Effects of Biotin on Glucotoxicity or Lipotoxicity in Rat Pancreatic Islets

H. Yoshikawa, Y. Tajiri, Y. Sako, T. Hashimoto, F. Umeda, and H. Nawata

Biotin (vitamin H) plays an important role as a cofactor in glucose or lipid metabolism. We showed that biotin potentiated glucose-induced insulin release in isolated rat islets, while biotin alone did not affect insulin release. Coculture with biotin in islets for 48 hours significantly enhanced glucose-induced insulin release or islet insulin content. Similarly, preproinsulin or pancreatic/duodenal homeobox-1 (PDX-1) mRNA was also enhanced in islets cultured with biotin for 48 hours. Furthermore, we measured effects of biotin on β-cell function under glucotoxic or lipotoxic states. In islets cultured with high glucose or palmitate for 48 hours, glucose-induced insulin release or islet insulin content deteriorated. Coculture with biotin significantly restored glucose-induced insulin release or islet insulin content together with the restoration of preproinsulin or PDX-1 mRNA. We conclude that biotin exerts its beneficial effects on β-cell dysfunction induced by glucose or free fatty acids probably through the enhancement of insulin biosynthesis.

IT IS WELL KNOWN that the diabetic state induces desensitization specific for glucose in pancreatic β cells.[1] Glucose infusion to nondiabetic rats leads to hyperglycemia and produces glucose desensitization (glucotoxicity).[2] Moreover, isolated rat islets cultured with high glucose showed similar glucose desensitization to in vivo experiments.[3,4] Glucose desensitization observed in those models mimics the loss of sensitivity to glucose observed in type 2 diabetes mellitus.[5-7] In most cases, type 2 diabetes mellitus is associated with obesity and increased levels of circulating free fatty acids.[8] In healthy individuals, the levels of free fatty acid usually range between 0.2 to 0.7 mmol/L, whereas in diabetics, those are higher and reach 1.0 mmol/L.[9-11] Zhou and Grill[12] first demonstrated the long-term inhibitory effects of free fatty acid on β-cell functions. Indeed, they showed that insulin release or content was impaired after a 48-hour exposure of islets to free fatty acid (lipotoxicity).[12]

Biotin (vitamin H: $C_{10}H_{16}N_2$), which is initially isolated from egg yolk as enzymatic yeast growth factor,[13] is a member of the vitamin B group and plays an important role in metabolism of carbohydrates, lipids, and proteins.[14] Recently, it has been reported that biotin administration improved insulin release by oral glucose load in biotin-deficient rats with no change of insulin content in pancreatic islets.[15] Maebashi et al[16] demonstrated that in type 2 diabetes mellitus biotin concentration in blood was low, and biotin administration restored normoglycemia. Sone et al[17] showed that biotin evoked glucose-induced insulin release in rat pancreatic islets. In the present study, we investigated effects of biotin on β-cell dysfunction caused by glucose or free fatty acid in rat pancreatic islets.

MATERIALS AND METHODS

Materials

Biotin was from FUSO Pharmaceutics Industries (Osaka, Japan). Glucose, histopaque, and Dulbecco's Modified Eagle's Medium (DMEM) were from Sigma Chemical (St Louis, MO). Rat insulin was from Cosmo Bio (Tokyo, Japan). Collagenase was from Boehringer Mannheim (Mannheim, Germany). Penicillin, streptomycin, and fetal calf serum (FCS) were from Life Technology (Grand Island, NY). Tissue culture flasks were from Falcon (Plymouth, England).

Animals

Female Wistar rats (150 to 250 g of body weight) were bred under pathogen-free conditions at the Kyushu University Animal Center, Fukuoka, Japan. They had free access to tap water and standard pelleted chow (Clea Japan, Tokyo, Japan). They were exposed to a 12-hour light (6 AM to 6 PM), 12-hour dark cycle. All experiments were approved by the ethics committee for animal experiments at the Faculty of Medicine, Kyushu University and performed according to the Guidelines for Animal Experiments of the Faculty of Medicine, Kyushu University, as well as Law No. 105 and Notification No. 6 of the Japanese Government.

