Biotin uptake by mouse and human pancreatic beta cells/islets: a regulated, lipopolysaccharide-sensitive carrier-mediated process

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Ghosal A, Sekar TV, Said HM. Biotin uptake by mouse and human pancreatic beta cells/islets: a regulated, lipopolysaccharide-sensitive carrier-mediated process. Am J Physiol Gastrointest Liver Physiol 307: G365–G373, 2014. First published June 5, 2014; doi:10.1152/ajpgi.00157.2014.—Biotin is essential for the normal health and function of pancreatic beta cells. These cells obtain biotin from their surroundings via transport across their cell membrane. Little is known about the uptake mechanism involved, how it is regulated, and how it is affected by internal and external factors. We addressed these issues using the mouse-derived pancreatic beta-TC-6 cells and freshly isolated mouse and human primary pancreatic beta cells as models. The results showed biotin uptake by pancreatic beta-TC-6 cells occurs via a Na⁺-dependent, carrier-mediated process, that is sensitive to des-thiobiotoin, as well as to pantothenic acid and lipote; the process is also saturable as a function of concentration (apparent $K_m = 22.24 \pm 5.5 \mu M$). These cells express the sodium-dependent multivitamin transporter (SMVT), whose knockdown (with doxycycline-inducible shRNA) led to a sever inhibition in biotin uptake. Similarly, uptake of biotin by mouse and human primary pancreatic islets is Na⁺-dependent and carrier-mediated, and both cell types express SMVT. Biotin uptake by pancreatic beta-TC-6 cells is also adaptively regulated (via transcriptional mechanism) by extracellular substrate level. Chronic treatment of pancreatic beta-TC-6 cells with bacterial lipopolysaccharides (LPS) leads to inhibition in biotin uptake. This inhibition is mediated via a Toll-Like receptor 4-mediated process and involves a decrease in membrane expression of SMVT. These findings show, for the first time, that pancreatic beta cells/islets take up biotin via a specific and regulated carrier-mediated process, and that the process is sensitive to the effect of LPS.

bland; pancreatic beta cells; biotin uptake mechanism; transport regulation

THE WATER-SOLUBLE VITAMIN BIOTIN, also known as vitamin B7 or vitamin H, is required for normal cellular functions, growth, and development. Biotin acts as a cofactor (as a carboxyl carrier) for five carboxylases that catalyze indispensable steps in intermediate metabolism including gluconeogenesis and fatty acid metabolism (reviewed in Refs. 34, 35). Emerging evidences also point to a role for biotin in normal immune function and in the regulation of gene expression (reviewed in Refs. 24, 29). In reference to the latter, biotin status has been shown to influence the expression of more than 2,000 human genes, at both the transcriptional and post-transcriptional levels (3, 30, 44). The affected genes include those that are critical for maintaining the differentiated phenotype of pancreatic beta cells, preservation of their mass (i.e., their proportion), and for insulin secretion (3, 30, 38, 44). In reference to the latter, the vitamin has been shown to increases the expression of proce-
measured in cells incubated in Krebs-Ringer buffer (pH 7.4 unless otherwise stated) at 37°C as described before (23); uptake was linear as a function of time for 8 min (data not shown). Uptake was expressed in term of milligram cellular protein per unit time (i.e., femtomoles per milligram protein per minute). Protein concentrations were determined using a Bio-Rad DC protein assay kit (Hercules, CA).

Preparation of mouse and human primary pancreatic islets for uptake studies. Mouse primary pancreatic islets were isolated by enzymatic digestion followed by Ficoll gradient centrifugation method as reported by us before (23). Briefly, mice (8–15 wk old) were euthanized using ketamine and xylazine (Intraperitoneal injection of 100 and 10 mg/kg, respectively). The pancreas was isolated, minced, and digested with collagenase IV (1 mg/ml) containing DNase I (0.1 mg/ml) for 30 min with occasional shaking at 37°C. After digestion, pancreatic tissues were filtered through nylon mesh and the filtrate was subjected to discontinuous Ficoll gradient (25, 23, 20, and 11%, respectively) for isolation of islets. Islets were collected from the interface of 20 and 11% of Ficoll, subsequently washed three times, and used for uptake studies as described before (23). Cell viability was assessed by using Trypan blue, with the result showing 85–90% viability.

