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ORIGINAL

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Effect of biotin on glucokinase activity, mRNA expression and insulin release in cultured beta-cells

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Abstract Biotin is known to influence hepatic glucokinase (GK) expression both at a transcriptional and at a translational level. The aim of the present paper was to investigate the effect of biotin on pancreatic GK. For this purpose, RIN1046-38 cells were cultured in the presence of different biotin concentrations for different times; thereafter, GK mRNA expression, GK activity and insulin release were studied. Results demonstrated that biotin has a biphasic effect on GK mRNA expression, being stimulatory after short-term treatment and inhibitory after long-term treatment. GK activity was increased after long-term treatment. Insulin release was not affected by biotin treatment. These data suggest that biotin may influence glucose metabolism also by acting directly at the level of beta-cells.

Key words Glucokinase · Pancreatic islets · Biotin

Introduction

Glucokinase (GK; EC 2.7.1.1), one of the isoenzymes of the hexokinase group (hexokinase type IV), catalyses the initial step in the utilization of glucose (phosphorylation at the sixth carbon) by the pancreatic beta-cells and by the liver. It differs functionally from the other hexokinases by its higher K_m and its greater specificity for glucose, and its lack of end-product inhibition by glucose-6-phosphate (G6P) [1, 2]. These functional properties, as well as its tissue distribution restricted only to liver and pancreatic beta-cells, have attracted considerable attention [3]. In liver GK plays a key role in the regulation of glucose uptake and production, while in beta-cells it regulates glucose utilization and hence insulin release, being considered the pancreatic 'glucose sensor' [4, 5].

Regulation of GK activity and gene expression is exerted by either nutritional or hormonal factors [6, 7]. In liver it has been demonstrated that GK activity and mRNA expression are dramatically modified in response to fasting and re-feeding, showing a threefold increase during re-feeding [8]; moreover, hepatic GK activity is reduced under insulin-deficient conditions (such as diabetes), returning to normal upon insulin administration [9]. In contrast, in pancreatic islets GK activity and mRNA expression are not influenced by fasting and re-feeding nor by insulin levels. Pancreatic GK is regulated rather by changes in glucose levels, as demonstrated by the observation that GK activity is reduced by 70% in insulinoma-bearing hypoglycaemic animals, and that GK activity returned to normal within 24 h after removal of the insulinoma [10].

The identification of two different promoters in the GK gene, located at least 12 kb apart, with the pancreatic control region located upstream from the hepatic one, provides a molecular basis for the differential tissue-specific regulation of hepatic and pancreatic GK [11–13].

It has been previously demonstrated that, in addition to nutritional and hormonal factors, hepatic GK in rats is also influenced by biotin levels. For example, the low enzymatic activity in liver of biotin-deficient rats is increased by biotin administration [14, 15]. In primary cultures of rat hepatocytes, the addition of biotin in the culture medium determines an increase in GK activity [16]. Moreover, biotin is also able to increase GK mRNA expression in rat liver [17]. The possible effect of biotin on pancreatic GK has not been studied.

The aim of the present study was to investigate whether or not biotin's effect on GK activity and gene expression is also exerted in beta-cells. The availability of pancreatic beta-cell lines expressing GK is a valuable tool for the study of the regulation of the enzyme. For this purpose, cultured beta-cells (RIN 1046-38) derived from a X-ray-induced rat insulinoma were used as the experimental model [18].

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Materials and methods

Cell cultures

RIN 1046-38 cells were cultured in M199 medium (Gibco, BRL) supplemented with 10% fetal calf serum (FCS), 2 mM glutamine, 50 IU penicillin and 50 µg/ml streptomycin (Flow) at 37°C in an atmosphere of 95% humidified air/5% CO_2. Cells were used at passage 15–25, during which the glucose responsiveness is maintained [18].

Cell treatment

Cells were seeded in culture medium in 6 multiwell plates at a density of 2×10^5 cells/ml for subsequent RNA extraction or glucose-phosphorylating activity assay, or in 24 multiwell plates at a density of 10^5 cells/ml for insulin release experiments. Biotin (kindly provided by Lifegroup SpA, Padova, Italy) was added to the cells at increasing concentrations (0.01, 0.1, 1 and 10 µM) for 24 or 72 h. At the end of the treatment period, the cells were processed for RNA extraction, glucose-phosphorylating activity assay or insulin release experiments.

