Glucose Stimulated Proinsulin Biosynthesis

Rates of Turn Off after Cessation of the Stimulus

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Summary. Proinsulin biosynthesis was stimulated progressively to a plateau level at 45 min by 5.5 mmol/l glucose. The rate of biosynthesis promptly decreased following cessation of either 15 or 60 min exposure to this concentration of glucose. Actinomycin D had no effect on the rate of decline. In contrast, exposure to 20 mmol/l glucose caused a prolonged increase in proinsulin biosynthesis which was still apparent at 75 min. After removal of the stimulus there was an initial decrease in the rate of proinsulin biosynthesis followed by persistently elevated rates. When proinsulin biosynthesis was stimulated by 20 mmol/l glucose in the presence of actinomycin D, there was a rapid switch off of biosynthesis and no persistent effect. Thus, two controls for regulation of proinsulin biosynthesis can be characterised: a prompt, rapidly reversible stimulation in response to low or high glucose concentrations and a persistent stimulation in response to high glucose concentrations. These two effects may represent the translational and transcriptional effects of glucose respectively.

Key words: Proinsulin biosynthesis, rat islets of Langerhans, sepharose-coupled insulin antibody, glucose, actinomycin D.

Islets of Langerhans respond to increased glucose concentrations with an increase in the rate of insulin secretion and an increase in the rate of proinsulin biosynthesis (1–12). After sustained periods of glucose stimulation, the insulin content of the islets falls so that proinsulin biosynthesis fails to keep pace with the rate of insulin release. Thus after the stimulation of insulin secretion the β -cells are faced with a for-

ward control mechanism in that proinsulin biosynthesis is stimulated in an attempt to make up for an anticipated loss of insulin from the cells. In addition there appears to be an effect to increase the rate of synthesis of mRNA [13–15]. This could conceivably lead to a more persistent increase in the rate of proinsulin biosynthesis after cessation of the glucose stimulus [16]. Thus far, little is known of the control of proinsulin biosynthesis, its forward control or the way it is turned on and off.

In the work presented here the major emphasis is on the rate at which proinsulin biosynthesis is turned off, after two different concentrations of glucose applied for either 15 or 60 minutes. An attempt was also made, by use of actinomycin D, to assess the importance of increased mRNA synthesis in the long-term control of β -cell insulin content.

Material and Methods

Islets of Langerhans were isolated by the collagenase digestion technique of Lacy and Kostianovsky [17] from the pancreases of Wistar derived rats [150–200 g] fed ad libitum. After isolation the islets were washed twice with Hank's solution containing 2.5 mmol/l glucose, gassed with 95% O₂, 5% CO₂ at pH 7.4. Then batches of 20 or 25 islets were distributed into siliconised vials. The Hank's solution was removed, under microscopic control, and replaced by 0.5 ml of Krebs-Ringer bicarbonate solution (KRB) containing 5 g/l bovine serum albumin 2.5 mmol/l glucose and a mixture of essential amino acids (Eagle-Hela) without leucine. The islets were kept under continuous gassing for 45 min in a Dubnoff metabolic shaking incubator at 37° C. They were then exposed to either 2.5, 5.5 or 20 mmol/l glucose for periods of 15 to 75 min. Incorporation of ³H-

leucine into proinsulin was measured for 15 min periods at different times after the start of incubations in the presence or absence of actinomycin D, 5 µg/ml. To study the rate of turn off of proinsulin biosynthesis, batches of 20–25 islets were incubated in KRB with 2.5 mmol/l glucose prior to stimulation with 5.5 or 20 mmol/l glucose for 15 or 60 min. ³H-leucine was included during the 15 min stimulus and during the last 15 min of the 60 min stimulus. This measures the extent of stimulation of proinsulin biosynthesis. Subsequent measurements of proinsulin biosynthesis were made by including ³H-leucine during each 15 min of the hour following the end of glucose stimulation, when the islets were returned to KRB with 2.5 mmol/l glucose. Incorporation was performed in the presence of 100 µCi L-4,5-³H-leucine (The Radiochemical Centre, Amersham, Bucks, U.K.). It has been shown previously that in 15 minutes, ³H-leucine is incorporated into proinsulin but not into insulin so that there is no loss of radioactivity into the medium [11].

