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RESEARCH ARTICLE

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Taurine rescues pancreatic β-cell stress by stimulating α-cell transdifferentiation

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Abstract

The semi-essential ubiquitous amino acid taurine has been shown to alleviate obesity and hyperglycemia in humans; however, the pathways underlying the antidiabetic actions have not been characterized. We explored the effect of chronic taurine exposure on cell biology of pancreatic islets, in degenerative type 1-like diabetes. The latter was modeled by small dose of streptozotocin (STZ) injection for 5 days in mice, followed by a 10-day administration of taurine (2% w/v, orally) in the drinking water. Taurine treatment opposed the detrimental changes in islet morphology and β-/α-cell ratio, induced by STZ diabetes, coincidentally with a significant 3.9 ± 0.7-fold enhancement of proliferation and 40 ± 5% reduction of apoptosis in β-cells. In line with these findings, the treatment counteracted an upregulation of antioxidant (*Sod1*, *Sod2*, *Cat*, *Gpx1*) and downregulation of islet expansion (*Ngn3*, *Itgb1*) genes induced by STZ, in a pancreatic β-cell line. At the same time, taurine enhanced the transdifferentiation of α-cells into β-cells by 2.3 ± 0.8-fold, echoed in strong non-metabolic elevation of cytosolic Ca^{2+} levels in pancreatic α-cells. Our data suggest a bimodal effect of dietary taurine on islet β-cell biology, which combines the augmentation of α-/β-cell transdifferentiation with downregulation of apoptosis. The dualism of action, stemming presumably from the intra- and extracellular modality of the signal, is likely to explain the antidiabetic potential of taurine supplementation.

KEYWORDS

apoptosis, Ca^{2+} dynamics, taurine, type 1 diabetes, α/β-cell transdifferentiation

Abbreviations: Arx, aristaless-related homeobox; Bax, BCL2-associated X protein; BCL2, B-cell CLL/lymphoma 2; DAPI, 4′,6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; GLP-1, glucagon-like peptide-1; Glut2, glucose transporter 2; Gpx1, glutathione peroxidase 1; IFN, interferon; IL, interleukin; NFKβ1, nuclear factor of kappa light polypeptide gene enhancer in β-cells 1; Ngn3, norvegicus neurogenin 3; Pax6, paired box 6; Pdx1, pancreatic and duodenal homeobox 1; SOD1, superoxide dismutase 1 (soluble); SOD2, superoxide dismutase 2, mitochondrial; STZ, streptozotocin; T1D, type 1 diabetes; TNF, tumor necrosis factor; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling.

1 | INTRODUCTION

Isolated from ox-bile almost 200 years ago,[1] a conditionally essential 2-aminoethylsulphonic acid (taurine)[2] has been found in tissues of most animal[3] but not plant[4] species. Originally viewed as a biochemically inert end-product of cysteine and methionine metabolism, taurine has been attracting a wide interest since the 1970s, due to its then-discovered antioxidant properties.[5] High cytosolic concentration of the amino acid (5–20 mM[6]) suggested its role in osmoregulation,[7] membrane excitability,[8–10] neurotransmission,[11] and mitochondrial Ca^{2+} uptake.[12] Extracellular taurine has been tipped as an inhibitory neurotransmitter[13] and a global antihyperglycemic[14] and insulinotropic[15,16] agent, with a perspective for use in, among others, diabetes therapy.[17]

Apart from its acute stimulatory effect on islet β-cells,[15] taurine has been reported to enhance glucose utilization and reduce oxidative stress in the liver,[18] counteract obesity,[19] and renal tubular cell hypertrophy[20] induced by chronic hyperglycemia.[21] In humans, taurine supplementation attenuated the detrimental complications of diabetes such as retinopathy, neuropathy, and nephropathy.[11,22,23] This pleiotropism is likely to stem from the effects in different compartments, cytosolic, and extracellular, with a clear implication to, among others, pancreatic islet biology. Acting intracellularly, taurine was reported to attenuate the impact of glucotoxicity on mitochondrial metabolism,[24] thereby enhancing the glucose-sensing ability of islet cells. At the same time, extracellular taurine is likely to activate $GABA_A$ and glycine[25,26] receptors on islet cells, thus acutely potentiating insulin[27] and suppressing glucagon[25,28] release.

The antioxidative and anti-inflammatory effect of chronic taurine supplementation on islets attained a particular prominence under β-cell stress.[29] In streptozotocin (STZ)-induced type 1 diabetes models, chronic administration of taurine was reported to improve the global phenotype such as glucose tolerance, presumably via enhancing glucose sensitivity of islet β-cells and/or altering their gene expression profile.[30] This de facto insulinotropic effect is balanced by reports of attenuation of excessive pancreatic hormonal output that has been suggested to underlie the anti-obesity effects of taurine treatment, which, in turn, would improve islet glucose sensitivity.[31]

In this study, we examine the direct impact of taurine on islet cell biology, in a mouse and clonal models of β-cell stress induced by STZ. Notably, the experiments were designed to avoid the alteration the global diabetic phenotype,[18] to exclude the contribution of the indirect factors, such as changes in insulin sensitivity or glycemia.[32] Thus, using histological and functional techniques, we demonstrate the ability of taurine to attenuate islet diabetic phenotype by decreasing β-cell apoptosis and inducing α-cell transdifferentiation, with α-cell-specific increase in cytosolic Ca^{2+} being one of potential underlying mechanisms.

2 | EXPERIMENTAL PROCEDURES

2.1 | Animals

All experiments were carried out under the UK Animals (Scientific Procedures) Act 1986 & EU Directive 2010/63EU and approved by the Ulster University Animal Welfare and Ethical Review Body (AWERB). Animals were maintained in an environmentally controlled laboratory at 22 ± 2°C with a 12 h dark and light cycle and given ad libitum access to standard rodent diet (10% fat, 30% protein, and 60% carbohydrate; Trouw Nutrition, Northwich, UK) and drinking water.

2.1.1 | $Glu^{CreERT2}$;ROSA26-eYFP mice

All of the studies, apart from $[Ca^{2+}]_{cyt}$ and ATP/ADP imaging, were performed using 10-week old male $Glu^{CreERT2}$;ROSA26-eYFP transgenic mice that allow pancreatic islet α-cell identification via the specific expression of YFP. Originally developed on the C57BL/6 background at the University of Cambridge,[33] the mouse colony was transferred to the animal facility at Ulster and genotyped to confirm Cre-ERT2 and ROSA26eYFP gene expression (Table 1). Three days prior to STZ dosing, mice were injected with tamoxifen (Sigma-Aldrich, Dorset, UK; 7 mg/mouse; i.p.) to activate the tissue-specific expression of yellow fluorescent protein (YFP) in pancreatic islet α-cells (Figure 1A).

2.1.2 | Diabetes model and taurine administration

Mice were kept under observation with standard diet and normal drinking water at least 5 days prior to the study, which included three groups of animals. To develop insulin-deficient diabetes,[34] two groups of mice ("STZ" and "STZ + tau," Figure 1) underwent a 5-day course of injections with a low dose of STZ (Sigma-Aldrich, Dorset, UK; 50 mg/kg body weight daily, i.p.), dissolved in 0.1 M sodium citrate buffer (pH 4.5). The control group ("Ctl," $n = 6$, Figure 1) received injections with the buffer alone. Taurine ("STZ + tau") was administered by inclusion of the amino acid into the drinking water (2% w/v)

TABLE 1 Primer sequence for PCR genotyping of $Glu^{CreERT2}$;ROSA26-eYFP mice.

Primers	Sequence
β-catenin (Housekeeping control, 220BP)	Forward: AAGGTAGAGTGATGAAAGTTGTT Reverse: CACCATGTCCTCTGTCTATTC
iCre002/003 fragment (Cre lines, 537BP)	Forward: GACAGGCAGGCCTTCTCTGAA Reverse: CTTCTCCACACCAGCTGTGGA
GLUCre-ERT2 (759BP)	Forward: CCACCTTCTAGAATGTGCCTG Reverse: CATCTGCATGCAAAGCAATATAGC
EYFP (442BP)	Forward: GACGTAAACGGCCACAAGTT Reverse: GGATCTTGAAGTTCGCCTTG

