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Journal of Nutritional Biochemistry 23 (2012) 306–312

Journal of Nutritional Biochemistry

Taurine supplementation restores glucose and carbachol-induced insulin secretion in islets from low-protein diet rats: involvement of Ach-M3R, Synt 1 and SNAP-25 proteins☆

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Received 17 June 2010; received in revised form 21 November 2010; accepted 13 December 2010

Abstract

Isolated islets from low-protein (LP) diet rats showed decreased insulin secretion in response to glucose and carbachol (Cch). Taurine (TAU) increases insulin secretion in rodent islets with a positive effect upon the cholinergic pathway. Here, we investigated the effect of TAU administration upon glucose tolerance and insulin release in rats fed on a normal protein diet (17%) without (NP) or with 2.5% of TAU in their drinking water (NPT), and LP diet fed rats (6%) without (LP) or with TAU (LPT). Glucose tolerance was found to be higher in LP, compared to NP rats. However, plasma glucose levels, during ipGTT, in LPT rats were similar to those of controls. Isolated islets from LP rats secreted less insulin in response to increasing glucose concentrations (2.8–22.2 mmol/L) and to 100 μmol/L Cch. This lower secretion was accompanied by a reduction in Cch-induced internal Ca^{2+} mobilization. TAU supplementation prevents these alterations, as judged by the higher secretion induced by glucose or Cch in LPT islets. In addition, Ach-M3R, syntaxin 1 and synaptosomal associated protein of 25 kDa protein expressions in LP were lower than in NP islets. The expressions of these proteins in LPT were normalized. Finally, the sarcoendoplasmatic reticulum Ca^{2+}-ATPase 3 protein expression was higher in LPT and NPT, compared with controls. In conclusion, TAU supplementation to LP rats prevented alterations in glucose tolerance as well as in insulin secretion from isolated islets. The latter effect involves the normalization of the cholinergic pathway, associated with the preservation of exocytotic proteins.

Keywords: Low-protein diet; Insulin secretion; Taurine supplementation; Cholinergic pathway; Ach-M3R; Synt 1; SNAP-25

1. Introduction

Insulin secretion is stimulated by nutrients, particularly glucose, and is regulated by hormonal and neuronal inputs [1,2].

Acetylcholine (Ach), released from cholinergic terminals in the neighborhood of the islet-cells [3,4], binds to Ach muscarinic receptor-3 (Ach-M3R), potentiating nutrient-induced insulin secretion by the activation of phospholipase (PL) C, which generates diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP3). DAG activates protein kinase (PK) C, which increases the efficiency of free cytosolic Ca^{2+} upon the exocytosis of the insulin containing granules, whereas IP3 releases Ca^{2+} from endoplasmic reticulum (ER), binding to its specific receptor [1]. In addition, PLC contributes to the insulin secretion not only when membrane receptors are activated but also when the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increases in response to glucose [5,6].

Isolated islets from rodents, submitted to the LP diet, during the gestational period or after weaning, showed impaired insulin secretion in response to nutrients. This is a consequence of several structural and functional adaptations of the islets to the poor environment, including; decreased numbers of beta-cells [7]; a reduction in GLUT2 gene expression and glucose metabolism [8,9]; diminished mitochondrial substrate oxidation [10,11]; lower Ca^{2+} uptake [12]; and reduced expression of $PLC_{\beta 1}$, PKC and PKA proteins [13,14].

TAU, 2-amino-ethanesulfonic acid, is abundant in the plasma and tissues of mammals, particularly in the pancreas [15,16]. Possible effects of TAU upon insulin secretion and action have been extensively reported [17–20]. In experimental models and in humans, TAU improved glucose tolerance and insulin action [20–24]. With regard to insulin secretion, it has been demonstrated that TAU acts on Ca^{2+} handling via the beta-cells, improving the accumulation of this cation by increasing Ca^{2+} voltage-sensitive protein expression [19,20]. In addition, TAU increases external and internal Ca^{2+} mobilization in mice islets via the PKA and PLC pathways [16]. In addition, TAU supplementation, during the prenatal period,

☆ Grants, sponsors, and funding sources: This study was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP); Conselho Nacional para o Desenvolvimento Científico e Tecnológico (CNPq); Instituto Nacional de Obesidade e Diabetes (CNPq/FAPESP) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

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doi:10.1016/j.jnutbio.2010.12.012

prevented the metabolic perturbations observed in fetuses and adult rats of dams that were submitted to a LP diet [17,18].

In this study, we investigated glucose homeostasis and pancreatic islets functionality in LP rats supplemented, or not, with TAU. We observed that LP rats were hypoinsulinemic, and developed a compensatory improvement in glucose tolerance. Isolated islets from these rats showed impaired glucose- and Cch-induced insulin secretion, probably due to lower Ach-M3R, syntaxin (Synt) 1 and synaptosomal associated protein of 25 kDa (SNAP-25) protein expressions. TAU supplementation of LP rats restored Ach-M3R, Synt1, SNAP-25 protein levels and increased sarcoendoplasmatic reticulum Ca^{2+}-ATPase (SERCA) 3 protein expression. These effects seem to be important for improving the stimulus-secretion coupling in the islets, normalizing glucose- and Cch-induced insulin secretion.

