Taurine supplementation modulates glucose homeostasis and islet function[☆]

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Abstract

Taurine is a conditionally essential amino acid for human that is involved in the control of glucose homeostasis; however, the mechanisms by which the amino acid affects blood glucose levels are unknown. Using an animal model, we have studied these mechanisms. Mice were supplemented with taurine for 30 d. Blood glucose homeostasis was assessed by intraperitoneal glucose tolerance tests (IPGTT). Islet cell function was determined by insulin secretion, cytosolic Ca^{2+} measurements and glucose metabolism from isolated islets. Islet cell gene expression and translocation was examined via immunohistochemistry and quantitative real-time polymerase chain reaction. Insulin signaling was studied by Western blot. Islets from taurine-supplemented mice had: (i) significantly higher insulin content, (ii) increased insulin secretion at stimulatory glucose concentrations, (iii) significantly displaced the dose-response curve for glucose-induced insulin release to the left, (iv) increased glucose metabolism at 5·6 and 11·1-mmol/L concentrations; (v) slowed cytosolic Ca^{2+} concentration ($[Ca^{2+}]i$) oscillations in response to stimulatory glucose concentrations; (vi) increased insulin, sulfonylurea receptor-1, glucokinase, Glut-2, proconvertase and pancreas duodenum homeobox-1 (PDX-1) gene expression and (vii) increased PDX-1 expression in the nucleus. Moreover, taurine supplementation significantly increased both basal and insulin stimulated tyrosine phosphorylation of the insulin receptor in skeletal muscle and liver tissues. Finally, taurine supplemented mice showed an improved IPGTT. These results indicate that taurine controls glucose homeostasis by regulating the expression of genes required for glucose-stimulated insulin secretion. In addition, taurine enhances peripheral insulin sensitivity.

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1. Introduction

Amino acids have been recognized as important signaling mediators in different cellular functions. Taurine (2-amino ethane sulphonic acid) is a conditionally essential amino acid for human and nonhuman primates [1], that is involved in many important biological functions including osmoregulation, inhibition of protein phosphorylation and calcium modulation [2,3].

Taurine is found at high concentrations within pancreatic islets [4]. Taurine reduces the rate of apoptosis [5] and acts on DNA synthesis, preventing abnormal development of the endocrine pancreas [6]. Previous reports have dealt with its effects on insulin secretion. In fetal rat islets, taurine increases glucose-stimulated insulin secretion and enhances

the action of some secretagogues, such as leucine or arginine [7,8]. In an animal model of fetal protein malnutrition, taurine supplementation normalized islet cell proliferation and insulin secretion [9]. Furthermore, in guinea pigs with hyperglycemia, taurine administration significantly decreased blood glucose levels [10]. Moreover, it has been shown that taurine increases glucose sensitivity of UCP2-overexpressing cells by enhancing mitochondrial metabolism [11], at least partially by acting on the mechanism for Ca^{2+} sequestration into the mitochondrial matrix [12]. Finally, taurine inhibits ATP-sensitive K^+ (KATP) channel activity in adult rat beta cells [13].

In addition, there is evidence indicating that taurine also has hypoglycemic properties due to the potentiation of the effects of insulin [14] and its interaction with the insulin receptor [15]. Taurine increases glycogen synthesis, glycolysis and glucose uptake in the liver and heart of adult rats [14,16]. In Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a model of Type 2 diabetes with insulin resistance, and in humans, taurine improves insulin sensitivity [17,18]. Finally, taurine antioxidant properties protect pancreatic beta-cells against stress oxidative-induced decrease in function observed in some pathophysiological conditions [19,20].

These findings indicate that taurine is involved in distinct central and peripheral processes necessary for the control of glucose homeostasis; however, the mechanisms by which the amino acid affects blood glucose levels are unknown. In this study we investigate these mechanisms.

2. Materials and methods

2.1. Experimental design

Male Swiss mice (OF1) 12–16 weeks old were used throughout the experiments. The mice were obtained from the breeding colony at Animal Center of Miguel Hernandez University. The mice were allowed free access to food and water and maintained on a 12-h light–dark cycle at 24°C and constant humidity. Body weight, food and water intake were recorder throughout the study. For the "in vivo" studies, two groups of animals were established: (i) Control group (Ctrl) (n=36): mice fed with standard rodent chow (Maintenance Diet 2014, Harlan Interfauna Iberica, Barcelona, Spain) and (ii) Taurine group (Tau) (n=40): mice fed with the same diet supplemented with 2.0% (w:v) taurine (Sigma, St. Louis, MO, USA) in the drinking water for 30 days, as previously published [21,22]. The rodent chow does not contain taurine. The content of cystine is 0.27%. Both groups of animals were studied in parallel and in the same laboratory. For the "in vitro" studies, islets of Langerhans were isolated by collagenase (Boehringer Mannheim, Mannheim, Germany) digestion as previously described [23] from mice fed with standard rodent chow. Average diameter of isolated islets used throughout the study was 125 μm. Isolated islets were cultured for 3 days in RPMI 1640 medium, pH 7.4, supplemented with 10% fetal calf serum, 100 IU/ml

penicillin, 0.1 mg/ml streptomycin (all from Invitrogen, Barcelona, Spain), 5.6 mmol/L glucose (Sigma) and 3 mmol/L taurine. To test the effects of acute taurine treatment, freshly isolated islets of Langerhans were used. Animals were killed by cervical dislocation. All experimental procedures involving animals followed the requirements of the Animal Welfare Act and the National Institute of Health guidelines. In addition, the local animal ethics approved the studies.

