Detrimental actions of metabolic syndrome risk factor, homocysteine, on pancreatic β-cell glucose metabolism and insulin secretion

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Abstract

Elevated plasma homocysteine has been reported in individuals with diseases of the metabolic syndrome including vascular disease and insulin resistance. As homocysteine exerts detrimental effects on endothelial and neuronal cells, this study investigated effects of acute homocysteine exposure on β-cell function and insulin secretion using clonal BRIN-BD11 β-cells. Acute insulin release studies in the presence of various test reagents were performed using monolayers of BRIN-BD11 cells and samples assayed by insulin radioimmunoassay. Cellular glucose metabolism was assessed by nuclear magnetic resonance (NMR) analysis following 60-min exposure of BRIN-BD11 cell monolayers to glucose in either the absence or presence of homocysteine. Homocysteine dose-dependently inhibited insulin release at moderate and stimulatory glucose concentrations. This inhibitory effect was reversible at all but the highest concentration of homocysteine. 13C-glucose NMR demonstrated decreased labelling of glutamate from glucose at positions C2, C3 and C4, indicating that the tricarboxylic acid (TCA) cycle-dependent glucose metabolism was reduced in the presence of homocysteine. Homocysteine also dose-dependently inhibited insulinotrophic responses to a range of glucose-dependent secretagogues including nutrients (alanine, arginine, 2-ketoisocaproate), hormones (glucagon-like peptide-1 (7–36)amide, gastric inhibitory polypeptide and cholecystokinin-8), neurotransmitter (carbachol), drug (tolbutamide) as well as a depolarising concentration of KCl or elevated Ca2+. Insulin secretion induced by activation of adenylate cyclase and protein kinase C pathways with forskolin and phorbol 12-myristate 13-acetate were also inhibited by homocysteine. These effects were not associated with any adverse action on cellular insulin content or cell viability, and there was no increase in apoptosis/necrosis following exposure to homocysteine. These data indicate that homocysteine impairs insulin secretion through alterations in β-cell glucose metabolism and generation of key stimulus-secretion coupling factors. The participation of homocysteine in possible β-cell demise merits further investigation.


Introduction

The sulphhydryl amino acid, homocysteine, is subject to three key metabolic fates: remethylation to form methionine, transsulphuration to form cysteine, or export into the circulation (Medina et al. 2001). The latter fate is fundamental to the development of hyperhomocystinaemia, where plasma homocysteine levels exceed 15 µM (Ueland et al. 2001). There are varying degrees of hyperhomocystinaemia, which are defined as mild (15–30 µM), moderate (30–100 µM) or severe (>100 µM). Most severe cases can occasionally give rise to almost millimolar concentrations of plasma homocysteine (Dalton et al. 1997). Deficiencies of the enzymes cystathionine β-synthase, needed for the conversion of homocysteine to cystathionine in the transsulphuration pathway (Dalton et al. 1997, Medina et al. 2001), and methylenetetrahydrofolate reductase (MTHFR), needed for the remethylation of homocysteine to methionine (Medina et al. 2001) are common hereditary causes of hyperhomocystinaemia and premature atherosclerosis. In fact, the prevalence of the 677TT mutation of the gene that encodes MTHFR is between 5 and 15% in most Caucasian populations, and leads to higher total homocysteine concentrations especially in subjects with marginal or suboptimal folate status (De Bree et al. 2002). Also, vitamin deficiencies, including vitamin B6, B12 and folate, which occur commonly within the general population, as well as deteriorating renal function (associated with the metabolic syndrome) significantly contribute towards

Elevated circulating homocysteine levels are observed clinically, and raised plasma homocysteine has recently been reported in obese subjects with defective insulin secretion and insulin resistance compared with normoinsulinemic obese subjects (Sanchez-Margalet et al. 2002). An increased prevalence of hyperhomocysteinaemia in patients with type 2 diabetes with pre-existing coronary vascular disease has also been reported (Okada et al. 1999, Becker et al. 2003), and hyperhomocysteinaemia is now well established as a risk factor for vascular disease (Majors et al. 1997), which is the most common cause of mortality in type 2 diabetes.

