EXPERIMENTAL STUDY

The direct effects of the angiotensin-converting enzyme inhibitors, zofenoprilat and enalaprilat, on isolated human pancreatic islets

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Abstract

Objective: Data from prospective studies suggest a significant reduction in the risk of new diabetes from drug therapies containing angiotensin-converting enzyme (ACE) inhibitors. Since the renin–angiotensin system (RAS) has been found locally in several tissues and cells, including pancreatic islets, we hypothesized that the positive metabolic effects of ACE inhibitors may be due to a beneficial action of these compounds on insulin-secreting β -cells.

Design and methods: Isolated human pancreatic islets were studied after 24 h of incubation with 22.2 mmol/l glucose, with or without the presence in the incubation medium of 0.5–6.0 mmol/l zofenoprilat or enalaprilat, ACE inhibitor drugs which differ by the presence of a sulphhydryl or a carboxyl group in their structural formula. Functional and molecular studies were then performed to assess insulin secretion, redox balance, mRNA and protein expression.

Results: Angiotensinogen, ACE and angiotensin type 1 receptor mRNA expression increased in islets cultured in high glucose; this was similarly prevented by the presence of either ACE inhibitor. As expected, preculture of human islets in high glucose determined a marked reduction in insulin secretion which was associated with enhanced oxidative stress, as shown by increased nitrotyrosine concentrations, and enhanced expression of protein kinase C β and NADPH oxidase. The presence of either of the ACE inhibitors counteracted several of the deleterious effects of high glucose exposure, including reduction of insulin secretion and increased oxidative stress; zofenoprilat showed significantly more marked effects.

Conclusions: These results showed that: (a) RAS molecules are present in human islets and their expression is sensitive to glucose concentration, (b) ACE inhibitors, and in particular zofenoprilat, protect human islets from glucotoxicity and (c) the effects of ACE inhibition are associated with decreased oxidative stress. Together, these findings provide evidence that the possible beneficial effects of ACE inhibitors in human diabetes are due, at least in part, to a protective action on pancreatic β -cells.

European Journal of Endocrinology 154 355–361

Introduction

Type 2 diabetes mellitus is a major health problem associated with excess morbidity and mortality (1, 2). As the incidence and prevalence of this disease are rapidly increasing (1, 2), improved prevention and treatment strategies should be considered as a key objective in the near future. It has recently been shown that lifestyle changes and various pharmacological treatments may indeed prevent or at least delay the onset of type 2 diabetes (3, 4).

Arterial hypertension is a clinical entity which is strongly associated with type 2 diabetes and may

precede the disease by several years (5). While anti-hypertensive agents such as diuretics or β -adrenoceptor antagonists may worsen glucose tolerance (6), newer anti-hypertensive agents exert neutral or even positive metabolic effects (6, 7). Interestingly, a number of recent large-scale clinical studies have reported a remarkably consistent reduction in the incidence of type 2 diabetes in hypertensive patients treated with either angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor antagonists for 3–6 years, compared with other anti-hypertensive drugs or placebo (7). In particular, the relative risk reduction averaged 34% in the Heart Outcomes Prevention

Evaluation study with ramipril compared with placebo (8), and 25% in the Study on Cognition and Prognosis in the Elderly with candesartan cilexetil compared with placebo (9).

The mechanisms by which ACE inhibitors have positive effects on glucose metabolism are not clear. In type 2 diabetes, alterations of post-receptor insulin signaling have been clearly demonstrated; these include abnormalities in phosphatidylinositol-3 kinase and protein kinase B signaling (10). These defects are accentuated by angiotensin II (11) and, therefore, inhibition of the renin-angiotensin system (RAS) by ACE inhibitors could have a favorable effect on insulin action. In addition, ACE inhibitors have been shown to favor blood flow through the microcirculation, which contributes to increased glucose uptake in skeletal muscle tissue (12), a mechanism that may also be present at the level of pancreatic islets (13). Indeed, in recent years, an intrinsic RAS has been found in the pancreas of several species including man (14–16). In particular, Lau *et al.* (17) have investigated the presence of the RAS in mouse pancreatic islets, and suggested its potential importance in the regulation of insulin secretion. Moreover, it was shown by immunohistochemistry that angiotensinogen is localized in human pancreatic islets (18), even though the possible functional role was not investigated.

