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Journal of Nutritional Biochemistry 21 (2010) 606–612
Journal of Nutritional Biochemistry

Biotin increases glucokinase expression via soluble guanylate cyclase/protein kinase G, adenosine triphosphate production and autocrine action of insulin in pancreatic rat islets☆

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Received 5 October 2008; received in revised form 3 March 2009; accepted 17 March 2009

Abstract

Besides its role as a carboxylase prosthetic group, biotin has important effects on gene expression. However, the molecular mechanisms through which biotin exerts these effects are largely unknown. We previously found that biotin increases pancreatic glucokinase expression. We have now explored the mechanisms underlying this effect. Pancreatic islets from Wistar rats were treated with biotin, in the presence or absence of different types of inhibitors. Glucokinase mRNA and 18s rRNA abundance were determined by real-time PCR. Adenosine triphosphate (ATP) content was analyzed by fluorometry. Biotin treatment increased glucokinase mRNA abundance approximately onefold after 2 h; the effect was sustained up to 24 h. Inhibition of soluble guanylate cyclase or protein kinase G (PKG) signalling suppressed biotin-induced glucokinase expression. The cascade of events downstream of PKG in biotin-mediated gene transcription is not known. We found that inhibition of insulin secretion with diazoxide or nifedipine prevented biotin-stimulated glucokinase mRNA increase. Biotin treatment increased islet ATP content (control: 4.68±0.28; biotin treated: 6.62±0.26 pmol/islet) at 30 min. Inhibition of PKG activity suppressed the effects of biotin on ATP content. Insulin antibodies or inhibitors of phosphoinositol-3-kinase/Akt insulin signalling pathway prevented biotin-induced glucokinase expression. The nucleotide 8-Br-cGMP mimicked the biotin effects. We propose that the induction of pancreatic glucokinase mRNA by biotin involves guanylate cyclase and PKG activation, which leads to an increase in ATP content. This induces insulin secretion via ATP-sensitive potassium channels. Autocrine insulin, in turn, activates phosphoinositol-3-kinase/Akt signalling. Our results offer new insights into the pathways that participate in biotin-mediated gene expression.
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Keywords: Biotin; cGMP; Glucokinase; Gene expression; Insulin signaling

1. Introduction

In the last few decades, an increasing number of vitamin-mediated effects have been discovered at the level of gene expression [1–3] in addition to their well-known roles as substrates and cofactor [4]. Examples are the lipophilic vitamins A and D that serve as ligand precursors of the hormone nuclear receptors superfamily and thus affect systemic functions [1–3]. Although little is known about water-soluble vitamins as genetic modulators, there is increasing number of examples of their effects on gene expression [5–8].

Biotin is a water-soluble vitamin that acts as a prosthetic group of carboxylases. Unrelated to this classic role as a carboxylase prosthetic group, biotin regulates gene expression at both the transcriptional and the translational level [6,8,9], and has a wide repertoire of effects on systemic processes such as development [10,11], immunity [12,13] and glucose metabolism [14,15]. Biotin affects gene expression at both the transcriptional and the posttranscriptional level. DNA microarray studies have aided in the identification of thousands of genes affected by biotin [16,17].

The first evidence that biotin affects glucose metabolism was reported by Dakshinamurti et al. [18] in biotin-deficient rats. They observed that glucose tolerance test curves in biotin-deficient rats were significantly higher than the curves of nondeficient rats [18]. They further demonstrated that these effects were the result of a reduction in hepatic glucokinase (EC 2.7.1.1) activity [19], and that the stimulatory effect of biotin occurs at the level of transcription [20]. This group also found that biotin decreases the transcription of hepatic phosphoenolpyruvate carboxykinase (EC 4.1.1.32), a key

☆ Supported by research grants from the Consejo Nacional de Ciencia y Tecnologia 44266-M and the Direccion General de Asuntos del Personal Academico, Universidad Nacional Autónoma de México IN201901 and IX229004. Alonso Vilches-Flores was a recipient of the CONACyT scholarship number 169971.
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doi:10.1016/j.jnutbio.2009.03.009

enzyme that stimulates glucose production by the liver [21]. In previous studies [22], we found that biotin stimulates the expression and activity of the pancreatic isoform of glucokinase, which is regulated differently than the hepatic isoenzyme [23]. We also found that insulin secretion increased in response to biotin [22]. Furthermore, studies by our group [22] and others [24,25] have found that the lack of biotin affects pancreatic islet functions.

The findings that biotin has stimulatory effects on the transcription of genes whose action favors glycemia reduction and decreases the transcription of gluconeogenic genes support other observations that indicate that pharmacological doses of biotin decrease hyperglycemia [26–30]. In genetically diabetic KK mice and in OLETF rats, biotin treatment lowered postprandial glucose concentration and improved tolerance to glucose [26,27]. Hyperglycemia reduction was also observed in both type 1 and type 2 diabetics treated with biotin [28,29]. In hemodialysis patients, pharmacological doses of biotin improved their oral glucose tolerance tests [30]. Moreover, commercially available drugs containing pharmacological amounts of biotin are currently used in the treatment of diabetes [31,32].

