A role for polyamines in glucose-stimulated insulin-gene expression

Nils WELSH

Department of Medical Cell Biology, Uppsala University, Biomedical Center, P.O.B. 571, S-751 23 Uppsala, Sweden

The aim of the present study was to evaluate the possible role for polyamines in the glucose regulation of the metabolism of insulin mRNA of pancreatic islet cells. For this purpose islets were prepared from adult mice and cultured for 2 days in culture medium RPMI 1640 containing 3.3 mM- or 16.7 mM-glucose with or without the addition of the inhibitors of polyamine biosynthesis difluoromethylornithine (DFMO) and ethylglyoxal bis(guanyldrazone) (EGBG). Culture at the high glucose concentration increased the islet contents of both insulin mRNA and polyamines. The synthesis of total RNA, total islet polyamines and polyamines associated with islet nuclei was also increased. When the combination of DFMO and EGBG was added in the presence of 16.7 mM-glucose, low contents of insulin mRNA, spermine and spermidine were observed. Total islet polyamine synthesis was also depressed by DFMO+EGBG, unlike islet biosynthesis of polyamines associated with nuclei, which was not equally decreased by the polyamine-synthesis inhibitors. Total RNA synthesis and turnover was not affected by DFMO+EGBG. Finally, actinomycin D attenuated the glucose-induced enhancement of insulin mRNA, and cycloheximide counteracted the insulin-mRNA attenuation induced by inhibition of polyamine synthesis. It is concluded that the glucose-induced increase in insulin mRNA is paralleled by increased contents and rates of polyamine biosynthesis and that an attenuation of the increase in polyamines prevents the increase in insulin mRNA. In addition, the results are compatible with the view that polyamines exert their effects on insulin mRNA mainly by increasing the stability of this messenger.

INTRODUCTION

It is generally agreed that glucose is the principal regulator of the insulin gene (Welsh, 1989). This nutrient is thought to increase insulin mRNA contents both *in vitro* and *in vivo*, and increases of as much as 10-fold have been observed on glucose stimulation (Brunstedt & Chan, 1982). The glucose effect is mediated by a combination of increased transcription of the insulin gene (Nielsen *et al.*, 1985) and a selective stabilization of insulin mRNA against degradation (Welsh *et al.*, 1985). The stimulatory effect of glucose on insulin-gene transcription may, in part, be mediated by cyclic AMP (Nielsen *et al.*, 1985; Welsh *et al.*, 1985). It has been shown that other nutrients also enhance insulin-gene expression (Welsh *et al.*, 1986) and that the insulin mRNA contents often correlate well with the rate of islet ATP generation (Welsh *et al.*, 1986; Spinas *et al.*, 1987; Eizirik *et al.*, 1988). Thus glucose may need to be metabolized in order to exert its effects on insulin-gene expression; however, the precise role of the putative factors produced by glucose metabolism in this mechanism remains unknown.

Polyamines are ubiquitous aliphatic cations present at millimolar concentrations in pancreatic β -cells (Hougaard *et al.*, 1986; Welsh & Sjöholm, 1988). It is now well established that polyamines modulate activities of RNA polymerases when studied in systems *in vitro* (Blair, 1985). The polyamines also influence the stability of RNA *in vitro* by binding directly to RNA or by interacting with enzymes that degrade RNA molecules (Karpetsky *et al.*, 1977). However, the findings of studies *in vivo* have been far from conclusive as far as a direct relationship between the metabolism of polyamines and that of RNA is concerned (Goyns, 1982). In a previous study we observed that inhibition of polyamine biosynthesis caused decreased insulin mRNA contents in mouse pancreatic islets *in vitro* (Welsh & Sjöholm, 1988). In view of this and the findings that glucose

increased the biosynthesis (Hougaard *et al.*, 1986) and cellular contents (Welsh & Sjöholm, 1988) of polyamines, I have studied the role of polyamines in glucose-stimulated insulin-gene expression.

