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Regulation by glucose of the biosynthesis of PC2, PC3 and proinsulin in (ob/ob) mouse Islets of Langerhans

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Abstract The prohormone convertases PC2 and PC3 have been shown to catalyze the processing of proinsulin to insulin in pancreatic β-cells. In these studies we have compared the effects of glucose on PC2 and PC3 biosynthesis in freshly isolated islets from normal and hyperglycemic (ob/ob) mice. In contrast to normal islets [Alarcón, et al. (1993) J. Biol. Chem. 268, 4276] the biosynthesis of both PC2 and PC3 is stimulated by glucose, parallel to the stimulation of proinsulin in the (ob/ob) islets. Inhibition of PC2 biosynthesis by glucose in normal islet non β-cells may obscure stimulation of PC2 biosynthesis in normal islet β-cells.

Key words: Prohormone convertase; β-Cell; Translational control; Precursor processing

1. Introduction

The biosynthesis and release of insulin from β-cells in the Islets of Langerhans is regulated by glucose [1,2] more specifically by the metabolism of glucose [3]. Insulin, like many hormones, is initially synthesized as a larger polypeptide and then post-translationally modified prior to secretion [4]. A new superfamily of serine endoproteases, all related to yeast kexin and subtilisin, has recently been described [5] and these proteases participate in the cleavage of prohormones, neuropeptides and other precursor proteins, predominantly at paired basic amino acids, to release a variety of products [6,7]. Two of these enzymes, the prohormone convertases PC2 and PC3, are responsible for the conversion of proinsulin to insulin [8,9]. Although both enzymes have been colocalized by immunocytochemistry in β-cells [10], recent studies have suggested that PC3 is more abundant than PC2 in β-cells [11]. On the other hand, non-β-cells in islets, primarily the α-cells, have been shown to express high levels of PC2 but no detectable levels of PC3 [12]. Alarcón and co-workers have shown that the biosynthesis of PC3, but not PC2, is regulated by glucose in cultured rat islets [13]. It is possible, however, that glucose stimulates the biosynthesis of PC2 in normal islet β-cells, but this effect is masked by opposing effects of glucose on the expression of PC2 in islet non-β-cells, particularly the α-cells. Thus, while the expression of PC2 in β-cells may be increased, it may be decreased in the α-cells resulting in no apparent change in its overall expression in whole islets.

To better understand the biosynthetic regulation of PC2 we have used freshly isolated islets from Bar Harbor obese mice. The morphology of islets in (ob/ob) mice differs markedly from that of normal islets, in that these islets contain a much higher ratio of β-cells to α-cells. Moreover, the activity of the α-cells is suppressed in the obese mouse by the hyperglycemia [14] . Therefore, (ob/ob) islets may demonstrate more clearly how the expression of PC2 is regulated in pancreatic β-cells.

2. Materials and methods

2.1. Antisera

PC2 antiserum referred to as PC2 pep 4 #3 bleed 10/26/92 was raised in rabbits using a peptide corresponding to amino acids 611–636 at the carboxyl terminus of PC2. This peptide was conjugated to KLH via an added Cys residue at its N-terminus and the conjugate was injected into rabbits, essentially as previously described [10]. PC3 antibodies 2B6 and 2B7 (kindly provided by Iris Lindberg) recognized the N-terminal region of mature PC3.

2.2. Animal models

Islets of Langerhans were isolated from 20–40 gram CD1 mice from Charles Rivers Labs and 40–60 gram KSJ (ob/ob) Bar Harbor mice from Jackson Labs as previously described [15].

2.3. Islet incubation

The islets were divided into batches of 150–200 and were preincubated at 37°C for 60 min in 0.2 ml of modified Hank's buffer containing either 0.5 mg/ml or 5 mg/ml glucose and all amino acids except Cys, Met, Leu, and Phe. The islets were then labeled for 45 min with 100 μCi each of [^{35}S]methionine, [^{35}S]cysteine, [^{3}H]leucine and [^{3}H]phenylalanine in 0.2 ml of modified Hank's buffer containing the same concentrations of glucose. In experiments 1–4 the islets were chased under similar conditions for varying periods of time. After incubation the islets were centrifuged briefly at 14,000 RPM and the labeling media was removed. The islets were washed once in Hank's buffer.

