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N-Acyl taurines trigger insulin secretion by increasing calcium flux in pancreatic β-cells

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ABSTRACT

Pancreatic β-cells secrete insulin in response to various stimuli to control blood glucose levels. This insulin release is the result of a complex interplay between signaling, membrane potential and intracellular calcium levels. Various nutritional and hormonal factors are involved in regulating this process. *N*-Acyl taurines are a group of fatty acids which are amidated (or conjugated) to taurine and little is known about their physiological functions. In this study, treatment of pancreatic β-cell lines (HIT-T15) and rat islet cell lines (INS-1) with *N*-acyl taurines (*N*-arachidonoyl taurine and *N*-oleoyl taurine), induced a high frequency of calcium oscillations in these cells. Treatment with *N*-arachidonoyl taurine and *N*-oleoyl taurine also resulted in a significant increase in insulin secretion from pancreatic β-cell lines as determined by insulin release assay and immunofluorescence ($p < 0.05$). Our data also show that the transient receptor potential vanilloid 1 (TRPV1) channel is involved in insulin secretion in response to *N*-arachidonoyl taurine and *N*-oleoyl taurine treatment. However our data also suggest that receptors other than TRPV1 are involved in the insulin secretion response to treatment with *N*-oleoyl taurine.

1. Introduction

Pancreatic β-cells secrete insulin in response to various triggers in order to control blood glucose levels (for review see [1]). The mechanism of insulin release is through an increase in the intracellular ATP/ADP ratio, which in turn closes ATP-sensitive K^+ channels on the cell surface and opens voltage-gated Ca^{2+} channels. The influx of Ca^{2+} through these channels increases the cytoplasmic free Ca^{2+} concentration, leading to exocytosis of insulin-containing vesicles. There is also a large body of evidence showing that transient receptor potential channels (TRP) may play a significant role in insulin release from pancreatic β-cells (for review see [2]). TRP channels form a large group of cation channels, with 28 mammalian members identified to date. Several members of the TRP channels [3], including TRPM5 expressed within the pancreatic islets of Langerhans, have been shown to mediate insulin secretion from pancreatic β-cell lines [4]. Other members of the TRP family known to function in regulation of insulin secretion are expressed in mouse, rat or human pancreas or islets, and in several β-cell lines, however there are a number of TRP proteins whose functions are currently unknown [2].

Recent studies have shown that the amino acid taurine (2-aminoethanesulfonic acid) regulates insulin release from pancreatic β-cell lines HIT-T15 (Syrian hamster) and RIN-m (rat islet) [5], with other chemicals such as capsaicin modulating insulin secretion in rat islet β-cells through activation of the transient receptor potential vanilloid 1 (TRPV1) [6]. A number of lipids, collectively known as *N*-acyl amino acids, have been identified in vivo and these lipids are various fatty acids that are amidated (or conjugated) to amino acids [7]. One group of these lipids identified is the *N*-acyl taurines (NATs). *N*-Acyl taurines were first detected in brain, liver and other tissues in mouse in 2004 [8]. *N*-Arachidonoyl taurine activates members of the TRP channels, specifically the transient receptor potential vanilloid receptors, TRPV1 and TRPV4 ion channels [9]. TRPV1 is a non-selective cation channel that mediates peripheral nociception and pain sensation and its expression has been detected in the rat insulinoma cell lines RIN and INS-1 [6]. TRPV1 has also been suggested as a central controller of both islet stress and T cell infiltration [10]. Expression of TRPV4 has been detected in mouse pancreas and MIN6 cells and may be involved in the increase in intracellular calcium in response to human islet amyloid polypeptide fibril formation [11]. Another *N*-acyl amino acid, *N*-arachidonoyl glycine (NAGly), has been identified as a novel insulin secretagogue in primary β-cells [12], although the insulinotropic action of NAGly in rat islet β-cells occurs via voltage-dependent Ca^{2+} channels (VDCC), rather than via activation mechanism of the TRPV1 channel [12].

