Effects of Glucose on Proinsulin Messenger RNA in Rats In Vivo

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SUMMARY

The purpose of these studies was to determine whether glucose, the principal regulator of insulin biosynthesis in mammals, controls synthesis through alterations in levels of proinsulin mRNA in whole animals. Rats were starved for 3 days and then either refed or injected with glucose or saline for 24 h. Glucose injection raised plasma glucose levels equivalent to levels seen with refeeding but provided less than 20% of caloric replacement. Pancreatic RNA was extracted and the relative concentration of proinsulin mRNA was determined by blot hybridization with a cloned rat proinsulin cDNA probe. In starved animals proinsulin mRNA levels were 15–20% that of fed controls. Glucose injection produced a specific three- to fourfold increase in proinsulin mRNA levels relative to total pancreatic RNA, within 24 h. The effect was measurable 2 h after glucose injection and appeared largely complete by 12 h. Actinomycin D blocked the glucose-induced increase in proinsulin mRNA.

These studies demonstrate effects of changes of plasma glucose on levels of proinsulin mRNA. Their rapidity of onset and large magnitude are comparable to effects of glucose on rates of insulin biosynthesis in isolated islets and suggest that insulin biosynthesis is regulated at least in part by levels of proinsulin mRNA. DIABETES *31*:624–629, July 1982.

Insulin is the major secretory protein of pancreatic islet beta-cells.[1] Its release is finely regulated by changes of plasma glucose concentrations between 2 and 20 mM.[2] Insulin biosynthesis is also regulated by changes in plasma glucose.[1,3–10] Best et al.[11] showed over 40 years ago that pancreatic insulin content was markedly depleted during several days of starvation in rats, and was restored toward normal by refeeding with carbohydrate or protein but not with fat. The study of glucose regulation of insulin biosynthesis was greatly facilitated by isolation of rat islets from pancreas, since islets comprise only 1% of total pancreatic tissue.[12] Glucose was shown to have a specific effect on proinsulin relative to total islet protein synthesis. Proinsulin synthesis increased from less than 5% of total protein synthesis at 2 mM to 20–30% at 20 mM.[4–7] An effect of glucose at the translational level was shown to occur immediately after addition of glucose to incubation media,[7] increasing the number of ribosomes active in protein synthesis by increasing initiation of all islet mRNA.[8] Another component of glucose-stimulated proinsulin synthesis appeared to involve increased mRNA synthesis. This component was observed only after an hour or more of incubation in high levels of glucose, was specific for proinsulin biosynthesis, and was blocked by the presence of actinomycin D.[7,9,10]

All the earlier studies only indirectly estimated proinsulin messenger RNA by using inhibitors of RNA synthesis and left unanswered the question of whether, under physiologic conditions, alterations in rates of insulin biosynthesis could be accounted for by changes in the level of proinsulin mRNA. We therefore measured the effects of fasting and refeeding on proinsulin mRNA levels by cell-free translation and cDNA-RNA hybridization.[13] A specific cloned probe complementary to proinsulin mRNA was used. Fasting decreased and refeeding increased proinsulin mRNA levels. Here we present studies which indicate that intraperitoneal glucose administration to starved rats specifically induced proinsulin mRNA, that this induction is rapid, and is dependent on synthesis of new proinsulin mRNA.

METHODS

Animals. All experiments were carried out on Sprague-Dawley rats weighing 150–200 g (Eldridge Laboratory Animals, St. Louis, Missouri). These animals were fed Purina Rat Chow ad libitum until the time of experiments.

Isolation of RNA from rat pancreas. Rats were weighed, marked, and divided into groups of 4–6 animals. All animals were fasted for 72 h and allowed free access to water.