Primary human pancreatic islets were obtained from healthy adult organ donors (National Disease Research Interchange, Philadelphia, PA). After receiving the islets, samples were centrifuged (1,500 rpm for 8 min), washed, and resuspended in KR buffer, then used for uptake/molecular investigations. Islet viability was checked by Trypan blue and found to be around 70%.

Quantitative real-time PCR. Total RNA samples were isolated using TRIzol (Invitrogen, Carlsbad, CA) method as described in the manufacture’s protocol. The isolated RNA samples were subjected to DNaseI treatment to remove DNA contamination, and first strand c-DNAs were synthesized using iScript reverse transcriptase kit (Bio-Rad). Relative gene expression was measured by quantitative real-time PCR using gene-specific primers, and levels were normalized to rat 18s rRNA. The initial rate of biotin uptake by pancreatic beta-T6 cells was significantly (P < 0.01) higher at physiological pH 7.4 than at pH 6.5 and 5.5 (as percentage to uptake at pH 7.4: 100 ± 0.88, 65.16 ± 3.19, 48.32 ± 4.46, respectively). We examined the effect of unlabeled biotin, its structural analog desthiobiotin, and that of lipote and pantethionic acid (all at 500 µM) on the initial rate of 3H-biotin (5 nM) uptake and observed a significant (P < 0.01 for all) inhibition in 3H-biotin uptake in the presence of all these compounds (as percentage to uptake at pH 7.4: 100 ± 3.84, 13.57 ± 2.44, 13.72 ± 4.61, 12.67 ± 3.71, and 11.57 ± 3.13 for control and in the presence of unlabeled biotin, desthiobiotin, lipote, and pantethionic acid, respectively).

Similar findings were obtained with freshly isolated primary mouse pancreatic islets in that the initial rate of biotin uptake was significantly (P < 0.05) higher at physiological pH 7.4 compared with pH 6.5 and 5.5 (11.1 ± 0.42, 8.73 ± 0.46, and 3.61 ± 0.65 fmol·mg protein⁻¹min⁻¹ in the presence and absence of Na⁺, respectively). The initial rate of biotin uptake by pancreatic beta-T6 cells was also pH dependent with a significantly (P < 0.01) higher uptake at pH 7.4 than at pH 6.5 and 5.5 (as percentage to uptake at pH 7.4: 100 ± 0.88, 65.16 ± 3.19, 48.32 ± 4.46, respectively). We examined the effect of unlabeled biotin, its structural analog desthiobiotin, and that of lipote and pantethionic acid (all at 500 µM) on the initial rate of 3H-biotin (5 nM) uptake and observed a significant (P < 0.01 for all) inhibition in 3H-biotin uptake in the presence of all these compounds (as percentage: 100 ± 3.84, 13.57 ± 2.44, 13.72 ± 4.61, 12.67 ± 3.71, and 11.57 ± 3.13 for control and in the presence of unlabeled biotin, desthiobiotin, lipote, and pantethionic acid, respectively).

RESULTS

Physiological Characterization of the Biotin Uptake Process of Pancreatic Beta Cells/Islets

General characteristics. Isosmotic replacement of Na⁺ in the incubation buffer with another monovalent cation (K⁺) led to a significant (P < 0.01) inhibition in the initial rate of biotin (5 nM) uptake by pancreatic beta-TC-6 cells (155 ± 13 and 20 ± 6 fmol·mg protein⁻¹min⁻¹ in the presence and absence of Na⁺, respectively). The initial rate of biotin uptake by pancreatic beta-TC-6 cells was also pH dependent with a significantly (P < 0.01) higher uptake at pH 7.4 than at pH 6.5 and 5.5 (as percentage to uptake at pH 7.4: 100 ± 0.88, 65.16 ± 3.19, 48.32 ± 4.46, respectively).
biotin uptake upon Na\(^+\) replacement [uptake of 2.46 ± 0.15 and 0.43 ± 0.9 fmol·mg protein\(^{-1}\)·min\(^{-1}\) (P < 0.05) in the presence and absence of Na\(^+\), respectively], and in the presence of unlabeled biotin [in percentage: 100 ± 5.3 and 14.51 ± 5.04 (P < 0.01) in the absence and presence of unlabeled biotin, respectively].