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The present study was carried out to examine if *N*-acyl taurines (specifically *N*-arachidonoyl taurine and *N*-oleoyl taurine) play a role in insulin secretion in pancreatic β-cells. We show that β-cell treatment with *N*-arachidonoyl taurine and *N*-oleoyl taurine leads to an increase of intracellular calcium ions $[Ca^{2+}]_i$ and directly activates insulin release from cells. Moreover, our results suggest that the TRPV1 channel, and channels other than TRP channels, are involved in the flux of $[Ca^{2+}]_i$ to the cytoplasm in response to *N*-acyl taurine treatment.

2. Materials and methods

2.1. Cell culture

Pancreatic β-cell line HIT-T15 from Syrian hamster or 832/13 INS-1 rat islets cell line (provided by Duke University, Durham, NC 27710) were used. HIT-T15 cells were cultured in RPMI-1640 medium supplemented with 10% dialyzed horse serum, 2.5% fetal bovine serum, 100 IU/ml penicillin, 100 IU/ml streptomycin and 2 mM L-glutamine. Insulin secreting 832/13 INS-1 cells (stably transfected with a plasmid coding for human Proinsulin [13]) were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 10 mM HEPES, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 IU/ml penicillin, and 100 IU/ml streptomycin. Cell lines were incubated under 95% O_2, 5% CO_2 at 37 °C. Media were changed twice weekly, and cells were trypsinized and passaged weekly. Early passages of the HIT-T15 cells were used in all experiments.

2.2. Measurement of free calcium ions $[Ca^{2+}]_i$ in β-cells

N-Arachidonoyl taurine and *N*-oleoyl taurine were purchased from Cayman Chemical Company (Ann Arbor, MI, USA) and dissolved in dimethylsulfoxide (DMSO) as a 10 mM stock.

The HIT-T15 cells and 832/13 INS-1 cell lines were grown in complete RPMI-1640 medium in 0.17 mm glass-bottomed cell culture dishes coated with poly-d-lysine (Mat Tek, Ashland, MA 01721, USA) for calcium ion measurements. When cells reached ~80% confluence, the medium was removed and replaced with fresh glucose-free RPMI-1640 medium or medium containing 3 mM glucose (basal glucose level) for INS-1 cells. The next day cells were washed twice with PBS and incubated for 40 min at 37 °C with a 1% solution of the probenecid fluorescent dye fluo-4 (Fluo-4 NW Calcium Assay Kit, Molecular Probes™, Invitrogen). For the free calcium assay, cells were treated with 10 μM *N*-arachidonoyl taurine or *N*-oleoyl taurine in assay buffer (3 ml of 1 M HEPES in 147 ml of 1× HEPES-balanced salt solution HBSS), or alternatively assay buffer alone (as a negative control). The intracellular calcium ($[Ca^{2+}]_i$) release was monitored using live-cell imaging. Throughout the experiment the cells were maintained in the microscope incubation chamber at 37 °C in 5% CO_2. Immediately following the addition of *N*-arachidonoyl taurine or *N*-oleoyl taurine, images were captured every third second for 5 min using a 60× oil immersion objective and an Axio Cam MRm, Zeiss using the Axiovision Cell Observer HS fluorescent microscope (Carl Zeiss). Images were further processed using Axiovision software (Carl Zeiss), ImageJ software (NIH) and Photoshop software (Adobe).