2.2. Amino acid determination

For taurine determination, 100 islet samples were deproteinized with 200 μl of 25% trichloroacetic acid. After centrifugation for 20 min at 10,000×g, 175 μl of the supernatant was analyzed on a Biochem 20 Plus Amino Acid Analyzer (Amersham Pharmacia, Piscataway, NJ, USA), using a PEEK (polyetheretherketone) cation exchange high-performance column with ninhydrin detection. Amino acids were quantified with a Biochrom 20 Control software version (Biochrom Cambridge, UK), using an amino acid standard (Sigma).

2.3. Glucose and taurine-tolerance test

The glucose (IPGTT) and taurine (IPTTT) intraperitoneal tolerance tests were performed after 30 days of taurine supplementation. For IPTTT, the same protocol as IPGTT was used; however, animals receipted only an intraperitoneal injection of taurine (0.2 g/kg body weight). After overnight fasting, blood samples (20 μl) were obtained by tail-snipping before (0 min), then 30, 60 and 120 min after an intraperitoneal injection of glucose (2 g/kg body weight) or taurine (0.2 g/kg body weight). Blood was obtained from the snipped tail and glucose measured with a portable glucose meter (Esprit, Bayer Diagnostic Europe, Dublin, Ireland). The glucose responses during the IPGTT or IPTTT were calculated via estimation of the total area under the glucose (G) curves using the trapezoidal method [24].

2.4. Insulin measurements

Insulin measurements were performed as previously described [23]. Briefly, fresh collagenase-isolated islets or cultured islets were incubated for 1 h at 37°C in fresh modified Krebs Ringer Bicarbonate Buffer (KRB) supplemented with 5.6 mmol/L glucose and 3% bovine serum albumin (BSA) (Sigma, St. Louis, MO). The medium was continuously bubbled with a mixture of O_2 (95%) and CO_2 (5%) for a final pH of 7.4. Islets were incubated in groups of 4 in 1 ml of KRB with 1% BSA plus the different glucose concentrations (5.6, 8.3, 11.1, 16.7 and 22.2 mmol/L) or 30 mmol/L KCl or 10 mmol/L taurine, for 30 min at 37°C. Insulin content and secretion was measured as previously described [23].

In addition, 15-μl aliquots of the same samples were stored at −80°C for protein determination by Bradford assay. Insulin was assayed by RIA using a kit from DPC (Los Angeles, CA, USA). Standard curves and experimental points were run in triplicate. The glucose concentration

producing a response that was 50% of the maximum (EC50) was calculated as the mean negative logarithm (pD2).

2.5. [Ca²⁺]i measurement

Fresh pancreatic islets were incubated with fura-2/AM (Invitrogen) (5 mol/L) for about 1 h at room temperature in standard medium of the following composition (pH 7.4): 145 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L MgCl2, 1 mmol/L CaCl2, 10 mmol/L HEPES and 10 mmol/L glucose. Islets were then washed with the same medium and placed in a chamber thermostatically regulated to 37°C, located on the stage of an inverted microscope (Nikon UK, Kingston, UK). Islets were then perfused with prewarmed (37°C) KRB supplemented with 3 mmol/L glucose. The solution was continuously gassed with 95% O_2 and 5% CO_2 to maintain pH at 7.4. A ratio image was acquired approximately every 3 s with an ORCA-100 CCD camera (Hammamatsu Photonics Iberica, Barcelona, Spain) in conjunction with a Lambda-10-CS dual filter wheel (Sutter Instrument, CA), equipped with 340-, 380- and 10-nm bandpass filters, and a range of neutral density filters (Omega Opticals, Stanmore, UK). Data were acquired by using the Aquacosmos version 2.0 (Hammamatsu). Otherwise, these experimental procedures were performed as previously described [25].

2.6. MTT assay

Freshly isolated islets were incubated for 1 h at 37°C in KRB supplemented with 5.6 mmol/L glucose and 3% BSA. The medium was continuously bubbled with a mixture of O_2 (95%) and CO_2 (5%) for a final pH of 7.4. Subsequently, C, N-diphenyl-N″-4,5-dimethylthiazol-2-yl-tetrazolium bromide (MTT) (Sigma) reduction was measured as previously described [26].

2.7. PDX-1 and insulin expression

To test PDX-1 localization, pancreatic islets were isolated from control and taurine groups as previously described [18], then cultured overnight in RPMI 1640 medium, pH 7.4, supplemented with 10% fetal calf serum, 100 IU/ml penicillin, 0.1 mg/ml streptomycin and 5.6 mmol/L glucose.