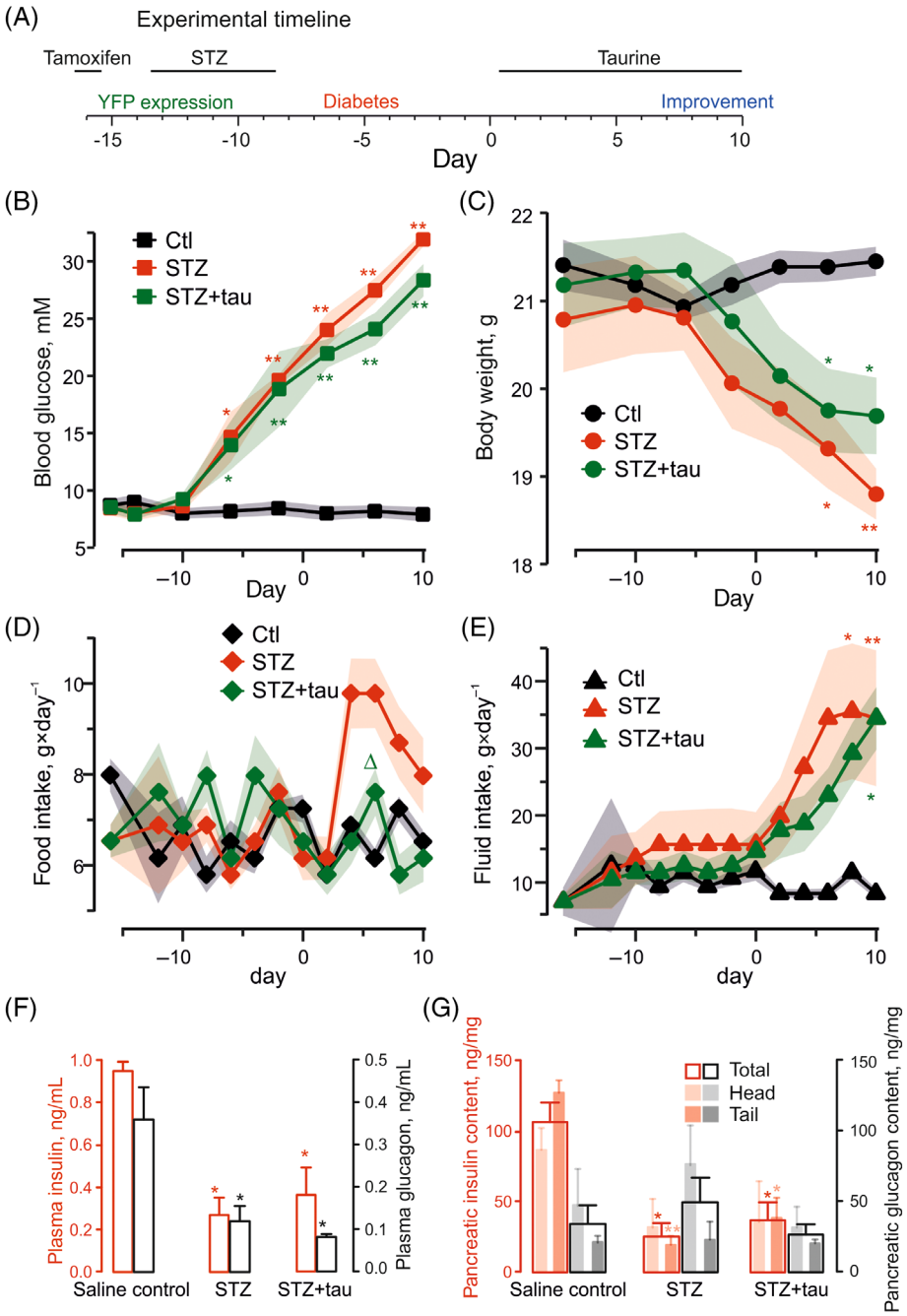

FIGURE 1 The global diabetic phenotype is unaffected by the chronic administration of taurine. (A) Experimental timeline. Taurine treatment starts on day 0. STZ is administered to model type 1 diabetes for 5 days, 5 days prior to the start of the taurine treatment. The ability of the latter to improve the diabetic phenotype is then assayed. (B–E) Fasting blood glucose (B), body weight (C), food (D) and fluid (E) intake of $Glu^{CreERT2}$; ROSA26-eYFP mice, following the administration of taurine and STZ treatment, as indicated, for groups of $n = 6$ mice each. "STZ," streptozotocin; "tau," taurine; "Ctl," saline control. (F,G) Plasma (F) and pancreatic (G) insulin (red) and glucagon (black) content. (F,G) Measurements were done on day 15, in different groups of mice, as indicated. $^*p < 0.05$, $^{**}p < 0.01$ compared to saline control group. $\Delta p < 0.05$ compared to streptozotocin-treated group.

throughout a 10-day span (days 1–10, Figure 1A). Dietary taurine was administered at a maximal concentration that produced no reported concomitant toxicity or decrease of glycemia in the STZ models.[18,35] Cumulative food and fluid intake, body weight and blood glucose were assessed daily or every 2 days. Non-fasting plasma insulin and glucagon were determined at the termination of the study. Pancreatic tissues were collected, divided longitudinally, and processed either for histology or measurement of hormonal content (day 10).

2.1.3 | Blood glucose and hormone measurements

Blood samples were collected from the tail vein of animals into ice-chilled heparin-coated microcentrifuge tubes. Blood glucose was measured using a portable Ascencia meter (Bayer Healthcare, Newbury, Berkshire, UK). For plasma insulin and glucagon, blood was collected in chilled fluoride/heparin-coated tubes (Sarstedt, Numbrecht, Germany) and centrifuged for 10 min at 16,000*g*. Plasma was extracted and stored at −20°C. For hormone determination from tissues, samples underwent acid-ethanol extraction (HCl: 1.5% v/v, ethanol: 75% v/v, H_2O: 23.5% v/v). Insulin concentrations were subsequently assessed by an in-house radioimmunoassay.[36] Plasma glucagon and pancreatic glucagon content were measured using glucagon ELISA (EZGLU-30 K, Merck Millipore), or RIA kit (250-tubes GL-32 K, Millipore, USA).

2.2 | Immunohistochemistry and imaging of fixed samples

Following the removal of pancreatic tissue, samples were fixed with 4% PFA for 48 h at 4°C. Fixed tissues were embedded and processed for antibody staining as described.[34] Briefly, tissue sections (7 μm) were blocked with 2% BSA, incubated with respective primary antibodies overnight at 4°C, and, subsequently, with appropriate conjugated secondary antibodies (Table 2). To stain nuclei, a final incubation was carried out at 37°C with 300 nM DAPI (Sigma-Aldrich, D9542). To assess cell proliferation and/or apoptosis, co-staining of mouse anti-insulin (Abcam, Cambridge, UK; 1:1000; ab6995) or guinea pig anti-glucagon (PCA2/4, 1:200; raised in-house) with rabbit anti-Ki-67 (1:200; Abcam ab15580) or TUNEL reaction mixture (Roche Diagnostics Ltd, UK) was used. YFP, tracing the α-cell lineage, was detected with a rabbit or goat anti-GFP (1:1000; Abcam, ab6556 or ab5450, respectively) (Table 2). The slides were imaged on an Olympus BX51 microscope, equipped with a 40×/1.3 objective. The multichannel fluorescence was visualized using DAPI (excitation 350 nm/emission 440 nm), FITC (488/515), and TRITC (594/610) filters and a DP70 camera controlled by Cell^F software (Olympus, UK).

TABLE 2 Primary and secondary antibodies used for immunohistochemistry.

	Dilution	Source
Primary antibody		
Mouse anti-insulin	1:1000	Abcam, ab6995
Guinea pig anti-glucagon	1:200	Raised in-house: PCA2/4
Rabbit (goat) anti-GFP	1:1000	Abcam, ab6556 (ab5450)
Rabbit anti-Ki-67	1:200	Abcam, ab15580
TUNEL enzyme	1:10	Sigma Aldrich 11,684,795,910
Secondary antibody		
Goat anti-mouse	1:400	Alexa Fluor 488, Invitrogen, UK
Goat anti-mouse	1:400	Alexa Fluor 594, Invitrogen, UK
Goat anti-guinea pig	1:400	Alexa Fluor 488, Invitrogen, UK
Goat anti-guinea pig	1:400	Alexa Fluor 594, Invitrogen, UK
Goat anti-rabbit	1:400	Alexa Fluor 488, Invitrogen, UK
Donkey anti-rabbit	1:500	Alexa Fluor 594, Invitrogen, UK
Donkey anti-goat	1:400	Alexa Fluor 488, Invitrogen, UK

2.3 | In vitro islet culture

Islets were isolated from the control group of Glu^{CreERT2}; ROSA26-eYFP mice (immunocytochemistry) or wild-type C57Bl/6 mice ($[Ca^{2+}]_{cyt}$ and ATP/ADP imaging) as described.[37] Briefly, bile duct was injected with cold Hanks' solution containing 0.1 mg/mL liberase (Sigma-Aldrich, Dorset, UK) and islets were picked with an automatic pipette following the digestion. Isolated islets were cultured in RMPI-1640 supplemented with 11 mM glucose, 10% (v/v) fetal bovine serum, 100 U/mL penicillin, 0.1 mg/mL streptomycin (all reagents from Gibco Life Technologies Ltd, Paisley, Strathclyde, UK).