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After this period, rats were injected with either 0.5 g of glucose in 1 ml of water every 4 h for 24 h or injected with an equal volume of 150 mM NaCl. A third group of rats was injected with saline every 4 h and fed ad libitum for the last 24 h of the experiment. Three hours after the last injection animals were anesthetized with sodium pentobarbital (30 mg/kg body wt), pancreases were excised, blotted, and excess fatty tissue and lymph nodes removed. In other experiments, rats were fasted 3 days, then injected with glucose every 4 h, and animals were killed at the time indicated in the figure legends. Pancreases were weighed and immediately homogenized 30 s at high speed with a Polytron (Brinkman Instruments, Westbury, New York) homogenizer in 10 ml of buffered 4 M guanidinium thiocyanate (Fluka, AG, Basel, Switzerland), 25 mM sodium citrate, pH 7.0, 0.1 M 2-mercaptoethanol (Eastman Kodak), and 0.33% anti-foam A (Sigma) according to the method of Chirgwin et al.[14] RNA from pancreas was purified as described by Chirgwin et al.[14] Samples were diluted with sterile water to a final concentration of approximately 5 mg/ml and stored at −70°C until further analysis.

RNA, DNA, protein, and glucose determinations. Aliquots (100 μl) were removed from the initial homogenate and precipitated on ice with 1 ml of 10% TCA at 4°C. RNA, DNA, and protein were separated. The amount of RNA was determined by absorbance at 260 nm and DNA by assay with diphenylalanine and protein by the method of Lowry, as described previously.[13] Glucose was determined on fresh heparinized whole blood by a standard glucose-oxidase technique.

Hybridization analysis. Pancreatic RNA from each rat (20 μg) was glyoxalated according to the method of McMaster and Carmichael.[15] Samples were electrophoresed on 2% agarose gels as previously described.[13] RNA in gels was transferred to diazophenylthio (DPT) paper and hybridized according to Wahl et al.[16] The 450-bp insert from pCRI 354 containing 354 bases of coding information[17] was removed from the parent plasmid pBR 322 by digestion with Hind III and electrophoresis on agarose gels. The insert was isolated from gels and found to contain less than 1 part pBR 322 in 10^5 parts insert by hybridization analysis. The purified insert labeled with ^{32}P deoxynucleotides by nick translation[18] to 3×10^6 cpm/mg was used as a probe. Filters were washed, air dried, and autoradiographs obtained[13] using Kodak XR5 film and a Dupont Lightning Plus intensifying screen. Hybridized bands on the DPT paper were identified by positioning the autoradiograph over the paper. Efficiency of transfer was examined in control experiments by electrophoresing duplicates of multiple samples and staining gels before or after transfer with Stainsall (Eastman-Kodak Co., Rochester, New York). Less than 20% of the most abundant species, 28s RNA, remained in the gel. Smaller species were not detectable in the gel after transfer. Insert from pCRI 354, labeled with ^{32}P by nick translation, was denatured in 50% DMSO, 1 M glyoxal, electrophoresed, and transferred, and counts bound to paper after prehybridization, hybridization, and posthybridization washes were determined. Sixty percent of the input counts bound to paper. Potential variation in signal caused by possible differences in efficiency of transfer, binding, and hybridization efficiency in each filter were monitored in the following manner. Fifty picograms of unlabeled insert from pCRI 354 treated with 1 mM glyoxal in 50% DMSO was electrophoresed in the extreme right- and -left-hand lanes of each experimental gel. If the counts bound to each insert sample in the same gel varied by more than 20%, the data from the rest of the gel were discarded and the samples rerun. Variations in counts obtained from different filters in the same experiment were normalized by comparing the counts bound to equal aliquots of insert from pCRI 354 on each filter. Because differences in the amount of background signal on different areas of the paper impaired precise quantitation by densitometric analysis, pieces were cut from directly above and below the area that bound the probe. Each paper was weighed on a microbalance to obtain a precise estimate of area. Each filter was counted to greater than 1000 counts above estimated background. Samples were corrected for background values by the following formula:

$$\text{CPM on hybridized filter} - \text{hybridized filter wt} \times \frac{1}{2}\left(\frac{\text{cpm background filter}_1}{\text{wt background filter}_1} + \frac{\text{cpm background filter}_2}{\text{wt background filter}_2}\right) = \text{cpm hybridized to proinsulin mRNA.}$$

