Bach1 deficiency protects pancreatic β-cells from oxidative stress injury

Keiichi Kondo,1 Yasushi Ishigaki,1 Junhong Gao,1 Tetsuya Yamada,1 Junta Imai,1 Shojiro Sawada,1 Akihiko Muto,2 Yoshitomo Oka,1 Kazuhiko Igarashi,2 and Hideki Katagiri1,3
1Division of Metabolism and Diabetes, Tohoku University Graduate School of Medicine, Sendai, Japan; 2Department of Biochemistry, Tohoku University Graduate School of Medicine, Sendai, Japan; and 3Japan Science and Technology Agency, CREST, Seiryo-machi, Aoba-ku, Sendai Japan

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Kondo K, Ishigaki Y, Gao J, Yamada T, Imai J, Sawada S, Muto A, Oka Y, Igarashi K, Katagiri H. Bach1 deficiency protects pancreatic β-cells from oxidative stress injury. Am J Physiol Endocrinol Metab 305: E641–E648, 2013. First published July 23, 2013; doi:10.1152/ajpendo.00120.2013.—BTB and CNC homology 1 (Bach1) is a transcriptional repressor of antioxidative enzymes, such as heme oxygenase-1 (HO-1). Oxidative stress is reportedly involved in insulin secretion impairment and obesity-associated insulin resistance. However, the role of Bach1 in the development of diabetes is unclear. HO-1 expression in the liver, white adipose tissue, and pancreatic islets was markedly upregulated in Bach1-deficient mice. Unexpectedly, glucose and insulin tolerance tests showed no differences in obese wild-type (WT) and obese Bach1-deficient mice after high-fat diet loading for 6 wk, suggesting minimal roles of Bach1 in the development of insulin resistance. In contrast, Bach1 deficiency significantly suppressed allooxan-induced pancreatic insulin content reduction and the resultant glucose elevation. Furthermore, TUNEL-positive cells in pancreatic islets of Bach1-deficient mice were markedly decreased, by 60%, compared with those in WT mice. HO-1 expression in islets was significantly upregulated in allooxan-injected Bach1-deficient mice, whereas expression of other antioxidative enzymes, e.g., catalase, superoxide dismutase, and glutathione peroxidase, was not changed by either allooxan administration or Bach1 deficiency. Our results suggest that Bach1 deficiency protects pancreatic β-cells from oxidative stress-induced apoptosis and that the enhancement of HO-1 expression plays an important role in this protection.

BTB and CNC homology 1; diabetes; insulin; pancreatic β-cells; oxidative stress; apoptosis

TYPE 2 DIABETES IS CAUSED BY COMPLEX INTERACTIONS BETWEEN insulin resistance in peripheral tissues and impaired insulin secretion from pancreatic β-cells (47). Exacerbation of oxidative stress (19) as well as endoplasmic reticulum stress (50) and hypoxic conditions (44) play key roles in the progressive deterioration of pancreatic β-cells during the development of diabetes. Pancreatic β-cells are a direct target of oxidative stress-mediated effects. Oxidants have been shown to negatively affect mitochondrial action in pancreatic β-cells, leading to impaired insulin secretion (25). However, pancreatic islets have very low intrinsic antioxidant enzyme capacity compared with other tissues (8), making islets more vulnerable to oxidative stress than other tissues. Therefore, both reducing oxidative stress and enhancing the antioxidative system may be beneficial strategies for preserving β-cell function.

On the other hand, oxidative stress also plays a crucial role in obesity-associated insulin resistance (46). Obesity increases reactive oxygen species (ROS) production via NADPH oxidase activation in adipose tissue (6). Excessive generation of ROS impairs glucose uptake into muscle (24) and adipose tissues (37) and is involved in the pathogenesis of hepatic steatosis (36), collectively leading to systemic insulin resistance. In addition, oxidative stress directly causes dysregulated production of adipocytokines such as adiponectin, plasminogen activator inhibitor-1, and monocyte chemotactic protein-1 (6), resulting in deterioration of insulin sensitivity. Thus, oxidative stress is closely related to obesity-associated insulin resistance and thereby contributes to the vicious cycle leading to the development of metabolic syndrome.