In the present study, we have confirmed the expression of RAS molecules in human pancreatic islets and showed that the presence of ACE inhibitors protected human islets from glucotoxicity, with differences between the agents used. Molecular studies were eventually performed to demonstrate that the positive effects of ACE inhibition on islet cells were due, at least in part, to an action on cellular redox balance.

Materials and methods

Pancreatic islet isolation

Pancreata were obtained from 15 non-diabetic multiorgan donors (age 52 ± 5 years, seven males and eight females, body mass index $25.0 \pm 0.6 \text{ kg/m}^2$), and isolated islets were prepared by collagenase digestion and density gradient purification (19–21); the work was approved by our local Ethics Committee.

Insulin secretion studies

Islet aliquots were cultured free floating for 24 h at 37°C in medium 199 (M199) culture medium under the following conditions: (a) control M199 culture medium containing 5.5 mmol/l glucose, (b) M199 culture medium containing 5.5 mmol/l glucose plus 0.5, 1.0, 3.0 or 6.0 mmol/l zofenoprilat or enalaprilat (kindly provided by Menarini Group, Florence, Italy); these are the respective active derivatives of zofenopril

and enalapril (two largely used ACE inhibitors), (c) M199 medium containing 22.2 mmol/l glucose and (d) M199 medium containing 22.2 mmol/l glucose plus 0.5, 1.0, 3.0 or 6.0 mmol/l zofenoprilat or enalaprilat. The major difference between the two ACE inhibitors that we used is the presence of a sulfhydryl group (zofenoprilat) or carboxyl (enalaprilat) group in their structure. Insulin secretion studies were performed as previously described (19–23). Following a 45-min preincubation period at 3.3 mmol/l glucose, islets of comparable size were kept at 37°C for 45 min in Krebs–Ringer bicarbonate solution (KRB) and 0.5% albumin, pH 7.4, containing 3.3 mmol/l glucose. At the end of this period, medium was completely removed and replaced with KRB containing either 3.3 or 16.7 mmol/l glucose. After an additional 45 min of incubation, the medium was removed. Samples (500 µl) from the different media were stored at –20°C until insulin concentrations were measured by IRMA (Pantec Forniture Biomediche, Turin, Italy).

Nitrotyrosine determination

Nitrotyrosine concentration was determined by an ELISA method as previously described (21). The procedure is based on the reaction of nitrotyrosine with a purified monoclonal anti-nitrotyrosine mouse IgG antibody (Upstate, Charlottesville, VA, USA), followed by incubation with a peroxidase-conjugated goat anti-mouse IgG secondary antibody and generation of the peroxidase reaction by tetra-methyl-benzidine (Micro-well peroxidase substrate; Sigma-Aldrich, St Louis, MO, USA).

Molecular studies: mRNA expression

mRNA expression was measured by RT reaction followed by competitive PCR. Total RNA was extracted by using the RNeasy Protect Mini Kit (QIAGEN, Hilden, Germany), and then handled as detailed elsewhere (19, 21). Quantification was performed by absorbance at A_{260}/A_{280} (ratio >1.65) nm in a Perkin-Elmer spectrophotometer, and RNA integrity was assessed after electrophoresis in 1.0% agarose gels by ethidium bromide staining. cDNA synthesis was performed from 1 µg total RNA, in the presence of dNTP mix (final concentration: 200 µM) and 200 µU of a thermostable reverse transcriptase (Superscript II, Invitrogen Corporation, Carlsbad, CA, USA). After the reaction, the RT fluid was added to a PCR mix (5 µl buffer 10×, 0.2 µM deoxynucleotide mix, 500 pmol specific sense and antisense primers and 2 U Taq Polymerase) in a final volume of 25 µl. The tubes were subjected to 35 cycles of amplification, including denaturation for 60 s at 95°C, hybridization for 60 s at 52–60°C and elongation for 90 s at 72°C.

