Biosynthetic Regulation of Endogenous Hamster Insulin and Exogenous Rat Insulin II in Transfected HIT Cells

GERALD GOLD, MICHAEL D. WALKER, DARRELL L. EDWARDS, AND GEROLD M. GRODSKY

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/11/1509/355582/37-11-1509.pdf by guest on 15 April 2024

To investigate mechanisms underlying biosynthetic regulation of an insulin gene, the rat insulin II gene was introduced into hamster β-cells (HIT) by cotransfection with the neomycin phosphotransferase–selectable marker. The insulin gene fragment was 2.2 kilobases (kb) in length and contained all exons, introns, and ~700 base pairs (bp) of 5′-flanking DNA and 300 bp of 3′-flanking DNA. The HIT cell was known to have endogenous hamster insulin production under regulation by glucose and dexamethasone. In a pool of stably transfected cells (HIT M62pR2), rat insulin II and hamster insulin were produced at comparable rates. Glucose (20 mM) stimulated cellular [^{3}H]leucine labeling of both hamster insulin and rat insulin II by approximately twofold. Addition of 10^{-6} M dexamethasone to media containing 11.1 mM glucose inhibited biosynthesis of both hamster insulin and rat insulin II by >90%. Thus, with both positive and negative biosynthetic regulation, changes in the cellular labeling of exogenous rat insulin II were qualitatively and quantitatively similar to those of the endogenous hamster insulin. These data suggest that the 2.2-kb rat insulin II gene fragment contained sufficient information for both expression and apparently "normal" biosynthetic regulation of exogenous rat insulin II (when compared with endogenous hamster insulin) in response to glucose and dexamethasone. *Diabetes* 37:1509–14, 1988

HIT cells are a line of simian virus 40–transformed Syrian hamster β-cells (1) that respond to several regulators of insulin release and, unlike other lines of transformed rodent β-cells, retain their ability to regulate rates of insulin secretion in response to physiologic concentrations of glucose (1–3). In HIT cells, glucose also increases levels of preproinsulin mRNA (4), overall rates of proinsulin biosynthesis, and cellular conversion of proinsulin to insulin (5).

HIT cells can take up and efficiently express added DNA; this property has been exploited to define the *cis*-elements located in the 5′-flanking sequences of the human and rat insulin I genes that are required for cell-specific transcription (6–8). However, these studies focused exclusively on the role of 5′-flanking sequences and none investigated the roles of other sequences in the gene nor the effects of biosynthetic regulators.

Glucose is a regulator that has both transcriptional and posttranscriptional effects on insulin biosynthesis (9). To approach the molecular basis for both transcriptional and posttranscriptional regulation, we inserted the intact rat insulin II gene, containing ~700 base pairs (bp) of 5′-flanking and 300 bp of 3′-flanking DNA, into HIT cells, which already possess a glucose-responsive mechanism for synthesizing, processing, and secreting insulin. The resulting pool of stably transfected cells was first screened for expression of rat insulin II and then tested for simultaneous biosynthetic regulation of both endogenous and exogenous insulins by glucose and dexamethasone. Biosynthetic rates of endogenous and exogenous insulins were equally responsive to these two regulators, suggesting that the 2.2 kilobase (kb) transfected rat insulin II gene fragment contains sufficient information for "normal" biosynthetic regulation in HIT cells.

MATERIALS AND METHODS

Construction of plasmid. The plasmid p.*rIns*II.RSVneo was constructed by inserting a 2.2-kb *Bam*HI fragment from pRCII (10) into the unique *Bam*HI site of pRSVneo (Fig. 1).

Transfection and selection of cell lines. HIT T15 (C1) cells were obtained from Dr. W. J. Rutter (University of California, San Francisco). They were cultured in Dulbecco's modified Eagle's medium containing 15% horse serum, 2.5% fetal calf serum, and penicillin (100 U/ml)/streptomycin (100 μmg/ml). Transfections were performed with the calcium phosphate method (11). Briefly, DNA-calcium precipitates

From the Department of Biochemistry, Lilly Research Laboratories, Indianapolis, Indiana; the Metabolic Research Unit, University of California, San Francisco, California; and the Department of Biochemistry, Weizmann Institute, Rehovot, Israel.

Address correspondence and reprint requests to Dr. Gerald Gold, Biochemistry 88/4, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285.