Transcription factor BTB and CNC homology 1 (Bach1) is a basic region leucine zipper transcription factor that suppresses oxidative stress responses (33). Bach1 forms a heterodimer with small Maf oncoproteins and binds to Maf recognition elements (MAREs) on the genome, thereby inhibiting transcription of oxidative stress-responsive genes, including heme oxygenase-1 (HO-1) (30), NADPH quinone oxidoreductase 1 (2), and thioredoxin reductase 1 (10). Expression of these genes is very low under normal conditions due to the dominant binding of Bach1 to MAREs. Once cells are subjected to oxidative stress, Bach1 loses its DNA-binding activity and is exported out of the nuclei. Instead of Bach1, NF-E2-related factor (Nfr) 2, another basic region leucine zipper transcription factor, rapidly accesses and binds to MAREs, resulting in activation of the transcription of oxidative stress-responsive genes (38). Indeed, in Bach1-deficient mice, HO-1 is constitutively expressed at higher levels in many tissues, indicating that Bach1 plays a major role in negative regulation of HO-1 expression (39).

It is particularly noteworthy that pharmacological enhancement of the HO-1 pathway reportedly exerts protective effects against the development of diabetes via several mechanisms (1). Upregulation of HO-1 protects pancreatic β-cells from oxidative injury induced by high glucose concentrations, resulting in preservation of insulin secretion in vitro (48). Administration of an HO-1 inducer decreases pancreatic superoxide contents, which in turn results in suppression of β-cell loss in nonobese diabetic (NOD) mice, a well-established model of type 1 diabetes (22). In addition to its effects on insulin secretion, HO-1 induction suppresses fat accumulation (21, 29) and insulin resistance (28) in obese rodents.

Previous studies have revealed Bach1-deficient mice to be protected from a wide range of pathological conditions, including myocardial infarction (51), atherosclerosis (45), steatohepatitis (12), intestinal injury (9), lung injury (43), keratinocyte differentiation (31), and neural tissue damage (16). Thus, Bach1 deficiency may protect mice against oxidative tissue damage. These findings prompted us to hypothesize that ablation of Bach1 would be a promising approach toward prevent-
ing the development of insulin resistance, insulin secretion impairment, and ultimately diabetes. Therefore, in this study, we examined the effects of Bach1 deficiency on glucose metabolism in two different murine models of diabetes, i.e., alloxan-induced pancreatic β-cell loss and high-fat diet (HFD)-induced insulin resistance.

METHODS

Animals. Animal studies were conducted in accordance with the institutional guidelines for animal experiments at The Center for Laboratory Animal Research, Tohoku University, which approved the experiments. Bach1-deficient mice were backcrossed with C57BL/6J mice (39). Wild-type (WT) and Bach1-deficient mice were kept on a 12:12-h light-dark cycle with free access to food and sterile water. For the purpose of oxidative stress loading of pancreatic β-cells, a 50 mg/kg dose of alloxan (2,4,5,6-tetraoxypyrimidine; Sigma-Aldrich, St. Louis, MO) was injected intravenously into fasted 8-wk-old mice (49). In detail, a 10 mg/ml alloxan-citrate solution was prepared with ice-cold 0.05 M citrate buffer (pH 4.3) immediately prior to injection. In the control group of mice, the same volume of citrate buffer was injected intravenously. Mice with HFD-induced obesity were obtained by 18-wk feeding of a HFD (32% safflower oil, 33.1% casein, 17.6% sucrose, and 5.6% cellulose) (14) beginning at 6 wk of age.

Analysis of glucose metabolism. Blood glucose was assayed with Antsense-III (Horiba Industry, Kyoto, Japan). Plasma insulin concentrations were determined using an ELISA kit (Morinaga, Tokyo, Japan). Glucose tolerance tests were performed on ad libitum-fed mice. The mice were given glucose (1 or 2 g/kg of body wt) intraperitoneally, followed by measurement of blood glucose levels. Insulin tolerance tests were performed on ad libitum-fed mice. The mice were injected intraperitoneally with human regular insulin (0.5 or 0.75 U/kg body wt; Eli Lilly, Kobe, Japan), followed by measurement of blood glucose levels (7).

Quantitative RT-PCR-based gene expression. Quantitative RT-PCR was performed as described previously (13). The relative amount of mRNA was calculated, employing GAPDH mRNA as the invariant control. The oligonucleotide primers are presented in Table 1.

Histological analysis. The pancreases from WT and Bach1-deficient mice were fixed with 10% formalin, embedded in paraffin, and sectioned. Sections were stained with hematoxylin and eosin (H & E).