Collectively, the above described results suggest that biotin uptake by mouse and human pancreatic beta cells/islets is mediated via a Na\(^+\)-dependent carrier-mediated mechanism.

**Kinetic parameter of biotin uptake by pancreatic beta cells.** Kinetic parameters of the biotin uptake process of pancreatic beta-TC-6 cells were determined by examining the initial rate of biotin uptake as a function of substrate concentration. The results showed that uptake includes a saturable component over the micromolar range (Fig. 1A) with a \(V_{\text{max}}\) and an apparent \(K_m\) of 55.1 ± 5.9 fmol·mg protein\(^{-1}\)·min\(^{-1}\) and 22.24 ± 5.5 \(\mu\text{M}\), respectively (Fig. 1A). Since studies by others (13, 45) have suggested the possible existence of another biotin uptake system that functions at the nanomolar range, we tested the existence of such a system by examining uptake as a function of that concentration range. No evidence for the existence of such a system was observed, as biotin uptake was linear over this range (Fig. 1B).

**Molecular identity of the biotin uptake system of pancreatic beta cells/islets.** The SMVT system is expressed in a variety of tissues (41). Here we examined whether mouse and human pancreatic beta cells/islets express the SMVT system at the mRNA levels. The results showed that SMVT is indeed expressed, albeit at different levels, in pancreatic beta-TC-6 cells and in mouse and human primary pancreatic islets. Human pancreatic islets showed more expression of SMVT than cultured and primary mouse pancreatic beta-TC-6 cells, respectively.

To examine the relative contribution of the SMVT system toward carrier-mediated biotin uptake by pancreatic beta-TC-6 cells, we used the approach of gene silencing with shRNA. We stably transfected the cells with doxycycline-inducible shRNA targeted to mouse SMVT. Gene knockdown was induced by incubating the transfected cells with doxycycline. The effectiveness of the gene silencing was verified by demonstrating a significant reduction (around 70%; \(P < 0.01\) for both) in expression of SMVT at the mRNA and protein levels in the shRNA transfected and induced cells compared with noninduced cells (Fig. 2, A and B). The consequence of knocking down the SMVT system on carrier-mediated biotin uptake was determined with the results showing ~60% (\(P < 0.01\)) inhibition in carrier-mediated biotin uptake in induced cells expressing shRNA compared with noninduced cells (Fig. 2C). These findings suggest that the SMVT system is the major biotin uptake carrier by pancreatic beta-TC-6 cells. Since SMVT also transports pantothenic acid, we tested if knocking down SMVT (with the same shRNA approach) also inhibits uptake of this substrate. The results indeed showed a significant decrease in \(^3\text{H}\)-pantothenic acid uptake (\(P < 0.05\)) in induced cells expressing shRNA compared with noninduced cells (Fig. 2D).

The human SLC5A6 (the gene that encodes SMVT) has two promoters (promoter 1 and 2; P1 and P2) with activity of P1 being higher than that of P2 in a number of tissues, as we reported before (8, 27). Thus we determined the relative activity of these two promoters in pancreatic beta-TC-6 cells [the human SLC5A6 5' -promoters are active in mice in vivo (27)]. The results showed a significantly (\(P < 0.01\)) higher P1 activity than P2 (Fig. 3), suggesting these cells utilize the former promoter to a greater extent than the latter in driving the transcription of this gene in pancreatic beta cells.

**Regulation of the Biotin Uptake by Pancreatic Beta Cells**

**Adaptive regulation by extracellular substrate level.** We examined whether the biotin uptake process of pancreatic beta cells is adaptively regulated by the prevailing vitamin level. We examined the initial rate of biotin uptake by pancreatic beta-TC-6 cells maintained in biotin-deficient medium and in biotin oversupplemented (100 \(\mu\text{M}\)) medium (see MATERIALS AND METHODS). The results showed a significantly (\(P < 0.01\)) higher \(^3\text{H}\)-biotin (5 \(\text{nM}\)) uptake in cells maintained in biotin-deficient medium compared with those maintained in biotin oversupplemented medium (Fig. 4A). Expression of the SMVT protein and mRNA were both found to be significantly (\(P < 0.01\) for both) higher in the cells that were maintained in the biotin-deficient medium compared with those in the oversupplemented medium.