2. Methods and materials

2.1. Materials

^{125}I human insulin was purchased from Genesis (São Paulo, SP, Brazil) and routine reagents were from Sigma Chemical (St. Louis, MO, USA).

2.2. Animals and groups

All experiments were approved by the ethics committee at Universidade Estadual de Campinas (UNICAMP). The studies were carried out on 21-day-old male Wistar rats obtained from the breeding colony at UNICAMP and maintained at 22±1°C, on a 12-h light–dark cycle, with free access to food and water. The rats were distributed into four groups: rats that received a diet containing 17% of protein without (NP) or with 2.5% of TAU in their drinking water (NPT) or rats submitted to an isocaloric diet containing 6% of protein [low-protein (LP) diet] without (LP) or with TAU supplementation (LPT). Diet composition was previously reported [25].

2.3. General nutritional parameters

Body weight was measured throughout the experimental period. At the end of the diet treatment and supplementation period, fasted and fed rats were decapitated, their blood was collected and plasma was stored at −20°C. Total plasma protein and plasma albumin were measured using standard commercial kits, according to the manufacturer's instructions (Laborlab, Guarulhos, SP, Brazil). Plasma glucose was measured using a glucose analyzer (Accu-Chek Advantage, Roche Diagnostic, Basel, Switzerland), insulin was measured by radioimmunoassay (RIA; as previously reported by Ref. [16]). Free fatty acids (FFA) were measured using an enzymatic kit (Wako; Richmond, VA, USA). This method is based on the acylation of coenzyme A (CoA) by the fatty acids in the presence of acyl-CoA synthetase. The acyl-CoA produced is oxidized by acyl-CoA oxidase and generates hydrogen peroxide, which is degraded by the peroxidase enzyme, which results in the oxidative condensation of 3-methy-*N*-ethyl-*N*(β-hydroxyethyl)-aniline with 4-aminoantipyrine, which forms a purple colored adduct that is measured by colorimetry at 550 nm.

2.4. TAU plasma levels

To measure TAU plasma concentrations, 200 μl of plasma was deproteinized by adding 200 μl of 25% trichloroacetic acid solution and was then centrifuged at 21,000×*g* for 10 min. Supernatant (100 μl) was collected and mixed with sample loading buffer (100 μl) (Biochrom 20 Reagent Kit, Cambridge, UK). An aliquot of 25 μl of the mixture was then resolved by liquid chromatography on a Biochrom 20 plus amino acid analyzer (Amersham Pharmacia, Piscataway, NJ, USA). Amino acid standards were analyzed first, followed by the samples. Amino acids were quantified using Biochrom 20 control software, version 3.05.

2.5. Intraperitoneal glucose tolerance test (ipGTT)

For ipGTT, blood glucose levels (Time 0) were measured (as previously described) in overnight fasted rats. A glucose load of 2 g/kg body weight was then administered by intraperitoneal injection, and additional blood samples were collected at 15, 30, 60, 120 and 180 min.

2.6. Islet isolation and static insulin secretion

Islets were isolated by collagenase digestion of the pancreas. For static incubations, five islets from each group were first incubated for 30 min at 37°C in Krebs–bicarbonate (KRB) buffer with the following composition: NaCl 115 mmol/L, KCl 5 mmol/L, $CaCl_2$ 2.56 mmol/L, $MgCl_2$ 1 mmol/L, $NaHCO_3$ 10 mmol/L, HEPES 15 mmol/L, supplemented with 5.6 mmol/L glucose, 3 g of bovine serum albumin (BSA) per liter, and equilibrated with a mixture of 95% O_2/5% CO_2 to give pH 7.4. This medium was then replaced with fresh buffer, and the islets were incubated for 1 h with 2.8, 8.3, 11.1, 16.7 or 22.2 mmol/L glucose. For carbachol (Cch)-stimulated insulin secretion, the islets were incubated with 11.1 mmol/L glucose plus 100 μmol/L Cch. At the end of the incubation period, the insulin content of the medium was measured by RIA.

2.7. Cytoplasmatic Ca^{2+} Oscillations

Fresh pancreatic islets were incubated with Fura-2 acetoxymethyl ester (5 μmol/L) for 1 h at 37°C in KRB buffer containing 5.6 mmol/L glucose, 0.3% BSA and pH 7.4. After this period, the islets were washed with the same medium and placed in a chamber that was thermostatically regulated at 37°C on the stage of an inverted microscope (Nikon UK, Kingston, UK). Islets were then perifused with Ca^{2+}-free KRB continuously gassed with 95% O_2/5% CO_2, pH 7.4, containing 11.1 mmol/L glucose plus 250 μmol/L of diazoxide and 10 mmol/L EGTA with or without 100 μM of Cch. A ratio image was acquired approximately every 5 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, USA), equipped with 340 and 380 nm, 10-nm bandpass filters and a range of neutral density filters (Omega Opticals, Stanmore, UK). Data were obtained using the ImageMaster3 software (Photon Technology International, NJ, USA).

