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Glucose regulates preproinsulin messenger RNA levels in a clonal cell line of simian virus 40-transformed B cells

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In HIT-T15 cells grown in the absence of glucose, Northern blot analysis of total RNA revealed a major 0.5 kb preproinsulin (ppI) mRNA transcript which co-migrated with the mature transcript from a human insulinoma. In 4 h tissue cultures, glucose (2–20 mM) stimulated HIT cell ppI mRNA levels in a markedly dose-dependent manner. Glucose-stimulated ppI mRNA was (i) inhibited by actinomycin D, suggesting that regulation may be in part transcriptional, and (ii) potentiated by agents known to activate B cell protein kinases. HIT cells represent a unique model for investigating long term regulation of insulin gene expression and biosynthesis.

Preproinsulin; mRNA; D-Glucose; Insulin synthesis; Protein kinase; (HIT-T15 cell, Pancreatic B cell)

1. INTRODUCTION

In pancreatic B cells, a major physiological regulator of both insulin biosynthesis and secretion is extracellular glucose. The events whereby glucose stimulates insulin release are beginning to be understood at a molecular level. In contrast, although the stimulatory effects of glucose on insulin biosynthesis are well established [1,2], the molecular mechanisms remain unclear. With the development of preproinsulin cDNA sequences, it is now feasible to study transcriptional regulation of insulin gene expression by glucose.

The clonal insulin-secreting B cell line HIT-T15 was derived from Syrian hamster islets by SV40 transformation [3] and has been extensively characterised in our laboratory [4]. In contrast to other B cell lines [5], HIT cells retain the essential insulin secretory characteristics of the normal differentiated B cell, including responsiveness to glucose [4,6]. In the Syrian hamster, as in humans [7], there is a single preproinsulin gene, whereas rats and mice possess two non-allelic genes [8]. Moreover, there is evidence that the two rat preproinsulin genes may be differentially regulated under stimulatory conditions [9]. Therefore, HIT cells potentially represent a more appropriate model for defining the molecular events regulating insulin biosynthesis. In the present study we have evaluated this potential and demonstrate regulation of HIT cell preproinsulin (ppI) mRNA levels by glucose, possibly at the level of transcription. Moreover there is evidence implicating protein kinases as putative mediators of glucose regulation.

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2. EXPERIMENTAL

2.1. *Materials*

Tissue culture medium RPMI 1640 and foetal calf serum were purchased from Gibco (Paisley, Scotland). [^{32}P]dATP (spec. act. >3000 Ci/mmol) and the cAMP radioimmunoassay kit was purchased from Amersham (Bucks, England). The

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phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA) was purchased from PL Biochemicals (Northampton, England). Forskolin was purchased from Calbiochem (Cambridge, England). X-ray film was purchased from Kodak (Liverpool, Merseyside, England). All other reagents were purchased from BDH or Sigma (Poole, Dorset, England).

2.2. *Methods*

HIT cells (clone T15) were grown at 37°C in RPMI 1640 supplemented with glucose (11 mM) antibiotics and 10% (v/v) foetal calf serum as described [4]. In all experiments, HIT cells were preincubated for 1 h in culture medium without glucose. Subsequently, insulin release and ppI mRNA were assayed following incubation for 4 h in culture medium supplemented with glucose and additions as listed in section 3. Insulin release was measured by radioimmunoassay [4]. Intracellular cAMP was assayed by radioimmunoassay using a commercial kit. ppI mRNA was assayed by Northern blotting followed by autoradiography and densitometry. Autoradiographic exposures were shown to be in the linear resolution range of the film (Kodak X-AR). The probe, a Syrian hamster cDNA (p Shins 1/HB101) [8] was a gift from Dr G.I. Bell, Chiron Corporation, Emeryville, CA, USA, and was labelled with [^{32}P]dATP by random hexanucleotide priming [10]. Total RNA was electrophoresed on agarose/formaldehyde gels [11], blotted to nitrocellulose filters and hybridized as described [12].

