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Control of insulin gene expression by glucose

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Northern-blot analysis was used to demonstrate that an increase in extracellular glucose concentration increased the content of preproinsulin mRNA 2.3-fold in the β-cell line HIT T15. A probe for the constitutively expressed glyceraldehyde-3-phosphate dehydrogenase was used as a control. Mannoheptulose blocked this effect of glucose. A stimulatory effect on preproinsulin mRNA levels was also observed in response to mannose and to 4-methyl-2-oxopentanoate. However, galactose and arginine were ineffective. Glucagon, forskolin and dibutyryl cyclic AMP also elicited an increase in HIT-cell preproinsulin mRNA. The ability of the 5′ upstream region of the preproinsulin gene to mediate the effect of glucose and other metabolites on transcription was studied by using a bacterial reporter gene technique. HIT cells were transfected with a plasmid, pOK1, containing the upstream region of the rat insulin-1 gene (−345 to +1) linked to chloramphenicol acetyltransferase (CAT). Co-transfection with a plasmid pRSVβ-gal containing β-galactosidase driven by the Rous sarcoma virus promoter was used as a control for the efficiency of transfection; expression of CAT activity in transfected HIT cells was normalized by reference to expression of β-galactosidase. Glucose caused a dose-dependent increase in expression of CAT activity, with a half-maximal effect at 5.5 mM and a maximum response of 4-fold. Mannoheptulose blocked this effect of glucose. Other metabolites (mannose, 4-methyl-2-oxopentanoate and leucine plus glutamine) were also able to increase insulin promoter-driven CAT expression, but galactose and arginine were ineffective. The stimulatory effect of glucose on CAT expression was not blocked by verapamil and was inhibited by increasing extracellular Ca^{2+} from 0.4 to 5 mM. Both dibutyryl cyclic AMP and forskolin caused an increase in insulin promoter-driven gene expression in the presence of 1 mM-glucose, but neither agent further increased the level of expression occurring in the presence of a maximally stimulating glucose concentration. The phorbol ester phorbol 12-myristate 13-acetate (PMA) also increased insulin promoter-driven CAT expression in the presence of 1 mM-, but not 11 mM-glucose. Staurosporine blocked the stimulatory effect not only of PMA but also of glucose and of dibutyryl cyclic AMP. We conclude that the 5′ upstream region of the insulin gene contains sequences responsible for mediating the stimulatory effect of glucose on insulin-gene transcription. The mechanism involves metabolism of the sugar, but does not appear to be mediated by intracellular Ca^{2+}; however, the data are consistent with the possible involvement of both protein kinase A and C.

INTRODUCTION

Insulin secretion from the β-cells of the islets of Langerhans is controlled primarily by the circulating glucose concentration. The stimulatory effect of glucose on insulin secretion is mediated by metabolism of the sugar within the β-cell (Ashcroft, 1980). The resultant increase in intracellular ATP/ADP ratio leads to closure of ATP-sensitive K^+ channels in the β-cell membrane (Ashcroft *et al.*, 1984); the consequent depolarization causes opening of voltage-dependent Ca^{2+} channels, and the ensuing influx of Ca^{2+}, and hence rise in intracellular Ca^{2+} concentration, couples the initial signal to the release process (Ashcroft & Ashcroft, 1989, 1990). Hormones or drugs which activate protein kinases A or C amplify the secretory response to a given level of glucose, primarily by increasing the sensitivity of the secretory system to Ca^{2+} (Hughes *et al.*, 1987; Ashcroft & Hughes, 1989).

It has long been known that glucose also increases the rate of insulin biosynthesis (Howell & Taylor, 1966), but the signalling pathway is much less well-defined than for secretion. Detailed studies of the specificity of the biosynthetic response indicated that, as for secretion, intracellular metabolism of the sugar was involved (Ashcroft *et al.* 1978; Lin *et al.* 1979). However, unlike secretion, the biosynthetic response is not dependent on Ca^{2+}; indeed, omission of extracellular Ca^{2+} augments glucose-stimulated insulin biosynthesis (Lin & Haist, 1973; Lin *et al.*, 1979).