2.4 | Immunocytochemistry

Isolated islets were cultured for 72 h in the medium as above containing 10 mM taurine or equivolumetric saline or RPMI-1640. To mimic diabetic conditions, the medium was supplemented with a mixture of cytokine factors (300 U/mL IL-1B, 300 U/mL IFNγ, 40 ng/mL TNFα, Gibco Life Technologies Ltd), as well as 0.25 mM sodium palmitate (Sigma-Aldrich, Gillingham, UK) and 25 mM glucose (BDH Chemicals Ltd, UK).[34]

2.5 | Imaging of cytosolic [Ca^{2+}] and ATP/ADP

For concurrent imaging of the two signals, isolated islets were dispersed gently into cell clusters that were plated on glass coverslips. Following the attachment of the clusters to the glass, ATP/ADP reporter Perceval[38,39] was expressed in the cells, using an adenoviral vector (10^5 IU/islet), for 36 h. Ca^{2+} reporter Indo-1 (2 μM, ThermoFisher, Loughborough, UK) was loaded prior to the imaging experiments for 20 min. The coverslip was immobilized inside the imaging chamber[40,41] and signals were imaged using a Zeiss AxioZoom.v16 system equipped with a 2.3×/0.56 objective. The fluorescent reporters were co-imaged from the same cell in a single-wavelength mode,[42] the excitation/emission wavelength being (nm): 490/535 (Perceval), 365/465 (Indo-1). The impact of extracellular taurine and glucose on islet cell metabolism and signaling was imaged at the equimolar concentration of 20 mM.

For real-time imaging of $[Ca^{2+}]_{cyt}$ from intact islets, a recombinant sensor GCaMP6f (Vector Biolabs, Malvern, PA, USA) was expressed in the cells, using an adenoviral vector (10^5 IU/islet), for 36 h. The islets were subsequently immobilized in the imaging chamber and signals imaged using a Zeiss LSM510-META confocal system equipped with a 20×/0.75 objective, at 488/535 nm.

Cell images were acquired every 30 s (wide-field) and 4 s (confocal microscope), at +37 °C, while the cells/islets were perifused continuously with bath solution containing, mM: 140 NaCl, 4.6 KCl, 2.6 $CaCl_2$, 1.2 $MgCl_2$, 1 NaH_2PO_4, 5 $NaHCO_3$, 10 HEPES (pH 7.4, with NaOH), 0.2% BSA, and glucose and agents as indicated (Figure 5B).

2.6 | BRIN-BD11 cell culture and qPCR

BRIN-BD11 rat clonal β-cells[43] were grown at 37°C in an atmosphere of 5% CO_2 and absolute humidity in RPMI-1640 tissue culture medium containing 2 mM L-glutamine, sodium pyruvate, 11.1 mM glucose, supplemented with 10% (v/v) fetal calf serum, 100 U/mL penicillin, 0.1 mg/mL streptomycin (all reagents from Gibco Life Technologies Ltd, Paisley, Strathclyde, UK). Cells were seeded at 200,000 cells/well in a six-well plate, allowed to attach for 6 hours and cultured for further for 12 h in the presence/absence of either STZ (5 mM) and/or taurine (20 mM). The expression of four groups of functional genes was investigated to assess changes in (i) proliferation, (ii) apoptosis, (iii) antioxidant defense, and (iv) islet cell transdifferentiation (Table 3). To that end, cells were washed with HBSS solution, lysed using Trizol, and centrifuged at 16,000*g* for 10 min at 4°C. mRNA was extracted from the supernatant using chloroform-isopropanol, and reverse-transcribed using a Superscript II reverse transcriptase—RNase H kit (Invitrogen, UK), at 42°C for 50 min. RT-PCR was performed using primers (Table 3) on a MiniOpticon two-color real-time PCR detection system (BioRad, UK). Data evaluation was performed using the ΔΔCt method, using *ACTB* (β-actin) as a housekeeping gene (Table 3).

2.7 | Data analysis and statistics

Images of fixed samples were analyzed using ImageJ software. All counts were determined in a blinded manner with 25–150 islets analyzed per treatment group, as indicated in the figure legends. Image processing for live-cell recordings (ROI mapping, intensity-vs.-time computation) was performed in Fiji, with subsequent time series data analysis done in IgorPro (Wavemetreics), as detailed in.[44] Statistical analysis was performed using GraphPad PRISM 5.0 (GraphPad, U.S.A.) and/or R4.1.0.[45] For cluster analysis (Figure 7F), we used the mean values for 25 phenotypic characteristics presented in this study (Figures 1–4), inclusive of global animal physiological indexes as well as proliferative, apoptotic and differentiation parameters from current study and our earlier works.[46–49] The analysis was performed using the *kmeans* function of the R core and visualized using the *factoextra* library. The optimal number of clusters was calculated using the elbow method. The data values are expressed as mean ± SEM. Comparisons between experimental groups of normally distributed (Shapiro–Wilk's normality test) data were assessed using Student's *t*-test (Figure 5C, Figure 6B) or (for multiple groups) one-way ANOVA with Bonferroni's post-hoc. For non-normally distributed data (Figure 1B–G), non-parametric analogue, Kruskal-Wallis ANOVA with Nemenyi post-hoc analysis, was utilized. The difference between groups was considered significant for $p < 0.05$.

TABLE 3 Primer sequence for gene expression studies in BRIN BD11 cell line.

Gene	Rat primer sequence
Proliferation	
Ngn 3	Forward: GCAGAGCAGATAAAGCGTGC Reverse: TCGCCTGGAGTAAATTGCGT
Integrin β1	Forward: CTCAATGATGCGTGCGGAAG Reverse: AAGGCGTTGGGAGTTACAGG
Apoptosis	
BCL2	Forward: TGTGGAGAGCGTCAACCGGGAG Reverse: ATCAAACAGAGGCCGCATGCTG
BAX	Forward: TGGACTTCCTCCGGGAGCGG Reverse: CTGGGGGCCTCAGCCCATCT
NFKβ1	Forward: CCTGGATGACTCTTGGGAAA Reverse: TCAGCCAGCTGTTTCATGTC
Anti-oxidants	
SOD1	Forward: ACGGGGTGCTGGTTTGCGTC Reverse: TTCAGCACGCACACGGCCTT
SOD2	Forward: TCCCAAGGGAAACACTCGGCTTT Reverse: AAACCACTGGGTGACATCTACCAGA
Catalase	Forward: CGTGCTGAATGAGGAACAGA Reverse: AGTCAGGGTGGACCTCAGTG
Gpx1	Forward: TCCCTGCGGGGCAAGGTACTAC Reverse: TTCGTTCTTGGCGTTCTCCTGATG
Transdifferentiation genes	
Glucagon	Forward: CTCAGCTCAGTCCCACAAGG Reverse: AGCTGCCTTGTACCAGCATT
Pax6	Forward: ACCTCCTCGTACTCCTGCAT Reverse: CCCATGGGCTGACTGTTCAT
Arx3	Forward: CTCTTCCGTTGGCTGTCCAT Reverse: GGAGGAAGAGAAGGTTGGGC
Insulin	Forward: TACCAGCATCTGCTCCCTCT Reverse: TGCTGGTTCAAGGGCTTTAT
Pdx1	Forward: GAACGCTGGAACAGGGAAGT Reverse: CCCCAGTCTCGGTTCCATTC
Glut2	Forward: CATTCGGAACAGGACCTGGAT Reverse: AGGTGCATTGATCACACCGA
Housekeeping gene	
β-Actin	Forward: CCACCATGTACCCAGGCATT Reverse: CGGACTCATCGTACTCCTGC

3 | RESULTS

3.1 | STZ-induced type 1 diabetic phenotype is unaffected by taurine administration

STZ treatment resulted in a progressive diabetic phenotype of the experimental animals, manifested in elevated levels of blood glucose (Figure 1B). Thus, fasting glucose concentration increased from 8.4 ± 0.6 mM (end of the STZ treatment) to 31.9 ± 0.8 mM ($p = 0.01$ vs. control) 19 days afterwards, in the STZ-treated mice, whereas the control animals displayed unaltered (8.7 ± 0.6 and 7.7 ± 0.5 mM, respectively) levels of glucose. A 10-day oral administration of taurine (2% w/v) had no significant impact on glycemia (28.4 ± 1.4 mM at the end of the study, $p = 0.04$ vs. control, $p = 0.74$ vs. the STZ group) (Figure 1B). The elevation of blood glucose strongly correlated with a decrease in the body weight (Figure 1C), from 20.8 ± 0.6 g at the end of the STZ treatment to 18.8 ± 0.3 g 19 days afterwards. Undetectable in the control group (Figure 1B), the ca.9% loss of weight in STZ mice was only mildly alleviated by the 10-day administration of taurine (21.3 ± 0.4 and 19.7 ± 0.4 g, before and after the treatment, respectively, $p = 0.03$ vs. control, $p = 0.9$ vs. STZ) (Figure 1C).