2.4. Immunoprecipitation of PC2 and PC3

Labeled islets were suspended in 300 μl of immunoprecipitation buffer (2.5 μg/ml poly-L-Lysine, 50 mM $NaHPO_4$, 1 mM EDTA, 0.3 mM PMSF, 0.1% Triton X-100, 0.5% nonidet P40 and 0.9% NaCl pH 7.4) sonicated, and centrifuged at 14,000 RPM for 2 min. The supernatant was transferred to a fresh tube and 2 μl of PC2 antiserum was added. The tubes were incubated overnight at 4°C. The next day 20 μl of IgG Sorb (The Enzyme Center, MA) was added to each tube and incubated on ice for 15 min. The tubes were then centrifuged for 5 min at 14,000 RPM. The supernatant was saved for PC3 immunoprecipitation; 2 μl of PC3 antiserum was added and the immunoprecipitation procedure repeated. The immunoprecipitated pellets were washed 5 times with immunoprecipitation buffer and centrifuged at 14,000 RPM for 2 min. Prior to the last wash the samples were transferred to fresh tubes. After boiling 5 min in loading buffer pellets were subjected to one-dimensional SDS electrophoresis on a 7.5% polyacrylamide gel and visualized by fluorography [16].

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2.5. Densitometric analysis

Fluorographs were analyzed on a LKB Ultrascan XL enhanced laser densitometer. Each band was scanned several times. The area under the peaks was calculated by weighing and then averaged together.

2.6. Proinsulin biosynthesis analysis

Prior to immunoprecipitation, 30 μl aliquots were taken from each islet lysate for insulin immunopurification using guinea pig anti-insulin antisera coupled to Affi-Gel 10 agarose beads (Bio-Rad) as previously described [10]. Proinsulin and insulin were resolved by gel filtration on a P-30 column.

3. Results

3.1. Glucoregulation of PC3, PC2 and proinsulin biosynthesis in normal mouse islets

Pancreatic islets isolated from CD1 mice were radiolabelled at low (0.5 mg/ml) or high (5.0 mg/ml) glucose concentration for 45 min and then chased for periods up to 4 h. PC2 and PC3 were sequentially immunoprecipitated from the islet lysates, using antisera specific for these enzymes, analyzed on SDS PAGE (Fig. 1, panel A), and quantitated using densitometric analysis (Table 1). Proinsulin was quantitated by counting the radioactive fractions from a P-30 gel filtration column (Fig. 1, panel B). The biosynthesis of both PC3 and proinsulin was markedly increased, 6.8- and 7.5-fold, respectively, when the islets were incubated at high glucose concentration (Fig. 1, panel A, Table 1). Furthermore, about 80% of the proinsulin was converted to insulin (Fig. 1, panel B) after 2 h of chase incubation. However, the biosynthesis of PC2 was not significantly increased by incubating these islets in high glucose (5 mg/ml) (Fig. 1, panel A), in confirmation of the results of Alarcón [13].