3. RESULTS AND DISCUSSION

Previous studies using rat of mouse islets of Langerhans or rat cell lines suggest that glucose regulation of insulin biosynthesis may be mediated through changes in the rates of both insulin gene transcription and translation of mature transcript. Translational regulation is rapid, occurring within minutes [13–15], and operates by (i) stimulating initiation of ppI synthesis; (ii) increasing the elongation rate of nascent ppI; (iii) stimulating transfer of initiated ppI mRNA transcript from the cytoplasm to microsomal membranes [16]. In contrast, long term regulation, occurring over hours to days, may be transcriptional and previous studies [14,17–19] have suggested that glucose may regulate insulin gene expression by modulating ppI mRNA levels.

Northern blot analysis of HIT cell RNA from cells grown without glucose revealed a 0.5 kb ppI transcript which co-migrated with mature

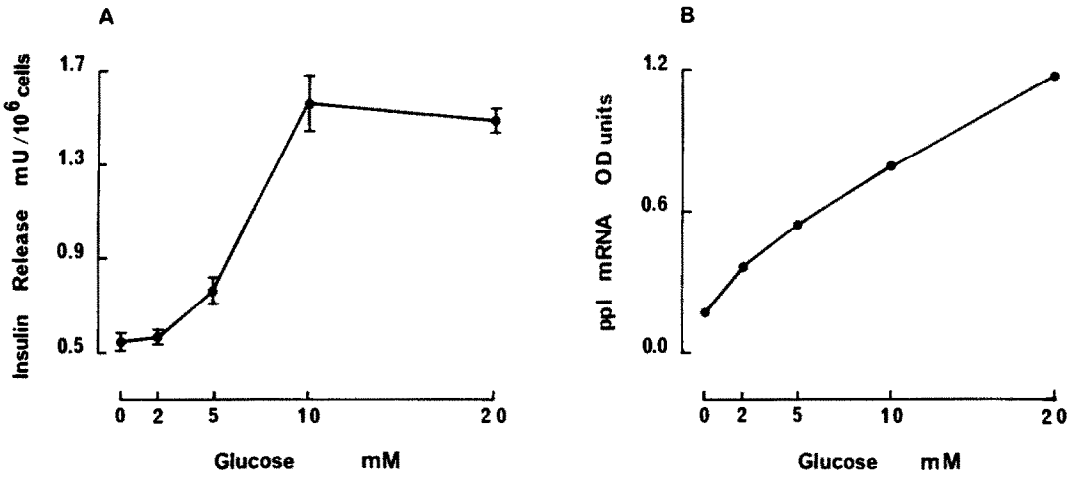

Fig.1. Effect of glucose on HIT cell insulin release and ppI mRNA. (A) HIT cell insulin release measured by radioimmunoassay after 4 h culture in RPMI in the absence or presence of glucose (2–20 mM). Data are presented as mean ± 95% confidence limits (n = 3). The dependence of insulin release on glucose concentration was significant by anovar ($P < 0.001$). (B) Dose-dependent stimulation of HIT cell ppI mRNA by glucose after 4 h culture. Data, determined by densitometry of Northern blots, are representative of duplicate experiments.

transcript produced by a primary human insulinoma. Incubation for 4 h in the presence of glucose (2–20 mM) stimulated accumulation of HIT cell ppI mRNA relative to controls incubated in the absence of glucose (fig.1B). Moreover, ppI mRNA accumulation was markedly dose-dependent with respect to glucose concentration: raising medium glucose induced progressive increments in ppI mRNA with an overall 4-fold stimulation between 2 and 20 mM glucose. Similarly, isolated rat islets incubated for 4 h in high glucose (28 mM) demonstrated a 5-fold elevation of ppI mRNA relative to low glucose (2.8 mM), although ppI mRNA was considerably reduced compared to non-incubated islets [19]. Moreover, in the same study the authors report both a 45% reduction in the translatable activity of islet mRNA from incubated islets and impaired proinsulin biosynthesis at low glucose. These findings are consistent with further observations that freshly isolated islets demonstrate acute rapid losses of ppI mRNA concomitant with reduced rates of proinsulin biosynthesis and require a recovery period in culture of up to 24 h [17,20]. This may explain in part why glucose-stimulated ppI mRNA accumulation was more dose-dependent in HIT cells than in 24 h cultured rat islets [21].

