Arachidonic acid, palmitic acid and glucose are important for the modulation of clonal pancreatic β-cell insulin secretion, growth and functional integrity

Gordon DIXON*, John NOLAN†, Neville H. McCLENAGHAN‡, Peter R. FLATT‡ and Philip NEWSHOLME*

*Department of Biochemistry, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland, †Department of Endocrinology, St. James’s Hospital, Dublin 8, Ireland, and ‡School of Biomedical Sciences, University of Ulster, Coleraine, N. Ireland, U.K.

ABSTRACT

Insulin-resistant states such as obesity can result in an increase in the function and mass of pancreatic β-cells, so that insulin secretion is up-regulated and Type II diabetes does not develop. However, expansion of β-cell mass is not indefinite and may well decrease with time. Changes in circulating concentrations of nutritional factors, such as fatty acids and/or glucose, may lead to a reduction in β-cell mass in vivo. Few previous studies have attempted to explore the interplay between glucose, amino acids and fatty acids with respect to β-cell mass and functional integrity. In the present study, we demonstrate that culture of clonal BRIN-BD11 cells for 24 h with the polyunsaturated fatty acid arachidonic acid (AA) increased β-cell proliferation and enhanced alanine-stimulated insulin secretion. These effects of AA were associated with significant decreases in the cellular consumption of D-glucose and L-alanine as well as decreased rates of production of nitric oxide and ammonia. Conversely 24 h exposure to the saturated fatty acid palmitic acid (PA) was found to decrease β-cell viability (by increasing apoptosis), increase the intracellular concentration of triacylglycerol (triglyceride), while inhibiting alanine-stimulated insulin secretion. These effects of PA were associated with significant increases in D-glucose and L-glutamine consumption as well as nitric oxide and ammonia production. However, L-alanine consumption was decreased in the presence of PA. The effects of AA, but not PA, were additionally dependent on glucose concentration. These studies indicate that AA may have a critical role in maintaining the appropriate mass and function of islet β-cells by influencing rates of cell proliferation and insulin secretion. This regulatory effect may be compromised by high circulating levels of glucose and/or PA, both of which are elevated in Type II diabetes and may impact upon dysfunctional and apoptotic intracellular events in the β-cell.

INTRODUCTION

Pancreatic β-cell hypertrophy and hyperplasia play a key role in compensation for the peripheral insulin resistance that is associated with obesity [1–3]. However, it is postulated that, in individuals genetically predisposed to Type II diabetes, a gradual decline in the ability of the β-cell to adapt to increasing functional demand

Key words: l-alanine, apoptosis, β-cells, fatty acid, d-glucose.

Abbreviations: AA, arachidonic acid; FCS, fetal calf serum; LC-CoA, long-chain acyl-CoA; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium; NEFA, non-esterified fatty acid; NO, nitric oxide; PA, palmitic acid.

Correspondence: Professor Philip Newsholme (e-mail philip.newsholme@ucd.ie).
leads to β-cell dysfunction and depletion of β-cell mass. Although the mechanisms involved in loss of adaptability are unclear, β-cell glucotoxicity, due to chronic hyperglycaemia, and β-cell lipotoxicity, due to elevated circulating non-esterified fatty acid (NEFA) levels, are considered to be important confounding factors [4–9].

Although substantially raised NEFA concentrations induce β-cell lipotoxicity, at lower concentrations NEFAs are essential for glucose-stimulated insulin secretion [10,11] and can modulate the insulinotropic activity of other classes of nutrients [12–14]. The underlying mechanisms by which NEFAs affect insulin secretion are largely unknown, but may involve the accumulation of cytoplasmic long-chain acyl-CoA (LC-CoA) esters. An elevation in LC-CoA esters may increase protein kinase C and/or phospholipase C activity, leading to increased diacylglycerol, opening of Ca$$^{2+}$$ channels and insulin secretion [13,15]. Alternatively, LC-CoA esters may stimulate insulin secretion by modulating the acylation of several proteins involved in regulating the activity of ion channels [16,17]. NEFAs may also modulate β-cell ion channel activity and thus insulin secretion by oxygenation of their metabolites via pathways such as the cyclo-oxygenase and lipo-oxygenase pathways [18].

