Glucose induces and leptin decreases expression of uncoupling protein-2 mRNA in human islets

James E.P. Brown, Steven Thomas[1], Janet E. Digby, Simon J. Dunmore*

Diabetes Research Group, Division of Biomedical Science, School of Applied Sciences, University of Wolverhampton, Wulfruna Street, Wolverhampton WV1 1SB, UK

Received 22 November 2001; accepted 15 January 2002

First published online 25 January 2002

Edited by Jacques Hanoune

Abstract Elevated islet uncoupling protein-2 (UCP-2) impairs β-cell function and UCP-2 may be increased in clinical obesity and diabetes. We investigated the effects of glucose and leptin on UCP-2 expression in isolated human islets. Human islets were incubated for 24 h with glucose (5.5–22 mmol/l) ± leptin (0–10 nmol/l). Some islet batches were incubated at high (22 mmol/l), and subsequently lower (5.5 mmol/l), glucose to assess reversibility of effects. Leptin effects on insulin release were also measured. Glucose dose-dependently increased UCP-2 expression in all islet batches, maximally by three-fold. This was not fully reversed by subsequently reduced glucose levels. Leptin decreased UCP-2 expression by up to 75%, and maximally inhibited insulin release by 47%, at 22 mmol/l glucose. This is the first report of UCP-2 expression in human islets and provides novel evidence of its role in the loss of β-cell function in diabetes.

Key words: β-Cell; Gene expression; Glucose toxicity; Type 2 diabetes

1. Introduction

Uncoupling protein-2 (UCP-2) is a homologue of UCP-1, a thermogenic mitochondrial protein expressed primarily in brown adipose tissue which uncouples respiration from ATP generation, and is expressed in many tissues including pancreatic islets [1]. Over-expression of UCP-2 in rat islets results in impaired glucose-stimulated insulin secretion (GSIS) [2]. Leptin has been shown to increase UCP-2 expression in rat islets [1] and this has been suggested as a possible mechanism for leptin's direct inhibition of insulin secretion, a phenomenon that has been observed in several studies [3,4] although a few have demonstrated stimulatory [5] or nil [6] effects of the hormone. It has been suggested [2] that UCP-2 decreases GSIS because it would be expected to reduce the cellular ATP/ADP ratio as a result of its proposed uncoupling properties. This would in turn reduce the capacity of increased glucose concentrations to stimulate insulin release through closure of the K_{ATP} channel.

In the present study we examined the effect of glucose and leptin on UCP-2 mRNA expression and insulin secretion in human islets. Initial studies (previously reported in brief [7]) provided a serendipitous indication that glucose alone is capable of increasing UCP-2 expression and we therefore studied this dose related effect which could provide an explanation for so-called glucose toxicity.

2. Materials and methods

2.1. Insulin release from human islets

Human islets isolated from pancreases of heart-beating donors, under both multi-centre and local ethical committee consents, were obtained from the islet transplant programmes at Leicester Royal Infirmary (UK) (batches designated HP) and Worcester Royal Infirmary (UK) (batches designated W). Islets were cultured in 24-well plates (100 islet equivalents (IE) per well), in RPMI 1640 medium containing 5.5 mmol/l D-glucose, 10% foetal bovine serum, 50 U/ml penicillin, 50 μg/ml streptomycin and 2 mmol/l L-glutamine, and allowed to attach for 48 h before treatments. Islets were then subsequently incubated in serum-free RPMI medium containing glucose (5.5, 11 or 22 mmol/l) and leptin (0, 0.1, 1, 5 and 10 nmol/l) for 20 h. Samples were collected, centrifuged at $500 \times g$ for 5 min to remove loose cells and assayed for insulin by standard radioimmunoassay.

2.2. UCP-2 mRNA expression

2.2.1. Incubations. Human islets from five different donors were incubated at 1000 IE/well in 6-well plates containing serum-free RPMI medium containing 5.5, 11 or 22 mmol/l glucose (G) for 24 h (in the case of two donors the 11 mmol/l G treatment was omitted because of limited islet availability). In a further series of experiments ('reversibility protocol'), islets from two separate donors were treated as before with the following modifications: (1) 5.5 mmol/l G, 24 h; (2) 22 mmol/l G, 24 h; (3) 5.5 mmol/l G 48 h; (4) 22 mmol/l G 48 h; (5) 22 mmol/l G 24 h followed by 24 h 5 mmol/l G.