In spite of the extensive effect of biotin on gene transcription and its present use as a pharmacological agent, the molecular mechanisms of the vitamin as genetic modulator are largely unknown. Two different mechanisms appear to be involved in the genetic effects of biotin: histone biotinylation [33–37] and a soluble guanylate cyclase signalling cascade [38–42]. Histone biotinylation has been implicated in heterochromatin structures, DNA repair, and mitotic chromosome condensation and lifespan [34–36]. On the other hand, guanylate cyclase activity is linked to gene expression of the enzymes involved in its function as a cofactor, such as carboxylases and holocarboxylase synthase [40], and to translation of the asialoglycoprotein receptor [38,39] and insulin receptor [39]. Solorzano-Vargas et al. [40] have proposed that biotinyl-AMP, an intermediate of holocarboxylase synthethase catalytic action, activates soluble guanylate cyclase. Activation of guanylate cyclase leads to increased production of cyclic guanosine monophosphate (cGMP) [41,42], which in turn stimulates protein kinase G (PKG). The cascade of events downstream guanylate cyclase/PKG in biotin-mediated transcription has not been investigated yet. In the present study, we sought to understand the mechanisms regulating biotin-induced pancreatic glucokinase expression.

2. Methods and materials

2.1. Reagents

Dulbecco's Modified Eagle Medium (DMEM) with 5.5 mmol/L glucose, antibiotic mixture (penicillin, streptomycin, amphotericin), dialyzed fetal bovine serum and d-biotin were purchased from Gibco/Invitrogen Corp. (Grand Island, NY, USA); collagenase P, glucose-6-phosphate dehydrogenase and hexokinase from Roche Diagnostics (Mannheim, Germany); wortmannin from Fluka/Sigma Aldrich (St. Louis, MO, USA); 8-Br-cGMP and ODQ (1-GH (1,2,4) oxadiazolo-(4,3-*a*) quinaxolin-1-one) from ICN Biomedicals, Inc. (Costa Mesa, CA, USA). The Akt inhibitor 1L-6-hydroxymethyl-chiro-inositol-2-((*R*)-2-*O*-methyl-3-*O*-octadecylcarbonate) was purchased from Alexis Biochemicals (San Diego, CA, USA); Reporter Lysis Buffer from Promega (Madison, WI, USA); TRIzol reagent, dNTP and M-MLV reverse transcriptase from Invitrogen (Carlsbad, CA, USA); ultrasensitive rat insulin enzyme-linked immunosorbent assay from ALPCO Diagnostics (Salem, NH, USA); Rp-cGMPS (Rp-8 (4-chlorophenylthio)-guanosine-3′,5′-cyclic monophosphothiorate), an inhibitor of PKG, diazoxide and nifedipine from Sigma (St. Louis, MO, USA); normal rabbit serum and serum anti-insulin were provided by Dr. Eulises Díaz (Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico). Immunoassay kit for insulin determination was purchased from ALPCO Diagnostics.

2.2. Animals

Wistar adult male rats (200–250 g) were obtained from the Biomedical Research Institute of the National Autonomous University of Mexico. Environmental conditions and light–dark cycles (12:12 h) were strictly controlled. Animals were handled according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, Washington, DC, 1996). All methods were approved by the Internal Council and the Animal Care Committee of The Biomedical Research Institute.

2.3. Islet culture

Overnight food-deprived Wistar adult male rats (200–250 g) were anaesthetized by intraperitoneal injection of sodium pentobarbital (Pfizer, Mexico City, Mexico), and their pancreas was removed. Finally, rats were killed by cervical dislocation. Pancreatic islets were isolated as described previously [22]. Batches of 400 islets were cultured in a biotin-free DMEM medium with low glucose (5.5 mmol/L), supplemented with biotin-free 5% dialyzed fetal bovine serum and 200,000 U/L penicillin G, 200 g/L streptomycin and 0.5 g/L amphotericin B. After 16 h of incubation at 37°C in a humidified atmosphere of 5% CO_2, cultured media were replaced and islets were treated or not with one of the following: vehicle (PBS); 0.01, 0.1, 1 μmol/L of d-biotin; or 1 μmol/L 8-Br-cGMP, at different times as indicated in the text. Inhibitors or antibodies were added 30 min before biotin or cGMP treatment. Concentrations of inhibitors were 50 μmol/L ODQ; 10 μmol/L Rp-cGMPS; 250 μmol/L diazoxide; 100 nmol/L wortmannin; 10 μmol/L PKB/Akt inhibitor; insulin antibody serum diluted 1:1000. Ten micromoles per liter of nifedipine was added 15 min before either d-biotin or 8-Br-cGMP treatment.