2.8. Western blotting

Isolated islets from NP, NPT, LP and LPT rats were solubilized in homogenization buffer containing: Tris pH 7.5 100 mmol/L, sodium pyrophosphate 10 mmol/L, sodium fluoride 100 mmol/L, EDTA 10 mmol/L, sodium vanadate 10 mmol/L, PMSF 2 mmol/L and Triton X-100 1%, final pH 8.08. The islets were disrupted using a sonicator (Brinkmann Instruments, Westbury, NY, USA), employing three 10-s pulses. The extracts were then centrifuged at 12,600×*g* at 4°C for 5 min to remove insoluble material. The protein concentration in the supernatants was assayed using the Bradford dye method [26], using BSA as a standard curve and Bradford reagent (Bio-Agency Lab., São Paulo, SP, Brazil). For sodium dodecyl sulfate gel electrophoresis and Western blot analysis, the samples were treated with a Laemmli sample buffer containing dithiothreitol. After heating to 95°C for 5 min, the proteins were separated by electrophoresis (40 μg protein/lane, 10% gels). Following electrophoresis, proteins were transferred to nitrocellulose membranes. The nitrocellulose filters were treated overnight with a blocking buffer (5% non-fat dried milk, Tris 10 mmol/L, NaC 150 mmol/L l and Tween-20 0.02%, final pH 7.5) and were subsequently incubated with a polyclonal antibody against SNAP-25 (1:1000; S-5187, Sigma Chemical St. Louis, MO, USA) or Ach-M3R [1:200, cat. sc 9108], PLCβ$_2$ [1:500; cat. (H-255) sc 9018], SERCA 3 (1:200, cat. sc 26507) and Synt 1 (1:200, cat. sc 12736). All later primary antibodies used were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Detection was performed using enhanced chemiluminescence (SuperSignal West Pico, Pierce, Rockford, IL) after 2-h incubation with a horseradish peroxidase-conjugated secondary antibody (1:10,000, Invitrogen, São Paulo, SP, Brazil). Band intensities were quantified by optical densitometry (Scion Image, Frederick, MD). After assaying the target proteins, Western blotting was repeated using an α-tubulin (1:1000, cat. T6199, Sigma, St Louis, MO, USA) antibody as an internal control.

2.9. Statistical analysis

Results are presented as means±S.E.M. for the number of determinations (n) indicated. The statistical analyses were carried out using two-way analysis of variance followed by the Newman-Keuls post-test ($P \leq .05$) and performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Animal features

Fig. 1 shows that body weight in LP rats was significantly lower even at 8 days after the onset of the diet, when compared to NP rats. The total body weight expressed by the area under the growth curve (AUC) during the experimental period was also lower in LP (18,015±1358 g.days^{-1}) than NP rats (34,118±1066 g.days^{-1}; $P<.001$). TAU supplementation did not alter this parameter in both supplemented groups (LPT 19,497±1493 and NPT 31,290±1931 g.days^{-1}). Rats that were fed on an LP diet showed decreased fed insulin, fasted albumin and total protein plasma levels ($P<.005$, $P<.001$ and $P<.01$, respectively; Table 1), whereas FFA was significantly higher in LP, compared with NP rats ($P<.02$). TAU supplementation did not prevent these alterations in LPT rats, whereas an improvement in fed insulin plasma levels was observed (Table 1). Finally, TAU plasma levels in fed supplemented rats were approximately 1.4 and 3.4 times higher in NPT and LPT, respectively, compared with NP rats ($P<.005$).

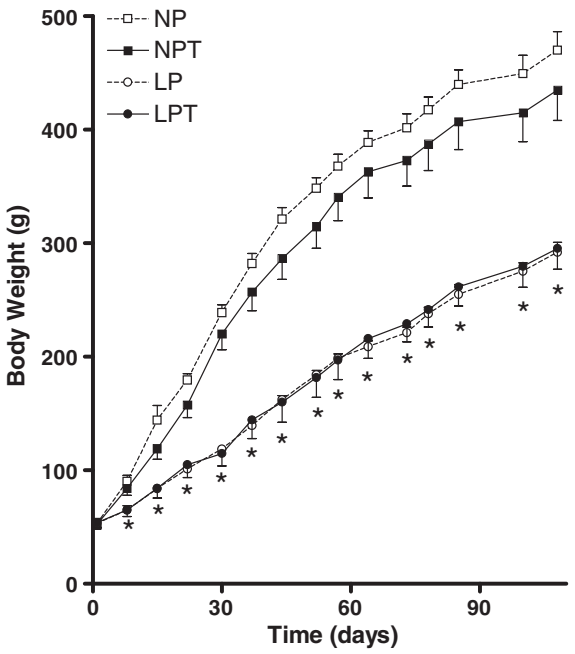

Fig. 1. Body weight during the feeding period was measured weekly in rats on an NP or LP diet without or with TAU supplementation from weaning until 110 days old. Values are means±S.E.M.; n=5 rats. *Significant difference of LP and LPT compared with NP and NPT groups (P<.05).