Homocysteine has been shown to exert detrimental effects on endothelial cells via the generation of reactive oxygen species (Blundell et al. 1996). Neuronal cells are also susceptible to the toxic effects of homocysteine via the production of free radicals as well as over stimulation of the N-methyl-D-aspartate (NMDA) receptor (Kim & Pae 1996, Lipton et al. 1997). In pancreatic β-cells, chronic exposure to elevated plasma glucose levels results in glycation of endogenous proteins, sustained secretory hyperactivity, and the eventual loss of glucose sensitivity and β-cell exhaustion (Malaisse 1991, 1994, Ling & Pipeleers 1996). Glucose can exert toxic irreversible effects on the β-cells mainly due to glycogen accumulation (Malaisse 1994), defective insulin gene expression (Harmon et al. 1999) and chronic oxidative stress (Tanaka et al. 1999). Prolonged exposure to high plasma lipid concentrations also exerts toxic effects on pancreatic β-cells (Prenkt & Corkey 1996), partly through the accumulation of intracellular triacylglycerol (steatosis) (Unger 1995, Dixon et al. 2004). Indeed, β-cells with a high proportion of saturated fatty acid-rich triglyceride demonstrated impaired insulin secretion, mitochondrial alterations and evidence of eventual lipoapoptosis (Unger et al. 1999, Dixon et al. 2004).

Although sustained elevations of blood glucose and lipids have been reported to exert detrimental effects on pancreatic β-cell function, the possible effects of raised plasma levels of homocysteine have not yet received due attention. In order to study the acute effects of homocysteine on the regulation of insulin secretion and pancreatic β-cell metabolic and functional integrity, we utilised clonal pancreatic glucose-responsive BRIN-BD11 β-cells (McClenaghan et al. 1996a). The present data demonstrate that acute exposure to moderate to high levels of homocysteine exerts detrimental effects on β-cell metabolism and insulin secretion.

**Materials and Methods**

**Chemicals**

Reagents of analytical grade and deionised water (Purite) were used. RPMI 1640 tissue culture medium, fetal bovine serum, and antibiotics were from GIBCO and 125I-bovine insulin was purchased from Amersham International plc (Amersham). L-Alanine, L-arginine, L-carnitine, D,L-glutamine, 2-ketoisocaproate, phorbol 12-myristate 13-acetate (PMA), potassium chloride and tolbutamide were from Sigma. All other chemicals were from BDH Chemicals (Poole, Dorset, UK).

**Cell culture**

Clonal BRIN-BD11 β-cells (passages 20–35), were grown at 37°C with 5% CO2 and 95% air in RPMI 1640 tissue culture medium containing 11·1 mM glucose and 0·3 g/l L-glutamine, and supplemented with 10% (v/v) fetal calf serum, 100 IU/ml penicillin and 0·1 mg/ml streptomycin. Homocysteine was added to the culture medium as indicated in the Tables and Figures. The cells were gently washed with Hanks balanced saline solution (HBSS) before detachment from the culture flask with the aid of 0·025% (w/v) trypsin containing 1 mM EDTA, and was utilised immediately. The generation and basic characteristics of BRIN-BD11 cells have been described elsewhere (McClenaghan et al. 1996a) and these cells exhibit appropriate responses to physiological and pharmacological agents including a diverse range of amino acids (McClenaghan et al. 1996b, McClenaghan & Flatt 1999).