MATERIALS AND METHODS

Chemicals

The chemicals were obtained from the following sources. Boehringer-Mannheim, Mannheim, Germany: collagenase, type CLS (EC 3.4.24.3). Statens Bakteriologiska Laboratorium, Stockholm, Sweden: Hanks' balanced salt solution. Flow Laboratories, Irvine Ayrshire, Scotland, U.K.: culture medium RPMI 1640, L-glutamine and donor calf serum. Amersham International, Amersham, Bucks., U.K.: [32 P]dCTP (sp. radioactivity 3000 Ci/mmol), [5,6- 3 H]uridine (40 Ci/mmol) and [1,4(n)- 3 H]-putrescine dihydrochloride (15–30 Ci/mmol). Pharmacia Fine Chemicals, Uppsala, Sweden: Ficoll 400. Sigma Chemical Co., St. Louis, MO, U.S.A.: spermidine, spermine, dansyl chloride, L-proline and Hepes. All other chemicals of analytical grade were obtained from E. Merck, Darmstadt, Germany.

Preparation and culture of isolated islets

Islets were isolated from the pancreas of male NMRI mice (Anticimex, Stockholm, Sweden) by a collagenase-digestion technique and separated from exocrine pancreatic cells by Ficoll-gradient centrifugation (Howell & Taylor, 1968; Lernmark *et al.*, 1976). The islets were subsequently picked free from exocrine tissue and maintained free-floating in tissue culture at 37 °C under a humidified atmosphere of air/5% CO₂. The culture medium was RPMI 1640 containing 10% (v/v) calf serum, 2 mM-L-glutamine, benzylpenicillin (100 units/ml) and streptomycin (0.1 mg/ml). The islets were cultured for 3 days in

Abbreviations used: DFMO, difluoromethylornithine; EGBG ethylglyoxal bis(guanyldrazone); PBS, phosphate-buffered saline (154 mM-Na⁺, 4 mM-K⁺, 140 mM-Cl⁻, 10 mM-HPO₄²⁻, pH 7.4).

3.3 mM-glucose and were then divided into groups of 100–200 islets each and cultured for another 24–48 h with additions of glucose, the inhibitors of polyamine synthesis, DFMO (5 mM) and EGBG (100 μ M), and actinomycin D and cycloheximide at concentrations of 5 μ g/ml and 10 μ g/ml respectively.

Polyamine contents

The islet spermidine and spermine contents were measured essentially as described previously (Seiler, 1983; Welsh & Sjöholm, 1988). Briefly, the polyamines were extracted from sonicated islet preparations with 0.3 M-HClO₄ and conjugated to dansyl chloride in acetone overnight. The dansylated conjugates were extracted from the water phase in toluene and applied to t.l.c. plates (HPTLC Fertigplatten, Kieselgel 60 F₂₅₄; E. Merck). The dansylated polyamines were separated by one run with ethyl acetate/cyclohexane (1:1, v/v), followed by two runs with diethyl ether/cyclohexane (2:3, v/v). The spots were identified under u.v. light, scraped off the plates and eluted in 400 μ l of methanol. The fluorescence intensities were measured in a Perkin-Elmer LS-5B fluorescence spectrophotometer.

Polyamine synthesis

[³H]Putrescine at 2 μ Ci/ml was added to the culture medium during the last 24 h of the culture period. For total islet polyamine biosynthesis, the islets in groups of 25 were washed in cold phosphate-buffered saline (PBS) and sonicated in 25 μ l of HClO₄. The polyamines were extracted, dansylated and separated on t.l.c. plates as described above. The specific radioactivity of [³H]-putrescine was determined in islets in groups of 300 which were cultured, labelled and homogenized as described above. The polyamines were dansylated, separated on t.l.c., and the putrescine spots were scraped off the plates and extracted with methanol. A sample was taken for liquid-scintillation counting, and the fluorescence intensity of the remaining volume was determined.