RNA preparation

Total RNA from RIN 1046-38 cells was prepared using RNAzol B method (Tel Test). RNA was quantified spectrophotometrically and stored in diethylpyrocarbonate (DEPC)-treated water at –70°C until use.

Semi-quantitative PCR (SQ-PCR) assay

Total RNA (1 µg/tube) was reverse transcribed by Moloney murine-leukemia virus (M-MLV) reverse transcriptase (Promega) (200 U/µg RNA) using 1 mM dNTP (Pharmacia) and 2.5 mM random hexamers (Pharmacia). The obtained cDNAs were PCR amplified in a thermal cycler (Perkin Elmer Cetus) using specific primers for the co-amplification of GK together with cyclophilin (p1B15), an ubiquitous and abundant eukaryotic protein, used as a reference [19]. The primers were designed in order to obtain a GK amplification product of 226 bp and a p1B15 amplification product of 158 bp (Table 1). The amplification mixture contained 2.5 U Taq DNA polymerase (Promega), 1.5 mM $MgCl_2$, 120 nM upstream and downstream primers for GK, 12 nM upstream and downstream primers for p1B15 and trace amounts of ^{32}P-dCTP (Amersham) in a final volume of 100 µl. The mixture was overlaid with 50 µl mineral oil (Sigma) and amplified for 28 cycles. Each amplification cycle consisted of a 45 s denaturation step at 94°C, 1 min annealing step at 62°C and 1 min elongation step at 72°C with a final elongation step at 72°C for 5 min. Preliminary experiments demonstrated that, under these conditions, the rate of amplification of GK and p1B15 remains constant between 25 and 31 cycles (not shown). DEPC-treated water aliquots were processed in parallel as a control. Triplicate samples of the amplification products were analysed on 2% (w/v) agarose gel (Biorad) in 0.5×Tris-borate-ethylene diamine tetra-acetic acid (EDTA) (TBE, Sigma). Bands corresponding to the two different amplification products (GK and p1B15) were excised from the gel, and the incorporated radioactivity was counted in a beta-counter (LKB). The relative amount of GK mRNA was determined by measuring the ratio between the radioactivity incorporated in the GK amplification product and that in the p1B15 amplification product [20]. Each experiment was done in triplicate.

Table 1 Primer sequences

Rat pancreatic glucokinase
us 5′ ATG GAG GCC ACC AAG AAG GAA AAG 3′
ds 5′ TTG GTT CCT CCC AGA TCT AAG GAG 3′
Δ=226 bp
Rat cyclophilin
us 5′ AGA AGG GCA TGA GCA TTG TGG AAG 3′
ds 5′ TGC TCT CCT GAG CTA CAG AAG GAA 3′
Δ=158 bp

Glucose-phosphorylating activity

Cells were detached by 0.05% trypsin/0.5 mM EDTA and pelleted. Cell pellets were resuspended and sonicated in 200 µl of ice-cold sonication buffer containing 20 mM K_2HPO_4, 1 mM EDTA, 110 mM KCl and 5 mM dithiothreitol (pH 7.7). The sonicated material was then centrifuged at 4°C for 15 min at 12 000×g. Supernatants were recovered and frozen for the subsequent glucose-phosphorylating activity assay. For this purpose, 4 µl of each supernatant were added to 100 µl assay buffer containing 50 mM Hepes HCl (pH 7.7), 100 mM KCl, 7.4 mM $MgCl_2$, 15 mM β-mercaptoethanol, 0.5 mM NAD^+, 0.05% bovine serum albumin (BSA), 2.5 µg/ml glucose-6-phosphate dehydrogenase, 5 mM ATP, and different glucose concentrations (0.03, 0.06, 0.12, 0.25 and 0.5 mM for measurements of hexokinase; 5, 7.5, 10, 15, 20, 25, 50, 65, 80 and 100 mM for measurements of GK activity). After 1 h at 30°C, the reaction was stopped by adding 1 ml of 500 mM sodium bicarbonate buffer (pH 9.4), and the fluorescence was then measured at 460 nm (excitation at 340 nm). V_{max} and K_m of hexokinase and GK activities were calculated in each experiment by the Eadie-Hofstee plot. To calculate GK activity, the V_{max} for hexokinase was subtracted from the activities measured at glucose concentrations >5 mM. Blanks were obtained by incubating either 0.5 or 100 mM glucose in the absence of adenosinetriphosphate (ATP). A standard curve was obtained by incubating 0.3–1.0 nmol glucose-6-phosphate and 1 nmol NADH with the assay reagents.