The stimulatory effect of glucose on insulin biosynthesis is specific in that the response is not ascribable solely to a general increase in protein synthesis (Ashcroft *et al.*, 1978). However, recent studies have shown that insulin is only one of a number of β-cell proteins whose synthesis is augmented by glucose (Guest *et al.*, 1991). The mechanism whereby insulin biosynthesis is increased by glucose is complex and not well understood. There are effects at the level of translation (Permutt & Kipnis, 1972*a*,*b*; Permutt, 1974; Welsh *et al.*, 1985). However, several studies have shown an increase in preproinsulin mRNA in response to glucose (Brunsted & Chan, 1982; Giddings *et al.*, 1982, 1985; Nielsen *et al.*, 1985; Hammonds *et al.*, 1987*a*,*b*); this response involves increases in both transcription rate (Efrat *et al.*, 1991) and mRNA stability (Welsh *et al.*, 1985).

The regulation of insulin-gene transcription is dependent on *cis*-acting sequences in the upstream non-coding region of the gene (for review see Philippe, 1991). Transfection of β-cell lines with fragments of the insulin-gene upstream region linked to a reporter gene have demonstrated that specific sequences exert both positive and negative effects on gene expression (Walker *et al.*, 1983; Edlund *et al.*, 1985; Nir *et al.*, 1986; Karlsson *et al.*, 1988, 1989; Crowe & Tsai, 1989; Takeda *et al.*, 1989; Whelan *et al.*, 1989; Cordle *et al.*, 1991). Studies with transgenic mice have also demonstrated the key role of upstream sequences in insulin-gene expression (Bucchini *et al.*, 1986; Selden *et al.*, 1986; Fromont-Racine *et al.*, 1990). *trans*-Acting factors specific to the β-cell, as well as non-β-cell-specific factors, have been demonstrated to bind to the upstream region of the insulin

Abbreviations used: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PMA, phorbol 12-myristate 13-acetate; CAT, chloramphenicol acetyltransferase.

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gene by gel-mobility shift and nuclease-protection assays (Ohlsson & Edlund, 1986; Boam & Docherty, 1989; Boam *et al.*, 1990; Karlsson *et al.*, 1990).

These studies showed that the insulin-gene enhancer region is involved in cell-specific expression of the insulin gene, but did not give information about the mechanism whereby glucose produces acute changes in transcription of the gene. Increased expression of the bacterial reporter gene has recently been shown to be evoked in islets of Langerhans transfected with a chimeric construct of the insulin-gene upstream region and chloramphenicol acetyltransferase (CAT) (German *et al.*, 1990). That study did not determine the possible involvement of glucose metabolism in the response, but the authors suggested that Ca^{2+} might mediate the effect of glucose on insulin-gene expression. In the present study we have sought to examine further the mechanism of glucose-stimulated insulin-gene expression. We have used the cloned β-cell line HIT-T15 (Santerre *et al.*, 1981), which we have previously shown retains the main features of glucose-stimulated insulin release (Ashcroft *et al.*, 1986) and also responds to glucose with increased rates of insulin biosynthesis and preproinsulin mRNA levels (Hammonds *et al.* 1987*a*; Gold *et al.*, 1988). The cells were transfected with a plasmid, pOK1 (Karlsson *et al.*, 1987), which contains a chimeric construct of the insulin-gene 5′-region linked to CAT; expression of the reporter gene was followed in response to glucose and other agents. A preliminary account of our findings has been published in abstract form (Goodison & Ashcroft, 1991).

MATERIALS

Phorbol 12-myristate 13-acetate (PMA) was obtained from P-L Laboratories, Northampton, Northants., U.K. Forskolin and staurosporine were from Calbiochem, Cambridge Bioscience, Cambridge, U.K. Nylon membranes (Hybond-N) were obtained from Amersham International, Amersham, Bucks., U.K. Fluorescent chloramphenicol (FASTCAT) was purchased from Molecular Probes Ltd., Eugene, OR, U.S.A. Tissue-culture materials were from Gibco, Paisley, Scotland, U.K. All other reagents were from Sigma Chemical Co., Poole, Dorset, U.K. Plasmid pOK1 and the control plasmid pRSVβ-gal were kindly supplied by Dr. O. Karlsson and Dr. T. Edlund, University of Umeå, Sweden. Hamster insulin cDNA (pSHins1/HB101) was kindly provided by Dr. G. I. Bell, University of Chicago, U.S.A. The cDNA probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was a gift from Dr. P. Schofield, University of Oxford.