Received for publication 14 January 1988 and accepted in revised form 2 June 1988.

were applied to cultures of HIT cells seeded 24 h previously at a density of 10^6 cells/10-cm dish. Four hours after addition of DNA, cells were exposed to a 20% glycerol shock for 2 min. Seventy-two hours later, cells were trypsinized and seeded into a new 10-cm dish at a 10-fold lower density (12). After 24 h, G-418 (Gibco, Chagrin Falls, OH) was added [final concn of active drug 0.25 mg/ml (10)]. Medium was subsequently changed every 3 days until colonies of cells became visible. Pools of transformed cells containing many colonies were trypsinized, seeded directly into a T-25 flask (Falcon, Oxnard, CA), and, until plated for an experiment, maintained continuously in RPMI-1640 medium containing 25 mM HEPES, 10% fetal calf serum, penicillin (100 U/ml)/streptomycin (100 μmg/ml), and 0.25 mg/ml active G-418. Sera, media, and penicillin/streptomycin were obtained from the Cell Culture Facility at the University of California, San Francisco.

Culturing and labeling of cells. T-25 tissue-culture flasks were inoculated with 0.3–0.5 × 10^6 HIT cells for labeling experiments. These cells were subsequently maintained in media without G-418. After 3 days of growth, incubation medium was aspirated from cells and replaced with fresh medium containing either specified concentrations of glucose [achieved by supplementing custom glucose-free RPMI-1640 medium with sterile glucose (50% wt/vol) and substituting dialyzed for regular fetal bovine serum] or 10^{-6} M dexamethasone (added from a 10^{-3} M ethanolic stock to medium containing 11.1 mM glucose). Labeling was done for the final 1- or 24-h experimental period by removing incubation medium and replacing it with 6 ml of fresh RPMI-1640 medium free of carrier leucine and supplemented with 63 μCi/ml (final) [^{3}H]leucine (Amersham, Arlington Heights, IL).

Immunopurification of total proinsulins and insulins. After labeling medium was discarded, the cell layer was washed with 5.0 ml of ice-cold Dulbecco's phosphate-buffered saline and then extracted for 14–16 h with 5.28 ml of ice-cold acid-ethanol [67 ml ethanol + 31 ml water + 1.4 ml concentrated hydrochloric acid + 3 mg bovine serum albumin (fraction V, Sigma, St. Louis, MO)] (13,14). All subsequent procedures were done at 4°C. Extracts were mixed with 50 μl 2 M ammonium acetate adjusted to pH 8.3 with ammonium hydroxide and, after 30 min, centrifuged 10 min at 1000 × *g*. Supernatants were decanted, readjusted to pH 5.3 with hydrochloric acid, and then mixed with 10 ml ethanol plus 20 ml ether. Three hours later, tubes were centrifuged for 10 min at 1000 × *g*; supernatants were discarded, and air-dried pellets were dissolved in 1.0 ml of buffered detergent [1% (wt/vol) sodium deoxycholate (Sigma) + 1% (wt/vol) Triton X-100 (Sigma) + 0.15 M sodium chloride + 50 μM leucine + 10 mM sodium phosphate, pH 7.4] (15). Insolubles were removed by centrifugation for 5 min in an Eppendorf centrifuge, and supernatants were transferred to tubes containing preformed antibody precipitates, which had been made previously by adding 4 μl of guinea pig anti-porcine insulin serum (Linco, Eureka, MO) and 100 μl of goat anti-guinea pig serum (heavy- and light-chain specific; Cooper, Malvern, PA) to 336 μl of buffered detergent. Precipitates were formed by incubating both antibodies together for 2 h at 37°C then an additional ≥4 h at 4°C. HIT cell samples were subsequently added and incubated for an additional 14–16 h at 4°C. HIT cell immune precipitates were layered over 100 μl 1 M sucrose plus buffered detergent in 1.5-ml Eppendorf tubes and centrifuged 5 min. Supernatants were aspirated and discarded, and pellets were washed three times with a total of 2.1 ml of buffered detergent by vortex mixing and centrifugation. Washed pellets were dissolved in 0.25 ml 1 M acetic acid by triturating and vortex mixing; samples were centrifuged 30 min later. Immunoprecipitated radioactivity was determined by liquid-scintillation counting (Packard Tri-Carb, Downers Grove, IL) after mixing 25 μl of supernatant with 5.0 ml Hydrofluor (National Diagnostic, Manville NJ); the remainder of each sample was frozen.