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**Fig. 1.** Uptake of biotin by pancreatic beta-TC-6 cells as a function of substrate concentration. Confluent monolayers were incubated at 37°C in Krebs-Ringer buffer, pH 7.4, in the presence of micromolar (1–50 \(\mu\text{M}\)) (A) and nanomolar (5–50 \(\text{nM}\)) (B) concentrations of biotin. Uptake by the carrier-mediated system was calculated as described in MATERIALS AND METHODS. Values are means ± SE of at least 3 separate uptake determinations. When not visible, error bars are smaller than the displayed symbol.
supplemented medium (Fig. 4, B and C). We further examined if the adaptive regulation in biotin uptake is mediated by induction of the SMVT promoters. For this we used the full-length human SMVT promoters P1 and P2 fused to the firefly luciferase reporter gene (27, 28). Promoter constructs were transfected into pancreatic beta-TC-6 cells and maintained for 96 h in biotin-deficient and oversupplemented medium. The result showed a significantly \((P \leq 0.01)\) higher promoter activity in cells maintained in the biotin-deficient medium compared with those in the oversupplemented medium (Fig. 4D, i and ii).

Possible regulation of biotin uptake in pancreatic beta cells by intracellular signaling pathways and by high glucose levels. We examined whether the process of biotin uptake by mouse pancreatic beta cells is regulated by intracellular signaling pathways, focusing on those pathways that regulate the uptake of other nutrients in a variety of other cellular systems [e.g., protein kinase A (PKA), \(\text{Ca}^{2+}/\text{calmodulin}\), protein tyrosine kinase (PTK), and protein kinase C (PKC)] (11; reviewed in Ref. 32). Using specific modulators of these regulatory pathways, the results showed that although modulators of PKA (dibutyryl cAMP and forskolin), \(\text{Ca}^{2+}/\text{calmodulin}\) (calmidazolium chloride), and PTK (genistein)-mediated pathways have no effect on biotin uptake by pancreatic beta-TC-6 cells (data not shown), the classic PKC activator phorbol 12-myristate 13-acetate (PMA), but not its negative control (4\(\alpha\)-PMA), caused a significant \((P < 0.05)\) inhibition (as percentage: 100\% – 6, 75.7\% – 3.5, and 106.1\% ± 6.7 for control, PMA-treated, and 4\(\alpha\)-PMA-treated cells, respectively).

Since biotin plays a role in normal pancreatic beta cells homeostasis and function (38), and high glucose levels affect

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### Fig. 2. Effect of knocking down the sodium-dependent multivitamin transporter (SMVT) system of pancreatic beta-TC-6 cells on carrier-mediated biotin and pantothenic acid uptake. Following induction of the knockdown with doxycycline (DOX) (see MATERIALS AND METHODS), the following were examined: level of expression of the SMVT mRNA (quantitative real-time PCR; expression was relative to beta-actin) (A), level of expression of the SMVT protein (Western blotting; expression was relative to respective beta-actin) (B), initial rate of carrier-mediated biotin uptake (C), and the effect of knocking down the SMVT system on carrier-mediated pantothenic acid uptake (another substrate of the SMVT system) (D). Inset in B shows a representative gel image. All values are means ± SE of at least 3 independent experiments. \(*P < 0.01.\)

### Fig. 3. Relative activities of the SLC5A6 promoters 1 and 2 in pancreatic beta-TC-6 cells promoters. Promoter constructs pGL3-P1-P2, pGL3-P1, and pGL3-P2 were transiently expressed in pancreatic beta-TC-6 cells followed (48 h) by determination of firefly luciferase activity. Activity of the latter was normalized relative to activity of simultaneously expressed Renilla luciferase and expressed as %. All values are means ± SE of at least 3 independent experiments. \(*P < 0.01\) in comparison of P1 to P2.
pancreatic physiology (9, 17, 18, 37), we also investigated the effect of prolonged exposure (96 h) of pancreatic beta-TC6 cells to a high glucose (26 mM) level on biotin uptake. The results, however, showed no effect of such a treatment on biotin uptake (as percentage: 100/110 and 114/110 in the presence of 5.5 and 26 mM glucose, respectively), suggesting that biotin uptake is not affected by hyperglycemia.

