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# Regulation and specificity of glucose-stimulated insulin gene expression in human islets of Langerhans

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The insulin response of cultured human islets of Langerhans was measured at both mRNA and polypeptide levels in response to natural and pharmacological stimuli. We report a dosage dependent stimulation of both mRNA levels and insulin secretion by extracellular glucose, and present evidence that islet responsiveness can be divided into two temporal phases: an early response, apparently under post-transcriptional control, and a late phase in which insulin messenger accumulates. Although glucose effects in man are similar to rodents, there are important differences, especially with respect to modulation of glucose stimulation by activators of β-cell protein kinases.

Insulin gene expression; Preproinsulin mRNA; Insulin secretion; cyclic AMP; Protein kinase; (Human pancreatic islet)

## 1. INTRODUCTION

Co-ordinated regulation of insulin gene expression, biosynthesis and release is essential to maintain the fuel sensor function of the pancreatic β-cell. Extracellular glucose is the major physiological regulator of both insulin biosynthesis and release. However, in contrast to the many studies on insulin release, there have been few on the molecular mechanisms regulating glucose stimulation of insulin biosynthesis and these have been entirely restricted to rodent models. It is important that these observations be extended to man. Although data are necessarily fragmentary, human insulin secretion is essentially similar to rodent models [1]. However, the molecular basis of insulin gene expression and biosynthesis is unknown in man, which restricts our understanding of a possible contribution of defects at this level to the pathophysiology of diabetes mellitus.

The clonal β-cell line HIT-T15 [2] is derived from the Syrian hamster which has, like humans, a single insulin gene [3]. In contrast, rats and mice possess two non-allelic insulin genes [4]. We have recently demonstrated a dose-dependent regulation of HIT-T15 insulin gene expression by glucose, and this may be in part transcriptional [5], and is modulated by activators of β-cell protein kinases. In the present study we have used similar techniques to address, for the first time, the molecular nature of control mechanisms regulating insulin gene expression in the human β-cell. We demonstrate that regulation of insulin gene expression is essentially similar in man and rodent in terms of responsiveness to glucose stimulation. However, it is apparent that there are differences between man and rodent with respect to both modulation of glucose stimulation by activators of β-cell protein kinases, and also in responsiveness to pharmacological stimuli.

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## 2. EXPERIMENTAL

### 2.1. *Materials*

Culture medium RPMI 1640, fetal calf serum and tissue culture dishes (Nunclon) were purchased from Gibco (Paisley, Scotland); [$^{32}$P]dATP (spec. act. > 3000 Ci/mmol) from Amersham (Bucks, England); the phorbol ester, 12-*O*-tetradecanoylphorbol 13-acetate (TPA), from PL Biochemicals (Northampton, England) and forskolin from Calbiochem (Cambridge, England). X-ray film was bought from Kodak (Liverpool, England). All additional reagents were purchased from Sigma or British Drug Houses (Poole, Dorset, England).

### 2.2. *Methods*

Human islets of Langerhans were isolated from pancreata of renal transplant donors by collagenase digestion as described [6]. Freshly isolated islets (approx. 1000/dish) were preincubated at 37°C in RPMI 1640 supplemented with glucose (11 mM), 10% (v/v) fetal calf serum and antibiotics for 48 h prior to further manipulation. Subsequently, insulin secretion and preproinsulin (ppI) mRNA were assayed following incubation in RPMI 1640 without glucose, supplemented as listed in section 3. All data are derived from duplicate or triplicate preparations of human pancreas. Insulin secretion was measured by radioimmunoassay [7]. ppI mRNA was assayed by Northern hybridization to human insulin cDNA, autoradiography and densitometry. The human insulin cDNA probe (pchinsl-19/HB101) [8] was a gift from Dr G.I. Bell (Chiron Corp., Emeryville, CA, USA) and was labelled with [$^{32}$P]dATP by nick-translation [9]. Total RNA was extracted by guanidinium isothiocyanate, separated on agarose-formaldehyde gels, blotted to nitrocellulose filters