2.2.2. RT-PCR. Total RNA from treated islets was extracted using a modified guanidinium thiocyanate–phenol method (TRIzol reagent; Sigma, Poole, Dorset, UK) and mRNA levels were quantified using a semi-quantitative RT-PCR method using oligo$(dT)_{15}$ primers and AMV reverse transcriptase (Hybaid, UK) for reverse transcription, and sequence-specific primers for UCP-2 (sense primer TCTGACCATGGTGCGTACTGA; antisense primer GACAATGGCATTACGAGCAAC) and the housekeeping gene β-actin (sense primer CGGGAAATCGTCGTGCGTGACAT; antisense primer GAACTTTGGGGGATGCTCGC) for cDNA amplification (using an annealing temperature of 60°C for 36 cycles). PCR products were electrophoresed through 2% agarose gel, stained with ethidium bromide and visualised under ultraviolet illumination. Band intensity was calculated densitometrically using SCION imaging software (NIH).

2.3. Statistical analysis

Comparisons between single pairs of data were made using Student's *t*-test for paired data. Multiple comparisons were made by one-way ANOVA with Dunnett's post-hoc analysis. In all cases $P \leq 0.05$ was taken as significant.

*Corresponding author. Fax: (44)-1902-322714.
E-mail address: s.dunmore@wlv.ac.uk (S.J. Dunmore).

[1] Present address: Cellular Pharmacology Group, School of Life Sciences, Keele University, Staffordshire, UK.

0014-5793/02/$22.00
PII: S0014-5793(02)02296-2

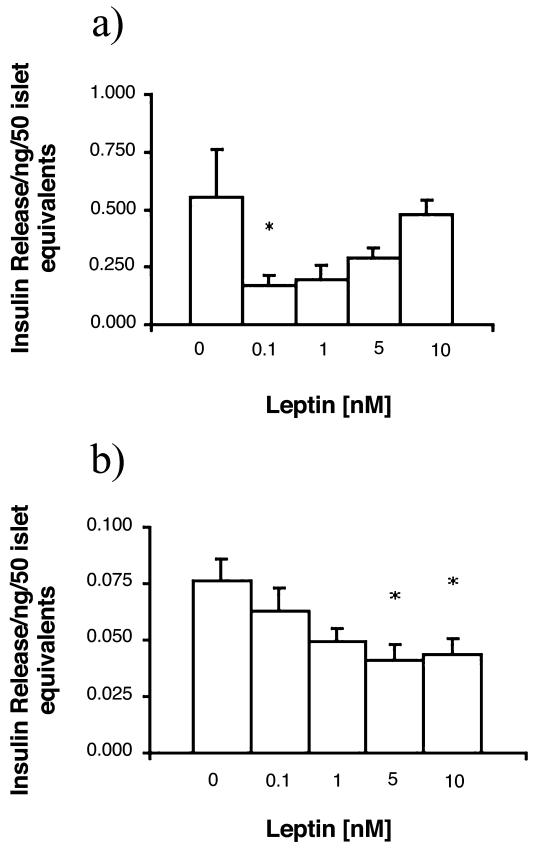

Fig. 1. Leptin inhibits insulin release from human islets. Recombinant human leptin caused a dose-dependent decrease in insulin release from human islets over 20 h. a: One batch (W100) exhibited a U-shaped response to leptin. b: Another three batches (representative sample batch HP598 shown) gave a linear reduction. Data expressed as means ± S.E.M., $n = 6–12$; *significant compared to control (0 leptin) $P < 0.05$ by ANOVA/Dunnett's post-hoc analysis.

3. Results

3.1. Effect of leptin on insulin release

Leptin had no significant effect on insulin release at low (5.5 mmol/l) or moderate (11 mmol/l) glucose concentrations on any of the batches of islets tested (results not shown). At high glucose levels leptin had a U-shaped effect in one batch of islets (with a significant maximal inhibition at 0.1 nmol/l leptin of 69%) but caused a dose-related inhibition in other batches (significant maximal inhibition at 5–10 nmol/l of 46–56%) (Fig. 1).

3.2. Effects of leptin on UCP-2 expression

In this series of experiments it was observed that there was comparatively little UCP-2 expression at low (5.5 mmol/l) glucose whereas at high (22 mmol/l) glucose there was a three-fold higher level of UCP-2 mRNA (Fig. 2). Leptin had no effect on UCP-2 expression at low glucose concentrations (results not shown). At high glucose concentrations, however, leptin induced a general dose-dependent reduction in UCP-2 mRNA expression from the high levels observed with 22 mmol/l glucose alone, although with some islet batches an initial stimulation was observed at the lowest (1.6 ng/ml) concentrations of leptin (Fig. 2).

