The role of insulin-like growth factor I in growth of diabetic rats

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Abstract. Insulin-deficient, streptozotocin-diabetic rats show severe metabolic disturbances and stop growing. Besides insulin, these animals also lack growth hormone and insulin-like growth factor-I. We examined whether or not growth parameters correlate with IGF-I serum levels in young rats with streptozotocin-diabetes of different severity. In the diabetic rats, blood glucose varied between 18.4 and 38.6 mmol/l (healthy controls between 6.1 and 9.3), IGF-I serum levels between 2.6 and 15.6 nmol/l (controls between 19.6 and 26.5), and serum insulin levels between 0.05 and 0.14 nmol/l (controls between 0.36 and 0.55). We found a highly significant linear correlation between IGF-I serum levels and the two investigated growth parameters, tibial epiphyseal width and longitudinal tibial bone growth. The finding that these indices of growth are strongly correlated with IGF-I serum levels in young rats with diabetes of different severity, suggests that IGF-I is a major determinant of growth. This is in keeping with our earlier demonstration that exogenously infused IGF-I promotes growth in diabetic rats.

Serum IGF-I levels are mainly controlled by growth hormone, insulin, nutrition, and age (1,2,3). Reduced serum IGF-I levels are found in hypophysectomized and in diabetic rats. Both conditions are accompanied by growth arrest. Beside growth hormone, hypophysectomized rats also lack the other pituitary hormones. Streptozotocin-diabetic rats are insulin-deficient and have low levels of GH and IGF-I (4,5).

In 1982, Schoenle et al. (6) demonstrated that subcutaneous infusion of human IGF-I during 6 days stimulated growth in hypophysectomized rats and thus provided evidence in favour of the somatomedin hypothesis (7). Subsequently, it was shown that infusion of recombinant human IGF-I during 18 days had a sustained growth-promoting effect in hypophysectomized rats (8).

Insulin treatment of streptozotocin-diabetic rats normalizes the metabolic condition, restores GH and IGF-I levels, and allows resumption of growth (9,10). Subcutaneously infused IGF-I imitates insulin with respect to growth promotion, but does not normalize blood glucose (9).

It was the purpose of this study to examine whether or not growth parameters correlate with IGF-I serum levels in rats with diabetes of different severity.

Material and Methods

Animals

Male Zur: SIV rats (outbred, Institut für Zuchthygiene, Universität Zürich, Switzerland) with a body weight between 115 and 125 g were used for the experiments. Two independent experiments were done. In the first we investigated metabolic and growth parameters from 9 rats (3 control and 6 diabetic rats), in the second the same parameters from 12 rats (4 control and 8 diabetic).

The rats had free access to food (Altromin, Lage, FRG) and water and were kept on a 12 h light and dark cycle. Body weight was recorded daily at 08.00 h. Food and water intake were approximated from weighed daily intake per cage (3 rats were in one standard cage). Diabetes was induced by iv injection of streptozotocin (gift from Ciba-Geigy AG, Basel, Switzerland), freshly dissolved in
0.9% saline (pH 3.5, adjusted with citric acid), at a dose of 90 mg per kg body weight. The rats were not fasted and were under sedation with diazepam (10 mg/kg ip, Hoffmann-La Roche, Basel, Switzerland). Onset of diabetes was determined by the Keto-Diabur-Test® (Boehringer, Mannheim, FRG). After 26 days of experimental diabetes, the rats were anesthetized with fentanyl-droperidol (2 ml/kg, Pitman-Moore, Washington, DC) and bled by aortic puncture. Blood glucose was immediately determined in a glucose analyzer (YSI model 23A, Yellow Springs Instr Comp, Yellow Springs, OH). The non-diabetic control rats were treated identically except that they received no streptozotocin.

Radioimmunoassays

Serum insulin was measured with a rat insulin radioimmunoassay kit (NovoBiolabs, Bagsvaerd, Denmark).

Endogenous IGF-I was separated from serum binding proteins by chromatography on Sep-Pak C$_{18}$ cartridges (Waters Associates, Milford, MA) according to the protocol supplied by the manufacturer and determined by radioimmunoassay (11,12). Purified native human IGF-I (kindly supplied by Dr. R. E. Humbel, Institute of Biochemistry, University of Zürich) served as a standard. Cross-reactivity of pure rat IGF-I (kindly supplied by Dr. M. Kobayashi, Fujisawa Pharmaceutical, Osaka, Japan) with the anti-human IGF-I antisera was around 25%.

Tibia preparation

Tibial epiphyseal width was measured according to Greenspan et al. (13). Briefly, the tibiae were prepared and split with a sharp razor blade in the mid-frontal plane. The bones were immersed overnight in 10% formalin (pH 7.0–7.4), rinsed in water, treated with acetone, washed again and stained with 2% AgNO$_3$ by exposure to light. Polaroid pictures were taken at 25-fold magnification.

Accumulated longitudinal tibial bone growth was determined by intravital tetracycline staining (14). Tetracycline (3.8 mg/kg Terravenos® Pfizer, Zürich, Switzerland) was injected ip. After 10 days, the rats were sacrificed, the undecalcified tibiae embedded in metacrylate, cut and abraded to 100 μm. Finally, the fluorescent tetracycline band in the bone was photographed at 400-fold magnification. The distance between the border of the epiphysis and the tetracycline band was measured. In some rats, fluorescent bands were missing as also reported by Hansson et al. (14). The reason for the absence of the bone label is not clear.

