GLUCOSE STIMULATION OF THE PROINSULIN SYNTHESIS IN ISOLATED PANCREATIC ISLETS WITHOUT INCREASING AMOUNT OF PROINSULIN mRNA

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1. Introduction

Since the pioneering work of Steiner et al. in 1967 [1,2] numerous papers have been published concerning the induction by glucose of proinsulin synthesis in pancreatic islets. However, the mechanism of glucose stimulation of proinsulin synthesis is still uncertain. From their observations that:

(i) Actinomycin D had little effect on the induction of proinsulin by glucose, at least during the early phase;

(ii) Glucose stimulation did not alter the overall rate of peptide chain elongation;

Permutt and Kipnis [3–5] assumed that the induction might be expressed at the translational level.

In order to clarify this problem, direct quantitation of the specific mRNA levels during the induction of proinsulin synthesis by glucose seemed desirable. Recent techniques of extraction and isolation of mRNA [6] and development of a cell-free protein synthesizing system [7] make it possible to compare the amount of a specific mRNA with the rate of synthesis of the corresponding protein in intact cells.

Data to be presented in this paper indicate that in isolated pancreatic islets of rats the level of proinsulin mRNA remains unchanged during the period of proinsulin induction up to 60 min. Further, the glucose-induced proinsulin synthesis is completely abolished by cycloheximide, but not by α-amanitin, an RNA polymerase II inhibitor. Neither agent had any apparent effect upon the mRNA level during the proinsulin synthesis. These findings strongly suggest that the induction of proinsulin synthesis by glucose is achieved by an increase in the availability of pre-existing proinsulin mRNA for translation rather than by an increase in the amount of proinsulin mRNA.

Abbreviations: SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid

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2. Experimental

2.1. *Materials*

Collagenase (CLS IV, 160 U/mg) was purchased from Worthington Biochemical Co., NJ; L-[^{3}H]-leucine (57.4 Ci/mmol) from New England Nuclear, Boston; α-amanitin and cycloheximide from Sigma, St Louis; guinea pig antiserum to bovine insulin from Miles-Yeda, Israel; fetal calf serum from Gibco, New York; horse heart cytochrome *c* from Boehringer, Mannheim.

2.2. *Preparation of pancreatic islets*

Pancreatic islets of Langerhans were prepared from male Wistar rats (250–300 g) by the collagenase digestion method [8]. All experiments were started at 10:00 a.m. using rats which had been maintained on commercial complete rat diet (laboratory chow).

2.3. *Extraction of islet nucleic acid*

About 150 islets were preincubated at 37°C for 60 min in 50 μl incubation medium of Krebs-Ringer bicarbonate solution [8,9] supplemented with 3.3 mM glucose, 11 amino acids (Arg, Cys, His, Ile,

Lys, Met, Phe, Thr, Trp, Tyr, Val, at 0.1 mM each), and 20% fetal calf serum. 50 μl of medium containing 10 μCi [^{3}H]leucine and 3.3 mM or 36.7 mM glucose was then added, and the incubation further continued at 37°C. The incubation was stopped by adding 2 ml ice-cold Hanks buffer, centrifuged at 2000 × *g* for 30 s, and the supernatant discarded. The islets were suspended in 0.5 ml 0.1 M Tris–HCl, pH 9, 0.01 M EDTA, 0.1 M dithiothreitol, 2% SDS (w/v), and an equal volume of phenol–chloroform–isoamyl alcohol (50:50:2) pre-equilibrated with 0.1 M Tris–HCl, pH 9, and 0.01 M EDTA. They were sonicated for 20 s at room temperature with the microtip of a Kontes Micro-Ultrasonic Cell Disrupter, Vineland, NJ. After removing a 100 μl sample for quantitation of proinsulin, islet nucleic acid was isolated from the remaining sonicated material as in [10,11]. About 150 islets yielded 4–6 μg nuclei acid.