METHODS

Cell culture

HIT cells (clone T-15) were propagated as a monolayer culture, grown in RPMI 1640 medium supplemented with 10% (v/v) foetal-calf serum and antibiotic/antimycotics in a humidified atmosphere of air/CO_2 (19:1) as previously described (Ashcroft *et al.*, 1986). Culture medium was replaced every 48 h, and cells were passaged weekly after detachment by trypsin/EDTA.

RNA extraction and Northern-blot analysis

For all experimental conditions, 80%-confluent 60 mm-diam. plates containing $(6–8) \times 10^6$ cells were preincubated in Hepes-buffered Krebs bicarbonate medium containing 5 mg of BSA/ml for 1 h. Subsequently cells were incubated for a further 4 h in fresh medium containing test agents. Cells were then washed in phosphate-buffered saline, lysed in guanidinium isothiocyanate (Chirgwin *et al.*, 1979) and total RNA was extracted by centrifugation through a CsCl gradient. Heat-denatured RNA (10 μg) was size-fractionated by electrophoresis through a 1.2% formaldehyde/agarose gel and transferred to a nylon membrane. Membranes were baked at 80 °C for 2 h and prehybridized for 4 h at 42 °C. Hybridizations were carried out at the same temperature for 18 h by adding oligonucleotide probes complementary to insulin mRNA and to the constitutively expressed GAPDH mRNA.

Oligonucleotides were labelled with [^{32}P]dATP by the random-prime method to a specific radioactivity of $(1–3) \times 10^9$ c.p.m./μg according to the manufacturers' instructions. Hybridized blots were washed in decreasing concentrations (5× to 0.5×) of SSC (0.15 M-NaCl/0.015 M-sodium citrate, pH 7) at 60 °C, and membranes were exposed to Kodak X-AR film with an intensifying screen at −70 °C. The resulting autoradiographs were quantified by scanning densitometry, and the values for preproinsulin mRNA were expressed relative to those for GAPDH mRNA in the same extract.

Transfection and analysis of chimeric gene expression

Each plate of HIT cells was co-transfected with 5 μg of test plasmid and 5 μg of internal control plasmid by the calcium phosphate co-precipitation technique (Graham & van der Eb, 1973) as modified by Luthman & Magnusson (1983). The test plasmid (pOK1) was derived from pUC18 by inserting a fragment containing 410 bp of rat insulin-1 gene 5′ flanking region upstream of the coding sequence of the bacterial CAT gene (Karlsson *et al.*, 1987). The plasmid used here was created by *Xba*1 digestion, yielding a plasmid which has the CAT gene driven by −345 bp to +1 bp of the insulin gene upstream region. pRSVβ-Gal was used as a control plasmid to allow for differences in transfection efficiency and cell number. Cells were allowed to recover in propagation medium for 24 h before replacement with fresh medium containing the additions stated. Low-glucose media were prepared from an RPMI 1640 constituent kit. In one series of experiments, the concentration of Ca^{2+} in the RPMI medium was increased from the usual value of 0.4 mM to 5 mM by addition of $CaCl_2$. After 24 h exposure to test agents, cell extracts were prepared as described by Gorman *et al.* (1982) and assayed for CAT and β-galactosidase enzyme activities; 25–100 μg of protein was used for measurement of CAT activity. We initially used [^{14}C]chloramphenicol as described by Edlund *et al.* (1985); latterly we used a fluorescent chloramphenicol substrate which was quantified by fluorimetry after acetylation products were separated by t.l.c. CAT activity measurements were performed in triplicate and were normalized against β-galactosidase values measured as described by Edlund *et al.* (1985).

Significance of data

All data are expressed as means ± S.E.M. for the numbers of observations shown. The significance of the observed differences was assessed by Student's *t* test.

RESULTS

Effects of glucose and other agents on levels of preproinsulin mRNA in HIT cells

The data are given in Table 1. Glucose (10 mM) increased the level of preproinsulin mRNA 2.3-fold. This response was blocked by mannoheptulose and was mimicked by the β-cell metabolite 4-methyl-2-oxopentanoate, but not by the non-metabolized sugar galactose. Increases in preproinsulin mRNA were also elicited by dibutyryl cyclic AMP, by forskolin (an activator of adenylate cyclase) and by glucagon.

