Glucose-Regulated Proinsulin Processing in Isolated Islets From Rat Pancreas

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We demonstrated previously that the conversion rate of proinsulin to insulin in pancreatic islets progressively increased after prolonged prior exposure to glucose (11 mM) and that this effect could be blocked by cycloheximide. This study was designed to characterize further the time course and regulation of the proinsulin conversion process. The effects of prior exposure to glucose on proinsulin conversion were dose dependent (K_m, ~7 mM glucose) and time dependent, taking ~3 h to reach the maximum rate. Glucose added at or after the subsequent [^{3}H]leucine pulse was ineffective. Mannoheptulose, added during a 3-h exposure with glucose (11 mM), prevented glucose-induced activation of the proinsulin conversion process. L-Leucine (20 mM) was as effective as 11 mM glucose in activating conversion, whereas 2-α-ketoisocaproic acid (20 mM) or phorbol ester (50 nM) had little effect. Activation of proinsulin conversion by a 24-h exposure to glucose (11 mM) was reversed by a subsequent 3-h prior exposure to cycloheximide. α-Amanitin, an inhibitor of mRNA synthesis, did not influence the glucose-induced activation of proinsulin conversion when present during a 3-h exposure to glucose; however, it completely inhibited glucose-stimulated conversion when present during 24 h exposure. Results suggest that activation of the proinsulin conversion process is regulated by glucose metabolism rather than the glucose molecule per se and that other, but not all, secretagogues are effective. Conversion may require prior synthesis of a pool of converting enzyme(s) or other regulatory proteins whose turnover is relatively rapid (~33 h) and whose mRNA is more stable (to 24 h). *Diabetes* 37:1426–31, 1988

In the pancreatic β cell, glucose is a major regulator for the gene expression (1–3), translational biosynthesis (4–6), and release (7,8) of insulin. It was previously accepted that glucose does not affect the conversion of proinsulin to insulin (9–14). However, this conclusion resulted from pulse-chase experiments in which glucose or inhibitors (e.g., mannoheptulose, cycloheximide) were added during or after the pulse labeling and indicated only that conversion of labeled hormone is not affected if these agents are acutely present after formation of proinsulin.

We observed recently that a 3-h prior exposure of freshly isolated islets to a high level of glucose (11 mM) accelerated the subsequent conversion of proinsulin to insulin (15). Furthermore, inhibition of total protein synthesis by cycloheximide, added during the 3-h exposure period, completely prevented glucose activation of the conversion process. We hypothesized that the amount of converting enzyme(s) (or proteins associated with the conversion process) reaching the secretory granule is determined by the amount of its prior synthesis into a pool made available in the endoplasmic reticulum or the Golgi apparatus. Two classes of enzymes may be required for the overall processing of proinsulin (16–18), endopeptidases, and an exopeptidase, possibly carboxypeptidase H. Because our method measured conversion from a 9000- to a 6000-M_r product, regulation at the level of the endopeptidases was suggested.

In this study, we characterized further the regulation of the conversion process, including the concentration and time requirement for glucose, and the effects of other β-cell secretagogues [L-leucine, 2-ketoisocaproic acid (KIC), and phorbol ester]. We also evaluated the stability of the proteins and mRNA produced after activation of the conversion process by using an inhibitor of protein synthesis, cycloheximide, or an inhibitor of RNA polymerase II, α-amanitin.

EXPERIMENTAL PROCEDURES

Materials. L-Leucine, KIC, HEPES, cycloheximide, mannoheptulose, and α-amanitin were from Sigma (St. Louis, MO). Phorbol 12-myristate 13-acetate was from P-L Biochemicals

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Received for publication 4 January 1988 and accepted in revised form 14 April 1988.

TABLE 1
Effect of glucose after pulse on proinsulin conversion

Chase	Proinsulin after 90-min pulse chase (%)	
	Incubation (40 min)	Prior exposure (140 min)
2 mM glucose	37.5 ± 8.0	11.2 ± 2.1
11 mM glucose	40.0 ± 6.5	11.2 ± 0.6

Islets were collected after incubation in HB104 + 11 mM glucose for 40 min or after prior exposure to glucose for an additional 140 min. Islets were washed with Krebs-Ringer bicarbonate (KRB) containing 11 mM glucose and labeled with [^{3}H]leucine for 20 min. After the labeling, islets were washed 3 times and chased in KRB (+0.2 mM leucine) containing 2 or 11 mM glucose for 70 min. Proinsulin and insulin were purified, and the percentage of tritiated proinsulin in islets was calculated as described in MATERIALS AND METHODS. n = 3.

(Milwaukee, WI). D-Glucose was from Mallinckrodt (St. Louis, MO). L-[4,5-^{3}H]leucine was obtained from Amersham (Arlington Heights, IL).