2.4. Messenger RNA analysis

After treatment, islets were collected and total RNA was extracted with TRIzol. RNA concentration was determined by absorbance at 260 nm, and its integrity confirmed by electrophoresis on 1% denaturing agarose gel. Single-stranded cDNA was synthesized from 0.5 μg of total RNA by reverse-transcription reaction with 500 U of M-MVL RT. Glucokinase relative expression was evaluated in real-time PCR with materials and methods supplied by Applied Biosystems. Twenty nanograms of cDNA was mixed with TaqMan universal PCR master mix, unlabeled PCR primers and TaqMan MGB probes (FAM dye-labeled), in a total volume of 25 μl. Forward and reverse primers sequence for *Ratus norvergicus* glucokinase were GACAAGGGCATCCTCCTCAATTGGA and CTAGA-CAAGGGCATCCTCCTCAATT, respectively. Real-time PCR was performed on an ABI Prism 7700 Sequence Detector (Foster City, CA, USA). The samples were analyzed in triplicate and corrected for the 18S ribosomal subunit used as internal standard. Standard curves were constructed from 0.1 to 10 ng of cDNA from untreated rat islets. Relative glucokinase expression was calculated from cycle threshold (Ct) values by the abundant relative quantification model of the ΔΔCt [43].

2.5. Glucokinase assay

Glucokinase activity was determined as reported previously [22]. Briefly, batches of approximately 450 islets for each experimental group were isolated and cultured as described above. After 16 h of incubation, cultured media were replaced and islets were treated with either vehicle (PBS) or 1 μmol/L of d-biotin. After 6 h of incubation, islets were harvested and centrifuged at 1200 rpm. Tissue pellets were lysed in 500 μl reporter lysis buffer, vortexed and cell membranes disrupted by three freeze–thaw cycles. The lysates were then centrifuged at 4°C for 1 h at 35,000×g, in a Beckman ultracentrifuge model Optima TLX. Supernatants were recovered and enzymatic activity was assayed using NAD as coenzyme. Glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* was used as coupling enzyme. Correction for hexokinase activity was applied by subtracting the activity measured at 0.5 mM glucose from the activity measured at 100 mM glucose.

2.6. Adenosine triphosphate determination

Islets were isolated and cultured as described above. Batches of 400 islets were preincubated in PBS without glucose during 10 min, and then the PBS solution was replaced by DMEM medium with 5.5 mmol/L glucose, treated with vehicle (PBS) or 1 μmol/L of d-biotin or 1 μmol/L 8-Br-cGMP for 30 min. Islets were then washed and sonicated, and adenosine triphosphate (ATP) content determined at 30°C by following the fluorescence signal of NADPH as described previously [44] in an RF5000 U fluorophotometer (Shimadzu, Kyoto, Japan). The excitation and emission wavelengths were 340 and 460 nm, respectively.

2.7. Insulin concentration assay

After 16 h of incubation, groups of 50 islets were washed with secretion buffer [22]. Then islets were incubated in DMEM medium with 5.5 mmol/L glucose, treated with vehicle (PBS), or 1 μmol/L of d-biotin or 1 μmol/L 8-Br-cGMP for 60 min. Insulin concentration in the media was analyzed using an ultrasensitive rat insulin enzyme-linked immunosorbent assay.

2.8. Statistical analysis

The PRISM 2.01 program (GraphPad, CA, USA) and Statview statistical analysis program v. 4.5 (Abacus Concepts, Berkeley, CA, USA) were used. Each result is expressed as the mean±S.E.M. of the number of experiments indicated in the text. Data