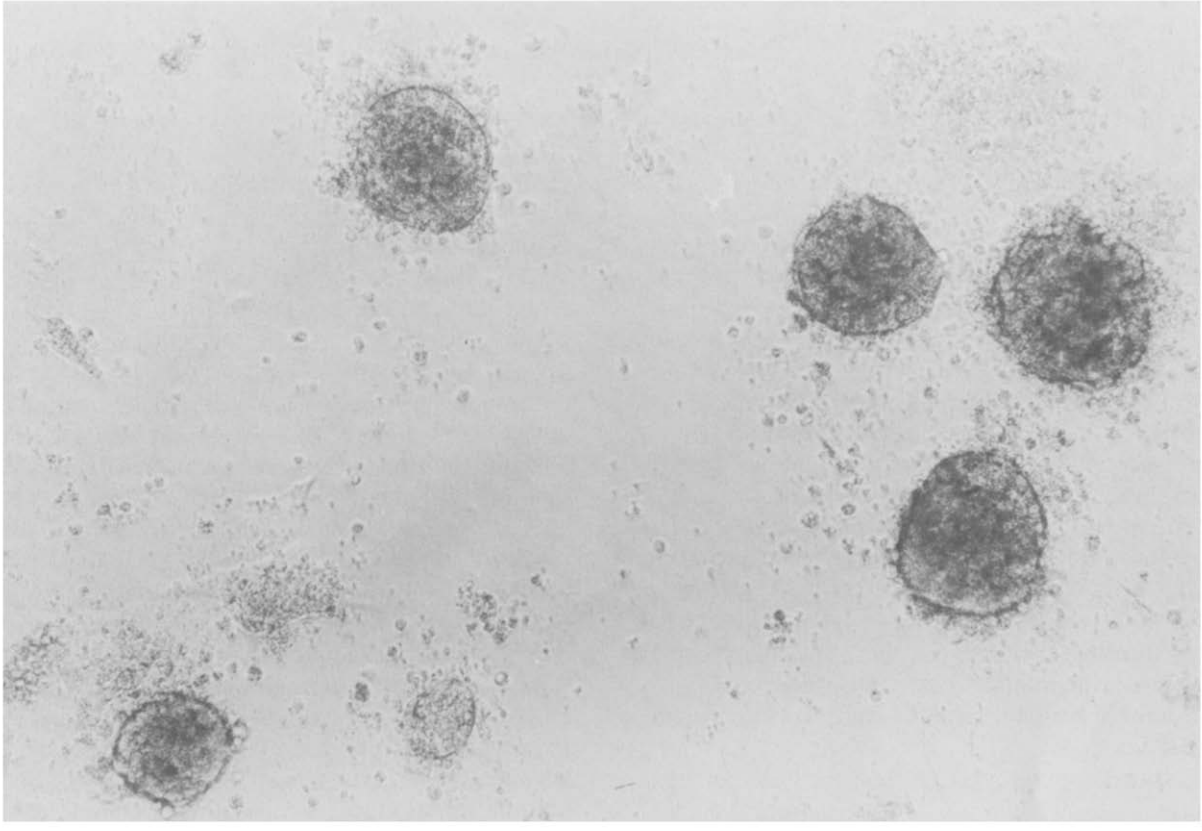

Fig.1. Human islets of Langerhans in tissue culture. Islets were isolated by collagenase digestion and cultured for 48 h in RPMI 1640. Magnification × 190.

and hybridized essentially as we have described for HIT-T15 cells [5]. ppI mRNA levels were subsequently normalised against a constitutively expressed mRNA (glyceraldehyde-3-phosphate dehydrogenase, GAP-DH) by rehybridization to GAP-DHcDNA as described [10].