3.2. Glucose tolerance

At the end of the experimental period, rats were submitted to an ipGTT. After glucose load, plasma glucose concentrations reached maximal levels at 15 min in all groups. The LP rats showed lower glucose values at 15, 30 and 60 min when compared with NP and NPT rats (P≤.05, P<.001 and P<.03, respectively; Fig. 2A). However, at these times, the plasma glucose levels of NPT were similar to NP rats. The total plasma glucose concentration (AUC), during the ipGTT in the LP group, was 44% lower than the NP group (P<.02; Fig. 2B). LP-supplemented rats showed similar total glucose plasma concentrations during the ipGTT to those of controls rats (Fig. 2B).

3.3. Glucose-induced insulin secretion

The insulin released by islets during static incubation, in response to crescent glucose concentrations (2.8 – 22.2 mmol/L), is shown in Fig. 3. Islets isolated from LP rats showed decreased insulin secretion in all glucose concentrations used, when compared with NP rat islets (P<.05). TAU supplementation increased insulin release in NPT and LPT groups, compared with their respective controls. It is worthy to note that the insulin secretion, in all stimulatory glucose concentrations used (8.3–22.2 mmol/L), in LPT, reached similar values to those found in NP islets.

Table 1
Blood glucose, plasma insulin, free fatty acids (FFA), albumin, total proteins levels and TAU in fasted and fed (#) NP and LP rats supplemented or not with TAU

	NP	NPT	LP	LPT
Glucose (mmol/L)	4.39±0.19	4.24±0.22	3.54±0.19	3.84±0.33
Glucose (mmol/L) #	4.78±0.12	4.87±0.10	5.10±0.08	5.22±0.27
Insulin (ng/mL)	1.19±0.18	1.48±0.23	0.71±0.14	0.93±0.19
Insulin (ng/mL) #	4.97±0.34[a]	3.56±0.52[a]	1.39±0.10[b]	3.31±0.70[a]
FFA (mmol/L)	0.82±0.08[a]	0.69±0.11[a]	1.21±0.14[b]	1.20±0.16[b]
Albumin (g/dL)	3.20±0.11[a]	3.18±0.05[a]	2.74±0.07[b]	2.67±0.04[b]
Total protein (g/dL)	6.81±0.04[a]	6.87±0.19[a]	5.35±0.24[b]	5.70±0.09[b]
TAU (μmol/mL)	2.07±0.22[a]	4.99±0.79[b]	1.99±0.64[a]	6.77±0.99[b]

Data are means±S.E.M. of 12 rats of each group. For TAU plasma level analysis, three rats of each group was used. Different superscripted letters[a,b] indicate statistical significance, P<.05.

3.4. Cch-induced insulin secretion and intracellular Ca^{2+} mobilization

Confirming previous observations [13], the insulin secretion in LP was lower than that of NP islets in the presence of 100 μmol/L Cch (P<.02; Fig. 4). However, the augmentation in insulin secretion, induced by the cholinergic agent, in LPT was similar to that found in NP rats (Fig. 4). In fact, the increment in the insulin secretion, induced by Cch in NPT islets, was even higher than in that obtained in the NP group (P<.01). In the next series of experiments, we analyzed intracellular Ca^{2+} mobilization in all groups of islets. For this purpose, 100 μmol/L Cch was added to a perifusion system with a Ca^{2+}-free medium, containing 11.1 mmol/L glucose, 250 μmol/L diazoxide and 10 mmol/L EGTA. Fig. 5A and D illustrates that LP islets showed a lower Cch-induced intracellular Ca^{2+} mobilization from internal stores, when compared with NP islets. The amplitude and AUC of $[Ca^{2+}]_i$ were significantly lower in LP than in NP islets (P<.02 and P<.05, respectively; Fig. 5C and F). TAU supplementation did not improve the amplitude and total amount of the cation in response to Cch in LPT islets (Fig. 5E), although the amplitude of the response was higher in NPT compared with NP islets (P<.05; Fig. 5C).

3.5. Ach-M3R, PLCβ_2, SERCA 3, Synt 1 and SNAP-25 protein expressions

Ach-M3R, Synt 1 and SNAP-25 protein expressions were 50% lower in islets from LP, compared with NP rats (P<.04, P<.005 and P<.05, respectively; Figs. 6A, C and D) whereas the levels of these proteins in LPT were similar to those of NP islets. In addition, SERCA 3 protein expression was higher in NPT and LPT, compared with NP and LP islets (P<.02 and P<.05, respectively; Fig. 6B). No differences in $PLC_{\beta 2}$ protein expression were observed between both islet groups (not shown).

4. Discussion

LP rats showed typical features of malnutrition including low body weight, low plasma levels of albumin, total proteins and insulin and higher FFA, whereas glucose was unaltered (Fig. 1 and Table 1). In LP rats, lower insulin plasma levels were observed during the oral GTT, despite normal glicemia. The normoglicemia of these rats was probably due to an increase in insulin sensitivity in peripheral tissues [27].