RESULTS

Quantitation of proinsulin mRNA with a cloned rat proinsulin cDNA. To determine whether changes of proinsulin mRNA could be quantitated by the RNA gel blotting procedure of Wahl et al.,[16] various amounts of total cytoplasmic RNA were electrophoresed on agarose gels, transferred to DPT paper, and hybridized with ^{32}P-labeled rat proinsulin cDNA as probe. An autoradiogram of a filter is seen in Figure 1A. Relative concentrations of proinsulin mRNA were estimated from RNA blots by counting the filters as described in METHODS. Decreasing amounts of pancreatic RNA were electrophoresed in lanes 1–4, Figure 1A. Counts bound to each lane are shown in Figure 1B. Over the range of concentrations studied, counts bound were directly proportional to RNA electrophoresed. To determine the reproducibility of this method, 8 μg of RNA was electrophoresed in each of lanes 5–8. The range of cpm bound to each sample was 15% (Figure 1B). These results are consistent with those from a number of laboratories in which relative changes in mRNA levels have been assessed with specific cloned probes.[19–23]

Regulation of proinsulin mRNA levels in starved rats by glucose. We have shown that proinsulin mRNA levels decrease during starvation in rats and increase to greater than control levels within 24 h of refeeding.[13] To determine whether changes in plasma glucose concentration are responsible for this modulation, glucose was given to 3-day-starved rats intraperitoneally for 24 h as described in METHODS. These animals were compared with 24-h-refed and starved animals. Body weight decreased 23% after 3 days of starvation (Table 1 and Figure 2A). During 24 h of refeeding or glucose injection, body weight increased significantly only in refed animals. Glucose injection raised blood glucose levels from 4.5 mM in starved animals to 9.0 mM measured 3 h after the last glucose injection (Figure 2B). Refeeding increased blood glucose levels to 7.6 mM. Pancreatic weights, DNA, and protein content of pancreases obtained from the three groups at the time of death were the same (Table 1). There was a marked increase in