For islet nuclear polyamine biosynthesis, nuclei were isolated by a rapid and simple method in order to minimize redistribution of nuclear polyamines during the preparation procedure. Islets in groups of 100 were labelled, washed and subsequently homogenized in a glass tissue homogenizer in 100 μ l of 250 mM-sucrose/2% Triton X-100/2 mM-EDTA/20 mM-Tris, pH 7.5 (0–4 °C). The homogenates were centrifuged at 12000 *g* for 10 s, and the resulting pellet was washed once with the homogenization buffer and re-pelleted. The pellet obtained consisted of essentially pure nuclei as assessed by microscopic inspection. The nuclei fraction was also characterized biochemically by determining the insulin content. Less than 3% of the total islet insulin content was recovered in the nuclear fractions, suggesting an insignificant contamination of nuclei with secretory granules. To the nuclei was added 25 μ l of 0.3 M-HClO₄ and the polyamines were extracted, dansylated and separated on t.l.c. plates as described above. After scraping off the spots, the incorporated radioactivity was determined by liquid-scintillation counting after addition of 1.5 ml of Scintillator 299 (Packard Instruments, Downers Grove, IL, U.S.A.).

Total RNA synthesis

After the culture period, islets in groups of 20 were incubated for 1 h in 100 μ l of a Krebs–Ringer bicarbonate-buffered salt solution (Krebs & Henseleit, 1932) containing 10 mM-Hepes, 1 mg of BSA/ml, 5 μ Ci of [³H]uridine and 16.7 mM-glucose. After the incubation, the islets were washed in cold PBS and sonicated in 100 μ l of redistilled water. To the homogenate was added 0.5 ml of 10% trichloroacetic acid, and the solutions were centrifuged for 10 min at 12000 *g*. The pellets were washed once with trichloroacetic acid and subsequently dissolved in 100 μ l of Soluene before liquid-scintillation counting.

Total RNA turnover

Groups of 200 pre-cultured islets were labelled for 3 h with [³H]uridine as described above. The islets were subsequently cultured for 24, 48, 72 and 96 h in RPMI 1640 (11.1 mM-glucose) with or without addition of 5 mM-DFMO + 100 μ M-EGBG. Groups of islets were taken at the different time points and the incorporated radioactivity was determined as above. The RNA half-lives (*t*_{1/2}) were estimated by assuming that the labelled RNA was degraded according to a first-order decay process. The percentage decline, expressed as a function of time, was then subjected to an exponential regression analysis in order to calculate the RNA half-life.

Insulin-mRNA content

For insulin-mRNA quantification, as determined by dot-blot analysis (Thomas, 1980; White & Bancroft, 1982), groups of 40–50 islets were washed in PBS immediately after the culture period and briefly sonicated in 200 μ l of 0.1 M-Tris (pH 7.5)/10 mM-EDTA/1% SDS. The sonicated islets were extracted with 200 μ l of phenol/chloroform/3-methylbutan-1-ol (25:24:1, by vol.). Nucleic acids were precipitated with potassium acetate and ethanol overnight at –20 °C. The precipitates were washed with 70% ethanol and subsequently treated with 1 M-glyoxal for 1 h at 50 °C, diluted with 4 vol. of 2 M-NaCl/0.3 M-sodium citrate and bound to Genescreen membranes (New England Nuclear) at 80 °C for 2 h. After pre-hybridization, the samples were hybridized at 42 °C with the appropriate probe (pRI-7) (Chan *et al.*, 1979), which had been labelled by using an oligonucleotide-labelling kit (Pharmacia). After hybridization, the filters were washed and then exposed at –70 °C to Hyperfilm-MP (Amersham) with an intensifying screen. The intensities of the spots thus obtained were determined by densitometry and expressed as optical density (o.d.).

Statistical analysis

Means $\pm$ S.E.M. were calculated, and comparisons between 3.3 mM-glucose and the other groups were performed by Student's paired *t* test.

RESULTS

Effects of glucose and inhibitors of polyamine biosynthesis on islet polyamine contents

Islets cultured for 2 days at 16.7 mM-glucose after a pre-culture period at 3.3 mM-glucose contained more spermine and spermidine than did those maintained throughout at the low glucose concentration (Table 1). A similar increase in spermine content was observed in islets cultured at the high glucose concentration with additional supplementation with DFMO. However, no significant increase in the spermidine contents was observed in this condition. Islets cultured in the presence of high glucose, DFMO and EGBG displayed unaltered polyamine contents as compared with islets cultured at the lower glucose concentration.