An interrelationship between TAU and glucose homeostasis has been previously reported [19,20,28]. In different experimental models of insulin resistance, TAU supplementation normalizes plasma insulin, glucose levels and insulin sensitivity [21–23,29,30]. The hypoglycemic effect of TAU was attributed to a possible interaction of the amino acid with the insulin receptor [19,31]. Here, we demonstrated that TAU also prevents alterations in glucose tolerance in LP rats that, at the contrary, show an increased sensitivity to insulin [27]. While no interference in plasma albumin, total proteins and FFA plasma levels was detected, the LPT rats seemed to be protected from this poor environment, since the hypoinsulinemia and compensatory increase in insulin sensitivity, described in LP rats, was not observed.

It is well known that islets isolated from LP rodents secrete less insulin in response to glucose, when compared with controls [25,27,32]. Here, we confirm these results and demonstrate that LPT rats had normal fed insulin plasma levels and that glucose-induced insulin secretion was normalized (Fig. 3).

The beneficial effect of the supplementation with TAU in LP rats has also been reported. It has been observed that the reduction in beta-cell proliferation in fetuses from dams, maintained on the LP diet

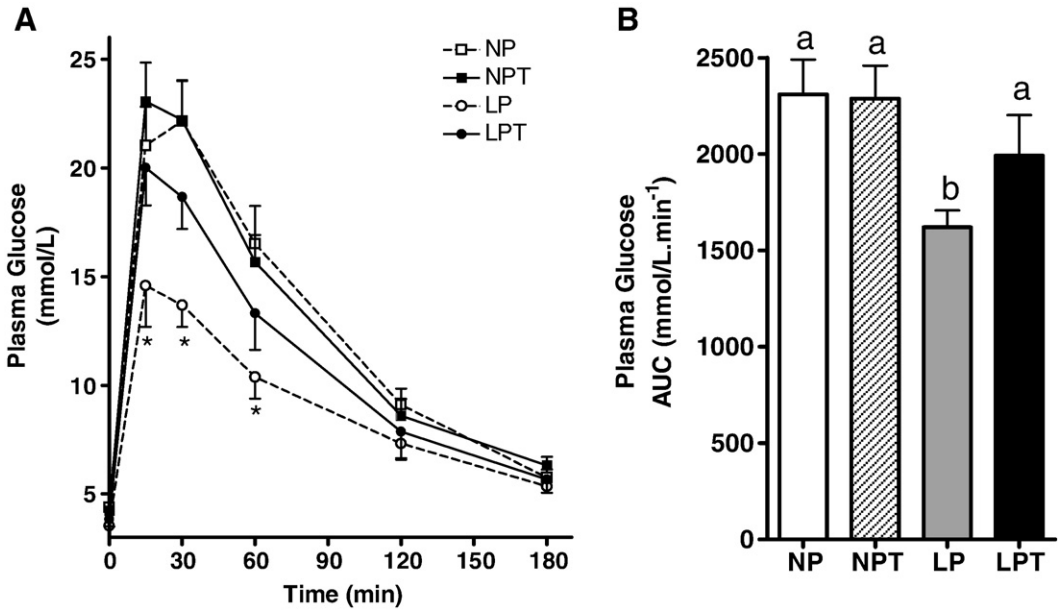

Fig. 2. (A) Changes in plasma glucose levels during ipGTT in LP, LPT, NP and NPT 110-days old rats. Total plasma glucose concentrations during the ipGTT, expressed by AUC (B). Data are means±S.E.M. obtained from 7–10 rats. * $P<.05$ indicates significant difference vs. NP and NPT groups. Different letters over the bars represent significant differences, $P<.05$.

during the gestational period, was prevented by TAU supplementation [17,18,33]. However, our study was the first to demonstrate the effect of TAU supplementation in rats submitted to the LP diet after weaning. It is known that TAU increases glucose-induced insulin release improving internal and external Ca^{2+} mobilization through the cholinergic and PKA pathways [16,20]. Since Cch-induced insulin secretion is reduced in LP rat islets [13], we decided to evaluate whether the cholinergic pathway was regulated by TAU supplementation in LP islets.

We observed that the smaller increment in insulin secretion, induced by Cch (Fig. 4), was accompanied by lower intracellular Ca^{2+} mobilization (Fig. 5) in LP, compared with NP islets. This defect in Ca^{2+} mobilization parallels a reduced expression of the Ach-M3R protein. TAU supplementation restored Cch-induced insulin release in LPT islets, possibly by the preservation of the Ach-M3R protein levels, but without modifications in internal Ca^{2+} handling (Figs. 4, 5 and Fig. 6).