## 3. RESULTS AND DISCUSSION

Following isolation, islets were maintained in culture for 48 h prior to further analysis. This was a necessary precaution taken to extract optimal information from scarce tissue, since freshly isolated rat islets demonstrate (i) acute rapid losses of ppI mRNA and impaired proinsulin biosynthesis, requiring a recovery period in tissue culture of up to 24 h [11,12], and (ii) a refractory secretory response to acetylcholine, which is restored only after at least 44 h in culture [13]. During the preincubation period, a majority of islets adhered firmly to the culture dish, facilitating subsequent manipulation (fig.1). After preincubation, islets were incubated in the presence (2–20 mM) or absence of glucose for 24 h. Northern hybridization of total RNA with human ppI cDNA revealed a 0.5 kb transcript, consistent with that expected of mature ppI mRNA, and similar to insulin messenger present in both primary human insulinoma [5] and post-mortem extracts of whole, frozen pancreas [14]. Glucose (2–20 mM) induced a 3.5-fold dose-dependent stimulation of human ppI mRNA concomitant with 5-fold stimulation of insulin secretion (fig.2a). Both ppI mRNA and insulin secretion demonstrated threshold levels of between 2 and 5 mM glucose and maximal rates of stimulation between 5 and 10 mM glucose. In contrast, between 0 and 2 mM glucose, basal insulin secretion rates were maintained whereas ppI mRNA levels fell by 35%. These results indicate that, at physiological concentrations of extracellular glucose, there is parallelism between ppI mRNA content and insulin secretion. However, the uncoupling between ppI mRNA and insulin secretion at low levels of extracellular glucose indicates that post-transcriptional control may also occur.

Next, we investigated the kinetics of stimulation induced by high (20 mM) relative to low (2 mM) glucose for 4, 12 or 24 h following preincubation

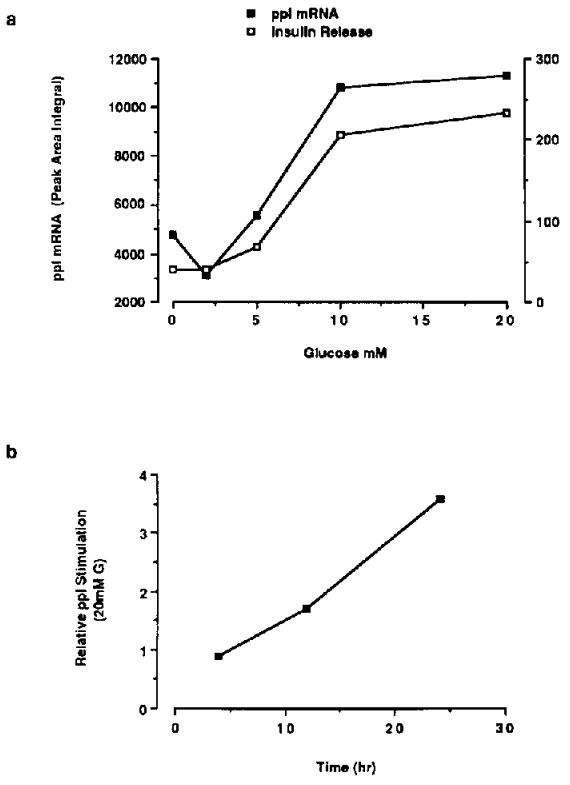


Fig.2. Effect of glucose on ppI mRNA and insulin secretion (a) Dose-dependent stimulation of ppI mRNA and insulin secretion by glucose. Islets were preincubated for 48 h, then exposed to glucose (2–20 mM) for a further 24 h. ppI mRNA was determined by densitometry of Northern blots and was normalised against GAP-DH mRNA; insulin secretion was measured by radioimmunoassay. Data are representative of triplicate preparations of human pancreas. (b) Time course of glucose stimulation of ppI mRNA. Islets were preincubated for 48 h, then exposed to high (20 mM) and low (2 mM) glucose for 4, 12, or 24 h. ppI mRNA was determined as above and is expressed as stimulation by high relative to low glucose. Data are representative of duplicate preparations of human pancreas.

(fig.2b). After 4 h there was no stimulation of ppI mRNA accumulation, and only 1.5-fold stimulation after 12 h, compared with a 3.5-fold increase after 24 h. In contrast, insulin secretion was stimulated 5-fold at all time points (not shown). This suggests that in culture, the immediate response of islets to glucose stimulation is predominantly secretory, with demands for insulin being met through depletion of existing stores of

proinsulin and/or ppI mRNA. This clearly allows for a short-term response which is both rapid and flexible, since transcription de novo must inevitably take longer.