The following primers (sense and antisense) were used to generate human angiotensinogen (AGT),

angiotensinogen-converting enzyme (ACE), angiotensin type 1 receptor (AT1) and β -actin cDNAs: for AGT, 5'-CAT ACA CCC CTT CCA CCT C-3' and 5'-GGA TTG CCT GTA GCC TGT C-3'; for ACE, 5'-GTC CTC CTG TTA CCC GA ACG-3' and 5'-GTT CTG CTC CTG CAG CA CTG-3' (209 bp); for AGT1, 5'-TTT GTG GTG GGA ATA TTT GG-3' and 5'-TGA TGA TGC AGG TGA CTT TG-3'; for β -actin, 5'-ACC AAC TGG GAG GAG ATG GAG-3' and 5'-CGT GAG GAT CTT CAT GAG GTA AGT C-3'. For NAPDH oxidase, we assessed mRNA expression of the essential subunit p22phox by using 5'-AAC GTT GAG GGG CAT CGT CG-3' as sense and 5'-GAT CTG CCC CAT GGT GAG GAC C-3' as anti-sense primers.

Molecular studies: protein expression

Protein kinase C (PKC) β 2 expression was determined by immunoblotting procedure as published previously (22); it was slightly modified to be applied to human islets. Five hundred hand-picked islets were washed extensively in ice-cold buffer (137 mmol/l NaCl, 120 mmol/l Tris, 1 mmol/l MgCl₂, 1 mmol/l CaCl₂ and 100 mmol/l Na₃VO₄, pH 7.6) and then incubated with a lysis solution (10% glycerol, 1% Nonidet P-40, 120 mmol/l NaCl, 100 mmol/l tetra-natriumdiphosphate-decahydrate 20 mmol/l Tris, 10 mmol/l Na₃VO₄, 100 mmol/l NaF, 10 mmol/l EDTA, 2 μ g/ml aprotinin, 5 g/ml leupeptin, 0.5 pg/ml pepstatin and 1 mmol/l phenylmethylsulfonyl fluoride, pH 7.4). Islets were vortexed, incubated for 60 min at 4°C on a rotating device and then centrifuged at 20 000 *g* for 90 min at 4°C. One aliquot of the supernatant was used to determine protein concentration by DC Protein Assay (BioRad Laboratories, Hercules, CA, USA). Anti-PKC β 2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was detected by direct immunoblotting in SDS PAGE/Tris acrilamide. Protein electrotransfer to nitrocellulose (Schleicher & Schuell Inc., Dassel, Germany) was performed in a buffer containing

192 mmol/l glycine, 25 mmol/l Tris, 3.4 mmol/l SDS and 20% methanol for 4 h at 480 mA. Nitrocellulose filters were blocked in Tris-buffered saline, 0.1% Tween-20 and 5% milk for 1 h at room temperature with rocking. Filters were incubated with anti-PKC β 2 total and activated (phospho-PKC β 2, Ser 660) antibody (Transduction Laboratories, Lexington, KY, USA), diluted in blocking solution for 20 h at 4°C, washed extensively and then incubated with appropriate secondary antibody. Bound antibodies were detected using procedures carried out according to the manufacturer's instructions (Amersham Biosciences, Amersham, Bucks, UK). Bands of interests were quantitated with a densitometer (GS 690; BioRad Laboratories) using MultiAnalyst/PC-PC software for Bio-Rad's Image Analysis Systems, version 1.02 (BioRad Laboratories) and all results were normalized for mg protein.

Statistical data analysis

Results are expressed as means $\pm$ S.E.M. for all groups. Data were analysed by the Student's *t*-test for unpaired comparison or by the two-way ANOVA method. For all comparisons, *P* < 0.05 was considered statistically significant.

Results

RAS molecule expression

RT-PCR experiments showed that angiotensinogen, ACE and angiotensin type 1 receptor mRNAs were expressed in isolated human islets (Fig. 1). In addition, it was shown that culturing the islets in the presence of 22.2 mmol/l glucose induced an increased expression of both mRNAs, which was normalized or reduced when zofenoprilat or enalaprilat was present in the culture medium (Fig. 1).

Figure 1 Angiotensinogen (AGT), ACE and angiotensin type 1 receptor (AT1) mRNA expression in control islets (Ctrl), islets exposed for 24 h to 22.2 mmol/l glucose and islets exposed for 24 h to 22.2 mmol/l glucose plus 6.0 mmol/l zofenoprilat or enalaprilat. The expressions increased significantly in islets cultured with high glucose (**P* < 0.01 vs Ctrl). The presence of either of the ACE inhibitors normalized/improved the expression of the RAS molecules (***P* < 0.05 vs 22.2 mmol/l glucose; ****P* < 0.05 vs 22.2 mmol/l glucose and 22.2 mmol/l glucose + zofenoprilat).