It is known that Ach-M3R is expressed in many central and peripheral tissues [34]. Ach-M3 knockout mice showed increased glucose tolerance, insulin sensitivity and decreased insulin secretion [35–37]. These behaviors are in accordance with the features observed in rodents submitted to a LP diet [25,27,32]. In addition, the consequences of undernutrition on the activity of the autonomic

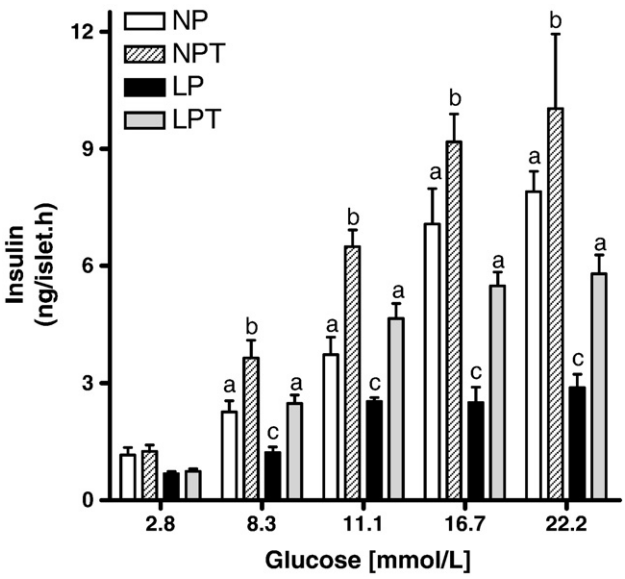

Fig. 3. Glucose-induced insulin secretion in islets from LP, LPT, NP and NPT rats treated for 90 days with their respective diets without or with TAU supplementation. Groups of five islets were incubated for 1 h with different glucose concentrations, as indicated. Each bar represents mean±S.E.M. of 6–9 groups of islets. Different letters over the bars represent significant differences between the glucose concentrations evaluated, $P<.05$.

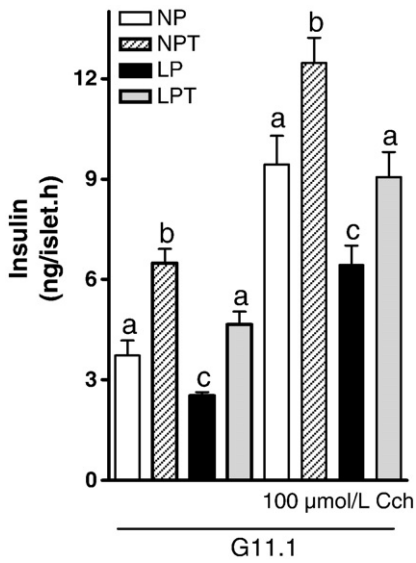

Fig. 4. Insulin secretion induced by 100 µmol/L Cch in isolated islets from LP, LPT, NP and NPT rats treated for 90 days with their respective diets without or with TAU supplementation. Islets were incubated for 1 h at 11.1 mmol/L glucose (G11.1), with or without Cch. Each bar represents mean±S.E.M. of 7–12 groups of islets. Different letters over the bars represent significant differences between the same stimulus, $P<.05$.