Isolation of Islets

Pancreatic islets were isolated by collagenase digestion as previously described.[18] Collagenase was injected into the common bile duct at a concentration of 2 mg/mL in 10 mL Hanks solution. The pancreas was digested at 37°C for 20 minutes. Islets were partially separated from exocrine tissue using gradient centrifugation (1,000 × *g*, 20 minutes at 4°C) in histopaque. Islets were transferred to DMEM containing 5.5 mmol/L glucose and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) and 10% FCS. Islets were cultured free floating at 37°C with an atmosphere of 5% CO_2/95% air for 24 hours (primary culture) to remove exocrine or other tissues.

Islet Culture

After the period of primary culture, islets were selected under microscope and transferred into tissue culture flasks. Culture was performed in DMEM containing with or without 27 mmol/L glucose, 250 μmol/L palmitate, and 1 μmol/L biotin. Islets were cultured for 48 hours at 37°C in an humidified atmosphere of 5% CO_2/95% air. Palmitate was dissolved in 95% ethanol before being added to the cultured media. The final concentration of ethanol in medium was 0.1% at the concentration of palmitate used in this study. The control condition of 0.1% ethanol during culture was included in each experiment.

Insulin Release

After the period of primary culture or each culture period, islets were preincubated at 37°C for 30 minutes in Krebs-Ringer bicarbonated (KRB) medium[19] with the following compositions: 143 mmol/L Na^+, 5.8 mmol/L K^+, 2.5 mmol/L Ca^+, 1.2 mmol/L Mg^{2+}, 124.1 mmol/L

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Cl^-, 1.2 mmol/L SO_4^{3-}, and 25 mmol/L CO_3^{2-}, pH 7.4, supplemented with 10 mmol/L HEPES, 0.2% bovine serum albumin (BSA), and 3.3 mmol/L glucose. Islets were selected after preincubation in batches of 3 islets in 300 μL KRB containing either 3.3 mmol/L or 27 mmol/L glucose with or without various concentrations of biotin. Final incubations were then performed at 37°C for 60 minutes in a water bath with continuous shaking and in an humidified atmosphere of 5% CO_2/95% air. At the end of incubation, aliquots of the incubation medium were removed for assay of insulin concentrations. Islets that had been exposed to 3.3 mmol/L glucose in the final incubation were retrieved for determination of islet insulin content.

Insulin Assay

Insulin was measured by radioimmunoassay (RIA) using rat insulin as standard. For the determination of islet insulin content, 3 islets were transferred into 200 μL acid-ethanol (0.18 mol/L HCl in 95% ethanol). Insulin was extracted overnight at 4°C after sonication as previously described.[20]

Measurement of mRNA Levels

Total RNA was extracted by the TRIzol isolation method (Life Technologies, Gaithersburg, MD) from approximately 100 isolated islets in each group of cultured islets. The mRNA levels of proteins (described previously) were measured by using semiquantitative polymerase chain reaction (PCR). Total RNA was reverse-transcribed by random priming using Avian Myeloblastosis Virus reverse transcriptase (RT) (first-strand DNA synthesis) according to the manufacturer's instructions. A total of 1 μL of RT reaction mix was amplified with primers in a total volume of 50 μL. Primers specific for preproinsulin are 5′-TGC CCG GGC TTT TGT CAA AC-3′ (sense) and 5′-CTC CAG TGC CAA GGT CTG AA-3′ (antisense),[21] those for pancreatic/duodenal homeobox-1 (PDX-1) are 5′-GAG CAG GAT TGT GCC GTA ACC-3′ (sense) and 5′-CTC AAA GTT TTC AGA AGC TCG-3′ (antisense),[21] and those for ß-actin are 5′-CGT AAA GAC CTC TAT GCC AA-3′ (sense) and 5′-AGC CAT GCC AAA TGT GTC AT-3′ (antisense).[22] The samples were amplified in 18 to 20 cycles for preproinsulin, 30 to 32 cycles for PDX-1 or ß-actin, using the following parameters: 92°C for 45 seconds, 55°C for 45 seconds, and 72°C for 1 minute. Aliquots (10 μL) of the PCR products were tested on 1% agarose gels. Gels were stained with ethidium bromide. Signals were quantified by scanning densitometry using NIH Image 1.56 software (NIH, Bethesda, MD).

