Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Steroid Biochemistry & Molecular Biology 89–90 (2004) 121–125
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

Expression of 25-hydroxyvitamin D_3-1α-hydroxylase in pancreatic islets☆

Rosemary Bland[a,*], Danijela Markovic[a], Claire E. Hills[a], Susan V. Hughes[b], Susan L.F. Chan[c], Paul E. Squires[a], Martin Hewison[b]

[a] *Department of Biological Sciences, Biomedical Research Institute, The University of Warwick, Coventry CV4 7AL, UK*
[b] *Division of Medical Sciences, Queen Elizabeth Hospital, Birmingham B15 2TT, UK*
[c] *Nottingham University Medical School, Institute of Cell Signalling, The University of Nottingham, University Park, Nottingham NG7 2RD, UK*

Abstract

A number of studies have suggested that Vitamin D has a potential role in the development/treatment of diabetes. These effects may be mediated by circulating levels of 1α,25$(OH)_2D_3$, but local production of 1α,25$(OH)_2D_3$, catalysed by the enzyme 25-hydroxyvitamin D_3-1α-hydroxylase (1α-OHase), is also likely to be important. RT-PCR analyses demonstrated that both isolated rat islets and MIN6 cells (mouse insulin-secreting cell line, characteristic of β cells) expressed 1α-OHase mRNA. The transcript in both cell types was similar to that seen in HKC-8 cells (a renal cell line, which expresses 1α-OHase). Western blot analysis and immunolocalisation identified 1α-OHase protein in MIN6 cells and human pancreatic tissue. In addition, suspensions of rat islets were able to convert [^{3}H]-25-hydroxyvitamin D_3 to [^{3}H]-1α,25$(OH)_2D_3$, demonstrating 1α-OHase activity. Both cell systems expressed the Vitamin D receptor and 1α,25$(OH)_2D_3$ (50 nM) evoked a rapid rise in $[Ca^{2+}]_i$ in MIN6 cells. This data clearly demonstrates islets are able to produce 1α,25$(OH)_2D_3$ and respond rapidly to treatment with 1α,25$(OH)_2D_3$. Therefore, we would postulate that local production of 1α,25$(OH)_2D_3$ maybe an important autocrine link between Vitamin D status and pancreatic function.

Keywords: 25-Hydroxyvitamin D_3-1α-hydroxylase; Vitamin D; 1α,25-Dihydroxyvitamin D_3; Islets; Diabetes; Calcium

1. Introduction

A number of in vitro and in vivo studies have indicated that Vitamin D may have a role in normal pancreatic function and the prevention/treatment of diabetes. Vitamin D deficient rats secrete insufficient insulin in response to a glucose challenge. Insulin secretion and β cell function is restored following correction of Vitamin D levels [1,2]. Recent data from two studies in Scandinavia have suggested a link between Vitamin D supplementation during pregnancy and protection of the offspring from type 1 diabetes [3,4]. However, the mechanism by which Vitamin D influences islet cell function is as yet unknown. Islets have been shown to express the Vitamin D receptor (VDR) and a Vitamin D response element has been recently identified in the human insulin receptor gene promoter [5,6]. Polymorphisms in the VDR have been linked with both type 1 and type 2 diabetes [7,8] and insulin secretion is impaired in the VDR KO mouse [9].Some of the actions of Vitamin D may be due to circulating levels of 1,25-dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$), but it is becoming increasingly apparent that local production of 1α,25$(OH)_2D_3$ is important, where it may regulate tissue function in a paracrine or autocrine fashion. Synthesis of 1α,25$(OH)_2D_3$ from 25$(OH)D_3$ is catalysed by the mitochondrial cytochrome P450 enzyme 25-hydroxyvitamin D_3-1α-hydroxylase (1α-OHase). Although classically located in the kidney, it is now known that 1α-OHase is expressed in a number of extra-renal tissues [10]. Additionally, polymorphisms in the Vitamin D binding protein have also been linked to type 2 diabetes [11] and this may indicate a decreased supply of 25$(OH)D_3$ to the pancreas. To examine the expression of 1α-OHase we studied isolated rat islets. We also utilised the mouse insulinoma cell line, MIN6, which is an insulin secreting cell line with characteristics similar to β cells and isolated islets [12].