In addition to the above short-term effects of fatty acids, longer-term exposure in vitro (18, 24 or 48 h) to high concentrations of NEFAs can inhibit glucose-induced insulin secretion and provoke apoptosis. NEFA-induced inhibition of insulin secretion may be mediated by down-regulation of glucose transport and oxidation [6], by inhibiting insulin biosynthesis [7,19] or by increasing cytoplasmic triacylglycerol (triglyceride) [20,21]. The deposition of high levels of triacylglycerol in islets is also believed to initiate apoptotic β-cell death by a ceramide/cyclooxygenase and lipo-oxygenase pathways [18].

Apart from a reduction in glucose metabolism, via a proposed Randle cycle [6,7], the effects of NEFAs on the consumption of other important β-cell nutrients have largely been ignored. In the present study, we have investigated the effects of palmitic acid (PA; a saturated fatty acid) on the consumption of other important β-cell nutrients, namely l-glutamine and l-alanine. Previous studies of NEFA-induced modulation of β-cell apoptosis or insulin secretion have used excessively high (0.5–2.0 mM) concentrations of NEFAs [6,8,13,14,24]. In the present study, we have used the much lower concentration of 100 μM NEFA in an attempt to investigate specific metabolic effects associated with β-cell function. We provide evidence that NEFA-induced changes in nutrient consumption as well as the prevailing extracellular glucose concentration may be determining factors in deciding the secretory response and functional integrity of the β-cell, via alterations in metabolism, NO formation, ammonia production and the accumulation of cytoplasmic triacylglycerol.

**MATERIALS AND METHODS**

**Reagents**

Culture media, antibiotics, fetal calf serum (FCS) and tissue-culture plastic were obtained from Gibco (Paisley, Scotland, U.K.). The CellTitre 96 non-radioactive cell proliferation assay {3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium [MTS] assay} was obtained from Promega (Wisconsin, IL, U.S.A.). The cell death detection ELISA kit and l-asparaginase were obtained from Roche Molecular Biochemicals (Mannheim, Germany). The rat insulin ELISA kit was obtained from Mercodia (Uppsala, Sweden). The triacylglycerol assay kit was obtained from Human (Wiesbaden, Germany). All other materials were purchased from Sigma–Aldrich (Poole, Dorset, U.K.).

**Culture of BRIN-BD11 cells**

Clonal insulin-secreting BRIN-BD11 cells were maintained in RPMI-1640 tissue culture medium with 10% (v/v) FCS, 0.1 % antibiotics (100 units/ml penicillin and 0.1 mg/ml streptomycin) and either 11.1 or 25 mM d-glucose, as indicated. The cells were maintained at 37°C in a humidified atmosphere of 5% CO2 and 95% air at pH 7.4, using a Forma Scientific incubator. The cells were cultured in 50–70 ml of tissue culture medium in T175 sterile tissue culture flasks. Cells were subsequently seeded into 96-well plates (4 × 10^5 cells/well) for assessment of cell viability and DNA fragmentation, into 24-well plates (1 × 10^5 cells/well) for insulin secretion experiments or into T25 sterile tissue culture flasks (4 × 10^6 cells/flask) for other experiments. Fatty acids were added to the cell culture medium to give a final concentration of 100 μM. Fatty acids were initially dissolved in ethanol, but the final concentration of ethanol in cell incubations was less than 0.05%. The final BSA concentration derived from FCS was 0.3%. The β-cells were then cultured for 24 h as above.

**Cell viability and DNA fragmentation**

Cell viability was determined by the MTS colorimetric assay, which is based on the ability of viable cells, but not dead cells, to reduce MTS into a formazan product, which is soluble in tissue culture medium [25]. The absorbance of the formazan at 490 nm can be measured directly from 96-well assay plates without additional processing. BRIN-BD11 cells cultured for 24 h were used to assess DNA fragmentation. The 96-well plate was centrifuged for 10 min at 200 g and cell pellets were resuspended in lysis buffer (Roche Molecular Biochemicals). Following
centrifugation to remove cell debris, the supernatant was transferred to a prepared microtitre plate for determination of DNA fragments using a cell death detection ELISA kit (Roche Molecular Biochemicals) according to the manufacturer’s instructions.