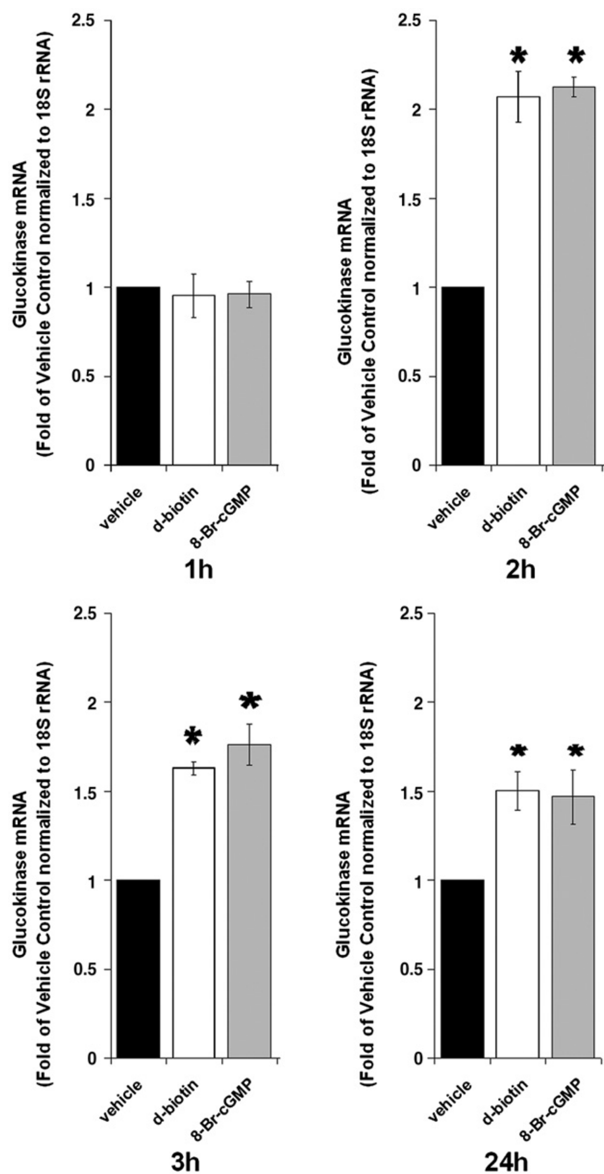

Fig. 1. Time course of the effect of either biotin or cGMP on glucokinase mRNA abundance. Islets were treated at different times in the presence of vehicle, 1 μmol/L d-biotin or 1 μmol/L 8-Br-cGMP. Data are expressed as fold change relative to that measured in control islets. Values are represented as mean±S.E.M. of three independent experiments. Each time group was normalized to its vehicle control. Bars with asterisk indicate significant differences between treatments ($P<.01$).

for the experiments in Figs. 1 and 2, as well as insulin concentration in the cultured media data, were analyzed using one-way ANOVA. Data presented in Table 1 and in Figs. 3–7 were tested using two-way ANOVA with interaction (treatments, inhibitors). Bonferroni correction was used for the *post hoc* detection of significant differences. Differences were considered significant at $P<.05$.

3. Results

3.1. Effect of biotin and cGMP on glucokinase mRNA

We compared the effect of biotin and of the nucleotide 8-Br-cGMP on glucokinase mRNA abundance. Biotin treatment produced a onefold increase of glucokinase mRNA abundance at 2 h compared with controls ($P<.01$) (Fig. 1). At 3 h, the increase was slightly reduced ($P<.01$) and maintained during the 24 h of treatment ($P<.01$). Incubation with 1 μmol/L 8-Br-cGMP induced increased glucokinase mRNA abundance of comparable magnitude and with similar kinetics

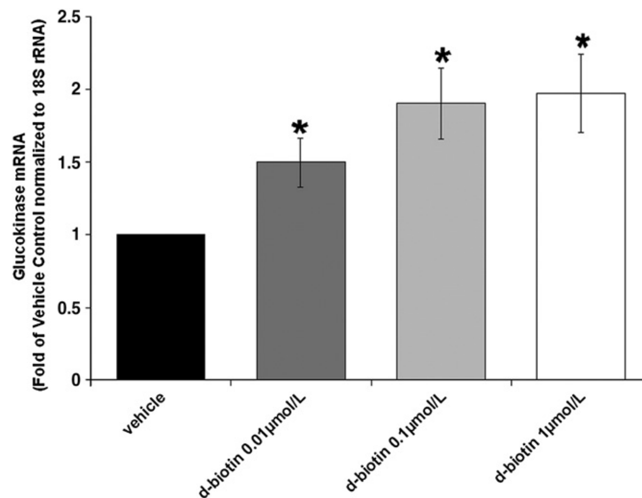

Fig. 2. Effect of different concentrations of biotin on glucokinase mRNA abundance. Islets were treated for 2 h in the presence of different concentrations of biotin. Data are expressed as fold change relative to that measured in control islets, and values are represented as mean±S.E.M. of four independent experiments. Bars with asterisk indicate significant differences between treatments ($P<.01$).

as biotin. We also assessed the effect of different concentrations of biotin on glucokinase mRNA expression (Fig. 2). We found that glucokinase mRNA abundance increased in response to 0.01, 0.1 or 1 μmol/L of biotin ($P<.01$). The stimulatory effect of either biotin or cGMP was also observed on glucokinase activity (control: 54.9±13; biotin: 93.7±17; cGMP: 109.9±14 pmol/h per islet; $n=3$).

3.2. Requirement of cGMP signaling

To assess the role of soluble guanylate cyclase activity in biotin signaling, we incubated pancreatic islets in a medium containing the guanylate cyclase-specific inhibitor ODQ (1-H-(1,2,4)oxadiozolo (4,3-a)quinoxalin-1-one). As shown in Table 1, the biotin response was inhibited in ODQ-treated islets, while the cGMP response was not affected ($P<.05$). To investigate whether the effect of biotin occurs through PKG, we preincubated pancreatic islets in the presence of the PKG inhibitor Rp-cGMPS (Rp-8(4-clorophenylthio)-guanosine-3′5′-cyclic monophosphoro thioate) before treatment with either biotin or cGMP. In the presence of 10 μmol/L Rp-cGMPS, neither biotin nor 8-Br-cGMP induced an increase of glucokinase mRNA abundance (Table 1). Our data indicate that soluble guanylate cyclase and PKG are involved in the effects of biotin. The cascade of events downstream of PKG in biotin-mediated gene transcription is not known. We next investigated the actions that ensue guanylate cyclase/PKG activation in biotin-induced glucokinase expression.