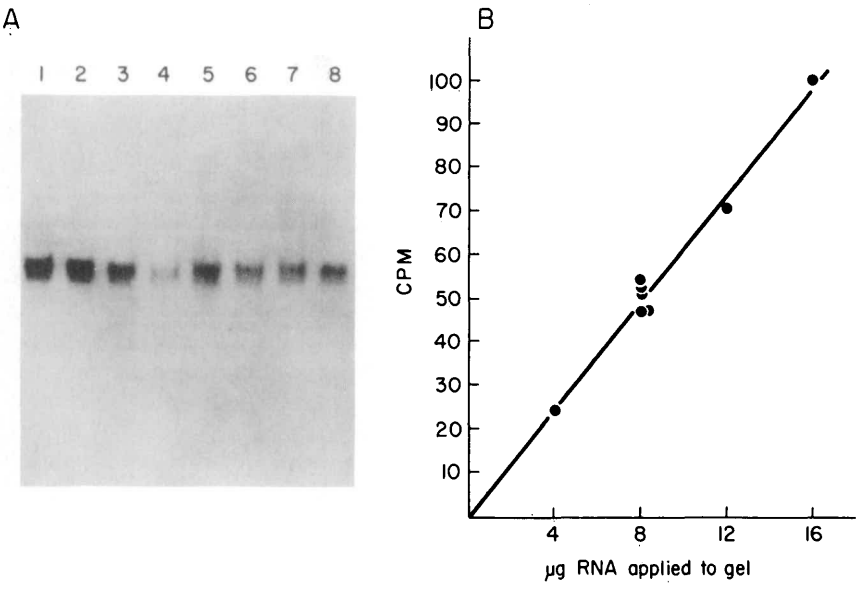

FIGURE 1. Quantitation of the relative concentration of proinsulin mRNA sequences by electrophoresis, blot transfer, and hybridization with ^{32}P-labeled proinsulin cDNA. RNA from rat pancreas was electrophoresed, transfered to DPT-filters, and hybridized as described in METHODS. An autoradiograph was obtained and cpm in each band determined (METHODS). A: Autoradiograph of a DPT-filter signal obtained by electrophoresis and transfer of 16 μg (lane 1), 12 μg (lane 2), 8 μg (lanes 3,5–8), and 4 μg (lane 4) of pancreatic RNA. B: ^{32}P-proinsulin cDNA bound (determined by Cerenkov counting), as a function of the RNA applied to the gel.

the RNA content in pancreases obtained from the refed animals but not in animals in whom either glucose or saline was injected.

Equal amounts of RNA from glucose-injected (Figure 3A and D), fasted (Figure 3B and E), and refed animals (Figure 3C and F) were electrophoresed and the relative concentration of proinsulin mRNA determined (N = 5 for each group). The relative abundance of proinsulin mRNA was three- to fourfold greater in the glucose-injected animals compared with either fasted or refed animals (Figure 2C and Table 1). When proinsulin mRNA content was calculated on the basis of total pancreatic RNA, it was determined that both glucose injection and refeeding increased proinsulin mRNA, since refeeding was associated with a four- to fivefold overall increase in pancreatic RNA. These data demonstrate that glucose produces a specific increase in proinsulin mRNA in the absence of the overall increase in pancreatic RNA seen with refeeding.

Time course of glucose induction and the effect of actinomycin D on proinsulin mRNA. To measure the time course of glucose induction of proinsulin mRNA in starved animals, groups of four animals were starved for 3 days, injected with glucose, and killed at the times indicated in Figure 4. Pancreatic RNA was isolated, and the relative concentration of proinsulin mRNA was compared with that present in nonstarved controls. Proinsulin mRNA levels decreased more than fivefold with starvation. Two hours after glucose injection there was a significant increase in proin-

TABLE 1
Effect of glucose injections or refeeding in 3-day-fasted rats on body weight, pancreatic weight, blood glucose, pancreatic DNA, pancreatic protein, RNA, and proinsulin mRNA

	Body weight (g)				
	Day 1	Day 3	Day 4	Pancreatic wt (g)	Blood glucose (mM)
Fasted	164.4 ± 8.4	127.6 ± 7.0	123.0 ± 8.3	0.508 ± 0.048	4.5 ± 0.6
Glucose injected	161.0 ± 7.5	126.0 ± 6.0	123.0 ± 6.4	0.529 ± 0.044	9.0 ± 1.0*†
Refed	164.0 ± 3.0	123.0 ± 4.0	142.0 ± 4.0*‡	0.679 ± 0.023	7.6 ± 0.4*

	Pancreatic content				
				Proinsulin mRNA cpm ^{32}P-labeled probe bound	
	DNA (mg)	Protein (mg)	RNA (mg)	Per mg RNA	Per pancreas
Fasted	2.19 ± 0.22	96 ± 10	6.96 ± 0.59	410 ± 17	3,098 ± 112
Glucose injected	2.05 ± 0.06	87 ± 8	8.51 ± 0.73	1,325 ± 94*	8,449 ± 724*
Refed	2.49 ± 0.11	113 ± 4	26.30 ± 2.51*	401 ± 174	15,339 ± 1,053*

See legend Figure 2. Glucose, DNA, protein, and RNA were determined as described in METHODS.
N = 5 data expressed as mean ± SEM.
* Significantly different from fasted values ($P < 0.01$).
† Measured approximately 3 h after last glucose injection.
‡ Increased over values on day 3 ($P < 0.01$).