**Preparation of BRIN-BD11 cells for nuclear magnetic resonance (NMR) analysis**

For NMR experiments, cells were grown in T175 flasks as described previously (Brennan et al. 2002). After a 20–min preincubation period in Krebs Ringer bicarbonate buffer with 1·1 mM glucose, the cells were incubated for 60 min with 16·7 mM [1-13C]glucose in the presence or absence of 250 µM homocysteine. After incubation, the buffer was removed and the cells were washed with ice-cold phosphate-buffered saline before addition of ice-cold perchloric acid (6%). The extracts of six culture flasks (1 × 10⁸ cells) were pooled and centrifuged at 1500 r.p.m. The resulting supernatant was neutralised with KOH (5 M and 0·1 M solutions) and the pellets were soaked overnight in 0·1 mM NaOH. The protein concentration was determined using the Lowry method (Lowry et al. 1951). The neutralised supernatant was centrifuged (1500 r.p.m.) and the supernatant was treated with Chelex-100 resin and then lyophilised.

**Assessment of glucose metabolism by NMR spectroscopy**

The lyophilised cell extracts were dissolved in 3 ml H₂O and then centrifuged at 1500 r.p.m. for 5 min. The supernatant was carefully removed and 10% D₂O was
added and the pH checked and adjusted when necessary with 0·1 M NaOH or 0·1 M HCl. An insert containing 1% v/v dioxane in water was used as an external signal intensity reference for quantification of the NMR spectra. Proton decoupled 13C spectra were acquired on a Bruker DRX 500 spectrometer using a 10 mm broadband probe as outlined elsewhere (Brennan et al. 2002). Typically, spectra were acquired with 32 K data points using 13 μs pulses (90° pulse angle), 260 ppm spectral width, 2·5 s relaxation delay and 12 000 scans. Chemical shifts in aqueous media were referenced to tetramethylsilane at 0 ppm. Exponential multiplications with 2 Hz line broadening, baseline corrections and manual integrations were performed using Bruker WINNMR software (Thermo Electron Corporation, Waltham, MA, USA). The amount of 13C in each resonance was evaluated by integration of the extract peaks and the corresponding peaks in the standard sample relative to the dioxane signal. Corrections for the natural abundance signal were made. To calculate the percentage enrichments, the total glutamate concentration was measured using enzymatic methods (glutamate dehydrogenase based assay; Roche Diagnostics).

Assessment of cell viability

Cell viability was determined using the neutral red assay following incubation with 1 ml neutral red buffer (KRB, 0·05 mg/ml neutral red) for 2 h at 37 °C. Neutral red buffer was gently removed and, following washing, 1 ml glacial acetic acid was added to release dye from viable cells. Aliquots (150 µl) were transferred to a 96-well plate and absorbance was measured at 540 nm. Cell viability was expressed as a percentage relative to control cells not exposed to homocysteine.

Analysis of cell apoptosis and necrosis

Following acute (20 min) exposure of BRIN-BD11 cells to various test reagents as described earlier, the cells were harvested and resuspended in phosphate-buffered saline (PBS) at a density of 0·5 × 106 cells/ml. A cocktail of acridine orange (100 µg/ml) and ethidium bromide (100 µg/ml) was prepared and 1 µl added to 25 µl cell suspension. Following mixing, the cells were allowed to sit at room temperature for 2 min and 10 µl cell suspension were placed on a glass slide and covered with a coverslip. The cells were then examined under a fluorescence microscope with an FITC filter at ×40 magnification. Cells were categorised as follows: (i) viable cells: bright green nucleus and intact structure, (ii) early apoptotic: bright green nucleus with condensed green chromatin, (iii) late apoptotic: orange nucleus and condensed orange chromatin, and (iv) necrotic: orange nucleus and intact structure.

Statistical analyses

Results are presented as means ± S.E.M or S.D., as indicated. Groups of data were compared using unpaired Student’s t-test. Differences were considered significant if P<0·05.

