Volume 223, number 2, 227–231 FEB 05236 November 1987

Differential kinetics of rat insulin I and II processing in rat islets of Langerhans

Mikhail L. Gishizky and Gerold M. Grodsky

Metabolic Research Unit, University of California, San Francisco, CA 94143, USA

Received 3 September 1987

Synthesis and processing of radiolabelled rat insulin I and II were studied by pulse-labelling freshly isolated rat islets with [^{3}H]leucine and chasing in 2 mM glucose for up to 270 min (which minimized insulin secretion, $<1\%$/h). Islet samples were taken during the chase period and analyzed for their rat insulin I and II content by high-performance liquid chromatography. Prior to 60 min chase rat insulin I accounted for $>85\%$ of the radiolabelled insulin present. With longer periods of chase, the relative percentage of rat insulin II progressively increased so that by completion of proinsulin to insulin processing the two labelled rat insulins were present in the same proportion as the relative immunoreactive content, approx. 60:40% insulin I/ insulin II. Thus, although islets synthesize the two insulins in proportion to their relative immunoreactive content, rat insulin I and II are processed with different kinetics.

Insulin; Enzyme processing; Enzyme synthesis; HPLC; (Islets of Langerhans, Rat)

1. INTRODUCTION

The importance of protein structure in regulating processing is supported by the observed differences in the rates of maturation of various secretory proteins in tumor [1,2] and normal cells [3]. Processing of secretory proteins involves multiple events including intracellular transport and enzymatic modification of the proteins. Each of these steps can in turn perform a regulatory function in the maturation process.

Rat islets of Langerhans contain two insulins (I,II) [4,5] that are products of non-allelic genes [6,7]. Translation of the two insulin mRNAs results in the synthesis of two preproinsulins differing by 7 amino acids. Processing of these peptides involves removal of the pre region and formation of proinsulins differing in 4 of 86 amino acids. The proinsulins are then transported from the ER, through the Golgi and sequestered into secretory granules [8]. Within the granules the proinsulins are cleaved to mature insulins I and II [9] which have identical A chains but differ by 2 amino acids in the B chain (positions 9,29 [7]).

Although freshly isolated islets from normal rats contain the two insulins in approx. 60% insulin I, 40% insulin II proportions [5,10], alterations in this distribution have been reported [11–13]. Furthermore, controversy exists as to whether glucose (the primary physiologic regulator of insulin synthesis and secretion) can differentially regulate the levels of the two hormones [5,11,13]. Thus rat islets of Langerhans offer a unique opportunity to investigate regulation of synthesis and processing of two highly homologous proteins in normal tissue. Experiments were designed to investigate: (i) the effect of acute glucose stimulation on the translational synthesis of the two insulins and (ii)

Correspondence address: G.M. Grodsky, Metabolic Research Unit, 1141-HSW, University of California, San Francisco, CA 94143, USA

Abbreviations: ER, endoplasmic reticulum; HPLC, high-performance liquid chromatography; IRI, insulin immunoreactivity

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

the temporal aspects of individual rat insulins I and II processing.

In this report we demonstrate that in freshly isolated islets from normal rats: (i) acute glucose exposure stimulates the synthesis of both insulins in proportion to their immunoreactive content and (ii) differences in the amino acid structure of the two proinsulins appear to confer a selectivity in the processing mechanism resulting in differential rates of rat insulin I and II maturation.

2. MATERIALS AND METHODS

Islets of Langerhans from adult male rats (Long Evans) were isolated by 3 sequential digestions of pancreas with collagenase followed by Percoll gradients as described [14]. All islet incubations were performed at 37°C in Krebs-Ringer bicarbonate buffer (KRB) containing 10 mM Hepes, pH 7.4, 0.1% bovine serum albumin and either 2 or 20 mM glucose. Groups of 40–150 islets were preincubated for 45 min in KRB containing 20 mM glucose (to stimulate translational synthesis of proinsulin [15,16]) then pulsed for 15–20 min with 0.25 mCi L-[4,5-^{3}H]leucine, also in 20 mM glucose KRB. Following the pulse, secretion of newly synthesized insulin was minimized by washing islets with 2 mM glucose KRB containing 0.2 mM cold leucine and incubating in this media for up to 275 min. During culture in 2 mM glucose KRB, islets secreted less than 1% of their total insulin content per hour. As part of another investigation (to be described elsewhere), a 40 min glucose stimulation was introduced at 150 min. Therefore, for this study, data used to calculate the rat insulin I and II percentages (at 190 and 290 min) were a result of combining islet and glucose-stimulated media measurements.