☆ Presented at the 12th Workshop on Vitamin D (Maastricht, The Netherlands, 6–10 July 2003).

* Corresponding author. Fax: +44-2476523701.
E-mail address: rosemary.bland@warwick.ac.uk (R. Bland).

0960-0760/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2004.03.115

2. Materials and methods

2.1. Cell culture

Rat islets were isolated following standard collagenase digestion. MIN6 cells (passage 38–44) and HKC-8 cells were maintained as previously described [13,14] For immunocytochemistry and microfluorimetry cells were seeded onto 3-aminopropyltriethoxysilane-coated glass coverslips.

2.2. Measurement of 1α-OHase activity

1α-OHase activity was assessed by incubating rat islets with 3.75 nM [^{3}H]-25(OH)D_3 for 4 h and 37 °C. Analysis of 1α,25$(OH)_2D_3$ production was performed using previously validated thin layer chromatography methods [14].

2.3. RNA extraction and RT-PCR

RNA was prepared using the GenElute Mammalian total RNA Kit (Sigma) according to the manufactures protocol. Reverse transcription (RT) of 1 μg of total RNA was performed using a Promega System. PCR primers specific for 1α-OHase and VDR were used [15,16].

2.4. Western blot analyses and immunolocalisation

Total cell lysates were subjected to SDS-PAGE (5 μg per lane) and electroblotted onto Immobilon P membrane as described previously [14]. Filters were analysed with specific polyclonal antibodies against the human VDR (Cambridge BioScience, UK) and the mouse 1α-OHase (The Binding Site Ltd., UK). Proteins were detected by enhanced chemiluminescent (Amersham). Immunohistochemistry and immunocytochemistry were performed using standard techniques [10,17]. Proteins were visualised by 3,3′diaminobenzidine or Alexa fluorescent secondary antibodies.

2.5. Single-cell microfluorimetry

MIN6 cell clusters were loaded with the Ca^{2+}-fluorophore Fura-2/AM (2.5 μM, 20 min, 37 °C). Experiments were carried out as previously described [18]. Cells were illuminated alternatively at 340 and 380 nm using Metafluor Imaging Workbench (Universal Imaging Corp. Ltd., Marlow Bucks, UK). Emitted light was filtered using a 510 nm long-pass barrier filter and detected using a CoolSnap HQ CCD camera (Roper Scientific). Changes in the emission intensity of Fura-2 expressed as a ratio of dual excitation were used as