Results

Effects of homocysteine on glucose-induced insulin release

Increasing the homocysteine concentration progressively from 0 µM to 1000 µM had a dose-dependent inhibitory effect on insulin secretion at both moderate (5·6 mM) glucose (Fig. 1A) and stimulatory (16·7 mM) glucose concentrations (Fig. 1B). At 5·6 mM glucose, 50 µM, 100 µM, 250 µM and 1000 µM homocysteine significantly (P<0·001) reduced insulin secretion by 37%, 37%, 57% and 90%, respectively. Similar effects were observed at 16·7 mM glucose, with a significant (P<0·001) inhibition of glucose-induced insulin release by 37%, 52%, 75% and 86%, respectively. These acute inhibitory effects were also observed in the absence of glucose but exposure to lower concentrations of homocysteine (12·5 µM and 25 µM) did not significantly alter insulin secretion in any
of the tests (data not shown). Notably, acute exposure of clonal BRIN-BD11 β-cells to concentrations of 0 µM to 100 µM homocysteine did not alter cell viability as evaluated by the neutral red assay (Table 1). The BRIN-BD11 cells showed no significant increases in apoptosis/necrosis when exposed to homocysteine and all cells exhibited a bright green nucleus and intact structure (data not shown). Interestingly, the inhibitory effects of homocysteine were reversible at 50, 100 and 250 µM homocysteine but not at 1000 µM homocysteine (Table 1). Additionally, there were no significant changes in cellular insulin content (66–88 ng/10⁶ cells) following acute exposure to homocysteine over this concentration range (data not shown).

**Effects of homocysteine on insulin secretory responses to amino and keto acids**

The effects of 50 µM, 100 µM, 250 µM or 1000 µM homocysteine on insulin secretory responses to alanine, arginine and 2-ketoisocaproate (KIC) at stimulatory (16·7 mM) glucose are given in Fig. 2A-D. As shown, alanine, arginine and KIC induced respective 3·4-, 2·7- and 2·0-fold \( P < 0·01 \) insulin secretory responses. Exposure to 50 µM homocysteine caused a significant inhibition of insulin output in response to these agents by 36% \( P < 0·001 \), 32% \( P < 0·01 \) and 36% \( P < 0·05 \), respectively. Similar effects were observed at higher concentrations of homocysteine with decreases for alanine, arginine and KIC of 36% \( P < 0·001 \), 35% \( P < 0·01 \) and 56% \( P < 0·01 \) at 100 µM homocysteine, 40% \( P < 0·001 \), 32% \( P < 0·01 \) and 58% \( P < 0·01 \) at 250 µM homocysteine, and 47% \( P < 0·001 \), 39% \( P < 0·001 \) and 54% \( P < 0·01 \) at 1000 µM homocysteine, respectively.

### Table 1

<table>
<thead>
<tr>
<th>HC (µM)</th>
<th>Cell viability following 20-min exposure to HC (%)</th>
<th>Insulin secretion following 20-min exposure to HC (ng/10⁶ cells/20 min)</th>
<th>Insulin secretion following 20-min recovery (ng/10⁶ cells/20 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100±0·3±1</td>
<td>2·70±0·18</td>
<td>2·0±0·11</td>
</tr>
<tr>
<td>50</td>
<td>103·6±3·3</td>
<td>1·69±0·11***</td>
<td>1·88±0·14</td>
</tr>
<tr>
<td>100</td>
<td>104·7±4·0</td>
<td>1·31±0·10***</td>
<td>2·0±0·12</td>
</tr>
<tr>
<td>250</td>
<td>99·3±4·8</td>
<td>0·66±0·05***</td>
<td>1·9±0·11</td>
</tr>
<tr>
<td>1000</td>
<td>97·5±4·0</td>
<td>0·37±0·02***</td>
<td>1·13±0·03***</td>
</tr>
</tbody>
</table>

All incubations were performed at 16·7 mM glucose. Values are means ± s.e.m. (\( n = 6 \)). *** \( P < 0·001 \) compared with incubations in the absence (0 µM) of homocysteine.
Effects of homocysteine on insulin secretory responses to tolbutamide, KCl and Ca²⁺

Insulin secretory responses to tolbutamide, a depolarising concentration of KCl, and raised Ca²⁺ levels were assessed at stimulatory (16.7 mM) glucose in the absence or presence of 50 to 1000 µM homocysteine (Fig. 3A-D). Tolbutamide caused a 1.7-fold (P<0.001) increase in insulin secretion that was significantly impaired at 100 µM, 250 µM and 1000 µM homocysteine by 27% (P<0.01), 48% and 53% (P<0.001), respectively. The insulin releasing effects of 30 mM KCl (5.2-fold, P<0.001) were inhibited (P<0.05-P<0.001) by homocysteine in a concentration-dependent manner by 16%-50%.