Although glucose regulates insulin biosynthesis and secretion, both processes may also function independently [2]: we also demonstrate dissociation between ppI mRNA content and insulin secretion with respect to both induction by glucose and modulation of glucose stimulation. Thus, like insulin biosynthesis [22], glucose-stimulated ppI mRNA accumulation showed a lower threshold compared with insulin release (fig.1). In contrast, ppI mRNA accumulation was further stimulated on raising medium glucose to 20 mM whereas insulin release, like biosynthesis [22], was saturated at 10 mM glucose (fig.1). The observation that insulin biosynthesis persists after glucose stimulation has ceased [2] may be related to this continued accumulation of ppI mRNA during periods of hyperglycaemic stress. In addition, dissociation between glucose-stimulated parameters was also evident in that whereas theophylline and bombesin potentiated HIT cell insulin release (fig.3), ppI mRNA levels remained unchanged (fig.4).

It has been suggested [20,21] that long term regulation of insulin gene expression by glucose occurs through simultaneous stimulation of transcription and inhibition of ppI mRNA degradation. Incubation of HIT cells for 4 h with the RNA polymerase inhibitor actinomycin D (1 μg/ml) reduced by over 50% the stimulatory effect of 10 mM glucose on ppI mRNA but had no effect on basal levels (fig.2). This suggests that glucose-stimulated HIT cell ppI mRNA accumulation may also be comprised of transcriptional or transcript-stabilising components. The latter possibility cannot be ruled out since actinomycin D is known to affect other cellular processes, including initiation of protein synthesis [23], and may therefore indirectly affect transcript stability. However, the observation that actinomycin D did not affect basal or glucose-stimulated insulin secretory rates implies that inhibition of glucose-stimulated ppI mRNA is not simply attributable to impairment of HIT cell viability. Resolution of this point awaits the development of a sensitive assay for ppI mRNA-specific transcription: the

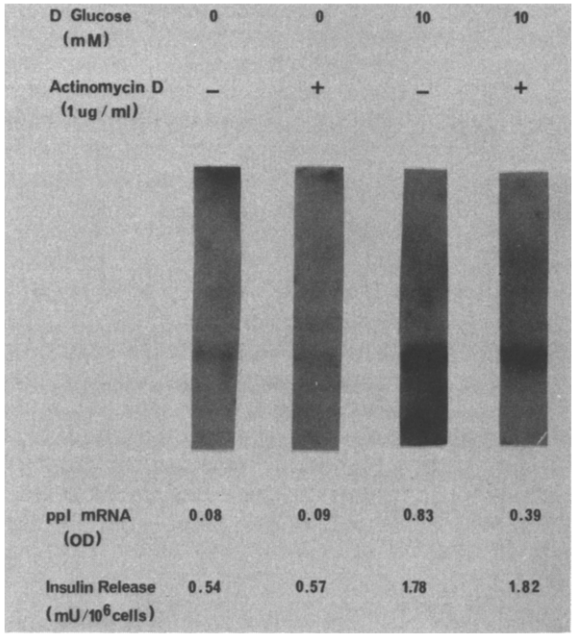

Fig.2. Effects of actinomycin D on glucose-stimulated HIT cell ppI mRNA and insulin release. After 4 h incubation in RPMI supplemented as shown, insulin release was measured by radioimmunoassay, ppI mRNA by Northern blotting followed by autoradiography and densitometry. Data presented are representative of duplicate experiments.