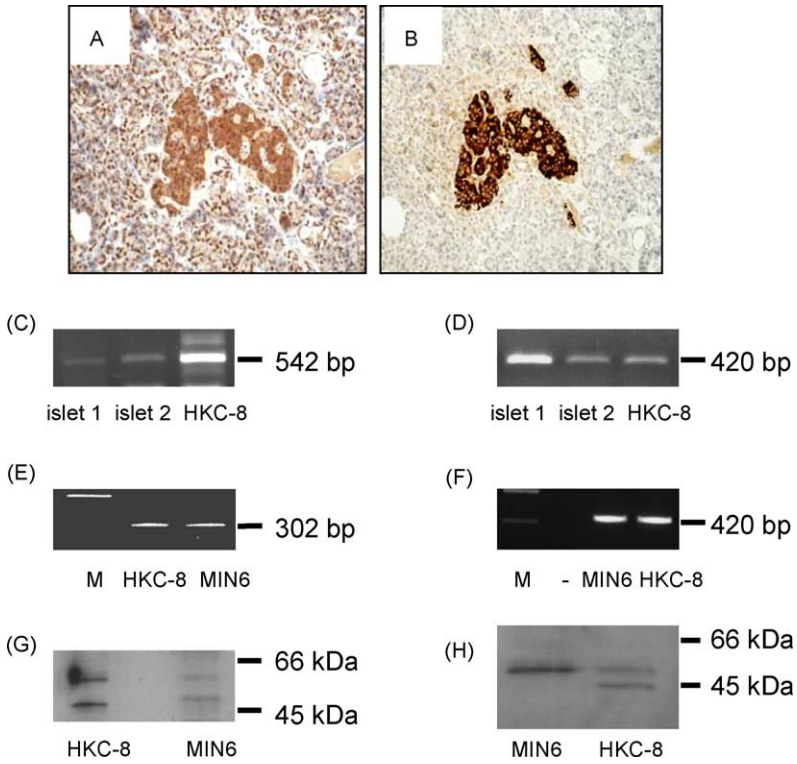

Fig. 1. Expression of 1α-OHase and VDR mRNA and protein in pancreatic islets. (A) Immunohisto-chemical analysis (IHC) of 1α-OHase. (B) IHC analysis of insulin in sections of human pancreata (positive staining brown, both X200). RT-PCR analysis indicated 1α-OHase and VDR mRNA in rat islets (C and D) and HKC-8 and MIN6 cells (E and F), respectively. Western blot analysis of protein confirmed the presence of 1α-OHase and VDR protein in both cell lines (G and H).

an indicator of changes in $[Ca^{2+}]_i$, using established procedures [19]. Data were collected every 3 s for multiple regions of interest in any one field of view. All records have been corrected for background fluorescence (determined from cell-free coverslip).

3. Results

3.1. Expression of 1α-OHase

Initial studies confirmed the expression of 1α-OHase protein in human pancreas, which was coincident with the expression of insulin (Fig. 1A and B). Further studies in isolated rat islets and MIN6 cells demonstrated 1α-OHase mRNA (Fig. 1C and E). RT-PCR revealed a similar size transcript to that seen in HKC-8 cells, a human proximal convoluted tubule cell line known to express 1α-OHase mRNA and protein [15]. Immunocytochemistry and western blot analysis demonstrated 1α-OHase protein expression in MIN6 cells (Figs. 1G and 2B). Although both 1α-OHase and insulin expression were cytoplasmic, profile data of the intensity of fluorescence suggested that expression did not overlap (Fig. 2). Analysis of $[^3H]$-25(OH)D_3 metabolism by intact rat islets demonstrated production of 1α,25(OH)$_2D_3$. Basal enzyme activity was calculated as 45 ± 12 (S.E.M.) fmoles of 1α,25(OH)$_2D_3$ produced per hour per 1000 islets. Further RT-PCR analysis demonstrated expression of VDR in rat islets and MIN6 cells (Fig. 1D and F). Immunocytochemistry (data not shown) and western blot analysis confirmed expression of VDR protein in MIN6 cells (Fig. 1H).

3.2. The effect of 1α,25(OH)$_2D_3$ on cytosolic calcium ($[Ca^{2+}]_i$) in insulin-secreting cells

At basal glucose concentrations (2 mM) the acute application of 1α,25(OH)$_2D_3$ (50 nM) evoked a marked rise in $[Ca^{2+}]_i$ (22/33 cells (67%) from four separate experiments) (Fig. 3). The response was slow in onset, with a delay between application of the agonist and the initial rise in $[Ca^{2+}]_i$ of 148 ± 21 s (mean ± S.E.M.) ($n = 22$ responsive cells). The response persisted beyond the removal of the stimulus in greater than 60% of the responsive cells examined. The amplitude of the 1α,25(OH)$_2D_3$-evoked change in $[Ca^{2+}]_i$

