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Effect of Streptozotocin Diabetes on Polysomal Aggregation and Protein Synthesis Rate in the Liver of Pregnant Rats and Their Offspring

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To study the effect of diabetes on hepatic protein synthesis and polysomal aggregation in pregnant rats, female rats were treated with streptozotocin prior to conception. Some animals were mated, and studied at day 20 of pregnancy, whereas, others were studied in parallel under non pregnant conditions. The protein synthesis rate measured with an "*in vitro*" cell-free system was higher in pregnant than in virgin control rats. It decreased with diabetes in both groups, although values remained higher in diabetic pregnant rats than in the virgin animals. The fetuses of diabetic rats had a lower protein synthesis rate than those from controls, although they showed a higher protein synthesis rate than either their respective mothers or virgin rats. Liver RNA concentration was higher in control and diabetic, pregnant rats than in virgin rats, and the effect of diabetes decreasing this parameter was only significant for pregnant rats. Liver RNA concentration in fetuses was lower than in their mothers, and did not differ between control and diabetic animals. The decreased protein synthesis found in diabetic animals was accompanied by disaggregation of heavy polysomes into lighter species, indicating an impairment in peptide-chain initiation.

KEY WORDS: diabetes; liver; polysome; pregnancy; protein synthesis; streptozotocin.

INTRODUCTION

Diabetes is known to decrease protein synthesis in several tissues, and changes in the level of polysomes and ribosomal subunits, as well as peptide-chain initiation have been implicated in this effect (reviewed in (1, 2)). Inhibition of protein synthesis in fast-twitch glycolytic muscles from diabetic rats, such as gastrocnemius and psoas, was due to both loss of tissue RNA and reduced translational efficiency, as a result of impairment in peptide-chain initiation (2–4). Protein

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synthesis is also inhibited in cardiac muscle from diabetic rats, although peptide-chain initiation is not impaired (2, 5). More controversial results have been obtained in liver, where both plasma and intracellular proteins are synthesized, and liver protein synthesis in diabetic rat has been reported as either unchanged (6) or impaired (7, 8).

Hyperinsulinemia (9–11) and insulin resistance (12–14) are characteristic features of late gestation which are known to affect protein synthesis, although it is not known how protein synthesis is modified during late pregnancy nor the effect of diabetes in this condition. Fetuses from streptozotocin diabetic rats become also diabetics as shown by their hyperglycemia and decreased circulating insulin levels, and although it is more than probable that protein synthesis is highly enhanced in the fetal liver (15), it is, however, not known how this pathway is modified in the fetus of the streptozotocin diabetic pregnant rat.

The aim of this paper was to study the effect of diabetes on hepatic protein synthesis during pregnancy and to examine whether it is associated with an impairment in peptide-chain initiation. The study has been performed not only in pregnant rats but also in their fetuses and age matched virgins. For the first time, our results clearly show that the diabetic condition produces inhibition of protein synthesis not only in pregnant rats but also in their fetuses. This inhibition is accompanied by an impairment in peptide-chain initiation.

MATERIALS AND METHODS

Materials

L-[4,5-^{3}H]leucine (70–170 Ci/mmol) was obtained from The Radiochemical Centre (Amersham, Buckinghamshire, U.K.) and RNAse-free sucrose from E. Merck (Darmstadt, F-R.G.). All other chemicals were obtained from commercial sources and were of the highest purity available.

Experimental Animals

Female Wistar rats from our colony, weighing 180–200 g were housed in a temperature-controlled room (21–23°) with 12-h light-dark cycles and fed a Purina Chow diet (Pamlab, Spain). After an overnight fast animals were treated i.v. with 40 mg/Kg streptozotocin dissolved in 50 mM citrate buffer pH 4.5; control rats were treated with the same volume of citrate buffer. Animals were refed and 48 h later a urinary glucose test was performed and only rats showing glucosuria were included in the study, along with the control rats. The diabetic animals were treated s.c. with bovine ultralente monocomponent insulin (Novo-Nordisk, Denmark) 1.5 IU/Day per 100 g body weight. After 7 days of treatment these rats were mated with normal males, and positive pregnancy was estimated by the appearance of sperm in vaginal smears. Insulin treatment was interrupted on day 0 of pregnancy until the 20 day of gestation when the animals were killed.