**Insulin secretion**

After 24 h of culture, the cells were washed twice with 1 ml of Krebs–Ringer bicarbonate buffer [115 mM NaCl, 4.7 mM KCl, 1.28 mM CaCl2, 1.2 mM KH2PO4, 1.2 mM MgSO4·7H2O, 10 mM NaHCO3 and 5 g/l BSA (pH 7.4)] containing 1.1 mM d-glucose. After a 40-min preincubation at 37 °C, buffer was removed and the cell monolayers were incubated in the same buffer containing 1.1 mM d-glucose in the absence or presence of 10 mM L-alanine. After incubation for 20 min at 37 °C, a portion (900 µl) of buffer was removed from each well and stored at −20 °C for measurement of insulin by Mercodia Ultrasensitive rat insulin ELISA kit, according to the manufacturer’s instructions.

**Cellular triacylglycerol content**

After 24 h of culture, cells were resuspended in 2 mM NaCl, 20 mM EDTA and 50 mM sodium phosphate (pH 7.4) and sonicated for 1–2 min. A portion (10 µl) of the homogenate was mixed with 10 µl of t-butyl alcohol and 5 µl of Triton X-100/methyl alcohol mixture (1:1, v/v) for the extraction of lipids. Triacylglycerol was measured by a commercial kit (Human, Wiesbaden, Germany) and expressed as µg/mg of protein.

**D-Glucose consumption**

The glucose concentration at the beginning and 24 h after culture was determined using a commercially available kit (Sigma–Aldrich). The glucose utilized over the 24 h period was calculated by subtracting the concentration at 24 h from that at 0 h. Data are expressed as µmol·mg of protein⁻¹·h⁻¹.

**L-Alanine consumption**

The alanine concentration in Krebs–Ringer bicarbonate buffer was determined before and after a 60-min incubation with BRIN-BD11 cells based on the oxidation of L-alanine to pyruvic acid and ammonia in the presence of NAD⁺ and alanine dehydrogenase. The increase in absorbance at 339 nm due to the formation of NADH was used to quantify the amount of L-alanine consumed, which was expressed as µmol·mg of protein⁻¹·h⁻¹.

**L-Glutamine consumption**

The glutamine concentration was determined at the beginning and 24 h after culture by its hydrolysis to glutamic acid and ammonium ions (NH₄⁺) in a reaction catalysed by asparaginase. NH₄⁺ generated combined with 2-oxoglutarate in the presence of NADH to form glutamic acid, NAD⁺ and water. The concentration of glutamine in the sample was quantified indirectly by measuring the decrease in absorbance at 340 nm due to the conversion of NADH into NAD⁺. The glutamine consumed over 24 h was converted into absolute amounts and expressed as nmol·mg of protein⁻¹·h⁻¹.

**Ammonia production**

Ammonia produced by BRIN-BD11 cells over 24 h of culture was determined by the reductive amination of 2-oxoglutarate, in the presence of ammonia (in sample), glutamate dehydrogenase and NADPH, to produce glutamic acid and NADP⁺. The concentration of ammonia in the sample was quantified, as it is proportional to a decrease in absorbance at 340 nm due to the oxidation of NADPH. Data are expressed as nmol·mg of protein⁻¹·h⁻¹.

**Nitrite production**

Deproteinized samples (150 µl) at the beginning and 24 h after culture were mixed with 75 µl of ice-cold 4,4′-diamino-di-phenylsulphone (14 mM in 2 M HCl), following the addition of 75 µl of N-(1-naphthyl)ethylenediamine (4 mM in H₂O), and the mixture was incubated at room temperature for 5 min before measurement of absorbance at 550 nm. Nitrite levels were calculated from NO₂⁻ standard curves (0.2–100 µM) generated in medium containing 10% (v/v) FCS. Data are expressed as nmol·mg of protein⁻¹·24 h⁻¹.

**Cellular protein and protein determination**

Cells were extracted by addition of lysis buffer [20 mM EDTA, 10 mM Tris/HCl (pH 8.0) and 0.5% Triton X-100]. The protein content was measured by the Bradford assay using BSA as a standard [26].

**Statistical analysis**

Results were expressed as means ± S.D. Analysis was performed by Student’s t test, and P < 0.05 was considered to be statistically significant.

