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Regulation of the biosynthesis of insulin-secretory-granule proteins

Co-ordinate translational control is exerted on some, but not all, granule matrix constituents

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The regulation of the biosynthesis of the insulin-secretory-granule matrix proteins insulin II, chromogranin A and carboxypeptidase H was studied in isolated rat islets of Langerhans. Islets were labelled with [^{35}S]-methionine, and incorporation into total protein was determined by trichloroacetic acid precipitation and that into specific proteins by immunoprecipitation followed by polyacrylamide-gel electrophoresis and fluorography. Islets incubated in the presence of 16.7 mM-glucose incorporated 3 times as much [^{35}S]-methionine into total protein as did islets incubated with 2.8 mM-glucose. The same conditions produced more than a 20-fold increase in incorporation into both proinsulin and chromogranin A, with no observable effect on carboxypeptidase H. The concentration-dependencies of the glucose-stimulated synthesis of chromogranin A and proinsulin were parallel, and in both cases the response to 16.7 mM-glucose was typified by an initial lag of 20 min, followed by a rapid activation to a new steady state over the ensuing 40 min. Synthesis of total protein, although activated to a lesser extent, responded with similar kinetics. Extracellular Ca^{2+} depletion did not affect the basal or glucose-stimulated biosynthesis of any of the proteins under investigation. Mannoheptulose (20 mM) abolished glucose-stimulated synthesis of insulin, chromogranin A and total protein, but had no effect on the synthesis of carboxypeptidase H. It is concluded that the biosynthesis of insulin and chromogranin A is regulated principally at the translational level by the same intracellular signal generated from the metabolism of glucose. Such regulation is not common to all insulin-secretory-granule proteins, since the synthesis of carboxypeptidase H was unaffected by the same stimulus.

INTRODUCTION

The insulin secretory granule of the pancreatic β-cell contains more than 100 different proteins, of diverse physiological function (for review see Hutton, 1984). Despite wide fluctuation in the rate of secretion under different dietary and physiological conditions, the number of insulin granules is maintained within relatively narrow limits, indicating that their biogenesis is a precisely regulated process. How co-ordinate synthesis and delivery of such a large number of different proteins to the site of granule assembly is achieved is a question of major importance in the understanding of the cellular biology of secretory processes.

The pancreatic β-cell is a useful model for such investigation, since changes in the rate of insulin biosynthesis can be rapidly induced by secretagogues such as glucose (Howell & Taylor, 1966). Such control is exerted principally at the level of translation of pre-formed mRNA (Permutt & Kipnis, 1972). We have used [^{35}S]methionine as a marker for newly synthesized proteins to investigate the biosynthesis of proinsulin II in parallel with chromogranin A and carboxypeptidase H, two other functionally distinct insulin-granule matrix proteins. Chromogranin A is a widely distributed secretory-granule protein in neuroendocrine tissues (Streider *et al.*, 1968; O'Connor, 1983; Wilson & Lloyd, 1984; Nolan *et al.*, 1985). Although its biological function is unknown, the observation that its primary sequence includes pancreastatin (Tatemoto *et al.*, 1986; Iacangelo *et al.*, 1986), a peptide which inhibits insulin secretion, suggests that it may function in the endocrine pancreas as a precursor to autocrine or paracrine regulators (Eiden, 1987; Huttner & Benedum, 1987; Hutton *et al.*, 1987*a*). In the pancreatic β-cell, it is initially synthesized as a 100 kDa precursor and proteolytically processed in parallel with proinsulin (Hutton *et al.*, 1987*b*). One of the major products of this conversion, the *N*-terminal protein betagranin, is co-secreted with insulin from isolated rat islets and a transplantable insulinoma (Sopwith *et al.*, 1984).

Carboxypeptidase H is a 53 kDa metallo-enzyme involved in the removal of *C*-terminal basic amino acids from a number of secretory proteins, including intermediates in the processing of proinsulin (Docherty & Hutton, 1983). It is found in the soluble fraction of purified insulin secretory granules (Davidson & Hutton, 1987), and its properties resemble those of the carboxypeptidase isolated from bovine adrenal medulla, brain and pituitary involved in enkephalin-precursor processing (Fricker & Snyder, 1983).

Our main objectives in the present investigation were to document the phenomenon of co-ordinate biosynthesis of insulin-granule proteins in the islets of Langerhans

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and to perform a preliminary investigation into the nature of the regulatory signals derived from glucose.

EXPERIMENTAL

Materials

Analytical-grade biochemicals were obtained from Sigma Chemical Co. or BDH Chemicals (both of Poole, Dorset, U.K.), unless specified otherwise.