A standard immunocytochemistry protocol was used to visualize PDX-1 and insulin. Islets were fixed with 4% paraformaldehyde for 4 min, washed with phosphate-buffered saline and permeabilized with 0.02% Triton X-100 overnight. Primary antibodies and dilutions were as follows: insulin mouse monoclonal (1:200; Sigma) and PDX-1 (1:200; Abcam, Cambridge, UK). Primary antibody localization was done using antirabbit FITC (fluorescein isothiocyanide; 1:80; Sigma) and antimouse TRITC (tetra-methylrhodamine isothiocyanate; 1:300; Sigma). Proper controls for secondary antibodies revealed no nonspecific staining. Samples were analysed using laser scanning microscopy (Zeiss LSM-510; Oberkochen, Germany).

2.8. Insulin receptor phosphorylation experiments

The abdominal cavities of anesthetized mice with 15 mg/kg body weight of sodium thiopental (Lilly, Indianapolis, IN) were opened, and the animals received an infusion of human recombinant insulin (Humulin R, Lilly) (0.2 ml, 1 µmol/L) or saline (0.2 ml) through the cava vein.

In the experiments to ascertain the acute effect of taurine, mice were treated iv with 0.2 ml of a solution containing 20 mg/ml taurine. After 90-s fragments (3.0×3.0×3.0 mm) of liver and skeletal muscle (soleus muscle) were excised and immediately homogenized in solubilization buffer at 4°C, containing 1% Triton X-100, 100 mmol/L Tris-HCl (pH 7.4), 100 mmol/L sodium pyrophosphate, 100 mmol/L sodium fluoride, 10 mmol/L EDTA, 10 mmol/L sodium orthovana-date, 2.0 mmol/L phenylmethylsulphonylfluoride and 0.1 mg/ml aprotinin (all from Sigma), using a Polytron PTA 20S generator (model PT 10/35; Brinkmann Instruments, Westbury, NY, USA). Insoluble material was removed by centrifugation for 20 min at 9,000×g in a 70. Ti rotor (Beckman Instruments, Palo Alto, CA, USA) at 4°C. The protein concentration of the supernatants was determined by Bradford assay. Aliquots of the resulting super-natants containing 5.0 mg of total protein were used for overnight immunoprecipitation using rabbit polyclonal anti-insulin receptor (sc-711, Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C, followed by SDS/PAGE (BioRad, Richmond, CA, USA), transfer to nitrocellulose membranes (Amersham, Aylesbury, UK) and blotting with mouse monoclonal antiphosphotyrosine antibodies (sc-508, Santa Cruz Biotechnology). In direct immunoblot experiments, 0.2 mg of protein extracts obtained from each tissue were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membranes and blotted with an anti-insulin receptor anti-body. Visualization of specific protein bands was performed by the incubation of the membranes with 125I-protein A (Amersham) followed by exposure to RX films (Amersham), as described [27].

2.9. Quantitative polymerase chain reaction

RNA was prepared with Trizol (Invitrogen) reagent according to the manufacturer's instructions and 2 µg of total RNA was reverse-transcribed using TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA). The cDNA was amplified by quantitative real-time polymerase chain reaction (PCR) using an ABI prism 7700 Sequence Detection System (Applied Biosystems). Quanti-tative real-time PCR reactions were performed in triplicate using 9 µl of cDNA, 10 µl of TaqMan Universal PCR Master Mix 2X and 1 µl of mix 20X of probe and primers in a final volume of 20 µl. Samples were amplified using the following thermal profile: (i) an initial incubation at 50°C for 2 min; (ii) an incubation at 95°C for 10 min and (iii) 40 cycles of denaturation at 95°C for 15 s followed by annealing and extension at 60°C for 1 min. Primers and probes for genes

Table 1
Effect of taurine supplementation on body weight, food and water intake, basal blood glucose and serum insulin concentrations of the Ctrl and Tau mice groups

Groups	Initial body weight (g)	Final body weight (g)	Food intake (g/day)	Taurine intake (mg/day)	Water intake (ml/day)	Basal blood glucose (mg/dl)	Basal serum insulin (ng/ml)
Ctrl	26±1	28±1	3.1±0.2	0	5.0±0.4	62±4	0.75±0.07
Tau	27±1	29±1	3.1±0.1	90±2.9	4.5±0.8	60±5	0.75±0.08

The data are represented as mean±S.E.M. of 10 mice from both groups.

were from Applied Biosystems Assay On-Demand Gene expression products: -actin (Mm00607939) housekeeping, Ins2 (Mm00731595_Gh), Pdx-1 (Mm00435565_m1), Glut-2 (Mm00446224_m1), Glucokinase (GK) (Mm00439129_m1), Sur-1 (Mm00803450_m1) and Pro-convertase (Pcsk-1 Mm00479023). Amplifications were normalized to -actin, and quantification of gene expression was performed using the $\Delta\Delta$Ct calculation, where Ct was the threshold cycle. The amount of target gene was normalized to -actin and relative to the calibrator (islet from control group) using the equation $2-\Delta\Delta$Ct.

2.10. Statistical analysis

Results are expressed as mean±S.E.M. Differences between pairs of means were addressed by the two-tailed Student's t test. Analysis of variance was used followed by the Tukey–Kramer test, when necessary, for multiple comparisons. Differences were considered significant for $P<.05$.