Untreated age-matched nondiabetic pregnant rats and virgin rats receiving the same treatments were studied in parallel.

Processing and Analysis of Samples

Approximately 500 mg of fresh liver were homogenized in buffer (1:2 w/v for fetuses and 1:2.5 for adult rats): 50 mM Hepes-KOH, pH 7.55; 4 mM magnesium acetate; 140 mM potassium acetate; 2.5 mM dithiothreitol and 0.32 M sucrose. The postmitochondrial supernatant (PMS) was obtained by centrifugation at 11,000 × g for 10 min and kept at −80°C until processed. RNA content was determined in homogenates by the dual-wavelength method of Munro & Fleck (16), and protein in PMS fractions by the method of Bradford (17).

Protein synthesis was measured in the PMS fractions according to Garcia *et al.* (15). The complete reaction system in a final volume of 50 μl contained: 50 mM Hepes-KOH, pH 7.55; 4 mM magnesium acetate; 140 mm potassium acetate; 2.5 mM DTT; 0.32 M sucrose; 1 mM ATP; 0.75 mM GTP; 30 mM phosphocreatine; 150 μg/ml creatine phosphokinase; 150 μg of PMS protein; all amino acids except leucine at 75 μM, 50 μM leucine and 10 μCi of L-[4,5-^{3}H]Leucine (75 Ci/mmol). 20 μl aliquots (duplicate) were processed after 45 min incubation at 30°C.

For the polysome profile determination, PMS fractions from the different experimental animals (50 μg of RNA) were layered on 4 ml of a 20%–50% linear sucrose gradient containing 20 mM Tris-HCl, pH 7.6; 3 mM magnesium acetate and 100 mM KCl. This continuous gradient was centrifuged at 40,000 rpm on a SW 60 Beckman rotor for 90 min. The ultraviolet absorption of the gradient was automatically recorded at 260 nm with a density gradient fractionator coupled to an Uvicord detector (LKB). The profiles were highly reproducible and the relative abundance of heavy polysomes (aggregates of 5 or more ribosomes) compared to the lighter species and the appearance of 60S subunit in a given profile were used for interpretation. All the procedure was performed at 4°C.

RESULTS AND DISCUSSION

Studies dealing with the effect of diabetes on hepatic protein synthesis are scarce and while profound changes were observed after a short-term diabetes (2 days) (2) in the muscle, one week was required after the induction of diabetes, before any changes in liver polysome profiles were observed (18). In the present work, the study is performed in the liver from animals with prolonged experimental diabetes (27 days) and the cell-free system utilized to measure protein synthesis, has been validated for liver tissue, and accurately reflects changes that occur "*in vivo*" (15).

Table 1. RNA content and protein synthesis rate in control and diabetic rats. RNA content and Protein synthesis rate were determined in homogenate and PMS fractions respectively, from the liver of control and diabetic rats as described in Material and Methods. Numbers represent the average ± SEM from 3-4 different animals per group

	Protein synthesis rate (pmoles/mg protein)		RNA content (mg/g tissue)	
	Control	Diabetic	Control	Diabetic
Virgin	38.2 ± 5.1^{a}	$18.5 \pm 3.3^{a*}$	6.57 ± 0.4^{a}	5.47 ± 0.4^{a}
Pregnant	60.2 ± 6.7^{b}	$38.2 \pm 2.2^{b*}$	9.45 ± 0.5^{b}	$7.40 \pm 0.5^{b*}$
Fetus	224.5 ± 29^{c}	$90.3 \pm 28^{c*}$	6.32 ± 0.7^{a}	$6.43 \pm 0.3^{a*}$

* represent significative differences ($P < 0.05$) between control and diabetic animals. Comparison between virgin and pregnant rats and the corresponding fetus was also made and different superscript letters for the group values means statistical differences between them (at least $P < 0.05$).