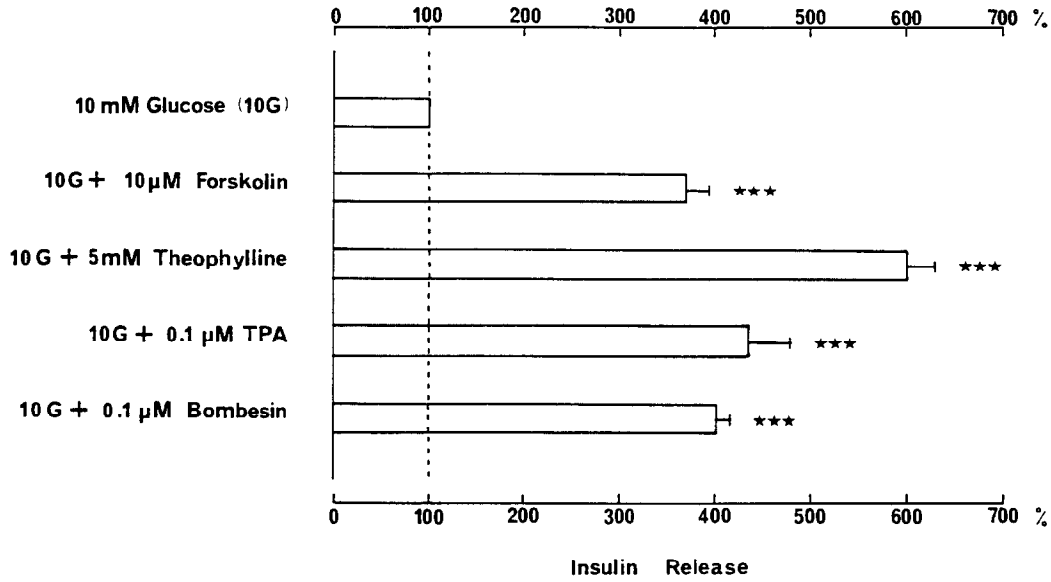

Fig.3. Modulation of glucose-stimulated insulin release. HIT cells were incubated for 4 h with the additions shown. Insulin release, measured by radioimmunoassay, is expressed as a percentage of the mean rate observed with 10 mM glucose. Data are given as mean ± SE (n = 3). The mean absolute rate of insulin release in controls (10 mM) was 1.7 ± 0.26 mU/10^6 cells. Significance (Student's t-test) of the differences between test and control (10 mM glucose) are: *** $P < 0.001$.

method employed in the above study [21] is inadequate in that it fails to distinguish between mature and therefore translatable ppI mRNA and the multiple precursor messengers characteristic of ppI genes [24].

Regulation of transcriptional activity by cAMP is well established in prokaryotes and has also been implicated in the control of several eukaryotic genes. The observation that addition of dibutyryl cAMP to isolated rat islets could partially mimic the stimulatory effect of glucose on ppI mRNA [21] suggests that regulation of insulin gene expression by glucose may also be mediated via cAMP. Moreover, in 7 day cultures of mouse islets, ppI mRNA was augmented to the same degree as glucose by leucine and ketoisocaproate, compounds which also increase cAMP [25]. In the present study, raising medium glucose to 10 mM induced a significant ($P < 0.01$, Student's t-test) increment in HIT cell cAMP (6.4 ± 0.5 to 9.8 ± 0.6 pmol cAMP/10^6 cells (n = 4)) concomitant with a 2-fold stimulation of ppI mRNA (fig.1B). In addition, incubation with the adenyl cyclase activator forskolin provoked marked potentiation of the effects of glucose on both cAMP (26.1 ± 4.7 pmol cAMP/10^6 cells (n = 4)) and ppI mRNA (2-fold, fig.4). These findings are consistent with a second messenger role for the cAMP system in the regulation of insulin gene expression. In contrast, the phosphodiesterase inhibitor theophylline failed to potentiate glucose-stimulated ppI mRNA (fig.4) despite augmenting HIT cell cAMP to a level similar to that induced by forskolin (28.3 ± 3.3 pmol cAMP/10^6 cells (n = 4)). However, the observed dissociation between cAMP and ppI mRNA with theophylline may be related to effects of theophylline additional to elevation of cAMP such as inhibition of poly(ADP-ribose) synthetase activity in nuclei [26].

cAMP-mediated regulation of insulin secretion occurs through activation of cAMP-dependent protein kinase [27,28]. Similarly, cAMP-dependent phosphorylation may be instrumental in the regulation of ppI mRNA levels, possible substrates including DNA-binding transcriptional factors and proteins regulating post-transcriptional processing or turnover of the mature transcript. In addition, fig.4 demonstrates 2-fold potentiation of glucose-