3. Results

3.1. Effect of taurine supplementation on metabolic characteristics of mice

Taurine supplementation did not modify food or water intake. Also, it had no effect on body weight, basal blood glucose and serum insulin concentrations (Table 1). However, during IPGTT, the blood glucose concentration at 30 and 60 min after glucose challenge was significantly lower in taurine group (267±9 and 149±13 mg/dl, respectively; $n=5$) than in control group (310±21 and 191±9 mg/dl, respectively; $n=5$) ($P<.05$; Fig. 1A). The mean total area under the glucose curve (G) in response to glucose load was lower in taurine group with respect to control group (17,961±856 vs. 21,503±1174, respectively; $n=5$; $P<.05$). In addition, blood glucose values after IPTTT (Fig. 1B) and the total area under the glucose curves (G) were similar in mice from taurine and control groups (10,346±564 vs. 10,950±231, respectively; $n=5$; P=NS).

3.2. Effect of taurine supplementation on insulin secretion

To test whether the better glucose homeostasis in taurine group reflected modifications in insulin secretion, the effects of taurine supplementation on glucose-induced insulin release were monitored. Levels of glucose-induced insulin secretion from islets in the taurine and control groups are

shown in Fig. 2A. The relationship between insulin secretion and glucose concentration was sigmoidal in both groups; however, the dose-response curve in islets from the taurine group showed a significant displacement to the left ($P<.001$) in the dose response (EC50=7.85±0.38 mmol/L; $n=5$) when compared with islets from the control group (EC50=10.83±0.10 mmol/L; $n=5$). A broader glucose response range was seen in the taurine group (Fig. 2A). In addition, islets from the taurine group showed increased insulin secretion ($P<.001$) at stimulatory glucose concentrations, when compared with the control group. In contrast, in the in vitro studies (Fig. 2B), no significant differences were found in the dose response when islets were cultured for three days in the absence (EC50=8.19±0.21 mmol/L; $n=5$) or presence (EC50=8.33±0.32 mmol/L; $n=5$) of taurine. Nevertheless, at all glucose concentrations tested in the in
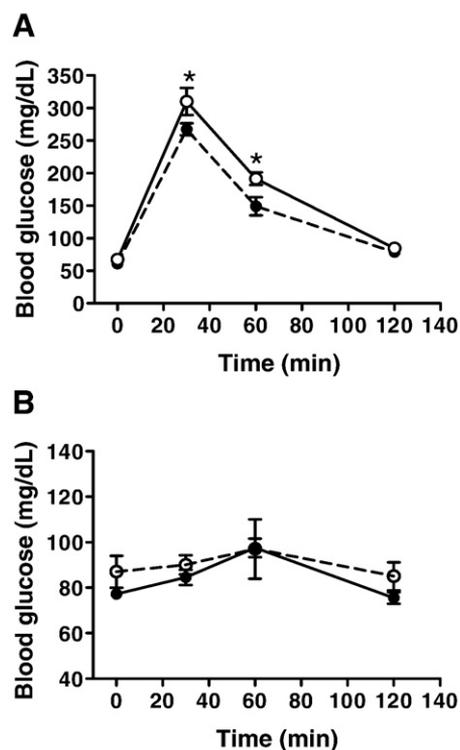

Fig. 1. Effect of taurine supplementation on glucose homeostasis. (A) Intraperitoneal glucose tolerance test on overnight fasted control mice (white circles) and taurine supplemented mice (black circles). (B) Intraperitoneal taurine tolerance test on overnight fasted control mice (white circles) and taurine supplemented mice (black circles). Values are expressed as means±S.E.M. of five experiments. *$P<.05$ when compared with taurine group.
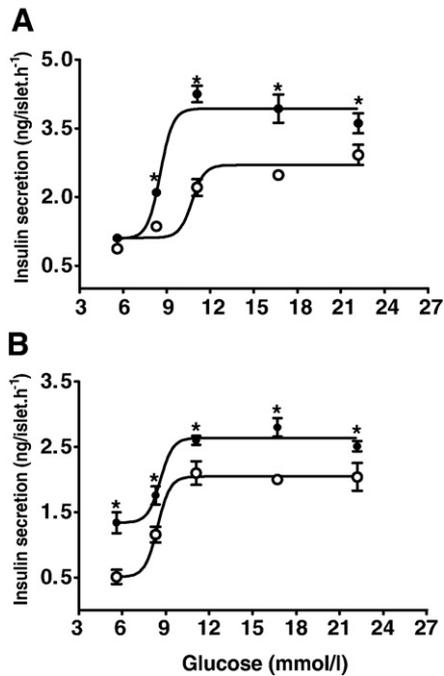
Fig. 2. Effect of taurine supplementation on glucose-induced insulin secretion. (A) Fresh islets from the control group (white circles) and the taurine group (black circles). (B) Islets, from mice not given additional taurine, cultured for three days in the absence (white circles) or presence of taurine (black circles). To perform the insulin secretion experiments, islets were incubated in batches of four for 30 min at 37°C in 1 ml of fresh KRB with 1% BSA plus different glucose concentrations. Values are expressed as means±SEM of five experiments. *$P<.001$ when compared with control group and islets cultured in the absence of taurine.