As shown in Table 1, the rate of protein synthesis was higher in the control pregnant rats as compared to virgin animals and much higher in the fetuses than in their mothers. The rate of protein synthesis was significantly lower in the diabetic liver of the three groups of animals studied, than in their respective controls. These effects are directly a consequence of the diabetic condition of the fetuses (or their mothers) rather than a direct action of the drug, since this was given to their mothers few days prior to conception. Also shown in Table 1, is the higher rate of protein synthesis in pregnant rats which may be related to their higher RNA content when compared to values in virgin animals; however, this is not the case in fetuses where in spite of their high protein synthesis, the RNA content is lower than in their mothers, indicating an effect of ribosomal activity. Protein synthesis inhibition in the diabetic virgin rats and in the fetuses from diabetic mothers is not accompanied by changes in the content of RNA (Table 1), which indicates that there is a reduction in the activity of the ribosomes. On the other hand, the diabetic pregnant showed a significantly lower RNA content as compared to their controls (Table 1) and this finding may be directly related to the reduced protein synthesis found in these animals.

Polysome profiles, the distribution of ribosomal aggregates by size, have been shown to be directly related to protein synthesis, suggesting that the rate of protein synthesis "*in vivo*" is dependent on the intactness and the amount of aggregation of the polysomes. Moreover, disaggregation of heavy polysomes (5 or more) into lighter polysomes or ribosomal subunits, is used as an index of the rate of protein synthesis and indicates that regulation of protein synthesis is being exerted at the level of initiation of polypeptide-chains (3, 18, 19). As shown in Figure 1, both in the diabetic virgin rats and fetuses from diabetic mothers, there is a clear decrease in the heavy polysomes and an increase in the lighter species, especially a marked shift to disomes in the diabetic virgin rats. These findings indicate that the inhibition of protein synthesis found in the diabetic condition in virgin rats and fetuses is accompanied by disaggregation of polysomes. The effects observed in the diabetic pregnant rats are less pronounced and an increase in 60S monomers, which is also found in their fetus, is also observed. The amount of heavy polysomes seems to be parallel to the rate of protein synthesis, being

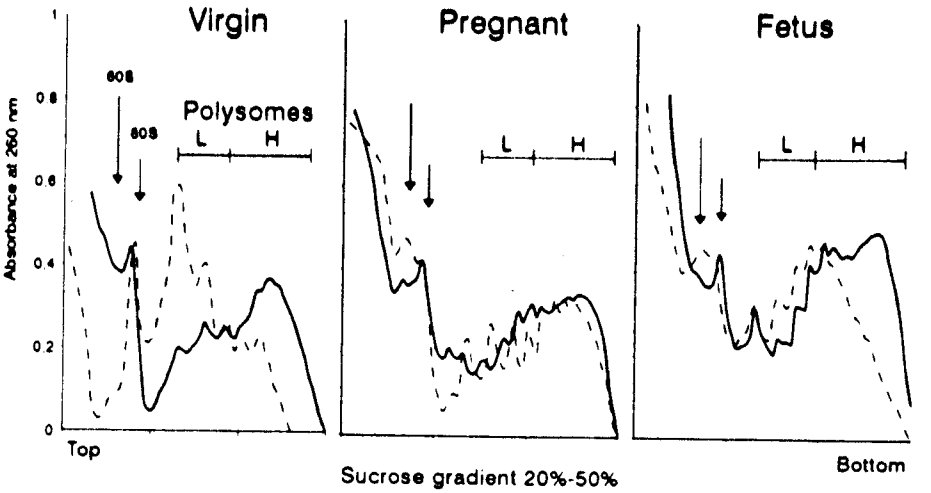

Fig. 1. Rat liver polysome profiles of control and diabetic animals. Polysome profiles were obtained as described in Methods. Four different preparations were studied and a representative profile from each experimental animal is shown. Control animals (——), diabetic animals (· · ·).

higher in the control fetuses than in their corresponding mothers and also higher in the control pregnant rats than in the control virgin rats. These differences are not modified in the diabetic animals.

Although preliminarily, these results show that hepatic protein synthesis is enhanced in pregnant rats, whereas it is decreased by diabetes in both pregnant and virgin animals. Interestingly, the protein synthesis rate in the liver of the fetus is much higher than in its mother and it is highly inhibited in those from diabetic mothers. The disaggregation of polysomes observed in diabetic animals, indicates that the impairment in protein synthesis is produced at the initiation level and work is now in progress to determine the precise mechanisms operating in the regulation of protein synthesis during pregnancy and diabetes.

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