Insulin release

Cells were washed twice at 37°C for 30 min with a glucose-free buffer containing 114 mM NaCl, 25.5 mM $NaHCO_3$, 10 mM Hepes, 2.5 mM $CaCl_2$, 4.7 mM KCl, 1.21 mM KH_2PO_4, 1.16 mM $MgSO_4$, 0.1% BSA (pH 7.2). Thereafter, cells were incubated for 1 h in the same buffer with different glucose concentrations (0, 0.1 mM or 2.8 mM). At the end of the incubation period, aliquots of the supernatant were collected and stored at –20°C for subsequent insulin radioimmunoassay.

Insulin radioimmunoassay

Insulin was determined by a dextran-charcoal method as previously described [21], using an anti-insulin antibody raised in guinea pig, porcine insulin standard (Sigma, St. Louis, Mo.) and ^{125}I-insulin from New England Nuclear (Boston, Mass.).

Statistical analysis

Data are presented as the average ±SEM. Statistical analysis was carried out by Student's t-test.

Results

GK mRNA expression

Figure 1 illustrates changes in GK mRNA expression in RIN 1046–38 cells after 24 h (Fig. 1A) or 72 h (Fig. 1B)

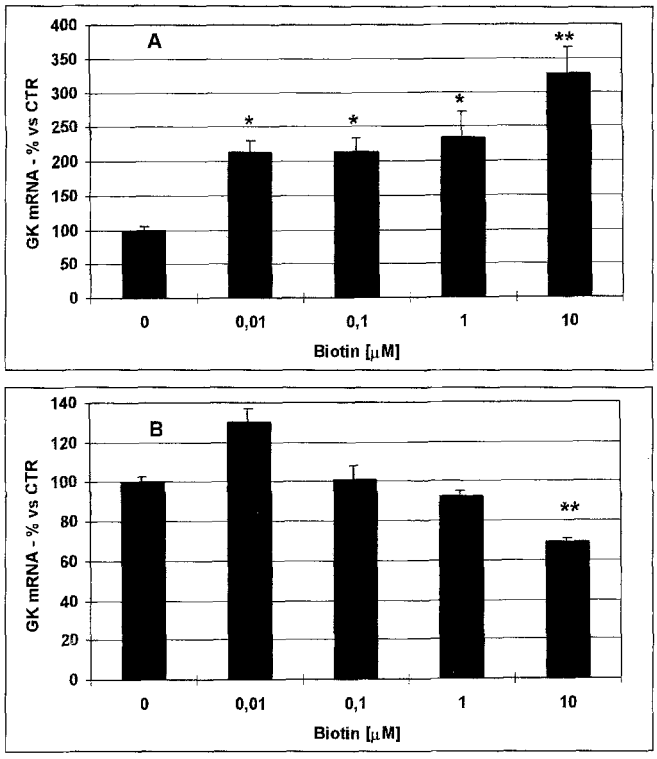

Fig. 1 Effect of 24 h (**A**) or 72 h (**B**) biotin treatment on glucokinase (GK) mRNA expression in RIN 1046-38 cells. Data are expressed as percent of control, where the control is GK mRNA expression in the absence of biotin treatment. *$P<0.05$; **$P<0.01$

biotin treatment. Biotin produced a dose-dependent increase of GK mRNA expression after 24 h (12.86%± 35.15%*, 13.23%±42.12%*, 33.35%±74.62%* and 126.80%±78.99%**, respectively, at 0.01, 0.1, 1 and 10 mM; *$P<0.05$; **$P<0.01$). Changes in GK mRNA expression after 72 h biotin treatment followed a biphasic pattern; a low dose of biotin (0.01 μM) stimulated GK mRNA expression (30.38%±12.23%), whereas higher doses produced a dose-dependent inhibition (–8.69%± 5.46% and –31.14±4.04%**, respectively, at 1 and 10 mM; **$P<0.01$).