The insulin response to raised extracellular Ca²⁺ (2.1-fold, P<0.01) was reduced at all homocysteine concentrations by 26%-76% (P<0.01-P<0.001).

Effects of homocysteine on insulin secretory responses to gut hormones

The gut hormones glucagon-like peptide 1 (7–36) amide (GLP-1), gastric inhibitory polypeptide (GIP) and cholecystokinin-8 (CCK-8) induced respective 1.9-, 1.4- and 1.5-fold (P<0.01-P<0.001) insulin secretory responses (Table 2). However, homocysteine at 50 µM, 100 µM, 250 µM or 1000 µM caused significant (P<0.01-P<0.001) concentration-dependent decreases in the
secretory effects of GLP-1 (by 39–93%), GIP (by 50–90%) and CCK-8 (by 34–83%) (Table 2).

**Effects of homocysteine on insulin secretory responses to carbachol, forskolin, and PMA**

As shown in Table 2, the cholinergic receptor agonist, carbachol, elicited a 1.4-fold \((P<0.001)\) increase in insulin secretion, which was inhibited by 28%–80% \((P<0.001)\) by homocysteine concentrations of 100 \(\mu M\) and above. Forskolin–induced insulin release \((4.1\text{-fold}, P<0.001)\) was inhibited by 57% \((P<0.001)\) only at the highest concentration of 1000 \(\mu M\) homocysteine. Higher concentrations of homocysteine \((250 \text{ and } 1000 \mu M)\) also inhibited by 25%–68% \((P<0.05\text{–}P<0.001)\) the actions of the protein kinase C activator, PMA, which by itself caused a 6.1-fold \((P<0.001)\) increase in insulin release (Table 2).

**Effects of homocysteine on glucose metabolism**

The major metabolites labelled after 60 min incubation with 16.7 mM \([1-^{13}C]\)glucose included glutamate labelled at positions C2, C3 and C4, lactate labelled at position C3, and alanine labelled at position C3 (Fig. 4). As shown in Table 3, homocysteine \((250 \mu M)\) significantly reduced the amount of glutamate labelled at positions C2, C3 and C4 by 15%, 14% and 21% respectively. The effects of homocysteine on lactate and alanine labelling were not significant. Also, there were no significant differences in \(^{13}C\) enrichment of glutamate \((\text{mean} \pm \text{s.d.}, \ldots)\).
The present study has examined the acute effects of homocysteine on pancreatic β-cell function and insulin secretion (Ashcroft & Rorsman 2004, Kulkarni 2004). In the presence of moderate (5·6 mM) and high (16·7 mM) glucose concentrations, homocysteine caused a significant dose-dependent inhibition of insulin secretion with initial effects observed at 50 μM. Since regulation of insulin secretion by glucose involves many steps from glucose metabolism to signal recognition and insulin discharge, the mechanism underlying the inhibitory effects of homocysteine on insulin secretion could involve many possible actions. However, it is notable that acute exposure to homocysteine in the present study did not affect cellular insulin content, cell viability or apoptosis/necrosis at any of the concentrations tested. Moreover, the inhibitory effects of homocysteine were reversible at all but the highest (1000 μM) homocysteine concentration.