2.4. *Quantitation of proinsulin mRNA*

Proinsulin mRNA was quantitated by its ability to direct the synthesis of preproinsulin in a wheat germ cell-free mRNA-dependent protein-synthesizing system as in [11]. The [^{3}H]leucine-labeled translation products were characterized by 15% SDS–polyacrylamide gel electrophoresis [11]. The gels were cut into 1 mm slices, and radioactivity was measured by scintillation spectrometry after extraction of gels with NCS tissue solubilizer (Amersham/Searle). The amount of [^{3}H]leucine incorporated into preproinsulin was quantitated from the sum of the radioactivity of the gel slices making up the preproinsulin peak and subtraction of the background (an assay with no nucleic acid added).

2.5. *Quantitation of newly synthesized proinsulin*

A 100 μl sample of the sonicated material (see above) was evaporated in a Vortex-Evaporator, Buchler Instruments, NJ. The dry material was dissolved in 0.5 ml distilled water containing 100 μg bovine insulin and 20 mM leucine. Protein was precipitated with 0.5 ml 20% TCA (w/v) washed with 10% TCA and ethyl ether, and then analyzed by 15% SDS–polyacrylamide gel electrophoresis [11]. The amount of newly synthesized proinsulin was calculated by summing up the [^{3}H]leucine radioactivity of the gel slices corresponding to the proinsulin peak.

3. Results

The identification and quantitation of proinsulin mRNA in the wheat germ system is illustrated in fig.1. The TCA-insoluble product gave a single major peak (fig.1A) which was identified to represent preproinsulin from the following evidence:

(i) The molecular weight of the major peak estimated with marker proteins was 11 500 [10,11];

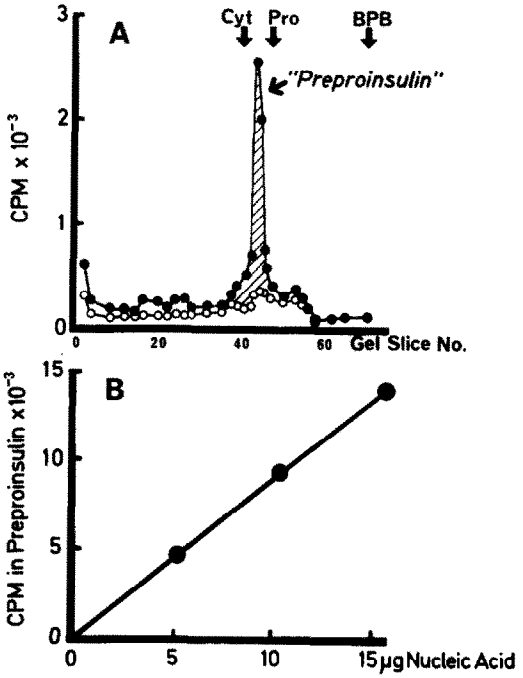

Fig.1A. SDS–polyacrylamide gel electrophoresis of the translation product of the wheat germ system with or without islet nucleic acid. Islet nucleic acid (10.5 μg) was translated in 50 μl wheat germ system at 25°C for 90 min. The translation was stopped by adding 20 μl RNase A solution (1 mg/ml) followed by incubation at 37°C for 20 min [11]. A 30 μl sample of the mixture was treated with TCA and the precipitate subjected to SDS–polyacrylamide gel electrophoresis [11]. (•) Incubation with 10.5 μg islet nucleic acid; (○) incubation without exogenous nucleic acid. Arrows indicate relative migration of: Cyt, horse heart cytochrome *c* (mol. wt 13 400); Pro, rat proinsulin (mol. wt 9000); BPB, bromophenol blue, run on parallel gels. Fig.1B. Islet nucleic acid concentration dependence for preproinsulin synthesis. The total amount of [^{3}H]leucine incorporated into preproinsulin corresponds to the sum of the radioactivity of the gel slices of the preproinsulin peak (shaded area in fig.1A) after subtraction of the background.

(ii) The immunoprecipitated material gave a single peak which corresponded to the major TCA-insoluble peak [10,11];
(iii) Immunoprecipitation was completely inhibited by bovine insulin [10,11].