Glucose-phosphorylating activity

GK K_m was 10.1±0.7 mM in RIN 1046–38 cells. Biotin treatment did not affect GK K_m at any concentration used (Table 2).

GK V_{max} measured in RIN 1046–38 cells was 12.4±1.1 nmol · h^{-1} · mg $prot^{-1}$. As shown in Table 2, low doses of biotin did not modify GK V_{max}, while higher doses significantly increased GK V_{max} (15.6±1.0 and 16.2±0.9* nmol · h^{-1} · mg $prot^{-1}$, respectively, in the presence of 1 and 10 mM biotin; *$P<0.01$) after 72 h treatment. No effect was observed after 24 h treatment.

Table 3 shows that hexokinase activity was not modified by biotin treatment at any concentration used.

Table 2 Kinetic characteristics of glucokinase in RIN 1046-38 cells treated with increasing biotin concentrations (data are mean ±SEM; n=4)

Biotin (M)	K_m (μM glucose)	V_{max} (nmol·h^{-1}mg $prot^{-1}$)
0	10.1 ± 0.7	12.4 ± 1.1
10^{-8}	9.2 ± 0.9	9.6 ± 0.9
10^{-7}	10.3 ± 0.5	10.1 ± 1.1
10^{-6}	10.6 ± 0.7	15.6 ± 1.0*
10^{-5}	9.8 ± 0.8	16.2 ± 0.9*

*$P<0.01$ vs control value in the absence of biotin

Table 3 Kinetic characteristics of hexokinase in RIN 1046-38 cells treated with increasing biotin concentrations (data are mean ±SEM; n=4)

Biotin (M)	K_m (μM glucose)	V_{max} (nmol·h^{-1}mg $prot^{-1}$)
0	35.2 ± 3.1	4.8 ± 0.8
10^{-8}	32.4 ± 4.3	4.7 ± 0.9
10^{-7}	36.0 ± 5.5	4.6 ± 1.2
10^{-6}	36.2 ± 4.1	4.7 ± 1.0
10^{-5}	36.6 ± 4.2	4.9 ± 0.9

Insulin release

Basal insulin release (in the absence of glucose) from RIN 1046–38 cells was 4.79±1.32 pg · ml^{-1} · h^{-1} and it increased by 85.98%±7.93% and by 98.04%±4.23%, respectively, in the presence of 0.1 and 2.8 mM glucose. Biotin treatment did not influence basal insulin release nor insulin release in the presence of 2.8 mM glucose at any concentration tested. Insulin release induced by 0.1 mM glucose was modestly increased by high-dose biotin treatment (12.32%±7.42% and 20.54%±9.57%, respectively, in the presence of 1 and 10 μM biotin; not statistically significant).

Discussion

It is known that the vitamin status influences glucose metabolism in man [22]. In particular, early studies reported that deficiency of vitamin B6, vitamin A or riboflavin are related to impaired glucose metabolism. Biotin is also involved in glucose metabolism, since it functions as a cofactor of pyruvate carboxylase and acetyl-CoA carboxylase, enzymes that are important for fatty acid synthesis and gluconeogenesis. These biotin-dependent enzymes are impaired under biotin-deficient conditions, leading to impaired gluconeogenesis [23]. In contrast, glycolytic enzymes are not influenced by biotin deficiency except for GK.

Biotin is known to regulate hepatic GK expression both at a transcriptional and at a translational level. In fact, it has been demonstrated that biotin increases the activity of

guanylate cyclase in primary cultures of rat hepatocytes [24, 25]. The increase in intracellular cGMP after biotin addition to the culture medium is followed by an increase in GK activity, suggesting a role of the cyclic nucleotide in the regulation of the enzyme at a translational level [16]. Moreover, Chauhan and Dakshinamurti demonstrated that biotin administered to starved rats induces a considerable and rapid increase of hepatic GK mRNA acting at a transcriptional level, as revealed by the nuclear run-on assay [17].