In 1-h–labeling studies, immune precipitates contained only combined proinsulins (data not shown). For these experiments, purification was stopped at this point because we lacked a method to separate hamster proinsulin from rat proinsulin II. In 24-h–labeling studies, immune precipitates contained both proinsulins and insulins. For the latter experiments, hamster insulin and rat insulin II were separated from one another with high-performance liquid chromatography (HPLC).

Separation of hamster and rat insulin II. Frozen immunopurified samples were thawed and then centrifuged for 5 min in an Eppendorf centrifuge; 150 μl of supernatant was removed for reverse-phase HPLC. The reverse-phase column (PEP-RP1, C-8), controller, spectrophotometer, and pumps were the 8800 series (Du Pont, Wilmington, DE). A linear acetonitrile-water gradient from 32 to 36% acetonitrile was run over 40 min; temperature was maintained between 23 and 24°C, and both acetonitrile and water contained 0.1% (vol/vol) trifluoroacetic acid plus 0.1% (vol/vol) morpholine. Radioactivity in [^{3}H]leucine-labeled hamster insulin and rat insulin II was determined in 1-ml fractions of column effluent

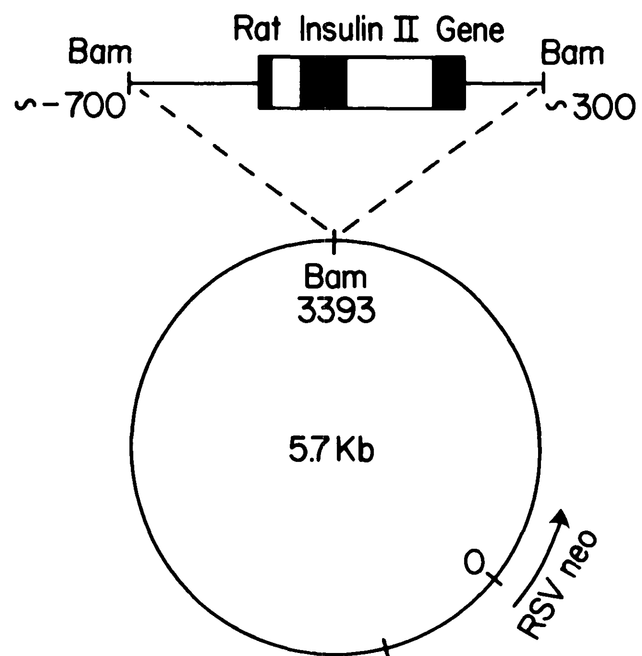

FIG. 1. Schematic of p.*rIns*II.RSVneo. Plasmid was contructed by inserting 2.2-kilobase *Bam*HI fragment from pRCII into unique *Bam*HI sites of pRSVneo.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/11/1509/355582/37-11-1509.pdf by guest on 15 April 2024

