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Regulation of hepatic expression of IGF I and fetal IGF binding protein mRNA in streptozotocin-diabetic rats

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Hepatic mRNA levels of insulin-like growth factor I (IGF I) and of the fetal, nonglycosylated 32 kDa IGF-binding protein (BP) were analysed in diabetic, diabetic insulin- and IGF I-treated rats as well as in age-matched, healthy control animals. IGF I mRNA levels are reduced in diabetic rats and increased by insulin treatment. In contrast, the infusion of IGF I does not significantly upregulate IGF I mRNA levels. Fetal IGF BP mRNA expression is very low in healthy control animals, but high levels are found in diabetic rats. Insulin therapy lowers fetal IGF BP mRNA levels, whereas IGF I has no effect. We propose that insulin is a major regulator of the 32 kDa IGF BP levels in adult rats.

Insulin-like growth factor I; mRNA; Protein, IGF-binding; (Streptozotocin-diabetic rat)

1. INTRODUCTION

Insulin-like growth factor I, a structural homologue of insulin, exerts growth-promoting activities in vitro and in vivo [1,2]. IGF I circulates in plasma tightly associated with high-affinity binding proteins [3,4]. Serum IGF I concentrations are strongly correlated with growth indices [5]. In streptozotocin-diabetic rats, growth is arrested and serum IGF I levels are markedly diminished [6,7]. The reduction of IGF I levels is accompanied by reduced binding protein levels [8]. This reduction of total binding capacity of serum is associated with a qualitative change of the pattern of binding proteins. The glycosylated forms with apparent molecular masses of 42–49 kDa, which are the predominant species in serum of control animals, virtually disappear, whereas the nonglycosylated, fetal, 32 kDa binding protein is more prominent (fig.6 in [8]). Treatment of diabetic rats with IGF I or insulin stimulates growth and increases serum IGF I levels [5–7]. The predominant production site of circulating IGF I appears to be the liver [9,10]. In addition, the liver might also produce the fetal binding protein in adult rats [10], however, nothing is known about its regulation. Recently, the cDNA of the fetal IGF BP has been cloned [11,12]. Therefore, we examined the hepatic mRNA expression of IGF I and of the fetal IGF BP in an animal model system consisting of healthy control, diabetic and insulin- or IGF I-treated diabetic rats.

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2. MATERIALS AND METHODS

2.1. *Animals*

Two strains of rats were used in separate groups of experiments: (i) Outbred Zur:SIV (Institut für Zuchthygiene, Universität Zürich) and (ii) inbred DA(RT1^a) purchased from the Zentralinstitut für Zuchthygiene, Hannover). Final body weight of outbred rats was 400 g, that of inbred rats being 200–230 g. All experiments were performed with both strains and the same qualitative results were obtained. To induce diabetes, 4-week-old inbred and outbred rats fasted for 24 h were injected i.v. with a dose of 80 or 100 mg/kg, respectively of streptozotocin (ZanosarR, Upjohn, Kalamazoo, MI). The body weight was recorded daily for 3 weeks and rats which continued to gain weight were excluded from further experiments. Diabetic rats were treated with 6 U/day of insulin (ProtaphanR, HM, Novo) using the NovoPen II or 300 μg/day of recombinant human IGF I (gift from W.R. Rutter, Chiron, Emeryville, CA) and J. Nuesch, Ciba-Geigy, Basel). IGF I was

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dissolved in 0.1 M acetic acid and 0.9% saline and applied s.c. using Miniosmotic pumps (Alzet 2001). Diabetic control rats were treated in the same way, except that the pumps were filled only with acetic acid and saline. Age-matched, healthy rats served as controls. After 7 days of treatment, the rats were killed by aorta puncture in deep anesthesia (2 ml/kg Innovar-Vet^R, Pitman-Moore, Washington, USA) and 1 g liver was immediately processed for RNA extraction.

2.2. *Determination of blood glucose, insulin and IGF I*

Blood glucose was determined immediately after aorta puncture using a YSI glucose analyser. Serum insulin was determined using a radioimmunoassay kit (NovoBiolabs, Bagsvaerd, Denmark). Immunoreactive IGF I was determined as in [8] using human IGF I and rat IGF I (gift from M. Kobayashi, Osaka [13]) as standards.

2.3. *mRNA isolation and Northern blotting*

Total liver mRNA was isolated using a standard guanidine hydrochloride extraction procedure [14]. Samples containing 20 μg total RNA were separated in a 1% agarose gel containing 2 M formaldehyde [15] and subsequently subjected to partial alkaline hydrolysis. RNA was neutralised and transferred to nitrocellulose (Schleicher and Schuell).

2.4. *DNA labeling and hybridization*

Isolated DNA fragments containing IGF I were labeled with 5-[α-^{32}P]deoxycytidine (Amersham) to a specific activity of $1-4 \times 10^9$ cpm/μg using a random primed DNA labeling kit from Boehringer (Mannheim). Hybridization [15] was performed at 42°C using 2×10^7 cpm/ml of a labeled probe. The filter was washed at 55°C in 0.2 × SSC and 0.1% SDS for 1 h with 3 changes. Labeled bands were visualised by autoradiography using Kodak X-Omat AR films in the presence of a Cronex lightning plus enhancer screen. The intensity of bands was determined using a Bio-Rad video densitometer (model 620). Hybridisation for fetal BP mRNA was performed as in [12].