vitro studies, taurine cultured islets showed significant higher values ($P<.001$) of insulin release (Fig. 2B). For the in vitro studies, the acute effect of 10 mmol/L taurine on 3 and 11.1 mmol/L glucose-induced insulin release was examined. No effect on 3 mmol/L glucose-induced insulin release was observed when islets were incubated in the presence or absence of taurine (1.04±0.34 and 0.65±0.20 ng/islet h^{-1} respectively, $n=6$). The same was observed with 11.1 mmol/L glucose-induced insulin release (5.73±1.17 and 5.55±0.59 ng/islet h^{-1} respectively, $n=6$). Additionally, the effects of taurine supplementation on KCl induced insulin release were studied. The insulin response to 30 mmol/L KCl is shown in Fig. 3. In the taurine group (Fig. 3A), KCl induced a significantly ($P<.001$) higher increase in insulin secretion (6.95±0.38 ng/islet h^{-1}, $n=12$) when compared with the control group (2.29±0.26 ng/islet h^{-1}, $n=12$). The same effect was observed for the in vitro studies (Fig. 3B). Islets cultured for 3 days with taurine elicited a significant ($P<.01$) increase in KCl-induced insulin release when compared with islets incubated in the absence of taurine (2.96±0.26 vs 2.06±0.17 ng/islet h^{-1} respectively, $n=12$). Insulin content in islets from mice supplemented with taurine and 72-h-cultured with taurine were also significantly higher ($P<.001$) than their respective controls (taurine group=107±6 vs. control group 60±5 and taurine cultured islets=84±12 vs.

control cultured islets=36±5 ng/μg protein; $n=5$). To note, that insulin content was measured in the presence of 5.6 mmol/L, and all islets had the same average size. In addition, cultured islets had less insulin than fresh islets. Finally, glucose metabolism was studied by MTT reduction test. Islets from the taurine group showed a significant increase ($P<.05$) in formazan production at 5.6 and 11.1 mmol/L glucose (187±8 and 246±50 OD 10^{-3}, 540 nm/10 islets; $n=10$) when compared with the control group (144±6 and 160±7 OD 10^{-3}, 540 nm/10 islets; $n=10$).

3.3. Effect of taurine on [Ca^{2+}]i

Cytosolic calcium is a moment-to-moment regulator of nutrient-induced insulin release. Thus, we tested whether taurine supplementation affected [Ca^{2+}]i. Cytosolic calcium response to glucose are shown in Fig. 4. Islet from the control group (Fig. 4A; 12 islets from four mice) showed the typical [Ca^{2+}]i oscillatory pattern in response to stimulatory glucose concentrations (11.1 and 16.7 mmol/L). However, islets from taurine group elicited slow [Ca^{2+}]I oscillation in response to the same stimulatory glucose concentrations (Fig. 4B; 14 islets from four mice). These slow [Ca^{2+}]i oscillations reached a significant higher amplitude value (0.34±0.05 fluorescence arbitrary units; $P<.001$) and slower frequency (frequency, 0.51±0.12 min^{-1}; $P<.001$) than the
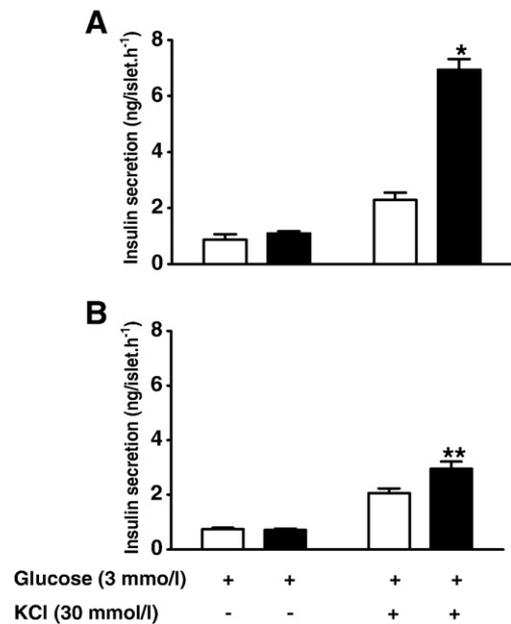

Fig. 3. Effect of taurine supplementation on KCl induced insulin secretion. (A) Fresh islets from control group (white bars) and taurine group (black bars). (B) Three days cultured islets in the absence (white bars) and presence of taurine (black bars). To perform the insulin secretion experiments, islets were incubated in batches of four for 30 min at 37°C in 1 ml of fresh KRB with 1% BSA and 3 mmol/l glucose, in the absence and presence of 30 mmol/L KCl. Values are expressed as means±S.E.M. of 12 experiments. *$P<.001$ when compared with islets from control group stimulated with KCl. **$P<.01$ when compared with islets cultured in the absence of taurine but in the presence of KCl.
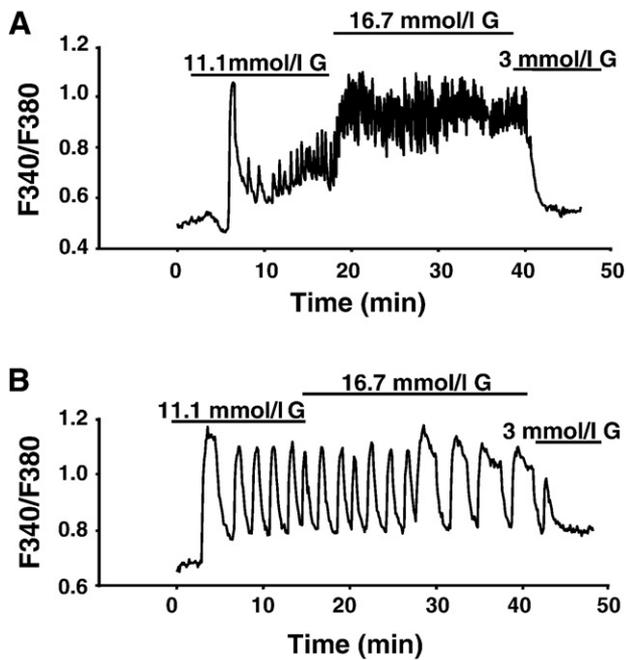
Fig. 4. Glucose-induced $[Ca^{2+}]$I changes in taurine group. Representative examples of the effects of different glucose concentrations (3, 11.1 and 16.7 mmol/L) on $[Ca^{2+}]$i in freshly isolated islets from control group (A) and taurine group (B).