Insulin secretion studies

Insulin secretion from isolated islets in response to 3.3 and 16.7 mmol/l glucose was studied after 24 h of exposure to 5.5 (control condition) or 22.2 mmol/l glucose. Insulin release at 3.3 mmol/l glucose did not differ between controls ($25.3 \pm 1.7 \mu\text{U/ml}$) and islets exposed to high glucose ($23.2 \pm 6.6 \mu\text{U/ml}$). However, the 16.7 mmol/l glucose challenge induced a higher insulin secretion from control islets ($67.4 \pm 6.0 \mu\text{U/ml}$) than from islets precultured with 22.2 mmol/l glucose ($29.5 \pm 6.7 \mu\text{U/ml}$, $P < 0.01$). In the presence of 5.5 mmol/l glucose, 24 h of culture with 0.5–6.0 mmol/l zofenoprilat or enalaprilat did not affect subsequent glucose-stimulated insulin secretion (with stimulation index values, i.e. the ratio of hormone release at 16.7 mmol/l glucose over hormone release at 3.3 mmol/l glucose, ranging from 2.1 ± 0.3 to 2.4 ± 0.3). Following 24 h of culture in the presence of 22.2 mmol/l glucose, both ACE inhibitors induced a concentration-related improvement of glucose-induced insulin release in response to acute stimulation (Fig. 2, $P < 0.01$ by two-way ANOVA). In fact, the stimulation index increased progressively from 1.1 ± 0.2 in islets precultured with 22.2 mmol/l glucose to 2.8 ± 0.2 and 1.9 ± 0.1 in islets pre-exposed to high glucose and 6.0 mmol/l zofenoprilat or enalaprilat respectively (Fig. 2). Compared with islets not exposed to ACE inhibition, the stimulation index was significantly higher with 1.0, 3.0 and 6.0 mmol/l zofenoprilat (all $P < 0.05$) and with 1.0, 3.0 and 6.0 mmol/l enalaprilat (all $P < 0.05$). In addition, at 3.0 and 6.0 mmol/l concentrations, improvements with zofenoprilat were significantly better than with enalaprilat (Fig. 2).

The role of oxidative stress

Nitrotyrosine (marker of oxidative stress) levels were respectively 7.1 ± 0.2 and $16.2 \pm 0.8 \text{ nmol/l}$ in control islets and islets exposed for 24 h to 22.2 mmol/l glucose (Fig. 3A, $P < 0.01$). Co-incubation with

0.5–6.0 mmol/l ACE inhibitor significantly decreased nitrotyrosine concentrations in islets cultured in high glucose (Fig. 3A, $P < 0.01$ by two-way ANOVA). Again, improvements with zofenoprilat were significantly more marked than with enalaprilat (Fig. 3A).

As shown in Fig. 3B, islet mRNA expression of NADPH oxidase increased following culture in high levels of glucose. This effect was prevented by zofenoprilat, but not by enalaprilat. The effect of zofenoprilat was already maximal at the concentration of 1.0 mmol/l, and this concentration was used to test the ACE inhibitors action on PKC $\beta 2$ protein expression (Fig. 4). In pancreatic human islets exposed for 24 h to 22.2 mmol/l glucose, a significant increase of phosphorylated PKC $\beta 2$ protein expression was observed, as compared with control islets (185.2 ± 12.4 vs 101.5 ± 5.4 arbitrary units of optical density (O.D.) respectively, $P < 0.01$), which corresponded to a two-fold higher phosphorylated over total protein ratio (Fig. 4). Culture of islets with high glucose and 1.0 mmol/l zofenoprilat (111.4 ± 11.2 O.D., $P < 0.05$ vs high glucose alone), but not enalaprilat (154.6 ± 17.2 O.D.) determined a marked reduction of phosphorylated PKC $\beta 2$ expression, with normalization of the phosphorylated over total protein ratio with the former (Fig. 4).