Incubation and preparation of islets. Islets from fed male Long-Evans rats (Simonsen, Gilroy, CA) were prepared by collagenase digestion (19) as modified in our laboratory (20). After freshly isolated islets were maintained in Krebs-Ringer bicarbonate containing HEPES (KRB) and 2 mM glucose for 1 h as described previously (21), they were prior exposed for 140 min with specially prepared HB104 medium (derivative of RPMI, fortified with amino acids, specific vitamins, nucleotides, and 0.7% human serum albumin with insulin deleted; Hana Biologics, Berkeley, CA) containing various concentrations of glucose, phorbol ester (50 nM) alone, or 2 mM glucose plus L-leucine (20 mM) or KIC (20 mM). Twenty millimoles KIC was reported to exert adverse effects on ionic fluxes; however, this concentration was highly effective on insulin secretion (22). We found that 25 mM KIC produced characteristic stimulation, potentiation, and desensitization of insulin release (23). In other experiments, cycloheximide (20 μg/ml), α-amanitin (40 μg/ml), or mannoheptulose (20 mM) was preincubated for various periods (see figures).

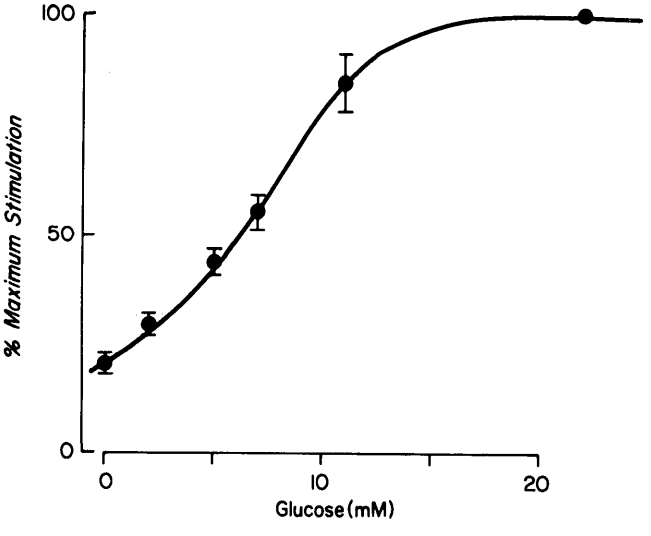

FIG. 1. Effect of concentration of preincubation prior exposure to glucose on proinsulin conversion. Islets were exposed to various concentrations of glucose in HB104 medium for 140 min before initiating 40-min incubation with 11 mM glucose followed by 90-min pulse chase (n = 4). Percent maximum stimulation of conversion after 90-min pulse-chase is expressed in relationship to that found for 22 mM glucose: (PI_{22}/PI_c) × 100, where PI_{22} is percent [^{3}H]proinsulin in 22 mM glucose (10.5 ± 1.8%) and PI_c is percent [^{3}H]proinsulin at various concentrations of glucose. Proinsulin synthesis was not significantly affected at any glucose concentration in exposure media.

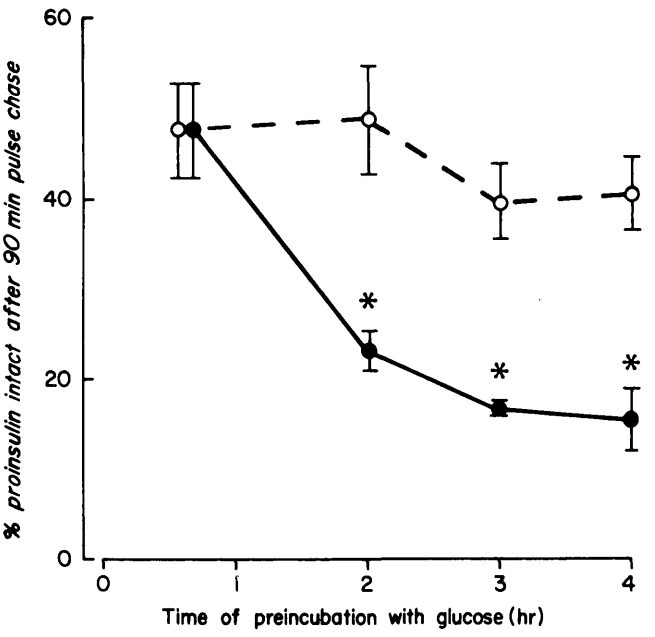

FIG. 2. Effect of time of prior exposure of islets to glucose on rates of proinsulin conversion. Islets were prior exposed to 2 (○) or 11 (●) mM glucose in HB104 medium at various times before initiating standard 40-min incubation and 90-min pulse chase. Proinsulin synthesis was not significantly affected by any exposure time. *P < .01 compared with values for 2 mM glucose; n = 4.