Presentation of Results

All results are presented as mean ± SEM of more than 4 experiments. Data were analyzed by Student's *t* test (paired) or 1-way analysis of variance (ANOVA) with Fisher's least significant difference test. *P* values less than .05 were considered to indicate significant differences.

RESULTS

Effects of Biotin on β-Cell Functions

A total of 1 μmol/L biotin enhanced 16.7 mmol/L or 27 mmol/L glucose-induced insulin release (Fig 1). However, the higher concentration of biotin alone did not enhance 3.3 mmol/L glucose-induced insulin release. In islets cultured with 1 μmol/L biotin for 48 hours, 27 mmol/L glucose-induced insulin release or islet insulin content were significantly enhanced (Fig 2). Furthermore, preproinsulin or pancreatic/duodenal homeobox-1 (PDX-1) mRNA increased in islets cultured with biotin for 48 hours (Fig 2).

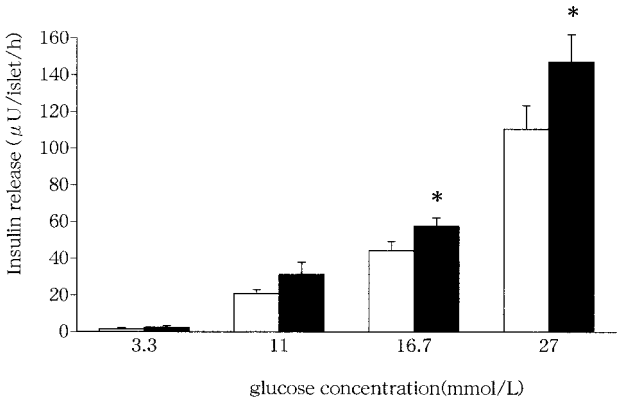

Fig 1. Insulin release at 3.3 to 27 mmol/L glucose with or without 1 μmol/L biotin for 1 hour. Data are expressed as mean ± SEM of 4 experiments. Open bar: islets incubated without biotin. Closed bar: islets incubated with biotin. *$P < .05$ *v* 0 μmol/L biotin.

Effects of Biotin on β-Cell Dysfunction Induced by Glucose or Free Fatty Acids

In islets cultured with 27 mmol/L glucose for 48 hours, 3.3 mmol/L glucose-induced insulin release was increased and 27 mmol/L glucose-induced insulin release was impaired, which showed glucose desensitization, and islet insulin content was markedly impaired. However, coculture with 1 μmol/L biotin partially enhanced 27 mmol/L glucose-induced insulin release. Similar results were obtained in islets cultured with 250 μmol/L palmitate for 48 hours. Coculture with 1 μmol/L biotin almost completely restored the impaired 27 mmol/L glucose-induced insulin release or islet insulin content to the level without palmitate (Fig 3). Similarly, preproinsulin or PDX-1 mRNA content of islets were also significantly impaired after a 48-hour culture period with high glucose or palmitate. Coculture with 1 μmol/L biotin again almost completely restored the aggravated mRNA content (Fig 4).

DISCUSSION

In this study, we showed that biotin alone did not affect insulin release, whereas biotin significantly enhanced high–glucose-induced insulin release. These findings are consistent with the fact that biotin plays an important role as a cofactor in glucose or lipid metabolism. It is suggested that enzymes activated by biotin may potentiate glucose-induced insulin release. Biotin affects 2 critical enzymes in glucose metabolism, glucokinase and phosphoenolpyruvate carboxykinase (PEPCK),[23,24] whereas PEPCK does not exist in islets.[25] It was reported that biotin enhanced cyclic guanosine monophosphate (GMP) level, which stimulates the synthesis of glucokinase in rat liver.[26] Romero-Navarro et al[27] demonstrated that coculture with biotin enhanced the enzyme activity of glucokinase and increased insulin release in rat islets. It is thus plausible that the enhancement of glucokinase induced by biotin may potentiate insulin release. Biotin is also essential as a cofactor of acetyl-CoA carboxylase or pyruvate carboxylase.[14] Acetyl-CoA carboxylase catalyzes the formation of manonyl-CoA, a metabolite that has been proposed to play an important role in the metabolic signal transduction for insulin secretion.[28] Pyruvate carboxy-