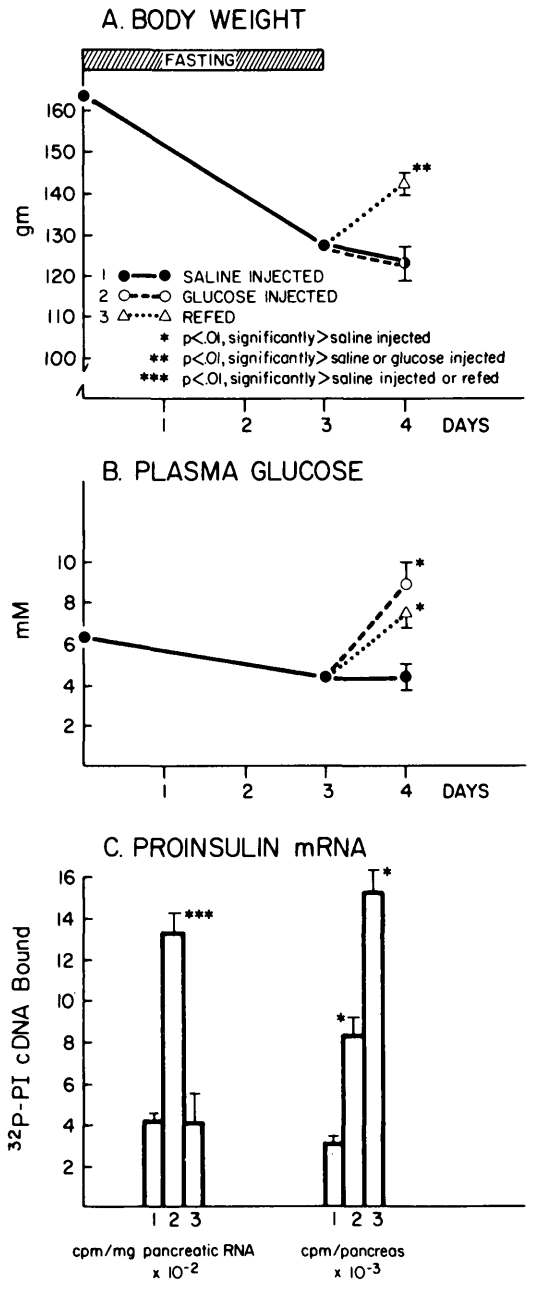

FIGURE 2. Effect of glucose injection or refeeding of starved rats on body weight, blood glucose, and relative concentrations of proinsulin mRNA. Rats were starved 3 days, then either refed or injected with glucose (0.5 g/ml) or saline (1 ml) every 4 h, for 24 h. In each group of rats (N = 5) the relative concentration of proinsulin mRNA was determined as described in METHODS, by hybridization of ^{32}P cDNA to the gel-blotted RNA, expressed as CPM hybridized/mg of RNA or CPM hybridized/pancreas.

sulin mRNA. The inductive effect of glucose appeared to plateau between 12 and 24 h; however, the level of proinsulin mRNA was still less than in fed controls.

To determine whether synthesis of new mRNA is necessary for the increment in proinsulin mRNA caused by increased plasma glucose, actinomycin D (1 mg/kg) was injected coincidently with glucose, and proinsulin mRNA was measured 6 h later (Table 2). Actinomycin D blocked the glucose-induced rise in proinsulin mRNA, while it had no effect on levels of proinsulin mRNA in saline-injected rats.