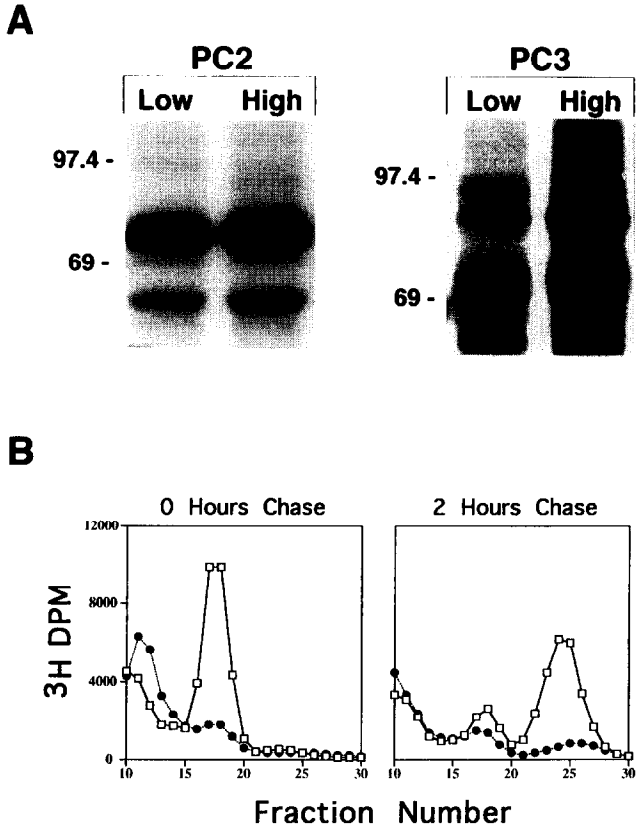

Fig. 1. Glucoregulation of PC2 and PC3 expression in normal mouse islets. Islets from CD1 mice were preincubated for 60 minutes, labeled with [^{35}S]Met, [^{35}S]Cys, [^{3}H]Leu, and [^{3}H]Phe for 45 min, and chased for 0 or 2 h in media containing 0.5 mg/ml or 5.0 mg/ml glucose. Islets were lysed and lysates were subjected to immunoprecipitation with PC2 and PC3 antisera, sequentially. (A) Immunoprecipitates were analyzed by SDS-PAGE and fluorography. *Low*: material immunoprecipitated from islets cultured in 0.5 mg/ml glucose, *High*: material immunoprecipitated from islets cultured in 5.0 mg/ml glucose. The migration of molecular weight markers is shown on the left side. (B) The conversion of proinsulin to insulin in islets incubated in 5.0 mg/ml glucose containing medium was analyzed by gel filtration. Proinsulin-related material was eluted in fractions 16–19, insulin in fractions 22–27. Solid circles represent radioactivity of lysates of islets incubated in 0.5 mg/ml glucose. Open squares represent radioactivity of lysates of islets incubated in 5.0 mg/ml glucose.

Table 1
Fold increase of PC2, PC3, and Proinsulin biosynthesis at high glucose concentration in normal and obese mouse islets

	Normal islets	Obese islets
Proinsulin	7.6 ± 2.1	9.8 ± 2.3
PC2 (SPC2)	1.0 ± 0.1	7.5 ± 2.7
PC3 (SPC3)	6.8 ± 2.2	8.9 ± 2.8

Results from 6 experiments with normal mouse islets, and 4 experiments with obese mouse islets are summarized. Numbers represent fold stimulation (mean ± S.E.) for the biosynthesis of the indicated proteins in islets incubated in 5.0 mg/ml versus 0.5 mg/ml glucose containing media.

3.2. Glucoregulation of PC2, PC3, and proinsulin biosynthesis in obese mouse islets

Pancreatic islets isolated from (ob/ob) mice were pulse labeled for 45 min in low (0.5 mg/ml) or high (5.0 mg/ml) glucose concentration. Because these islets were only labeled for 45 min and not chased, very little conversion of proinsulin to insulin was observed (Fig. 2, panel C). However, the biosynthesis of both proinsulin and PC3 increased 6.8- and 7.6-fold, respectively, similar to the observed increases in their synthesis in the normal mouse islets (Fig. 2, panels A, B, & C; Table 1). Furthermore, the biosynthesis of PC2 increased 7.5-fold when the (ob/ob) islets were incubated at high glucose concentration (5 mg/ml) (Fig. 2, Table 1). The increase of PC2 by high glucose concentration in these islets correlates well with the increased biosynthesis of both PC3, 8.9-fold, and proinsulin 9.8-fold.

4. Discussion

Both PC2 and PC3 have been shown to process pro-opimelanocortin (POMC) at paired basic residues resulting in α-melanocyte stimulating hormone (αMSH), corticotropin-like intermediate, γ-lipotropin, β-endorphin-(1–31), and 18 kDa NH_2-terminal fragment [17,18]. In a study by Birch et al. the mRNA levels of both PC2 and PC3 were coordinately regulated with POMC using dopamine agonists and antagonists, resulting in full cleavage of POMC at the paired basic residues [19]. Like POMC, insulin is synthesized as a prohormone and then processed to the active hormone in the secretory granules [20]. The biosynthesis and secretion of insulin from pancreatic β-cells is regulated by the uptake and metabolism of glucose in these cells [3]. The effect of glucose on proinsulin biosynthesis results from stimulation of both translation, as well as increases in insulin mRNA [2,21]. Furthermore, it has been shown that the rapid translational effect of glucose also affects the synthe-