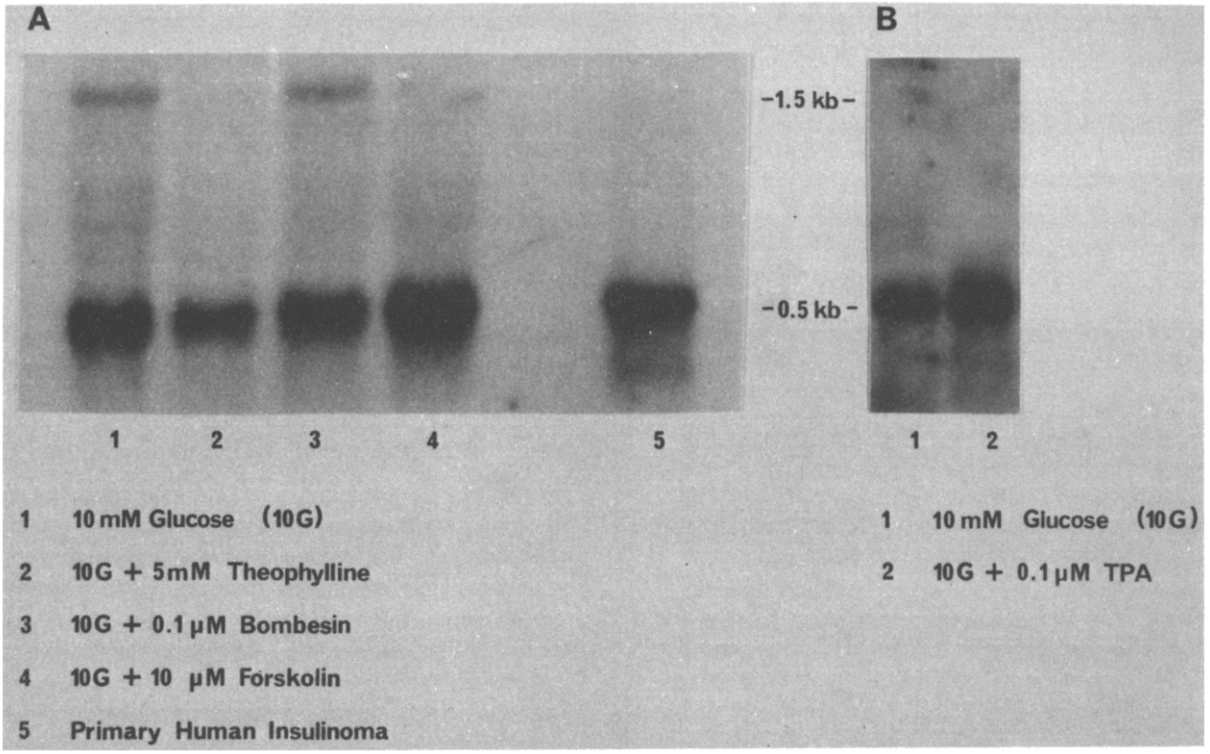

Fig.4. Modulation of glucose-stimulated ppI mRNA accumulation. Autoradiographs demonstrating Northern blot analysis of HIT cell ppI mRNA are shown for 2 separate experiments. Densitometry revealed 2-fold potentiation of glucose-stimulated ppI mRNA by both forskolin (A4) and TPA (B2). Theophylline (A2) and bombesin (A3) proved ineffective. A positive control is provided by a primary human insulinoma (A5) producing mature insulin gene transcript. Autoradiographs are representative of duplicate blots.

stimulated HIT cell ppI mRNA accumulation evoked by a phorbol ester known to activate Ca^{2+}-phospholipid-dependent protein kinase (C kinase) in B cells [29]. In contrast, bombesin which induces phosphatidylinositol turnover [30] and therefore presumably activates C kinase via diacylglycerol, did not affect ppI mRNA content (fig.4). The role of protein kinase activity in the regulation of insulin gene expression clearly warrants further investigation.

We conclude from these studies that glucose regulates ppI mRNA levels in HIT cells and that the mechanism of action of glucose may be mediated in part through protein kinases. HIT cells represent a unique model system for investigating long-term regulation of insulin gene expression and biosynthesis.