Collected samples were immediately frozen in microcentrifuge tubes then lyophilized over a 4-h period. As described elsewhere (in preparation), following lyophilization, samples were redissolved and extracted in 1 M acetic acid then twice frozen, thawed, sonicated and centrifuged for 5 min in an Eppendorf microfuge. 50–100 μl aliquots of the supernatant were subjected to gradient elution reverse-phase HPLC using a Dupont PEP-R C-8 column with a 32–35% acetonitrile/water gradient over 35 min and a flow rate of 1 ml/min. Both mobile phases (water and acetonitrile) contained 0.1% trifluoroacetic acid and 0.1% morpholine. Absorbance, radioactivity and insulin immunoreactivity [17] were measured in 1 min fractions (fig.1). In addition, 100 μl aliquots from 20 min and 290 min chase time samples were affinity purified [14] and total insulin related radioactivity (insulin + proinsulin) determined.

3. RESULTS

Fig.1 is a schematic HPLC elution profile demonstrating the separation of the two rat insulins from [^{3}H]leucine labelled islets. Rat insulin I and II are resolved as two major peaks of absor-

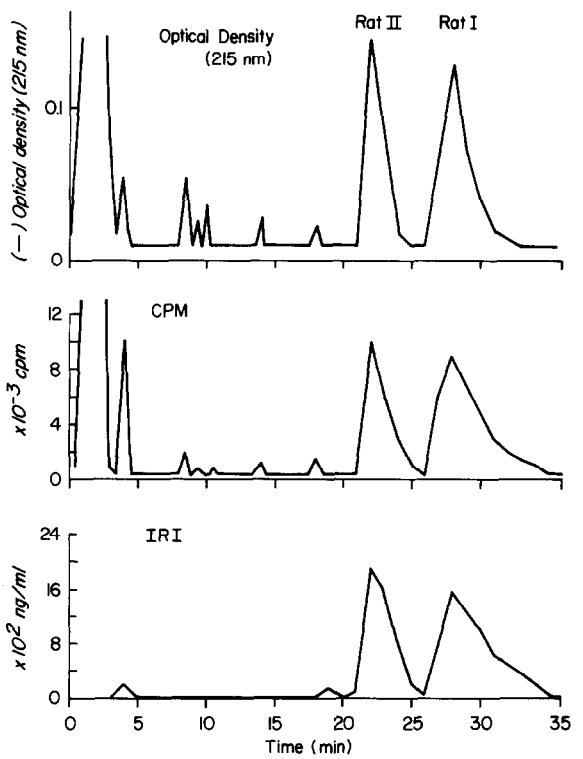

Fig.1. Schematic representation of rat insulin I and II separation in pulse-chased islets using reverse-phase HPLC with a 32–35% acetonitrile gradient over 35 min. The top panel depicts the absorbance elution recording at a wavelength of 215 nm. The middle panel represents the radioactivity elution profile and the bottom panel is the corresponding IRI measurements for each fraction. Under these conditions rat insulin II elutes first at 21–25 min followed by a broader rat insulin I peak at 27–34 min.

bance (at 215 nm) corresponding to major peaks of both radioactivity and IRI. The identity of each insulin peak was determined (in preparation) by using islets labelled with [^{35}S]methionine (only rat insulin II contains methionine in its amino acid structure [7]; this label appeared solely in the peak eluting at 21–25 min). The purity of each peak was confirmed using polyacrylamide gel electrophoresis fluorography [18]. This analysis revealed that the fractions within each peak contained a single radioactive band with a molecular mass identical to that of purified insulin standards. In all samples analyzed, rat insulin I and II peaks accounted for greater than 83% of the total IRI in each original sample. Under these conditions, using HPLC purified labelled insulin I and II standards, recovery of radioactivity for both insulins was greater than 95% while the recovered IRI ranged between 78 and 93%.