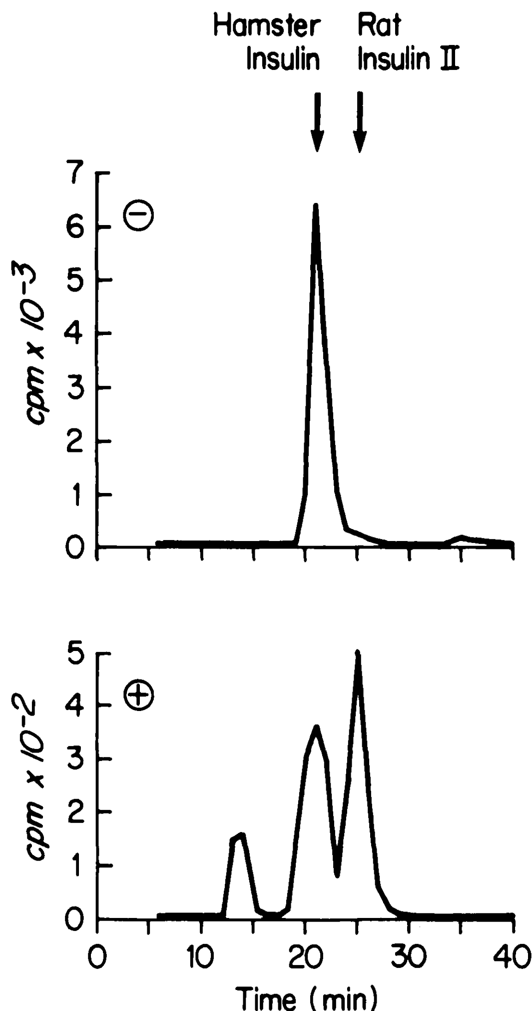

FIG. 2. Separation of hamster insulin and rat insulin II by high-performance liquid chromatography. Insulins were separated by reverse-phase chromatography with 32–36% acetonitrile-water gradient and PEP-RP1 C-8 column. Elution positions of authentic hamster insulin and rat insulin II were determined with extracts of hamster pancreas and [^{35}S]methionine-labeled rat islets, respectively. *top*, HIT m484 R2; *bottom*, HIT m62 pR2.

by liquid-scintillation counting in 5 ml Hydrofluor. Biosynthetic human insulin (2.5 μg; a gift from Lilly, Indianapolis, IN) was routinely added with each sample as an optical density standard; peaks of optical density for hamster or rat insulin II could not be detected on the scale of labeling experiments. With select samples, a standard of authentic Syrian hamster insulin was also added. This sample was prepared from frozen Syrian hamster pancreas (Pel-Freez, Rogers, AZ) by extraction and immunopurification as previously described (13,14). Elution positions of both human insulin and hamster insulin were determined by optical density at 215 nm. Elution position of rat insulin II was determined with an immunopurified standard prepared from rat islets incubated with [^{35}S]methionine, which labels rat insulin II but not rat insulin I.

RESULTS

In parental cells or negative clones of transfected cells (e.g., HIT M484R2), there was a single peak of labeled protein that eluted in the position of authentic Syrian hamster pancreatic insulin (Fig. 2). In positive cells (e.g., HIT M62pR2), there were two additional peaks of radioactivity (Fig. 2). One peak eluted at 25 min and was cochromatographic with authentic rat insulin II. There was no evidence for a subpopulation of pooled cells that synthesize atypical or incorrectly processed rat insulin II; however, there was in most, but not all, preparations an unidentified second additional peak, which eluted at 13–14 min. This unidentified peak had a molecular weight of 6000 on Bio-Gel P-30–size gel chromatography (same as insulin) and was not converted to authentic rat insulin II by carboxypeptidase B treatment (data not shown). This same protein peak was also found after isolation of insulins from [^{3}H]leucine-labeled normal rat islets, was probably produced during isolation rather than during biosynthesis, and may represent an oxidation of the B-29 methionine residue of rat insulin II.

The effects of glucose concentration (48 h) on [^{3}H]leucine labeling of immunopurified cellular proinsulins (1-h pulse) and on combined cellular proinsulins plus insulins (24-h pulse) are shown in Fig. 3. With a 1-h pulse and glucose concentrations in culture from 0 to 20 mM, biosynthetic labeling of combined immunopurified hamster proinsulin and rat proinsulin II increased progressively with increasing glucose concentration. Half-maximal effect occurred at ~1 mM glucose, and maximal fivefold stimulation (20 vs. 0 mM glucose) occurred between 10 and 20 mM glucose. Little if any immunoprecipitable radioactivity was secreted during this 1-h labeling period (5), so immunoprecipitated labeled cellular proteins represent total proinsulin biosynthesis.

Stimulatory effects of glucose on biosynthetic labeling of combined cellular proinsulins plus insulins were less clear-cut with a 24-h pulse-labeling experiment; cellular labeling