**RESULTS**

**Effects of fatty acids and glucose on cell growth and apoptosis**

The initial experiments were aimed at determining the effects of AA and PA on the viability and growth of the BRIN-BD11 cells using the MTS assay. The MTS assay is a rapid and convenient method of determining viable cell number in proliferation [27]. The normal growth of BRIN-BD11 cells in RPMI-1640 medium supplemented with 10% (v/v) FCS resulted in an approximate doubling of cell number after 24 h (from
100 ± 3 % at 0 h to 186 ± 10 % at 24 h; Figure 1). Increasing the glucose concentration to 25 mM had no significant effect on the 24-h growth rate. Addition of 100 µM PA in the presence of 11.1 mM glucose significantly inhibited the 24-h growth rate by approx. 75 % (P < 0.001; Figure 1). Increasing the glucose concentration from 11.1 to 25 mM slightly attenuated this inhibitory effect of PA. Conversely, 100 µM AA significantly stimulated (P < 0.001) β-cell growth by 1.9-fold at 11.1 mM glucose, but this effect was completely lost at 25 mM glucose (Figure 1).

β-Cell apoptotic DNA fragmentation was not influenced by increasing the glucose concentration in culture from 11.1 to 25 mM (Figure 2). Addition of PA (100 µM) significantly increased (P < 0.01) apoptotic DNA fragmentation approx. 2-fold after 24 h of culture at 11.1 mM glucose (Figure 2). Increasing the glucose concentration to 25 mM partially attenuated the increase in apoptosis (Figure 2). However, AA (100 µM) had no effect on the level of apoptosis as determined by DNA fragmentation at either glucose concentration (Figure 2).

**Effects of fatty acids and glucose on insulin secretion**

In a previous study [28], it was demonstrated that maximal stimulation of insulin secretion from BRIN-BD11 cells was achieved by addition of 10 mM L-alanine. Basal insulin secretion (1.1 mM D-glucose) following culture at 11.1 mM D-glucose for 24 h was 9.6 ± 1.4 ng·mg of protein⁻¹·20 min⁻¹ (Figure 3A). Addition of L-alanine (10 mM) stimulated insulin secretion by 4.2-fold (Figure 3A). Culture with PA (100 µM) in the presence of 11.1 mM glucose for 24 h significantly increased basal insulin secretion by 1.8-fold, but significantly inhibited alanine-stimulated insulin secretion by 36 % (Figure 3A).

Increasing the glucose concentration in culture to 25 mM in the absence of fatty acid significantly increased (P < 0.05) basal insulin secretion by 1.5-fold, but had no effect on L-alanine-stimulated insulin secretion (Figure 3B). Addition of 100 µM PA at this elevated glucose concentration also increased basal secretion and decreased alanine-stimulated insulin secretion compared with 11.1 mM glucose in the absence of fatty acid. However, the effect of PA at 25 mM glucose on basal and alanine-stimulated insulin secretion was not significantly different from that at 11.1 mM glucose (Figure 3).

Culture with AA (100 µM) at 11.1 mM D-glucose augmented basal insulin secretion by 1.8-fold and enhanced alanine-stimulated insulin secretion by 1.3-fold compared with culture with 11.1 mM D-glucose alone (Figure 3A). In the presence of 25 mM glucose, AA significantly increased (P < 0.05) basal insulin secretion by 1.5-fold, but inhibited alanine-stimulated insulin secretion by 26 % (Figure 3B). Accordingly, culture with AA and 25 mM glucose did not affect basal insulin secretion, but significantly reduced the alanine response by 42 % compared with cells cultured with AA at 11.1 mM glucose (Figure 3).

**Effects of fatty acids and glucose on intracellular triacylglycerol stores**

Culture in 11.1 mM glucose in the absence of fatty acid produced measurable quantities of intracellular triglyceride after 24 h (8.0 ± 2.2 µg·mg of protein⁻¹·24 h⁻¹), which was slightly, but not significantly,
Effects of a 24 h exposure to fatty acids and glucose on cellular D-glucose and L-alanine consumption

Metabolism of glucose and amino acids are closely linked to the regulation of insulin secretion, and NEFAs are known to regulate glucose consumption [24,29,30]. The consumption of glucose over culture for 24 h at 11.1 mM glucose was found to be 8.2 ± 0.9 μmol·mg of protein⁻¹ · h⁻¹ (control). Increasing the glucose culture concentration to 25 mM had no significant effect on the consumption of glucose (Table 1).