Laser microdissection. Coronal cryostat sections (10 μm) of the pancreas were placed on PEN-coated slides (Leica Microsystems, Wetzlar, Germany). Laser microdissection was carried out on a Leica AS LMD (Leica Microsystems). Immediately after microdissection of each islet, the samples were stored at −80°C until RNA purification (15).

Table 1. Oligonucleotide primers used in quantitative RT-PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>HO-1 forward</td>
<td>5′-ATGGCCTGACCTTCGTACAGAG-3′</td>
</tr>
<tr>
<td>HO-1 reverse</td>
<td>5′-ATTTCCGCAAACGAGACTGTTG-3′</td>
</tr>
<tr>
<td>GAPDH forward</td>
<td>5′-TGAAGTCTGCTGTTGAGCC-3′</td>
</tr>
<tr>
<td>GAPDH reverse</td>
<td>5′-CCATCTGGGCGCTTGAATCT-3′</td>
</tr>
<tr>
<td>Catalase forward</td>
<td>5′-AGGTGTTAGGACGAGGAGGA-3′</td>
</tr>
<tr>
<td>Catalase reverse</td>
<td>5′-CCAGGTTGTTCCTTGGCACT-3′</td>
</tr>
<tr>
<td>SOD forward</td>
<td>5′-GGTCGTTCAAGATTGCTCT-3′</td>
</tr>
<tr>
<td>SOD reverse</td>
<td>5′-GCCAGTTAGCTTACCATGCC-3′</td>
</tr>
<tr>
<td>Gpx forward</td>
<td>5′-GCTGCCGAGCTTCTTGGAGA-3′</td>
</tr>
<tr>
<td>Gpx reverse</td>
<td>5′-GCTGCCGAGCCTTCTTGGAGA-3′</td>
</tr>
<tr>
<td>p22 phox forward</td>
<td>5′-CCATTCCGAGCTGTTGAGCCA-3′</td>
</tr>
<tr>
<td>p22 phox reverse</td>
<td>5′-TGGTTAGCTTCTTGGAGA-3′</td>
</tr>
<tr>
<td>gp91phox forward</td>
<td>5′-GTGCGCAGTTAGCTGTTGAGA-3′</td>
</tr>
<tr>
<td>gp91phox reverse</td>
<td>5′-GGTTGAGATGCTTCTTGGAGA-3′</td>
</tr>
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HO-1, heme oxygenase; SOD, superoxide dismutase; Gpx, glutathione peroxidase.

Apoptosis detection. At 24 h after alloxan injection, DNA fragmentation associated with apoptosis was detected in situ by the addition of nucleotides to free 3′-hydroxyl groups in DNA. Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining was performed using an in situ cell death detection kit (Roche, Mannheim, Germany). The results are expressed as the number of TUNEL-positive cells per islet, as reported previously.

Pancreatic insulin contents. Pancreases were suspended in cold acid ethanol (1.5% HCl in 75% ethanol), minced with scissors, and left at −20°C for 48 h, with sonication every 24 h. Insulin contents in the acid ethanol supernatant were determined with an ELISA kit (Morinaga) (41).

Statistical analysis. All statistical analyses were performed with the Statistical Package for the Social Sciences version 15.0 (SPSS Japan, Tokyo, Japan). All data were tested for normality by the Kolmogorov-Smirnov test. When data were distributed normally, the statistical significance of differences was assessed using the unpaired t-test and one-way ANOVA, followed by Tukey post hoc analyses. When data were not distributed normally, the statistical significance of differences was judged based on P values, using the Mann-Whitney U-test.

RESULTS

Bach1 deficiency exerted minimal effects on glucose metabolism under standard feeding conditions. First, we measured body weights and blood glucose levels until 12 wk of age, followed by analysis of glucose metabolism under standard feeding conditions. Body weights were similarly increased and fasting blood glucose levels were also similar in WT and Bach1-deficient mice (Fig. 1A). In addition, neither blood pressure nor plasma insulin differed significantly between the two groups at 12 wk of age (Fig. 1B). Furthermore, glucose and insulin tolerance tests revealed that Bach1 deficiency affected neither systemic glucose tolerance nor insulin sensitivity (Fig. 1, C and D). Histological findings with H & E staining revealed no apparent differences in pancreatic islet morphology between WT and Bach1-deficient mice (Fig. 1E). In addition, pancreatic insulin contents were also similar in Bach1-deficient and WT mice (Fig. 1F). Thus, Bach1 deficiency had no significant effects on systemic glucose metabolism under standard feeding conditions.