3.3. Requirement of insulin secretion

Previous reports (including our own) have reported that biotin [22,24,25] and cGMP [45,46] induce insulin secretion. Since autocrine

Table 1
Effect of biotin and cGMP on glucokinase mRNA abundance, in the presence of inhibitors of soluble guanylate cyclase and PKG

	Without inhibitors	50 μmol/L ODQ	10 μmol/L Rp-cGMPS
Vehicle	1±0.16	1.12±0.12	1.01±0.15
d-Biotin (1 μmol/L)	2.25±0.12 *	1.16±0.24	0.91±0.26
8-Br-cGMP (1 μmol/L)	2.06±0.28 *	2.27±0.16 *	0.97±0.13

Data are expressed as fold change relative to that measured in control islets, and values represent the mean±S.E.M. of three independent experiments.
* Indicates significant differences compared with vehicle ($P<.05$).

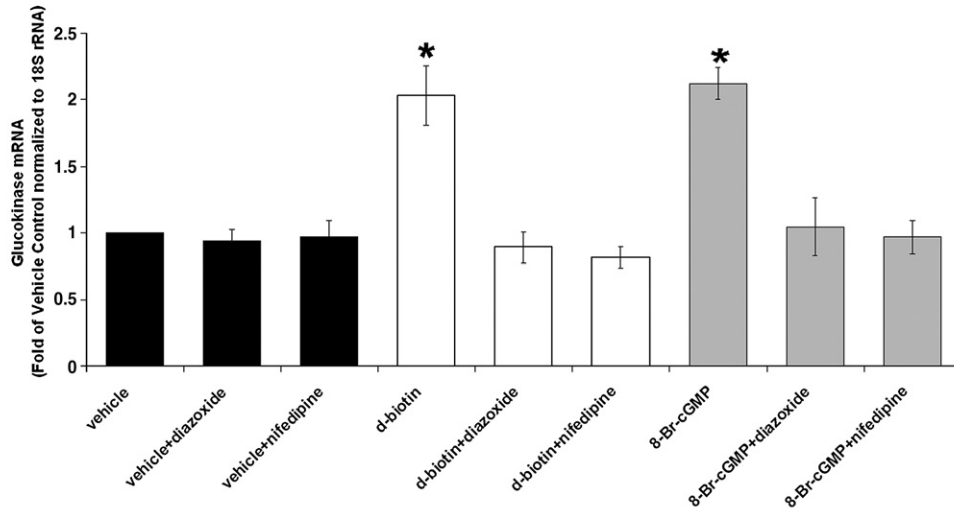

Fig. 3. Effect of either diazoxide or nifedipine on biotin- or cGMP-induced glucokinase mRNA abundance. Islets were treated with vehicle or diazoxide or nifedipine before adding either d-biotin or 8-Br-cGMP during 2 h. Data are expressed as fold change relative to that measured in control islets, and values represent the mean±S.E.M. of four independent experiments. Bars with asterisk indicate significant differences between treatments ($P<.01$).

insulin is involved in pancreatic glucokinase expression [47] we investigated whether the effect of biotin on glucokinase mRNA abundance is mediated by an increase in insulin release. As expected, we found that after 1 h of incubation islets treated with either biotin or cGMP significantly increased ($P<.01$) insulin concentration in the cultured media (control: 0.64±0.11 nmol/L; biotin-treated: 1.60±0.57 nmol/L; 8-Br cGMP-treated: 1.58±0.60 nmol/L; $n=3$). Inhibitors of insulin secretion prevented biotin-stimulated glucokinase mRNA increase. Islets pretreated with diazoxide, an ATP-sensitive K^+ channel opener, did not increase glucokinase mRNA abundance in response to either biotin or 8-Br-cGMP at 2 h (Fig. 3). We also investigated the effect of nifedipine, an L-type voltage-operated calcium channel blocker (Fig. 3). The results showed that islets treated with biotin increased glucokinase mRNA abundance; however, no effect of biotin or cGMP on glucokinase mRNA expression was observed on nifedipine-incubated islets (Fig. 3). Neither diaxozide nor nifedipine modified basal glucokinase mRNA abundance in islets incubated with vehicle at 2 h.

3.4. Adenosine triphosphate content

The classic stimulus-secretion coupling pathway that mediates glucose-induced insulin secretion involves an increase in ATP production [48]. Hence, we evaluated whether the treatment with biotin modified ATP content. Results showed that after 30 min of incubation biotin treatment significantly increased ($P=.01$) ATP content (Fig. 4); a similar response was observed in response to cGMP ($P<.01$).

To assess whether biotin activation of the cGMP/PKG signalling pathway leads to an increase in ATP content, we preincubated pancreatic islets in the presence of the PKG inhibitor Rp-cGMPS before treatment with either biotin or cGMP. Inhibition of PKG activity prevented the increase in ATP content in response to both biotin and cGMP treatment (Fig. 4).