Effects of glucose and inhibitors of polyamine biosynthesis on total islet polyamine biosynthesis

Glucose induced no alteration in the specific radioactivity of [³H]putrescine. The mean specific radioactivity of [³H]-putrescine from two separate experiments at 3.3 mM-glucose was 480 $\pm$ 60 c.p.m./pmol, and at 16.7 mM-glucose it was 410 $\pm$ 160 c.p.m./pmol. However, it was increased 2-fold by DFMO (940 $\pm$ 200 c.p.m./pmol) and approx. 8-fold by EGBG + DFMO (3600 $\pm$ 1000 c.p.m./pmol).

There was an approx. 4-fold stimulation of spermine and spermidine synthesis in islets cultured at 16.7 mM-glucose as

Table 1. Effects of glucose and inhibitors of polyamine biosynthesis on spermine and spermidine contents

Isolated adult mouse pancreatic islets were pre-cultured at 3.3 mM-glucose for 3 days and subsequently cultured for 2 days under the four different conditions given in the Table. Islet polyamine contents (pmol/100 islets) were determined spectrofluorimetrically after separation on t.l.c. and are expressed as means $\pm$ S.E.M. for five separate experiments: * $P < 0.05$ for a chance difference versus 3.3 mM-glucose, by Student's paired t test.

Islet culture	Content (pmol/100 islets)	
	Spermine	Spermidine
3.3 mM-glucose	210 $\pm$ 30	120 $\pm$ 10
16.7 mM-glucose	310 $\pm$ 20*	210 $\pm$ 40*
16.7 mM-glucose + DFMO	360 $\pm$ 60*	170 $\pm$ 60
16.7 mM-glucose + DFMO + EGBG	250 $\pm$ 25	150 $\pm$ 40

Table 2. Effects of glucose and inhibitors of polyamine biosynthesis on total islet polyamine biosynthesis

Islets were cultured as described in Table 1. [3 H]Putrescine was present in the culture media during the last 24 h of the culture period. The islets were washed and the islet polyamines were separated on t.l.c. The spots were scraped off and the radioactivity was determined by scintillation counting. Values are means $\pm$ S.E.M. for five separate experiments: * $P < 0.05$, *** $P < 0.001$, for chance differences versus 3.3 mM-glucose-cultured islets, by Student's paired t test.

Islet culture	10 ⁻² $\times$ Polyamine synthesis (c.p.m./24 h per 25 islets)	
	Spermine	Spermidine
3.3 mM-glucose	4.4 $\pm$ 0.6	21 $\pm$ 4.5
16.7 mM-glucose	16 $\pm$ 1.3***	75 $\pm$ 4.8***
16.7 mM-glucose + DFMO	28 $\pm$ 2.4***	111 $\pm$ 11***
16.7 mM-glucose + DFMO + EGBG	7.0 $\pm$ 0.8*	32 $\pm$ 5.7

compared with 3.3 mM-glucose (Table 2). This effect appeared to be even further potentiated by DFMO (Table 2). However, when corrected for the observed difference in specific radioactivity of the labelled precursor, the islets cultured in 16.7 mM-glucose and those cultured in 16.7 mM-glucose + DFMO exhibited similar rates of polyamine biosynthesis. In the presence of DFMO + EGBG, the spermidine and spermine synthesis rates appeared similar to that of 3.3 mM-glucose-cultured islets (Table 2). The means of the DFMO + EGBG group should probably be divided by 8, leading to a considerably lower rate of polyamine biosynthesis, since the specific radioactivity of putrescine was 8-fold higher in the DFMO + EGBG group.

Effects of glucose and inhibitors of polyamine biosynthesis on biosynthesis of polyamines associated with nuclei

Islets cultured at 16.7 mM-glucose displayed a 2-fold increase in spermine synthesis and a 3-fold increase in synthesis rates of nuclear spermidine as compared with 3.3 mM-glucose (Table 3). The stimulatory effect of glucose was not affected by DFMO.