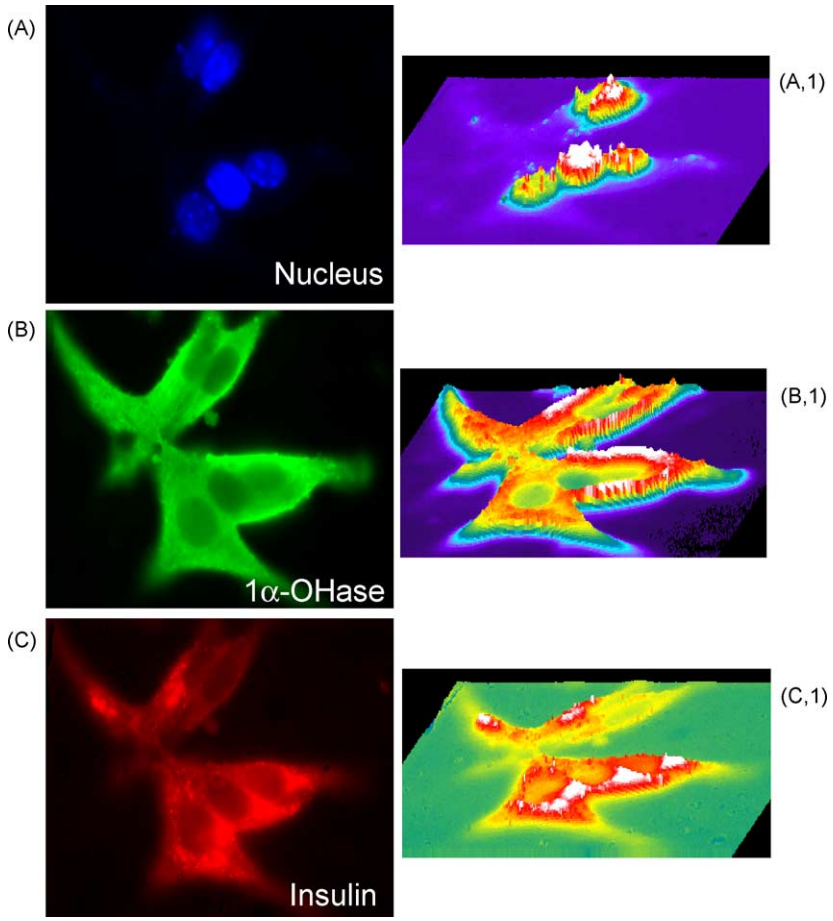

Fig. 2. Immunocytochemical analysis of 1α-OHase and insulin in MIN6 cells. (A) cells stained with DAPI indicating the nucleus. (B) Indicates positive staining for 1α-OHase. (C) Demonstrates insulin. Panels A,1; B,1 and C,1 are profile data of the intensity of fluorescence for DAPI, 1α-OHase and insulin staining, respectively and indicate that 1α-OHase and insulin are cytoplasmic, but not co-localised.

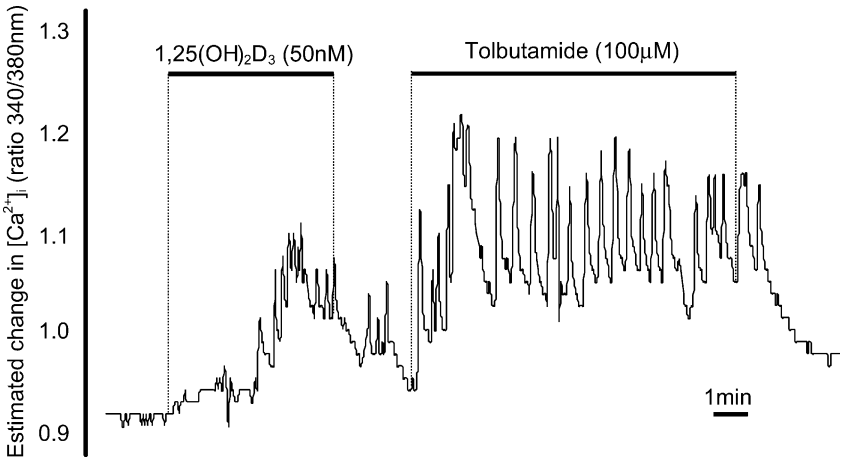

Fig. 3. 1α,25(OH)$_2$D$_3$ elevated $[Ca^{2+}]_i$ in insulin-secreting cells. The response was slow in onset, but once initiated persisted in the continued presence of the agonist. The sulphonylurea tolbutamide elicited a characteristic rapid increase in $[Ca^{2+}]_i$ that oscillated above a mean elevated plateau.