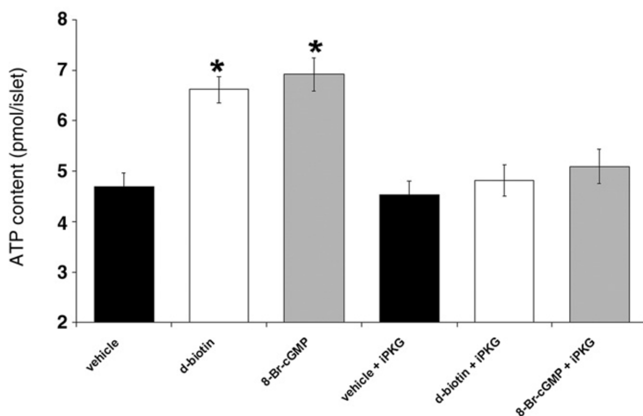

Fig. 4. Effect of either biotin or cGMP on ATP content, in the presence or absence of PKG inhibitor. Islets were treated with either vehicle (PBS) or PKG inhibitor for 30 min before addition of d-biotin or 8-Br-cGMP. ATP content was determined after 30 min of incubation. Values represent the mean±S.E.M. of five independent experiments. Bars with asterisk indicate significant differences between treatments. P values: biotin=.0006; cGMP<.0001.

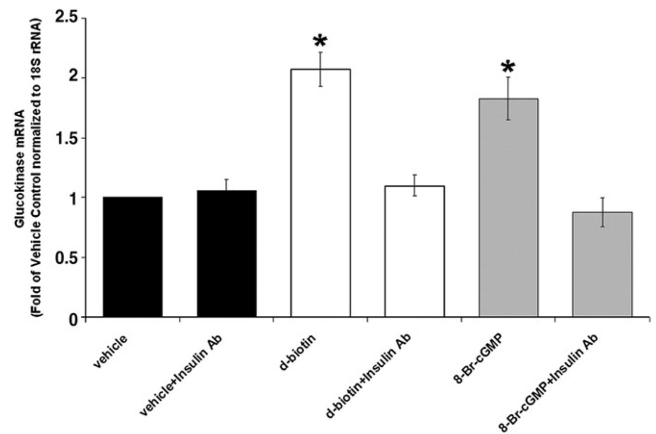

Fig. 5. Effect of insulin neutralization in culture media on either biotin- or cGMP-induced glucokinase mRNA abundance. Islets were treated with either serum (vehicle) or anti-insulin serum during 30 min before adding either d-biotin or 8-Br-cGMP during 2 h. Data are expressed as fold change relative to that measured in control islets, and values represent the mean±S.E.M. of three independent experiments. Bars with asterisk indicate significant differences between treatments. P values: biotin=.0163; cGMP=.032.

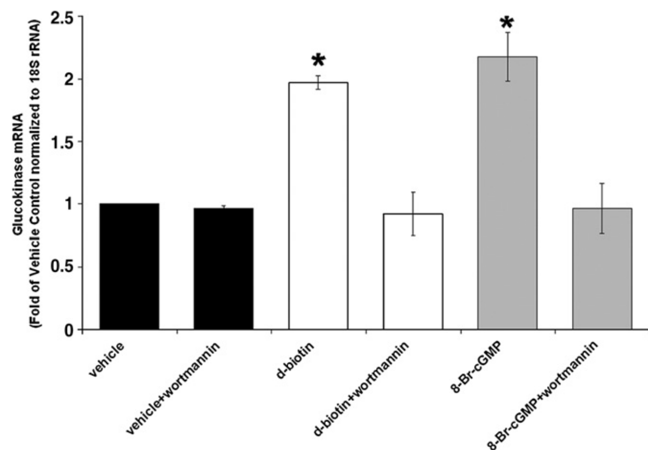

Fig. 6. Effect of phosphoinositol-3 kinase activity inhibition on either biotin- or cGMP-induced glucokinase mRNA abundance. Islets were treated with either vehicle (PBS) or wortmannin during 30 min before either d-biotin or 8-Br-cGMP was added to the cultures for 2 h. Data are expressed as fold change relative to that measured in control islets. The values represent the mean±S.E.M. of four independent experiments. Bars with asterisk indicate significant differences between treatments (*P*<.01).

3.5. Requirement of autocrine action of insulin

Insulin antibodies added to the culture media inhibited the response to biotin (Fig. 5). Similarly, insulin antibodies suppressed the stimulatory effect of cGMP on glucokinase mRNA abundance (Fig. 5). We next assessed the role of insulin signalling in biotin-induced glucokinase. Pancreatic islets cultured in the presence of 100 mmol/L wortmannin, a phosphoinositol-3 kinase activity inhibitor, did not increase glucokinase mRNA abundance in response to either biotin or cGMP treatment (Fig. 6). We also analyzed the involvement of the serine/threonine kinase PKB/Akt activity in biotin-induced glucokinase mRNA. Islets treated with either biotin or 8-Br-cGMP increased glucokinase mRNA abundance; however, inhibition of PKB/Akt activity prevented the induction of glucokinase mRNA (Fig. 7).

4. Discussion

The molecular mechanisms by which biotin regulates gene expression are largely unknown. Biotin increases pancreatic glucokinase activity and mRNA abundance, as reported previously [22]. In this study, we explored the mechanisms involved in biotin-induced glucokinase gene expression.