In Bach1-deficient mice, HO-1 is constitutively expressed at very high levels in many tissues, such as the liver, lungs, and heart (39). We next examined expression of HO-1 in organs/tissues responsible for glucose metabolism. As shown in Fig. 1G, HO-1 expression was markedly increased not only in the liver but also in pancreatic islets and white adipose tissue.

Bach1 deficiency minimally impacts obesity and insulin resistance. Pharmacological HO-1 upregulation reportedly prevents the development of diabetes by both maintaining pancreatic β-cell function (48) and decreasing adiposity and insulin resistance (21). Therefore, we next examined metabolic effects of Bach1 deficiency on insulin resistance in models of HFD-induced obesity. First, WT and Bach1-deficient mice were started on a HFD at 18 wk of age. In Bach1-deficient mice, body weights were similarly increased and blood glucose was elevated during the 6 wk of HFD feeding compared with WT mice (Fig. 2A). Furthermore, neither glucose tolerance nor systemic insulin sensitivity (Fig. 2B) differed between WT and Bach1-deficient mice after 6 wk of HFD loading. Fasting insulin levels were similarly increased during the 18 wk of HFD feeding in the two groups (Fig. 2C). These findings
Fig. 1. Transcription factor BTB and CNC homology 1 (Bach1) deficiency exerted minimal effects on glucose metabolism under standard feeding conditions. A: body weight and fasting blood glucose of wild-type (WT) (○; n = 12) and Bach1-deficient mice (KO) (●; n = 12) from 6 to 12 wk of age. B: blood pressure at 8 wk of age in WT (open bar; n = 16) and Bach1-deficient mice (black bar; n = 8). Fasting plasma insulin at 12 wk of age in WT (open bar; n = 12) and Bach1-deficient mice (black bar; n = 12). C: intraperitoneal glucose (2 mg/kg) tolerance tests at 10 wk of age in WT (○; n = 7) and Bach1-deficient mice (●; n = 7). D: insulin (0.5 U/kg) tolerance tests at 12 wk of age in WT (○; n = 9) and Bach1-deficient mice (●; n = 8). E: hematoxylin and eosin staining of pancreatic islets of WT (left) and Bach1-deficient mice (right) at 12 wk of age. F: insulin contents adjusted by pancreatic weights of WT (open bars) and Bach1-deficient mice (black bars). Data are presented as means ± SE. *P < 0.05 and **P < 0.01, assessed by unpaired t-test.
suggest minimal roles of Bach1 in the development of obesity and insulin resistance.

**Bach1 deficiency suppressed alloxan-induced pancreatic β-cell injury.** Next, to examine the effects of Bach1 deficiency on pancreatic β-cell injury induced by oxidative stress, we administered alloxan to Bach1-deficient mice at 8 wk of age. Alloxan is an oxidative stress agent that is relatively specific to pancreatic β-cells, and its administration is widely accepted as a model of diabetes caused by β-cell loss (18). When a 50 mg/kg dose of alloxan was administered to WT mice intravenously, fasting blood glucose gradually rose, reaching ~200 mg/dl on day 6 after alloxan administration was started (Fig. 3A). In contrast, fasting blood glucose levels were significantly lower in Bach1-deficient than in WT mice. In addition, glucose tolerance tests on day 6 after alloxan administration was started revealed that glucose elevation after a glucose load was consistently suppressed in Bach1-deficient mice compared with WT mice (Fig. 3B). Furthermore, pancreatic insulin contents were significantly higher, approximately twofold, in Bach1-deficient than in WT mice (Fig. 3C). Thus, Bach1 deficiency protected mice from alloxan-induced injury of pancreatic β-cells.

Oxidative stress is one of the most important causes of pancreatic β-cell death during the development of diabetes (19), and many studies have shown relationships between β-cell loss and ROS accumulation-induced pancreatic β-cell apoptosis (35). Therefore, to determine the effect of Bach1 on alloxan-induced β-cell apoptosis, we measured the number of TUNEL-positive cells in pancreatic islets 24 h after alloxan administration. At this time point, pancreatic islet sizes were similar in the two groups of mice (Fig. 4, A, C, E, G, and J). TUNEL-positive pancreatic β-cells were quite rare in both types of mice in the absence of alloxan injection (Fig. 4, B and F). Although TUNEL-positive cells were clearly detected in islets of both types of alloxan-treated mice (Fig. 4, D and H), the number of TUNEL-positive cells in Bach1-deficient mice was markedly decreased, being 60% less than in WT mice (Fig. 4I). These findings indicate that Bach1 deficiency protected pancreatic β-cells from oxidative stress-induced apoptosis.