In addition, the major peak was found to be indistinguishable from the [^{14}C]leucine-labeled peak of preproinsulin which was translated in the same system using purified proinsulin mRNA from a rat B-cell tumor*.

As shown in fig.1B, the radioactivity incorporated into preproinsulin was proportional to the amount of nucleic acid added to the wheat germ system.

In further studies we compared the time course of proinsulin synthesis with the corresponding mRNA level (fig.2). Two series of experiments were run in parallel, one using a low (3.3 mM) and the other a high (20 mM) dose of glucose. [^{3}H]Leucine incorporation into proinsulin as a function of incubation time is shown in fig.2A. When glucose was increased from 3.3–20 mM, the rate of incorporation increased 3–4-fold. In contrast, the level of proinsulin mRNA remained essentially unchanged irrespective of glucose concentrations (fig.2B). The possibility that the translation of mRNA might be inhibited or activated by some factors co-extracted with the mRNA was excluded by the experiments documented in table 1. As may be seen mixing of purified proinsulin mRNA* from rat B-cell tumor with islet nucleic acid resulted in the additive formation of [^{3}H]preproinsulin by the cell-free protein synthesizing system.

Effect of cycloheximide and α-amanitin on proinsulin synthesis and on proinsulin mRNA level are shown in table 2. Cycloheximide completely inhibited the glucose-induced rise in proinsulin synthesis. Under conditions in which the extranucleolar RNA synthesis was completely inhibited by α-amanitin [12,13], we found no effect on the glucose-induced proinsulin synthesis during the 60 min incubation. Both cycloheximide and α-amanitin had essentially no effect on the proinsulin mRNA level in pancreatic islets of rats.

* Rat proinsulin mRNA was purified from poly(A)-containing RNA, prepared from streptozotocin-nicotinamide induced B-cell tumor as described previously [11], by two cycles of sucrose density gradient centrifugation. Details will be published shortly

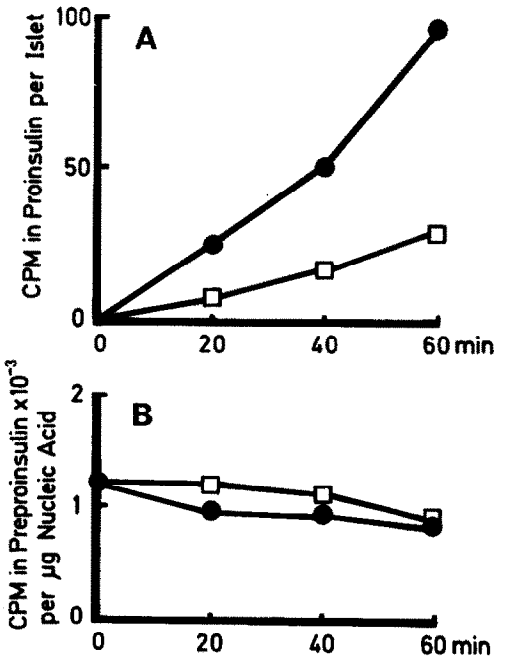

Fig.2A. Time course of the induction of proinsulin synthesis in islets. About 150 preincubated islets were incubated at 37°C for the indicated time in 100 μl medium containing 10 μCi [^{3}H]leucine and 3.3 mM (□) or 20 mM (•) glucose and the sonicated material of the incubated islets was further treated as described under section 2. The TCA-insoluble material prepared from a 100 μl sample of the sonicated material was analyzed by SDS–polyacrylamide gel electrophoresis. The amount of newly synthesized proinsulin was taken as the sum of the [^{3}H]leucine radioactivity of the proinsulin peak in the electrophoresis profile. Fig.2B. Time course of the proinsulin mRNA level in islets. Islet nucleic acid prepared from a 900 μl aliquot of the sonicated material as described in fig.2A, was translated in the wheat germ system. Proinsulin mRNA of islets incubated in the presence of 3.3 mM (□) or 20 mM (•) glucose was quantitated by the same procedure as described in fig.1.