The STZ treatment started impacting the intake of fluid or food by the mice 14 days after its start (day 0, Figure 1D,E), corresponding to the time-point when the blood glucose increased over 15 mM (Figure 1B). The intake of both fluid and food was elevated from that point, in the STZ-treated groups (Figure 1D,E). Whilst having no significant effect on the fluid intake (Figure 1E), corresponding well to the lack of the reversal in hyperglycemia (Figure 1B), taurine was able to alleviate the increase in food intake (6.2 ± 0.4, $p = 0.02$. vs. 9.8 ± 0.6 g in the STZ group on day 6) (Figure 1D).

The STZ treatment resulted in a substantial decrease in non-fasting terminal plasma insulin levels, measured on day 10 (0.23 ± 0.09 vs. 0.92 ± 0.05 ng/mL in STZ treated and control groups, respectively, $p = 0.02$), with glucagon levels following a similar trend (0.13 ± 0.06 vs. 0.37 ± 0.07 ng/mL respectively, $p = 0.04$) (Figure 1F).

In line with the effect on plasma insulin levels (Figure 1F), STZ substantially decreased pancreatic insulin content (24.38 ± 11.24 vs. 105.2 ± 13.03 ng/mg of tissue in control, $p = 0.03$), acting predominantly on the islets located in the "tail" of the pancreata (Figure 1G). No significant effect on the glucagon content was recorded. Likewise, no impact of taurine on the pancreatic content of either hormone was observed (Figure 1F,G, $p = 0.9$ vs. the STZ group in both cases).

3.2 | Chronic taurine treatment counteracts changes in the islet cellular composition induced by STZ

The phenotypic profile and blood glucose measurements (Figure 1B–G) were echoed by the decrease in the average cross-section area of islets isolated from the STZ-

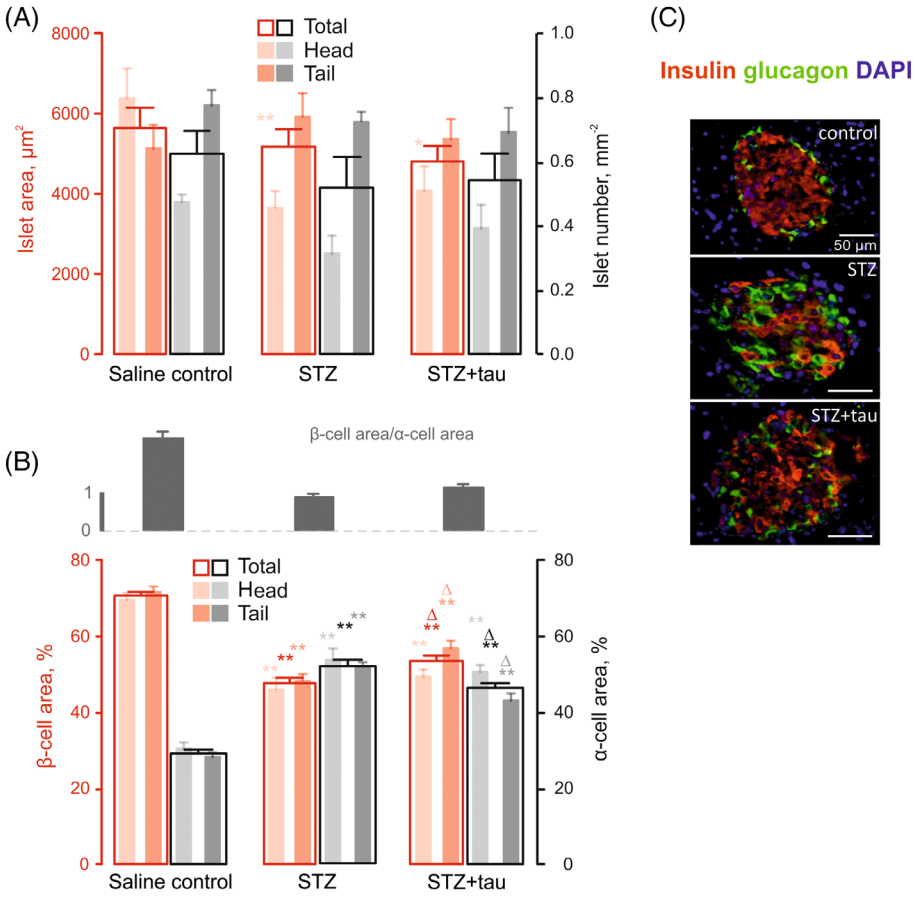

FIGURE 2 Diabetic phenotype is associated with changes in the islet composition. (A) Islet area (red, $n = 150$ (75 from the head +75 from the tail) islets from six mice) and number (black, $n = 150$ islets from six mice). (B) β- (red, $n = 150$ islets from six mice) and α-cell (black, $n = 150$ islets from six mice) percentage among the islet cells in response to the administration of STZ on the background of the taurine treatment in Glu^{CreERT2};ROSA26-eYFP mice, as indicated, analyzed on day 10. Gray bars in (B) represent the net difference in the percentage of β- and α-cells. (C) Representative immunostaining of mouse pancreatic sections for DAPI (blue), glucagon (green) and insulin (red). *$p < 0.05$, **$p < 0.01$ compared to saline control group. Δ$p < 0.05$ compared to streptozotocin treated group. Scale bars: 50 µm.

treated mice, with taurine having no effect (red in Figure 2A). At the same time, we were unable to detect any significant alteration in the islet numbers per mm^2, within any of the groups (black in Figure 2A), possibly due to substantial variability of this characteristic.

STZ-treatment produced a significant reduction in the relative β-cell area (red/insulin+ in Figure 2B,C) and an increase in the relative α-cell area (black/glucagon+ in Figure 2B,C). Remarkably, taurine partially rescued the effects of the STZ treatment on β- and α-cell fractions, resulting in small but significant differences in the percentage of β-cells (54 ± 1% vs. 46 ± 3% in STZ mice, $p = 0.04$) and α-cells (44 ± 1% vs. 54 ± 2% in STZ mice, $p = 0.04$) (Figure 2B,C).

3.3 | Taurine decreases apoptosis and increases the proliferation of β-cells

Used in small repeated doses, STZ has been shown to induce apoptosis specifically in pancreatic β-cells.[50] In line with this report, we observed almost a five-fold (1.9 ± 0.3 vs. 0.4 ± 0.1% in control mice) increase in the percentage of β-cells (insulin+) expressing an apoptosis marker, TUNEL, in mice treated with STZ (red in Figure 3A). The β-cell apoptosis was significantly reversed by taurine (red, Figure 3A), whereas none of the STZ-treated groups displayed any changes in apoptosis frequency in α-cells (glucagon+, black, Figure 3A).

The pro-apoptotic effect of the STZ treatment was not associated with any increase in the percentage of proliferating β-cells, probed via Ki-67 staining (red in Figure 3B). It did however produce a 3.5-fold increase in the fraction of proliferating α-cells (black in Figure 3B). Whilst having little effect on α-cell proliferation (black in Figure 3B), taurine was able to augment proliferation of β-cells by the factor of four (red in Figure 3B).

3.4 | Chronic taurine supplementation enhances α-/β-cell transdifferentiation induced by the STZ treatment

The key feature of the Glu^{CreERT2}; ROSA26-eYFP mice is the inducible expression of YFP specifically in pancreatic α-cells, which was accomplished at the beginning of the experimental protocol, via the injection of tamoxifen (Figure 1A). Stained with anti-glucagon antibodies, 26 days post-induction of the targeted YFP expression,