3.3. Effect of glucose on UCP-2 expression

Further studies, initiated following the above observation that UCP-2 expression appeared to be higher in islets exposed to high glucose, demonstrated over several islet batches that this was a highly consistent effect. In all batches UCP-2 mRNA expression was dose-dependently increased by exposure to elevated glucose concentrations (Fig. 3). The threshold concentration at which this occurred varied between batches (e.g. expression appeared maximal at 11 mmol/l in HP614, but the effect was still increasing at this glucose concentration in other batches (HP602 and HP617)). The maximal increase was approximately three-fold ($P = 0.027$).

3.4. Reversibility of UCP-2 mRNA increase

Islets incubated in 22 mmol/l glucose for 24 h and then for a further 24 h at 5.5 mmol/l glucose still exhibit elevated levels of UCP-2 mRNA when compared with those incubated for the whole 48 h in 5.5 mmol/l, although the level is lower than that observed in islets incubated for 48 h in 22 mmol/l glucose (Fig. 4).

4. Discussion

This is the first report of UCP-2 expression in human islets. In addition this is the first study to demonstrate the novel finding that exposure to hyperglycaemic environments results in increased expression of UCP-2, although a recent preliminary report has described similar findings in rat islets [8]. Given that human islets are notoriously variable due to the wide variation in donor characteristics (e.g. age, disease, metabolic status, cause of death) the effect appears to be impressively consistent, occurring in all seven batches tested. Whilst the mechanism of glucose induction of UCP-2 is unclear as yet, the effect suggests a possible mechanism whereby the

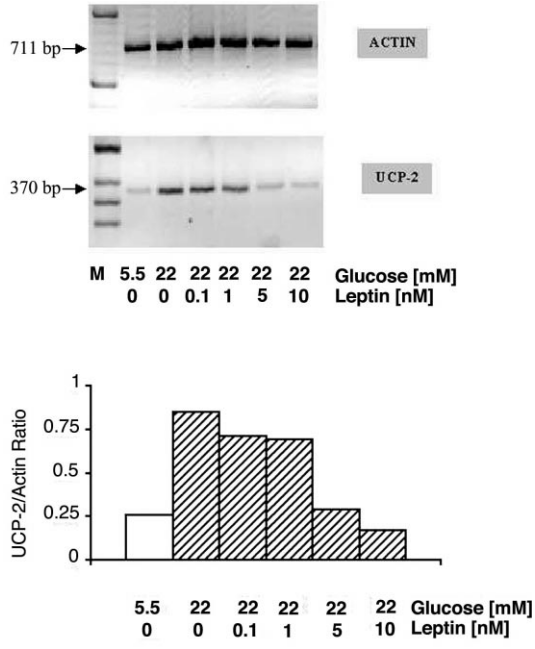

Fig. 2. Leptin inhibits UCP-2 mRNA expression induced by high glucose. Leptin caused a dose-dependent inhibition of UCP-2 mRNA expression which was elevated at 22 mmol/l glucose. Data shown are a representative sample from three sets of similar data obtained from different batches of human islets.

phenomenon of 'glucose toxicity' could occur. Prolonged exposure to high concentrations of glucose has been observed to impair GSIS in vivo (e.g. [9]) and in vitro (e.g. [10]). The work of Chan et al. [2,11] showing that over-expression of UCP-2 in rat islets causes impairment of GSIS coupled with the present findings would imply that glucose exposure causes an increase in UCP-2 expression which in turn impairs GSIS. UCP-2 is likely, by means of its probable ability to uncouple ATP production from respiration, to reduce cellular ATP/ADP ratios leading to a greater opening of K_{ATP} channels and thus reduced insulin secretion.

UCP-2 has also been reported to have pro-apoptotic properties ([12], also G. Williams (Keele University, UK), personal communication); the recent report that high glucose induces apoptosis in human islets [13] lends support to our suggestion that UCP-2 might have a role in this process. The possible uncoupling role of UCP-2 is controversial: the protein has been reported to cause proton leakage in transfected yeast [14] but it has been suggested that this is an artefact not observed with physiological levels of UCP-2 [15]. Chan et al. [11] have demonstrated however that, when over-expressed by adenovirus methodology, UCP-2 does lower mitochondrial membrane proton gradients and reduce ATP/ADP ratios. A recent report from the group of Lowell [16] confirms that UCP-2 knock-out mice have raised islet ATP and increased GSIS. Interestingly, the same study also demonstrated an increase in UCP-2 levels in leptin-deficient ob/ob mice; this is consistent with our finding that leptin prevents high glucose elevation of UCP-2 since in the absence of leptin this effect of hyperglycaemia would not be inhibited.