4. Discussion

The present experiments with isolated rat pancreatic islets show that the proinsulin mRNA level remains unchanged during the induction of proinsulin synthesis by glucose. The induction by glucose is completely arrested in the presence of cycloheximide, but not by α-amanitin. The level of proinsulin mRNA was not significantly influenced in the presence of these inhibitors. These results suggest that glucose

Table 1
Effect of islet nucleic acid on purified proinsulin mRNA translation

Additions	Nucleic acid added (μg)	[^{3}H]Preproinsulin (cpm)	
		Synthesized	Theoretical
Islet nucleic acid (L)	10.7	8623	
Islet nucleic acid (H)	8.45	5948	
Proinsulin mRNA	0.028	5901	
Islet nucleic acid (L) + proinsulin mRNA	10.7 0.028	13 730	14 524
Islet nucleic acid (H) + proinsulin mRNA	8.45 0.028	11 225	11 849

About 500 preincubated islets were incubated at 37°C for 60 min in 200 μl medium containing 3.3 mM (L) or 20 mM (H) glucose, and islet nucleic acid was prepared as described under section 2. In the combination experiments, purified proinsulin mRNA* from rat B-cell tumor and islet nucleic acids (L) and (H) were mixed as indicated and translated in the wheat germ cell-free system

Table 2
Effect of α-amanitin and cycloheximide on the induction of proinsulin synthesis and on the proinsulin mRNA level in islets

Treatment	Proinsulin synthesized (cpm/islets)	Islet nucleic acid extracted (μg)	Proinsulin mRNA level (Preproinsulin synthesized) (cpm/μg islet nucleic acid)
Experiment 1			
Glucose	63.5	4.11	929 (100)
Glucose + α-amanitin	54.8	3.71	872 (94)
Glucose + cycloheximide	0	3.28	1094 (118)
Experiment 2			
Glucose	71.9	4.00	1195 (100)
Glucose + α-amanitin	90.6	3.81	1207 (101)
Glucose + cycloheximide	0	4.00	1216 (102)

About 130 preincubated islets were incubated at 37°C for 60 min in 100 μl medium containing 10 μCi [^{3}H]leucine, 20 mM glucose and 1 μg α-amanitin or 2.5 μg cycloheximide. α-Amanitin and cycloheximide were added to the preincubation medium 15 min before the incubation. Proinsulin mRNA and newly synthesized proinsulin in the incubated islets were quantitated as in fig.2. The numbers in parentheses represent the percentage of the radioactivity with glucose alone

regulates proinsulin synthesis during the first 60 min by enhancing the availability of pre-existing proinsulin mRNA for translation rather than by increasing the amount of proinsulin mRNA. A possible stimulatory effect of glucose on the synthesis of some precursor form of proinsulin mRNA cannot be excluded. In fact, the rate of glucose-induced proinsulin synthesis was reported [3] to be essentially unaffected by actinomycin D during the first 45 min, but becomes blocked during the 2nd hour of incubation. This actinomycin-sensitive portion of proinsulin synthesis may be due to the newly synthesized proinsulin mRNA. As shown in table 2, in the presence of α-amanitin or cycloheximide, the proinsulin mRNA does not decay significantly during the first 60 min incubation, suggesting the apparent longevity of proinsulin mRNA in pancreatic islets.

Numerous recent observations indicate that the rate of protein synthesis such as of ovalbumin [14,15], α- and β-globin chains [16], tyrosine aminotransferase [17–19], tryptophan oxygenase [18,20], growth hormone [21,22], phosphoenolpyruvate carboxykinase [23], immunoglobulin [24], and phosphogluconate dehydrogenase [25] is closely correlated with the amount of mRNA coding for these proteins. On the other hand rapid changes in the rate of production of particular proteins may, in part, also be induced by mechanisms involving translational control [26]. The experimental data of this paper represent an interesting additional example for the occurrence of 'translational control' in a mammalian system.

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