Immunoadsorbents. A mouse monoclonal antibody (3B7) raised to human proinsulin, which recognizes epitopes within the rat insulin structure (Davidson *et al.*, 1988), was partially purified by $(NH_4)_2SO_4$ precipitation and then coupled to CNBr-activated Sepharose (Pharmacia, Stockholm, Sweden) by the method of Axen *et al.* (1967). The resulting immunoadsorbent had a binding capacity of approx. 100 μg of insulin/ml of swollen gel.

Antiserum was raised in guinea pigs to betagranin purified from a transplantable rat insulinoma (Chick *et al.*, 1977) as previously described (Hutton *et al.*, 1988). An immunoglobulin fraction was prepared by Protein A–Sepharose affinity chromatography (Axen *et al.*, 1967), and coupled to CNBr-activated Sepharose, resulting in a substitution of 3.2 mg of IgG/ml of swollen gel.

Antiserum was raised in rabbits to carboxypeptidase H isolated from rat insulinoma (Davidson & Hutton, 1987), and this was processed in a manner identical with that for anti-betagranin antisera, to yield an immunoadsorbent with a substitution of approx. 2.6 mg of IgG/ml swollen gel.

Preparation of islets

Pancreatic islets of Langerhans were isolated from 7–12-week-old New England Deaconess Hospital rats by a modification of the method of Lacy & Kostianovsky (1967). Briefly, four to six rats were killed and the pancreata distended via the pancreatic duct with 8 ml of ice-cold modified Hanks saline [(Hanks & Wallace, 1949); 20 mM-Hepes (pH 7.4), 137 mM-NaCl, 5.4 mM-KCl, 1.67 mM-$MgSO_4$, 0.34 mM-Na_2HPO_4, 4.2 mM-$NaHCO_3$, 6.3 mM-$CaCl_2$] containing 2.5 mg of collagenase (type I; Sigma)/ml. The pancreata were excised and then individually shaken (100 strokes/min) in 2.5 cm × 9.0 cm polypropylene/polystyrene 'Universal' tubes (Sterilin, Feltham, Middx., U.K.) at 37 °C, for 20–30 min. The partially disaggregated tissue was suspended in 10 ml of ice-cold modified Hanks saline containing 0.1% bovine serum albumin, and drawn up and down six to ten times through a 6.5 cm 14-gauge hypodermic-syringe needle until a homogeneous suspension was obtained. This was centrifuged for 10 s at 600 rev./min in an MSE bench-top centrifuge ($r_{max.}$ = 12.5 cm), and the pelleted material was subjected to two further cycles of resuspension and centrifugation and then filtered through a 6.5 cm-diam. plastic tea strainer (0.5 mm mesh). Each filtrate was centrifuged for 10 s at 1200 rev./min (MSE bench-top centrifuge) and the pellets were resuspended in 5 ml of Histopaque-1119 (Sigma) in Universal tubes, overlayered with 8 ml of Histopaque-1077, followed by 5 ml of modified Hanks saline containing 0.1% albumin. The tubes were centrifuged for 25 min at 2500 rev./min (MSE bench-top centrifuge), and islets were recovered from the saline/Histopaque-1077 interface and washed twice in an incubation medium consisting of modified Krebs bicarbonate buffer (120 mM-NaCl, 5 mM-KCl, 1 mM-$MgSO_4$, 2.5 mM-$CaCl_2$, 24 mM-$NaHCO_3$), containing 20 mM-Hepes (pH 7.4), 0.1% albumin and 2.8 mM-glucose.

Radioisotopic labelling

Batches of 100–200 islets were preincubated in 100 μl of incubation media containing 2.8–16.7 mM-glucose and other additions, as indicated, for various time periods at 37 °C under O_2/CO_2 (19:1) in 1.5 ml-capacity sealed microcentrifuge tubes (Alpha, Eastleigh, Hants., U.K.). Islets were then recovered by centrifugation for 10 s at 800 *g* (MSE Microcentaur micro-centrifuge) and resuspended in 100 μl of the same pre-warmed (37 °C) media containing 150 μCi of [^{35}S]methionine (1400 Ci/mmol; SJ235; Amersham International, Amersham, Bucks, U.K.). The incubations were terminated after 20 min by addition of ice-cold incubation medium, containing 2 mM-methionine, followed by centrifugation for 10 s at 3300 *g*. The islets were sonicated for 15 s at 25 W (MSE Sonifier) in 200 μl of lysis buffer, consisting of 25 mM-$Na_2B_4O_7$ (pH 9.0), 3% (w/v) albumin, 1% Tween 20, 1 mM-phenylmethanesulphonyl fluoride, 0.1 mM-E-64 [*trans*-epoxysuccinyl-L-leucylamido-(4-guanidino)butane], 1 mM-EDTA, 20 μM-leupeptin and 0.1% NaN_3. The lysates were centrifuged for 5 min at 13000 *g* and the supernatants retained.