2.3. Analysis of insulin secretion from 832/13 INS-1 cells by immunohistochemistry

The 832/13 INS-1 cells were cultured for 2 days on 0.17 mm glass coverslips (Mentzel). The cell culture medium was replaced with low glucose (3 mM) containing RPMI-1640 medium and the cells grown overnight. The following day cells were rinsed with HBSS and further washed in HBSS for 2 h. After washing cells were treated for 2 h with (a) glucose 0 mM, 3 mM and 18 mM; (b) 10 μM *N*-arachidonoyl taurine or *N*-oleoyl taurine dissolved in DMSO; or (c) pre-treated with 100 μM of capsazepine or 20 μM Ruthenium Red (Sigma Aldrich, Sweden) for 1 h followed by treatment with 10 μM *N*-arachidonoyl taurine or *N*-oleoyl taurine for 2 h. The cells were fixed and processed for fluorescence microscopy using a rabbit anti-insulin antibody (1:350; Abbiotec, San Diego, CA 92126, USA) by overnight staining at 4 °C. Cells were incubated for 1 h with a secondary goat anti-rabbit Alexa Fluor 488 nm antibody (Invitrogen) and DNA was stained for 5 min with 4′,6-diamidino-2-phenylindole (Sigma Aldrich, Sweden). Coverslips were placed on a drop of anti-fade reagent ProLong Gold (Invitrogen, OR, USA). Images were captured with a 100× oil immersion objective using a Cool Cube 1 CCD camera (Metasystem, Althusheim, Germany) coupled to a Nikon Eclips E800 (Nikon, Tokyo, Japan) fluorescence microscope. 2D images were captured by the ISIS image analysis system (version 5.3, Metasystem).

2.4. Insulin secretion assay

Pancreatic β-cells 832/13 INS-1 were plated in 12-well plates for measurement of insulin release. When cells reached 80–90% confluence, the medium was changed and assays were carried out the next day. Cells were rinsed with HBSS, and further washed in HBSS for 2 h at 37 °C. After washing, cells were treated with 10 μM *N*-arachidonoyl taurine or *N*-oleoyl taurine for 1 h or cells were pre-treated for 1 h with 100 μM capsazepine or 20 μM Ruthenium Red, followed by 2 h incubation with 10 μM *N*-arachidonoyl taurine or *N*-oleoyl taurine as outlined above. As a control, cells were treated with 0 mM, 3 mM and 18 mM glucose diluted in HBSS (2 ml/well) and incubated for an additional 2 h. The supernatants were collected and clarified via centrifugation, and insulin levels were determined using a Rat Insulin Enzyme Immunoassay Kit (SPI-Bio, Bertin Pharma, France). Experiments were performed in duplicate and repeated three times.

2.5. Statistical analysis

One-way ANOVA was used to evaluate differences between treatments, and statistical significance was determined with post-hoc Bonferroni's Multiple Comparison Test ($p < 0.05$). Graphpad Prism 5.0 software was used for statistical analysis.

3. Results

3.1. N-Arachidonoyl taurine and N-oleoyl taurine induce a high frequency of Ca^{2+} oscillations in pancreatic β-cells

To investigate the effect of *N*-arachidonoyl taurine ($C_{20:4}$-taurine) and *N*-oleoyl taurine ($C_{18:1}$-taurine) in pancreatic β-cells, HIT-T15 and 832/13 INS-1 cells were treated with the fluorescent calcium sensitive dye fluo-4 as outlined in Section 2. Following incubation cells were exposed to assay buffer or treatment with *N*-acyl taurines and monitored for 5 min taking pictures every 3rd second. The assay buffer served as a negative control and HIT-T15 did not respond to treatment with assay buffer alone, with no flashes, sparks or pulsations evident and no significant increase in $[Ca^{2+}]_i$ was observed (Fig. 1 and Supplemental Fig. S1A). Treatment of HIT-T15 cells with 10 μM *N*-arachidonoyl taurine led to a high frequency of oscillations and the signal was transmitted quickly throughout the cells and pulsations occurred (Fig. 1 and Supplemental Fig. S1B). A similar response was detected following treatment with 10 μM *N*-oleoyl taurine, however the frequency of flashes was not as high compared to cells treated with *N*-arachido-