Table 3. Effects of glucose and inhibitors of polyamine biosynthesis on biosynthesis of polyamines associated with islet-cell nuclei

Islets were cultured as described in Table 1. [3 H]Putrescine was present in the culture media during the last 24 h of the culture period. The islets were homogenized in 2% Triton X-100, and nuclei were pelleted by centrifugation. The polyamines were separated on t.l.c., and the amount of radioactivity was determined by scintillation counting. Values are means $\pm$ S.E.M. for 9 experiments: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ for chance differences versus 3.3 mM-glucose by Student's paired t test.

Islet culture	10 ⁻² $\times$ Polyamine synthesis (c.p.m./24 h per 100 islets)	
	Spermine	Spermidine
3.3 mM-glucose	4.2 $\pm$ 1.0	10 $\pm$ 2.3
16.7 mM-glucose	7.7 $\pm$ 1.7**	25 $\pm$ 4.6**
16.7 mM-glucose + DFMO	15 $\pm$ 2.4***	36 $\pm$ 7.3**
16.7 mM-glucose + DFMO + EGBG	8.6 $\pm$ 2.2*	23 $\pm$ 3.7**

Table 4. Effects of glucose and inhibitors of polyamine biosynthesis on insulin mRNA contents, total RNA synthesis and turnover

Isolated adult mouse pancreatic islets were pre-cultured at 3.3 mM-glucose for 3 days and subsequently cultured for an additional 2 days under the four different conditions given in the Table. The insulin mRNA contents of the islets were determined by using the dot-blot technique and optical densitometry. Total RNA synthesis rates were quantified by scintillation measurement of trichloroacetic acid-precipitated islet homogenates labelled with [3 H]uridine. Values are given as means $\pm$ S.E.M. for five separate experiments: * $P < 0.05$ and ** $P < 0.01$ for chance differences versus control islets by Student's paired t test.

Islet culture	Insulin mRNA content (o.d./40 islets)	Total RNA synthesis (d.p.m./h per 5 islets)
3.3 mM-glucose	159 $\pm$ 41	1810 $\pm$ 350
16.7 mM-glucose	268 $\pm$ 20*	2550 $\pm$ 440*
16.7 mM-glucose + DFMO	276 $\pm$ 31*	2390 $\pm$ 310**
16.7 mM-glucose + DFMO + EGBG	121 $\pm$ 28	3000 $\pm$ 340*

Without correction for differences in specific radioactivity, DFMO + EGBG evoked a stimulatory effect similar to that of 16.7 mM-glucose only (Table 3). This is in variance with the results obtained on total polyamine biosynthesis (Table 2), which indicated a more pronounced inhibition induced by DFMO + EGBG. This comparison is not dependent on the specific radioactivity of putrescine.

Effects of glucose and inhibitors of polyamine biosynthesis on insulin mRNA contents, total RNA synthesis and turnover

Both 16.7 mM-glucose alone and 16.7 mM-glucose supplemented with DFMO induced increased contents of insulin mRNA as compared with 3.3 mM-glucose (Table 4). Islets cultured with addition of both DFMO and EGBG contained unaltered insulin mRNA contents compared with low-glucose culture. The total RNA-synthesis rates, however, were significantly stimulated in all high-glucose-cultured groups as compared with those cultured at a low glucose concentration. The total

Table 5. Effects of actinomycin D and cycloheximide on insulin mRNA contents

Isolated pancreatic islets were pre-cultured at 3.3 mM-glucose for 3 days and subsequently cultured for an additional 24 h under the three different conditions given above. During this 24 h culture period, the islets were further divided into equal groups and supplemented with actinomycin D (Am D, 5 µg/ml) and cycloheximide (Ch, 10 µg/ml) as given in the Table. The insulin mRNA contents of the islets were determined by using the dot-blot technique and optical densitometry. The values from 7–11 separate experiments are given above as means $\pm$ S.E.M.: * $P < 0.05$ for chance difference versus corresponding control by Student's paired t test.

