin secretion from RINm5F cells. Metabolism 1997;46:656–660
6. Cabrera-Valladares G, German MS, Matschinsky FM, Wang J, Fernandez-Mejia C. Effect of retinoic acid on glucokinase activity and gene expression and on insulin secretion in primary cultures of pancreatic islets. Endocrinology 1999;140:3091–3096
7. Blumentrath J, Neye H, Verspohl JH. Effects of retinoids and thiazolidinediones on proliferation, insulin release, mRNA, GLUT 2 transporter protein and mRNA of INS-1 cells. Cell Biochem Funct 2011;19:159–169
8. RavnekJær K, Boergesen M, Rubi B, Larsen JK, Nielsen T, Frikridsken J, Maechler P, Mandrup S. Peroxisome proliferator-activated receptor α (PPARα) potentiates, whereas PPARγ attenuates, glucose-stimulated insulin secretion in pancreatic β-cells. Endocrinology 2005;146:3266–3276
9. Kastner P, Mark M, Leid M, Gansmuller A, Chin W, Grondona JM, DeCimino D, Krezel W, Dierich A, Chambon P. Abnormal spermatogenesis in RXRα/α mice: inhibition of the mouse pdx-1 gene activity by antisense RNA expression in pancreatic β-cells. Endocrinology 1996;8:1007–1018
10. Blanco JC, Dey A, Leid M, Minucci S, Park BK, Jurutka PW, Haussler MR, Ozato K. Inhibition of ligand induced promoter occupancy in vivo by a dominant negative RXR. Genes Cells 1996;1:209–221
11. Gossen M, Freundlieb S, Bender G, Müller G, Hillen W, Bujard H. Regulated overexpression of interleukin 11 in the lung: use to dissociate development-dependent and -independent phenotypes J Clin Invest 1997;100:2501–2511
12. Gaskill BA, Lightfoot RM, Brown HR. The RXR agonist LG100268 causes hepatomegaly, improves glycaemic control and decreases cardiovascular risk and cachexia in diabetic mice suffering from pancreatic β-cell dysfunction. Diabetologia 1999;42:545–554
13. Leibowitz MD, Ardecky RJ, Boehm MF, Broderick CL, Carfagna MA, Crombie DL, D’Arrigo J, Eigen GF, Paul MM, Grese TA, Havel H, Hein NJ, Heyman RA, Jolley D, Klausing K, Liu S, Mais DE, Mapes CM, Marschke KB, Michellys PY, Montrose-Radzadze C, Ogilvie KM, Pascual B, Rungta D, Tyhonas JZ, Urcan MS, Wardlow M, Yumibe N, Reifel-Miller A. Biological characterization of a heterodimer-selective retinoid X receptor modulator: potential benefits for the treatment of type 2 diabetes. Endocrinology 2006;147:1044–1053
14. Lottmann H, Vanselow J, Hessabi B, Walther R. The Tet-On system in insulin-secreting cells. Diabetes 2004;53(Suppl. 1):S60–S65
15. Hellemans K, Kerckhofs K, Hannaert JC, Martens G, Van Veldhoven P, Pipeleers D. Peroxisome proliferator-activated receptor α-retinoid X receptor agonists induce β-cell protection against palmitate toxicity. FEBS J 2007;274:6094–6105
16. Kim HI, Ahn YH. Role of peroxisome proliferator-activated receptor-γ in the glucose-sensing apparatus of liver and β-cells. Diabetes 2004;53(Suppl. 3):S75–S78
17. Zhao C, Dahman-Wright K, Liver X receptor in cholesterol metabolism. J Endocrinol 2010;204:233–240
18. Sugden MC, Holness MJ. Role of nuclear receptors in the modulation of insulin secretion in lipid-induced insulin resistance. Biochem Soc Trans 2008;36:891–900
19. Cheo SS, Choi AH, Lee JW, Kim KH, Chung JJ, Park J, Lee KM, Park KG, Lee IK, Kim JB. Chronic activation of liver X receptor induces β-cell apoptosis through hyperactivation of lipogenesis: liver X receptor-mediated lipotoxicity in pancreatic β-cells. Diabetes 2007;56:1534–1543
20. Wente W, Brenner MB, Zitzer H, Gromada J, Efkanov AM. Activation of liver X receptors and retinoid X receptors induces growth arrest and apoptosis in insulin-secreting cells. Endocrinology 2007;148:1834–1849
21. Efkanov AM, Sewing S, Bokvist K, Gromada J. Liver X receptor activation stimulates insulin secretion via modulation of glucose and lipid metabolism in pancreatic β-cells. Diabetes 2004;53(Suppl. 3):S75–S78
22. Kawaguchi R, Yu J, Honda J, Hu J, Whitelegge J, Ping P, Wiita P, Bok D, Sun H. A membrane receptor for retinol binding protein mediates cellular uptake of vitamin A. Science 2007;315:820–825
23. Ishihara H, Asano T, Tsukuda K, Katagiri H, Inukai K, Anai M, Kikuchi M, Yazaki Y, Miyazaki J, Oka Y. Overexpression of hexokinase I but not GLUT1 glucose transporter alters concentration dependence of glucose-stimulated insulin secretion in pancreatic beta-cell line MIN6. J Biol Chem 1994;269:3081–3087
24. Goldenstein SA, Bockenhauer D, O’Kelly I, Zilberberg N. Potassium leak channels and the KCNK family of two-P-domain subunits. Nat Rev Neurosci 2002;3:175–184
25. Matsuoka TA, Zhao L, Artert I, Jarrett HW, Friedman D, Means A, Stein R. Members of the large Maf transcription family regulate insulin gene expression in islet β-cells. Mol Cell Biol 2003;23:6049–6062
26. Warnecke M, Oster H, Revelli JP, Alvarez-Bolado G, Eichele G. Abnormal development of the locus coeruleus in Nar2(Nv2f6)-deficient mice impairs the functionality of the forebrain clock and affects nociception. Genes Dev 2005;19:614–625
27. Bar Y, Riss HA, Knoller S, Ouziel-Yahalom L, Erfat S. HES-1 is involved in adaptation of adult human beta-cells to proliferation in vitro. Diabetes 2008;57:2413–2420
28. Fujikura J, Hosoda K, Ikawa H, Tomita T, Noguchi M, Masuzaki H, Tanigaki K, Yabe D, Honjo T, Nakao K. Notch/Rh4-p signaling prevents premature endocrine and ductal cell differentiation in the pancreas. Cell Metab 2006;3:59–65
29. Henseleit KD, Nelson SB, Kuhlbrodt K, Hennings JC, Ericson J, Sander M. NKKX transcription factor activity is required for alpha- and beta-cell development in the pancreas. Development 2005;132:3139–3149