Discussion

Proteins of the RAS in human islet cells have been previously detected by several authors (14–18). Here we have confirmed that RAS molecules are indeed expressed in human islets. Interestingly, we observed that angiotensinogen, ACE and angiotensin type 1 receptor expression increased following islet culture in a high glucose concentration, an effect which was prevented, at least in part, by ACE inhibition (Fig. 1). These findings are in agreement with previous work performed with other cell types, showing that high glucose can stimulate angiotensinogen gene expression and

Figure 2 Insulin secretion (expressed as stimulation index, i.e. the ratio of glucose-stimulated release over basal release) from islets exposed for 24 h to 5.5 mmol/l glucose (open bars), 22.2 mmol/l glucose (solid bars) and islets exposed for 24 h to 22.2 mmol/l glucose plus 0.5–6.0 mmol/l ACE inhibitor (zofenoprilat (shaded bars) or enalaprilat (grey bars)). Both ACE inhibitors improved insulin release ($*P < 0.05$ vs 22.2 mmol/l glucose), but improvements with zofenoprilat were significantly better ($\$P < 0.05$ vs respective enalaprilat concentrations). $\#P < 0.01$ vs 5.5 mmol/l glucose.

Figure 3 (A) Nitrotyrosine levels in islets exposed for 24 h to 5.5 mmol/l glucose (empty bars), 22.2 mmol/l glucose (solid bars) and islets exposed for 24 h to 22.2 mmol/l glucose plus 0.5–6.0 mmol/l ACE inhibitor (zofenoprilat (shaded bars) or enalaprilat (grey bars)). Both ACE inhibitors reduced nitrotyrosine levels ($*P < 0.05$ vs 22.2 mmol/l glucose), but improvements with zofenoprilat were significantly better ($\$P < 0.05$ vs respective enalaprilat concentrations). $\#P < 0.01$ vs 5.5 mmol/l glucose **(B)** p22-phox NADPH oxidase subunit mRNA expression in islets exposed for 24 h to 5.5 mmol/l glucose (empty bars), 22.2 mmol/l glucose (solid bars) and islets exposed for 24 h to 22.2 mmol/l glucose plus 0.5–6.0 mmol/l ACE inhibitor (zofenoprilat (shaded bars) or enalaprilat (grey bars)). Both ACE inhibitors reduced p22-phox NADPH oxidase subunit mRNA expression ($*P < 0.05$ vs 22.2 mmol/l glucose), but improvements with zofenoprilat were significantly better ($\$P < 0.05$ vs respective enalaprilat concentrations). $\#P < 0.01$ vs 5.5 mmol/l glucose.

enhance renin activity and angiotensin II production (23, 24), and that these events are prevented by inhibiting RAS (25). Within the cell, both high glucose and angiotensin II can synergistically act to induce oxidative stress. Indeed, increased glucose concentration promotes the production of reactive oxygen species (ROS) through many mechanisms, including mitochondrial and non-mitochondrial pathways (26). Among them, toxic glucose levels induce PKC β 2 activation (27, 28) which, in turn, leads to enhanced NADPH oxidase activity on the one side (with increased H_2O_2 production) and to expression of RAS molecules on the other side (29). Since angiotensin II is known to directly activate the PKC–NADPH oxidase pathway, this leads to further ROS production (30, 31). In this complex scenario, inhibition of the RAS might therefore have several positive effects.

In the present study we found that 24 h of exposure of isolated islets to a high glucose concentration induced a condition of glucose unresponsiveness to subsequent stimulation with the exose (Fig. 2). This confirms many previous data showing the deleterious effects of exposing islet cells to an increased glucose level for prolonged periods of time (26, 32). In addition, we observed that islet cell dysfunction was associated with increased oxidative stress, as suggested by

enhanced nitrotyrosine concentration and increased expression and/or phosphorylation of PKC β 2 and NADPH oxidase (Figs 3 and 4). The presence of ACE inhibitors did not affect islet function following 24 h of incubation at 5.5 mmol/l glucose. Interestingly, however, the presence of ACE inhibitors counteracted several of the deleterious effects of prolonged high glucose exposure on insulin release (Fig. 2).