**Effect of bacterial LPS on biotin uptake by pancreatic beta cells.** Gut microbiota has recently been proposed as an environmental factor that increases the risk of metabolic disorders such as diabetes mellitus. Patients with Type 2 diabetes have altered gut microbial diversity and an increase in the level of circulating bacterial LPS, which is associated with a low-grade endotoximia (4, 16, 43). LPS has been shown to exert deleterious effects on the health/function of pancreatic beta cells, and it does so via Toll-Like receptor 4 (TLR4)–mediated mechanism (1). The effect of LPS on biotin uptake by pancreatic beta cells is not known and, therefore, was examined using pancreatic beta-TC-6 cells. The results showed that exposure (24 h) of the cells to clinically relevant LPS concentrations (1 μg/ml) (1, 42) lead to a significant and concentration-dependent inhibition in the initial rate of biotin uptake (Fig. 5A) (LPS did not affect cell viability as indicated by Trypan blue analysis where >95% of the treated cells were found to be viable). Since LPS exerts its effect via TLR4, we examined the effect of inhibiting the expression of this receptor by expressing a dominant negative mutant on the ability of LPS to inhibit biotin uptake by pancreatic beta-TC-6 cells (see MATERIALS AND METHODS). The results showed that such a treatment leads to significant (*P* < 0.01) protection against the inhibitory effect of LPS on biotin uptake by these cells (Fig. 5B).

To determine if the inhibitory effect of LPS is mediated via inhibition in the expression of SMVT protein and mRNA, we examined the effect of LPS treatment of pancreatic beta-TC6 cells by Western blotting and quantitative PCR, respectively. The results showed LPS treatment to have no effect on either of these parameters (Fig. 6, A and B). We also examined the effect of LPS on activity of the SLC5A6 promoters transfected into pancreatic beta-TC-6 cells and again observed no effect (Fig. 6C, i and ii). These findings suggest that the inhibitory
function of the endocrine pancreas, little is known about how
take up biotin, how the process is
important for normal health/function of pancreatic beta cells.

The vitamin plays a role in the expression of genes that are
critical for maintaining the differentiated phenotype of pancreatic beta cells and in preserving their mass; it also plays a role
in insulin secretion (3, 30, 39, 44) and in expression of glucokinase (30, 39) and Pdx1 (44), proteins that favor function
and preservation of pancreatic beta cells (2, 21). Despite
the importance of biotin for the normal homeostasis and
function of the endocrine pancreas, little is known about how
cells of this important organ take up biotin, how the process is
regulated, and how internal and external factors affect the
uptake process. Our objectives in the current investigations
were to address these issues, and we used cultured mouse-
derived pancreatic beta-TC-6 cells and complemented the findings with studies using freshly isolated mouse and human primary (native) pancreatic islets. The results showed the uptake of \(^{3}\text{H}\)-biotin by pancreatic beta-TC-6 cells to be \(\text{Na}^{+}\)-dependent and carrier-mediated as indicated by the inhibition caused by unlabeled biotin and its structural analog desthiobiotin, and by the saturation in biotin uptake as a function of concentration. Similarly, uptake of biotin by native freshly isolated primary mouse and human pancreatic islets was \(\text{Na}^{+}\)-dependent and carrier-mediated in nature. As seen with other cell types of the digestive system, biotin uptake by pancreatic beta cells was also inhibited by pantothentic acid and lipoate, thus pointing to possible involvement of the SMVT in the uptake process. Indeed, mouse and human pancreatic beta cells/islets were found, by Western blotting and qualitative PCR, to express the SMVT system. Studies on biotin uptake in certain other cellular systems have suggested possible involvement of another (non-SMVT) biotin uptake carrier that operates in the nanomolar range (13, 45), but no such evidence were found in the current studies. The latter was shown by the lack of saturation in the initial rate of biotin uptake over the nanomolar range, and by the SMVT shRNA knockdown approach which established a major role for the SMVT system in biotin uptake by pancreatic beta cells.