The regulation of GK mRNA and activity in pancreatic beta-cells has been poorly investigated. Recently, it was shown that dexamethasone and retinoic acid increase pancreatic GK mRNA and activity, and that T3 has no effect on pancreatic GK activity, while it decreases GK mRNA expression [26]. Nothing is known about the role of biotin on pancreatic GK.

RIN 1046-38 cells, a rat insulinoma cell line, express GK mRNA and protein corresponding to the size of the GK pancreatic isoform [27]. This cell line is also glucose-responsive, and therefore it has been used in the present study in order to investigate in parallel the role of biotin on GK mRNA expression, GK activity and insulin release.

Our data demonstrate that biotin treatment for 24 h enhances GK mRNA expression in a dose-dependent manner in cultured RIN 1046-38 cells. After 72 h treatment GK mRNA is still increased in cells treated at a low dose, while it is inhibited in cells treated at a high dose.

GK V_{max} is not modified after 24 h treatment, while after 72 h it is significantly increased in the presence of high-dose biotin. It is tempting to suggest that GK activity at 72 h is increased as a consequence of the mRNA accumulation observed at 24 h. The increased GK activity would in turn determine a negative feed-back effect at the level of mRNA, leading to the reduction observed at 72 h.

Kinetic data indicate that biotin does not affect enzyme affinity for the substrate. It has to be noted that biotin action seems to be specific for GK, as no detectable modifications could be observed in the kinetic properties of hexokinase.

Glucose-induced insulin release was modestly enhanced by high-dose biotin treatment in the presence of non-maximal glucose concentrations.

Previous studies demonstrated that biotin is able to improve glucose tolerance and insulin resistance in non-biotin-deficient, genetically diabetic KK rats by increasing glucose utilization [23]. Biotin administration to non-insulin-dependent diabetic patients lowered plasma glucose levels without modification of insulin secretion; moreover, in the same patients serum biotin levels were lower than in control subjects and inversely correlated with fasting plasma glucose levels [29]. A possible interpretation of biotin's mechanism of action is the stimulation of pyruvate carboxylase and therefore the induction of glucose utilization by entry into the tricarboxylic acid cycle. Our results are consistent with the hypothesis that, in our in vitro system, biotin is able to enhance pancreatic GK mRNA expression with a consequent increase of protein expression, leading to a modest improvement of glucose-induced insulin release.

These data suggest that biotin could influence glucose metabolism also by acting directly at the level of beta-cells.

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ANNOUNCEMENTS

L'Associazione per L'Aiuto ai Giovani Diabetici bandisce un Concorso per una Borsa di Studio sul Diabete di Tipo 1 nei Settori di Prevenzione, Cura e Complicanze

L'importo della Borsa di Studio, della durata di un anno, è di L.30.000,000 al lordo delle ritenute fiscali di legge e sarà conferita a un laureato di nazionalità italiana che non abbia compiuto i 35 anni al 31.7.96 e che presenti un programma di ricerca originale da svolgersi in Italia o all'estero.

Il candidato non dovrà avere un rapporto di lavoro dipendente e non dovrá usufruire contemporaneamente di altre Borse di Studio.

Nella domanda in carta libera dovranno essere indicati: indirizzo completo, data e luogo di nascita, codice fiscale.

Dovranno assere allegati alla domanda:

- Certificato di cittadinanza Italiana
- Certificato di laurea e votazioni degli esami
- Curriculum vitae
- Curriculum scientifico
- Progetto di ricerca che includa una relazione economica.

Le domande saranno vagliate da una Commissione Giudicatrice, nominata dall'Associazione, che le esaminerà in sede congiunta e l'attribuzione della Borsa di Studio dovrà assere approvata del Consiglio Direttivo dell'Associazione il cui giudizio è insindacabile.

Il vincitore sarà avvisato con lettera raccomandata e la Borsa di Studio verrà consegnata durante una manifestazione pubblica.