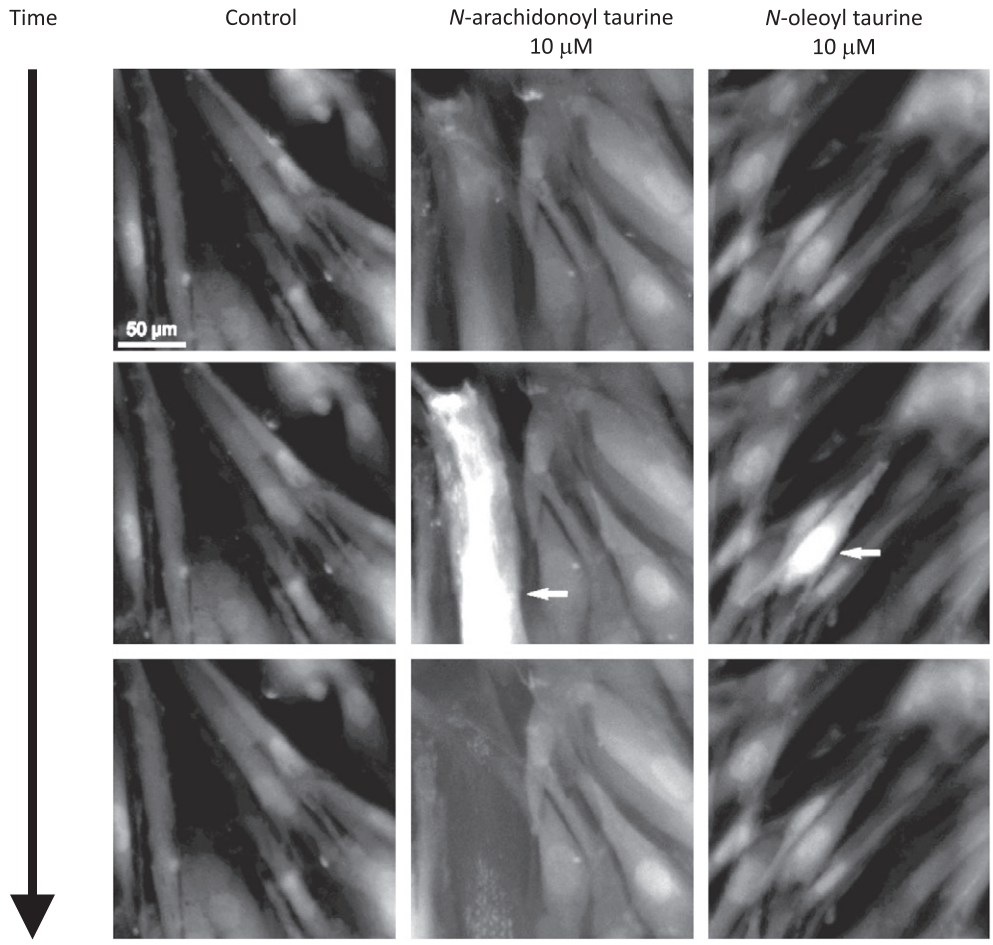

Fig. 1. *N*-Arachidonoyl taurine and *N*-oleoyl taurine induce a high frequency of Ca^{2+} oscillations in pancreatic β-cells. HIT-T15 cells were cultured overnight in glucose-free RPMI-1640 medium. Cells were treated with 10 μM *N*-arachidonoyl taurine or *N*-oleoyl taurine, followed by live-cell fluorescent microscopy during 5 min. Representative pictures from repeated experiments with images acquired every third second for 5 min are shown. Representative pictures for each treatment series cover a 36 s time span with 18 s delays between pictures. The arrows indicate signaling cells. Scale bar is 50 μm. The exposure time was 100 ms.

noyl taurine (Supplemental Fig. S1C). The data demonstrate that *N*-arachidonoyl taurine and *N*-oleoyl taurine increased the level of free calcium ions in the cytosol of the pancreatic β-cells HIT T15 (Supplemental Figs. S1B and S1C).

Similar experiments were carried out on 832/13 INS-1 cells and a flux of calcium ions was detected in 832/13 INS-1 cells (Supplemental Figs. S2A–S2C). 832/13 INS-1 cells were incubated in 3 mM glucose before the addition of *N*-acyl taurines and spontaneous very low frequent pulsations were observed continuously in all dishes containing 832/13 INS-1 cells. However treatment with *N*-acyl taurines extensively increased the number of cells showing free calcium ion flux in cytosol compared to control cells (Supplemental Figs. S2B and S2C).