0.19 ± 0.02 (mean ± S.E.M.) ($n = 22$ responsive cells) was 59% of that evoked by the sulphonylurea tolbutamide (100 μM; 0.32 ± 0.03 (mean ± S.E.M.) ($n = 9$ responsive cells), $P < 0.05$ (unpaired *t*-test).

4. Discussion

Previous studies have clearly suggested that Vitamin D may have an important role in the normal function of pancreatic islets. Although the mechanisms involved are not fully understood, the pancreas responds to circulating levels of 1α,25(OH)$_2$D$_3$ [2]. Pancreatic tissue expresses the VDR [5] and VDR immunoreactivity in MIN6 cells appeared to be predominantly located in the nucleus, although expression was more diffuse than that seen in HKC-8 cells where the VDR was found exclusively in the nucleus (data not shown). However, evidence from other extra-renal tissues would indicate that the local production of 1α,25(OH)$_2$D$_3$ may regulate tissue function in an autocrine or paracrine fashion [16,20]. Initial RT-PCR, immunohistochemistry and western blot analyses confirmed the presence of 1α-OHase in islets.

The acute application of 1α,25(OH)$_2$D$_3$ evoked a marked increase in cytosolic Ca^{2+} in insulin-secreting MIN6 cells. The characteristic response profile was one of oscillatory Ca^{2+}-transients above a mean elevated plateau. Although the onset of the evoked rise in $[Ca^{2+}]_i$ was relatively slow, as compared to that evoked by the sulphonylurea, tolbutamide, the changes were sufficiently rapid to suggest a non-genomic mechanism of action. Rapid changes in Ca^{2+} have been seen previously in other islet models [21] and are more likely to reflect small changes in local concentrations of 1α,25(OH)$_2$D$_3$ rather than gross circulating levels which will remain relatively constant.

We would therefore like to postulate that both the expression and activity of 1α-OHase in islets and rapid response of insulin-secreting cells to 1α,25(OH)$_2$D$_3$ would indicate that local production of 1α,25(OH)$_2$D$_3$ may be an important mediator of islet function.

Acknowledgements

Microfluorimetric equipment was funded by Diabetes UK (PES; BDA:RD01:0002216). Professor J.-I. Miyazaki (University of Osaka, Japan) kindly provided the MIN6 cell line. CEH is a BBSRC funded PhD Student.

References

[1] B.S. Chertow, W.I. Sivitz, N.G. Baranetsky, M.B. Cordle, H.F. DeLuca, Islet insulin release and net calcium retention in vitro in Vitamin D-deficient rats, Diabetes 35 (7) (1986) 771–775.
[2] C. Cade, A.W. Norman, Rapid normalization/stimulation by 1,25-dihydroxyvitamin D$_3$ of insulin secretion and glucose tolerance in the Vitamin D-deficient rat, Endocrinology 120 (1987) 1490–1497.
[3] L.C. Stene, J. Ulriksen, P. Magnus, G. Joner, Use of cod liver oil during pregnancy associated with lower risk of type I diabetes in the offspring, Diabetologia 43 (9) (2000) 1093–1098.
[4] E. Hypponen, E. Laara, A. Reunanen, M.-R. Jarvelin, S.M. Virtanen, Intake of Vitamin D and risk of type 1 diabetes: a birth-cohort study, Lancet 358 (2001) 1500–1503.
[5] J.A. Johnson, J.P. Grande, P.C. Roche, R. Kumar, Immunohistochemical localization of the 1,25 (OH)$_2$D$_3$ receptor and calbindin D28k in human and rat pancreas, Am. J. Physiol. 267 (1994) 356–360.
[6] B. Maestro, N. Davila, M.C. Carranza, C. Calle, Identification of a Vitamin D response element in the human insulin receptor gene promoter, J. Steroid Biochem. Mol. Biol. 84 (2–3) (2003) 223–230.
[7] M.A. Pani, M. Knapp, H. Donner, J. Braun, M.P. Baur, K.H. Usadel, K. Badenhoop, Vitamin D receptor allele combinations influence genetic susceptibility to type 1 diabetes in Germans, Diabetes 49 (3) (2000) 504–507.
[8] J.R. Ortlepp, J. Lauscher, R. Hoffmann, P. Hanrath, H.G. Joost, The Vitamin D receptor gene variant is associated with the prevalence of type 2 diabetes mellitus and coronary artery disease, Diab. Med. 18 (10) (2001) 842–845.