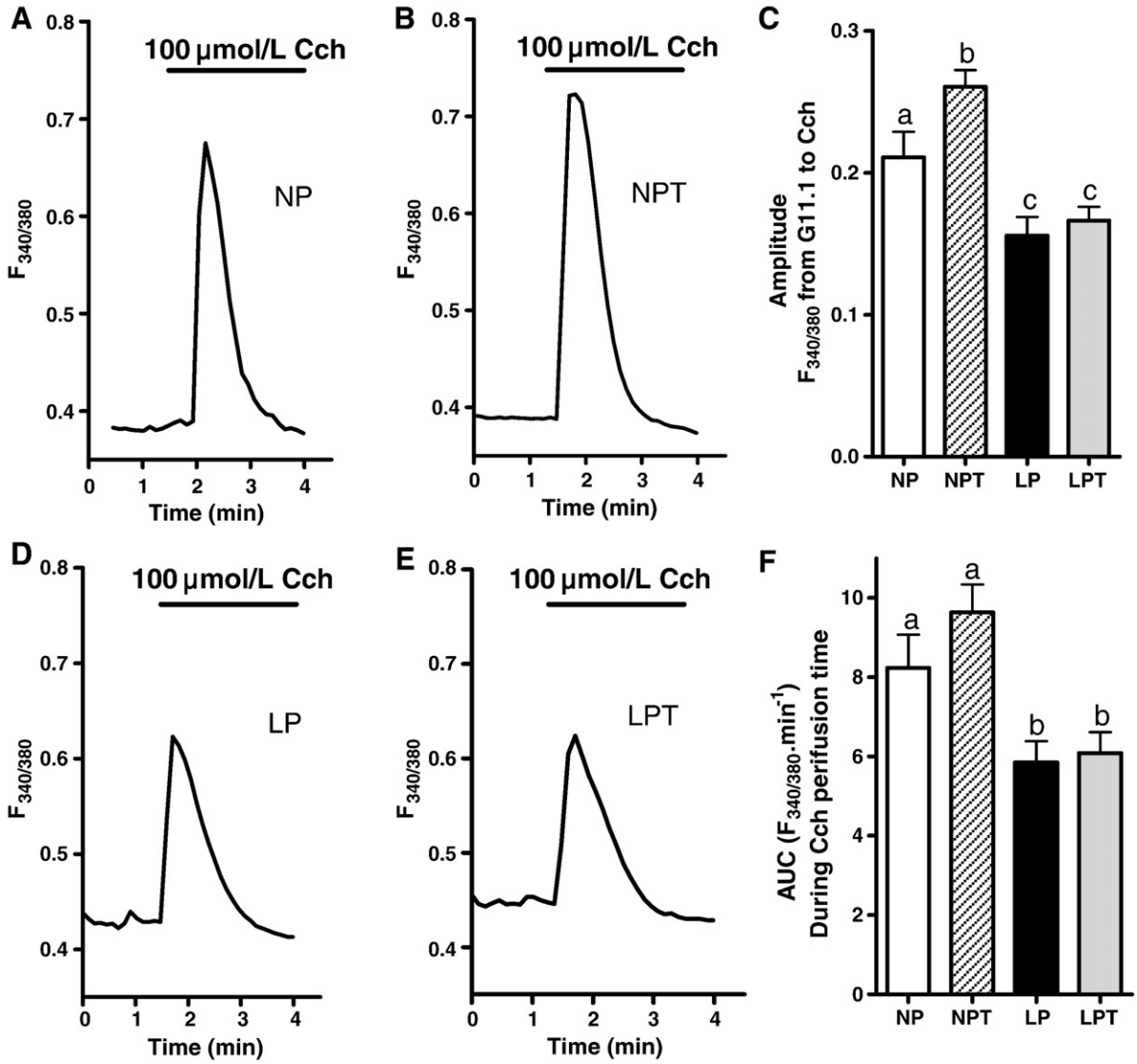

Fig. 5. Representative curves of Cch (100 µmol/L) induced internal Ca^{2+} mobilization in islets isolated from NP (A), NPT (B), LP (D) and LPT (E) rats treated for 90 days with their respective diets without or with TAU supplementation. (C) Amplitude and AUC (E) of the $[Ca^{2+}]_i$ in response to Cch. The experiments were performed in a perifusion system in a Ca^{2+}-free medium containing: 11.1 mmol/L glucose (G11.1), 250 µmol/L diazoxide, and 10 mmol/L EGTA. Values are the ratio of F340/F380 registered for each group. Data are means±S.E.M. obtained from 10–12 independent experiments. Different letters indicate a significant difference (P<.05).

nervous system have been reported. In the basal postabsortive state, after a glucose load, malnourished rats showed a decrease in the firing rates of the vagus nerve and lower insulin plasma levels [38]. Decreased parasympathetic activity was also reduced in malnourished children [39]. Thus, it is plausible that the Ach-M3R signaling pathway may be systematically disrupted in LP rats, which contributes to their metabolic phenotype. A possible action of TAU in the preservation of whole body parasympathetic activity through the maintenance of the Ach-M3R expression may contribute to normal glucose tolerance and improvement of metabolism in LP supplemented rats.

Sequestration of Ca^{2+} in the ER by SERCA, and its subsequent release, plays an important role in the control of $[Ca^{2+}]_i$, which regulates insulin secretion. Since Cch-induced Ca^{2+} mobilization was reduced in LP islets, we measured SERCA 3 protein expression to verify whether the ER Ca^{2+} stores were normal. However, no difference in SERCA 3 protein content was observed. Interestingly, TAU supplementation increased islet SERCA 3 protein expression in both LPT and NPT groups (Fig. 6C). It is known that SERCA 3 becomes operative when $[Ca^{2+}]_i$ rises, where it is required for normal $[Ca^{2+}]_i$ oscillations in response to glucose [40]. In islets isolated from Goto-Kakizaki (GK) rats, a nonobese spontaneous model of Type 2 diabetes, there is a significantly reduction in SERCA 3 gene expression, and this may account for the defect in Ca^{2+} signaling in these islets [41]. The relationship between TAU and Ca^{2+} homeostasis has been documented. In cardiac cells, TAU is an agonist of the Ca^{2+} current at low $[Ca^{2+}]_i$, whereas at high $[Ca^{2+}]_i$, TAU inhibits Ca^{2+} influx [42]. In isolated rat liver mitochondria, TAU enhanced Ca^{2+} uptake via the uniport system [43]. TAU also increased the expression of the beta 2 subunit of the Ca^{2+} voltage-sensitive channels in mice pancreatic islets [20]. Our study showed, for the first time, that this amino acid regulates SERCA 3 expression, indicating another important site of interaction between TAU and Ca^{2+} homeostasis.

It is known that the docking and exocytosis of vesicles containing insulin granules depend on soluble *N*-ethylmaleimide-sensitive factor attached protein receptor (SNARE) proteins, composed of Synt and SNAP-25, both of which are plasma membrane components (t-SNARE) and synaptobrevin, a membrane vesicle component (v-SNARE) [44]. Their deficiency was reported to contribute to the