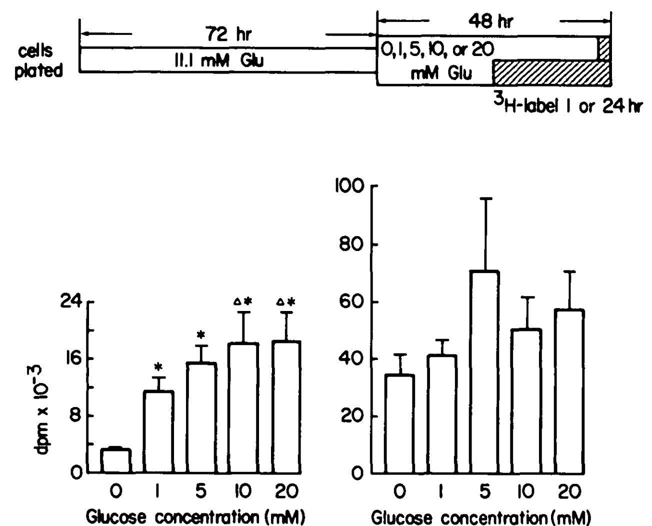

FIG. 3. Effects of glucose (48 h) on biosynthetic labeling of total immunoreactive proinsulins (1-h pulse) and immunoreactive proinsulins plus insulins (24-h pulse). HIT M62pR2 cells were labeled with [^{3}H]leucine (47th–8th or 24th–48th h), and at end of pulse (48th h), cellular hormones were purified by series of chemical procedures ending with precipitation with guinea pig anti-porcine insulin. Numbers represent trichloroacetic acid–precipitable radioactivity for 4 1-h–labeling experiments (*left*) and 3 24-h–labeling experiments (*right*) done on separate days. **P* < .01 vs. 0 mM glucose; Δ*P* < .05 vs. 1 mM glucose.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/11/1509/355582/37-11-1509.pdf by guest on 15 April 2024

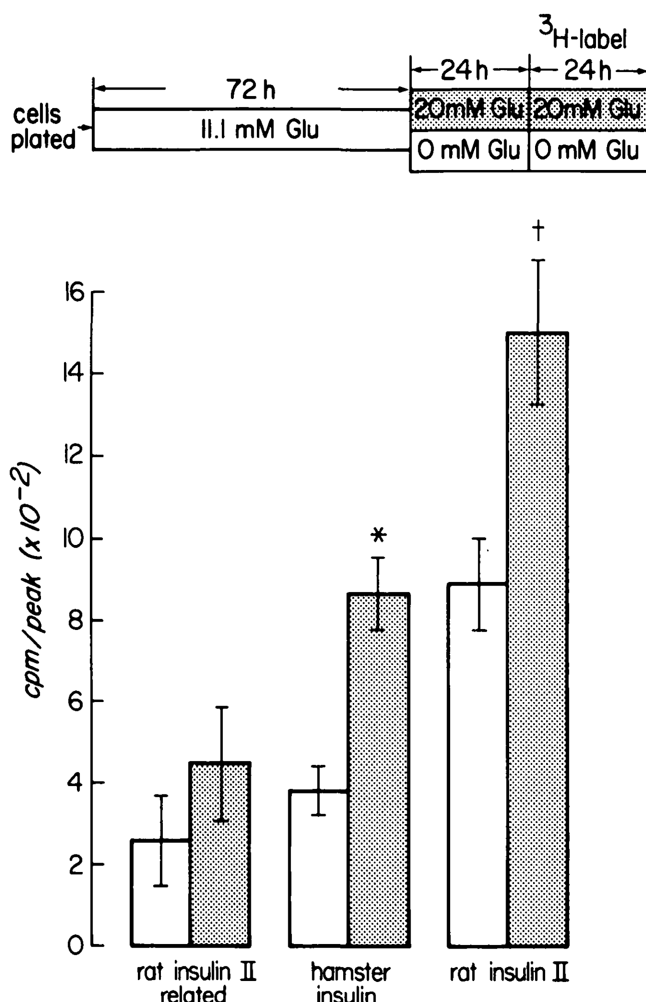

FIG. 4. Effects of glucose (48 h) on biosynthesis of cellular hamster insulin and rat insulin II (24-h pulse). HIT M62pR2 cells were labeled for 24 h (24th to 48th h) with [^{3}H]leucine, and at end of pulse (48th h), cellular hormones were purified by series of chemical procedures including precipitation with guinea pig anti-porcine insulin. Hamster insulin and rat insulin II were separated by reverse-phase high-performance liquid chromatography with acetonitrile-water gradient and C-8 column. *Bars* represent means ± SE for 5 independent experiments. *$P < .005$; †$P < .025$.

of proinsulins plus insulins approximately doubled (not statistically significant) with increasing glucose concentrations. Although 1- and 24-h–labeling experiments were done under identical conditions, cells labeled continuously for 24 h contained far less than 24 times as much immunoprecipitated radioactivity as cells labeled for 1 h; glucose-stimulated cells had only a 2.8- to 4.6-fold increment with 24- vs. 1-h–pulse labeling. Note that cellular immunoreactive radioactivity represents only a fraction of labeled precursor and hormone that remained stored in the cell; because extracellular proteolysis of hormones occurs in HIT cell cultures, secreted hormones were excluded from analysis. These cellular data suggest that glucose-stimulated HIT cells have a relatively rapid turnover of cellular hormone, which is consistent with known effects of glucose on both insulin biosynthesis and secretion (1–3).