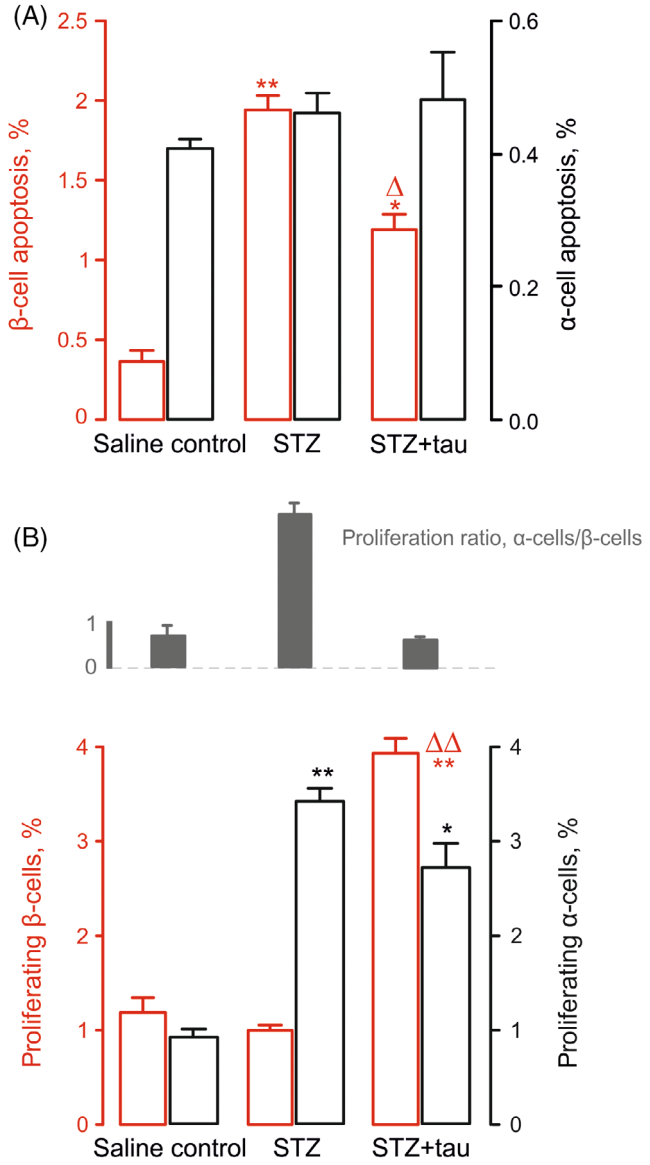

FIGURE 3 Taurine decreases apoptosis, increases proliferation of β-cells. (A,B) Percentage of primary β-cells (red, $n = 60$ islets from six mice each) and α-cells (black, $n = 60$ islets from six mice) in islets of Glu^{CreERT2};ROSA26-eYFP mice undergoing apoptosis (A), as determined by TUNEL staining, or proliferation (B), Ki-67 staining, in response to the administration of STZ on the background of the taurine treatment, as indicated. *$p < 0.05$, **$p < 0.01$ compared to saline control group. Δ$p < 0.05$ and ΔΔ$p < 0.01$ compared to STZ-treated group.

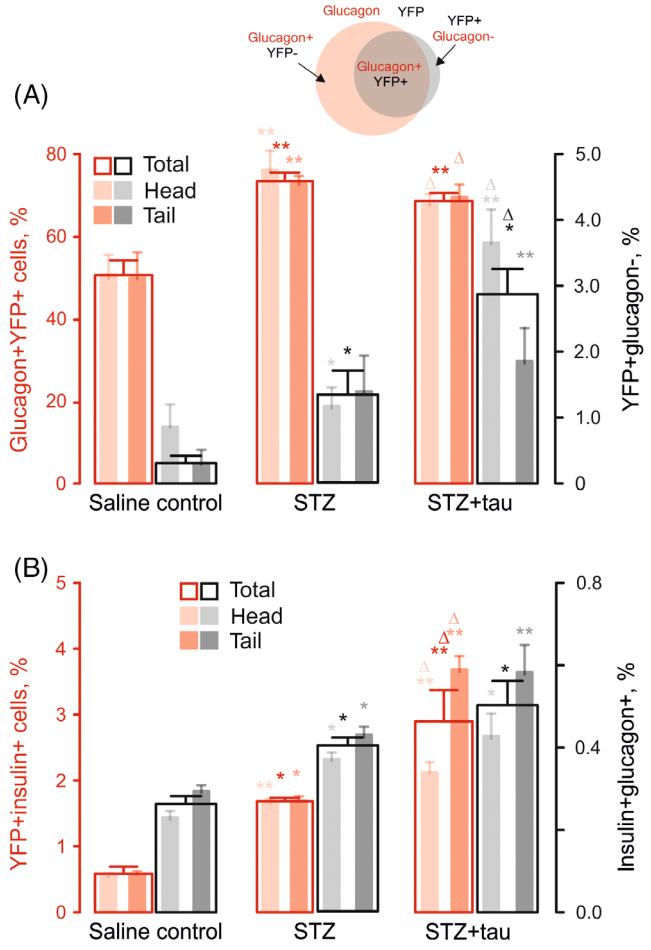

FIGURE 4 Taurine promotes α-cell transdifferentiation into β-cells. Percentage of the YFP-expressing cells detected within α- (A, red, $n = 60$ islets from 60 mice) and non-α-cells (A, black, $n = 60$ islets from six mice each) after administration of STZ to the Glu^{CreERT2};ROSA26-eYFP mice and subsequent treatment with taurine, as indicated, visualized at the termination of the study (Day 10). Percentage of insulin+ β-cells (B, red, $n = 60$ islets from 60 mice) and double-positive (insulin+glucagon+) cells (B, black, $n = 60$ islets from 60 mice) detected at the same time-point as in panel (A). *$p < 0.05$, **$p < 0.01$ compared to saline control group. Δ$p < 0.05$ compared to STZ-treated group.

the islets from the control mouse group had only a small fraction of YFP+ cells that did not express glucagon (0.3 ± 0.1%), which was increased five-fold by the STZ treatment (1.6 ± 0.4%, $p = 0.04$ vs. control) and further expanded by taurine (2.9 ± 0.6%, $p = 0.02$ vs. other groups) (black in Figure 4A).

Very low in the control animals (0.7 ± 0.2%), the percentage of the YFP + insulin+ cells in the STZ-treated mice was 2.5 times higher (1.8 ± 0.1%, $p = 0.03$) than in the control group (red in Figure 4B). Just as in the case of YFP + glucagon- cells, taurine (2.9 ± 0.5%) further significantly potentiated the commitment of the YFP+ cells towards the insulin lineage (red, Figure 4B). The effect was more prominent within islets residing in the tail of the pancreas, that has a different developmental origin to the head of the gland.[51] The differentiation of YFP+ α-cells towards β-cells was reflected in the increased percentage of bi-hormonal cells (black in Figure 4B). The size of this small cell subpopulation was mildly increased by the STZ treatment (0.32 ± 0.02% vs. 0.27 ± 0.05% in the control group, $p = 0.03$), and further expanded by taurine (0.37 ± 0.03%, $p = 0.02$ vs. control) administration (black, Figure 4B).

3.5 | Taurine promotes α-cell differentiation in vitro and acutely elevates cytosolic Ca^{2+} in isolated islet cells

The in vivo commitment of the YFP+ cells towards the insulin+ lineage, induced by the combination of STZ and taurine, was confirmed in islets isolated from non-treated $Glu^{CreERT2}$; ROSA26-eYFP mice (Figure 5A). Although the chronic in vitro exposure to the cytokine and fatty acids mixture, deemed to mimic the diabetic condition (see Experimental procedures), failed to increase the percentage of YFP + insulin+ islet cells (2.9 ± 0.2% vs. 2.7 ± 0.1% in the RPMI-1640 control, $p = 0.5$), the inclusion of 10 mM taurine in the medium expanded this fraction significantly (4.5 ± 0.3%, $p = 0.03$ vs. control and diabetic groups).

Having established the ex vivo model for taurine effect in islets, we subsequently probed potential mechanisms of taurine effect in islets on the acute timescale. To that end, we imaged cytosolic Ca^{2+} ($[Ca^{2+}]_{cyt}$) dynamics

