Regulation of circulating levels of IGF-I in pregnant rats: changes in nitrogen balance correspond with changes in serum IGF-I concentrations

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Abstract

Serum IGF-I concentrations in rats decrease significantly in late pregnancy. To determine if the reduction in serum IGF-I concentrations is attributable to circulating GH or maternal nutritional status, we investigated the effect of treatment with recombinant human GH (rhGH: 100 µg/rat per day) on IGF-I concentrations during late pregnancy, and evaluated the relationship between maternal nitrogen balance and IGF-I concentrations. Serum IGF-I concentrations and maternal nitrogen balance ((nitrogen intake)-(nitrogen content in faeces and placenta)) were measured by RIA and the Dumas method. In non-pregnant rats treated with rhGH for 3 days, serum IGF-I concentrations (835·4 ± 59·5 ng/ml; P<0·01) were significantly greater than in those animals treated with saline (319·6 ± 95·6 ng/ml). In the pregnant rats, however, there was no significant difference in serum IGF-I between those treated with rhGH (151·1 ± 43·0 ng/ml) and those treated with saline (142·0 ± 39·9 ng/ml) from day 17 to 19 of pregnancy. Maternal nitrogen balance in the pregnant rats increased significantly from day 4 to day 10 of pregnancy (169·5 ± 57·4 and 196·1 ± 33·4 mg/day, respectively; P<0·05) compared with non-pregnant controls (31·9 ± 19·9 mg/day) and decreased markedly from day 12 of pregnancy (79·8 ± 60·1 mg/day; P<0·05) onwards, to 14·9 ± 47·8 mg/day on day 20 of pregnancy (P<0·01), significantly different from the value on day 10 of pregnancy. The mean difference in maternal nitrogen balance between pregnant and non-pregnant rats was positively correlated (r=0·87, P<0·01) with the mean difference in maternal IGF-I concentrations, using linear regression analysis. These results support the conclusion that the circulating concentration of IGF-I in pregnant rats is associated with the change in nitrogen balance, but not with circulating GH.


Introduction

Circulating insulin-like growth factor-I (IGF-I) is the major anabolic agent responsible for tissue growth, mediating the effects of growth hormone (GH) (Schoenle et al. 1982). Nutrition, in addition to circulating concentrations of GH (Zapf et al. 1981, Luna et al. 1983), have profound effects upon serum IGF-I concentrations in humans (Clemmons et al. 1981, Merimee et al. 1982, Phillips & Unterman 1984) and rats (Phillips & Young 1976). Moreover, serum IGF-I concentrations can be used as a clinical index of nutritional status in nutritionally deprived patients (Clemmons et al. 1985).

Circulating IGF-I concentrations change in pregnancy in a variety of species. Serum IGF-I concentrations in women with an uncomplicated pregnancy are increased in the third trimester and decline rapidly in the early puerperium (Furlanetto et al. 1978, Wilson et al. 1982, Gargosky et al. 1990). These concentrations are regulated by variant GH secreted by the placenta, but not by the pituitary (Caufriez et al. 1990). In rats, the reduction in serum IGF-I concentrations (Davenport et al. 1990a, Gargosky et al. 1990, Donovan et al. 1991) is observed in late pregnancy, although the circulating concentration of rat GH (rGH) (Terry et al. 1977, Carlsson et al. 1990, Kishi et al. 1991) has an inverse association with serum IGF-I concentrations throughout pregnancy. There are several reports about hormones from the GH–prolactin family in rat placenta (Daughaday et al. 1979, Robertson et al. 1991, Lin et al. 1997), but the effect of these hormones on maternal IGF-I concentrations is unknown at present. Maternal IGF-I concentrations under protein (Pilistine et al. 1984) or food (Monaco & Donovan 1996) restriction in pregnant rats have also been reported. Nevertheless, the association between the reduction in serum IGF-I concentrations and maternal nutritional status in pregnant rats remains to be elucidated.

In this study, we aimed to determine if the reduction in serum IGF-I concentrations during pregnancy in rats is attributable to circulating GH or maternal nutritional...
status. The effect of treatment with recombinant human GH (rhGH) on IGF-I concentrations in pregnant rats was investigated and a possible relationship between the change in nitrogen balance and the reduction in IGF-I concentrations was evaluated.