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REFERENCES

[1] Permutt, M.A. (1981) in: The Islets of Langerhans (Cooperstein, S.J. and Watkins, D. eds) pp.75–95, Academic Press, New York.
[2] Campbell, I.L., Hellquist, L.N.B. and Taylor, K.W. (1982) Clin. Sci. 63, 449–455.
[3] Santerre, R.F., Cook, R.A., Crisel, R.M.D., Sharp, J.D., Schmidt, R.J., Williams, D.C. and Wilson, C.P. (1981) Proc. Natl. Acad. Sci. USA 78, 4339–4343.

[4] Ashcroft, S.J.H., Hammonds, P. and Harrison, D.E. (1986) Diabetologia 29, 727–733.
[5] Praz, G.A., Halban, P.A., Wollheim, C.B., Blondel, B., Strauss, A.J. and Renold, A.E. (1983) Biochem. J. 210, 345–352.
[6] Hill, R.S. and Boyd, A.E. iii (1985) Diabetes 34, 115–120.
[7] Bell, G.I., Pictet, R.L., Rutter, W.J., Cordell, B., Tischer, E. and Goodman, H.M. (1980) Nature 284, 26–32.
[8] Bell, G.I. and Sanchez-Pescador, R. (1984) Diabetes 33, 297–300.
[9] Kakita, K., Giddings, S. and Permutt, M.A. (1982) Proc. Natl. Acad. Sci. USA 79, 2803–2807.
[10] Feinberg, A.P. and Vogelstein, B. (1984) Addendum Anal. Biochem. 137, 266–267.
[11] Lehrach, H., Diamond, D., Wozney, M. and Boedtke, H. (1977) Biochemistry 16, 4743–4749.
[12] Thomas, P.S. (1980) Proc. Natl. Acad. Sci. USA 77, 5201–5205.
[13] Itoh, N. and Okamoto, H. (1980) Nature 283, 100–102.
[14] Permutt, M.A. and Kipinis, D.M. (1972) J. Biol. Chem. 247, 1194–1199.
[15] Permutt, M.A. and Kipinis, D.M. (1972) J. Biol. Chem. 247, 1200–1207.
[16] Welsh, M., Scherberg, N., Gilmore, R. and Steiner, D.F. (1986) Biochem. J. 235, 459–467.
[17] Brundstedt, J. and Chan, S.J. (1982) Biochem. Biophys. Res. Commun. 106, 1383–1389.
[18] Giddings, S.J., Chirgwin, J.M. and Permutt, M.A. (1982) Diabetes 31, 624–629.
[19] Giddings, S.J., Chirgwin, J.M. and Permutt, M.A. (1985) Diabetes Res. 2, 71–75.
[20] Welsh, M., Nielsen, D.A., Mackrell, A.J. and Steiner, D.F. (1985) J. Biol. Chem. 260, 13590–13595.
[21] Nielsen, D.A., Welsh, M., Casadabam, M.J. and Steiner, D.F. (1985) J. Biol. Chem. 260, 13585–13589.
[22] Ashcroft, S.J.H., Bunce, J., Lowry, M., Hansen, J.E. and Hedeskov, C.J. (1978) Biochem. J. 174, 517–526.
[23] Goldstein, E.J. and Penman, S.J. (1973) J. Mol. Biol. 80, 243–254.
[24] Steiner, D.F., Chan, S.J., Welsh, J.M. and Kwok, S.C.M. (1985) Annu. Rev. Genet. 19, 463–484.
[25] Welsh, M., Brundstedt, J. and Hellerstrom, C. (1986) Diabetes 35, 228–231.
[26] Kitamura, A., Tanigawa, Y., Okamoto, S., Miyake, Y. and Shimoyama, M. (1981) Biochim. Biophys. Acta 677, 63–68.
[27] Christie, M.R. and Ashcroft, S.J.H. (1984) Biochem. J. 218, 87–89.
[28] Christie, M.R. and Ashcroft, S.J.H. (1985) Biochem. J. 227, 727–736.
[29] Harrison, D.E., Ashcroft, S.J.H., Christie, M.R. and Lord, J.M. (1984) Experientia 40, 1075–1084.
[30] Swope, S.L. and Schonbrunn, A. (1984) Proc. Natl. Acad. Sci. USA 81, 1822–1826.