In addition, using the laser microdissection procedure, we evaluated mRNA expression of genes related to oxidative stress. Alloxan administration-induced HO-1 upregulation in islets was enhanced in Bach1-deficient mice (Fig. 4K). In contrast, expression of other antioxidant enzymes, e.g., catalase, superoxide dismutase, and glutathione peroxidase, showed no significant changes in response to either alloxan administration or Bach1 deficiency (Fig. 4K). These findings, taken together, suggest that enhancement of HO-1 expression plays a major role in the antioxidant effect of Bach1 deficiency. In addition, expression of p22phox and gp91phox, both of which are subunits of NADPH oxidase, was decreased in pancreatic islets of alloxaнтreated Bach1-deficient mice compared with those of alloxan-treated WT mice (Fig. 4L). These findings suggest that decreased ROS production is also involved in pancreatic β-cell protection in Bach1-deficient mice.

**DISCUSSION**

The present study showed the effect of Bach1 deficiency on β-cell protection against acute loading of a chemical oxidant, alloxan, that reportedly induces apoptosis in pancreatic β-cells. Bach1 deficiency preserved insulin content and suppressed apoptosis of pancreatic islet cells, resulting in amelioration of alloxan-induced hyperglycemia. Thus, Bach1 in pancreatic β-cells is involved in oxidative stress-induced β-cell dysfunction and apoptosis, which may underlie the development of both type 1 and type 2 diabetes. In the present study, Bach1 deficiency decreased the alloxan-induced apoptosis of pancreatic islet cells. This protective effect against oxidative stress resulted in suppression of hyperglycemia in Bach1-deficient mice. These results indicate that Bach1 deficiency-induced HO-1 upregulation prevented pancreatic β-cell deterioration induced by oxidative stress.

A putative mechanism underlying Bach1 deficiency-induced protection of pancreatic β-cells is HO-1 upregulation. HO-1 has been identified as a ubiquitous stress protein upregulated in many cell types by various stimulants, including hemolysis, inflammatory cytokines, oxidative stress, and heat shock (26).
HO-1 expression is induced by the Nrf2 system, which is suppressed in the absence of oxidative stress stimuli (17). HO-1 is the rate-limiting enzyme in the heme degradative pathway that catalyzes the oxidation of heme into biliverdin, carbon monoxide (CO), and free iron. CO as well as biliverdin and its metabolite bilirubin have antioxidant activities in vivo (32). Thus, HO-1 plays a crucial role in protection from oxidative stress (5). Induction or transgenic overexpression of HO-1 reportedly renders pancreatic islets resistant to apoptosis in islet transplantation models (34) and NOD mice (11). Administration of an HO-1 inducer decreases pancreatic superoxide contents, leading to suppression of β-cell reduction in NOD mice (22). In the present study, we observed that Bach1 deficiency induced constitutive expression of HO-1 in pancreatic islets. In addition, alloxan-induced HO-1 upregulation was markedly promoted in Bach1-deficient mice. In contrast, other antioxidant enzymes were not significantly upregulated in pancreatic islets by either Bach1 deficiency or alloxan administration. Taken together, these observations suggest that HO-1 plays an important role in the antioxidant effects of Bach1 deficiency. Since p22phox expression is reportedly upregulated by ROS (3), the observed p22phox downregulation suggests decreased ROS levels in pancreatic islets of Bach1-deficient mice. Thus, antioxidant effects induced by Bach1 deficiency may further suppress ROS production. In addition, expression of gp91phox, which reportedly plays a major role in ROS production in pancreatic β-cells (23), was downregulated in pancreatic islets of Bach1-deficient mice. Although the mechanism whereby Bach1 deficiency suppresses gp91phox expression is unclear, suppression of ROS production by downregulation of NADPH oxidase may also contribute to the pancreatic β-cell protection mediated by Bach1 deficiency. Thus, Bach1 inhibition by pharmacological strategies is a potential therapeutic target for pancreatic β-cell protection against oxidative stress, although pharmacological reagents that suppress Bach1 expression and/or function have not yet been developed.