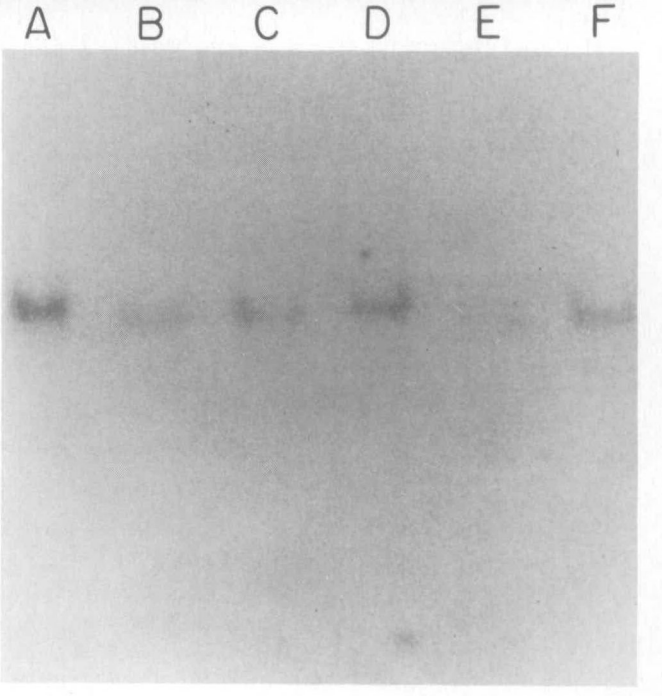

FIGURE 3. Analysis of the relative concentration of proinsulin mRNA from fasted rats either glucose injected, saline injected, or refed for 24 h hybridized with ^{32}P-PI cDNA. RNA (20 μg) was treated with glyoxal (1 M), electrophoresed on 2% agarose gels, transferred to DPT paper, hybridized to ^{32}P-rat proinsulin cDNA, and an autoradiograph obtained as described in METHODS. Exposure time was 96 h. RNA from glucose-injected animals in lanes A and D, 4-day-fasted animals in lanes B and E, refed animals in lanes C and F.

DISCUSSION

The data in this study demonstrate that glucose alters the level of proinsulin mRNA in vivo and strongly suggest that insulin biosynthesis is regulated by changes in levels of proinsulin mRNA. This conflicts with interpretations of data reported by Itoh et al.[24,25] They concluded that insulin biosynthesis was regulated mainly at the level of translation. In their studies, proinsulin mRNA levels were measured in isolated islets of Langerhans after 1 h of incubation with varied

FIGURE 4. Pancreatic RNA obtained from fed, starved, and starved-glucose-injected animals at the times indicated was analyzed for proinsulin mRNA content as in Figure 1 and METHODS. N = 4 ± SEM. Data are presented as the ^{32}P-PI cDNA cpm (× 10^{-2}) hybridized less background values (determined as described in METHODS). Levels of proinsulin mRNA in all glucose-injected groups are significantly greater than values in starved animals at the P < 0.05 level.

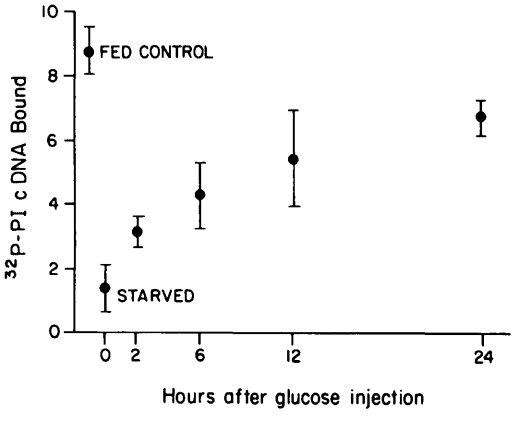

TABLE 2
Effect of actinomycin on the glucose-induced rise in proinsulin mRNA levels

	Actinomycin D	Blood glucose (mM)	Weight (mg)	RNA (mg)	DNA (mg)	Protein (mg)	Proinsulin mRNA (cpm × 10^{-2})
Glucose	−	11.3 ± 0.8*	554 ± 30	10.30 ± 0.93	2.98 ± 0.16	79.9 ± 3.3	123 ± 12‡
	+	44.2 ± 8.8†	459 ± 46	8.33 ± 1.62	2.47 ± 0.37	94.1 ± 5.8	64 ± 13
Saline	−	5.1 ± 0.8	500 ± 17	9.31 ± 1.32	2.37 ± 0.13	94.5 ± 7.3	52 ± 21
	+	6.3 ± 0.2	465 ± 29	8.21 ± 1.03	2.18 ± 1.03	82.5 ± 7.5	53 ± 14

Four groups of four 160-g rats were fasted 4 days. At 0 h, 4-day-fasted rats were injected intraperitoneally with 1 mg/kg actinomycin D or with vehicle only and ½ g glucose or an equal volume of sterile saline. The glucose injection was repeated at 4 h and the animals were killed at 6 h. Plasma glucose, pancreatic weight, RNA, DNA, protein, and proinsulin mRNA were determined as described in METHODS.