Islet labeling and measurement of conversion rate of proinsulin to insulin. After prior exposure to glucose or other agents, islets were incubated for 40 min in fresh HB104 medium containing 11 mM glucose to permit the maximum effect of glucose on the translation of proinsulin mRNA and proinsulin synthesis (24,25). This incubation was omitted only in the α-amanitin experiments. Islets were then washed three times and pulse labeled with 400 μCi/ml [^{3}H]leucine (60 Ci/mM) for 20 min in the KRB media containing 11 mM glucose. They were then chased for various periods in the same media, to which 0.2 mM leucine and different glucose concentrations had been added. Proinsulin and insulin were separately purified from islets extracted with 75% acid alcohol by anti-insulin affinity and Bio-Gel P-30 column chromatography as previously described (26,27). The radioactivity was determined by liquid-scintillation counting.

TABLE 2
Effects of mannoheptulose during prior exposure to glucose on subsequent conversion of proinsulin to insulin

Mannoheptulose during prior exposure	Proinsulin after 90-min pulse chase (%)
0 mM	15.0 ± 0.5
20 mM	49.1 ± 5.0

Isolated islets were prior exposed to HB104 medium + 11 mM glucose for 140 min in the presence or absence of 20 mM mannoheptulose. Islets were then incubated for 40 min in the 11 mM glucose-HB104 media and pulse chased for 90 min. n = 4.

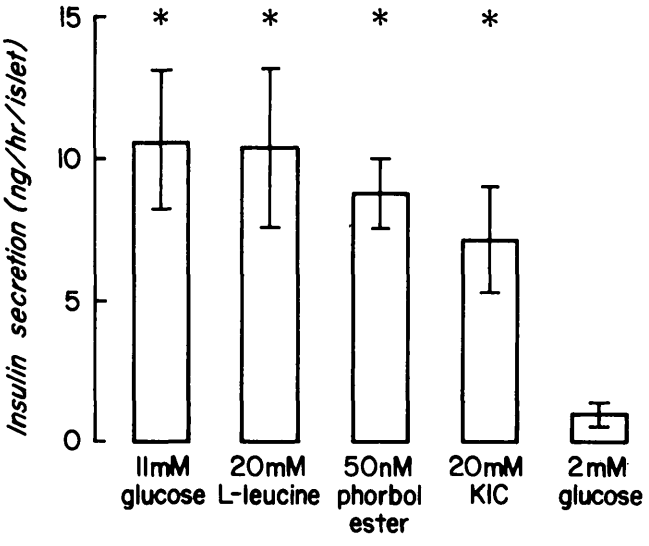

FIG. 3. Effects of 2-α-ketoisocaproic acid (KIC), L-leucine, phorbol ester, and glucose on insulin secretion from isolated islets. Islets were exposed for 140 min in HB104 with 20 mM KIC or 20 mM L-leucine + 2 mM glucose; 50 nM phorbol ester alone; or 2 or 11 mM glucose. n = 4. *P < .05 compared with values for 2 mM glucose.

Measurements of relative radioactivity in proinsulin and insulin were corrected for the loss of C-peptide by multiplying radioactivity eluting in the insulin peak by 11/6 (26). It was previously shown, under these conditions, that conversion of proinsulin to insulin assumed pseudo–first-order kinetics by a 60-min chase (26) and that measurement of the percentage of proinsulin intact at a constant time point within 1 h thereafter reflected the conversion rate.

Insulin assay. Insulin in medium was assayed as immunoreactive insulin with rat insulin standards, as described (28).

Statistical analysis. All values are reported as means ± SE, and differences were assessed with one-way analysis of variance or paired Student's t test. Experiments in duplicate or triplicate on a given day were taken as n = 1.

RESULTS

Table 1 shows the effects of glucose added after the pulse on proinsulin conversion. Islets were tested with either a 40-min incubation with 11 mM glucose (insufficient to activate conversion) or after prior exposure to glucose for 140 min to activate conversion. In either case, the presence of glucose after the labeling did not affect the conversion rate of proinsulin.

The effect of various glucose concentrations added during the prior exposure period on subsequent proinsulin conversion is shown in Fig. 1. The curve shows a sigmoidal dependence on prior glucose concentration, with a half-maximum effect at ~7 mM glucose.

The effect of prior exposure to glucose (11 mM) was time dependent (Fig. 2). Two hours of exposure accelerated the conversion rate to ~70% of maximal; near-maximal conversion was achieved by 3–4 h exposure.

The effect of mannoheptulose, present during the prior exposure period, was examined to determine if glucose metabolism is required for the activation of proinsulin conversion. Mannoheptulose was subsequently removed from the system by washing before the 40-min prepulse incubation; subsequent total synthesis of labeled proinsulin was not affected in mannoheptulose-pretreated islets [1700 ± 95 vs. 1850 ± 155 counts/min (cpm) per islet, untreated vs. treated]. Islets prior exposed to 11 mM glucose and mannoheptulose for 140-min failed to show the accelerated conversion caused by glucose alone (Table 2). In separate experiments in which several time points (30, 60, and 90 min) of chase were used, and in agreement with a previous report (14), the increased conversion rate was not affected if mannoheptulose was added after the labeling period ($t_{1/2}$ 20 ± 2 vs. 18 ± 4 min for untreated vs. treated islets, respectively).