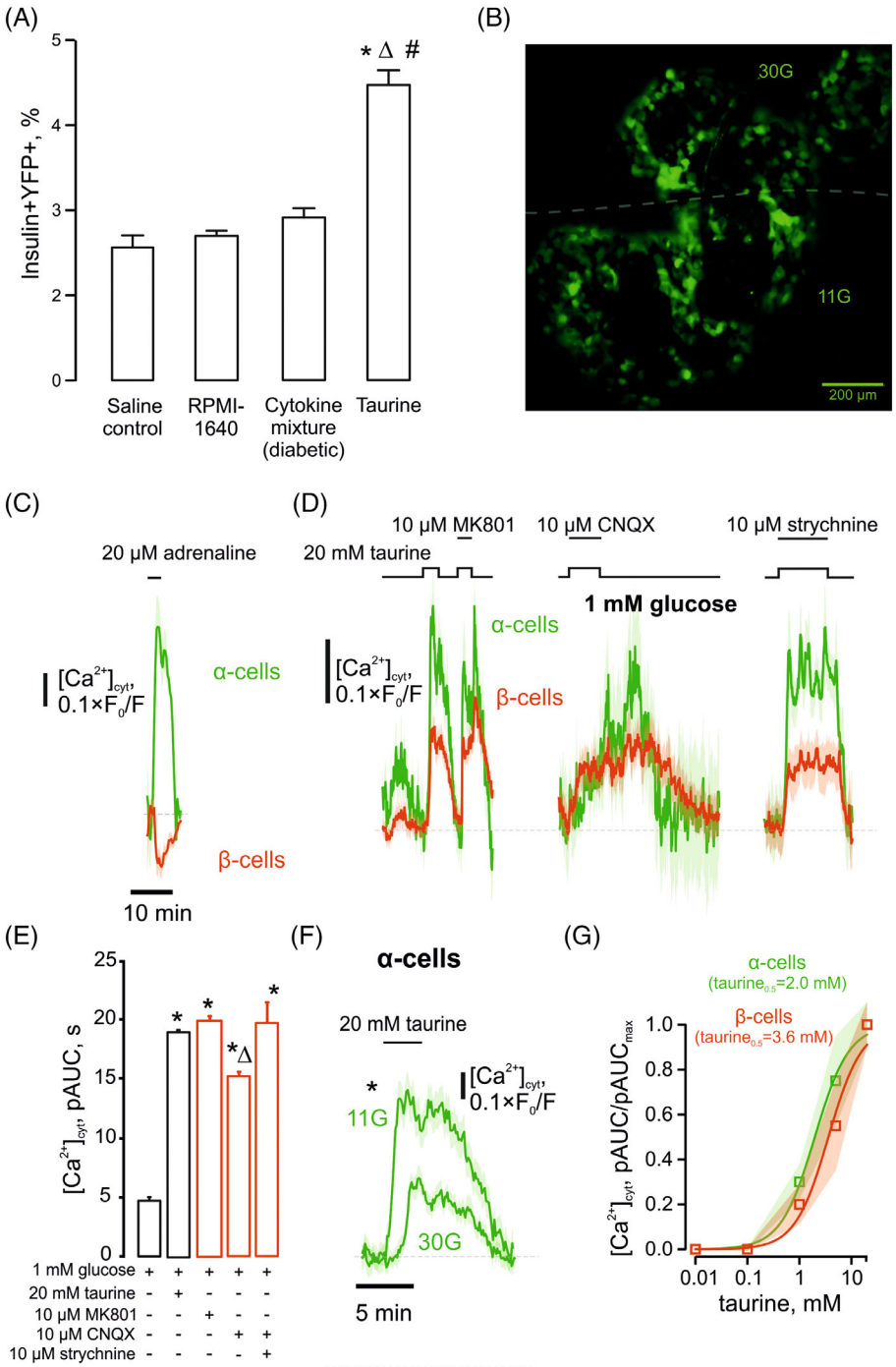

FIGURE 5 Legend on next page.

in intact islets using confocal microscopy (Figure 5B), with α-cells being identified by their positive $[Ca^{2+}]_{cyt}$ response to 10 μM adrenaline (Figure 5C).[37] In our hands, the application of taurine triggered a reversible elevation of $[Ca^{2+}]_{cyt}$ in α- and β-cells (Figure 5D). Whilst the elevation of $[Ca^{2+}]_{cyt}$ argued against the involvement GABA signaling that has an inhibitory effect, in α-cells,[25,27] potential role of glycine or glutamate signaling was tested by applying specific inhibitors of glycine (strychnine[52]), NMDA (MK801[52]), AMPA (CNQX[53]) receptors (Figure 5D). Ca^{2+} dynamics quantified as partial area under the curve, pAUC,[54] demonstrated that neither of the inhibitors was able to cancel the response to taurine in α- or β-cells, whereas CNQX produced a small but significant attenuation of the response to taurine (Figure 5E). Of note, 48-h pre-culturing in high (30 mM) glucose attenuated the impact of taurine on $[Ca^{2+}]_{cyt}$ in α- (Figure 5F) but not in β-cells (not shown).

We have furthermore quantified the dependence of the intensity of islet cell $[Ca^{2+}]_{cyt}$ dynamics on the dose of extracellular taurine, by imaging the former in real-time and quantifying as pAUC.[54] In some cases, taurine was able to elicit $[Ca^{2+}]_{cyt}$ dynamics at as little as 100 uM whereas 1 mM of taurine produced a palpable effect in most of the cells, with a dose-dependent progression to 10 mM (Figure 5G).

We investigated potential role of cell metabolism in taurine signaling as well as its selectivity towards α-cells, by co-imaging the impact of taurine stimulus on $[Ca^{2+}]_{cyt}$ and ATP/ADP ratio in islet cells (Figure 6A). The two signals were imaged simultaneously in real time from a population of islet cells, arranged in clusters, with α-cells being identified by their positive $[Ca^{2+}]_{cyt}$ response to 10 μM adrenaline, as above. The addition of 20 mM taurine produced a significantly higher elevation of cytosolic Ca^{2+} level in α-cells (red trace in Figure 6B) than in β-cells, a trend that was palpable in intact islets (Figure 5C). At the same time, taurine failed to elevate cytosolic ATP/ADP ratio (green trace in Figure 6B), which was a prerequisite of Ca^{2+} influx induced by 20 mM glucose in β-cells (Figure 6B).

3.6 | Taurine treatment rewires β-cell gene expression

Assessed in BRIN-BD11 β-cell line, taurine enhanced cell proliferation and attenuated apoptosis (Figure 7). We used this model, bearing its close similarity to primary rodent β-cells[43] and human β-cell lines,[55] to probe the changes in the expression of key β-cell genes induced by STZ or taurine treatment, alone or in a combination. 12-h exposure to STZ significantly attenuated the expression (dark blue) of the "islet expansion" genes (*Ngn3*, *Itgb*), alongside *Ins*, *Bcl2*, and *Pdx1*. The STZ treatment instead upregulated (dark red) the "antioxidant" genes (*Sod1*, *Sod2*, *Gpx1*, *Cat*) as well as *NFKβ* and *Pax6* (Figure 7E). Remarkably, the addition of taurine to STZ reversed these tendencies, whereas taurine alone had practically reciprocal effect to that of STZ on the expression of the selected genes (Figure 7E). The expression profile of the cells exposed to the combination of STZ and taurine clustered closely to that of the saline control (Figure 7E).

Having conducted several studies on the STZ-induced mouse model of diabetes utilizing a comparable experimental design,[46–49,56] we performed a meta-analysis of the animal phenotype and islet biology across these studies, aimed at dissecting the relationship of taurine to other potential and actual antidiabetic drugs from purely empirical point. In our hands, the effects provoked by taurine clustered (k-means) closely with that developed

FIGURE 5 Taurine chronically promotes α-cell differentiation in vitro and acutely depolarises islet cells. (A) Percentage of YFP+ cells expressing insulin in the islets ($n = 50$ per condition) isolated from $Glu^{CreERT2}$;ROSA26-eYFP mice ($n = 3$) and subsequently cultured in the medium containing control (saline or RPMI-1640), a mixture of cytokines, mimicking the diabetic condition, and taurine. *$p < 0.05$ versus saline control, #$p < 0.05$ versus RPMI-1640, Δ$p < 0.05$ RPMI-1640 versus cytokine mixture. (B) Confocal image of isolated pancreatic islets, pre-cultured for 48 h in 11 mM ("11G") or 30 mM glucose ("30G"), as indicated with a dashed line. The islet groups were co-imaged simultaneously. (C) Ca^{2+} dynamics induced by 10 μM adrenaline was used to differentiate α-cells from β-cells, as detailed in.[37] (D) Ca^{2+} dynamics in response to 20 mM taurine in the presence/absence of the inhibitors of NMDA (MK801), AMPA (CNQX) glutamate and glycine (strychnine) receptors, imaged in isolated islets ($n = 5$ mice) using laser scanning confocal microscopy. α-Cells were identified by the positive response to adrenaline. (E) Quantification of the representative data on the pharmacology taurine effect from panel *D*, in terms of the partial area under the curve (pAUC),[54] for α- ($n = 102$, $n = 102$, $n = 26$) and β-cells ($n = 257$, $n = 257$, $n = 42$) for MK801, CNQX and strychnine, respectively. Δ$p < 0.05$ versus taurine +3 mM glucose, *$p < 0.05$ versus 3 mM glucose alone. (F) Comparison of the taurine responses in α-cells from islets pre-cultured at 11 mM ($n = 102$) or 30 mM ($n = 63$) glucose, for 48 h, as indicated. *$p < 0.05$ versus time-averaged intensity recorded in α-cells pre-cultured at 11 mM glucose. (G) Dose–response relationship between extracellular taurine concentration and $[Ca^{2+}]_{cyt}$ dynamics, quantified as partial area under the curve (see Experimental Procedures) from the real-time confocal recordings (as in C), for α- ($n = 34$) and β-cells ($n = 74$). The lines represent the Hill equation fit with $taurine_{0.5} = 2.0$, $h = 1.3$ (α-cells), $taurine_{0.5} = 3.6$, $h = 1.4$ (β-cells).