* $P < 0.01$ saline only.
† $P < 0.01$ glucose + actinomycin D.
‡ $P < 0.05$ saline ± actinomycin D.

amounts of glucose. Their data confirmed previous reports that glucose has a rapid, i.e., within 1 h, effect on translation of total islet protein synthesis, but did not address selective induction of insulin biosynthesis by glucose seen only during longer incubation periods. The most important difference in their studies and the current one is that this represents the first demonstration of the effect of glucose on proinsulin mRNA in vivo. Extrapolation of results with cultured islets to events in whole animals must obviously be taken with caution, especially since we have noted an accelerated degradation of proinsulin mRNA in islets cultured for 4 h (manuscript in preparation).

During a brief period of starvation in rats, proinsulin mRNA levels fall to less than 20% of that found in fed control animals (Figure 4).[13] Following 24 h of refeeding, messenger RNA levels are returned to greater than control values. In the present study, we determined that injection of glucose at levels sufficient to raise the plasma glucose, but providing less than 20% of caloric replacement,[26] also stimulated a rapid increase in the level of proinsulin mRNA. These data suggest that changes in rates of insulin biosynthesis are in large part due to an increase in amount of insulin mRNA sequences and not to some posttranscriptional control that makes preexisting proinsulin mRNA sequences more biologically active.

The changes in proinsulin mRNA levels during starvation and refeeding or following glucose injection correlate closely with changes in insulin biosynthetic capacity in islet tissue.[3–8,13] The parallel between the effects of glucose on mRNA levels reported here and on rates of insulin biosynthesis in isolated islets suggests that levels of proinsulin mRNA may reflect accurately insulin biosynthetic activity in a variety of physiologic and pharmacologic situations. While the levels of mRNA may not be rate limiting for the synthesis of a specific protein, there does appear to be a good correlation between mRNA levels and synthetic rates in most biologic systems examined. These include induction of transferrin synthesis by iron,[27] estrogen and progesterone regulation of ovalbumin and conalbumin,[28] prolactin induction of casein and lactalbumin,[29] and insulin regulation of albumin synthesis,[30] among others.

The increase in proinsulin mRNA could be due to increased transcription of insulin genes or to decreased degradation of proinsulin mRNA. The rapid (2 h) increase in proinsulin mRNA sequences produced by glucose strongly suggests that increased transcription is involved in this response. An increase in half-life would play a significant role only if the half-life of proinsulin mRNA in the starved state was considerably less than 2 h. The finding that actinomycin D largely blocks the glucose-induced increment in proinsulin mRNA in whole animals is also consistent with an effect on transcription. Effects of actinomycin on other RNA-dependent processes cannot be excluded in these experiments.

More direct estimation of rates of transcription and half-life required direct measurement in vitro. While we have found an effect of glucose on levels of insulin mRNA when large numbers of islets are incubated per sample (500) in vitro (manuscript in preparation), the small amounts (20–50 μg) of RNA recoverable have precluded more detailed analysis.

These data demonstrate that changes in plasma glucose concentrations affect levels of insulin mRNA and suggest that proinsulin mRNA levels ultimately determine biosynthetic capacity of insulin. Superimposed upon these long-term effects on proinsulin mRNA are more rapid modulations of protein synthesis at the translational level, previously shown to be at the level of initiation.[8] The data presented here thus confirm earlier speculation[7] that glucose regulation of insulin biosynthesis occurs at the level of transcription or posttranscriptional processing, as well as at the level of translation.

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