[9] U. Zeitz, K. Weber, D.W. Soegiarto, E. Wolf, R. Balling, R.G. Erben, Impaired insulin secretory capacity in mice lacking a functional Vitamin D receptor, FASEB J. 17 (3) (2003) 509–511.
[10] D. Zehnder, R. Bland, M.C. Williams, R.W. McNich, A.J. Howie, P.M. Stewart, M. Hewison, Extra-renal expression of 25-hydroxyvitamin D_3 1α-hydroxylase, J. Clin. Endocrinol. Metab. 86 (2001) 888–894.
[11] L.J. Baier, A.M. Dobberfuhl, R.E. Pratley, R.L. Hanson, C. Bogardus, Variations in the Vitamin D-binding protein (Gc locus) are associated with oral glucose tolerance in non-diabetic Pima Indians, J. Clin. Endocrinol. Metab. 83 (8) (1998) 2993–2996.
[12] J. Miyazaki, K. Araki, E. Yamato, H. Ikegami, T. Asano, Y. Shibasaki, Y. Oka, K. Yamamura, Establishment of a pancreatic beta cell line that retains glucose-inducible insulin secretion: special reference to expression of glucose transporter isoforms, Endocrinology 127 (1) (1990) 26–32.
[13] S.J. Persaud, P.M. Jones, H.M. Roderigo-Milne, A.M.J. Buchan, P.E. Squires, Calcium-dependent translocation of cytosolic phospholipase A_2 in pancreatic β cells, Biochem. Biophys. Res. Commun. 300 (4) (2003) 889–893.
[14] R. Bland, E.A. Walker, S.V. Hughes, P.M. Stewart, M. Hewison, Constitutive expression of 25-hydroxyvitamin D_3-1α-hydroxylase in a transformed human proximal tubule cell line: evidence for direct regulation of Vitamin D metabolism by calcium, Endocrinology 140 (1999) 2027–2034.
[15] R. Bland, D. Zehnder, S.V. Hughes, P.M. Ronco, P.M. Stewart, M. Hewison, Regulation of Vitamin D-1α-hydroxylase in a human cortical collecting duct cell line, Kidney Int. 60 (2001) 1277–1286.
[16] M. Hewison, L. Freeman, S.V. Hughes, K.N. Evans, R. Bland, A.G. Eliopoulos, M.D. Kilby, P.A.H. Moss, R. Chakraverty, Differential regulation of Vitamin D receptor and its ligand in human monocyte-derived dendritic cells, J. Immunol. 170 (2003) 5382–5390.
[17] P.E. Squires, R.M. Meloche, A.M.J. Buchan, Bombesin-evoked gastrin release and calcium signaling in human antral G cells in culture, Am. J. Physiol. 276 (1999) G227–G237.
[18] P.E. Squires, R.F.L. James, N.J.M. London, M.J. Dunne, ATP-induced intracellular Ca^{2+} signals in isolated human insulin-secreting cells, Pflugers Arch. 427 (1994) 181–183.
[19] B. Grynkiewicz, M. Poenie, R.Y. Tsien, A new generation of Ca^{2+} indicators with greatly improved fluorescence properties, J. Biol. Chem. 260 (1985) 3440–3450.
[20] D. Zehnder, R. Bland, R.S. Chana, D.C. Wheeler, P.M. Stewart, M. Hewison, 1,25-dihydroxyvitamin D_3 synthesis in human endothelial cells is regulated by inflammatory cytokines: a novel autocrine determinant of vascular cell adhesion, J. Am. Soc. Nephrol. 13 (2002) 621–629.
[21] I. N Sergeev, W. B Rhoten, 1,25-dihydroxyvitamin D_3 evokes oscillations of intracellular calcium in a pancreatic beta cell line, Endocrinology 136 (7) (1995) 2852–2861.