**Discussion**

The present study has examined the acute effects of homocysteine on pancreatic β-cells, with specific regard to actions on insulin release and the regulation of secretory function. To date, the effects of homocysteine on pancreatic β-cell function have not received much attention. This is perhaps surprising given that homocysteine has detrimental effects on a number of different cell lineages (Blundell et al. 1996, Kim & Pae 1996, Lipton et al. 1997), is elevated in subjects with insulin resistance (Sanchez-Margalet et al. 2002) and occurs at a higher prevalence in type 2 diabetic subjects, especially those with impaired renal function (Buysschaert et al. 2000). Diabetes is a complex disorder and a major disease of the so-called metabolic syndrome. As such, the diabetic group encompasses individuals with elevated homocysteine combined with poor metabolic control (Drzewoski et al. 2000), pre-existing vascular disease (Okada et al. 1999, Becker et al. 2003), nephropathy (Stabler et al. 1999, Buysschaert et al. 2000) or gestational diabetes (Seghieri et al. 2003).

Glucose is considered to be the prime regulator of pancreatic β-cell function and insulin secretion (Ashcroft & Rorsman 2004, Kulkarni 2004). In the presence of moderate (5·6 mM) and high (16·7 mM) glucose concentrations, homocysteine caused a significant dose-dependent inhibition of insulin secretion with initial effects observed at 50 μM. Since regulation of insulin secretion by glucose involves many steps from glucose metabolism to signal recognition and insulin discharge, the mechanism underlying the inhibitory effects of homocysteine on insulin secretion could involve many possible actions. However, it is notable that acute exposure to homocysteine in the present study did not affect cellular insulin content, cell viability or apoptosis/necrosis at any of the concentrations tested. Moreover, the inhibitory effects of homocysteine were reversible at all but the highest (1000 μM) homocysteine concentration.
To gain further insight into the inhibitory mechanisms of homocysteine, effects on the secretory actions of other amino and 2-keto acids were examined. Homocysteine caused a concentration-dependent inhibition of insulin secretion induced by alanine, arginine and KIC. Alanine and KIC are both readily metabolised by the β-cell (Hutton et al. 1980, Ashcroft et al. 1987, Yada 1994, Brennan et al. 2002). Alanine also causes membrane depolarisation due to electrogenic Na⁺ transport (Dunne et al. 1990), while arginine induces insulin secretion via direct depolarisation of the plasma membrane (Ashcroft et al. 1987, Yada 1994). Alanine additionally enhances glucose metabolism via the tricarboxylic acid (TCA) cycle in the β-cell (Brennan et al. 2002). This raises the possibility that the inhibitory effect of homocysteine on the combined actions of alanine plus glucose may be mediated through impairment of alanine-enhanced glucose metabolism.

Consistent with the idea that homocysteine might also affect later steps in stimulus-secretion coupling, this sulphur-containing amino acid impaired the insulin secretory response to tolbutamide. This drug binds to the sulphonylurea receptor subunit (SUR1) of the KᵦᵥP channel complex on the β-cell membrane, depolarises the plasma membrane and opens voltage-gated Ca²⁺ channels (Ashfield et al. 1999). Furthermore, insulin secretion induced by direct membrane depolarisation with 30 mM KCl or elevation of cytoplasmic Ca²⁺ by high extracellular Ca²⁺ were inhibited concentration-dependently by homocysteine.

Additional experiments examined possible inhibitory actions of homocysteine on various amplification pathways of insulin release. Interestingly, even 50 μM homocysteine caused marked reductions in insulin secretory responses to GLP-1 and GIP, with almost complete abolition of secretion at higher concentrations. These gut hormones are known to activate adenylyl cyclase leading to increased levels of cAMP and activation of protein kinase A (McClenaghan & Flatt 1999). Insulin secretion induced by forskolin, a powerful activator of adenylyl cyclase, was also impaired by homocysteine, albeit at higher concentrations. Insulin releasing actions of agents, such as CCK-8 and carbachol, working through the phospholipase C–protein kinase C pathways were also disrupted by acute exposure to homocysteine. Pharmacological activation of protein kinase C and stimulation of secretion by PMA was also reduced at higher homocysteine concentrations. It should be considered that these amplifying pathways normally synergise with key metabolic stimulus–secretion coupling factors to enhance insulin secretion in response to hormonal or drug stimuli.