Effects of glucose and other agents on insulin promoter-driven CAT expression in transfected HIT cells

Fig. 1 shows that glucose caused a marked and concentration-dependent increase in CAT activity in HIT cells transfected with

Table 1. Effect of glucose and other agents on preproinsulin mRNA levels in HIT cells

HIT T15 β-cells were cultured with the agents shown for 4 h as described in the Methods section. Preproinsulin mRNA levels were measured by Northern-blot analysis on total RNA and quantified by autoradiography and densitometry. The values for preproinsulin mRNA were calculated relative to those for GAPDH mRNA in the same extracts and are expressed here as a percentage of the value in the absence of additions, given as means ±S.E.M. for the numbers of observations shown in parentheses: [a] significantly greater than value in the absence of glucose ($P < 0.001$); [b] significantly less than value in the presence of 10 mM-glucose ($P < 0.001$).

Conditions		
Glucose concn. (mM)	Other addition	Preproinsulin mRNA (% of control)
0	–	100±11 (9)
10	–	216±27 (9)[a]
10	15 mM-mannoheptulose	88±28 (6)[b]
0	10 mM-4-methyl-2-oxopentanoate	198±13 (6)[a]
0	10 μM-forskolin	231±39 (6)[a]
0	1 mM-dibutyryl cyclic AMP	248±18 (6)[a]
0	50 nM-glucagon	206±24 (6)[a]
0	15 mM-galactose	85±9 (6)

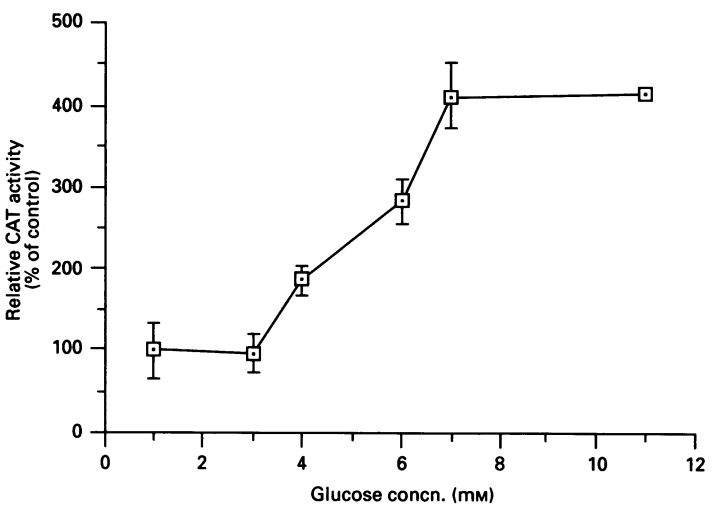

Fig. 1. Dose-dependent stimulation by glucose of insulin promoter-driven CAT expression in HIT cells

HIT T15 β-cells were co-transfected with pOK1, containing the 5′ upstream insulin-gene region driving the reporter gene CAT, and pRSVβ-gal as described in the Methods section. Then 24 h after transfection the cells were exposed to various concentrations of glucose for 24 h, before extraction and assay of CAT and β-galactosidase activities. The values for CAT activity are expressed relative to the β-galactosidase activity in the same cells. The data are plotted as means ±S.E.M. for three independent observations.

a chimeric construct of the insulin 5′ upstream region linked to CAT. The response was most sensitive to changes in glucose concentration between 3 and 7 mM, with half-maximal enhancement at 5.5 mM-glucose and a maximum response of 4-fold.

Table 2 shows that mannoheptulose abolished the effect of glucose on CAT expression. Other nutrients (mannose, 4-methyl-2-oxopentanoate and the combination of leucine plus glutamine) mimicked the effect of glucose. However, neither galactose nor arginine stimulated insulin promoter-driven gene expression.