In the absence of sufficient experimental material, direct analysis of insulin gene transcription by isolated human β-cell nuclei is not yet practicable. However, early observations inferring a regulatory role for glucose relied on the use of inhibitors [15] and were subsequently confirmed by the development of direct assays for insulin messenger [16]. By using specific inhibitors we can

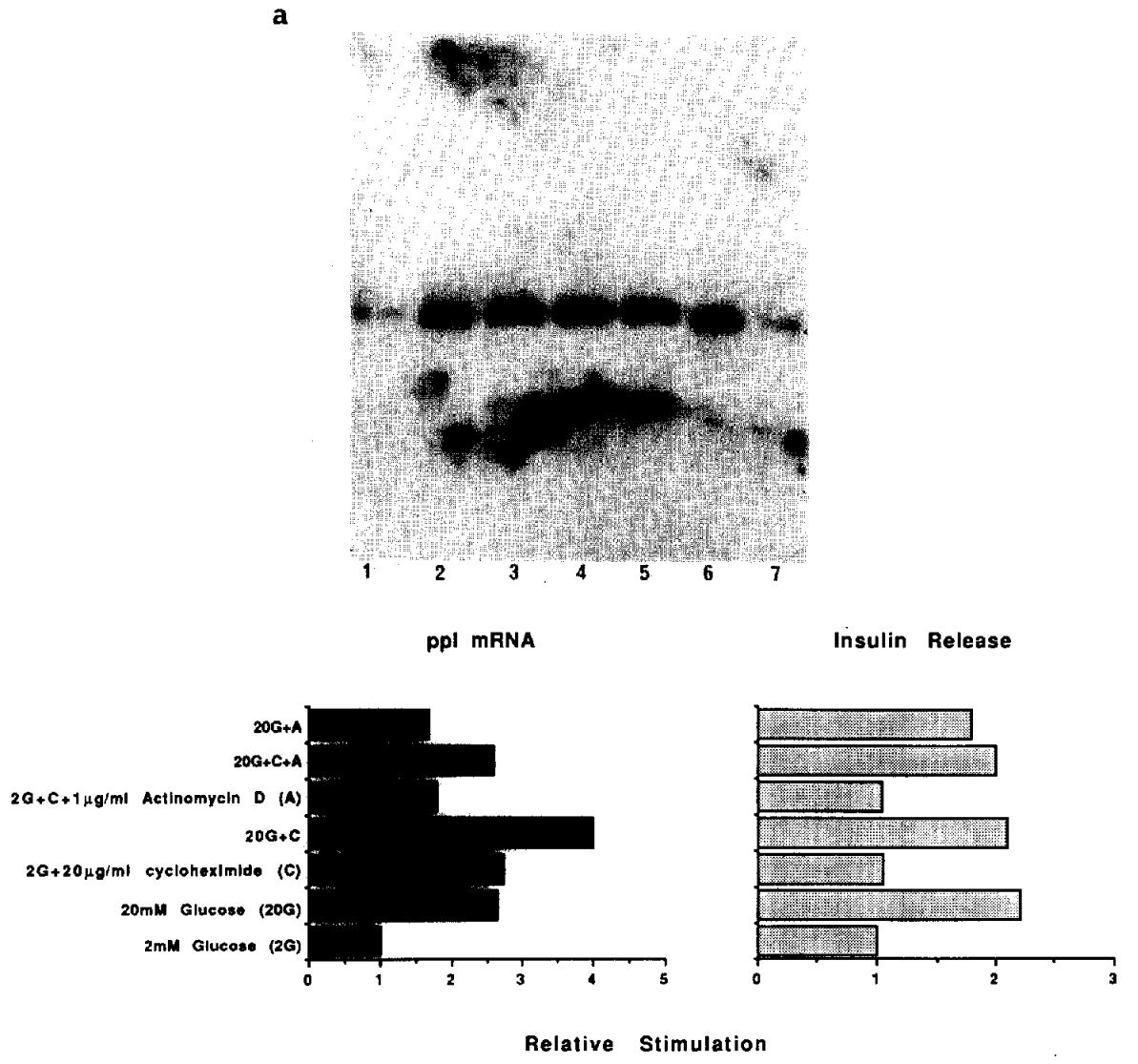


Fig.3. Modulation of glucose-stimulated ppI mRNA and insulin secretion by inhibitors of RNA and protein synthesis. Islets were preincubated for 48 h, then exposed to agents as listed for a further 24 h. ppI mRNA was determined by densitometry of Northern blots and was normalised against GAP-DH mRNA; insulin secretion was measured by radioimmunoassay. Data are representative of duplicate preparations of human pancreas. (a) Autoradiograph demonstrating Northern blot analysis of ppI mRNA; lane 1, 2 mM glucose (2G); lane 2, 20 mM glucose (20G); lane 3, 2G + 20 μg/ml cycloheximide (C) + 1 μg/ml actinomycin D (A); lane 4, 20G + C; lane 5, 2G + C; lane 6, 20G + C + A; lane 7, 20G + A. (b) Relative stimulation of ppI mRNA and insulin secretion.

now present evidence which suggests that more long-term regulation of human insulin gene expression by glucose is mediated at both transcriptional and post-transcriptional levels. Incubation of human islets with 20 μg/ml cycloheximide (which inhibits protein synthesis by over 90% in rat islets [15]) induced marked augmentation of ppI mRNA in the presence of both high and low glucose concentrations (fig.3), suggesting that synthesis of a negative peptide modulator of transcript levels (perhaps insulin itself), feeds back to regulate mRNA abundance via modulation of messenger half-life or transcription, or even both. The RNA polymerase inhibitor actinomycin D, inhibited cycloheximide-induced ppI mRNA accumulation at both