Hepatic and Renal Protein Synthesis in Normal, Diabetic and Ketotic Chinese Hamsters

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Summary. Two aspects of protein synthesis were studied *in vitro* in tissue extracts prepared from normal, diabetic and ketotic Chinese hamsters. Firstly, the activity to support polyuridylic acid (poly U)-dependent polyphenylalanine synthesis from ^{14}C -phenylalanine-tRNA was measured in the liver ribosomes and no significant difference was observed between diabetic hamsters (0.310 ± 0.007 nCi/min/mg ribosomal proteins) and their controls (0.325 ± 0.015), or between ketotic hamsters (0.256 ± 0.013) and their controls (0.274 ± 0.018). Secondly, activity in protein synthesis with endogenous mRNA's was followed in the postmitochondrial supernatant (PMS) fraction after the removal of endogenous amino acid pools by gel filtration. The rate of amino acid incorporation was measured in both liver and kidney extracts with ^{14}C -leucine, lysine, serine, or glycine as labels. The initial rates of incorporations in the diabetic liver PMS ($\text{Leu} = 8.01 \pm 1.64$ pmole/min/mg protein; $\text{Lys} = 2.90 \pm 0.56$; $\text{Ser} = 3.40 \pm 0.60$; $\text{Gly} = 3.34 \pm 0.66$),

and in the ketotic PMS ($\text{Leu} = 8.48 \pm 2.06$; $\text{Lys} = 3.49 \pm 0.82$; $\text{Ser} = 4.11 \pm 0.80$; $\text{Gly} = 3.73 \pm 0.81$) were similar to those in the matched controls ($\text{Leu} = 8.60 \pm 1.06$; $\text{Lys} = 3.14 \pm 0.37$; $\text{Ser} = 3.70 \pm 0.54$; $\text{Gly} = 3.38 \pm 0.37$). Likewise, the amino acid incorporations in the diabetic kidney PMS ($\text{Leu} = 4.38 \pm 1.09$; $\text{Lys} = 1.26 \pm 0.28$; $\text{Ser} = 1.98 \pm 0.29$; $\text{Gly} = 1.81 \pm 0.32$), and in the ketotic kidney PMS ($\text{Leu} = 5.39 \pm 1.29$; $\text{Lys} = 1.79 \pm 0.4$; $\text{Ser} = 1.9 \pm 0.18$; $\text{Gly} = 1.97 \pm 0.15$) were not significantly different from those in the normals ($\text{Leu} = 5.32 \pm 0.56$; $\text{Lys} = 1.60 \pm 0.19$; $\text{Ser} = 2.06 \pm 0.22$; $\text{Gly} = 2.02 \pm 0.18$). It is concluded that the diabetic and ketotic Chinese hamsters retain normal activity in the reported two aspects of protein synthesis.

Key words: Spontaneous diabetes, streptozotocin, ribosomes, hepatic and renal protein synthesis Chinese hamsters, postmitochondrial supernatant, polyuridylic acid-dependent polyphenylalanine synthesis

Several groups of investigators have reported a decrease in the capacity of microsomal preparations from the livers of chemically or insulin antibody-induced diabetic rats [1–6]. In these acutely insulin-deficient animals, the hepatocytes had low polysome content [2, 3] associated with disorganization and loss of rough endoplasmic reticulum [5]. Using crude hepatic postmitochondrial supernatant (PMS) fractions, Pain demonstrated a direct inhibitory effect of acute insulin deficiency at the level of peptide chain initiation [6]. The hepatic supernatant protein content and the levels of several hepatic enzymes were found to be in excess in the Chinese hamsters with long term diabetes [7]. Severe renal ultrastructural changes, particularly in thickening of the capillary basement membranes, were also reported in the diabetic Chinese hamsters [8]. Therefore, it is of importance to study the protein synthesis apparatus in both liver and kidney of the Chinese hamsters. This report summarizes our studies on the capacity of hamster liver and kidney PMS preparations to incorporate amino acids and of liver ribosomes to support poly U-directed polyphenylalanine synthesis.

Materials and Methods

Animals

The animals ranged in age from 7 to 18 months and were selected from the Upjohn colony. The "ketotic" hamsters showed positive results on Ketostix® and the "diabetic" hamsters were negative

to Ketostix. Both groups showed Testape® values of 4+ within two months after birth and consistently thereafter. All these animals were matched with nondiabetic animals of the same age and sex.