Trichloroacetic acid precipitation

Incorporation of [^{35}S]methionine into total protein was determined by mixing 10 μl of islet lysate with 1.5 ml of 1.5 M-H_2O_2/1 M-NaOH for 5 min at room temperature, followed by addition of 2 ml of ice-cold 25% (w/v) trichloroacetic acid and a further 10 min incubation at 4 °C. The precipitated material was collected on to 2.5 cm-diam. glass-fibre discs (GF/F; Whatman, Maidstone, Kent, U.K.), which were then washed with 5 ml of 10% trichloroacetic acid and air-dried. The filters were immersed in 4 ml of Optiphase HiSafe II scintillation cocktail (FSA Laboratory Supplies, Loughborough, Leics., U.K.) and the radioactivity was determined by liquid-scintillation counting.

Immunoprecipitation

Immunoprecipitation of the various proteins under study was performed sequentially in the order given below. Preliminary studies showed that in each case removal of the protein was quantitative and that the order in which the immunoprecipitations were performed was inconsequential.

Proinsulin. Islet lysates [(0.5–1.5) × 10^6 d.p.m. of trichloroacetic acid-precipitable material in 200 μl of lysis buffer] were incubated for 1 h at room temperature in 1.5 ml-capacity micro-centrifuge tubes with 50 μl of a 100 mg/ml suspension of Cowan-strain *Staphylococcus aureus* cells. The samples were then centrifuged for 5 min at 13000 *g*, and the supernatants were combined with 100 μl (packed gel) of 3B7 immunoadsorbent and mixed overnight at 4 °C. The immunoadsorbent was recovered by centrifugation for 10 s at 13000 *g*, and the supernatant was used for the immunoisolation of other cellular proteins (see below). The immunoadsorbent was then washed with 4 × 1 ml of lysis buffer, 2 × 1 ml of 50 mM-Tris/HCl (pH 7.5) containing 150 mM-NaCl, 1% Triton

X-100, 1% deoxycholate, 0.1% SDS and 5 mM-EDTA, and then with 2 × 1 ml of distilled water. The samples were then eluted with 2 × 1 ml of 25% (v/v) acetic acid, freeze-dried and reconstituted in 50 μl of 2.5 mM-Tris/HCl (pH 8.6) containing 8 M-urea and 0.001% Bromophenol Blue. Alkaline-urea/polyacrylamide-gel electrophoresis was performed by a modification of the method of Perrie & Perry (1970). Slab gels (15 cm × 15 cm × 0.15 cm) were polymerized from 7.5% (w/v) acrylamide and 0.2% *NN'*-methylenebisacrylamide containing 8 M-urea and 12.5 mM-Tris/80 mM-glycine (pH 8.6). Gels were pre-run in 12.5 mM-Tris/80 mM-glycine (pH 8.6) for 600 V·h, the upper tank buffer was replaced and the samples were loaded and electrophoresed for 1000 V·h. Gels were subsequently shaken for 2 × 5 min in acetic acid, and then for 2 h in 20% (w/v) 2,5-diphenyloxazole in acetic acid, rinsed for 30 min under cold running tap water, vacuum-dried and then exposed to Cronex 4 X-ray film (Dupont, Stevenage, Herts., U.K.) for 6–72 h at −70 °C. Incorporation of radioactivity into specific protein bands was determined by densitometric scanning (Chromoscan III; Joyce–Loebl, Gateshead, U.K.) of suitably exposed fluorographs.

Chromogranin A. The supernatant obtained after insulin immunoprecipitation was incubated overnight at 4 °C with 50 μl (packed gel) of anti-betagranin immunoadsorbent and washed as detailed above. Samples were eluted with 2 × 100 μl of 20 mM-HCl, and the eluates were freeze-dried and reconstituted in 50 μl of 125 mM-Tris/HCl (pH 6.8) containing 2% (w/v) SDS, 0.25 M-sucrose, 5 mM-EDTA, 65 mM-dithiothreitol and 0.005% Bromophenol Blue, then heated at 100 °C for 5 min. Electrophoresis was performed on slab gels (15 cm × 15 cm × 0.15 cm) polymerized from 15% (w/v) acrylamide and 0.08% *NN'*-methylenebisacrylamide, by using the discontinuous buffer system of Laemmli (1970). Fluorography was performed as described above for periods of 3–14 days. Molecular-size calibration was achieved with ^{14}C-labelled lysozyme, β-lactoglobulin, α-chymotrypsinogen, ovalbumin, bovine serum albumin, phosphorylase *b* and myosin heavy chain (BRL, Paisley, Scotland, U.K.).