At the end of the incorporation period the islets were washed twice with ice-cold KRB solution and 2 ml of glycine buffer 0.2 mol/l (pH 8.8) plus 2.5 g/l human serum albumin. The islets were sonicated for 40 seconds (Branson Sonic Power Company, Danbury, Conn., U.S.A.) and the mixture centrifuged at 4000 × g for 30 minutes. This procedure gives identical recovery to acid-ethanol extraction according to the method of Davoren [18] as shown previously [10, 11]. A 0.5 ml aliquot of the supernatant was then placed on 0.9 × 2.0 cm columns of CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) covalently linked to anti-insulin antibodies [12, 19–21] and equilibrated with 0.2 mol/l glycine buffer 2.5 g/l HSA pH 8.8. The antibodies were purified prior to the linking reaction by ammonium sulphate precipitation from guinea-pig anti-porcine insulin antiserum kindly provided by Dr. P.H. Wright, Indianapolis, Ind. The samples were left on the columns overnight at 4° C and the radioactivity not incorporated into immunoreactive proteins washed away with 20 ml of glycine buffer. Radioactivity incorporated into proinsulin was eluted with 5 ml of 1 mol/l acetic acid containing 2.5 g/l of bovine serum albumin. For measurement of ³H-leucine incorporation in proinsulin a 1 ml aliquot of the acetic acid eluate was mixed with 6 ml of Instagel in plastic vials and assayed by scintillation spectrometry.

The total binding capacity of different batches of the affinity columns was never less than 20 µg insulin per column and in the experiments only 0.2 to 0.3 µg insulin was applied. Furthermore, when 1 µg insulin samples were applied in 0.5 ml portions, 96% retention of the insulin was achieved. Recovery of this

insulin after elution by 5 ml acetic acid was essentially complete. The affinity of the antibodies used for pork proinsulin was 65% of that for rat insulin (rat proinsulin affinity is not known). Specificity of the affinity binding was assessed by the addition of ¹²⁵I-albumin, ¹²⁵I-glucagon and ³H-leucine to the anti-insulin antibody columns. 100% of these materials was eluted with the glycine buffer and none with the acetic acid. Control columns prepared with the ammonium sulphate precipitated fraction from normal guinea-pig serum were also tested with ¹²⁵I-insulin. (Na¹²⁵I used to prepare the labelled compounds was from Eidg. Institut für Reaktorforschung, Würenlingen). Again, 100% elution with glycine buffer was achieved.

Statistical data were obtained by paired analysis using Student's t-test.

Results

1. The Onset of Stimulation of Proinsulin Biosynthesis (Fig. 1)

Isolated islets were incubated in 2.5 mmol/l glucose for 45 minutes before measurement of ³H-leucine incorporation into proinsulin in a medium glucose concentration of 2.5, 5.5 or 20 mmol/l. ³H-leucine incorporation was determined in each of five 15 minute periods between 0 and 75 minutes following the 45 minute equilibration period. Under control conditions, 2.5 mmol/l glucose throughout, no change in the rate of ³H-leucine incorporation into proinsulin occurred from 0–75 minutes. When the glucose concentration was changed to 5.5 mmol/l glucose ³H-leucine incorporation increased progressively up to between 45 and 60 minutes, and then remained constant. With change to 20 mmol/l glucose, proinsulin biosynthesis was again progressively increased with time and appeared to be still increasing from 45 to 75 minutes.

2. The Rates of Proinsulin Biosynthesis after Cessation of a Glucose Stimulus

For these studies two concentrations of glucose (5.5 and 20 mmol/l) were employed and the effects of brief stimulation, 15 minutes, and a long stimulation, 60 minutes, were examined. The rates of proinsulin biosynthesis after 15 and 60 minutes of stimulation by 5.5 mmol/l glucose are shown in Figure 2. With the short stimulus, 5.5 mmol/l glucose stimulated proinsulin biosynthesis by 66%. Because of this mild stimulation the rate at which the stimulated biosynthesis was turned off cannot be described precisely.

Fig. 1. Time course of the increase in the rate of proinsulin biosynthesis following stimulation by 5.5 and 20 mmol/l glucose. Vertical lines represent $\pm$ SEM. P values for all significant differences from the appropriate control (2.5 mmol/l) measurements are shown.

Fig. 2. Time course of the decrease of proinsulin biosynthesis following stimulation by 5.5 mmol/l glucose for periods of 15 and 60 min.