3.2. Treatment of 832/13 INS-1 cells with N-arachidonoyl taurine and N-oleoyl taurine results in insulin secretion

Pancreatic β-cells 832/13 INS-1 have previously been shown to release insulin in response to intracellular levels of glucose higher than 3 mM [13]. We therefore treated 832/13 INS-1 cells with 0 mM, 3 mM and 18 mM glucose to confirm insulin release. When the cells were incubated with 0 mM glucose or 3 mM glucose there was no major decrease of cytoplasmic insulin levels as judged by fluorescence intensity (Fig. 2A and B). As expected, treatment of 832/13 INS-1 cells with 18 mM glucose led to exocytosis of a substantial amount of insulin as judged by reduced fluorescence (Fig. 2C).

Interestingly, when cells were treated with 10 μM *N*-arachidonoyl taurine, a significant amount of insulin was secreted as judged by reduced fluorescence (Fig. 2D) and the amount of insulin secreted was similar to that observed in 832/13 INS-1 cells incubated with 18 mM glucose (compare Fig. 2C and D). Similarly, treatment of 832/13 INS-1 cells with 10 μM *N*-oleoyl taurine resulted in an extensive amount of insulin secretion (Fig. 2E).

3.3. Pancreatic β-cells secrete insulin in response to treatment with N-acyl taurines

To quantify changes in insulin secretion induced by *N*-acyl taurines and to examine if insulin secretion is mediated through activation of TPR channels, especially TRPV1 channels, levels of insulin secreted were measured using an insulin secretion assay.

As a control, 832/13 INS-1 cells were treated with 3 mM and 18 mM glucose and treatment with 18 mM glucose resulted in a 50% increase in insulin secretion (Fig. 3 $p < 0.05$). 832/13 INS-1 cells were also treated 10 μM *N*-arachidonoyl taurine and *N*-oleoyl taurine, which resulted in a significant increase in the level of secreted insulin ($p < 0.05$) (Fig. 3), which was even higher (19% and 9% respectively) than insulin secretion following treatment with 18 mM glucose.

To examine the possible molecular mechanism of insulin release from 832/13 INS-1 cells by *N*-acyl taurines, cells were treated with two inhibitors of TRPV channels, as TRPV1 and TRPV4 have been suggested as receptors for *N*-arachidonoyl taurine [9]. Caps-