Carboxypeptidase H. The supernatant obtained after chromogranin A immunoprecipitation was subjected to immunoprecipitation with anti-carboxypeptidase H immunoabsorbent, followed by SDS/polyacrylamide-gel electrophoresis and fluorography as detailed above.

Fluorographic calibration

Calibration strips were constructed for use as an internal fluorographic standard, by using ^{14}C-labelled *Escherichia coli* proteins. Overnight cultures of *E. coli* TG1 were maintained in minimal media containing 250 μCi of ^{14}C-labelled amino acids (50 μCi/ml; CFB.104: Amersham International)/ml. Cellular proteins were recovered by trichloroacetic acid precipitation, solubilized in 1 ml of 125 mM-Tris/HCl (pH 6.8) containing 2% (w/v) SDS, 0.25 M-sucrose and 5 mM-EDTA and mixed in varying proportions with 3 ml portions of 15% (w/v) acrylamide and 1% Acrylaide cross-linker (FMC Bioproducts; Rockland, ME, U.S.A.). The solutions were polymerized in 1 cm layers in a 15 cm × 15 cm × 0.15 cm slab gel. The gel was impregnated for fluorography as described above, and then dried and cut into 15 cm × 1 cm strips, each containing 13 sections ranging from 1.5 to 100 d.p.m./mm^2. Such calibration strips were exposed in conjunction with each fluorograph and subjected to densitometric scanning. This provided a means of internal standardization and cross-reference between differently exposed fluorographs of the same or different immunoprecipitations.

RESULTS

Incorporation of radioactivity into islet proteins

Islets incubated in the presence of 2.8 mM-glucose incorporated 0.18 ± 0.02% (mean ± S.E.M., $n = 6$) of the [^{35}S]methionine from the initial media into acid-precipitable material. Under these conditions, 1% of the incorporated radioactivity appeared in proinsulin, and approx. 0.01% was incorporated into chromogranin A

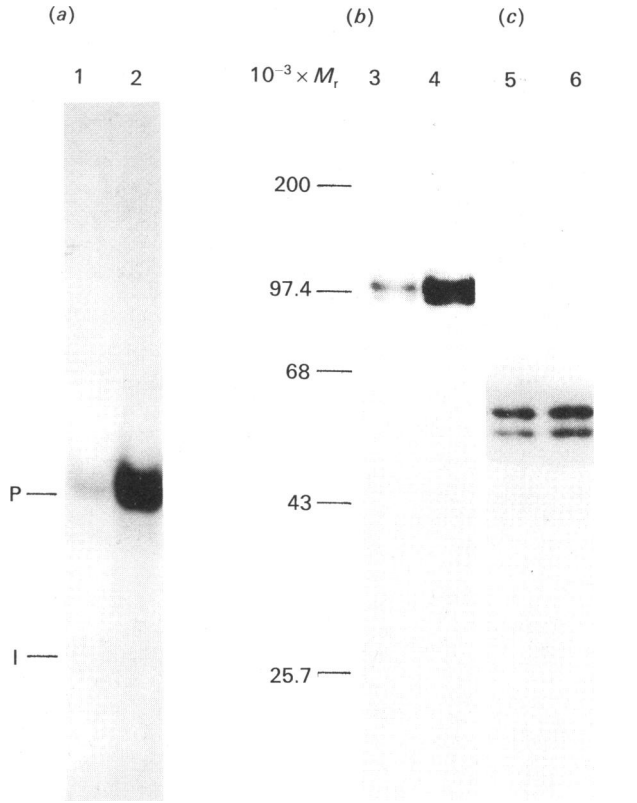

Fig. 1. Immunoprecipitation of proinsulin, chromogranin A and carboxypeptidase H

Islets were preincubated for 40 min and then labelled for 20 min with [^{35}S]methionine. The concentration of glucose in the medium was maintained throughout at either 2.8 mM-glucose (tracks 1, 3 and 5) or 16.7 mM-glucose (tracks 2, 4 and 6). Radioactivity associated with proinsulin (*a*; tracks 1 and 2), chromogranin A (*b*; tracks 3 and 4) and carboxypeptidase H (*c*; tracks 5 and 6) was immunoprecipitated and then subjected, in the case of proinsulin, to alkaline-urea/polyacrylamide-gel electrophoresis, and for chromogranin A and carboxypeptidase H to SDS/polyacrylamide-gel electrophoresis, followed by fluorography (see the Experimental section for further experimental details). The positions of migration of proinsulin (P), insulin (I) and molecular-size standards are indicated.