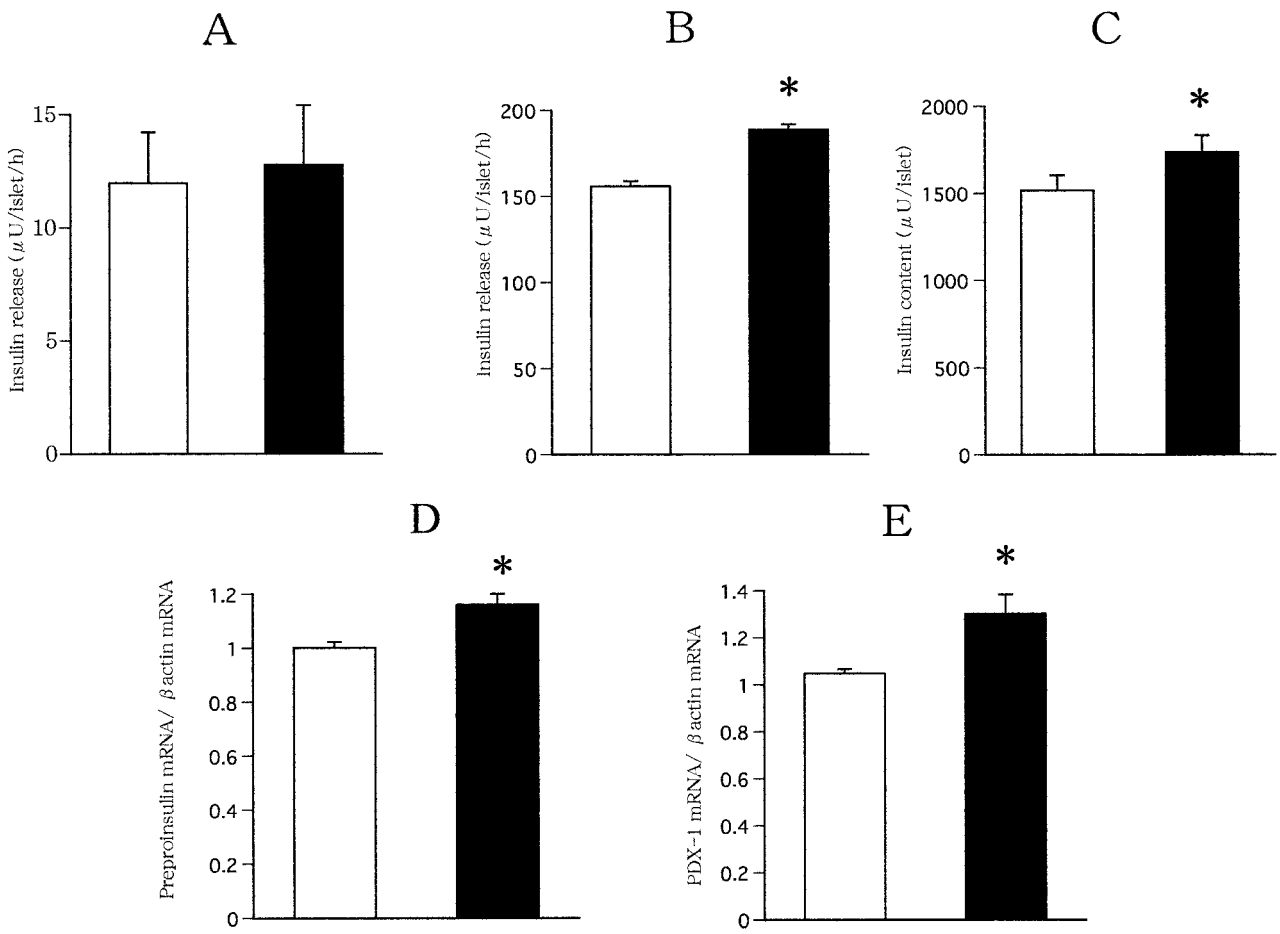

Fig 2. Glucose-induced insulin release, insulin content, and preproinsulin or PDX-1 mRNA content in islets cultured with or without biotin for 48 hours. (A) Represents 3.3 mmol/L glucose-induced insulin release. (B) Represents 27 mmol/L glucose-induced insulin release. (C) Islet insulin content. (D) Preproinsulin mRNA. (E) PDX-1 mRNA. Open bar: islets cultured without biotin. Closed bar: islets cultured with 1 μmol/L biotin. Data are expressed as mean ± SEM of 4 experiments. *$P < .05$ *v* islets without biotin.