Under the incubation conditions illustrated in fig.2 (which we previously reported to result in a net proinsulin to insulin conversion half-life of 50 min [14]) the relative appearance of labelled rat insulin I and II occurs with different kinetics. At early chase times when initial conversion of labelled proinsulin to insulin begins, rat insulin I is the predominant radioactive insulin; prior to the 60 min chase time it accounts for greater than 85% of the radioactive insulin present. At later chase times, with continued proinsulin to insulin conversion, the relative proportion of rat insulin II increases so that by 150 min chase, when insulin maturation nears completion, the intra-islet radioactive insulin distribution approaches the IRI distribution we find in freshly isolated untreated islets (insulin I = 58.4%, insulin II = 41.6%, SE ± 2.8%). Furthermore, similar amounts of total radioactive IRI (insulins + proinsulins; after correction for loss due to C peptide excision) were recovered at the beginning and end of these experiments (3.3×10^{-3} cpm at 20 min, 3.5×10^{-3} cpm at 290 min) indicating that the proinsulins must have been originally synthesized in a 60:40% I/II proportion. Thus, differential biosynthesis or degradation of rat insulin I or II cannot explain the observed differences in the disproportionate amounts of the two insulins at early chase times.

4. DISCUSSION

The stimulatory effect of glucose on total insulin synthesis has long been recognized, however, controversy exists as to its relative effect on regulating

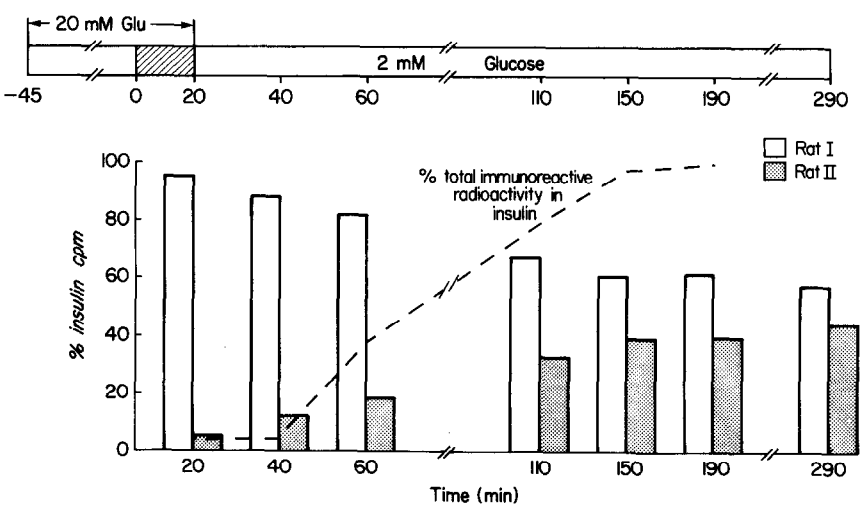

Fig.2. Effect of time on percent contribution of labelled rat insulin I and II (bars) to the total labelled insulin. The bar graph depicts the percent of rat insulin I and II at each time. The dashed line represents the total percent of newly synthesized insulin appearance over time. Percentage is calculated and normalized to the amount of radioactive IRI at the 20 min (0%) and 290 min (100%) chase times. Similar amounts of radioactive IRI were present at both these time points (after correction for the loss of radioactivity due to C peptide excision at 290 min).

rat insulin I and II biosynthesis. Previously investigators, using electrophoresis to separate the two insulins, reported that incubating freshly isolated islets in 16.0 mM glucose for 4 h resulted in an 8-fold greater synthesis of rat insulin I than II [11,12]. Subsequently, others observed that in islets cultured overnight, glucose stimulates the synthesis of both insulins to the same extent [13]. In this study we report that acute exposure (45 min preincubation) of freshly isolated islets to 20 mM glucose (which at such short times is known to act primarily at the translational level), stimulates net biosynthesis of the two rat insulins in approximately a 60% insulin I to 40% insulin II manner. This rat insulin I/II distribution is the same as the IRI content of untreated freshly isolated islets ([5,10] and personal observation) and is consistent with the previously reported in vivo effect of glucose on insulin I and II mRNA levels [19].

We also observed that rat insulin I and II maturation occurs with different kinetics. These data indicate that events involved in processing of the two insulins are differentially affected by differences in their amino acid structure.

In hepatoma cells [1,2] and hepatocytes [3], the regulatory step in determining the maturation rate of each protein appears to be its rate of transport from the ER. Recently the presence of a specific 3 amino acid sequence has been demonstrated to confer retention of a protein within the ER [20]. These studies have supported the contention that protein transport from the ER to the Golgi apparatus is a highly regulated process and occurs via a yet uncharacterized receptor(s) recognition mediated mechanism [21].