Table 2. Effect of nutrients on insulin promoter-driven CAT expression in transfected HIT cells

The ability of glucose and other agents to modulate insulin promoter-driven CAT expression in transfected HIT β-cells was determined as described in the legend to Fig. 1. Data are given as means ±S.E.M. for the numbers of observations in parentheses: [a] significantly greater than value in the presence of 1 mM-glucose ($P < 0.001$); [b] significantly less than control value at the same concentration of glucose ($P < 0.001$).

Conditions		
Glucose concn. (mM)	Other addition(s)	Relative CAT activity (% of control)
1	–	100±10 (27)
11	–	302±35 (27)[a]
11	15 mM-mannoheptulose	73±12 (6)[b]
1	15 mM-4-methyl-2-oxopentanoate	320±19 (6)[a]
1	15 mM-leucine+15 mM-glutamine	343±20 (3)[a]
1	11 mM-mannose	289±46 (6)[a]
1	15 mM-galactose	84±28 (3)
1	15 mM-arginine	50±5 (3)[b]

Table 3. Effects of verapamil and increased extracellular Ca^{2+} on insulin promoter-driven CAT expression in transfected HIT cells

The ability of glucose and other agents to modulate insulin promoter-driven CAT expression in transfected HIT β-cells was determined as described in the legend to Fig. 1. The Ca^{2+} concentration of the RPMI medium was increased from the normal value of 0.4 mM to 5 mM by addition of $CaCl_2$, as indicated. Data are given as means ±S.E.M. for the numbers of observations in parentheses: [a] significantly greater than with 1 mM-glucose ($P < 0.001$); [b] significantly less than with 0.4 mM-Ca^{2+} at the same glucose concentration ($P < 0.05$).

Glucose concn. (mM)	Conditions		Relative CAT activity (% of control)
	Ca^{2+} (mM)	Other addition(s)	
1	0.4	–	100±10 (27)
11	0.4	–	302±35 (27)[a]
11	0.4	100 μM-verapamil	256±41 (12)[a]
1	5	–	70±17 (6)
11	5	–	125±42 (9)[b]

The results of experiments to examine the possible role of Ca^{2+} in glucose-stimulated gene expression are shown in Table 3. The stimulation elicited by glucose was not inhibited by the Ca^{2+}-channel blocker verapamil, but was markedly inhibited when the concentration of extracellular Ca^{2+} was increased from 0.4 mM (the standard concentration of Ca^{2+} in RPMI 1640 medium) to 5 mM.

The involvement of the cyclic AMP system was tested. Table 4 shows that both dibutyryl cyclic AMP and forskolin caused an increase in insulin promoter-driven gene expression in the presence of 1 mM-glucose. Neither agent further increased the level of expression occurring in the presence of a maximally stimulating glucose concentration.

Studies to examine the possible effects of modulation of protein kinase C activity are also shown in Table 4. PMA increased insulin promoter-driven CAT expression in the presence of 1 mM but not 11 mM-glucose. Staurosporine, an inhibitor of protein kinase C, blocked the stimulatory effect not only of PMA but also of glucose and of dibutyryl cyclic AMP.

Table 4. Effects of modulators of protein kinase A or C on insulin promoter-driven CAT expression in transfected HIT cells

The ability of agents modulating protein kinase A or C to affect insulin promoter-driven CAT expression in transfected HITβ-cells was determined as described in the legend to Fig. 1. Data are given as means ± S.E.M. for the numbers of observations in parentheses: [a] significantly greater than value in the presence of 1 mM-glucose alone ($P < 0.001$); [b] significantly less than corresponding value in the absence of staurosporine ($P < 0.001$); [c] significantly greater than value in the presence of 1 mM-glucose ($P < 0.001$; not significant versus 11 mM-glucose).

Conditions		
Glucose concn. (mM)	Other addition(s)	Relative CAT activity (% of control)
1	–	100 ± 10 (27)
1	1 mM-dibutyryl cyclic AMP	247 ± 56 (6)[a]
1	10 μM-forskolin	221 ± 21 (9)[a]
1	100 nM-PMA	222 ± 19 (12)[a]
1	100 nM-staurosporine	100 ± 10 (12)
1	100 nM-PMA + 100 nM-staurosporine	89 ± 12 (9)[b]
1	1 mM-dibutyryl cyclic AMP + 100 nM-staurosporine	130 ± 5 (3)[b]
11	–	302 ± 35 (27)[a]
11	1 mM-dibutyryl cyclic AMP	340 ± 27 (6)[c]
11	10 μM-forskolin	318 ± 31 (9)[c]
11	100 nM-TMA	300 ± 35 (9)[c]
11	100 nM-staurosporine	93 ± 5 (3)[b]