fast glucose-induced $[Ca^{2+}]$i oscillations (0.14±0.06 fluorescence arbitrary units and frequency, 1.9±0.4 min^{-1}) observed in islets from the control group. Moreover, in the presence of 16.7 mmol/L glucose, taurine supplementation provoked slower $[Ca^{2+}]$I oscillations (frequency, 0.20±0.08 min^{-1}) when compared with 11.1 mmol/L glucose.

3.4. Effects of taurine supplementation on PDX-1 localization

The transcription factor PDX-1 is required for the expression of insulin and other proteins involved in the stimulus–secretion coupling in beta-cells; thus, we analyzed by immunocytochemistry the localization of PDX-1 in pancreatic islets from the taurine and control group (Fig. 5). In pancreatic islets obtained from control mice, PDX-1 was less present in the nucleus (Fig. 5A; n=7 isolated islets of 5 control mice). However, in pancreatic islets from taurine group, the expression of PDX-1 in the nucleus was evident (Fig. 5B; n=7 isolated islets of five mice from the taurine group).

3.5. Effect of taurine supplementation on expression of genes required for glucose-induced insulin secretion

To assess whether increased PDX-1 expression in the nucleus contributes to modifications in the expression of genes involved in the stimulus–secretion coupling, the expression of Glut-2, GK, Sur-1, insulin, Pdx-1, Pcsk-1 together with PDX-1 was monitored by quantitative PCR in islets from taurine and control group (Fig. 6). The level of expression of all genes studied were significantly increased

(P<.05 and P<.01) in islets derived from taurine group (n=8) when compared with islets from control group (n=7).

3.6. Taurine supplementation increases basal an insulin-induced tyrosine phosphorylation of the insulin receptor

To investigate the effect of chronic taurine treatment upon the molecular activation of the insulin receptor, control and taurine-treated mice were acutely injected with saline or insulin. Protein concentration, together with the tyrosine phosphorylation status of the insulin receptor, was then evaluated in skeletal muscle (Fig. 7A) and liver (Fig. 7B). Taurine supplementation did not modify insulin receptor expression in skeletal muscle (n=4) or liver (n=4). However, both basal and insulin stimulated tyrosine phosphorylation of the insulin receptor were significantly increased, (P<.01) in
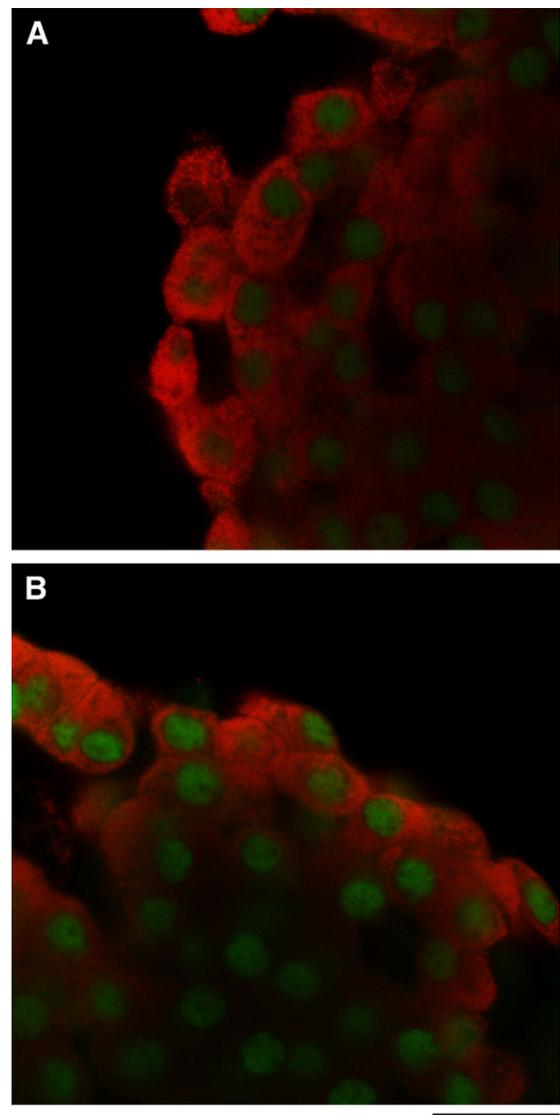