The role of UCP-2 as a modulator of GSIS is becoming increasingly apparent; a recent study suggested that exposure of rat islets to elevated non-esterified fatty acids, known to have both long-term (inhibitory) and short-term (stimulatory) effects on GSIS, increased UCP-2 protein expression [17]. Other reports have suggested that UCP-2 may have a protective function against glucotoxicity since it might be expected to reduce levels of reactive oxygen species in the cells [18].

It is significant that the increase in UCP-2 does not appear to be wholly reversible within 24 h, and this would imply that

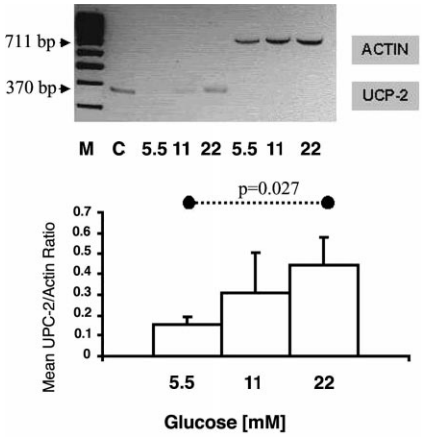

Fig. 3. Glucose induction of UCP-2. 24 h incubation with 5.5 ($n=5$), 11 ($n=3$), or 22 ($n=5$) mmol/l glucose causes a dose-dependent increase in UCP-2 mRNA measured by semi-quantitative RT-PCR (data shown as mean UCP-2/actin mRNA band density ratio ±S.E.M.). A representative RT-PCR gel is shown (one of five similar results). 11 mmol/l glucose was only tested in three of five batches due to limitations of islet supply.

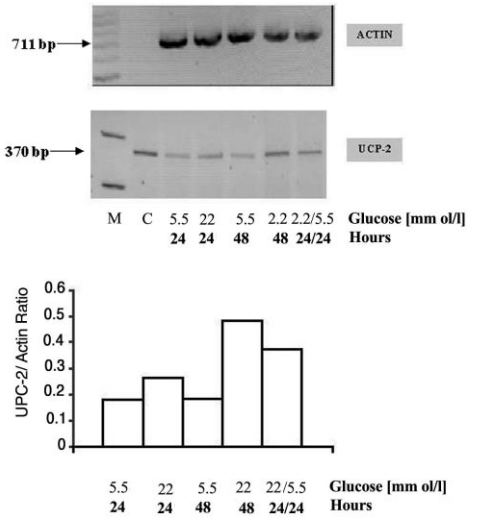

Fig. 4. Glucose induction of UCP-2 is not fully reversible. The 24 h increase in UCP-2 mRNA expression induced by 22 mmol/l glucose is not fully reversed when islets subsequently incubated in 5.5 mmol/l glucose for a further 24 h. Data shown are representative of two similar results.

the high glucose induction of UCP-2 expression and consequent impairment of GSIS may be a persistent effect. In order to provide support for our hypothesis, future work will need to focus on levels of UCP-2 protein following hyperglycaemic conditions using, for example, Western blotting and immunohistochemistry.

The finding that leptin inhibits insulin secretion is consistent with most previous studies (e.g. [3,4]), but not all [5,6]. The U-shaped response observed in one batch of islets is interesting and has also been reported in rat islets [19]: it is possible that this phenomenon would have been observed in other batches had the dose range been extended since human islets are likely to vary considerably in their dose response given the variability of donor previously referred to. Such a U-shaped response could explain the variability of leptin's effects reported in the literature. One possible explanation could lie in the cytokine-like nature of leptin's interaction with its receptor which requires homo-oligomerisation of the receptor by its ligand for activation of the JAK/STAT signalling pathway (discussed in review [20]). High leptin levels could saturate the receptor, preventing homo-oligomerisation.

Our data showing suppression of UCP-2 by leptin are at variance with those obtained by Unger and co-workers [1] who found that leptin increased UCP-2 mRNA. There were a number of differences in the experimental approaches however, since we used human, rather than rodent, islets and we observed the suppressive effect at high glucose concentrations. The present findings do not support the suggestion that leptin may inhibit insulin release and contribute to the pathogenesis of type 2 diabetes by increasing UCP-2.

In conclusion, we have demonstrated a highly reproducible effect of high glucose on UCP-2 gene expression which, in conjunction with the increasing body of published data suggesting a role for UCP-2 in modulation of glucose-stimulated insulin secretion and possibly β-cell apoptosis, suggests an important role for this protein in the mechanism of glucose toxicity. UCP-2 has potential as a pharmaceutical target in the prevention and treatment of β-cell failure in diabetes.