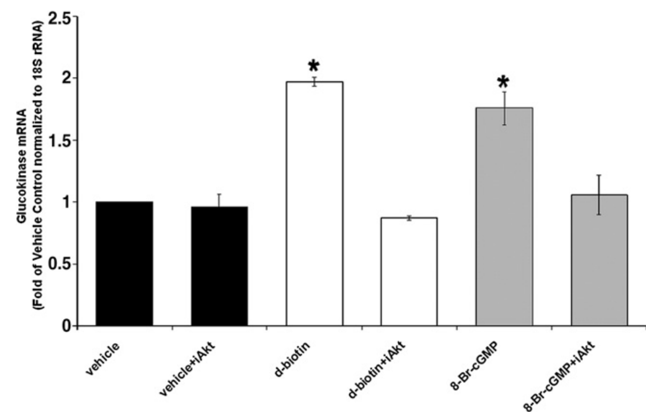

Fig. 7. Effect of PKB/Akt inhibition on either biotin- or cGMP-induced glucokinase mRNA abundance. Islets were preincubated with either vehicle (PBS) or with PKB/Akt inhibitor during 30 min before treatment either d-biotin or 8-Br-cGMP, for 2 h. Data are expressed as fold change relative to that measured in control islets, and values represent the mean±S.E.M. of three independent experiments. Bars with asterisk indicate significant differences between treatments (*P*<.01).

We found that activation of guanylate cyclase and PKG is part of the signalling involved in the stimulatory effect of biotin on pancreatic glucokinase expression. This mechanism is similar to the one involved in posttranscriptional regulation of proteins such as asialoglycoprotein and insulin receptor [39], as well as in mRNA expression of holocarboxylase synthetase (EC 6.3.4.10) [40], carboxylases [40,49] or sodium-dependent multivitamin transporter [50]. Interestingly, Spence and Koudelka [41] found that the effect of biotin on hepatic glucokinase activity was preceded by an increase in cGMP intracellular concentration. Although the involvement of soluble guanylate cyclase and PKG was not demonstrated in the mentioned studies, the increase in cyclic nucleotide suggests that this signalling pathway may also participate in biotin-induced hepatic glucokinase activity.

The cascade of events that follow guanylate cyclase/PKG activity in biotin-mediated gene transcription is unknown. We found that insulin secretion is required in biotin-induced glucokinase expression: inhibition of insulin secretion by preventing the closure of ATP-sensitive K^+ channels with diaxozide or by blocking L-type voltage-activated Ca^{2+} channels abolished biotin-induced effects on pancreatic glucokinase expression (Fig. 3). We also showed that either biotin or 8-Br-cGMP increased ATP content (Fig. 4). The observed increase in ATP is consistent with observations indicating that biotin and cGMP augment the production of this nucleotide as well as glucose utilization. In RINm5F insulinoma cells, Laychock et al. [45,46] found that 8-Br-cGMP increases glucose utilization in isolated pancreatic islets. In islets stimulated with biotin, Sone et al. [51] found that biotin increases ATP and CO_2 production and that the glucose oxidation rate was proportional to the effects observed in insulin secretion. They also found in liver mitochondria that the state-3 respiratory rate increased in response to biotin [51]. Since the classic stimulus-secretion coupling pathway that mediates glucose-induced insulin release

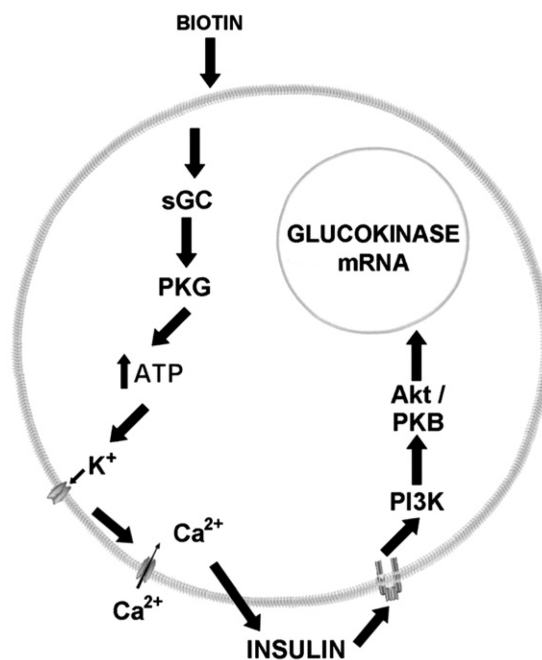

Fig. 8. Proposed model of the pathways involved in biotin-induced pancreatic glucokinase expression. We propose that the induction of pancreatic glucokinase mRNA by biotin involves guanylate cyclase and PKG activation, which leads to an increase in ATP content. This induces insulin secretion via ATP-sensitive potassium channels. Autocrine insulin, in turn, activates phosphoinositol-3-kinase/Akt signalling.

involves the closure of ATP-sensitive K^+ channel as a result of the enhanced ATP/ADP ratio generated by glucose oxidation [48], these studies [45,51] and our present results suggest that the effect of biotin on islets involves insulin secretion, mediated by increased glucose oxidation rates that in turn enhance ATP production.