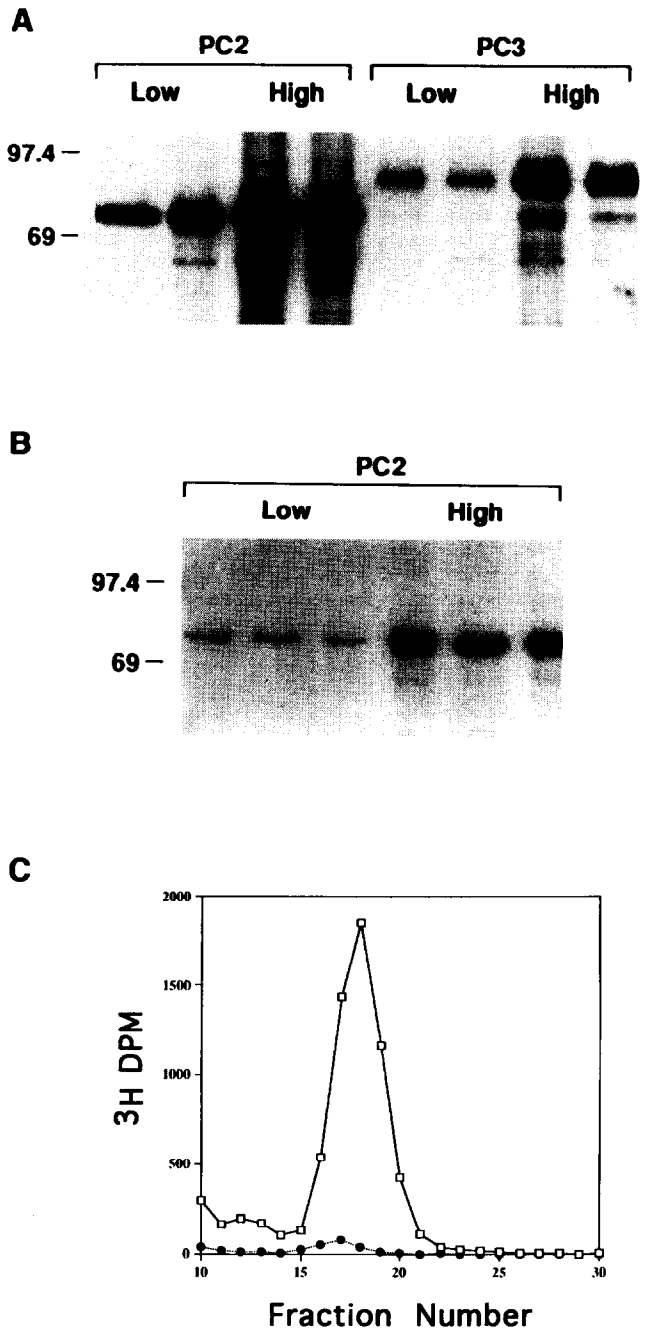

Fig. 2. Glucoregulation of PC2, PC3, and proinsulin expression in obese mouse islets. Islets from KSJ (ob/ob) Bar Harbor mice were preincubated for 60 min, and labeled with [^{35}S]Met, [^{35}S]Cys, [^{3}H]Leu, and [^{3}H]Phe for 45 min, in media containing 0.5 mg/ml or 5.0 mg/ml glucose. Islets were lysed and lysates were subjected to immunoprecipitation with PC2 and PC3 antisera, sequentially. (A) Immunoprecipitates were analyzed by SDS-PAGE and fluorography. (B) Immunoprecipitated PC2 material from another experiment. 10% aliquots of islet lysates were immunoprecipitated with anti-insulin antiserum. Immunoprecipitates were analyzed by gel filtration and scintillation counting. Proinsulin peaks are shown in (C). Solid circles represent radioactivity of islets incubated at low glucose concentration and open squares represent islets incubated at high glucose concentration.

sis of some 100 other proteins in the islets [22]. More specifically, the biosynthesis of 25 out of 32 major proteins localized to insulin secretory granules is increased by glucose [22]. It has also been shown that the conversion of proinsulin to insulin is mediated by the two prohormone convertases PC2 and PC3 [8,9]. Since the rate of conversion of proinsulin is largely unaffected by glucose (for example see Fig. 1B) it is likely that the rates of biosynthesis of the converting enzymes must be regulated in parallel with that of proinsulin. In a previous study, the biosynthesis of PC3 was found to be regulated parallel to that of proinsulin in normal rat pancreatic islets [13] and this observation has been confirmed in the present studies. However, both of these studies appear to indicate that the biosynthesis of PC2, the other endoprotease responsible for the conversion of proinsulin to insulin, is not glucoregulated.

Recent immunoblotting studies have shown that PC3 is more abundant in islets than PC2 [10] and more specifically that PC3 is much more abundant in β-cells than PC2 [11]. On the other hand, PC2 is present at very high levels in the α-cells [11,12], where it acts as the major converting enzyme responsible for the conversion of proglucagon to glucagon and these cells do not express detectable levels of PC3 [12]. Glucagon is secreted from the α-cells during hypoglycemia and directs glycogenolysis and gluconeogenesis in the liver [12], raising the blood glucose level by opposing the action of insulin. It is possible that the biosynthesis of PC2 is increased in the α-cells at low glucose concentration and thus obscures any stimulus to the biosynthesis of PC2 at high glucose concentration. Because the levels of PC2 are so high in the α-cells relative to the β-cells no detectable changes in the biosynthesis of PC2 in whole islets would be seen. On the other hand, PC3 is not expressed in the non β-cells of the islets of Langerhans and thus the regulation by glucose of PC3 biosynthesis in whole islets is a direct reflection of its expression in the β-cells.