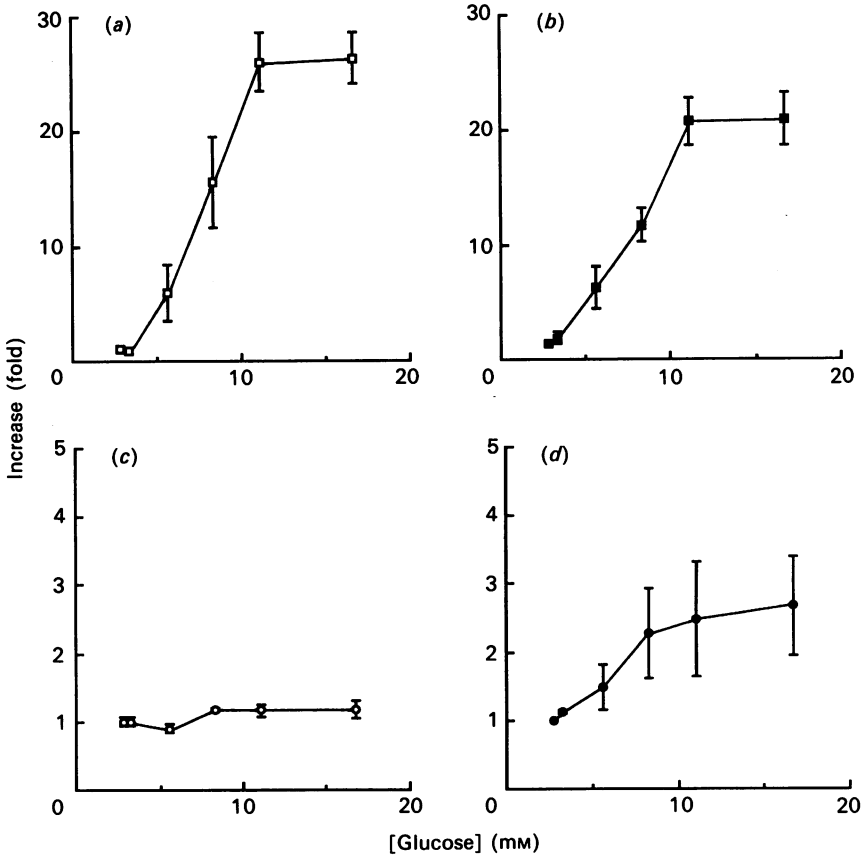

Fig. 2. Glucose-dependency of the biosynthesis of proinsulin, chromogranin A, carboxypeptidase H and total protein

Islets were preincubated for 40 min at the indicated glucose concentrations and then labelled for 20 min under the same conditions with [^{35}S]methionine. The radioactivity associated with proinsulin (*a*), chromogranin A (*b*) and carboxypeptidase H (*c*) was determined by immunoprecipitation, followed by polyacrylamide-gel electrophoresis and fluorography. Incorporation of radioactivity into total protein (*d*) was determined by liquid-scintillation counting of trichloroacetic acid precipitates. All results are means ± S.E.M. for three to six independent observations, and are expressed relative to the incorporation at 2.8 mM-glucose (proinsulin 50 d.p.m./islet, chromogranin A 0.5 d.p.m./islet, carboxypeptidase H 15 d.p.m./islet and total protein 5000 d.p.m./islet).

and 0.3% into carboxypeptidase H. The percentage incorporation of [^{35}S]methionine into proinsulin is lower than that with [4,5-^{3}H]leucine (Permutt & Kipnis, 1972; Ashcroft *et al.*, 1978), since only one of the two rat proinsulins (II) contains methionine, and then only a single residue (Smith, 1966; Clark & Steiner, 1969).

A 20 min radiolabelling period was routinely used in this investigation so that maximal incorporation of radioactivity could be achieved without the complicating factor of extensive post-translational proteolysis. Thus 3B7 antibodies principally immunoprecipitated proinsulin (Fig. 1*a*), betagranin antibodies a chromogranin A-like protein of approx. 100 kDa (Fig. 1*b*) and carboxypeptidase H antibodies a doublet of 53–56 kDa (Fig. 1*c*). The multiple forms of carboxypeptidase H are thought to arise from co- and post-translational glycosylation (H. W. Davidson, P. C. Guest & J. C. Hutton, unpublished work).

Regulation of [^{35}S]methionine incorporation into proinsulin

Islets incubated in the presence of 16.7 mM-glucose incorporated 0.57 ± 0.07% ($n = 6$) of the [^{35}S]methionine from the initial media into protein. This represented a 3-fold increase compared with the incorporation at 2.8 mM-glucose (Fig. 2*d*). Incorporation into proinsulin under these conditions was increased by more than 25-fold (Figs. 1*a* and 2*a*) and represented 9% of the total radioactivity incorporated into islet protein. Analysis of the glucose-dependency of proinsulin labelling revealed a threshold for stimulation of 3.3–5.6 mM-glucose, with half-maximal stimulation at approx. 8 mM-glucose and maximal response at 11.1–16.7 mM-glucose (Fig. 2*a*).