Effects of glucose concentration on cellular labeling of hamster insulin and rat insulin II after 24 h were determined after reverse-phase HPLC (Fig. 4). As seen for total immunoprecipitated hormone in Fig. 3, labeling of both endogenous hamster insulin and exogenous rat insulin II (and rat insulin II–related protein) was approximately doubled with 20 vs. 0 mM glucose.

Effects of a pharmacologic concentration of dexamethasone on biosynthesis of individual insulins were determined with glucose-stimulated (11.1 mM) cells labeled for 24 h (Fig. 5). We previously reported that 10^{-6} M dexamethasone severely inhibited glucose-stimulated endogenous proinsulin biosynthesis in unmodified HIT cells (5). With transfected HIT M62pR2 cells, glucose-stimulated labeling of hamster insulin and rat insulin II (and rat insulin II–related protein) was also strongly inhibited (>90%) by 10^{-6} M dexamethasone, and percent inhibition for exogenous rat insulin II was not significantly different from that for endogenous hamster insulin. Thus, with dexamethasone, there was coordinate negative biosynthetic regulation of both endogenous cellular hamster insulin and exogenous rat insulin II in HIT M62pR2 cells.

DISCUSSION

Biosynthetic regulation in the established HIT cell line has many points of similarity with regulation in normal pancreatic β-cells (16–18). In unmodified HIT cells, Hammonds et al. (4) demonstrated specific glucose stimulation of preproinsulin mRNA within 4 h, and we have shown that glucose stimulated, and dexamethasone inhibited, proinsulin synthesis between 4 and 48 h (5). For the latter experiments, bio-

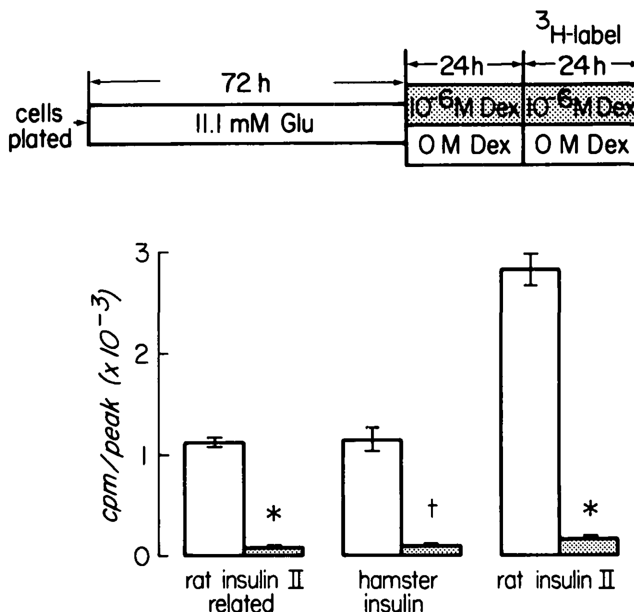

FIG. 5. Effects of 10^{-6} M dexamethasone (48 h) on biosynthesis of cellular hamster insulin and rat insulin II (24-h pulse). HIT M62pR2 cells were labeled for 24 h (24th to 48th h) with [^{3}H]leucine, and at end of pulse (48th h), cellular hormones were purified by series of chemical procedures including precipitation with guinea pig anti-porcine insulin. Hamster insulin and rat insulin II were separated by reverse-phase high-performance liquid chromatography with acetonitrile-water gradient and C-8 column. *Bars* represent means ± SE for 6 independent experiments. *$P < .001$; †$P < .005$.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/11/1509/355582/37-11-1509.pdf by guest on 15 April 2024

synthetic changes did not simply represent a global effect; both glucose and dexamethasone regulated biosynthesis of proinsulin to a significantly greater extent than that of total cellular protein (5).