Figure 4 PKC β 2 protein expression expressed as the ratio between phosphorylated over total protein, in control islets (Ctrl), islets exposed for 24 h to 22.2 mmol/l glucose (22.2 G) and islets exposed for 24 h to 22.2 mmol/l glucose plus 1.0 mmol/l zofenoprilat (zof) or enalaprilat (enal), showing normalization of the ratio with zofenoprilat exposure. $*P < 0.05$ vs Ctrl.

Intriguingly, whereas both zofenoprilat and enalaprilat seemed to have a similar effect in reducing high glucose-induced activation of RAS (Fig. 1), the former had more marked positive effects on β -cell function, possibly due to a more potent antioxidative action (Figs 3 and 4). This is in agreement with previous observations on human umbilical vein endothelial cells and in hypertensive patients (33–35), and could be due to the presence of a sulphhydryl group in the structure of zofenoprilat (36). In this regard, the relatively small antioxidant activity of enalaprilat, as shown in our study, does not involve the PKC–NADPH oxidase pathway, which was completely unaffected by the drug. Another potential factor influencing the different results obtained with the two ACE inhibitors is their marked difference in lipophilia (37) and therefore their capability to penetrate into tissues and cells.

Our findings raise a number of questions. For example, the positive effects of ACE inhibition were seen after prolonged incubation with 22.2 but not 5.5 mmol/l glucose. It therefore remains to be established at which threshold of glucose concentration such effects are elicited. In addition, although we used ACE inhibitor concentrations similar to those used previously in *in vitro* experiments (33), dose–effect relationship studies need to be performed to better clarify at which concentration the action of zofenoprilat and/or enalaprilat begins. Furthermore, it is not known whether direct exposure of human islets to angiotensin II has some deleterious effects *per se*, or if angiotensin II receptor antagonists may have actions potentially synergistic with those of zofenoprilat. Finally, although zofenoprilat has been successfully used in clinical trials to treat arterial hypertension and some of its complications (37), the possible role of zofenoprilat in preventing type 2 diabetes has never been investigated in perspective studies.

In conclusion, the results of the present study have shown that molecules of the RAS are expressed in human islets, and are sensitive to the concentration of glucose. In addition, we found that ACE inhibition can protect human islets from the functional damage induced by prolonged exposure to high glucose. In this regard, zofenoprilat was more effective than enalaprilat. Finally, our findings have indicated that the positive effects of ACE inhibition were due, at least in part, to a reduction of oxidative stress. Altogether, the reported evidence suggests that the beneficial action of ACE inhibitors in human diabetes is due, to some extent, to the protective effects of these compounds on pancreatic β -cells.

Acknowledgements

This study has been supported in part by funding from the Ministero Italiano Università e Ricerca Scientifica

(Cofin 2003–2004) and Ministry of Health. The authors thank Dr Stefano Evangelista from Menarini Ricerche and Dr Mario Gori from Laboratori Guidotti SpA (Pisa, Italy) for providing the molecules tested in the study.