DISCUSSION

The 5′ region of the insulin gene contains sequences which regulate cell-specific expression of insulin (see review by Philippe, 1991). In addition to such long-term control, studies of insulin biosynthesis (for review see Welsh, 1989) have indicated that transcriptional control of the insulin gene is also exerted by the availability of glucose and other factors. In the present study we have employed a bacterial reporter gene technique to determine whether the insulin-gene upstream region mediates glucose-regulated gene transcription.

The SV40-transformed hamster β-cell line (HIT-T15) used here is a ready source of glucose-responsive β-cells, well characterized in terms of insulin secretion and biosynthesis (Ashcroft *et al.*, 1986; Gold *et al.*, 1987). HIT cells have also been widely used for previous studies defining sequences involved in cell-specific transcription of the insulin gene (Edlund *et al.*, 1985; Karlsson *et al.*, 1987, 1989).

To confirm the suitability of the HIT cell line for this study, we first measured steady-state levels of preproinsulin mRNA under various conditions. As previously reported (Hammonds *et al.*, 1987*a*), glucose had a significant stimulatory effect on preproinsulin mRNA levels. Furthermore, this effect of glucose was inhibited by mannoheptulose, an inhibitor of β-cell glucose phosphorylation (Ashcroft, 1980). This suggests that glucose needs to be metabolized via glucose 6-phosphate for the stimulatory signal to be perceived. This requirement for metabolism, which has been well established for both secretion and biosynthesis of insulin (Ashcroft, 1980), is further supported by our observations that an increase in HIT-cell preproinsulin mRNA levels is evoked by 4-methyl-2-oxopentanoate, the deamination product of leucine, but not by the non-metabolized sugar galactose. These data agree with findings in islets of Langerhans (Welsh *et al.*, 1986*a*).

Reagents which raise intracellular cyclic AMP, thereby activating protein kinase A, enhance glucose-stimulated insulin release (Ashcroft & Hughes, 1989), and glucose itself is also known to raise cyclic AMP levels in HIT cells (Hammonds *et al.*, 1987*a*). In agreement with previous observations (Hammonds *et al.*, 1987*a*; Gold *et al.*, 1987), we found here that HIT cells also responded to agents activating protein kinase A with increased preproinsulin mRNA levels.

To obtain information on the DNA sequence involved in these effects on transcription, we transfected HIT cells with a chimeric plasmid construct, pOK1, containing the −345 to +1 bp upstream region of the rat insulin-1 gene driving the bacterial reporter gene CAT.

An increase in glucose concentration from 1 to 11 mM increased normalized CAT activity in transfected HIT cells 4-fold, with a half-maximal response at 5.5 mM-glucose. This dose/response curve is similar to that found for the dependence on glucose concentration of insulin secretion by HIT cells (Ashcroft *et al.*, 1986). As was observed for preproinsulin mRNA levels, the effect of glucose on transcription was inhibited by mannoheptulose. The sugar mannose, the amino acid leucine and its metabolite 4-methyl-2-oxopentanoate also stimulated CAT expression, again parallel to their effects on insulin-gene transcription. Incubation with the potent secretagogue arginine did not stimulate CAT expression, consistent with the failure of arginine to stimulate insulin biosynthesis (Anderson, 1976) and the fact that arginine-stimulated insulin release does not require metabolism of the amino acid (Charles *et al.*, 1982). Indeed, in our studies arginine significantly decreased CAT expression. This may result from the inhibitory effect of exposure to arginine on β-cell protein synthesis resulting from inhibition of neutral amino acid uptake (Lin, 1977). We therefore conclude that, as for insulin secretion, glucose stimulates insulin-gene expression as a consequence of intracellular metabolism of the sugar.