Preparation of Ribosomes

The animals were killed by decapitation; the livers were quickly excised and placed immediately in cold *Medium A* (0.25M sucrose, 0.1M KCl, 0.005M Mg-acetate, 0.006M mercaptoethanol and 0.05M Tris-Cl, pH 7.5). The livers were minced and homogenized in 2 volumes of *Medium A* in a glass homogenizer fitted with a Teflon-pestle. After centrifugation at $6,000 \times g$ for 10 min, the supernatant was decanted and further centrifuged at $20,000 \times g$ for 10 min. To each ml of supernatant collected, 0.15 ml of 10% sodium deoxycholate was added. After standing in ice for 1 hr with occasional stirring, the solution was centrifuged at $105,000 \times g$ for 2 hrs. The ribosomal pellets were suspended and homogenized in a small volume of buffer containing 0.35M sucrose, 0.07M KCl, 0.004M MgCl_2 , 0.006M mercaptoethanol and 0.05M Tris-Cl, pH 7.8. Protein was determined according to Lowry *et al.* [9] and adjusted to a concentration of 3.0 mg/ml.

Preparation of Rat Liver "pH 5 Enzyme"

Freshly excised livers from Upj = TUC (SD) spf rats were homogenized in 2.3 volumes of cold *Medium B* (0.35M sucrose, 0.035M KHCO_3 , 0.004M MgCl_2 and 0.025M KCl). The homogenate was centrifuged at $15,000 \times g$ for 10 min and the supernatant was

diluted with 3 volumes of cold *Medium C* (0.90M sucrose, 0.004M $MgCl_2$ and 0.025M KCl). The sample was centrifuged at $100,000 \times g$ for 70 min and the supernatant was diluted further with equal volume of *Medium C* and slowly adjusted to pH 5.0 by adding dropwise cold 1.0M acetic acid. The mixture was stirred for 1 h at 4°C. The precipitates collected were washed once with *Medium C* and dissolved in *Medium B*. The preparation was frozen in small aliquots.

Preparation of Postmitochondrial Supernatant (PMS)

Freshly excised livers or kidney cortices from the Chinese hamsters were homogenized in 2 volumes of *Medium A* and centrifuged at $10,000 \times g$ for 10 min. One ml of supernatant was filtered through a Sephadex G25 column (0.9 x 30 cm) equilibrated with *Medium A*. The materials collected in the excluded volume were designated as the PMS fraction and assayed immediately for activity to incorporate amino acids into proteins. Protein content in PMS was determined according to Lowry *et al.* [9].

Protein Synthesis Assay

The preincubation medium for poly U-dependent polyphenylalanine synthesis contained 0.2 ml of *reaction mixture A* (0.39M sucrose, 0.12M Tris-Cl, pH 7.5, 0.012M $MgCl_2$, 0.17M KCl, 2.4mM ATP, 0.24mM GTP, 0.0146M mercaptoethanol, 2.4mM phosphoenolpyruvate and 20 μg pyruvate kinase), 0.1 ml ribosome suspensions and 0.15 ml rat liver "pH 5 enzyme". After preincubation at 37°C for 30 min, 0.1 ml of *reaction mixture B* (4.5mM ATP, 0.45mM GTP, 4.5mM $MgCl_2$, 4.5mM phosphoenolpyruvate, 10 μg pyruvate kinase, 90 μg poly U, 7 μg ^{14}C -phenylalanine-tRNA, 1.6 $\mu Ci/mg$) was added to the sample. The reaction was allowed to proceed at 37°C and terminated by transferring 0.1 ml aliquots to a test tube containing 0.1 ml of 10 mg/ml bovine serum albumin and immediately followed by adding 2.0 ml cold 10% TCA containing 2mM unlabelled phenylalanine.

The postmitochondrial supernatant fractions prepared from liver and kidney cortex were assayed for amino acid incorporating activity with ^{14}C -labelled glycine, leucine, lysine or serine. The reaction mixture was made of 0.1 ml of PMS fraction, 0.11 ml of 0.12 mM ^{14}C -labelled amino acid in *Medium D* (0.1M KCl, 0.005M Mg-acetate, 0.006M mercaptoethanol, 0.05M Tris-Cl, pH 7.5), 0.11 ml of 0.12 mM 19 other amino acids in *Medium D* and 0.11 ml of *Medium D* containing 4 mM ATP, 0.8 mM GTP, 20 mM phosphoenolpyruvate and 100 μg pyruvate kinase. The reaction was allowed to proceed at 37°C and terminated as mentioned above with 10% TCA containing 2 mM unlabelled specific amino acid.