References

- 1 Zimmet P. The burden of type 2 diabetes: are we doing enough? *Diabetes Metabolism* 2003 **4** 6S9–6S18.
- 2 Winer N & Sowers JR. Epidemiology of diabetes. *Journal of Clinical Pharmacology* 2004 **44** 397–405.
- 3 Williamson DF, Vinicor F & Bowman BA. Centers For Disease Control And Prevention Primary Prevention Working Group. Primary prevention of type 2 diabetes mellitus by lifestyle intervention: implications for health policy. *Annals of Internal Medicine* 2004 **140** 951–957.
- 4 Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA & Nathan DM. Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *New England Journal of Medicine* 2002 **346** 393–403.
- 5 El-Atat F, McFarlane SI & Sowers JR. Diabetes, hypertension, and cardiovascular derangements: pathophysiology and management. *Current Hypertension Reports* 2004 **6** 215–223.
- 6 Sowers JR. Treatment of hypertension in patients with diabetes. *Archives in Internal Medicine* 2004 **164** 1850–1857.
- 7 Scheen AJ. Prevention of type 2 diabetes mellitus through inhibition of the renin–angiotensin system. *Drugs* 2004 **64** 2537–2565.
- 8 Sleight P. The HOPE study (heart outcomes prevention evaluation). *Journal of Renin Angiotensin Aldosterone Systems* 2000 **1** 18–20.
- 9 Papademetriou V, Farsang C, Elmfeldt D, Hofman A, Lithell H, Olofsson B, Skoog I, Trenkwalder P & Zanchetti A. Study on cognition and prognosis in the elderly. Stroke prevention with the angiotensin II type 1-receptor blocker candesartan in elderly patients with isolated systolic hypertension: the Study on Cognition and Prognosis in the Elderly (SCOPE). *Journal of the American College of Cardiologists* 2004 **44** 1175–1180.
- 10 Carvalho CR, Thirone AC, Gontijo JA, Velloso LA & Saad MJ. Effect of captopril, losartan, and bradykinin on early steps of insulin action. *Diabetes* 1997 **46** 1950–1957.
- 11 Sowers JR. Insulin resistance and hypertension. *American Journal of Physiology: Heart Circulation Physiology* 2004 **286** H1597–H1602.
- 12 Donnelly R. Angiotensin-converting enzyme inhibitors and insulin sensitivity: metabolic effects in hypertension, diabetes, and heart failure. *Journal of Cardiovascular Pharmacology* 1992 **20** (Suppl 11) S38–S44.
- 13 Kampf C, Lau T, Olsson R, Leung PS & Carlsson PO. Angiotensin II type 1 receptor inhibition markedly improves the blood perfusion, oxygen tension and first phase of glucose-stimulated insulin secretion in revascularised syngeneic mouse islet grafts. *Diabetologia* 2005 **48** 1159–1167.
- 14 Leung PS. Roles of the renin–angiotensin system and its blockade in pancreatic inflammation. *International Journal of Biochemistry Cell Biology* 2005 **37** 237–238.
- 15 Carlsson PO. The renin–angiotensin system in the endocrine pancreas. *Journal of the Pancreas* 2001 **2** 26–32.
- 16 Leung PS. The peptide hormone angiotensin II: its new functions in tissues and organs. *Current Protein Peptide Science* 2004 **5** 267–273.
- 17 Lau T, Carlsson PO & Leung PS. Evidence for a local angiotensin-generating system and dose-dependent inhibition of glucose-stimulated insulin release by angiotensin II in isolated pancreatic islets. *Diabetologia* 2004 **47** 240–248.