lase plays a key role in the conversion of pyruvate to oxaloacetate and in generating cytosolic nicotinamide adenine dinucleotide phosphate (NADPH) in β cells through a pyruvate-malate shuttle.[29] Thus, the enhancement of those enzymes induced by biotin might increase glucose- induced insulin release in the early culture period because insulin accumulation was increased, and islet insulin content was decreased in islets cultured with biotin for 8 hours (data not shown). Further studies are necessary to solve the mechanism by which biotin potentiates glucose-induced insulin release.

On the other hand, after a 48 hour-culture period, coexistence of biotin enhanced glucose-induced insulin release or insulin content. Coculture with biotin also enhanced PDX-1 or preproinsulin mRNA, suggesting biotin's effects on insulin biosynthesis. Our results are in line with a previous report that biotin produced a modest and significant increase of insulin mRNA.[27] It has been reported that when the rate of glucose phosphorylation is increased, glycolysis is accelerated and the insulin promoter is activated.[30] Therefore, the augmentation of glucose phosphorylation produced by biotin induction might account for the increase of insulin biosynthesis observed in this study.

We further investigated the effects of biotin on glucotoxicity or lipotoxicity. Low–glucose-induced insulin release was increased and high–glucose-induced insulin release or islet insulin content was significantly decreased in high–glucose-cultured islets. Similar results were obtained in palmitate-cultured islets. Gene expressions of preproinsulin or PDX-1 were also decreased in high–glucose- or palmitate-cultured islets for 48 hours. These results imply that β-cell dysfunction caused by exposure to glucose or palmitate is attributable to the inhibition of insulin biosynthesis. Olson et al[31] reported that chronic exposure of β-cell lines to high–glucose-impaired insulin gene promoter activity accompanied the decreases of PDX-1 binding activity. Gremlich et al[32] also reported that the exposure of isolated islets to palmitate for 48 hours decreased PDX-1 and insulin mRNA levels. Those reports were in line with our results. Coculture with biotin modestly, but significantly, improved the impaired insulin release or islet insulin content in high–glucose- or palmitate-cultured islets, together with the restoration of preproinsulin or PDX-1 mRNA content. These results indicate that potent effects of biotin on glucotoxicity or lipotoxity are probably through the enhancement of insulin biosynthesis.