However, the rate decreased such that at 45–60 minutes after removal of the glucose stimulus, proinsulin biosynthesis was not significantly different from non-stimulated rates. With the 60 minute stimulus, 5.5 mmol/l glucose increased proinsulin biosynthesis by 220% relative to control. When the glucose stimulus was removed the rate of biosynthesis declined rapidly so that at 15–30 minutes the rate was only 40% above, and not significantly different from, the control value. The rapid dissolution of elevated rates of proinsulin biosynthesis after 5.5 mmol/l glucose were in contrast to the results obtained after

stimulation with 20 mmol/l glucose. These results are shown in Figure 3. A 15 minute stimulation by 20 mmol/l glucose raised proinsulin biosynthesis by more than 80%. Following removal of the glucose little decline in proinsulin biosynthesis occurred so that after 60 minutes the rate was significantly raised by 60%. This failure to turn off rapidly after stimulation by 20 mmol/l glucose is seen clearly in the results of the 60 minute experiments. Proinsulin biosynthesis was stimulated by 260%. The rate declined in the first 15 minutes after removal but the rate of decline then slowed so that after 60 minutes

Fig. 3. Time course of the decrease of proinsulin biosynthesis following stimulation by 20 mmol/l glucose for periods of 15 and 60 min

Fig. 4. Time course of the increase in the rate of proinsulin biosynthesis following stimulation by 5.5 and 20 mmol/l glucose in the presence of actinomycin D (5 µg/ml)

the rate of biosynthesis was still significantly raised by 150%. Again, it is clear that 20 mmol/l glucose caused a persistent stimulatory effect upon proinsulin biosynthesis. In contrast short or long stimulation with 5.5 mmol/l glucose had only a transient effect on biosynthesis.

3. The Effects of Actinomycin D on the Onset of Proinsulin Biosynthesis

In order to define glucose control of proinsulin biosynthesis by an immediate rapid effect and by a longer term effect, probably via induction of messenger RNA, the studies were repeated in the presence of actinomycin D (5 µg/ml).

In Figure 4 it can be seen that with 2.5 mmol/l glucose in the presence of actinomycin D, proinsulin biosynthesis remained constant from 0–75 minutes. Comparison with Figure 1 shows that actinomycin D had no effect upon the rate of proinsulin biosynthesis. Similarly, actinomycin D had no effect upon the progressive stimulation of biosynthesis by 5.5 mmol/l glucose. When 20 mmol/l glucose was studied, however, a late effect of actinomycin D was detected. Proinsulin biosynthesis was stimulated, as expected, to 45 minutes but thereon no further stimulation was seen. Comparison with Figure 1 shows that the stimulation of proinsulin synthesis by 20 mmol/l glucose beyond 45 minutes was blocked.

Fig. 5. Time course of the decrease of proinsulin biosynthesis following stimulation by 5.5 mmol/l glucose for periods of 15 and 60 min in the presence of actinomycin D (5 µg/ml)

Fig. 6. Time course of the decrease of proinsulin biosynthesis following stimulation by 20 mmol/l glucose for periods of 15 and 60 min in the presence of actinomycin D (5 µg/ml)

4. The Rates of Proinsulin Biosynthesis after Cessation of a Glucose Stimulus Applied in the Presence of Actinomycin D

After a 15 min stimulus with 5.5 mmol/l glucose, the stimulated rate of proinsulin biosynthesis returned to basal levels at 45–60 min (Fig. 5). Similar results were obtained when the effects of actinomycin D on the rates of proinsulin biosynthesis following stimulation with 5.5 mmol/l glucose for 60 min were studied. Again, the marked stimulation of proinsulin biosynthesis rapidly returned to basal values as they had in the absence of actinomycin D.

When 20 mmol/l glucose was the stimulus for increased synthesis, effects of actinomycin D were

apparent (Fig. 6). With the 15 minute stimulus in the presence of actinomycin D, proinsulin biosynthesis had returned to basal values at 45–60 min. This is in contrast to the persistence of stimulated biosynthesis seen in the absence of the inhibitor (see Fig. 3).

A similar lack of persistence of the stimulatory effect of 20 mmol/l glucose in the presence of actinomycin D was seen even when the glucose exposure was for 60 min (Fig. 6). At 45–60 min after cessation of the glucose stimulus, proinsulin biosynthesis was significantly lower than in the absence of actinomycin D. At this time the rate of biosynthesis was only 58% above control compared with 150% above in the absence of actinomycin D.

Discussion

Proinsulin biosynthesis in islets of Langerhans occurs at a rate which is slower than the rate at which insulin can be released when the islets are stimulated. Thus to ensure adequate supplies of insulin for release, the islet relies on a biosynthetic process which is continually active. This is reflected in a left shift in the glucose dose response relationship for stimulation of biosynthesis relative to release [8, 12], and in a persistence of stimulated proinsulin biosynthesis after cessation of the stimulus [16]. By these means the islets have a reserve of insulin to cope with sustained stimulation of release. The precise mechanisms regulating insulin content are unknown and a feed-back control of biosynthesis by the insulin content appears not to be a dominant mechanism [12]. Rather, the stimuli for insulin release promptly stimulate proinsulin biosynthesis at a time when the insulin content is not significantly changed. This lack of feed-back control by insulin content is seen particularly in experiments performed in the absence of calcium in the medium. Under these conditions, glucose can stimulate proinsulin biosynthesis in the absence of an effect on insulin release and therefore without decrease of insulin content [22].