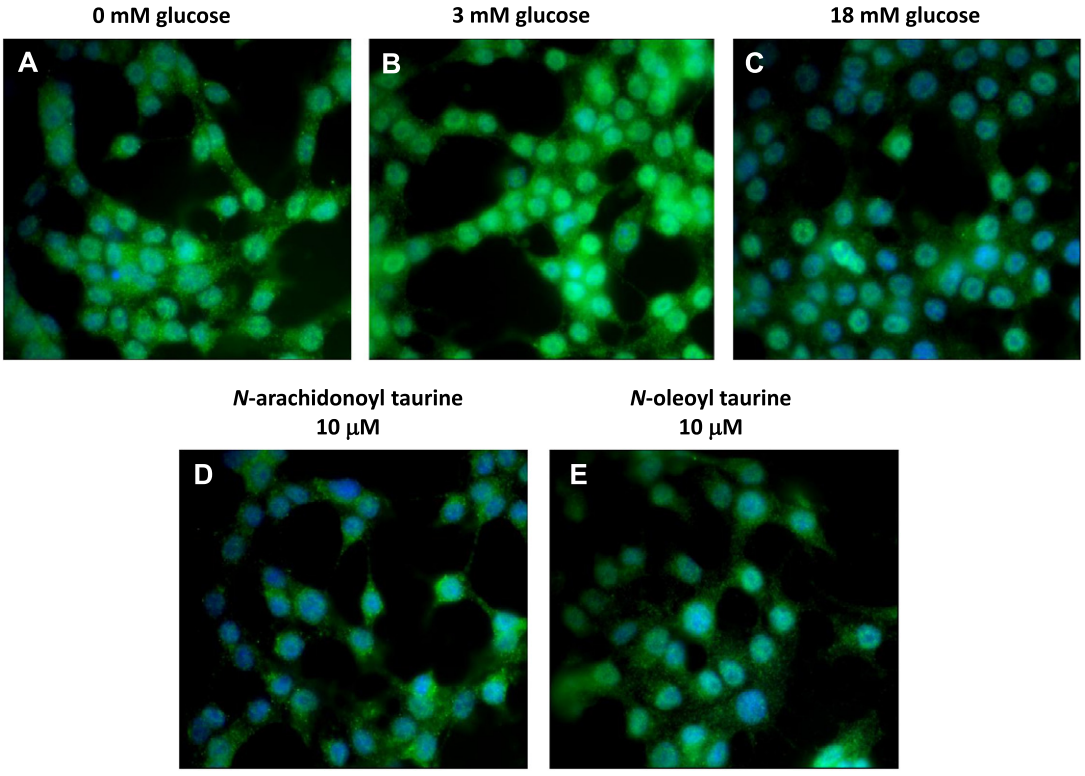

Fig. 2. Transient receptor potential vanilloid 1 (TRPV1) is involved in insulin secretion from 832/13 INS-1 cells in response to treatment with *N*-arachidonoyl taurine and *N*-oleoyl taurine. 832/13 INS-1 pancreatic β-cells were grown overnight in low glucose medium and treated with 0 mM, 3 mM or 18 mM glucose (A–C) or cells were treated with 10 μM *N*-arachidonoyl taurine (D) or 10 μM *N*-oleoyl taurine (E). Cells were fixed and stained for insulin using a rabbit anti-insulin antibody (green). Nuclei were counterstained with DAPI (blue) and cells were fixed. Images were captured at 100× magnification. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

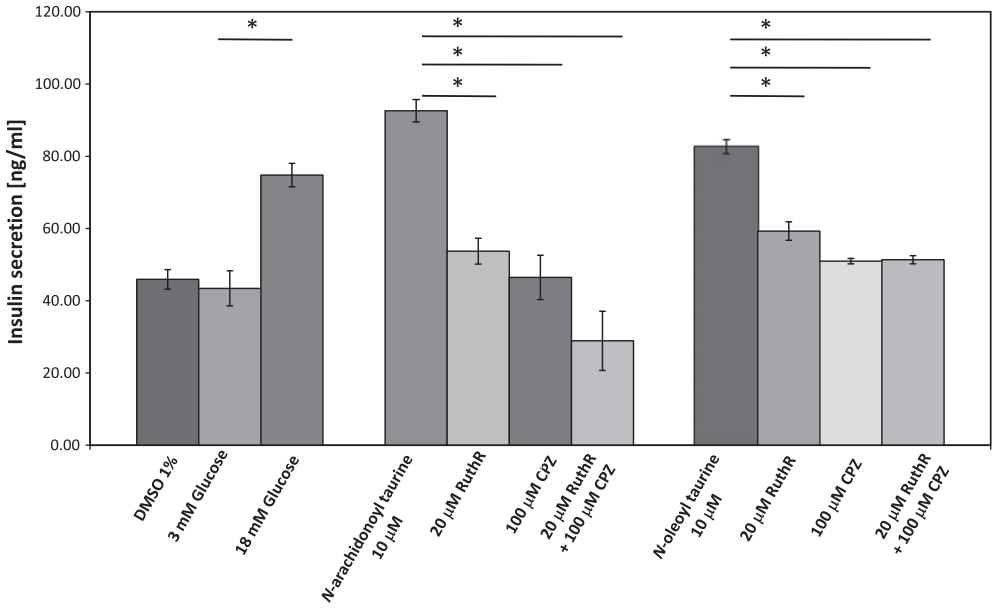

Fig. 3. Insulin secretion in 832/13 INS-1 cells is regulated by treatment with *N*-acyl taurines. 832/13 INS-1 cells were grown in media containing 0 mM glucose (1% DMSO), 3 mM glucose or 18 mM glucose. Alternatively 832/13 INS-1 cells were treated with 10 μM *N*-arachidonoyl taurine or 10 μM *N*-oleoyl taurine for 2 h. 832/13 INS-1 cells were pre-incubated with 100 μM capsazepine (CPZ) and/or 20 μM Ruthenium Red (RuthR) for 1 h and subsequently treated with 10 μM *N*-arachidonoyl taurine or 10 μM *N*-oleoyl taurine for 2 h as indicated. Levels of insulin secreted were detected by insulin enzyme immunoassay using an anti-insulin antibody.