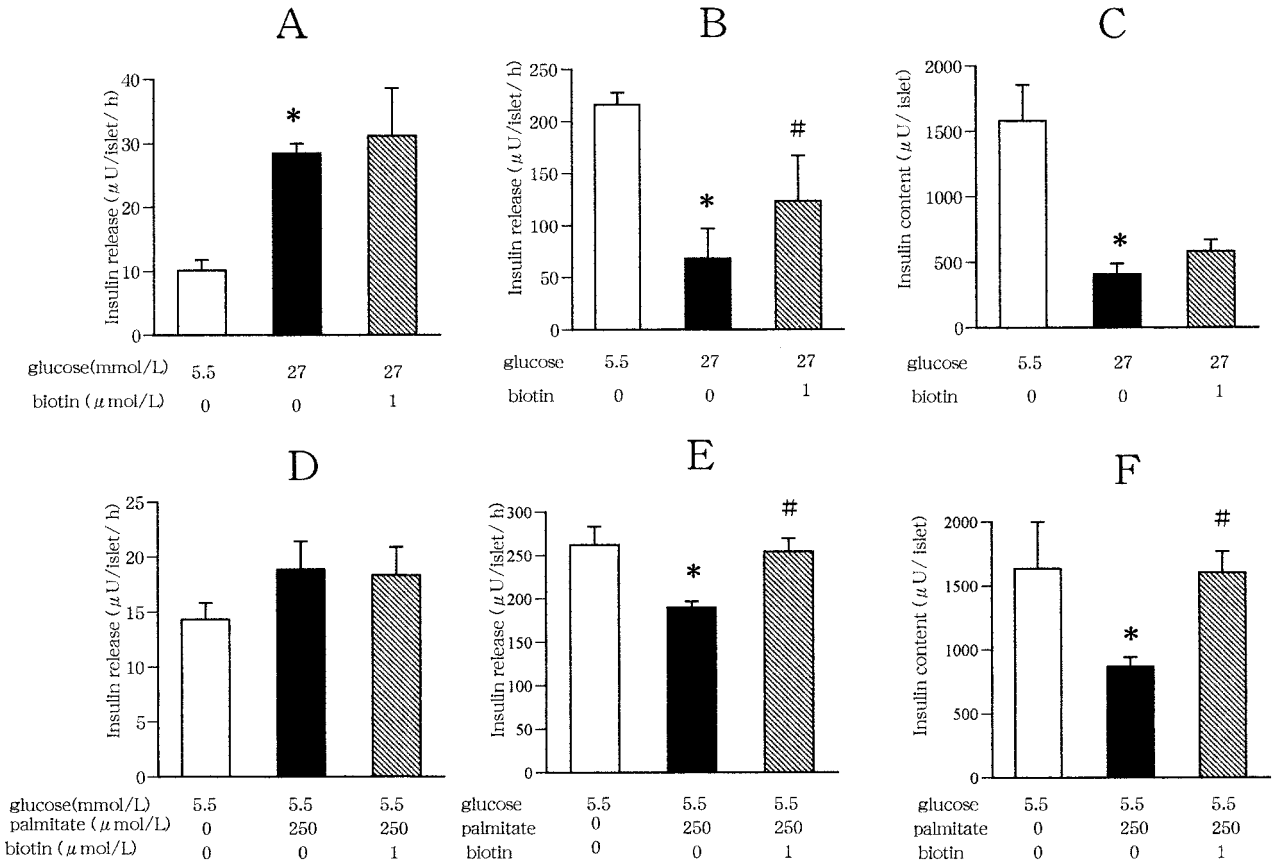

Fig 3. Glucose-induced insulin release and insulin content in islets cultured with 27 mmol/L glucose or 250 μmol/L palmitate and with or without 1 μmol/L biotin for 48 hours. (A and D) Represents 3.3 mmol/L glucose-induced insulin release. (B and E) Represents 27 mmol/L glucose-induced insulin release. (C and F) Represents islet insulin content. Open bar: islets cultured with 5.5 mmol/L glucose alone. Closed bar: islets cultured with 27 mmol/L glucose or 250 μmol/L palmitate. Hatched bar: islets cultured with 27 mmol/L glucose or 250 μmol/L palmitate plus 1 μmol/L biotin. Mean ± SEM of 4 experiments with biotin. Data are expressed as mean ± SEM of 4 experiments. *$P < .05$ *v* islets with 5.5 mmol/L glucose alone. #$P < .05$ *v* islets cultured with 27 mmol/L glucose or 250 μmol/L palmitate.