Chemical HO-1 inducers reportedly improve insulin resistance in murine obesity models (21, 28, 29). Unexpectedly, however, Bach1 deficiency had no impact on either insulin resistance or obesity even after long-term HFD loading. Thus, in contrast to acute induction of HO-1, congenital prolonged high expression of HO-1 might have less impact on insulin sensitivity. HO-1 is an inducible protein and is thought to act as a defense mechanism against acute oxidative injuries such as inflammation, ischemia, and radiation. Because the cytoprotective function of HO-1 is manifested by reaction products such as bilirubin and CO, amounts of the substrates heme and/or oxygen could be the limiting factor in HO-1 function (42). It is possible that depletion of free heme (i.e., not bound to proteins) due to constitutive HO-1 expression would cancel the effects on insulin resistance in Bach1-deficient mice. This may also explain the differential effects of Bach1 deficiency on acute injury of β-cells and chronic development of insulin resistance. In addition, differences in HO-1 expression levels may contribute to the diversity of effects in various tissues/organs. According to an HO-1 transgenic study, low levels (2- to 5-fold) of HO-1 overexpression are protective, whereas moderate levels (10- to 15-fold) do not modify the cell injury caused by hyperoxia, and high-level (≥15-fold) overexpression actually exacerbates the damage (40). In the present study, the HO-1 expression levels in Bach1-deficient mice were increased 12-fold in the liver and 30-fold in white adipose tissue, whereas the increase was sixfold in pancreatic islets. Thus, constant and markedly elevated HO-1 expression may deplete the necessary heme substrate, resulting in minimal effects of Bach1 deficiency on metabolism. It has been reported recently that sustained expression of HO-1 in cultured cells leads to profound changes to cellular iron homeostasis (20). Such adaptive changes may also suppress the protective function of HO-1. Alternatively, a pathway(s) other than HO-1 may exacerbate HFD-induced insulin resistance in Bach1-deficient mice, thereby cancelling out the beneficial effects of HO-1. For instance, Bach1 was reported to inhibit p53 transcriptional activity (4). Overexpression of p53 in adipose tissue reportedly causes insulin resistance via elevated inflammatory responses (27). Collectively, chronic HO-1 elevation showed minimal effects on the development of insulin resistance induced by high-fat feeding. Thus, the proper levels and duration of pharmacological HO-1 induction may be critical to mediating beneficial effects of HO-1.
Fig. 4. Suppression of apoptosis of pancreatic islet cells and upregulation of HO-1 expression in alloxan-injected KO mice. Alloxan or citrate buffer (control) was injected into 8-wk-old WT and KO mice. A–H: hematoxylin and eosin staining and TUNEL staining of pancreases taken 24 h after injection of 50 mg/kg alloxan. Paired adjacent slices were used for hematoxylin and eosin and TUNEL staining. A and B: WT control. C and D: alloxan-injected WT. E and F: KO control. G and H: alloxan-injected KO mice. I and J: nos. of TUNEL-positive cells/islet slice and mean islet areas in alloxan-injected mice. The average islet count was 12/slice for WT and 14/slice for Bach1-deficient mice. WT (open bars; n = 36) and KO islets (black bars; n = 42) were measured. K: quantitative RT-PCR for HO-1, catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx) of pancreatic islets 2 h after alloxan or citrate buffer injection. WT control (left open bars; n = 4), KO control (left black bars; n = 4), alloxan-injected WT (right open bars; n = 5), and alloxan-injected KO mice (right black bars; n = 6) were studied. L: quantitative RT-PCR for p22phox and gp91phox of pancreatic islets 2 h after alloxan. Alloxan-injected WT (open bar; n = 4) and alloxan-injected KO mice (black bar; n = 6) were studied. Data are presented as means ± SE. *P < 0.05, assessed by Mann-Whitney U-test (I) and 1-way ANOVA, followed by Tukey post hoc analyses (K); **P < 0.01, assessed by unpaired t-test (L).
Protec7ive e7ects of Bach1 deficic7y in pancreacic β-cells

K. K., J.G., and S.S. performed the experiments; K.K., J.G., and S.S. analyzed the data; K.K. prepared the figures; Y.I., Y.O., K.I., and H.R. contributed to the conception and design of the research; Y.I. and H.K. drafted the manuscript; T.Y., J.I., A.M., and H.K. interpreted the results of the experiments.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

K.K., J.G., and S.S. performed the experiments; K.K., J.G., and S.S. analyzed the data; K.K. prepared the figures; Y.I., Y.O., K.I., and H.R. contributed to the conception and design of the research; Y.I. and H.K. drafted the manuscript; T.Y., J.I., A.M., and H.K. interpreted the results of the experiments.

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