The precipitates collected were suspended in 0.5 ml of 0.5M glycine-NaOH, pH 10.4 and incubated at 37°C for 2 h. Two ml of cold 10% TCA containing

2mM unlabelled specific amino acid was added to the mixture. The precipitates were further washed twice with 2 ml 10% TCA, dissolved in 1.0 ml NCS Tissue Solubilizer® (Amersham/Searle) and counted in 15 ml Bray's solution [10]. Quenching was corrected by channel ratio method [11].

Results

Poly U-Dependent Polyphenylalanine Synthesis

The liver ribosomes used in this study were isolated in the presence of sodium deoxycholate and the ribosomes were further preincubated for 30 min in order to ensure that they were devoid of any residual endogenous mRNA activities. The diabetic and ketotic animals chosen for this experiment had shown consistent 4+ (Testape®) glucosuria for at least 8 months and their fasting blood sugar levels on the day of the experiment ranged from 160 to 600 mg%. The rate of poly U-dependent polyphenylalanine synthesis in the liver ribosomes was assayed with ^{14}C -phenylalanine-tRNA as reactants and the TCA-precipitable products were extensively washed and treated with alkali to break down the reactants. In each experiment, a time course was run and the linear portion was used to calculate the initial rate of incorporation. In a study with six pairs of Chinese hamsters, the rate of ^{14}C -phenylalanine incorporation in the liver ribosomes of the diabetics was not significantly different from their matched controls (Table 1). Similarly, the liver ribosomes of ketotic Chinese hamsters and of their controls yielded similar results in poly U-dependent polyphenylalanine synthesis (Table 2).

Rate of Amino Acid Incorporations in the Postmitochondrial Supernatant (PMS) Fractions

PMS preparations from livers and kidneys of hamsters, devoid of amino acid pools by means of gel filtration, were assayed for the rate of incorporation of ^{14}C -leucine, ^{14}C -lysine, ^{14}C -serine and ^{14}C -glycine into proteins. In both liver and kidney PMS fractions, leucine was incorporated at the highest rate and the other three amino acids at similar rates (Table 3). The liver PMS showed higher protein synthetic activity than the kidney fraction and the liver/kidney ratios were highest for lysine (2.07 ± 0.11) and lowest for leucine (1.67 ± 0.08). In the study with five sets of matched Chinese hamsters, the hepatic and renal protein synthetic activities, measured in terms of the rate of incorporation of leucine, lysine, serine or glycine in the PMS fractions, were found to be similar in diabetic, ketotic and control animals (Table 3).

Discussion

The purpose of this study is to determine if the diabetic and ketotic Chinese hamsters are defective

in hepatic and renal protein synthesis whereof two aspects were examined: the ability of hepatic ribosomes to support poly U-dependent polyphenylalanine synthesis and the activity of hepatic and renal PMS to incorporate amino acids into proteins.

The experimental results obtained in this study on the rate of transfer of phenylalanine from phenylalanine-tRNA into acid-insoluble products indicated

in amino acid incorporation [12] and its ability to carry out peptide chain initiations [6]. ^{14}C -labelled leucine, lysine and serine were selected because they represent different types of amino acids with individual biosynthetic pathways. Glycine was also included since it is present in an exceptionally large proportion in renal glomerular basement membrane which rates special attention due to its involvement in diabetic

Table 1. *Poly U-dependent polyphenylalanine synthesis by diabetic and control Chinese hamster liver ribosomes*

Type	No. animals	Age	Sex	Fasting Blood Glucose	Rate of ^{14}C -phenylalanine Incorporation ^a
		months		mg% $\pm$ S.E.M. ^b	nCi/min/mg ribosomal proteins $\pm$ S.E.M. ^b
Control	6	10–18	4F2M	88.5 $\pm$ 7.8	0.325 $\pm$ 0.015
Diabetic	6	10–18	4F2M	224 $\pm$ 25.4	0.310 $\pm$ 0.007

^a Calculated from the linear portion of a time course carried out as described in "Materials and Methods" at 0, 1, 2, 3 min.