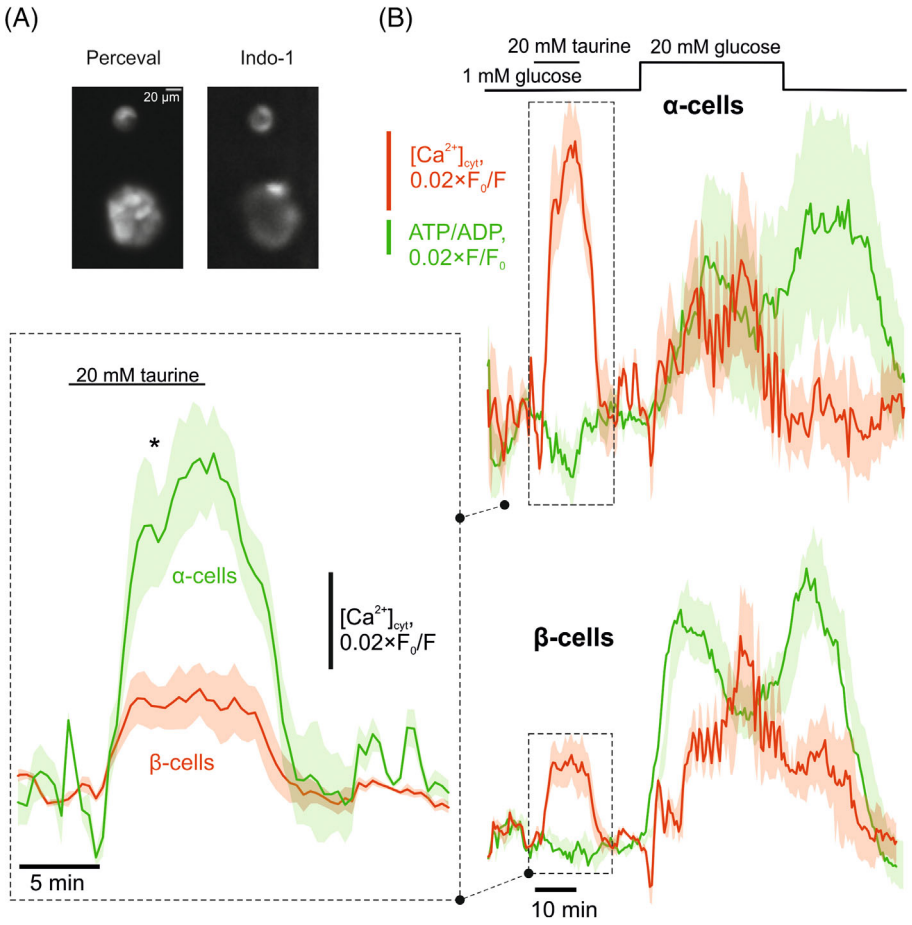

FIGURE 6 Taurine chronically promotes α-cell differentiation in vitro and acutely depolarises islet cells. (A) Wide-field images of dispersed islet clusters expressing/preloaded with spectrally compatible fluorescent reporters of ATP/ADP (Perceval, green channel) and Ca^{2+} dye (Indo-1, cyan channel). (B) ATP/ADP (green) and Ca^{2+} (red) dynamics in response to taurine and elevated glucose imaged in dispersed cells from mouse pancreatic islets ($n = 5$ mice) using wide-field zoom microscopy. α-Cells were identified by the positive response to adrenaline in the Ca^{2+} channel. *Inset*: comparison of Ca^{2+} responses imaged in α- (green, $n = 106$) and β-cells (red, $n = 212$). *$p < 0.05$ versus for time-averaged intensity recorded in β-cells.

after liraglutide and sitagliptin (Figure 7F) treatments, on the STZ background.

4 | DISCUSSION

Alongside the multimodal role of taurine in central nervous system,[57] the amino acid has been tipped for a role in type 2 diabetes pathophysiology in humans, as arguably impaired absorption of taurine[5,58] alters its signaling in diabetes.[59] We report a dual effect of taurine, aimed at the replenishment of the pool of pancreatic islet β-cells under the condition of a severe stress that was modeled by the STZ treatment. According to our data, chronic dietary supplementation of this key component of popular drinks augmented the proliferation/apoptosis ratio of the β-cell fraction and enhanced the re-programming of the α-cell fraction.

4.1 | Diabetic phenotype, islet morphology, proliferation, apoptosis

Multiple low-dose injections of STZ induced a severe diabetes reflected in the elevated fluid and food intake and hyperglycemia alongside decreased body weight.[46,48] The diabetic phenotype was unaffected by a relatively low-dose[60] dietary administration of taurine, as we deemed to separate potential direct effects of the latter on pancreatic islet biology and physiology from the indirect ones, mediated by the changes in such systemic factors as glucose or insulin. Notably, circulating glucagon levels were also decreased (Figure 1F) by STZ-induced depletion of the β-cell population (Figure 2B), a trend observed in our earlier reports.[47–49] This finding may reflect potential secondary damage of the α-cell population in the early stages of the STZ model with subsequent failure of the "resting" α-cell pool[41] to restore the systemic levels of glucagon. At the same time, as the STZ treatment had only a modest impact on the size of the islet α-cell population (Figure 2A,B), the impaired secretion of glucagon could have stemmed from a defect in glucose-sensing by α-cells[61] or indirect effects linked to the time-dependent apoptotic peaks within the β-cell fraction.[50] The latter are believed to upregulate glucagon release in models utilizing higher doses of STZ,[62,63] or allowing for longer progression of the phenotype.[63,64]

Notably, taurine administration was able to reverse the tendency for the β-cell loss (Figure 2B), evidently by decreasing apoptosis (Figure 3A) and increasing