To investigate how proinsulin biosynthesis is regulated in response to glucose stimulation of different intensity and duration, and particularly with regard to the persisting increased rates of biosynthesis after the end of glucose stimulation, a study both of onset and offset of proinsulin biosynthesis was performed. It can be concluded from the results that with a change from 2.5 mmol/l glucose to either 5.5 or 20 mmol/l a prompt stimulation of proinsulin biosynthesis occurs which is similar up to 30 min for both concentrations of glucose. Beyond 30 min, however, there was a difference. With 5.5 mmol/l glucose present the rate of proinsulin biosynthesis levelled off whereas with 20 mmol/l glucose the rate continued to increase. As this continued increase in the rate of proinsulin synthesis was blocked by actinomycin D it is likely that the initial increase in rate after change to high glucose is a post-transcriptional effect on the biosynthetic process while the continued increase beyond 30 min is a transcriptional effect. This presumed increase in the amount of mRNA for proinsulin biosynthesis, which appears some 30–45 min after exposure to high glucose, was first described by Permutt and Kipnis [15].

From the results on the rate at which stimulated proinsulin biosynthesis was turned off, it can be concluded that the post-transcriptional effect on biosynthesis, which results in prompt stimulation, is equally promptly turned off. In contrast, the transcriptional effect results in a persistent stimulation of proinsulin

biosynthesis. For example, with 5.5 mmol/l glucose as the stimulus, whether applied for 15 or 60 min, stimulated proinsulin biosynthesis rapidly returned to normal rates when the glucose stimulus was stopped. This is particularly well seen in Figure 2 after 60 min exposure. Comparison with Figure 3 reveals that whether high glucose exposure was for 15 or 60 min the stimulated rate of proinsulin biosynthesis was maintained and still significantly elevated 60 min after cessation of the stimulus. Furthermore, it can be seen in Figure 3 that the two components of the stimulatory process (transcriptional and post-transcriptional) can be detected from the rates of decline of stimulated biosynthesis when 20 mmol/l glucose is replaced with 2.5 mmol/l. Thus, there is a rapid initial fall, presumably due to the switching off of the post-transcriptional effect, followed by a stable plateau of still elevated biosynthesis presumably due to the increased amounts of mRNA as a result of increased transcription. That this conclusion seems likely is shown by the fact that in the presence of actinomycin D, 20 mmol/l glucose stimulated biosynthesis promptly and that this stimulation was rapidly abolished after reduction of the glucose concentration to 2.5 mmol/l.

In conclusion, it appears that the β -cells of islets of Langerhans react to high glucose concentrations and anticipated high rates of insulin release by transcriptional and post-transcriptional effects to stimulate proinsulin biosynthesis. 20 mmol/l glucose, but not 5.5 mmol/l glucose, will elicit these two effects. Small increases in glucose concentration as tested here by changing from 2.5 mmol/l to 5.5 mmol/l glucose cause only the post-transcriptional stimulatory effect on biosynthesis. The post-transcriptional effect is rapid in onset and rapidly dissipated after removal of the glucose stimulus. The transcriptional effect to increase the mRNA content of the β -cells has an onset time some 30 min after exposure to high glucose and results in a persistent elevation of proinsulin biosynthesis. The full duration of this persistent effect was not determined in this study. This system of forward control of β -cell insulin content appears to be primarily dependent upon the glucose concentration. For example, high (20 mmol/l) glucose for only 15 min resulted in a persistent elevation of the rate of proinsulin biosynthesis whereas 5.5 mmol/l glucose for 60 min did not. The effect of duration of exposure to high glucose concentration, after maximum rates of biosynthesis have been achieved, on the duration of elevated proinsulin biosynthesis remains to be examined.

Acknowledgements. These studies were supported by the Swiss National Science Foundation (grants nos 3.1060.73 and 3.774.076).