I candidati dovranno inviare la domanda in 8 copie, entro il 31.7.96 a:

Associazione per l'Aiuto ai Giovani Diabetici
Via Foscolo 3, I-20121 Milano, Tel.: 02 876404,
Fax: 02 878177

6th Meeting of the European Association for the Study of Diabetes (EASDEC)

Vienna, Austria, 31 August–1 September 1996

The title of the meeting will be "Update of Research in Diabetic Eye Disease" and the programme will include keynote lectures on risk factors for diabetic retinopathy, new perspectives on the role of growth factors in its pathogenesis and recent advances in vitreo-retinal surgery for advanced diabetic eye disease. There will be ample space for oral and poster free communications, proposals for collaborative multicentre activities, and interactive discussion.

Information: Organizing Secretariat Mondial Congress, Faulmanngasse 4, A-1040 Vienna, Austria, Tel. +43 15 88 04-0, Fax: +43 15 86 91 85

32nd Annual Meeting of the European Association of the Study of Diabetes (EASD)

Vienna, Austria, 1–5 September 1996

Location: Austria Center Vienna

Information: Professor G. Schernthaner, EASD 1996, c/o Conference Secretariat, Mondial Congress, Faulmanngasse 4, A-1014 Vienna, Austria, Tel.: 43/1/5 88 04-0, Fax: 43/1/5 35 60 16

III Course on the Non-Obese Diabetic (NOD) Mouse

London, 9–13 September 1996

The NOD mouse is probably the best animal model to study the pathogenesis and preventive therapy of Type 1 diabetes and other autoimmune conditions. The Diabetes Department at St. Bartholomew's Hospital is organizing the third course aimed at those investigators who wish to learn more about these spontaneously diabetic mice as an investigative tool. The course will last for a period of one week, and is aimed at both newcomers to research involving animals as well as more experienced researchers who have not previously worked with NOD mice.

Topics: This year the course is designed to cater for the specific requirements of the participants and the programme will be finalized according to the proposals made by those selected to participate. Therefore, in your application please specify the topic with the area of NOD mouse research you would particularly like to learn about.

At team of Internationally known experts will be among the teachers of the course.

Course organizers: P. E. Beales and P. Pozzilli

Cost including catering: £ 695 (Accommodation can be arranged in the Halls of Residence at an extra cost of £ 19.50 per night including breakfast.)

Information: Dr. PE Beales, III Course on the NOD mouse, ARTEM Unit, Department of Diabetes and Metabolism, St. Bartholomew's Hospital, London EC1A 7BE, UK, Tel.: +44-71-6 01 85 89, Fax: +44-71-6 01 74 49

3rd EFES Postgraduate Clinical Endocrinology Course

Turin, Italy, 17–19 October 1996

EFES Executive Committee:
President: L. Martini (Milan, Italy)
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Local Scientific Organizing Committee: F. Camanni (Chairman), E. Ghigo (Chairman), E. Arvat, E. Ciccarelli, M. Maccario (Division of Endocrinology – University of Turin)

Aims: The Course will address some of the latest advances in endocrinology and metabolism. Basic knowledge for endocrinological clinical practice will be introduced along with new diagnostic and therapeutic approaches to endocrinological and metabolic diseases. The Course is intended for senior/trainee endocrinologists, practising endocrinologists and diabetologists as well as internists.

Scientific program: The Course will last two and a half days. It will begin on Thursday, October 17, 1996 at 12.00 p.m. and will end on Saturday, October 19 at 6.30 p.m. A number of leading European scientific authorities will be invited as keynote speakers to present reviews of endocrine topics. Beside main lectures, interactive workshops are foreseen in which participants will be invited to present case reports to be selected for discussion. To ensure that each participant derives the maximum benefit, the number of places available for participants is limited. A certificate of attendance issued by the Executive Committee of the European Federation of Endocrine Societies will be delivered to registrants completing the Course.

Registration fee: The full registration will be Lit. 500.000 (including VAT) and a reduced fee will be available for trainee endocrinologists. The fee will include attendance to all scientific sessions, handout material, a welcome reception, and coffee breaks.

Information: Prof. F. Camanni or Prof. E. Ghigo, Divisione di Endocrinologia, Ospedale Molinette, C.so Dogliotti, 14-10126 Torino – Italy, Telephone: 39-11-6 96 31 56, Fax 39-11-6 64 74 21