high and low glucose by 43% and 32%, respectively, suggesting some transcriptional involvement. In addition, glucose-stimulated ppI mRNA accumulation was also inhibited by 25% in the presence of actinomycin D. The effects of these inhibitory compounds at the concentrations used are unlikely to reflect impaired viability since insulin secretory rates were not affected. This latter observation also suggests that the initial secretory requirements of the β-cell are met through mobilisation of proinsulin stores and are not restricted by mRNA availability.

The effects of activators of β-cell protein kinases on glucose-stimulated effects were also investigated (fig.4). The adenylate cyclase activator forskolin provoked a 1.5-fold potentiation of the effects of glucose on ppI mRNA accumulation and insulin secretion. This suggests that positive control of glucose-regulated insulin gene activity in man may be mediated in part by β-cell cAMP, which activates cAMP-dependent protein kinase (cAMP-PrK). In contrast, there was an inhibition of human insulin messenger accumulation by activators of $Ca^{2+}$-phospholipid-dependent protein kinase (protein kinase C-PKC). The phorbol ester TPA, which activates β-cell PKC directly [17], inhibited glucose-stimulated ppI mRNA accumulation by 50%, despite inducing a 2-fold potentiation in the rate of insulin secretion. Furthermore bradykinin, which activates PKC indirectly (via phosphoinositide turnover) in human fibroblasts [18], inhibited glucose-stimulated ppI mRNA without affecting insulin secretion. This indicates a further level of regulation of human insulin biosynthesis: negative modulation of insulin gene expression by PKC. However, it also demonstrates an important difference between man and rodent, because, in contrast, our previous studies with TPA in HIT-T15 cells [5] have indicated positive modulation of insulin gene expression by PKC.