The link between metabolism and transcription is unknown. The only previous report of glucose-modulated rat insulin-1 driven reporter gene expression (German *et al.*, 1990) used dispersed fetal-rat islets transfected by electroporation. It was reported that, in this system, Ca^{2+} appeared to play a key role in the transcriptional response to glucose. Our findings do not support this conclusion. In our study, co-incubation with the Ca^{2+}-channel blocker verapamil did not significantly decrease the response to glucose in transfected HIT cells; moreover, raising extracellular Ca^{2+} to levels which enhance glucose-mediated insulin release markedly decreased reporter gene expression in response to high glucose. We do not know why our findings differ from those of German *et al.* (1990). However, our conclusion that increased intracellular Ca^{2+} does not mediate effects of glucose on insulin-gene expression accords with previous studies on insulin biosynthesis showing that extracellular Ca^{2+} is not required for glucose to stimulate insulin biosynthesis (Lin & Haist, 1973; Lin *et al.* 1979; Welsh *et al.*, 1988), and indeed exerts an inhibitory effect on insulin biosynthesis (Lin *et al.* 1979). Nor does an increase in intracellular Ca^{2+} elicited by tolbutamide lead to increased insulin biosynthesis (Taylor & Parry, 1967; Lin & Haist, 1969; Duran Garcia *et al.*, 1976). Furthermore, diazoxide, which blocks glucose-induced elevation of intracellular Ca^{2+}, does not inhibit glucose-stimulated insulin biosynthesis (Lin & Haist, 1973). We suggest therefore that stimulation of insulin-gene transcription by glucose and other metabolites is not mediated by increased intracellular Ca^{2+}.

Agents activating protein kinase A stimulated CAT reporter gene expression in the transfected β-cell system, as in the preproinsulin mRNA studies. A role for cyclic AMP has been demonstrated in both basal and induced transcriptional activity in a number of genes (for review see Montminy *et al.*, 1990); a cyclic AMP-responsive element, which has been defined in the rat

insulin-1 promoter (Philippe & Missotten, 1990), is known to be intact in the constructs used in this study. Co-incubation with glucose and protein kinase A activators did not show an additive effect, but resolution of a synergistic action was not possible, as HIT-cell viability is adversely affected by long-term incubations without glucose. Since glucose increases cyclic AMP in HIT cells (Hammonds *et al.*, 1987*a*), it is therefore possible that cyclic AMP mediates effects of glucose on insulin-gene transcription.

Activation of protein kinase C also elicits marked effects on insulin secretion (Hughes *et al.*, 1990). We found that incubation with staurosporine, an inhibitor of protein kinase C, completely abolished CAT expression in response not only to glucose but also to dibutyryl cyclic AMP. These data suggest a pathway in which protein kinase A activity is either regulated by or dependent on protein kinase C activity, although this conclusion must be regarded as tentative, since staurosporine is not absolutely specific for protein kinase C. The phorbol ester PMA, which is known to stimulate protein kinase C activity directly, has been shown to be effective in increasing insulin release (Ashcroft *et al.*, 1986) and insulin-gene transcription (Hammonds *et al.*, 1987*a*) in HIT cells. Incubation of transfected HIT cells with PMA stimulated CAT expression to a similar extent as did glucose; the stimulation by PMA was prevented by staurosporine. Interpretation of the findings with PMA is difficult, however, since PMA, in addition to stimulating protein kinase C acutely, also causes down-regulation of protein kinase C in HIT cells (Hughes *et al.*, 1990).

The present findings are based on the use of the rat insulin-1 gene regulatory region in a different rodent species cell line. However, the insulin-gene 5′ flanking region shows great similarity between species, and there is evidence both that β-cell-specific transcription of the human insulin gene occurs in rodent cell lines (Boam *et al.*, 1990) and that rat insulin-1 and -2 regulatory regions operate in transgenic mice (Hanahan, 1985; Efrat & Hanahan, 1987). It is therefore likely that *trans*-acting factors responsible for regulating transcriptional activity of the insulin gene are highly conserved between mammals. Nevertheless, differences may exist in sequence specificities, and regulation of transcriptional factors may be mediated by different signal pathways. Transfected HIT cells represent a useful system for delineation of those DNA sequences and protein factors that are responsible for modulation of insulin-gene expression by alterations in metabolic flux. Characterization of such factors and their regulation may help to determine the pathways involved in linking extracellular signals to the insulin gene.

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