Preincubation of islets for periods of 0–100 min in the presence of 2.8 mM-glucose did not affect the incorporation of radioactivity into proinsulin over a subsequent 20 min labelling period (Fig. 3*a*). Also, no significant increase in incorporation was detected during the first 20 min after the glucose concentration in the medium was increased from 2.8 to 16.7 mM. After this time, however, the extent of incorporation increased markedly, to reach a new steady state at approx. 60 min (Fig. 3*a*).

In the presence of 20 mM-mannoheptulose and 16.7 mM-glucose, the extent of proinsulin labelling was decreased to 10.5 ± 3.6% ($n = 6$; $P < 0.001$, Student's t test for unpaired groups) of the value obtained with 16.7 mM-glucose alone (Table 1). Removal of extra-

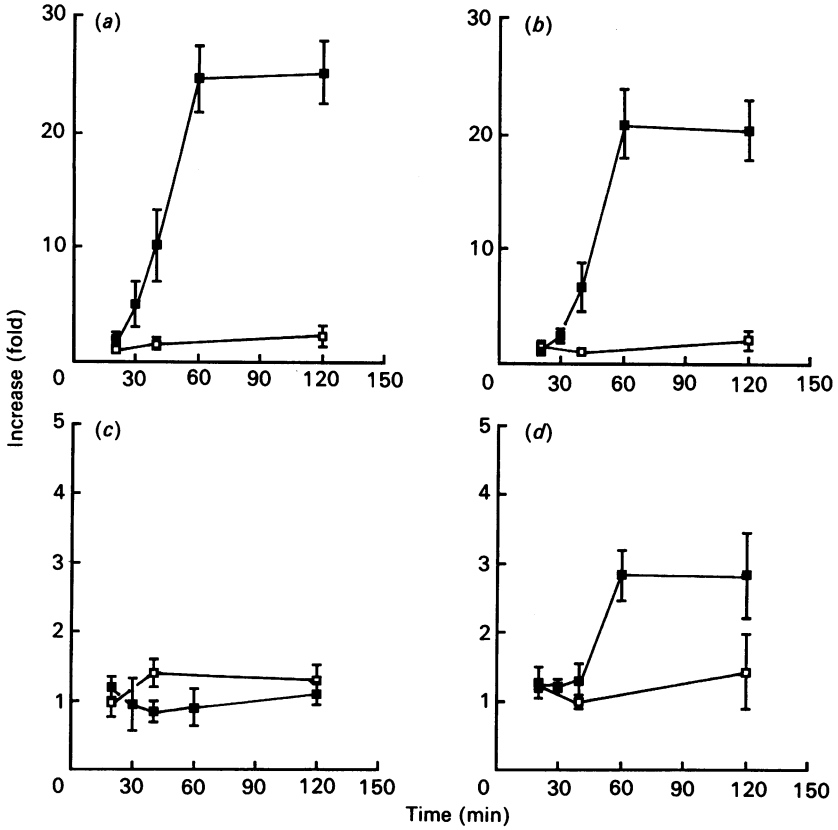

Fig. 3. Time-dependency for activation of biosynthesis of proinsulin, chromogranin A, carboxypeptidase H and total protein

Islets were preincubated for 0, 10, 20, 40 or 100 min at either 2.8 mM-glucose (□) or 16.7 mM-glucose (■) and then labelled for 20 min under the same conditions with [^{35}S]methionine. The radioactivity associated with proinsulin (*a*), chromogranin A (*b*) and carboxypeptidase H (*c*) was determined by immunoprecipitation, followed by polyacrylamide-gel electrophoresis and fluorography. Incorporation of radioactivity into total protein (*d*) was determined by liquid-scintillation counting of trichloroacetic acid precipitates. All results are means ± S.E.M. for three to six independent observations, and are expressed relative to the incorporation at 2.8 mM-glucose without preincubation (proinsulin 40 d.p.m./islet, chromogranin A 0.3 d.p.m./islet, carboxypeptidase H 10 d.p.m./islet and total protein 4000 d.p.m./islet).

Table 1. Effects of extracellular Ca^{2+} depletion and mannoheptulose on the biosynthesis of proinsulin, chromogranin A, carboxypeptidase H and total protein

Islets were preincubated for 40 min and then labelled for 20 min with [^{35}S]methionine. The medium was maintained throughout in the presence of 16.7 mM-glucose with the indicated modifications. The radioactivity associated with proinsulin, chromogranin A and carboxypeptidase H was determined by immunoprecipitation, followed by polyacrylamide-gel electrophoresis and fluorography. Incorporation of radioactivity into total protein was determined by liquid-scintillation counting of trichloroacetic acid precipitates. All results are expressed as means ± S.E.M. for six independent observations and are expressed relative to the incorporation at 2.8 mM-glucose (proinsulin 80 d.p.m./islet, chromogranin A 0.9 d.p.m./islet, carboxypeptidase H 25 d.p.m./islet and total protein 10000 d.p.m./islet).