Islet culture	Insulin mRNA (o.d./40 islets)		
	Control	Am D	Ch
3.3 mM-glucose	0.43 $\pm$ 0.04	0.41 $\pm$ 0.07	0.59 $\pm$ 0.17
16.7 mM-glucose	1.52 $\pm$ 0.28	0.94 $\pm$ 0.27*	1.00 $\pm$ 0.30
16.7 mM-glucose + DFMO + EGBG	0.81 $\pm$ 0.15	1.22 $\pm$ 0.25	1.31 $\pm$ 0.13*

RNA half-life ($t_{1/2}$) measured in islets cultured without inhibitors of polyamine biosynthesis was 42.7 $\pm$ 2.9 h, and the corresponding value for islets cultured with DFMO + EGBG was 43.6 $\pm$ 6.2 h ($P > 0.05$, $n = 4$).

Effects of actinomycin D and cycloheximide on insulin mRNA contents

No effects were observed of actinomycin D and cycloheximide on insulin mRNA contents of islets cultured at 3.3 mM-glucose only (Table 5). However, at 16.7 mM-glucose actinomycin D significantly decreased the insulin mRNA levels. There was no significant effect of cycloheximide. Islets cultured in the presence of the inhibitors of polyamine biosynthesis responded to cycloheximide with an increased insulin mRNA content. No statistically significant effect was observed in the actinomycin D group.

DISCUSSION

In the present study, we have used the inhibitors DFMO and EGBG to attenuate the glucose-induced increase in polyamine biosynthesis. DFMO specifically and irreversibly inhibits ornithine decarboxylase (Pegg, 1986), which in the β -cell leads to a depletion of putrescine and a partial decrease in spermidine (Welsh & Sjöholm, 1988). When DFMO and EGBG are used in combination, the enzyme *S*-adenosylmethionine decarboxylase, which indirectly controls the synthesis of spermidine and spermine, is inhibited also, leading to a decrease in the islet spermine content (Welsh & Sjöholm, 1988). Since EGBG is not completely specific, it cannot be ruled out that some of its effects may be due to other actions besides inhibition of *S*-adenosylmethionine decarboxylase (Seppänen *et al.*, 1984). Nevertheless, the viability of islet cells treated with EGBG does not seem to be impaired, as assessed by determinations of oxygen-uptake rates and ATP, protein and DNA contents (Welsh & Sjöholm, 1988).

Before addition of the inhibitors of polyamine biosynthesis, all islets were cultured at a low glucose concentration. This period of glucose deficiency leads to low contents of insulin mRNA (Brunstedt & Chan, 1982) and polyamines (Welsh & Sjöholm, 1988), and the mechanisms by which high glucose concentrations stimulate β -cell function can be easily characterized. The present findings show that the glucose-induced increase in insulin mRNA is paralleled by increased contents of spermidine and spermine

and that DFMO attenuated the increase in spermidine. After addition of inhibitors of ornithine decarboxylase, slowly proliferating cells dramatically increase the synthesis and half-life of spermine, which leads to maintained high levels of the polyamine (Pegg, 1986). In the present study, this compensatory mechanism was, however, counteracted by the combination of EGBG + DFMO, which prevented the glucose-induced rise in spermine. It should, however, be pointed out that DFMO + EGBG did not deplete the islet content of spermine below that of the low-glucose-cultured islets.

By fluorescence microscopy, it has been demonstrated that polyamines are mainly localized to the secretory granules of pancreatic β -cells (Hougaard *et al.*, 1986). Thus determinations of total islet polyamine contents may not be representative for the islet nucleus, which is the site of insulin-mRNA transcription. Unfortunately, the polyamine contents of islet nuclei are difficult to determine, since large quantities of islet tissue are needed. However, by assessing islet biosynthesis of polyamines associated with nuclei, it was found in the present study that glucose stimulated the accumulation of newly synthesized polyamines in this organelle. That the synthesis rates of polyamines associated with nuclei appeared less depressed than the total polyamine synthesis rates in islets treated with DFMO + EGBG may indicate that polyamines are preferentially translocated to this organelle. If this is the case, the inhibitors of polyamine synthesis used at present may not have adequately attenuated a glucose-induced rise in the nuclear polyamines. Therefore the present investigation probably does not address the role of polyamines in processes occurring in islet nuclei, such as transcription of the insulin gene.