To better understand the differential glucoregulation of PC2 and PC3 we used a model which contains mostly β-cells. Hyperglycemic (ob/ob) mice possess islets that are enlarged and have a highly altered morphology. They are composed primarily of β-cells and contain only a few α-cells that are suppressed in activity compared to the α-cells of normal islets [14]. Through short pulse radiolabelling experiments it was clear that the biosynthesis of PC2 was indeed positively regulated by glucose in these islets, an indication of the predominance of the β-cells. Furthermore, the biosynthesis of PC2 paralleled the biosynthesis of both PC3 and proinsulin in these islets. We thus can conclude that PC2 is glucoregulated in normal β-cells, just as are PC3 and proinsulin. This result also suggests that glucose levels may exert opposing effects on the expression of PC2 in the α- and β-cells of the islets of Langerhans. It is also possible that the apparent lack of carboxypeptidase H stimulation by glucose in normal islets [23] may have a similar basis.

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References

[1] Hedeskon, C.J. (1980) Physiol. Rev. 60, 442–509.
[2] Welsh, M., Sherberg, N., Gilmore, R. and Steiner, D.F. (1986) Biochem. J. 235, 459–467.
[3] Ashcroft, S.J.H. (1980) Diabetologia 18, 5–15.
[4] Docherty, K. and Steiner, D.F. (1982) Annu. Rev. Physiol. 44, 625–638.
[5] Smeekens, S. (1993) Biotechnology 11, 182–186.

[6] Lindberg, I. (1991) Endocrinology 5, 1361–1365.
[7] Steiner, D.F., Smeekens, S.P., Ohagi, S. and Chan S.J. (1992) J. Biol. Chem. 267, 23435–23438.
[8] Bennett, D.L., Bailyes, E.M., Nielsen, E., Guest, P.C., Rutherford, N.G., Arden, S.D. and Hutton, J.C. (1992) J. Biol. Chem. 267, 15229–15236.
[9] Bailyes, E.M., Shennan, K.I.J., Seal, A.J., Smeekens, S.P., Steiner, D.F., Hutton, J.C. and Docherty, K. (1992) Biochem. J. 285, 391–394.
[10] Smeekens, S.P., Montag, A.G., Thomas, G., Albiges-Rizo, C., Carroll, R., Benig, M., Phillips, L.A., Martin, S., Ohagi, S., Gardner, P., Swift, H.H. and Steiner, D.F. (1992) Proc. Natl. Acad. Sci. USA 89, 8822–8826.
[11] Neerman-Arbez, M., Cirulli V. and Halban, P.A. (1994) Biochem. J. 300, 57–61.
[12] Rouillé, Y., Westermark, G., Martin S.K. and Steiner D.F. (1994) Proc. Natl. Acad. Sci. USA 91, 3242–3246.
[13] Alarcón, C., Lincoln B. and Rhodes, C.J. (1993) J. Biol. Chem. 268, 4276–4280.
[14] Westman, S. (1968) Diabetologia 4, 141–149.
[15] Lernmark, A., Nathans, A. and Steiner, D.F. (1976) J. Cell Biol. 71, 606–608.
[16] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Labs, Plainview, NY), 2nd Edn.
[17] Rhodes C.J., Thorne, B.A., Lincoln, B., Nielsen E., Hutton, J.C. and Thomas, G. (1993) J. Biol. Chem. 268, 4267–4275.
[18] Zhou A., Bloomquist, B.T. and Mains, R. (1993) J. Biol. Chem. 268, 1763–1769.
[19] Birch, N.P., Tracer, H.L., Hakes, D.J. and Loh, P.Y. (1991) Biochem. Biophys. Res. Commun. 179, 1311–1319.
[20] Bailyes, E.M., Bennett, D.L. and Hutton, J.C. (1991) Enzyme 45, 301–313.
[21] Nielsen, D.A., Welsh, M., Casadaban, M.J. and Steiner, D.F. (1985) J. Biol. Chem. 260, 13585–13589.
[22] Guest, P.C., Bailyes, E.M., Rutherford, N.G. and Hutton, J.C. (1991) Biochem. J. 274, 73–78.
[23] Guest, P.C., Rhodes, C.J. and Hutton, J.C. (1989) Biochemistry R 257, 431–437.