At 11.1 mM glucose, PA increased glucose consumption by almost 2-fold (P < 0.001), whereas a 24 % decrease (P < 0.01) was observed in the presence of AA and 11.1 mM glucose (Table 1). The effects of PA at 25 mM glucose were similar, but glucose consumption was significantly increased by 1.6-fold (P < 0.01) in cells cultured in AA at 25 mM glucose (Table 1).

The consumption of L-alanine over 60 min (from an initial incubation concentration of 10 mmol/l) at 11.1 mM glucose was 3.2 ± 0.2 μmol·mg of protein⁻¹ · h⁻¹ (control; Table 1). Increasing the glucose concentration to 25 mM had no effect on the L-alanine consumption.

AA and PA inhibited the consumption of L-alanine by 25 % and 52 % respectively, at 11.1 mM glucose.
Table 2  Long-term effects of AA and PA on the consumption of l-glutamine and the production of ammonia

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Glucose concentration</th>
<th>l-Glutamine consumption</th>
<th>Ammonia production</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>11.1 mM</td>
<td>228.3 ± 24.7</td>
<td>376.9 ± 32.4</td>
</tr>
<tr>
<td>AA (100 µM)</td>
<td>11.1 mM</td>
<td>218.6 ± 32.9</td>
<td>247.1 ± 40.1</td>
</tr>
<tr>
<td>PA (100 µM)</td>
<td>11.1 mM</td>
<td>437.8 ± 45.8*</td>
<td>521.6 ± 69.1*</td>
</tr>
<tr>
<td>None</td>
<td>25 mM</td>
<td>251.3 ± 28.3</td>
<td>412.1 ± 30.6</td>
</tr>
<tr>
<td>AA (100 µM)</td>
<td>25 mM</td>
<td>278.2 ± 43.2</td>
<td>400.0 ± 33.8§</td>
</tr>
<tr>
<td>PA (100 µM)</td>
<td>25 mM</td>
<td>406.9 ± 38.8‡</td>
<td>502.4 ± 38.5‡</td>
</tr>
</tbody>
</table>

Increasing the glucose culture concentration to 25 mM did not significantly attenuate the effects of the fatty acids on l-alanine consumption (Table 1).

Effects of 24 h exposure to fatty acids and glucose on cellular l-glutamine consumption and ammonia production

l-glutamine is important in the β-cell for the synthesis of proteins, glutathione and γ-aminobutyric acid (GABA) and as an oxidative substrate [31]. However, ammonia, a product of glutamine metabolism, is known to cause reversible inhibition of glucose-stimulated insulin secretion [32].

The consumption of l-glutamine by BRIN-BD11 cells in medium containing 2 mM l-glutamine and 11.1 mM D-glucose was 228.3 ± 24.7 nmol · mg of protein⁻¹ · h⁻¹ (Table 2). Increasing the culture glucose concentration to 25 mM had no significant effect on the consumption of l-glutamine.

The consumption of l-glutamine was similarly unaffected by AA at 11.1 mM glucose, but PA induced a significant 1.9-fold increase (P < 0.001). AA significantly inhibited ammonia production by 34 % (P < 0.01) in the presence of 11.1 mM glucose, whereas PA increased the production of ammonia by 1.4-fold (P < 0.01) compared with 11.1 mM glucose alone (Table 2).

Increasing the culture glucose concentration to 25 mM had no effect on l-glutamine consumption in the presence of AA. However, the decrease in ammonia production induced by AA in the presence of 11.1 mM glucose was completely attenuated by 25 mM glucose (Table 2). There was no effect of PA on l-glutamine consumption or ammonia production in the presence of 25 mM glucose compared with the effects of PA at 11.1 mM glucose.

Effects of fatty acids and glucose on NO generation

Cytokine-induced generation of NO is believed to play a role in the inhibition of insulin secretion and the destruction of the β-cell by inducing DNA damage [33]. To determine whether the generation of NO participated in the effects observed with AA and PA, the concentration of nitrite in the media of cells incubated in the absence and presence of fatty acid was determined. Nitrite is the stable end product of NO oxidation and thus its accumulation in cell media can be taken as an indicator of NO generation.