It is clear from these various secretory studies that acute exposure to homocysteine for 20 min is sufficient to cause a significant general impairment of physiological insulin secretion. As this study uses D,L-homocysteine, detrimental effects on insulin secretion may be observed with the naturally occurring l-isomer at lower concentrations. A major question from this work concerns the possible mechanism underlying the adverse effects of homocysteine on β-cell function. It is interesting that homocysteine in association with deazaadenosine has been shown to inhibit phospholipid methylation reactions in pancreatic islets (Best et al. 1984, Saceda et al. 1984). While inhibition of methylation may impair insulin secretion, it seems unlikely that this can account for widespread actions of homocysteine on pancreatic β-cells.

A recent study has shown that homocysteine is transported into cells mainly via the cyst(e)ine transporters (Hultberg 2004). Accordingly, a much more plausible explanation, given the principal role of glucose in determining the secretory activity of the agents tested, is that homocysteine interferes with glucose metabolism in the β-cell. Glucose is rapidly metabolised in the β-cell and enters the TCA cycle via both pyruvate dehydrogenase and pyruvate carboxylase. As the intermediates of the cycle increase in concentration, anaplerotic production of specific amino acids will increase, for example glutamate from 2-oxoglutarate. By monitoring the fate of [1-¹³C]glucose using NMR, we found that homocysteine reduced the production of glutamate labelled at positions C2, C3 and C4 from glucose. This indicates a reduction in the flux through the TCA cycle (Malloy et al. 1990). This decrease was not a result of homocysteine itself acting as a substrate for the TCA cycle (the specific enrichment of glutamate carbons 2, 3 or 4 was not altered by homocysteine) or a reduced uptake of glucose. The presence of homocysteine did not alter the production of labelled lactate and alanine from glucose, indicating that the effects

Table 3 ¹³C-labelled glutamate, lactate, and alanine obtained in cell extracts after incubation of BRIN-BD11 cells with 16·7 mM [1-¹³C]glucose in the absence or presence of 250 μM homocysteine

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Glu C2</th>
<th>Glu C3</th>
<th>Glu C4</th>
<th>Lactate C3</th>
<th>Alanine C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8·0 ± 0·6</td>
<td>8·8 ± 0·4</td>
<td>26·6 ± 1·9</td>
<td>14·4 ± 7·6</td>
<td>5·9 ± 2·0</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>6·8 ± 0·3*</td>
<td>7·6 ± 0·8*</td>
<td>20·9 ± 2·5*</td>
<td>12·0 ± 6·3</td>
<td>4·5 ± 0·7</td>
</tr>
</tbody>
</table>

Values are reported as mean (nmol/mg protein) ± s.d. (n=4). *P < 0·05 compared with control.
of homocysteine are downstream of pyruvate production. Thus, it appears that homocysteine may impact on key regulatory steps of the TCA cycle in β-cells possibly via NAD+ depletion and reduction of glucose oxidation, as suggested by reduction in the production of C4 glutamate. These effects will restrain insulin secretion induced by a wide variety of agents (Blennow et al. 1979), and since the inhibitory effects were also observed in the absence of glucose it is possible that homocysteine inhibits basal TCA cycle flux from other endogenous sources (glycogen, glutamate, fatty acids) and therefore basal insulin secretion. The time-dependent effects of homocysteine on ATP turnover were not assessed in this study but would provide an interesting area for future research.

In conclusion, the present study demonstrates that homocysteine exerts deleterious effects on insulin secretion possibly mediated through alterations in β-cell glucose metabolism and generation of key stimulus–secretion coupling factors. Although this study reports novel findings on the in vitro effects of homocysteine on clonal beta cells, future investigations assessing in vivo effects of homocysteine on the function of normal pancreatic islets together with associated metabolic effects are merited.

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