^b Standard error of the mean.

Table 2. *Poly U-dependent polyphenylalanine synthesis by ketotic and control Chinese hamster liver ribosomes*

Type	No. animals	Age	Sex	Fasting Blood Glucose	Rate of ^{14}C -phenylalanine Incorporation ^a
		months		mg% $\pm$ S.E.M. ^b	nCi/min/mg ribosomal proteins $\pm$ S.E.M. ^b
Control	6	10–17	4F2M	89.6 $\pm$ 2.5	0.274 $\pm$ 0.018
Ketotic	6	10–17	4F2M	362 $\pm$ 51.8	0.256 $\pm$ 0.013

^a Calculated from the linear portion of a time course carried out as described in "Materials and Methods" at 0, 2, 5, 10 min.

^b Standard error of the mean.

Table 3. *Rate of amino acid incorporations in liver and kidney postmitochondrial supernatant fractions of control, diabetic, and ketotic Chinese hamsters*

Type ^a	Blood glucose	Rate of amino acid incorporations ^b							
		liver				kidney			
		Leu	Lys	Ser	Gly	Leu	Lys	Ser	Gly
	mg% $\pm$ S.E.M. ^b	pmole/min/mg protein $\pm$ S.E.M. ^c				pmole/min/mg protein $\pm$ S.E.M. ^c			
Control	108.2 $\pm$ 7.9	8.60 $\pm$ 1.06	3.14 $\pm$ 0.37	3.70 $\pm$ 0.54	3.38 $\pm$ 0.37	5.32 $\pm$ 0.56	1.60 $\pm$ 0.19	2.06 $\pm$ 0.22	2.02 $\pm$ 0.18
Diabetic	285.6 $\pm$ 37.1	8.01 $\pm$ 1.64	2.90 $\pm$ 0.56	3.40 $\pm$ 0.60	3.34 $\pm$ 0.66	4.38 $\pm$ 1.09	1.26 $\pm$ 0.28	1.97 $\pm$ 0.29	1.81 $\pm$ 0.32
Ketotic	305.8 $\pm$ 30.6	8.48 $\pm$ 2.06	3.49 $\pm$ 0.82	4.11 $\pm$ 0.80	3.73 $\pm$ 0.81	5.39 $\pm$ 1.29	1.79 $\pm$ 0.40	1.95 $\pm$ 0.18	1.97 $\pm$ 0.15

^a Each group contained five Chinese hamsters, male, 7–9 months of age.

^b Calculated from the linear portion of a time course carried out as described in "Materials and Methods" at 0, 1, 2, 3 min.

^c Standard error of the mean.

no difference in the activity of hepatic ribosomes between diabetic hamsters and their matched controls or between ketotic hamsters and their normal counterparts. This finding agrees with the reported observation that bound liver ribosomes from normal and alloxan-induced diabetic rats showed similar protein synthetic activity as directed by poly U [5].

The PMS system was chosen to study the overall protein synthetic capacity because of its high activity

complications [13]. The renal PMS fractions of diabetic or ketotic Chinese hamsters, however, showed similar rate of incorporation of amino acids as those of the normal animals. The failure to observe a high rate of glycine incorporation in the kidney extracts, relative to those of leucine, lysine and serine, points to the fact that little glomerular basement membrane-like proteins were made in the PMS system prepared from kidney cortex. The remarkable similarity in the

relative rates of incorporation of the four amino acids suggests homology in the proteins produced in the renal and hepatic PMS fractions.

Analogous to the findings in the kidney extracts, there was no significant difference among normal, diabetic and ketotic liver PMS fractions in the rate of incorporation of amino acids per unit weight of PMS proteins. However, Pain had reported a reduced ability to incorporate leucine and phenylalanine into proteins, in terms of incorporation per unit ribosome, by PMS preparations from livers of streptozotocin-induced diabetic rats [6]. The discordance may arise from the difference in units selected to express the activity of amino acid incorporation in these two reports. Alternatively, the disagreement may be due to an existing difference in the effects on protein synthetic capacity between streptozotocin-induced diabetes in rats and spontaneous diabetes in the Chinese hamsters. It is concluded from the present study that no detectable defect, regarding the two reported aspects of protein synthesis, is evident in the diabetic or ketotic Chinese hamster.

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