With transfected HIT M62pR2 cells, 10 and 20 mM glucose stimulated biosynthesis ([^{3}H]leucine labeling for 1 h) of immunopurified combined proinsulins approximately fivefold above levels observed with 0 mM glucose. Individual species of proinsulins were not analyzed in these experiments because the reverse-phase HPLC system described here was not developed to separate hamster and rat proinsulins. Separate determination of hamster insulin and rat insulin II biosynthesis required experiments with sufficient time for cellular conversion of radioactive precursors to mature cellular insulins. Consequently, biosynthetic effects of glucose were tested with a 24-h period of labeling.

Total immunoprecipitable radioactivity (combined cellular proinsulins, processing intermediates, and insulins) showed a less distinct (only twofold) biosynthetic stimulation of glucose, probably due to ongoing glucose-stimulated secretion of labeled cellular hormones through this period (1–3). Nonetheless, the twofold biosynthetic stimulation (20 vs. 0 mM glucose) of total immunoprecipitable radioactivity was mirrored in a twofold stimulation of cellular labeling of both hamster insulin and rat insulin II.

Translational and processing regulation (including sequestration of peptide inside the lumen of endoplasmic reticulum, transport to the Golgi apparatus and then to secretory granules, and final conversion to insulin) of rat insulin II also appeared to occur both correctly (without evidence of aberrant or misprocessed forms) and at the same rate as that of endogenous hamster insulin. In rat islets, mature rat insulin I has been reported to appear more rapidly than rat insulin II (19).

The effects of physiologic concentrations of glucocorticoids in vitro are controversial (20–23). Pharmacologic concentrations of glucocorticoids clearly inhibit insulin secretion and biosynthesis in both isolated islets (20) and HIT cells (1,5). In HIT M62pR2 cells, 10^{-6} M dexamethasone inhibited biosynthetic labeling of both endogenous hamster insulin and exogenous rat insulin II to a similar extent. Glucocorticoids, via receptor complexes, can bind specific tRNA species and thereby impede protein translation; they can also specifically alter rates of gene transcription (24). Thus, either a transcriptional or a posttranscriptional mechanism could be responsible for regulation of these cells. The human or rat insulin genes do not contain the glucocorticoid consensus sequence for positive stimulation (25).

Because biosynthetic regulation of exogenous rat insulin II by glucose and dexamethasone was essentially the same as that of endogenous hamster insulin, we conclude that the genetic information necessary for quantitative mediation of these regulatory effects is contained on the 2.2-kb transfected rat insulin II gene fragment. It has recently been shown that transgenic mice containing a 12.3-kb fragment of the human insulin gene also secreted both immunoreactive insulin and human C-peptide normally in response to glucose (26). We emphasize that our observations do not distinguish whether regulatory sites, which are specific for glucose or glucocorticoid action on the insulin genes, are affected or if these genes are susceptible to less specific glucose- and glucocorticoid-regulatory sites, which may affect the entire protein-synthetic machinery of the cell. However, we also emphasize that all information to date indicates that on introduction of sufficient genetic information into an appropriate recipient cell, regulated production and secretion of a foreign insulin can be achieved.

ACKNOWLEDGMENTS

We thank Dr. Harry Nick for plasmid pRCII and Dr. Bruce Howard for pRSVneo.

This work was supported in part by National Institutes of Health Grant DK-01410 to G.M.G. and a Career Development Award to M.D.W. from the Juvenile Diabetes Foundation.