The production of nitrite from β-cells cultured at 11.1 mM glucose (35.4 ± 3.0 nmol · mg of protein⁻¹ · 24 h⁻¹) or 25 mM glucose (33.9 ± 3.1 nmol · mg of protein⁻¹ · 24 h⁻¹) was comparable (Figure 5). Addition of PA at 11.1 or 25 mM glucose significantly increased (P < 0.01) the rate of nitrite production by approx. 1.4-fold (Figure 5). In contrast, AA significantly decreased (P < 0.01) the production of nitrite at 11.1 mM glucose by 35 % (Figure 5); however, AA did not affect the production of nitrite at 25 mM glucose.

DISCUSSION

In the present study, we have shown that 24 h exposure to a relatively low concentration of the polyunsaturated fatty acid AA stimulated β-cell growth and insulin secretion in BRIN-BD11 cells. In contrast, a similar period of exposure to the saturated fatty acid PA inhibited β-cell growth, impaired insulin secretion and induced cell death by apoptosis. Disturbances in glucose metabolism have been implicated in PA-mediated β-cell dysfunction and death via the so-called Randle cycle effect, where increased fatty acid oxidation caused a reciprocal decrease in glucose oxidation [7,34]. Since glucose oxidation has been critically linked to the insulin-secreting potency...
of the hexose, impairment of glucose-stimulated insulin secretion would result. Indeed, PA-induced reductions in glucose oxidation and utilization have been reported in association with decreases in ATP, insulin content, insulin secretion and the up-regulation of apoptotic genes [6,19,24]. However, the involvement of the classic Randle cycle in the demise of β-cells is questioned in the present study. Thus increases, rather than decreases, in BRIN-BD11 cell glucose consumption were associated with apoptosis and impairment of insulin secretion following long-term exposure to PA. This enhancement of glucose metabolism may be mediated by an increased activity of hexokinase induced by elevated levels of LC-CoA esters, which augment glucose flux and oxidation [35]. Conversely, the stimulatory effect of AA on cell growth in the present study was accompanied by decreases in glucose consumption. The mechanism linking the suppression of glucose consumption to β-cell growth by AA is unclear, but may involve the inhibition of several genes encoding proteins involved in glucose metabolism and fatty acid biosynthesis, as polyunsaturated fatty acids are known to inhibit the expression of glucokinase, acetyl-CoA carboxylase and stearoyl-CoA desaturase in the liver [36]. The finding that culture of clonal β-cells in 25 mM glucose inhibited the effects of AA on β-cell growth and glucose consumption observed at 11.1 mM glucose may support this hypothesis.

Not only does exogenous AA enhance insulin secretion from pancreatic β-cells (Figure 3 and [37]), but exogenous AA may be an important mediator of glucose-induced insulin secretion, since stimulatory glucose concentrations induce phospholipid hydrolysis leading to increases in non-esterified AA in β-cells [38]. The use of l-alanine as an insulin secretagogue in the present study was based on previous observations [28] that this amino acid is a potent insulin secretagogue for the BRIN-BD11 cell line. In the present study, both basal and l-alanine-stimulated insulin secretion were significantly increased following 24 h of culture with AA. This potentiation in insulin secretion is likely to be mediated by the transport and accumulation of intracellular non-esterified AA. Elevated levels of non-esterified AA may then amplify the glucose-induced Ca2+ signal by promoting Ca2+ influx into the β-cells via the opening of voltage-dependent Ca2+ channels [39,40]. This may, in turn, potentiate insulin secretion by increasing Ca2+-dependent processes such as protein kinase C activation and exocytosis. The beneficial effects of AA on insulin secretion were probably not related to an AA-dependent increase in insulin content of the β-cells, as in vivo rat studies have demonstrated that dietary supplementation with oils rich in n−3, n−6 or n−9 fatty acids did not alter pancreatic islet insulin content [41].

The inhibition of l-alanine-stimulated insulin secretion by PA may be mediated by increases in the level of cytoplasmic triacylglycerol. Previous reports [20,22,42] have demonstrated a positive correlation between the level of intracellular triacylglycerol in β-cells and the inhibition of insulin secretion as well as stimulation of β-cell apoptosis. In the present study, cellular triacylglycerol was significantly elevated following culture with PA in the presence of 11.1 or 25 mM glucose or with AA at 25 mM glucose. These increases in triacylglycerol correlated well with inhibition of l-alanine-stimulated insulin secretion, thereby supporting the hypothesis of Lee et al. [42].