The effect of exposure to two nonglucose fuel secretagogues, leucine and KIC, on insulin secretion and on subsequent conversion of proinsulin is shown in Fig. 3 and Table 3. Although 20 mM L-leucine was as effective in activating the conversion rate as 11 mM glucose, KIC had a minor effect, if any, on this process. Insulin release during exposure to these compounds, however, was stimulated by both (Fig. 3). Phorbol ester (50 nM), which is a direct activator of protein kinase C (29), also stimulated insulin release (Fig. 3) but had no effect on proinsulin conversion (Table 3).

To determine if activated proinsulin conversion was reversible, as well as the stability of the activating protein(s) involved, islets underwent prior exposure for 24 h in 11 mM glucose, conditions which fully activated the conversion process (15; Fig. 4). They were then incubated with or without cycloheximide for 3 h and were subsequently pulse chased. The apparent $t_{1/2}$ for conversion of cycloheximide-treated islets was partially reversed to ~30 min, from the ~15 min for glucose-activated untreated islets (Fig. 4).

α-Amanitin, an inhibitor of gene transcription, was used to evaluate the stability of mRNA of converting enzymes or other proteins involved in conversion. Addition of α-amanitin to islets during a 24-h prior exposure to 11 mM glucose blocked accelerated conversion; addition of α-aminitin during a shorter 3-h exposure had little, if any, inhibitory effect (Fig. 5). α-Amanitin had little effect on proinsulin synthesis, although some inhibition of total islet protein synthesis was observed at 24 h (Fig. 6).

TABLE 3
Effect of prior exposure to 2-α-ketoisocaproic acid, L-leucine, or phorbol ester on subsequent conversion of proinsulin to insulin

Prior exposure conditions	Proinsulin after 90-min pulse chase (%)
2 mM glucose	44.2 ± 5.4
11 mM glucose	15.7 ± 2.2*
2 mM glucose + 20 mM KIC	31.2 ± 6.7†
2 mM glucose + 20 mM L-leucine	17.7 ± 2.2*
50 nM phorbol ester	41.8 ± 4.2†

Isolated islets were prior exposed to HB104 medium for 140 min with 2 or 11 mM glucose; 20 mM 2-ketoisocaproic acid (KIC) or 20 mM L-leucine + 2 mM glucose; or 50 nM phorbol ester alone. Islets were then processed as described in Table 2. n = 4–6.
*P < .01 compared with values for 2 mM glucose.
†NS vs. 2 mM glucose. Proinsulin synthesis after prior exposure with different agents and after 40-min incubation in 11 mM glucose was not significantly different.