- 18 Lam KY & Leung PS. Regulation and expression of a renin-angiotensin system in human pancreas and pancreatic endocrine tumours. *European Journal of Endocrinology* 2002 **146** 567–572.
- 19 Lupi R, Dotta F, Marselli L, Del Guerra S, Masini M, Santangelo C, Patanè G, Boggi U, Piro S, Anello M, Bergamini E, Mosca F, Di Mario U, Del Prato S & Marchetti P. Prolonged exposure to free fatty acids has cytostatic and pro-apoptotic effects on human pancreatic islets: evidence that beta-cell death is caspase mediated, partially dependent on ceramide pathway, and Bcl-2 regulated. *Diabetes* 2002 **51** 1437–1442.
- 20 Marchetti P, Lupi R, Federici M, Marselli L, Masini M, Boggi U, Del Guerra S, Patanè G, Piro S, Anello M, Bergamini E, Purello F, Lauro R, Mosca F, Sesti G & Del Prato S. Insulin secretory function is impaired in isolated human islets carrying the Gly(972) → Arg IRS-1 polymorphism. *Diabetes* 2002 **51** 1419–1424.
- 21 Marchetti P, Del Guerra S, Marselli L, Lupi R, Masini M, Pollera M, Bugliani M, Boggi U, Vistoli F, Mosca F & Del Prato S. Pancreatic islets from type 2 diabetic patients have functional defects and increased apoptosis that are ameliorated by metformin. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 5535–5541.
- 22 Hug BA, Ahmed N, Robbins JA & Lazar MA. A chromatin immunoprecipitation screen reveals protein kinase C beta as a direct RUNX1 target gene. *Journal of Biological Chemistry* 2004 **279** 825–830.
- 23 Hsieh TJ, Fustier P, Zhang SL, Filep JG, Tang SS, Ingelfinger JR, Fantus IG, Hamet P & Chan JS. High glucose stimulates angiotensinogen gene expression and cell hypertrophy via activation of the hexosamine biosynthesis pathway in rat kidney proximal tubular cells. *Endocrinology* 2003 **144** 4338–4349.
- 24 Hsieh TJ, Zhang SL, Filep JG, Tang SS, Ingelfinger JR & Chan JS. High glucose stimulates angiotensinogen gene expression via reactive oxygen species generation in rat kidney proximal tubular cells. *Endocrinology* 2002 **143** 2975–2985.
- 25 Leiter LA & Lewanczuk RZ. Of the renin-angiotensin system and reactive oxygen species Type 2 diabetes and angiotensin II inhibition. *American Journal of Hypertension* 2005 **18** 121–128.
- 26 Robertson RP, Harmon J, Tran PO, Tanaka Y & Takahashi H. Glucose toxicity in beta-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. *Diabetes* 2003 **52** 581–587.
- 27 Park JI, Grant CM, Attfield PV & Dawes IW. The freeze-thaw stress response of the yeast *Saccharomyces cerevisiae* is growth phase specific and is controlled by nutritional state via the RAS-cyclic AMP signal transduction pathway. *Applied and Environmental Microbiology* 1997 **63** 3818–3824.
- 28 Kaneto H, Suzuma K, Sharma A, Bonner-Weir S, King GL & Weir GC. Involvement of protein kinase C beta 2 in c-myc induction by high glucose in pancreatic beta-cells. *Journal of Biological Chemistry* 2002 **277** 3680–3685.
- 29 Kapor-Drezgic J, Zhou X, Babazono T, Dlugosz JA, Hohman T & Whiteside C. Effect of high glucose on mesangial cell protein kinase C-delta and -epsilon is polyol pathway-dependent. *Journal of American Society of Nephrology* 1999 **10** 1193–1203.
- 30 Zhang Y, Bloem LJ, Yu L, Estridge TB, Iversen PW, McDonald CE, Schrementi JP, Wang X, Vlahos CJ & Wang J. Protein kinase C betaII activation induces angiotensin converting enzyme expression in neonatal rat cardiomyocytes. *Cardiovascular Research* 2003 **57** 139–146.
- 31 Shaw S, Wang X, Redd H, Alexander GD, Isaacs CM & Marrero MB. High glucose augments the angiotensin II-induced activation of JAK2 in vascular smooth muscle cells via the polyol pathway. *Journal of Biological Chemistry* 2003 **278** 30634–30641.
- 32 Lupi R, Del Guerra S, Tellini C, Giannarelli R, Coppelli A, Lorenzetti M, Carmellini M, Mosca F, Navalesi R & Marchetti P. The biguanide compound metformin prevents desensitization of human pancreatic islets induced by high glucose. *European Journal of Pharmacology* 1999 **364** 205–209.
- 33 Cominacini L, Pasini A, Garbin U, Evangelista S, Crea AE, Tagliacozzi D, Nava C, Davoli A & LoCascio V. Zofenopril inhibits the expression of adhesion molecules on endothelial cells by reducing reactive oxygen species. *American Journal of Hypertension* 2002 **15** 891–895.
- 34 Napoli C, Sica V, de Nigris F, Pignalosa O, Condorelli M, Ignarro LJ & Liguori A. Sulfhydryl angiotensin-converting enzyme inhibition induces sustained reduction of systemic oxidative stress and improves the nitric oxide pathway in patients with essential hypertension. *American Heart Journal* 2004 **148** e5.
- 35 Evangelista S & Manzini S. Antioxidant and cardioprotective properties of the sulphhydryl angiotensin-converting enzyme inhibitor zofenopril. *Journal of International Medical Research* 2005 **33** 42–54.
- 36 Ranadive SA, Chen AX & Serajuddin ATM. Relative lipophilicities and structural pharmacological considerations of various angiotensin-converting enzyme (ACE) inhibitors. *Pharmaceutical Research* 1992 **9** 1480–1486.
- 37 Borghi C, Bacchelli S, Degli Esposti D & Ambrosioni E. A review of the angiotensin-converting enzyme inhibitor, zofenopril, in the treatment of cardiovascular diseases. *Expert Opinion in Pharmacotherapy* 2004 **5** 965–977.

Received 20 May 2005

Accepted 7 November 2005