azepine is a chemical compound that inhibits transient receptor potential vanilloid type 1 (TRPV1) [14,15], and 832/13 INS-1 cells were incubated in the presence of 100 μM capsazepine followed by treatment with 10 μM *N*-acyl taurine. However, cells incubated with the TRPV1 channel inhibitor capsazepine, followed by treatment with *N*-arachidonoyl taurine or *N*-oleoyl taurine significantly decreased insulin secretion. 832/13 INS-1 cells treated with capsazepine and *N*-arachidonoyl taurine resulted in a 50% reduction

in insulin secretion ($p < 0.05$) compared to treatment with *N*-arachidonoyl taurine alone, whereas incubation with capsazepine followed by treatment with *N*-oleoyl taurine resulted in a 38% decrease in insulin secretion ($p < 0.05$) compared with *N*-oleoyl taurine treatment alone (Fig. 3). To determine if *N*-acyl taurines promote insulin release through TRP channels other than TRPV1, Ruthenium Red (RuthR) an inhibitor of several TRP channels and therefore not specific only for TRPV1 [16,17] was used. Interestingly, incubation with the unspecific TRP channel inhibitor RuthR followed by treatment with *N*-arachidonoyl taurine or *N*-oleoyl taurine significantly reduced the levels of secreted insulin to 55% and 72% of *N*-arachidonoyl taurine or *N*-oleoyl taurine alone respectively ($p < 0.05$). Additionally, the combined treatment of 832/13 INS-1 cells with both capsazepine and RuthR followed by treatment with *N*-arachidonoyl taurine resulted in a synergistic effect in reducing insulin secretion to 30% of that secreted compared to treatment with *N*-arachidonoyl taurine alone ($p < 0.05$). Interestingly, the combined treatment with both inhibitors did not further affect insulin secretion in response to *N*-oleoyl taurine treatment compared to treatment with the two inhibitors alone. In addition, the presence of both inhibitors capsazepine or RuthR, whether separately or combined and followed by treatment with *N*-arachidonoyl taurine significantly reduced the level of insulin secreted to a level comparable with 3 mM glucose incubations, which is in the physiological range of glucose in 'resting' β-cells.

Collectively, these data indicate that TRP channels, especially TRPV1 mediates insulin secretion in 832/13 INS-1 cells in response to *N*-arachidonoyl taurine and *N*-oleoyl taurine. However our data also point to the involvement of receptors other than TRP channels being involved in triggering insulin secretion in 832/13 INS-1 cells in response to *N*-oleoyl taurine treatment.

4. Discussion

The cellular events underlying insulin secretion are well documented and many of the key steps have been identified at the molecular level. It is known that continuous changes in $[Ca^{2+}]_i$ that occur in the form of oscillations (pulsations of free calcium ions) are necessary for insulin release from β-cells in the pancreas. The frequency of oscillations in calcium signals are essential to regulate insulin secretion and so far at least three different types of Ca^{2+} oscillations have been described [18,19]. The increase in free calcium ions has been observed in the form of oscillations and it has been shown that pulsate oscillating signals of Ca^{2+} lead to significant increase of insulin release. Our results now show that *N*-arachidonoyl taurine and *N*-oleoyl taurine increase the level of free calcium ions in the cytosol of the pancreatic β-cells HIT-T15 and 832/13 INS-1 by inducing calcium oscillations, resulting in insulin secretion. We further investigated the mechanism for insulin secretion mediated by *N*-acyl taurines. Our data demonstrate that *N*-arachidonoyl taurine stimulates insulin release via activation of the transient receptor potential channel 1 (TRPV1). Previous data has shown that *N*-arachidonoyl taurine activates the TRPV1 and TRPV4 channels in CHO cells ($EC_{50} \sim 28$ μM (11–70 μM, 95% confidence limits) and 21 μM ($EC_{50} \sim 3$–130 μM, 95% confidence limits) respectively, whereas no activity was identified with the cannabinoid receptors CB1 and CB2 or nuclear receptors such as peroxisome proliferator-activated receptors [9]. Interestingly, *N*-oleoyl taurine may partially signal through TRPV1 but it is likely that other receptors are involved in calcium ion flux and insulin secretion. Recently it has been shown that *N*-oleoyl dopamine (OLDA), a cannabinoid, activates the endogenously expressed G-protein coupled receptor GPR119 and results in enhanced insulin secretion in HIT-T15 cells and GPR119-transfected RIN-5F cells [20]. Whether GPR119 is activated by *N*-oleoyl taurine remains unknown. Our data point to the involvement of receptors other than TRP channels in free calcium ion mobilization stimulated by *N*-oleoyl taurine in pancreatic β-cells.