Proinsulin biosynthesis is regulated by a variety

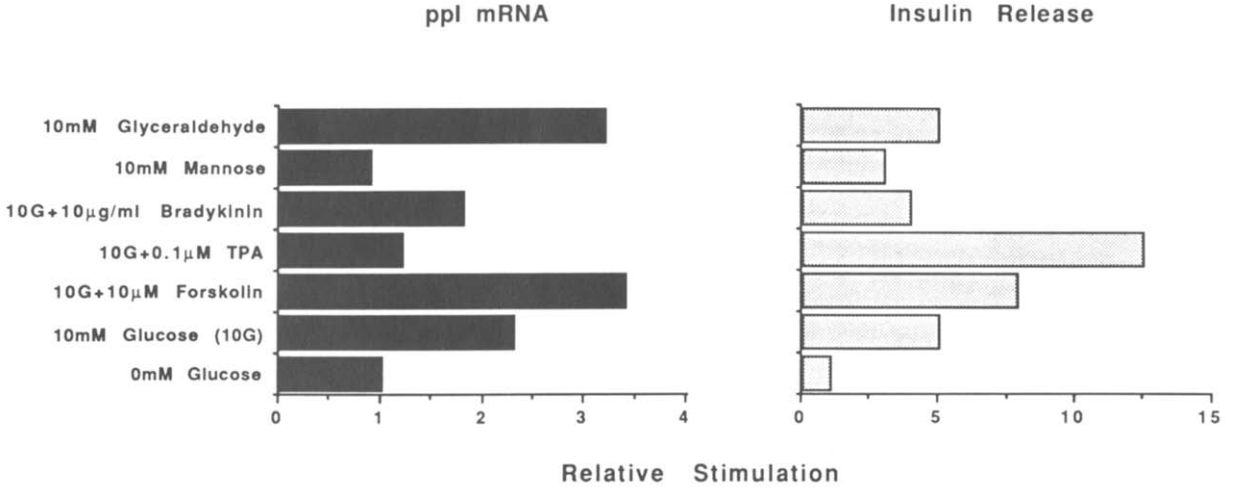


Fig.4. Modulation and specificity of glucose-stimulated ppI mRNA and insulin secretion. Islets were preincubated for 48 h, then exposed for a further 24 h to either (i) agents known to affect β-cell protein kinase or (ii) metabolisable sugars other than glucose. ppI mRNA was determined by densitometry of Northern blots and was normalised against GAP-DH mRNA; insulin secretion was measured by radioimmunoassay. Data are representative of duplicate preparations of human pancreas.

of additional agents in rodents [19]. The specificity of glucose regulation in humans was therefore considered in relation to effects of other metabolisable sugars. At equivalent concentrations (10 mM), glyceraldehyde proved an even more potent stimulator of human ppI mRNA than glucose. In contrast, mannose was ineffective, although both sugars induced a 3-fold stimulation of insulin secretion. Although these effects are unlikely to be of physiological significance, the failure of mannose to affect human insulin messenger was unexpected because, in rat islets, it parallels the effects of glucose on both insulin secretion and biosynthesis (as measured by radio-labelled leucine incorporation) [19]. A comprehensive survey of agents affecting human insulin biosynthesis at a molecular level is clearly essential to evaluate the usefulness of rodent models.

These studies demonstrate a dose-dependent stimulation of human insulin gene expression by extracellular glucose, the major physiological regulator of insulin biosynthesis and release. In the short-term, a pool of stored proinsulin and/or unused mRNA allows a very rapid and flexible response, whereas in the long-term transcriptional and post-transcriptional controls become important. In addition, pharmacological stimulation was effected by glyceraldehyde. The effects of extracellular glucose are essentially similar to those previously reported in rodents [5,11,12,20], as is a possible second messenger role for cAMP [5,21, 22]. However, there were differences between man and rodent with respect to both the effects of activators of PKC and also the specificity of glucose stimulation. These differences may represent important limitations of rodent models and require delineation.

In the rodent $\beta$-cell, cAMP regulates insulin secretion by modulating cAMP-PrK activity [23,24]. In addition, our laboratory has previously shown cAMP-PrK phosphorylation in human islets in response to glucose stimulation [25], and this represents a possible site for positive control of insulin gene expression in man. Moreover, the effects of forskolin in human islets essentially parallel those we have previously reported in HIT-T15 cells [5]. Therefore, HIT-T15 cells may provide sufficient experimental material to allow identification of either insulin gene sequences or related protein substrates associated with regulation of gene activity mediated by cAMP-PrK phosphorylation.

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