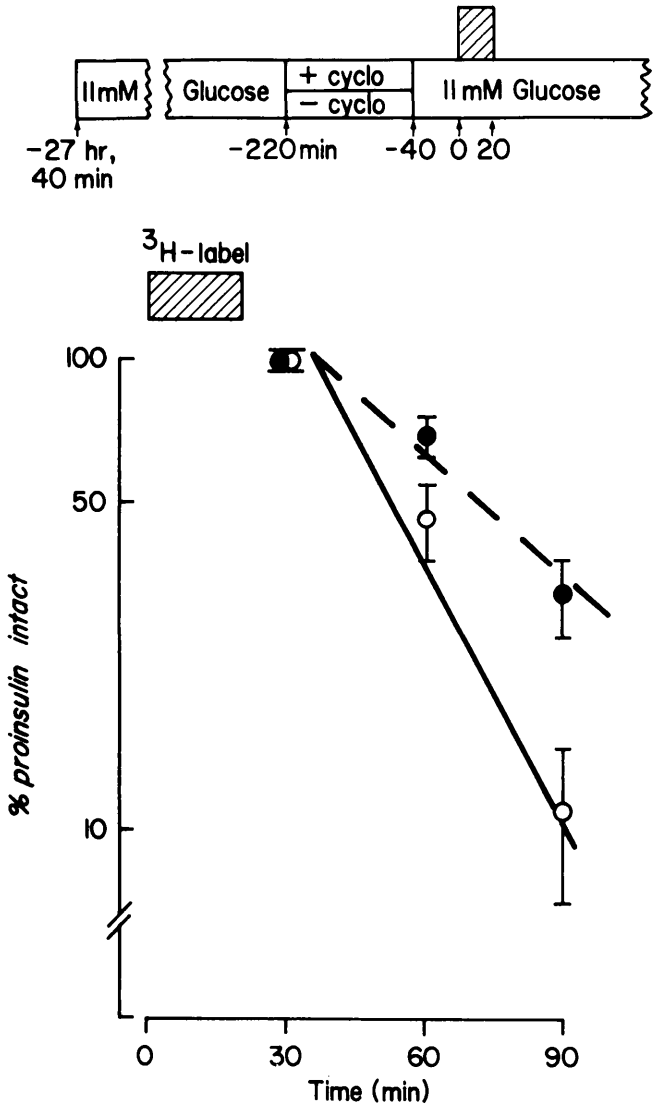

FIG. 4. Effect of cycloheximide on reversing glucose-stimulated conversion. Islets were prior exposed to HB104 + 11 mM glucose for 24 h to enhance conversion and were then incubated with (●) or without (○) 20 μg/ml cycloheximide for additional 3 h. Islets were subsequently labeled with [^{3}H]leucine as indicated. Slopes of lines were determined by linear regression. $t_{1/2}$ conversion: untreated glucose-enhanced islets, 15.2 ± 2 min; treated glucose-enhanced islets, 30.7 ± 5 min. $P < .001$ by paired t test ($n = 4$); normal islets with only 42 min incubation with glucose, 70 min (15). Proinsulin synthesis of treated islets was not different from untreated islets.

DISCUSSION

The freshly isolated islets used in this study appear viable because they exhibit similar insulin-release patterns, including time-dependent potentiation and subsequent desensitization, as obtained with the perfused pancreas, in which the vascular system is intact (30). They also maintain glucose-sensitive insulinogenesis throughout experimental periods of 24 h (15).

Regulation of proinsulin conversion rate by glucose. Conversion of proinsulin to insulin increased progressively with increasing prior glucose concentration. Because the K_m for glucose-stimulated conversion (~7 mM) was in the same range as both insulin release (8 mM) and biosynthesis (6 mM) (31), the glucose-sensor mechanism may have some common features for all three processes. Mannoheptulose blocked glucose-stimulated conversion, indicating that for this process, as for synthesis and secretion, intracellular glucose metabolism is required. This study extends our previous observation and shows that near-maximal activation of conversion by glucose (11 mM) required 3–4 h. Glucose activation of the conversion process may be relatively specific for the β-cell and for proinsulin; 21-h culture with a high level of glucose did not change the processing of pancreatic polypeptide in canine pancreas (32). Because cycloheximide, added during prior exposure to glucose, prevented accelerated proinsulin conversion (15), we had proposed that glucose increases a pool of proteins (converting enzymes?) that are copackaged with proinsulin during formation of granules in the Golgi apparatus. If so, these results suggest that glucose (11 mM) requires ~3 h to fill that pool.

We, as well as others (9–14), find that glucose has no effect on conversion when added during and after the [^{3}H]leucine pulse. Therefore, glucose probably does not affect conversion once proinsulin synthesis has been initiated or after proinsulin is concentrated in the secretory granule,

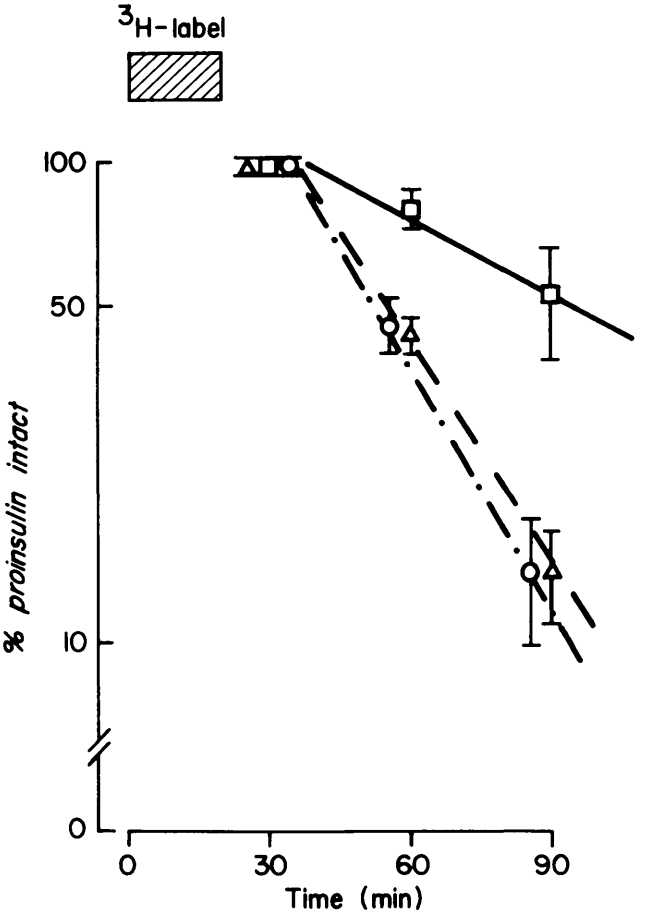

FIG. 5. Effects of α-amanitin during exposure to glucose for 3 or 24 h on subsequent proinsulin conversion. Isolated islets were prior exposed to HB104 + 11 mM glucose with α-amanitin for 3 (△) or 24 (□) h before the 20-min pulse with [^{3}H]leucine. Control (○) represents exposure with 24-h glucose alone; exposure with glucose for 3 h gave similar results (see Fig. 2) and was deleted for clarity. Slopes of line were determined by linear regression ($n = 4$). $t_{1/2}$ for conversion after 3 h with α-amanitin (18.7 ± 0.9 min) and that after 24 h without α-amanitin (15.7 ± 0.7 min) was similar but significantly different from the $t_{1/2}$ at 24 h with α-amanitin (68.3 ± 20.4 min; $P < .05$).