Following delineation of the mechanism of biotin uptake by pancreatic beta cells/islets, we examined potential regulation of the uptake process by extracellular and intracellular factors. Biotin uptake by pancreatic beta-TC-6 cells was shown to be adaptively regulated by the prevailing biotin level in the extracellular environment, and was higher in cells maintained under a biotin-deficient condition compared with those maintained in the presence of a high level of biotin. This adaptive regulation in biotin uptake was associated with parallel changes in the level of expression of the SMVT protein and mRNA, as well as in the activity of the \(\text{SLC}5\text{A}6\) promoters. These findings suggest possible involvement of a transcriptional mechanism(s) in mediating the observed adaptive response in pancreatic beta cells biotin uptake process. We also examined possible regulation of the pancreatic beta cells biotin uptake process by specific intracellular regulatory pathways and found that specific modulators of the PKA, PTK, and \(\text{Ca}^{2+}/\text{calmodulin}\) pathways had no effect on biotin uptake. On the other hand, a role for the PKC-mediated pathway was suggested by the effect of the PKC modulator PMA (but not by its negative control \(4\alpha\)-PMA) on biotin uptake by pancreatic beta cells. It is interesting to mention here that biotin uptake by intestinal epithelial cells is also under the regulation of this intracellular regulatory pathway, suggesting that different cells may use the same regulatory mechanism to regulate their biotin uptake (32).
The intestinal microbiota appears to be an important environmental factor that influences the risk of metabolic disorders like diabetes mellitus (4). Studies have also shown that subjects with Type 2 diabetes have altered microbiota diversity and an enrichment in gram-negative bacteria (which express LPS) (16, 43). Indeed there is an increase in the level of LPS in the circulation of those subjects and a low-grade endotoxemia, which are believed to play a role in the onset of the metabolic disorders (1). Pancreatic beta cells express significant levels of TLR4 which make them sensitive to the effect of LPS (10, 14, 40). Circulating LPS binding to TLR4 leads to activation of the nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB), p38 mitogen-activated protein kinases (p38 MAPK), activator protein 1 (AP-1), and interferon-inducible inflammatory gene expression (1, 14). We observed that chronic exposure of pancreatic beta-TC-6 cells to LPS lead to a significant inhibition in biotin uptake. This inhibition was not associated with changes in the level of expression of the total cellular SMVT protein, nor changes in level of its mRNA or activity of its gene promoters, but rather due to a decrease in the level of expression of the SMVT protein at the cell membrane as demonstrated by biotinylation assay. This is similar to what

Fig. 6. Effect of exposure of pancreatic beta cells to LPS on molecular parameters of SMVT. Pancreatic beta-TC-6 cells were pre-exposed to LPS (1 μg/ml for 24 h) followed by determination of level of expression of SMVT protein (A) and mRNA (B) levels and activity of SLC5A6 promoter promoters 1 and 2 (C, i and ii). D shows the result of the biotinylation assay. Level of expression of biotinylated membranous SMVT was normalized relative to total SMVT in the cellular homogenate. Inset: a representative Western blot showing level of expression of SMVT at the cell membrane and in the total cellular homogenate. All methodologies are described in MATERIALS AND METHODS. Data are means ± SE of 3 independent experiments and expressed as % relative to simultaneously performed untreated controls. *P < 0.05.
others have seen in studies on the inhibitory effects of LPS on the serotonin transporter in intestinal epithelial cells (22).

In summary, our studies show for the first time that biotin uptake by mouse and human pancreatic beta cells/islets is mediated via a regulated carrier-mediated mechanism that involves the SMVT system. This study also shows that the bacterial LPS inhibits biotin uptake by reducing the amount of the SMVT protein that is expressed at the cell membrane.

REFERENCES