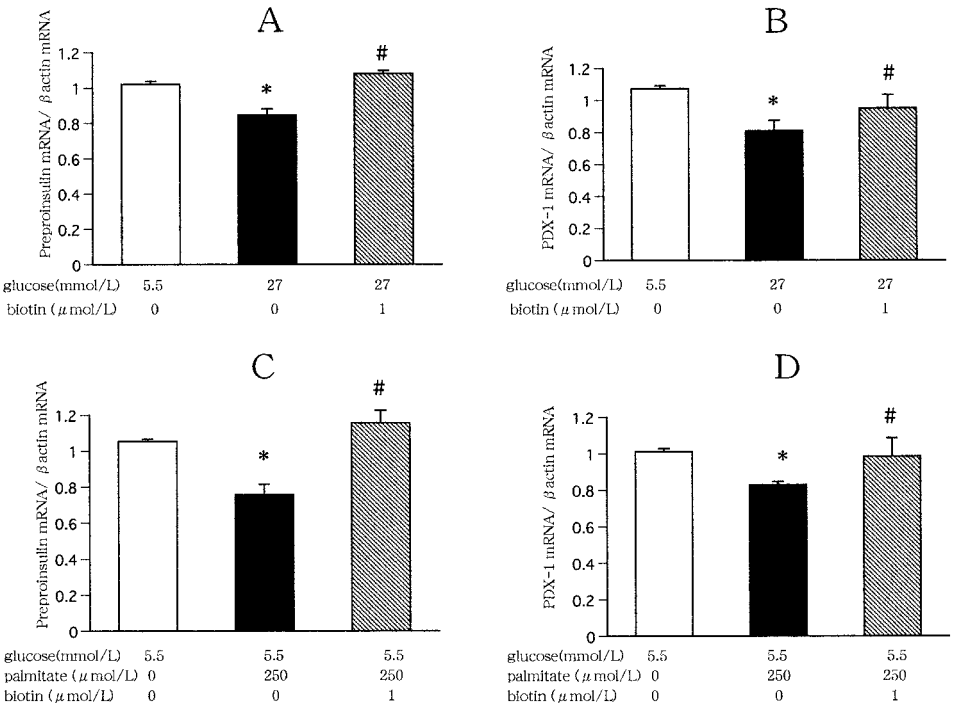

Fig 4. Quantification of preproinsulin, PDX-1 mRNA expression by semiquantitative RT-PCR in islets cultured for 48 hours. (A and C) Represent preproinsulin mRNA. (B and D) Represent PDX-1 mRNA. Open bar: islets cultured with 5.5 mmol/L glucose alone. Closed bar: islets cultured with 27 mmol/L glucose or 250 μmol/L palmitate. Hatched bar: islets cultured with 27 mmol/L glucose or 250 μmol/L palmitate plus 1 μmol/L biotin. Mean ± SEM of 4 experiments with biotin. *$P < .05$ *v* islets with 5.5 mmol/L glucose alone. #$P < .05$ *v* islets cultured with 27 mmol/L glucose or 250 μmol/L palmitate.

A defect in biotin metabolism could contribute to diabetes. Maebashi et al[16] demonstrated that in patients with type 2 diabetes mellitus serum biotin concentrations were significantly lower than that in control subjects, and that there was an inverse correlation between serum biotin concentration and fasting blood glucose level. On the other hand, biotin treatment could ameliorate the diabetic state. It has been demonstrated that biotin administration improved the impaired glucose tolerance in Otsuka Long-Evans Tokushima Fatty (OLETF) rats (a spontaneously model rat with type 2 diabetes mellitus).[33] It is possible that biotin also improves insulin resistance. In clinical studies, Coggeshall et al[34] demonstrated that a pharmacologic dose of biotin (16 mg/day for 1 week) lowered the fasting blood glucose concentration in patients with type 1 diabetes mellitus during insulin withdrawal. Similar results were obtained in patients with type 2 diabetes mellitus, in which fasting blood glucose levels were decreased by 45% by the administration of biotin in a pharmacologic dose for 1 month.[16] These findings suggest therapeutic effects of biotin for the treatment of patients with diabetes mellitus.

In this study, we used 1 μmol/L concentration of biotin, which is much higher than the generally accepted plasma concentration of less than 0.01 μmol/L.[35] Preliminary experiments (data not shown) indicate that coculture with 0.1 μmol/L biotin enhanced insulin release in glucose-desensitized islets. Maebashi et al[16] reported that the serum biotin level in normal subjects was 0.098 μmol/L and in diabetic patients, it was lower (0.056 μmol/L). They also reported that the patients with diabetes mellitus who take 9 mg biotin daily showed improvement of blood glucose. Many investigators who are engaged in the effects of biotin on insulin release in other aspects, used 1 μmol/L biotin in in vitro experiments.[26,27,36] Therefore, we selected that concentration in the current experiments.

In conclusion, biotin has its beneficial effects on glucotoxicity or lipotoxicity in pancreatic islets probably through the enhancement of insulin biosynthesis. Further investigations are necessary to confirm the therapeutic effects of biotin for diabetic patients because in vitro studies have all used concentrations of biotin that far exceed values measured in plasma or pancreatic tissue.

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