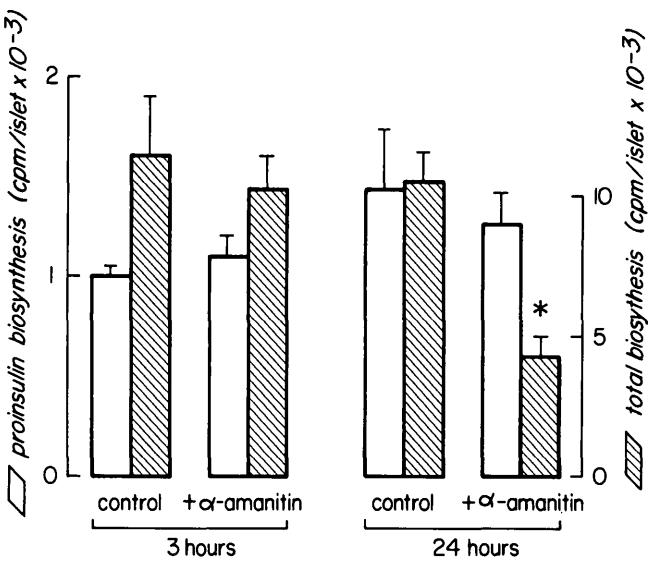

FIG. 6. Effects of α-amanitin on synthesis of proinsulin and total protein after 3 or 24 h prior exposure. Islets were exposed to HB104 medium containing 11 mM glucose in presence or absence of 40 μg/ml α-amanitin for 3 or 24 h, as in Fig. 5. To measure proinsulin synthesis, islets were washed with Krebs-Ringer bicarbonate containing 11 mM glucose and pulse-labeled with [^{3}H]leucine for 20 min without chase. Islets were homogenized and extracted, and proinsulin was purified by anti-insulin affinity chromatography as described. In separate aliquots, total protein synthesis was determined after precipitation of islet proteins by homogenization in 10% trichloroacetic acid (n = 4). *P < .01 compared with 24-h control.

even though glucose can decrease granular pH, or increase granular calcium thereby theoretically activating proteolytic enzymes (33–36).

Possibly, glucose could increase conversion of proinsulin by increasing its transport rate from endoplasmic reticulum to the secretory granules. Although this increase could contribute to the overall enhanced conversion observed, activation of conversion itself appears to be the predominant site of regulation. In other studies we (37) and others (38,39) have used antimycin A to inhibit transport and found the process to be complete in 40–60 min and only mildly affected by glucose. In another study we determined changes in conversion as the first-order disappearance of [^{3}H]proinsulin measured from 60 min after a [^{3}H]leucine pulse (15). Although a decrease in transport to <40–60 min would shorten the time at which first-order conversion of [^{3}H]proinsulin begins, model analysis shows these changes would not affect the later first-order disappearance rate from which the $t_{1/2}$ for conversion was calculated (37). Finally, cycloheximide, which inhibits protein synthesis and glucose activation of conversion (15), is reported not to affect intracellular transport of proinsulin (40).

The observed increased conversion by glucose may result, at least partly, from an increase in an enzyme similar to procathepsin B (18). Under the same activation conditions, an increase in synthesis of this enzyme (measured by immunoprecipitation with polyclonal anti-procathepsin-like antibody) has been reported (37).

Alternatively, a more recent study suggests the involvement of two β-cell endopeptidases, which differ in their specificities for the cleavage sites in proinsulin and in their pH and calcium dependencies (36). Both are insensitive to group-specific inhibitors of serine, suggesting they are distinct from procathepsin. Our data suggest that synthesis of one or both of these enzymes could be increased by prolonged exposure to glucose.

Effectiveness of other agents. Glucose is not the sole metabolizable secretagogue that stimulates proinsulin conversion to insulin. L-Leucine (20 mM) was as effective as glucose (11 mM), whereas the secretagogue KIC was relatively ineffective. Phorbol ester alone, which at the concentration used was as effective a secretagogue as 11 mM glucose, also did not affect conversion. Thus, increased insulin release per se is not required for enhanced conversion of proinsulin.

Turnover of proteins involved in proinsulin conversion. The fully activated proinsulin conversion rate produced by prior exposure of islets to 11 mM glucose for 24 h was reversed by the addition of cycloheximide for 3 h when introduced before the subsequent pulse chase. This result suggests that the loss of proteins involved in conversion was relatively rapid. If this reflects a degradation of proteins, it is faster than that for insulin, 0.5–1.0%/h (41). The possibility that glucose accelerates the synthesis of rapidly turning over proteins that regulate the sorting of the converting enzyme to the secretory granule cannot be excluded.