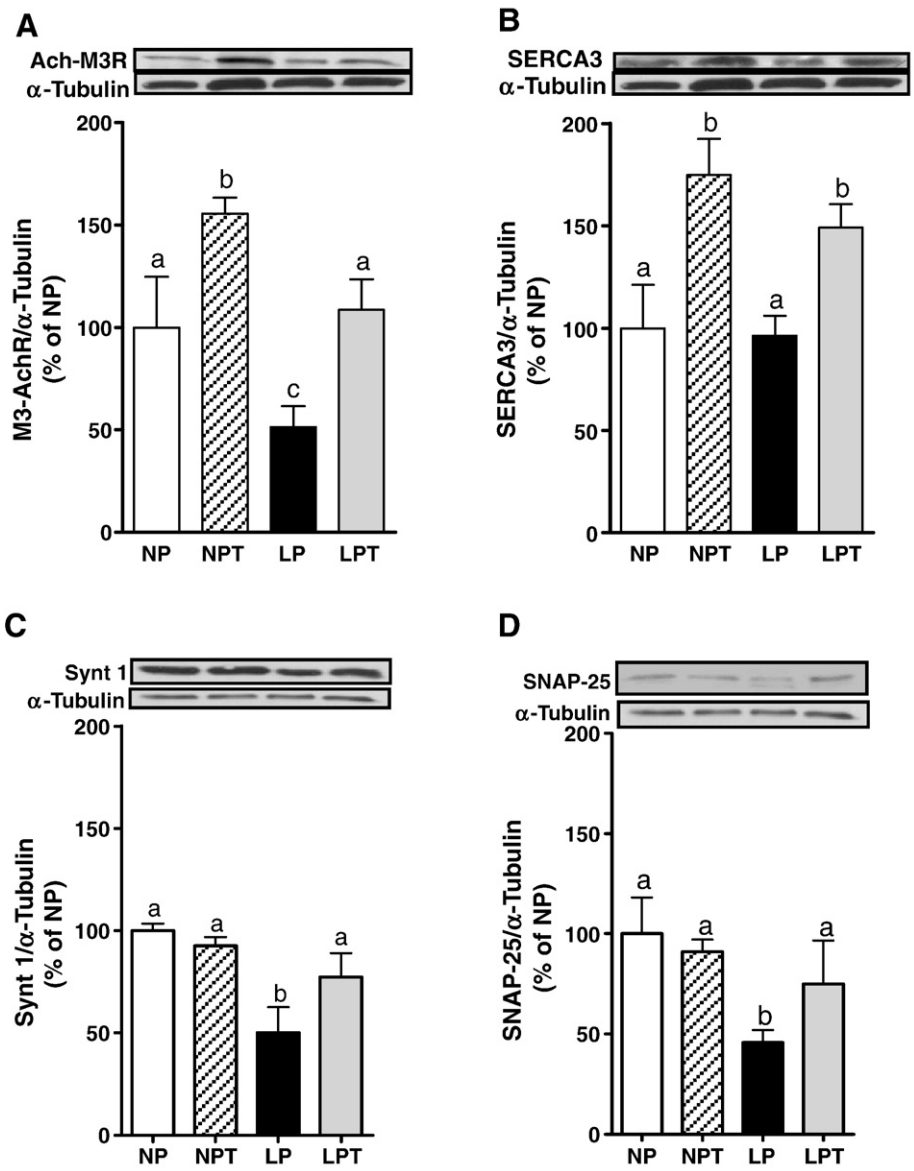

Fig. 6. Ach-M3R, SERCA 3, Synt 1 and SNAP-25 protein expressions in islets from NP, NPT, LP and LPT rats treated for 90 days with their respective diets without or with TAU supplementation. Protein extracts were processed for Western blot detection of M3-AchR (A), SERCA 3 (B), Synt 1 (C), SNAP-25 (D) and α-tubulin (internal control). The bars represent the means±S.E.M. of the values, determined by optical densitometry (n=3–6). Different letters indicate significant difference (P<.05).

impaired insulin secretion observed in GK rats [45]. In addition, neutralization of Synt 1 and SNAP-25 with specific antibodies in rat beta-cells resulted in reduced insulin exocytosis [46]. Synt 1 and SNAP-25 protein expression is decreased in LP islets (Fig. 6C and D), and these alterations, together with a decreased internal Ca^{2+} mobilization (Fig. 5), may account for the lower glucose and Cch-induced insulin secretion in LP islets.

Ach-M3R agonists and Ca^{2+} activate PLC, which increases IP3 and DAG production [1,5]. The latter messenger, together with Ca^{2+}, activates PKC, which phosphorylates SNAP-25 in beta-cell lines [47], neurons and other endocrine cells, increasing Ca^{2+}-triggered exocytosis [48,49]. Thus, we suggest that TAU supplementation improved insulin secretion in LPT islets maintaining adequate Ach-M3R, Synt 1 and SNAP-25 protein levels. These effects may contribute to a better cholinergic activity, resulting in PKC activation and an improvement in SNARE complex formation in these islets.

In conclusion, this study confirms and extends previous data regarding LP diet rats. The lower insulin secretion, in response to glucose and Cch, in the islets of these rats was due, at least in part, to a decreased Ach-M3R, Synt 1 and SNAP-25 protein expression. TAU supplementation preserved the normal glucose tolerance in LP rats and the beta-cell responsiveness to glucose, possibly by the maintenance of an adequate cholinergic pathway flux, together with the normalization of the t-SNARE complex and increased SERCA 3 protein levels. All these alterations contribute to a better coupling between stimulus and insulin secretion without any modification in internal Ca^{2+} handling in LPT islets.

Acknowledgments

We are grateful to Nicola Conran for English editing.

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