References

- Howell, S. L., Taylor, K. W.: Effects of glucose concentration on incorporation of ^3H -leucine into insulin using isolated mammalian islets of Langerhans. *Biochim. Biophys. Acta* **130**, 519–521 (1966)
- Steiner, D. F., Cunningham, D. D., Spigelman, L., Aten, B.: Insulin biosynthesis: evidence for a precursor. *Science* **157**, 697–700 (1967)
- Lin, B. J., Haist, R. E.: Insulin biosynthesis: effects of carbohydrates and related compounds. *Can. J. Physiol. Pharmacol.* **47**, 791–801 (1969)
- Clark, J. L., Steiner, D. F.: Insulin biosynthesis in the rat: demonstration of two proinsulins. *Proc. Natl. Acad. Sci. U. S. A.* **62**, 278–285 (1969)
- Morris, G. E., Korner, A.: The effect of glucose on insulin biosynthesis by isolated islets of Langerhans in the rat. *Biochim. Biophys. Acta* **208**, 404–413 (1970)
- Tanese, T., Lazarus, N. R., Devrim, S., Recant, L.: Synthesis and release of proinsulin and insulin by isolated rat islets of Langerhans. *J. Clin. Invest.* **49**, 1394–1404 (1970)
- Lin, B. J., Nagy, B. R., Haist, R. E.: Effect of various concentrations of glucose on insulin biosynthesis. *Endocrinology* **91**, 309–311 (1972)
- Pipeleers, D. G., Marichal, M., Malaisse, W. J.: The stimulus-secretion coupling of glucose induced insulin release XIV. Glucose regulation of insular biosynthetic activity. *Endocrinology* **93**, 1001–1011 (1973)
- Schatz, J., Maier, V., Hinz, M., Nierle, C., Pfeiffer, E. F.: Stimulation of ^3H -leucine incorporation into the proinsulin and insulin fraction of isolated pancreatic mouse islets in the presence of glucagon, theophylline and cyclic AMP. *Diabetes* **22**, 433–441 (1973)
- Trueheart, P. A., Maldonado, A., Kaelin, D., Renold, A. E., Sharp, G. W. G.: Proinsulin synthesis in islets of Langerhans from Spiny mice (*Acomys Cahirinus*). *Diabetologia* **12**, 463–470 (1976)
- Maldonado, A., Trueheart, P. A., Renold, A. E., Sharp, G. W. G.: Effects of streptozotocin in vitro on proinsulin biosynthesis, insulin release and ATP content of isolated rat islets of Langerhans. *Diabetologia* **12**, 471–481 (1976)
- Maldonado, A., Renold, A. E., Sharp, G. W. G., Cerasi, E.: Glucose-induced proinsulin biosynthesis: role of cyclic AMP. *Diabetes* **26**, 538–545 (1977)
- Jarrett, R. J., Keen, H., Track, N. S.: Glucose and RNA synthesis in mammalian islets of Langerhans. *Nature* **213**, 634–635 (1967)
- Jarrett, R. J., Track, N. S., Keen, H.: Ribonucleic acid metabolism and insulin synthesis. In: S. Falkmer, B. Hellman, I. B. Täljedal (Eds.): *Structure and metabolism of the pancreatic islets*. Wenner-Gren Center Int. Symp. Ser. **16**, 371–379. New York: Pergamon Press 1970
- Permutt, M. A., Kipnis, D. M.: Insulin biosynthesis. I. On the mechanism of glucose stimulation. *J. Biol. Chem.* **247**, 1194–1199 (1972)
- Zucker, P., Logothetopoulos, J.: Persisting enhanced proinsulin-insulin and protein biosynthesis (^3H -leucine incorporation) by pancreatic islets of the rat after glucose exposure. *Diabetes* **24**, 194–200 (1975)
- Lacy, P. E., Kostianovsky, M.: Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* **16**, 35–39 (1967)
- Davoren, P. R.: The isolation of insulin from a single cat pancreas. *Biochim. Biophys. Acta* **63**, 150–153 (1962)
- Akunuma, Y., Kuzuya, T., Hazashi, M., Ide, T., Kuzuya, N.: Immunological reactivity of insulin to Sepharose coupled with insulin antibody: its use for the extraction of insulin from serum. *Biochem. Biophys. Res. Commun.* **38**, 947–953 (1970)
- Berne, C.: Anti-insulin serum coupled to Sepharose 4B as a tool for the investigation of insulin biosynthesis in the B-cells of obese hyperglycemic mice. *Endocrinology* **97**, 1241–1247 (1975)
- Bone, A. J., Taylor, K. W.: Metabolic adaptation to pregnancy shown by increased biosynthesis of insulin in islets of Langerhans isolated from pregnant rats. *Nature* **262**, 501–502 (1976)
- Pipeleers, D. G., Marichal, M., Malaisse, W. J.: The stimulus-secretion coupling of glucose-induced insulin release. XV. Participation of cations in the recognition of glucose by the β -cell. *Endocrinology* **93**, 1012–1018 (1973)

*Received: November 7, 1977,
and in revised form: January 12, 1978*

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