Stability of mRNA for proteins responsible for activated proinsulin conversion. The conversion of proinsulin was not inhibited by a 3-h prior exposure of islets with α-amanitin, but α-amanitin completely blocked the activated conversion when presented for 24 h. In contrast, α-amanitin had little effect on the proinsulin synthesis rate during 24 h. Thus, mRNA for either converting enzymes or protein involved in conversion was probably less stable than that for insulin mRNA. This little change of proinsulin synthesis when islets were exposed to α-amanitin for 24 h supports other investigations concluding that the half-life of insulin mRNA is ~60 h in the presence of elevated glucose (3). The observations that glucose causes near-maximal activation of conversion at 3 h by a process requiring protein synthesis (inhibited by cyclohexamide) but not dependent on mRNA synthesis (insensitivity to α-amanitin at 3 h) indicates that much of the glucose regulation of proinsulin-to-insulin conversion is at the translational level of synthesis of the regulatory protein(s).

ACKNOWLEDGMENTS

We thank Hana Biologics (Alameda, CA) for supplying the media used in this study.

This work was supported by NIH Grant DK-01410 and the Upjohn Company.

REFERENCES

1. Giddings SJ, Chirgwin J, Permutt MA: Effects of glucose on proinsulin messenger RNA in rats in vivo. *Diabetes* 31:624–29, 1982
2. Nielsen DA, Welsh M, Casadaban MJ, Steiner DF: Control of insulin gene expression in pancreatic B-cells and in an insulin-producing cell line, RIN-5F cells. I. Effects of glucose and cyclic AMP on the transcription of insulin mRNA. *J Biol Chem* 260:13585–89, 1985
3. Welsh M, Nielsen DA, MacKrell AJ, Steiner DF: Control of insulin gene expression in pancreatic B-cells and in an insulin-producing cell line, RIN-5F cells. II. Regulation of insulin mRNA stability. *J Biol Chem* 260:13590–94, 1985
4. Itoh N, Okamoto H: Translational control of proinsulin synthesis by glucose. *Nature* (Lond) 283:100–102, 1980
5. Itoh N, Ohshima Y, Nose K, Okamoto H: Glucose stimulates proinsulin synthesis in pancreatic islets without a concomitant increase in proinsulin mRNA synthesis. *Biochem Int* 4:315–21, 1982
6. Welsh M, Scherberg N, Gilmore R, Steiner DF: Translational control of