Although the mechanism underlying the insulinotropic effect of l-alanine in islets remains controversial [29,43], the high consumption rate by β-cells suggests that metabolism of this amino acid is important for β-cell function (Table 1). AA significantly decreased the consumption of l-alanine and glucose by BRIN-BD11 cells incubated in 11.1 mM glucose. This may suggest a role for AA in down-regulating nutrient transport and metabolism. However, addition of PA resulted in inhibition of l-alanine-stimulated insulin secretion and an inhibition of l-alanine consumption, but an increase in the rate of glucose consumption. These changes may reflect induction of pathways leading to cellular dysfunction and apoptosis, which may be associated with enhanced glucose consumption as the cell attempts to defend itself. Consistent with this view, glucose consumption has been inversely correlated with apoptosis in human neutrophils [44]. Increasing the extracellular glucose concentration to 25 mM enhanced glucose consumption in the presence of either PA or AA, perhaps due to enhanced lipid and carbohydrate metabolism in the presence of the different fatty acids. Metabolism may be stimulated for different reasons; for example, promotion of insulin secretion and cell growth was stimulated by AA, but defense mechanisms against apoptosis were promoted in the presence of PA. l-Glutamine utilization was unaffected by the addition of AA, but it was enhanced almost 2-fold by PA. l-Glutamine has been shown to protect rat neutrophils from spontaneous apoptosis in vitro [45]. The novel finding in the present paper of enhanced rates of D-glucose and l-glutamine utilization in the presence of PA may be associated with generation of metabolites involved in cellular defence, such as glutathione, ATP and NADPH. These factors may therefore have a completely different function from their traditional involvement in mechanisms related to the stimulation of insulin secretion and cell growth.

In the present study, the increased consumption of glucose induced by PA at 11.1 mM glucose was accompanied by enhanced l-glutamine consumption plus an elevation in NH4+ production. NH4+ production may be associated with product inhibition of glutamic acid dehydrogenase. Alternatively, increased production of NH4+ may be responsible for the observed inhibition of alanine-stimulated insulin secretion, since NH4+ has been reported [32] to inhibit glucose-stimulated insulin
secretion by reducing the concentration of pyridine nucleotides. Conversely, \( \text{NH}_4^+ \) production was decreased and \( \text{l}-\text{alanine}-\text{stimulated insulin secretion was enhanced} \) in BRIN-BD11 cells exposed to AA in the presence of 11.1 mM glucose. Thus AA, by decreasing glucose consumption, may also attenuate the regulatory role of glucose on glutaminolysis, thereby increasing insulin secretion through activation of glutamic acid dehydrogenase.

Finally, \( \beta \)-cell apoptosis and inhibition of alanine-stimulated insulin secretion by long-term exposure to PA was associated with significant increases in nitrite production. Conversely, decreases in nitrite production were correlated with potentiation of \( \beta \)-cell growth and insulin secretion with exposure to AA. However, increasing the glucose culture concentration from 11.1 to 25 mM inhibited alanine-stimulated insulin secretion and the effects of AA on NO production and \( \beta \)-cell growth. This may suggest that the stimulatory effect of exogenous AA observed at 11.1 mM glucose on \( \beta \)-cell growth and \( \text{l}-\text{alanine}-\text{induced insulin secretion may be mediated by a mechanism involving a reduction in NO production.} \) This may be achieved by stimulation of urea synthesis (arginase and NO synthase will compete for available l-arginine) in the presence of elevated extracellular glucose concentrations as reported previously [46].

In conclusion, the findings of the present study suggest that adequate levels of extracellular AA may play a key role in the compensatory increases in \( \beta \)-cell mass and insulin secretion associated with Type II diabetes. Furthermore, sufficient levels of AA may protect against PA-mediated \( \beta \)-cell apoptosis and insulin secretory demise, possibly by regulating glucose, glutamine and alanine metabolism as well as the production of \( \text{NH}_4^+ \) and NO by the \( \beta \)-cell. Alternatively, high concentrations of PA and/or glucose, both of which are elevated in Type II diabetes, may compromise the beneficial effects of AA on \( \beta \)-cell function by stimulating dysfunctional and apoptotic mechanisms as described previously [6,7,19,24].

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