3. RESULTS AND DISCUSSION

3.1. *Metabolic parameters*

Streptozotocin-treated animals were severely diabetic and stopped gaining weight. Respective growth and metabolic parameters of a typical experiment using inbred rats are summarized in table 1. The average weights at the beginning and end of the treatment period with insulin or IGF I are listed whereas glucose, insulin and IGF I levels were determined at the end of the treatment period. Diabetic animals were characterised by very high glucose levels, low insulin and IGF I levels and complete growth arrest. Insulin treatment lowered blood glucose, increased IGF I levels and stimulated weight gain. The blood glucose was even lower than in controls because the diabetic rats had received a bolus injection of insulin only 2 h prior to being killed. IGF I-treated animals remained severely diabetic and had high blood glucose and low insulin levels, but nevertheless gained weight. IGF I serum levels in IGF I-treated animals are difficult to interpret, since these animals contain a mixture of residual rat and infused human IGF I, whereas the standards used in the immunoassay were either rat or human IGF I. We do not believe that this poses a real problem, since IGF I values observed were well above those of healthy controls with either rat or human IGF I as standard. Age-matched, healthy control rats do not gain weight during the treatment period, because they have already reached their final body weight during the period between the administration of streptozotocin and the beginning of treatment of diabetic rats. Very similar results were obtained with outbred rats except that age-matched healthy controls (final body weight 400 g) continued to grow during the whole period of the experiment.

3.2. *Hepatic IGF I mRNA levels*

Total hepatic RNA was analysed by Northern blotting. A representative gel of IGF I mRNA of a few outbred animals is shown in fig.1. The most prominent band seen migrates at 8 kb, and in addi-

Table I

Metabolic and growth parameters determined from control, diabetic, insulin-treated and IGF I-treated diabetic rats

	Initial weight (g)	End weight (g)	Glucose (mmol/l)	Insulin (ng/ml)	IGF I (h) (ng/ml)	IGF I (r) (ng/ml)
Controls	196 ± 8	205 ± 7.1	7.0 ± 0.3	0.94 ± 0.17	180 ± 18	1235 ± 171
Diabetic	134 ± 11	135 ± 4.6	21.9 ± 1.7	0.34 ± 0.05	56 ± 7.3	413 ± 85
Diabetic (ins)	131 ± 11	171 ± 8.3	2.95 ± 1.8	>10	146 ± 12	885 ± 138
Diabetic (IGF)	128 ± 7.4	149 ± 16.2	22.2 ± 2	0.28 ± 0.04	446 ± 100	3073 ± 991

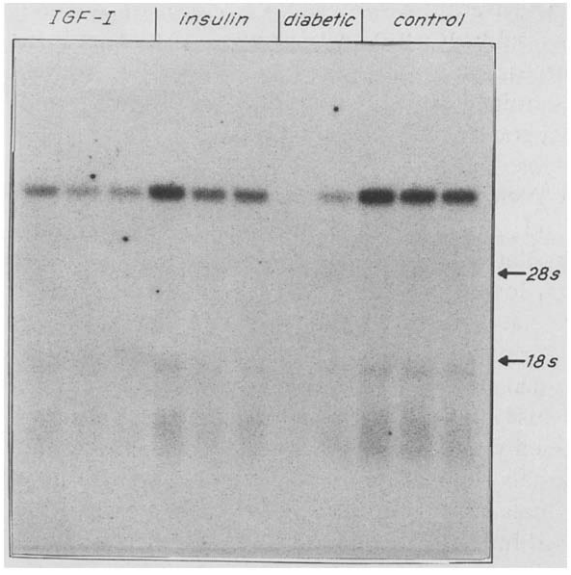

Fig.1. Northern blot of liver IGF I mRNA from control, diabetic, insulin-treated and IGF I-treated diabetic rats. Age-matched, healthy rats (control), streptozotocin-diabetic rats (diabetic), insulin-treated, diabetic rats (insulin) and IGF I-treated diabetic rats (IGF I) were analysed for the presence of hepatic IGF I mRNA as described in section 2.