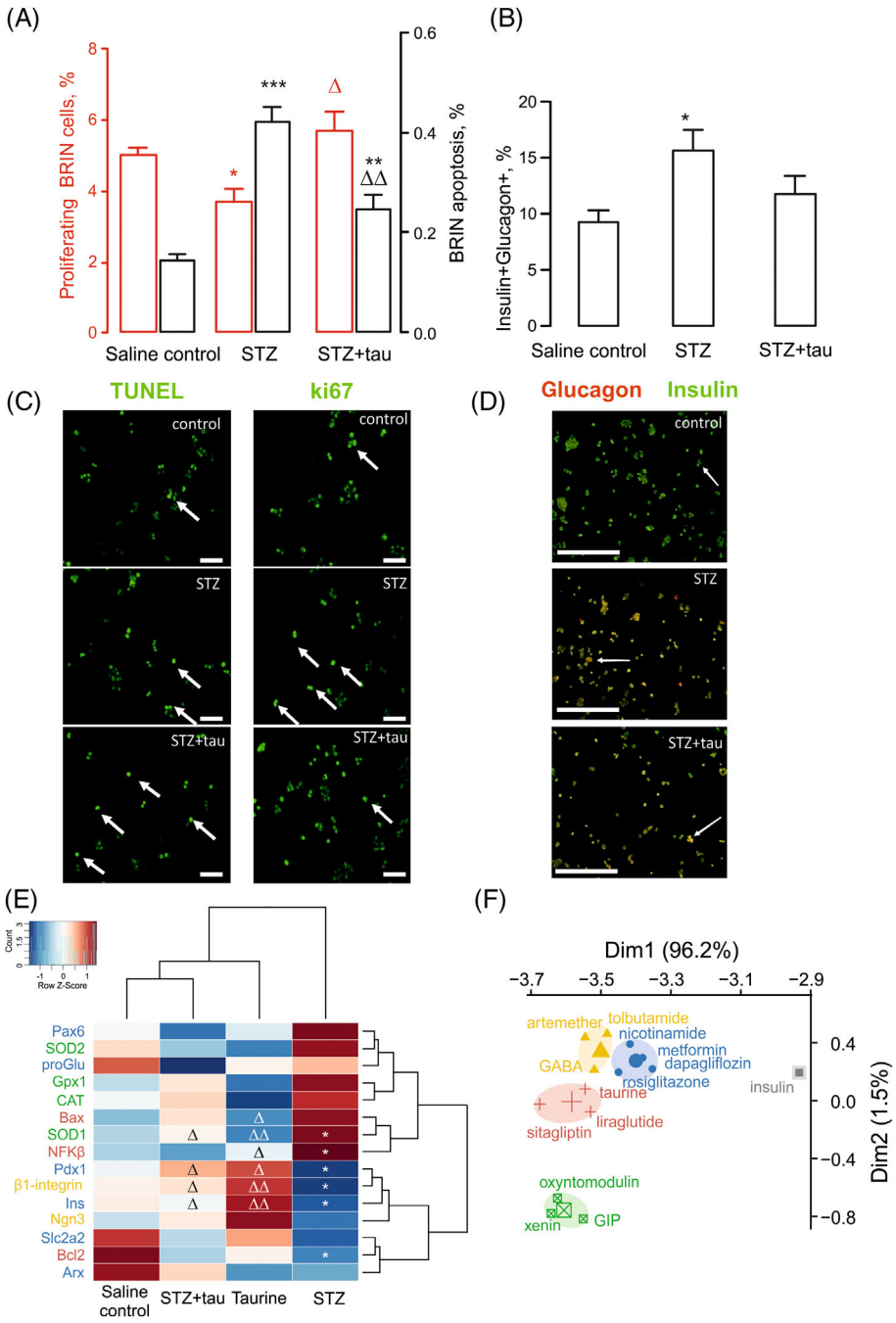

FIGURE 7 Taurine attenuates changes in gene expression of pancreatic β-cells induced by STZ administration. (A–D) Taurine effect is reliably reproduced in β-cell line, BRIN BD11. Percentage of BRIN BD11 β-cells undergoing apoptosis ($n = 60$, black) or proliferation ($n = 60$, red), as determined by TUNEL and Ki-67 staining, respectively, in response to the administration of STZ on the background of the taurine treatment, as indicated, after a 12-h culture. (B) Percentage of BRIN-BD11 cells cultured for 12 h in RPMI medium with different supplements (as indicated, detailed in Experimental procedures) co-expressing insulin and glucagon ($n = 150$ cells). *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ compared to saline control group. Δ$p < 0.05$ and ΔΔ$p < 0.01$ compared to STZ-treated group. (C,D) representative immunofluorescence images for the data in panels (A), (B), respectively. Arrows indicate insulin+glucagon+ cells. (E) Gene expression pattern in BRIN-BD11 cells cultured for 12 h in RPMI medium with different supplements (as indicated, detailed in BRIN-BD11 cell culture and qPCR) presented as a hierarchical clustering heatmap. Four groups of genes were detected, reflecting pancreatic islet expansion (Ngn3, β1-integrin), apoptosis (Bax, Bcl2, NFKβ), oxidative stress (SOD1, SOD2, CATalase, Gpx1) and (trans)differentiation (Pax6, Pdx1, Arx, Glut2, Ins, proGlu). *$p < 0.05$ compared to the control RPMI-1640 media; Δ$p < 0.05$ and ΔΔ$p < 0.01$ compared to the medium containing STZ (diabetic control) ($n = 3$). (F) K-means clustering of the animal phenotype and islet biology features investigated upon administration of taurine, oxyntomodulin, xenin, GIP,[46] insulin, GABA, nicotinamide,[49] tolbutamide, metformin, rosiglitazone,[48] dapagliflozin, liraglutide, sitagliptin[47] and artemether (in press) on the background of the STZ treatment.

proliferation (Figure 3B) in this population of islet cells, in line with earlier reports from endo-[65,66] and exocrine[67] pancreatic tissue. The proliferation of α-cells, elevated by STZ injections, was not affected by taurine administration (Figure 3B).

4.2 | Expression of insulin by YFP+ cells

The beta-cell-selective antiapoptotic/proliferative effect of taurine was, however, contrasted by acquisition of insulin by the YFP+ α-cells that was further enhanced via the supplementation of the amino-acid (Figure 4B). Although taurine has been shown to enhance cell differentiation in some tissues, for example, mesenchymal[68] or neural[69] stem cells, opposite effects were reported in other systems, such as smooth muscle[70] or aortic valve interstitial cells.[71]

4.3 | Acute taurine signaling is not linked to the oxidative metabolism

The effect on α-cell differentiation was further strengthened by acute impact of taurine on the cytosolic Ca^{2+} dynamics that was moderately specific to the α-cell population (Figure 5B). The conventional stimulus for insulin secretion from islet cells is systemic glucose, which directly impacts cytosolic levels of ATP in β-[72] and α-cells.[73] ATP, in turn, inhibits the ATP-sensitive K+ conductance[74] thereby driving the plasma membrane potential into the range of activation of voltage-gated Ca^{2+} channels,[75] initiating regenerative electrical spiking and Ca^{2+} entry into the cytosol. It is plausible to expect some amino acids, such as highly abundant in plasma glutamine or L-alanine, to act in a way similar to glucose, via oxidation in mitochondria[76] and subsequent elevation of cytosolic ATP. In our hands, 20 mM of taurine was unable to impose any immediate stimulation on the energy metabolism in β- or α-cells at 1 mM glucose suggesting the lack of enzymatic machinery for this acute effect (Figure 5B). Instead, we report a small reduction of cytosolic ATP/ADP ratio, in response to taurine, (Figure 5B), likely required for handling the elevated Ca^{2+}.[77] Thus, contrary to the earlier reports of its K_{ATP} channel-dependence,[78] our findings suggest that the acute effect of taurine on $[Ca^{2+}]_{cyt}$ dynamics in islet cells is likely to be attributed receptor-mediated signaling impacting Ca^{2+} homeostasis or the Na^+-coupling of the import of the amino acid into the β-cell (see below). The latter mechanism would explain the differences between the effect of taurine on α- and β-cells (Figure 5B), in terms of higher input resistance of α-cells, at low (1 mM) glucose.[79]

4.4 | Can the acute effect of taurine be mediated by an ionotropic receptor?

With no specific receptors identified to date,[80] the neuronal inhibitory effect of taurine is believed to be exerted via the low-affinity activation of ionotropic $GABA_A$ ($GABA_AR$, EC_{50} for taurine ranging from 6 μM to10 mM, depending on the subunit composition)[80] and glycine (GlyR, EC_{50} = 153 μM)[81] receptors. Both receptors, representing Cl^- channels gated by extracellular ligands, are expressed on the membrane of islet β-cells[26] and α-cells.[25,28] The reported affinities of the $GABA_AR$ and GlyR to taurine are compatible with systemic levels of this predominantly exogenous[6] amino acid that range from 50 μM[82] to 690 μM,[83] depending on the dietary taurine supplementation. Notably, this range fits well the dose-dependence of the dynamics of $[Ca^{2+}]_{cyt}$ on extracellular taurine (Figure 5E).

Whilst the inhibitory modality of the signaling in neurons is linked to the millimolar cytosolic levels of $[Cl^-]$ in these cells (6 mM,[84]), much lower than those reported for pancreatic β-cells (30 mM,[85]). In line with this, taurine has been reported to acutely stimulate secretion of insulin from clonal pancreatic β-cells[86] and glucagon from isolated islets,[87] likely by depolarising the plasma membrane of the target cells.

The observed stimulatory effect of taurine on α-cell Ca^{2+} dynamics (Figure 5B) is at odds with inhibitory Cl-channel-mediated signaling reported in these cells by some authors.[27,28] At the same time, the lack of sensitivity to the inhibition of glycine receptors by strychnine (Figure 5D) rules out potential role of stimulatory glycine signaling.[25,88] Our findings furthermore rule out the interaction with NMDA[89] or AMPA receptors that were reported to be preferentially expressed by α-cells.[90] Despite acknowledging the hypothetical involvement of other ionotropic receptors, our results suggest alternative avenues for taurine import into α-cells, such as co-transport with Na^+, a mechanism which has been recently demonstrated to play a key role in islet δ-cell signaling.[91]

4.5 | Chronic effect of taurine on α- and β-cells: Intracellular mechanism(s)?

Rapid excretion rate of taurine suggests that its chronic effect is attained via the cytosolic compartment that contains as much as 10-fold higher level of the polar hydrophilic amino acid than the plasma.[6] Despite lacking the machinery for taurine oxidation (Figure 5B), islet cells are likely to benefit from its ability to inhibit the generation of reactive oxygen species and suppress the toxicity of oxidative agents,[92] which may in part explain the

antiapoptotic/proliferative effect of the amino acid on β-cells (Figure 3A).

Finally, taurine may affect islet biology indirectly, by enhancing the duodenal solubility of bile acids. The taurine-dependent neutralization of bile acids may potentially lead to enhanced release of the incretin GLP-1 by the duodenum.[93] In line with the role of GLP-1 was the observed phenotype that had parallels to that reported earlier for a synthetic long-acting GLP-1 mimetic, liraglutide (Figure 7F).[47] At the same time, the notable proximity of the phenotypical profile induced by taurine to that of GABA (Figure 7F) treatment, the dual liraglutide-GABA association could reflect the differential effect of taurine on islet cell subpopulations, α- and β-cells.

4.6 | Conclusions

A lifestyle-dependent factor, systemic taurine is modulated by the perturbations in its daily uptake[17] (ranging between 40 and 400 mg[94]) induced by disturbances of metabolism, such as metabolic disease[95] and age.[96] Our findings, suggesting two distinct mechanisms whereby taurine supplementation may ameliorate the islet phenotype in diabetes, leave open a question of interaction between the two pathways as well as the optimal dosage of the supplement. Apart from potential use for diabetes therapy, taurine-based signaling could be one of the targets of diabetic transformation thereby underlying functional demise of islets in diabetes.

AUTHOR CONTRIBUTIONS

Dipak Sarnobat: Methodology and investigation; data curation; visualization. **R. Charlotte Moffett**: Conceptualization; supervision; methodology. **Jinfang Ma**: Methodology and investigation; data curation. **Peter R. Flatt**: Conceptualization; supervision; writing; reviewing; editing. **Neville H. McClenaghan**: Conceptualization; supervision; writing; reviewing; editing. **Andrei I. Tarasov**: Data curation; investigation; visualization; conceptualization; writing; reviewing; editing.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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