Fig. 5. Effect of taurine supplementation on PDX-1 localization. Representative confocal images showing insulin (red) and PDX-1 (green) localization in pancreatic islets cultured in 5.6 mmol/L glucose from the control group (A) and the taurine group (B). Scale bars, 20 μm.
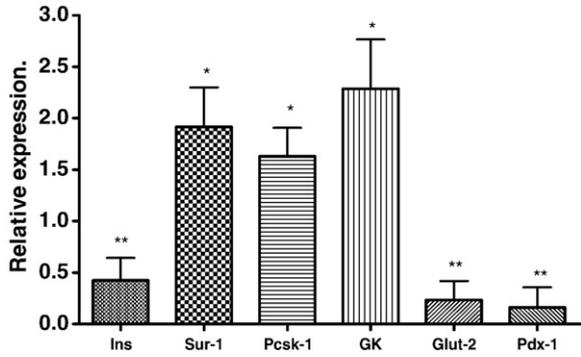
Fig. 6. Effect of taurine supplementation on expression of genes required for glucose-induced insulin secretion. Expression of *insulin* (ins), *PDX-1*, *Glut-2*, *GK*, *Pcsk-1* and *Sur-1* genes by real-time PCR. Bars indicate relative levels of expression determined by the expression 2−Ct for each of the studied genes. Thus, the level of expression of all genes studied in control group was 0. Values are expressed as means±S.E.M. *$P<.01$ when compared with control group. **$P<.05$ when compared with control group.

the taurine group ($n=4$) when compared with the control group ($n=4$), in both skeletal muscle and liver tissues.

3.7. Acute treatment with taurine induces the tyrosine phosphorylation of the insulin receptor

Since basal levels of tyrosine phosphorylation of the insulin receptor were increased in skeletal muscle and liver

tissues of the taurine group, we evaluated the role of an acute bout of taurine upon insulin receptor activation. Thus, control mice received a single iv dose of insulin or taurine and the insulin receptor protein, and tyrosine phophorylation levels were subsequently determined. We found that taurine administration was able to significantly increase the tyrosine phosphorylation of the insulin receptor ($P<.001$) ($n=4$), in both skeletal muscle (Fig. 7C) and liver tissues (Fig. 7D), when compared with saline infusion. Furthermore, a single acute dose of taurine did not modify insulin receptor expression in skeletal muscle ($n=4$) or liver tissues ($n=4$).

4. Discussion

During the last years, several studies have shown that taurine is involved in different central and peripheral processes necessary for the control of glucose homeostasis. However, the key events underlying taurine effects on blood glucose levels remain unknown. In mammals, glucose homeostasis requires tight regulation of insulin synthesis and secretion (central mechanisms), with peripheral metabolic effects of insulin. The present study indicates that taurine modulates blood glucose homeostasis (Fig. 1A) acting through both central and peripheral mechanisms.
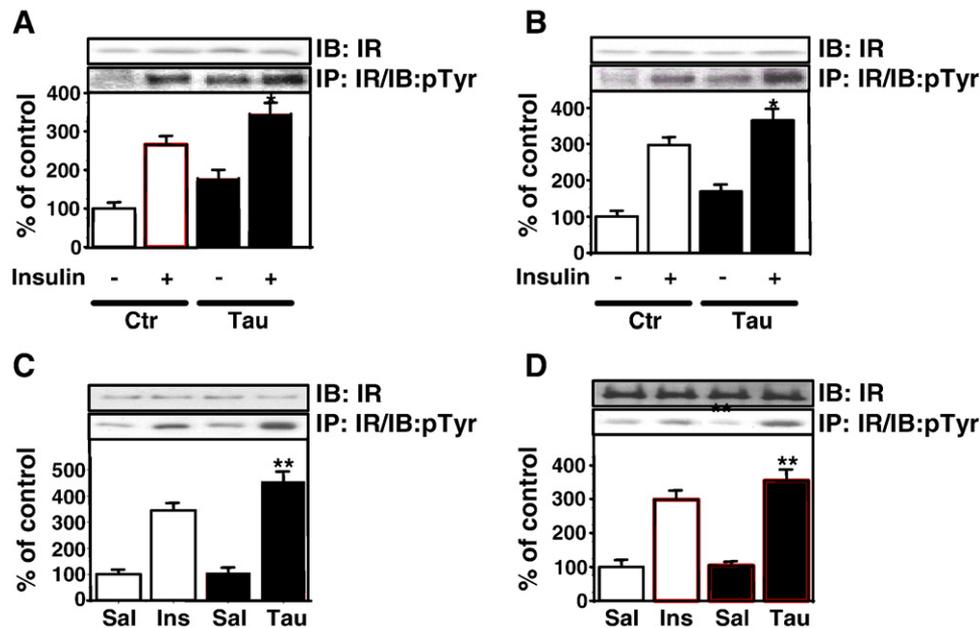