Acknowledgements: We are grateful to Dr Sue Swift (Leicester Royal Infirmary Islet Transplant Group) and Dr Karen Heald (Worcester Royal Infirmary Islet Transplant Group) for the supply of human islets.

References

[1] Zhou, Y.-T., Shimabukuro, M., Koyama, K., Lee, Y., Wang, M-Y., Trieu, F., Newgard, C.B. and Unger, R.H. (1997) Proc. Natl. Acad. Sci. USA 94, 6386–6390.
[2] Chan, C.B., MacDonald, P.E., Saleh, M.C., Johns, D.C., Marbàn, E. and Wheeler, M.B. (1999) Diabetes 48, 1482–1486.
[3] Kulkarni, R.N., Wang, Z.L., Wang, R.M., Hurley, J.D., Smith, D.M., Ghatei, M.A., Withers, D.J., Gardiner, J.V., Bailey, C.J. and Bloom, S.R. (1997) J. Clin. Invest. 100, 2729–2736.
[4] Emilsson, V., Liu, Y.L., Cawthorne, M.A., Morton, N.M. and Davenport, M. (1997) Diabetes 46, 313–316.
[5] Tanizawa, Y., Okuya, S., Ishihara, H., Asano, T., Yada, T. and Oka, Y. (1997) Endocrinology 138, 4513–4516.
[6] Leclercq-Meyer, V. and Malaisse, W.J. (1998) Mol. Cell. Endocrinol. 141, 111–118.
[7] Brown, J.E.P., Thomas, S., Digby, J.E. and Dunmore, S.J. (2001) Diabetes 50 (Suppl. 1), 363.
[8] Patane, G., Anello, M., Piro, S., Purrello, F. and Rabuazzo, A.M. (2001) Diabetes 50 (Suppl. 1), 363.
[9] Harmon, J.S., Gleason, C.E., Tanaka, Y., Oseid, E.A., Hunter-Berger, K.K. and Robertson, R.P. (1999) Diabetes 48, 1995–2000.
[10] Moran, A., Zhang, H.J., Olson, L.K., Harmon, J.S., Poitout, V. and Robertson, R.P. (1997) J. Clin. Invest. 99, 534–539.
[11] Chan, C.B., De Leo, D., Joseph, J.W., McQuaid, T.S., Ha, X.F., Xu, F., Tsushima, R.G, Pennefather, P.S., Salapatek, A.M. and Wheeler, M.B. (2001) Diabetes 50, 1302–1310.
[12] Voehringer, D.W., Hirschberg, D.L., Xiao, J., Lu, Q., Roederer, M., Lock, C.B., Herzenberg, L.A., Steinman, L. and Herzenberg, L.A. (2000) Proc. Natl. Acad. Sci. USA 97, 2680–2685.
[13] Federici, M., Hribal, M., Perego, L., Ranalli, M., Caradonna, Z., Perego, C., Usellini, L., Nano, R., Bonini, P., Bertuzzi, F., Marlier, L.N., Davalli, A.M., Carandente, O., Pontiroli, A.E., Melino, G., Marchetti, P., Lauro, R., Sesti, G. and Folli, F. (2001) Diabetes 50, 1290–1301.
[14] Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., Bouillaud, F., Seldin, M.F., Surwit, R.S., Ricquier, D. and Warden, C.H. (1997) Nature Genet. 15, 269–272.
[15] Stuart, J.A., Harper, J.A., Brindle, K.M., Jekabsons, M.B. and Brand, M.D. (2001) J. Biol. Chem. 276, 18633–18639.
[16] Zhang, C., Baffy, G., Perret, P., Krauss, S., Peroni, O., Grujic, D., Hagen, T., Vidal-Puig, A.J., Boss, O., Kim, Y., Zheng, X.X., Wheeler, M.B., Shulman, G.I., Chan, C.B. and Lowell, B.B. (2001) Cell 105, 745–755.
[17] Lameloise, N., Muzzin, P., Prentki, M. and Assimacopoulos-Jeannet, F. (2001) Diabetes 50, 803–809.
[18] Arsenijevic, D., Onuma, H., Pecqueur, C., Raimbault, S., Manning, B.S., Miroux, B., Couplan, E., Alves-Guerra, M.C., Goubern, M., Surwit, R., Bouillaud, F., Richard, D., Collins, S. and Ricquier, D. (2000) Nature Genet. 26, 435–439.
[19] Pallett, A.L., Morton, N.M., Cawthorne, M.A. and Emilsson, V. (1997) Biochem. Biophys. Res. Commun. 238, 267–270.
[20] Kieffer, T.J. and Habener, J.F. (2000) Am. J. Physiol. Endocrinol. Metab. 278, E1–E14.