insulin biosynthesis: evidence for regulation of elongation, initiation and signal-recognition-particle-mediated translational arrest by glucose. *Biochem J* 235:459–67, 1986
7. Gold G, Grodsky GM: The secretory process in B cells of the pancreas. In *Cell Biology of the Secretory Process*. Cantin M, Ed. New York, Karger, 1984, p. 359–88
8. Hedeskov CJ: Mechanism of glucose-induced insulin secretion. *Physiol Rev* 60:442–509, 1980
9. Steiner DF: Proinsulin and the biosynthesis of insulin. *N Engl J Med* 280:1106–12, 1969
10. Baird N: In vitro regulation of proinsulin synthesis in isolated rat islets. *Diabetes* 20 (Suppl. 1):332, 1971
11. Steiner DF, Kammler W, Clark JL, Cryer PE, Robenstein AH: The biosynthesis of insulin. In *Handbook of Physiology*. Sect. 7, vol. 1. Steiner DF, Freinkel N, Eds. Baltimore, MD, Williams & Wilkins, 1972, p. 175–98
12. Pipeleers DG, Marichal M, Malaisse WJ: The stimulus-secretion coupling of glucose-induced insulin release. XV. Glucose regulation of insulin biosynthetic activity. *Endocrinology* 93:1001–11, 1973
13. Andersson A, Westman J, Hellerstrom C: Effects of glucose on the ultrastructure and insulin biosynthesis of isolated mouse pancreatic islets maintained in tissue culture. *Diabetologia* 10:743–53, 1974
14. Jain K, Logothetopoulos J: Secretion of insulin in a perifusion system and conversion of proinsulin to insulin by pancreatic islets from hyperglycemic rats. *Diabetes* 26:650–56, 1977
15. Nagamatsu S, Bolaffi JL, Grodsky GM: Direct effects of glucose on proinsulin synthesis and processing during desensitization. *Endocrinology* 120:1225–31, 1987
16. Steiner DF, Kemmler W, Tager HS, Peterson JD: Proteolytic processing in the biosynthesis of insulin and other proteins. *Fed. Proc* 33: 2105–15, 1974
17. Docherty K, Steiner DF: Post-translational proteolysis in polypeptide hormone biosynthesis. *Annu Rev Physiol* 44:625–38, 1982
18. Steiner DF, Docherty K, Carroll R: Golgi/granule processing of peptide hormone and neuropeptide precursors: a minireview. *J Cell Biochem* 24:121–30, 1984
19. Lacy PE, Kostianovsky M: Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* 16:35–39, 1967
20. Grodsky GM, Schmid-Formby F: Kinetic and quantitative relationships between insulin release and ^{65}Zn efflux from perifused islets. *Endocrinology* 117:704–10, 1985
21. Bolaffi JL, Nagamatsu S, Harris, J, Grodsky GM: Protection by thymidine, an inhibitor of poly ADP-ribosylation on streptozotocin inhibition of insulin secretion. *Endocrinology* 120:2117–22, 1987
22. Hutton JC, Sener A, Herchuelz A, Atwater I, Kawayu S, Boschers AC, Somers G, Devis G, Malaisse WJ: Similarities in the stimulus-secretion coupling mechanisms of glucose and 2-keto acid-induced insulin release. *Endocrinology* 106:203–19, 1980
23. Bolaffi JL, Bruno L, Heldt A, Grodsky GM: Characteristics of desensitization of insulin secretion in fully in vitro systems. *Endocrinology*. 122:1801–809, 1988
24. Permutt MA, Kipnis DM: Insulin biosynthesis. I. On the mechanism of glucose stimulation. *J Biol Chem* 247:1194–99, 1972
25. Kailen D, Renold AE, Sharp GWG: Glucose stimulated insulin biosynthesis: rates of turn off after cessation of the stimulus. *Diabetologia* 14:329–35, 1978
26. Gold, G, Landahl HD, Gishizky ML, Grodsky GM: Heterogeneity and compartmental properties of insulin storage and secretion in rat islets. *J Clin Invest* 69:554–63, 1982
27. Gold G, Pou J, Nowlain RM, Grodsky GM: Effects of monensin on conversion of proinsulin to insulin and secretion of newly synthesized insulin in isolated rat islets. *Diabetes* 33:1019–24, 1984
28. Levin SR, Grodsky GM, Hagura R, Smith D: Comparison of the inhibitory effects of diphenylhydantoin and diazoxide upon insulin secretion from the isolated perfused pancreas. *Diabetes* 21:856–62, 1972
29. Nishizuka Y: Turnover of inositol phospholipids and signal transduction. *Science* 225:1365–70, 1984
30. Bolaffi JL, Heldt A, Lewis LD, Grodsky GM: The third phase of in vitro insulin secretion: evidence for glucose insensitivity. *Diabetes* 35:370–73, 1986
31. Maldonato A, Renold AE, Sharp GWG, Cerasi E: Glucose-induced proinsulin biosynthesis: role of islet cyclic AMP. *Diabetes* 26:538–45, 1977
32. Schwartz TW: Cellular peptide processing after a single arginyl residue: studies of the common precursor for pancreatic polypeptide and pancreatic icosapeptide. *J Biol Chem* 262:5093–98, 1987
33. Hutton JC: Secretory granules. *Experientia* 40:1091–98, 1984
34. Orci L, Ravazzola M, Amherdt M, Madsen O, Perrelet A, Vassalli J-D, Anderson RGW: Conversion of proinsulin to insulin occurs coordinately with acidification of maturing secretory vesicles. *J Cell Biol* 103:2273–81, 1986
35. Docherty K, Carroll RJ, Steiner DF: Conversion of proinsulin to insulin: involvement of a 31,500 molecular weight thiol protease. *Proc Natl Acad Sci USA* 79:4613–17, 1982
36. Davidson HW, Rhodes CJ, Hutton JC: Intraorganellar calcium and pH control proinsulin cleavage in the pancreatic β cell via two distinct site-specific endopeptidases. *Nature* (Lond) 333:93–96, 1988
37. Nagamatsu S, Grodsky GM: Analysis of proinsulin conversion activated by prior glucose: evidence that glucose stimulates synthesis of the conversion enzyme. *Biochem Biophys Res Commun* 148:1418–24, 1987
38. Clark JL, Steiner DF: Insulin biosynthesis in the rat: demonstrations of two proinsulins. *Proc Natl Acad Sci USA* 62:278–85, 1969
39. Kemmler W: B. Insulin synthesis in B-cells. I. Role of proinsulin in insulin biosynthesis. In *Handbook of Experimental Pharmacology. Insulin II*. Vol. 32, pt. 2. Hassalblatt A, Bruchhausen F, Eds. Berlin, Springer-Verlag, 1975, p. 17–56
40. Orci L, Ravazzola M, Amherdt M, Madsen O, Vassalli J-D, Perrelet A: Direct identification of prohormone conversion site in insulin-secreting cells. *Cell* 42:671–81, 1985
41. Halban PA, Wollheim CB: Intracellular degradation of insulin stores by rat pancreatic islets in vitro: an alternative pathway for homeostasis of pancreatic insulin content. *J Biol Chem* 255:6003–6006, 1980