The molecular mechanism involved in the effect of biotin on ATP content has not been determined. We showed that PKG activity is indispensable to increase ATP content in response to either biotin or cGMP (Fig. 4). These results demonstrate for the first time that biotin participates in fuel metabolism via cGMP/PKG signalling.

Our results also indicate that biotin-induced glucokinase expression requires autocrine insulin signalling and PI3K/Akt signalling. We found that the effect of biotin on treated islets was suppressed by (a) neutralization of insulin secreted with insulin antibodies added to the culture medium (Fig. 5); (b) inhibition of phosphoinositol-3 kinase activity (Fig. 6); and (c) inhibition of PKB/Akt activity (Fig. 7). Consistent with our findings, studies by Leibiger et al. [47] found that autocrine insulin signalling activates the transcription of pancreatic glucokinase through the activation of the B-type insulin receptor, phosphoinositol-3 kinase class II-like activity and PKB/Akt activity [47]. Recent investigations provide evidence that autocrine insulin is used as a signalling pathway in several processes that include gene transcription, translation, calcium flux, and insulin secretion [52–54], survival of isolated islets [53,55] and suppression of a constitutive apoptotic program in beta cells [56]. The effect of pharmacological concentrations of biotin on autocrine insulin action suggests that the vitamin may be of therapeutic importance in the first stages of type 1 diabetes and in the culturing and preservation of islets prior to transplantation.

To our knowledge, two studies have explored which cGMP target proteins mediate the effect of biotin: De la Vega and Stockert [38,39] have pinpointed that biotin, via soluble guanylate cyclase activity and PKG, increases phosphorylation of alpha-COP, a protein of the COPI trans-acting complex factor that regulates translation and participates in asialoglycoprotein receptor and insulin-receptor translation [39]. In HepG-2 cells, biotin deficiency increased the abundance of proteins involved in tyrosine kinase-mediated signalling [57]. However, this study did not demonstrate whether the increased abundance of tyrosine kinase cascade proteins actually mediates biotin signalling. The design of the present study, using blockers and inhibitors of different pathways, allowed us to identify proteins whose activity is involved in biotin signalling. Nonetheless, this approach possesses some limitations given that inhibitors may lack specificity. Also, our studies cannot rule out the possibility that, along with the events described herein, increased translation of insulin receptor [39] may account for the effect of biotin on pancreatic glucokinase expression. Further studies will be designed to generate additional insights into biotin-dependent cell signalling.

In Fig. 8, we depict our findings on the effect of biotin on glucokinase expression. We propose that biotin signalling involves guanylate cyclase/PKG activation, which triggers ATP production. The increase of ATP induces insulin secretion via ATP-sensitive potassium channels. Insulin, in turn, activates PI3K/Akt signalling, which increases pancreatic glucokinase mRNA expression.

Farsighted observations of Dakshinamurti [18,19,58] in 1968/1970 suggested that biotin possesses insulin-like effects. In our work, we demonstrated that insulin secretion and insulin-receptor tyrosine kinase PI3K/Akt signalling are involved in the effects of biotin. This finding, along with studies by De la Vega and Stockert [39] showing that biotin increases insulin receptor translation, lends molecular support to the insulin-like effects of biotin [19].

The reference dietary intake (RDI) of biotin for adults is 30 μg/day [59]. Several studies have demonstrated that biotin deficiency affects glucose metabolism [18,19,22,24,25], indicating that biotin dietary intake is required to maintain glucose homeostasis. On the other hand, pharmacological doses of biotin (milligrams) favor glucose homeostasis [26–30] and are currently used in the treatment of diabetes [31,32]. Pharmacologic supplementation of biotin of 40× the RDI (i.e., 1.2 mg/day) for 14 days reaches serum concentrations in a range of 9.4–47.7 nmol/L [60]. Present and previous results [22] show that these concentrations increase both glucokinase activity and mRNA abundance in cultured islets. How biotin-induced pancreatic glucokinase contributes to glucose homeostasis in diabetic models is currently being investigated in our laboratory.

Knowledge of the molecular mechanisms of different vitamins has opened new perspectives that form a connection between nutritional signals and biological functions. The best documented examples are those involving the lipophilic vitamins A and D. Extensive research on these vitamins has pointed to the broad spectrum of actions in which they participate and has made it possible to develop new therapeutic agents [61,63] against numerous diseases including diabetes [62,63]. In contrast, the study of the biological effects of biotin has been scarcely studied. Understanding the mechanisms of action of biotin is essential to determine its use as a therapeutic agent in the battle against diabetes, a major global health problem. The present report contributes to the understanding of the biotin signalling pathways that participate in gene transcription and in glucose metabolism.

Acknowledgments

The authors are grateful to Dr. Eulises Díaz (National Institute of Nutrition Salvador Zubirán, Mexico City) for providing anti-insulin antibodies and to Dr. Sumiko Morimoto for technical assistance. We are indebted to Dr. Marco Antonio Cerbón for valuable discussions throughout these studies and to Dr. Antonio Velázquez, Dr. Martha Perez-Armendariz and Dr. Albert Zlotnik for critical reading of this manuscript. We also thank Isabel Pérez Montfort for correcting the English version of the manuscript.

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