REFERENCES

1. Santerre RF, Cook RA, Crisel RMD, Sharp JD, Schmidt RJ, Williams DC, Wilson CP: Insulin synthesis in a clonal line of simian virus 40-transformed hamster pancreatic beta cells. *Proc Natl Acad Sci USA* 78:4339–43, 1981
2. Hill RS, Boyd AE III: Perifusion of a clonal cell line of simian virus 40-transformed beta cells. 1. Insulin secretory dynamics in response to glucose, 3-isobutyl-1-methylxanthine and potassium. *Diabetes* 34:115–20, 1985
3. Ashcroft SJH, Hammonds P, Harrison DE: Insulin secretory responses of a clonal cell line of simian virus 40-transformed B cells. *Diabetologia* 29:727–33, 1986
4. Hammonds P, Schofield PN, Ashcroft SJH: Glucose regulates preproinsulin messenger RNA levels in a clonal cell line of simian virus 40-transformed B cells. *FEBS Lett* 213:149–54, 1987
5. Gold G, Qian R-L, Grodsky GM: Insulin biosynthesis in HIT cells: effects of glucose, forskolin, IBMX, and dexamethasone. *Diabetes* 37:160–65, 1988
6. Walker MD, Edlund T, Boulet AM, Rutter WJ: Cell-specific expression controlled by the 5′-flanking region of insulin and chymotrypsin genes. *Nature* (Lond) 306:557–61, 1983
7. Walker MD, Edlund T, Boulet AM, Rutter WJ: DNA elements controlling cell specific expression of insulin and chymotrypsin genes. In *Molecular Biology of Development*. Davidson EH, Firtel RA, Eds. New York, Liss, 1984, p. 481–90
8. Edlund T, Walker MD, Barr PJ, Rutter WJ: Cell-specific expression of the rat insulin gene: evidence for role of two distinct 5′-flanking elements. *Science* 230:912–16, 1985
9. Welsh M, Scherberg N, Gilmore R, Steiner DF: Translational control of insulin biosynthesis: Evidence for regulation of elongation, initiation and signal-recognition-particle-mediated translation arrest by glucose. *Biochem J* 235:459–67, 1985
10. Cate RL, Chick W, Gilbert W: Comparison of the methylation patterns of the two rat insulin genes. *J Biol Chem* 258:6645–52, 1983
11. Graham FL, Van der Eb AJ: A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 52:456–67, 1973
12. Southern PJ, Berg P: Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. *J Mol Appl Genet* 1:327–41, 1982
13. Davoren PR: The isolation of insulin from a single cat pancreas. *Biochim Biophys Acta* 63:150–53, 1962
14. 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
15. Lomedico PT, Saunders GF: Preparation of pancreatic mRNA: cell-free translation of an insulin-immunoreactive polypeptide. *Nucleic Acids Res* 3:381–91, 1976
16. Steiner DF, Kemmler W, Clark JL, Oyer PE, Rubenstein AH: The biosynthesis of insulin. In *Handbook of Physiology. Endocrinology*. Vol I. Steiner DF, Freinkel N, Eds. Baltimore, MD, Waverly, 1972, p. 175–98
17. Gold G, Grodsky GM: The secretory process of B cells of the pancreas. In *Cell Biology of the Secretory Process*. Cantin M, Ed. Montreal, Canada, Karger, 1984, p. 359–88
18. Steiner DF, Chan SJ, Welsh JM, Kwok SC: Structure and evolution of the insulin gene. *Annu Rev Genet* 19:463–84, 1985
19. Gishizky ML, Grodsky GM: Differential kinetics of rat insulin I and II processing in rat islets of Langerhans. *FEBS Lett* 223:227–31, 1987
20. Brunstedt J, Nielsen JH: Direct long-term effect of hydrocortisone on

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/11/1509/355582/37-11-1509.pdf by guest on 15 April 2024

insulin and glucagon release from mouse pancreatic islets in tissue culture. *Acta Endocrinol* 96:498–504, 1981
21. Billaudel B, Mathias PCF, Sutter BCJ, Malaisse WJ: Inhibition by corticosterone on calcium inflow and insulin release in rat pancreatic islets. *J Endocrinol* 100:227–33, 1984
22. Khatin MS, Mahmood HA, Gumaa KA, Hellerstrom C: Effects of steroid hormones on DNA synthesis and insulin production of isolated foetal rat pancreatic islets in tissue culture. *Med Biol* (Helsinki) 62:210–13, 1984
23. Pierluissi J, Navas FO, Ashcroft SJH: Effect of adrenal steroids on insulin release from cultured rat islets of Langerhans. *Diabetologia* 29:119–21, 1986
24. Ali M, Vedeckis WV: The glucocorticoid receptor protein binds to transfer RNA. *Science* 235:467–70, 1987
25. Yamamoto KR: Steroid receptor regulated transcription of specific genes and gene networks. *Annu Rev Genet* 19:209–52, 1985
26. Selden RF, Skoskiewicz MJ, Howie KB, Russell PS, Goodman HM: Regulation of human insulin gene expression in transgenic mice. *Nature* (Lond) 321:525–28, 1986

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/11/1509/355582/37-11-1509.pdf by guest on 15 April 2024