Proteolysis is another potential site of regulation. Converting enzymes are present in many cell types and may represent a ubiquitous set of proteins [22]. Transfection of the insulin gene into non-insulin producing secretory tumor cells has demonstrated that non B-cells are capable of synthesizing and converting proinsulin to insulin [23]. However, the observation that a single amino acid substitution at position 10 within the B chain (His to Asp), which is not at the proinsulin cleavage point, results in a hyperproinsulinemic state in vivo [24] suggests that enzymes responsible for proinsulin to insulin conversion in normal B-cells (pro cathepsin B? [25,26]) exhibit selectivity toward particular proinsulin conformations.

In summary, our data support the theory that, in islets, acute glucose exposure stimulates the synthesis of the two insulins to the same extent, in proportion to their basal immunoreactive content and reported level of mRNA. Furthermore, amino acid substitutions within the proinsulins result in different kinetics of rat insulin I and II maturation. The mechanisms responsible for these differences and their regulation remain to be determined.

ACKNOWLEDGEMENTS

M.L.G. is the recipient of a grant from the Achievement Rewards for College Scientists Foundation, Inc. (Northern California Chapter). This work was supported by the National Institutes of Health (grant DK01410). We are indebted to Dr Gerald Gold for discussions and assistance throughout this study and thank Terri Arnold for her contribution in preparing this manuscript.

REFERENCES

[1] Lodish, H.F., Kong, N., Snider, M. and Strous, G.J.A.M. (1983) Nature 304, 80–83.
[2] Ledford, B.E. and Davis, D.F. (1983) J. Biol. Chem. 258, 3304–3308.
[3] Fries, E., Gustafsson, L. and Peterson, P.A. (1984) EMBO J. 3, 147–152.
[4] Smith, L.F. (1966) Am. J. Med. 40, 449–455.
[5] Clark, J.L. and Steiner, D.F. (1969) Proc. Natl. Acad. Sci. USA 62, 278–285.
[6] Cordell, B., Bell, G., Tischer, E., De Noto, F.M., Ulrich, A., Pictet, R., Rutter, W.J. and Goodman, H.M. (1979) Cell 18, 533–543.
[7] Lomedico, P., Rosenthal, N., Efstratiadis, A., Gilbert, N., Kolodner, R. and Tizard, R. (1979) Cell 18, 545–558.
[8] Orci, L. (1985) Diabetologia 28, 528–546.
[9] Orci, L., Ravazzola, M., Amherdt, M., Madsen, O., Perrelet, A., Vassalli, J.-D. and Anderson, R.G.W. (1986) J. Cell Biol. 103, 2273–2279.
[10] Rall, L.B., Pictet, R.L. and Rutter, W.J. (1979) Endocrinology 105, 835–841.
[11] Kakita, K., Giddings, S. and Permutt, M.A. (1982) Proc. Natl. Acad. Sci. USA 79, 2803–2807.
[12] Kakita, K., O'Connell, K. and Permutt, M.A. (1982) Diabetes 31, 841–845.
[13] Rhodes, C.J., Lucas, C.A. and Halban, P.A. (1987) FEBS Lett. 215, 179–182.
[14] Gold, G., Landahl, H.D., Gishizky, M.L. and Grodsky, G.M. (1982) J. Clin. Invest. 69, 554–563.

[15] Itoh, N. and Okamoto, H. (1980) Nature 283, 100–102.
[16] Nagamatsu, S., Bolaffi, J.L. and Grodsky, G.M. (1987) Endocrinology 120, 1225–1231.
[17] Lundquist, I., Fanska, R. and Grodsky, G.M. (1976) Endocrinology 99, 1304–1312.
[18] Skinner, M. and Grizwald, M.D. (1983) J. Biochemistry 209, 281–284.
[19] Giddings, S., Swyers, J. and Carnaghi, L. (1986) Diabetes 35, suppl.1, 44A (abstr.).
[20] Munro, S. and Pelham, H.R.B. (1987) Cell 48, 899–907.
[21] Kelly, R.B. (1985) Science 230, 25–32.
[22] Steiner, D.F., Docherty, K. and Carroll, R. (1984) J. Cell. Biochem. 24, 121–130.
[23] Moore, H.-P., Walter, M.D., Lee, F. and Kelly, R.B. (1983) Cell 35, 531–538.
[24] Chan, S.J., Seino, S., Gruppuso, P.A., Schwartz, R. and Steiner, D.F. (1987) Proc. Natl. Acad. Sci. USA 84, 2194–2197.
[25] Docherty, K., Carroll, R.J. and Steiner, D.F. (1982) Proc. Natl. Acad. Sci. USA 79, 4613–4617.
[26] Docherty, K., Hutton, J.C. and Steiner, D.F. (1984) J. Biol. Chem. 259, 6041–6044.