Results

The data presented here were obtained from two sets of experiments with a total number of 14 diabetic and 7 control rats. All rats had body weights between 115 and 125 g before induction of diabetes. A wide variation in body weight between 126 and 313 g was observed at the end of the experiment. Fig. 1 depicts representative examples of daily body weight gain from 3 diabetic and 3 control rats during the observation period.

Table 1 lists metabolic indices, serum insulin and IGF-I levels from diabetic and control rats. IGF-I serum levels were decreased in diabetic rats. They ranged from 2.6 to 15.6 nmol/l as compared with levels from 19.6 to 26.5 nmol/l in healthy controls.

Plotting tibial epiphyseal width as a function of the IGF-I serum level revealed a highly significant linear correlation (Fig. 2, r = 0.95, p < 0.01).

Longitudinal tibial bone growth was impaired in the diabetic rats. The rates of longitudinal bone growth correlate linearly with the corresponding IGF-I serum levels (Fig. 3, r = 0.97, p < 0.01). Since there appears to be a continuous transition from the healthy to the severely diabetic state, one can...
assume that the age-matched control rats show the same correlation between bone growth rate or epiphyseal width and IGF-I serum levels as the diabetic rats.

**Discussion**

Insulin, growth hormone and IGF-I are three major anabolic hormones. Impairment of metabolism caused by insulin deficiency in rats is accompanied by the loss of secretory peaks of GH, by low IGF-I serum levels, and by growth arrest (4,5). Insulin treatment normalizes the metabolic disturbance, restores the GH secretory pattern and the IGF-I serum levels, and allows resumption of growth (9,10). In contrast, GH-treatment of diabetic rats fails to raise low IGF-I serum levels and has no growth-promoting effects on bone and cartilage (9). Since IGF-I is mainly produced by the liver under the influence of GH (15,16), this responsiveness to GH must be impaired in diabetes. In fact, IGF-I secretion by the diabetic rat liver was found to be markedly reduced (17–19). Apart from the liver, other tissues, particularly bone and cartilage, synthesize and secrete IGF-I under the influence of GH (20–24). This mechanism involved in autocrine/paracrine stimulation of growth may also be impaired in the streptozotocin-diabetic rats and restored by insulin-treatment. It is likely that insulin acts indirectly via an increase in IGF-I synthesis on growth of diabetic rats, by means of restoring GH secretion and the responsiveness of the liver to GH. Evidence for this was provided by the finding that subcutaneous infusion of IGF-I stimulated growth of insulin-deficient diabetic rats despite persisting hyperglycemia (9).

In the study by Scheiwiller et al. (9), serum IGF-I levels of insulin-deficient rats treated with insulin correlated with body weight gain and tibial epiphyseal width. In this study, diabetic rats with
widely varying body weight gain were used as a model for diabetes of different severity. It may not appear justified to correlate a dynamic index like a growth parameter with an end point measurement of a serum hormone level. However, in a first set of experiments we had established that IGF-I levels decrease during the first two weeks of diabetes and then remain constant. Serum insulin levels at the time of sacrifice were not related to the severity of growth impairment. Although low insulin levels in the presence of hyperglycemia reflect the severely impaired insulin release capacity of the beta-cells, single insulin determinations may not be an adequate measure for the severity of diabetes. A better measure would be the integrated insulin release over 24 hours. The latter probably determines the serum IGF-I levels which show a highly significant linear correlation with the studied growth parameters. These results are in agreement with the finding that exogenous IGF-I stimulates the growth of diabetic rats and support the notion that IGF-I is an important mediator of the growth promoting effects of growth hormone (hypophysectomized rat, 6,8) and insulin (diabetic rat, 9).

Correlation between longitudinal tibial bone growth during 10 days as determined by intravital tetracycline staining and endogenous IGF-I serum level in diabetic (•) and control (▲) rats from two experiments. The full line represents a linear regression within the diabetic rats only (correlation coefficient r = 0.86), the broken line between both groups (r = 0.97). The equations are as follows: Full line: y = 17.4x−469.2, r = 0.86, N = 11, p < 0.01. Broken line: y = 13.9x−247.5, r = 0.97, N = 17, p < 0.01. IGF-I levels are expressed in equivalents of a human IGF-I standard.

Fig 2.

Correlation between tibial epiphyseal width and endogenous IGF-I serum levels in diabetic (•) and control (▲) rats from two experiments. The full line represents a linear regression within the diabetic rats only (correlation coefficient, r = 0.81). The broken line assumes a continuous transition between the healthy and the diabetic state and therefore draws a linear regression within both groups (r = 0.95). The equations are as follows: Full line: y = 1.9x + 56.6, r = 0.81, N = 14, p < 0.01. Broken line: y = 1.8x + 62.9, r = 0.95, N = 21, p < 0.01. IGF-I levels are expressed in equivalents of a human IGF-I standard (the molecular weight of human IGF-I is 7649).

Fig 3.

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References


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