Following our identification of *N*-acyl taurines as novel insulin secretagogues in this study, we suggest a hypothetical model to explain the molecular mechanism of the insulin secretion mediated by *N*-arachidonoyl taurine and *N*-oleoyl taurine. Transient receptor potential vanilloid type 1 (TRPV1) is a ligand-gated non-selective cation channel whose activation by *N*-acyl taurines, especially *N*-arachidonoyl taurine, leads to Ca^{2+} influx, resulting in membrane depolarization [21] which will result in the closing of K^+ ATP-dependent channels. The depolarization also leads to activation of voltage-gated Ca^{2+} channels. These signaling cascades will result in a rise in intracellular calcium. The increased uptake of calcium will activate intracellular calcium by Ca^{2+}-induced Ca^{2+} release (CICR) processes in the endoplasmic reticulum (ER) in many impulsive cells, such as muscle cells, nerve cells, and β-cells [22,23]. Following short pulses of calcium influx into the cytoplasm from the external cell environment as well as from intracellular stores, the pancreatic β-cells will start secreting insulin. The presence of *N*-arachidonoyl taurine and *N*-oleoyl taurine will result in the activation of TRPV1, TRP channels or other G-protein coupled receptors and may trigger the above cascade reactions through release of free calcium ions to the cytosol, which will finally result in insulin exocytosis.

The physiological functions of *N*-acyl taurines have not been well studied to date. *N*-Acyl taurines have recently been identified as being anti-proliferative in prostate cancer PC-3 cells [24]. Two enzymatic pathways for the production of *N*-acyl taurines have been identified: (a) the acyl-CoA:amino acid *N*-acyltransferase (ACNAT1) enzyme identified in mouse peroxisomes that can conjugate saturated fatty acids to taurine [25] and (b) the bile acid-CoA:amino acid *N*-acyltransferase (BAAT) enzyme localized in liver that can conjugate long- and very long-chain saturated and unsaturated fatty acids to taurine ([26] and O'Byrne et al., manuscript in preparation). A comprehensive study of the levels of *N*-acyl taurines in mouse tissues was recently carried out, showing that *N*-acyl taurines (chain lengths $C_{16:0}$, $C_{18:0}$, $C_{18:2}$, $C_{20:4}$, $C_{22:0}$ and $C_{22:6}$) are present in plasma, brown adipose tissue, heart, white adipose tissue, spleen, lung, testis, kidney and liver [27]. The latter study also suggested the existence of a transport system for *N*-acyl taurines that transfers these lipids from liver to other tissues, e.g. spleen [27]. Therefore *N*-acyl taurines produced at one particular site could potentially be transported by the plasma to signal insulin secretion from pancreatic β-cells. Levels of *N*-arachidonoyl taurine in vivo can also be regulated by cyclooxygenases (COXs) and lipoxygenases (LOXs), as the latter two enzymes can to oxygenate *N*-arachidonoyl taurine form taurine-conjugated signaling molecules [28].

In summary, our results suggest a potential therapeutic function of taurine conjugated signaling lipids, the *N*-acyl taurines, in regulating of insulin secretion from pancreatic β-cells. *N*-Acyl taurines may therefore be of great interest to pharmacologists because of their potential stimulative role in insulin secretion.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.11.026.

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