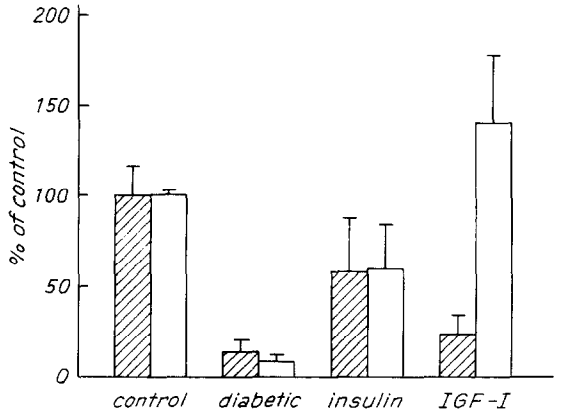

Fig.2. Comparison of relative levels of liver IGF I mRNA and IGF I in serum from healthy control, diabetic, insulin-treated and IGF I-treated diabetic rats. IGF I mRNA levels (hatched bars) and serum IGF I levels (unfilled bars) were determined in age-matched, healthy rats (control), diabetic rats (diabetic), insulin-treated (insulin) and IGF I-treated (IGF I) diabetic rats. Means and SD were determined from 6 animals and values of controls were taken as 100%. Values derived from other experimental groups were expressed as % of control.

tion there are minor bands of 2 kb and a broad band of 1–1.5 kb. The predominance of the large 8 kb message indicates that the RNA isolates were highly intact. Previous authors also reported the appearance of several messages, the smaller ones, however, being more abundant [16,17]. We did not observe more than 10–15% of the total IGF I mRNA as low molecular mass species.

Fig.2 shows a quantitative estimate of liver IGF I mRNA and serum IGF I levels in an experiment with outbred animals. In order to compare hepatic IGF I mRNA and IGF I serum levels six animals were used per group. Values of healthy controls were taken as 100% and all other experimental groups were expressed as % of control. In this group of animals growth restoration by insulin treatment was 77% and by IGF I 57% when compared to healthy control rats. In agreement with previous studies, insulin treatment resulted in an increase of serum IGF I concentrations [5]. There was a correlation between serum levels of IGF I and hepatic IGF I mRNA levels in diabetic as well as in insulin-treated diabetic animals. In IGF I-treated rats serum IGF I concentrations were very high but there was no significant increase of IGF I mRNA when compared to the diabetic group. These results further emphasize that the liver is the major source of IGF I in serum and that serum levels are regulated via regulation of IGF I mRNA in the liver. In contrast to insulin, IGF I infusions do not significantly affect hepatic IGF I mRNA levels and, hence, endogenous IGF I does not mimic the effects of insulin at that level. Growth restoration by infused IGF I must, therefore, result from a direct effect of infused IGF I on target organs.

3.3. *Hepatic fetal IGF BP mRNA levels*

Hepatic mRNA was further probed for the presence of the fetal IGF binding protein message. The upper part of fig.3 shows a typical Northern blot for a selection of a few animals. A single mRNA species of length 1.6 kb is observed. The lower part of fig.3 demonstrates quantitation of the respective signal. In healthy inbred rats we observed only a very small amount of fetal IGF-binding protein mRNA (see fig.3) whereas no message was detectable with RNA of outbred rats (not shown). In contrast, in diabetic animals of both rat strains there was a large increase in fetal IGF BP mRNA levels, that was again brought

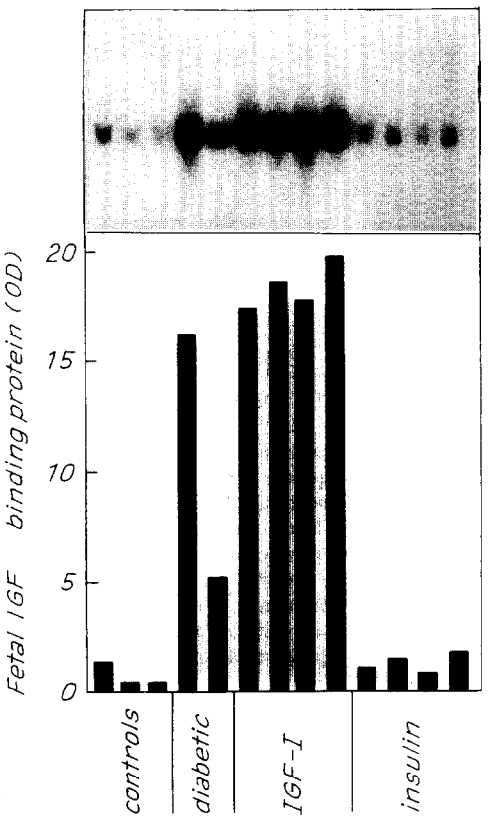

Fig.3. Northern blot of hepatic fetal IGF BP RNA. Age-matched, healthy rats (controls), streptozotocin-diabetic rats (diabetic), insulin-treated (insulin) and IGF I-treated (IGF I) diabetic rats were analysed for the presence of hepatic fetal BP mRNA. (Upper) Northern blot and (lower) relative absorbances of the bands.

back to normal by insulin treatment. In contrast, the infusion of IGF I did not reduce fetal IGF BP message in diabetic animals. These results show that in situations where insulin concentrations are low (diabetic rats, IGF I-treated diabetic rats) fetal BP mRNA levels are high, whereas in situations where insulin levels are high (healthy rats, insulin-treated diabetic rats) this mRNA species is very rare. Therefore, we conclude that insulin in vivo either directly or via insulin mediated mechanisms such as growth hormone induction, regulates fetal IGF BP expression. Recent in vitro results derived from isolated hepatocytes support the notion that insulin has a direct effect on the mRNA level of the fetal IGF BP (in preparation).

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