	Incorporation (relative to 2.8 mM-glucose)			
Modification	Proinsulin	Chromogranin A	Carboxypeptidase H	Total protein
–	28.2±0.3	21.3±2.6	1.1±0.3	2.7±0.7
+EGTA (1 mM)	29.0±4.9	23.2±3.4	1.1±0.1	2.5±0.6
+Mannoheptulose (20 mM)	2.9±1.0	0.9±0.3	0.8±0.2	1.2±0.1

cellular Ca^{2+} at this same glucose concentration failed to affect proinsulin labelling (Table 1). Results quantitatively similar to these have been reported previously (Howell & Taylor, 1966; Lin & Haist, 1973; Pipeleers *et al.*, 1973; Ashcroft *et al.*, 1978).

Regulation of [^{35}S]methionine incorporation into chromogranin A

The incorporation of radioactivity into chromogranin A, like that into proinsulin, showed a sigmoidal dependence on glucose concentration, with a threshold above 3.3 mM-glucose, half-maximal stimulation at 8.5 mM-glucose and maximal effects at 11.1–16.7 mM-glucose (Fig. 2*b*). Chromogranin A labelling was increased more than 20-fold in the presence of 16.7 mM-glucose, compared with the incorporation at 2.8 mM-glucose (Figs. 1*b* and 2*b*), and represented approx. 0.1% of the total radioactivity incorporated into islets.

Preincubation of islets for 0–100 min at 2.8 mM-glucose did not affect chromogranin A labelling over the subsequent 20 min labelling period (Fig. 3*b*). At 16.7 mM-glucose, however, incorporation increased markedly after a 20 min lag, to reach a new steady state at approx. 60 min.

Mannoheptulose (20 mM) in combination with 16.7 mM-glucose decreased chromogranin A labelling to 4.6±2.1% ($n = 6$; $P < 0.001$, Student's t test for un-

paired groups) of the value obtained at 16.7 mM-glucose (Table 1), but no significant effect was observed after the removal of extracellular Ca^{2+} from the incubation media (Table 1).

The results for all of the above parameters, when expressed relative to the response at 2.8 mM-glucose, were virtually identical with those obtained for proinsulin.

Incorporation of [^{35}S]methionine into carboxypeptidase H

In contrast with the observations made for proinsulin and chromogranin A, there was no change in the overall labelling of carboxypeptidase H at glucose concentrations in the range 2.8–16.7 mM (Figs. 1*c* and 2*c*). As a consequence, labelling of this protein relative to total protein was decreased in response to increasing concentrations of glucose. At 16.7 mM-glucose, 0.1% of the radioactivity incorporated into islet protein appeared in carboxypeptidase H, representing a 3-fold decrease compared with the incorporation at 2.8 mM-glucose.

No change was observed in the incorporation of radioactivity into carboxypeptidase H when islets were preincubated for 0–100 min and subsequently labelled for 20 min, in the presence of either 2.8 mM- or 16.7 mM-glucose (Fig. 3*c*). No effect of mannoheptulose (20 mM) or extracellular Ca^{2+} depletion was observed on carboxypeptidase H labelling (Table 1).

Regulation of [^{35}S]methionine incorporation into total islet protein

The effect of glucose on the incorporation of [^{35}S]methionine into total islet protein resembled the incorporation into proinsulin and chromogranin A, showing a sigmoidal dependence on glucose concentration with a threshold above 3.3 mM-glucose, and half-maximal and maximal stimulation at 7.5 mM- and 11.1–16.7 mM-glucose respectively (Fig. 2*d*). Preincubation for 0–100 min in the presence of 2.8 mM-glucose did not affect the incorporation of radioactivity into islet protein during a subsequent 20 min labelling period (Fig. 3*d*). However, incorporation increased after 30 min in the presence of 16.7 mM-glucose, to reach the maximum at 60 min.

In the presence of 20 mM-mannoheptulose and 16.7 mM-glucose, the incorporation of radioactivity into islets was decreased to $43.3 \pm 5.9\%$ ($n = 6$; $P < 0.1$, Student's t test for unpaired groups) of the control value at 16.7 mM-glucose alone, whereas the removal of extracellular Ca^{2+} at the same glucose concentration had no effect (Table 1).