The glucose-induced rise in synthesis of insulin mRNA and total RNA remained unaffected in islets treated with DFMO, suggesting that the glucose effect was not mediated by increased spermidine contents themselves. However, the finding that insulin mRNA levels did not increase, when the glucose-induced rise in both polyamines was prevented, implicates spermine alone, or the combination of spermine and spermidine, as a possible mediator of parts of the glucose effect. It is unlikely that EGBG plus DFMO exerted a general and non-specific effect on the islet RNA metabolism, since the rates of total RNA synthesis and turnover were not affected by the inhibitors. Interestingly, it has been reported that insulin mRNA contents decrease rapidly in islets during the first day after isolation with collagenase (Brunstedt & Chan, 1982; Giddings *et al.*, 1985). These findings, taken together with our previous finding that islet polyamine contents were lowered during the first 2 days of culture (Welsh & Sjöholm, 1988), are compatible with the view that polyamines play a role in the regulation of insulin mRNA contents.

It can be envisaged that spermine mediated the stimulatory effect of glucose by increasing insulin gene transcription, by decreasing insulin mRNA turnover, or by a combination of both mechanisms. Since direct measurements of insulin mRNA synthesis and insulin mRNA turnover rates are difficult to perform, owing to limited availability of tissue, we have instead used the inhibitor of RNA synthesis, actinomycin D, and the inhibitor of protein synthesis, cycloheximide, to assess these parameters indirectly. The lack of effect of cycloheximide at 3.3 mM- and 16.7 mM-glucose was somewhat surprising, since it has been reported that this compound increases insulin mRNA contents in human islets *in vitro* (Hammonds *et al.*, 1987). The latter finding was interpreted to reflect an inhibition of the synthesis of an insulin-mRNA-degrading factor. Alternatively, inhibition of protein synthesis in mouse islets may lead to depletion of protein factors needed for RNA transcription, which counteracts any insulin-mRNA-stabilizing effects. Interestingly, EGBG-cultured islets showed increased insulin mRNA contents in the presence of cycloheximide, supporting the notion of high rates of insulin

mRNA degradation in these islets, which in this situation is prevented by cycloheximide treatment, thus overcompensating for any other adverse effects of cycloheximide on insulin-gene expression. Actinomycin D inhibits RNA synthesis, leading to lower RNA contents. In the present study, actinomycin D exerted no effect on insulin mRNA contents at a low glucose concentration and decreased insulin mRNA at a high glucose concentration. This is in concordance with a previous study (Welsh *et al.*, 1985) in which it was suggested that the lack of effect at the low glucose concentration was due to similar decreases in the rate of transcription and of degradation of insulin mRNA induced by actinomycin D. It was further suggested that, at the high glucose concentration, the effect on transcription predominated and the net effect therefore would be a decreased content. The lack of effect of actinomycin D on insulin mRNA contents in the DFMO + EGBG-treated islets does not contradict a situation of high turnover in these cells of the insulin mRNA, which is stabilized by actinomycin D to an extent equal to the inhibition of transcription of the insulin gene.

In conclusion, spermine appears to mediate, at least in part, the stimulatory effect of glucose on the expression of the insulin gene, possibly by protecting the mRNA against degradation. Our results do not, however, exclude the possibility of an effect of spermine on mRNA transcription. Further attempts are necessary to determine whether spermine acts specifically on insulin mRNA or whether other messengers are similarly affected. Spermine may exert specific effects in the pancreatic β -cell, since the content of this polyamine is markedly higher than in most other cell types (Hougaard *et al.*, 1986).

DFMO and EGBG were generously given by Dr. Peter P. McCann, of Merrell Dow Research Center, Cincinnati, OH, U.S.A., and Dr. Juhani Jänne, University of Helsinki, Finland, respectively. I thank I.-B. Hallgren for skilful technical assistance. This study was supported by grants from the Swedish Medical Research Council (12X-109, 13P-8441),

the Swedish Diabetes Association, the Swedish Society for Medical Research and the Hoechst Foundation.

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Received 23 February 1990/29 May 1990; accepted 20 June 1990