Fig. 7. Effect of taurine on insulin signal transduction and on the expression and tyrosine phosphorylation of the insulin receptor. Analysis of insulin-induced tyrosine phosphorylation of the insulin receptor (IR) in the skeletal muscle (A) and liver (B) of control group (white bars) and taurine group (black bars). Analysis of taurine-induced tyrosine phosphorylation of the insulin receptor (IR) in the skeletal muscle (C) and liver (D) after an acute dose of taurine. For tyrosine phosphorylation studies, insulin (+) (0.2 ml, 1 μmol/L) or a similar volume of saline (−) were injected. For acute dose experiments, 0.2 ml of saline (sal), insulin (ins; $10^{−6}$ mol/L) or taurine (tau; 0.165 mmol/L) were injected. Products were injected through the cava vein and after 90-s fragments of skeletal muscle and liver were obtained for protein extraction. Samples containing 5.0 mg were used for immunoprecipitation (IP) with anti-IR antibodies. The immunoprecipitates were separated by SDS-PAGE, transferred to nitrocellulose membranes and immunoblotted (IB) with antiphosphotyrosine (pY) antibodies. Blots from panels (A) and (C) were obtained by immunoblotting nitrocellulose transfers of 0.2 mg total protein extracts, separated by SDS-PAGE, with anti-insulin receptor antibodies. Respective bands were quantified by digital densitometry. For all experiments the results are presented as means±S.E.M. of $n=4$. *$P<01$ when compared with control group. **$P<001$ when compared saline infusion.

Glucose-induced insulin secretion and its potentiation constitute the principal mechanism of insulin release. Glucose is transported by the glucose transporter (Glut-2) into the pancreatic β-cell. Metabolism of glucose increases adenosine triphosphate (ATP) production (and the ATP-to-adenosine diphosphate ratio), closing KATP channels, resulting in membrane depolarization, opening of the voltage-dependent Ca^{2+} channels and generation of $[Ca^{2+}]i$ oscillations, which in turn triggers insulin granule exocytosis [23]. Our data show that taurine acts on several stages of the so-called stimulus–secretion coupling process. PDX-1 regulates a number of genes involved in β-cell function, including insulin [28], Glut-2 [29] and GK (glucokinase) [30]. In addition, it has been recently shown that glucose activation of PDX-1 leads to phosphorylation and activation of a cytoplasmic form of PDX-1, which then becomes translocated to the nucleus [31]. Moreover, it has been clearly demonstrated that PDX-1 is required for the glucose-dependent regulation of insulin RNA production [31]. As is shown in Fig. 6, islets from animals supplemented with taurine exhibited significantly increased levels of PDX-1 expression. Significantly, taurine supplementation also elicited a PDX-1 increased expression in the nucleus (Fig. 5).

Thus, the dual effect on PDX-1 expression and localization could be responsible for observed increase in insulin content, as well as the higher expression levels of insulin, Glut-2, GK, Sur-1 and Pcsk-1 genes (Fig. 6). In this regard, it has been reported that PDX-1 transactivates Glut-2 [29] and GK [32].

Glut-2 is the protein that facilitates glucose diffusion through the plasma membrane, while GK is the first rate-limiting step in the glucose metabolism. In effect, GK functions as a glucose sensor by controlling the rate of entry of glucose into the glycolytic pathway. A significantly higher expression of GK (Fig. 5) could explain the increased glucose metabolism (MTT reduction data) observed in islets from taurine-supplemented mice, within the range of action of GK.

Thus, taurine induces an increase in PDX-1 expression in the nucleus. This accounts for the higher expression of Glut-2, Sur-1 and GK that we observed. Increased GK expression, in combination with higher levels of Glut-2 and Sur-1, would then result in: (i) the displacement to the left of the glucose dose-response curve (Fig. 2); (ii) the decrease in the glucose threshold (Fig. 2) and (iii) the increase in the dynamic range of the system in term of $[Ca^{2+}]I$ oscillations (Fig. 4).

The effects of taurine on KCl-induced insulin release (Fig. 3) are consistent with previous published data indicating that taurine causes a dose-dependent inhibition of KATP channel [13]. This inhibition potentiates the depolarizing effects caused by potassium. Moreover, taurine binding to KATP channel can activate the electrical oscillator and contribute to the slow glucose-induced cytosolic Ca^{2+} oscillatory pattern (Fig. 4) such as we observed in islets from taurine supplemented mice.

As it is shown in Fig. 1A, taurine-supplemented animals showed lower blood glucose concentrations after an IPGTT. Beside the effects of taurine on β-cell stimulus–secretion coupling, it is important to consider the action of taurine on the peripheral metabolic effects of insulin. In this regard, three studies, two in OLETF rats [17,33] and one in high-fructose fed insulin resistant rats [34] have shown that taurine supplementation increase insulin sensitivity. In these studies, the authors proposed two possible explanations for the improvement in insulin sensitivity: (i) a taurine antioxidant effect and (ii) a taurine lipid-lowering effect. However, a role of taurine in insulin signalling cannot be excluded. In fact, other amino acids, such as leucine, also appear to modulate insulin signalling and glucose usage by skeletal muscle [35]. In Fig. 7A and B, we show that taurine supplementation significantly increases both basal and insulin stimulated tyrosine phosphorylation of the insulin receptor, in skeletal muscle and liver tissues.

In conclusion, our results suggest that taurine supplementation modulates glucose homeostasis via β-cell stimulus–secretion coupling and enhancing peripheral insulin sensitivity. The first pathway appears to depend on the regulation of the expression of genes required for glucose-stimulated insulin secretion, while the second pathway appears due to a direct effect of taurine on the insulin signaling process.

Acknowledgments

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