DISCUSSION

Previous studies of insulin-granule biogenesis at the molecular level have been confined principally to the regulation of insulin biosynthesis. Control of insulin biosynthesis by secretory stimuli, at the level of transcription and mRNA stability, has been documented over periods ranging from 24 h and longer *in vitro* (Brunstedt & Chan, 1982; Nielsen *et al.*, 1985; Welsh *et al.*, 1985). The magnitude of the response, however, is modest in comparison with other secretory tissues, representing at best a 3-fold increase in biosynthesis. In contrast, short-term regulation at the translational level can increase insulin biosynthesis up to 20-fold. Such regulation involves changes in the rate of initiation of mRNA translation (Morris & Korner, 1970; Permutt & Kipnis, 1972; Itoh *et al.*, 1978), release of signal-recognition-particle-mediated arrest (Welsh & Welsh, 1986) and stimulation of peptide-chain elongation rate (Welsh *et al.*, 1986).

Since the matrix components of the granule are presumably lost during exocytosis, the question is raised of how secretory-granule composition and function are maintained in the face of changing rates of secretion. We have addressed this question by considering the regulation of the biosynthesis of two insulin-granule matrix proteins on the same time scale over which the major effects on insulin biosynthesis are observed. One factor that should be considered in the interpretation of these data is that the proteins in question, chromogranin A and carboxypeptidase H, are also found in islet cells other than the β-cell (Hutton *et al.*, 1988; P. C. Guest, L. Orci & J. C. Hutton, unpublished work). However, since the β-cell is a major site of concentration of these proteins and it constitutes approx. 70% of the islet-cell mass, it is probable that the phenomena observed relate mainly to the function of the β-cell.

The incorporation of radioactivity into chromogranin A paralleled that of insulin under all conditions studied. The synthesis of both molecules was stimulated to a similar extent over a wide range of glucose concentrations, and both required at least 20 min before activation of biosynthesis. A similar lag has been reported previously for the synthesis of (pro)insulin (Permutt & Kipnis, 1972; Ashcroft *et al.*, 1978). This delay may reflect the activation of factors which couple the stimulatory signal to translational events in the cell, or the need for the synthesis of cofactors required for efficient translation (Jarret *et al.*, 1967; Track *et al.*, 1968).

As with proinsulin, the metabolism of glucose was necessary for the generation of the stimulatory signal for chromogranin A biosynthesis, as indicated by the inhibitory effect of mannoheptulose, which blocks the conversion of glucose into glucose 6-phosphate in islets (Ashcroft & Randle, 1970). Such inhibition was not secondary to the inhibition of glucose-stimulated secretion of insulin (Ashcroft & Randle, 1970), since removal of Ca^{2+} from the incubation medium, which blocks glucose-induced secretion (Lin & Haist, 1973; Pipeleers *et al.*, 1973), failed to affect the biosynthesis of either proinsulin or chromogranin A.

In contrast with these findings, the incorporation of radioactivity into another insulin-granule matrix protein, carboxypeptidase H, was unaffected by glucose under short-term conditions. In this case the metabolism of glucose by the cell appeared to be inconsequential, since mannoheptulose had no effect on carboxypeptidase H synthesis.

One implication of the present results is that the composition of the secretory granule must change depending on the intensity and duration of stimulation, since the synthesis of some proteins is unaffected and that of others is increased by the same stimulus. It follows, for example, that prolonged stimulation may result in a relative decrease in the secretory-granule content of carboxypeptidase H. It is unlikely in this instance, however, that this would have any effect on prohormone processing, since the activity in the

endocrine pancreas exceeds by several orders of magnitude that required to account for the rates of conversion in the intact cell (J. C. Hutton, unpublished work). It may be argued that, since this enzyme is required for the processing of other islet proteins, which share the secretory pathway at least as far as the trans-Golgi, it would be inappropriate to couple its biosynthesis to that of proinsulin. On the other hand, the synthesis of proteins such as chromogranin A may need to be tightly coupled to that of insulin, since their activities may be expressed extracellularly through derived peptides.

These and previous studies (Grimaldi *et al.*, 1987) indicate that there is a subset of insulin-secretory-granule proteins whose synthesis is stimulated to a similar extent by glucose. This includes both granule matrix and membrane constituents. The synthesis of another subset, represented by carboxypeptidase H, is not regulated by glucose. Whether this applies to non-granule proteins was not directly examined in the present study. However, the observation that the trichloroacetic acid-precipitable radioactivity was increased 3-fold, and yet only 10% of this increase was attributable to insulin-granule constituents, indicates that the synthesis of many other islet proteins may be controlled by a similar mechanism.

The intriguing question which applies to this glucose-stimulated protein subset is how is such control mediated at the molecular level of the translational machinery and what, if any, structural features are common among the RNAs encoding them?

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