Materials and Methods

Animals

The study procedure was approved by the Committee of Animal Experimentation at Kobe University School of Medicine, and animals were cared for in compliance with the NRC Guide for the Care and Use of Laboratory animals (National Research Council 1985). Rats of the Wistar strain at 8 weeks of age (220–280 g) (Clea Japan, Inc., Osaka, Japan) were bred in a temperature-controlled environment with a 12-h light : 12-h darkness cycle and the day of sperm detection in a vaginal smear was taken as day 1 of pregnancy. Standard food pellets containing 32.03 mg/g nitrogen and water were freely available to all rats. From day 2 to day 20, three to seven rats were decapitated every other day for the collection of samples. Sera were stored at −20 °C. Any fetuses and placenta were weighed and frozen immediately for storage at −20 °C. In a metabolic cage, four pregnant rats were housed individually. Body weight, food intake, urine volume and faeces weight were measured daily. Some urine and faeces were stored at −20 °C. Virgin female rats at 8 weeks of age (220–280 g) served as non-pregnant controls.

GH treatment in pregnant rats

Five of the pregnant rats were treated with rhGH (100 µg/rat per day), provided by Sumitomo Pharmaceuticals (Osaka, Japan), or an equivalent volume of saline, by daily s.c. injections from day 17 to day 19. On day 20, the animals were decapitated and sera were collected for subsequent IGF-I measurement. In some experiments, non-pregnant rats were treated with an equivalent volume of rhGH or saline for 3 days.

IGF-I measurement

Highly purified recombinant IGF-I and polyclonal IGF-I antisera were provided by Fuzisawa Pharmaceutical Co. Ltd (Osaka, Japan). Iodination with [125I]-labelled Na was performed using the chloramine T method (Hunter & Greenwood 1962) to a specific activity of 100–120 µCi/µg. [125I]-labelled IGF-I was separated from unincorporated iodine using Sephadex G 25 fine (Pharmacia, Uppsala, Sweden) gel filtration; 60–70% of the [125I]-labelled IGF-I was bound at a 1 : 10 000 dilution of the antiserum provided. Serum immunoreactive IGF-I was measured by RIA according to the method of Hintz et al. (1982), after removal of IGF binding proteins (IGFBPs) from the samples by Seppak C18 cartridge chromatography (Daughaday et al. 1986).

Nitrogen balance measurement

Samples of placenta and fetus were homogenised with normal saline. Nitrogen content was measured in these homogenised samples, in addition to faeces and urine, using the Dumas method (Bellomonte et al. 1987). Maternal nitrogen balance was defined as follows: (maternal nitrogen balance) = (nitrogen intake) – (nitrogen content in the faeces and urine) – (nitrogen content in fetuses and placenta).

Statistical analysis

Statistical analysis was performed using the unpaired Student’s t-test or regression analysis as appropriate. Statistical significance was accepted at the <0·05 level.

Results

Serum IGF-I concentrations in pregnant rats

Serum IGF-I concentrations until day 12 (388·2 ± 102·7 ng/ml, means ± s.d.) were unchanged compared with those in non-pregnant controls (327·6 ± 92·8 ng/ml). The concentrations on day 14 (220·3 ± 88·8 ng/ml) decreased significantly compared with those in non-pregnant controls (P<0·05). This decrease was observed until pregnancy was terminated (Fig. 1).

Serum IGF-I concentrations in pregnant and non-pregnant rats treated with rhGH

Serum IGF-I concentrations (835·4 ± 59·5 ng/ml; P<0·01) were significantly greater in non-pregnant rats
treated with rhGH than in those treated with saline (319.6 ± 95.6 ng/ml). In the pregnant rats, however, serum IGF-I concentrations after treatment with rhGH (151.1 ± 43.0 ng/ml) showed no significant difference from those after treatment with saline (142.0 ± 39.9 ng/ml) (Table 1).

### Table 1 Effects of hGH treatment on serum IGF-I concentrations in pregnant rats. Values are means ± S.D.

<table>
<thead>
<tr>
<th>Serum IGF-I conc. (ng/ml)</th>
<th>Non-pregnant controls</th>
<th>Pregnant rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>319.6 ± 95.6</td>
<td>142.0 ± 39.9</td>
</tr>
<tr>
<td>GH</td>
<td>835.4 ± 59.5*</td>
<td>151.1 ± 43.0</td>
</tr>
</tbody>
</table>

*p < 0.001 compared with non-pregnant controls treated with saline by unpaired Student's t-test.

Maternal nitrogen balance in pregnant rats

Maternal nitrogen balance in the pregnant rats significantly increased from day 4 (169.5 ± 57.4 mg/day; *P < 0.05) to day 10 (196.1 ± 33.4 mg/day; *P < 0.05) compared with that in the non-pregnant controls (31.9 ± 19.9 mg/day). The balance decreased markedly from day 12 (79.8 ± 60.1 mg/day; *P < 0.05) onwards, to a value of 14.9 ± 47.8 mg/day on day 20 (*P < 0.01 compared with that on day 10) (Fig. 2). The association between maternal IGF-I concentrations and nitrogen balance was examined using linear regression analysis. The mean difference in maternal nitrogen balance between pregnant rats and non-pregnant controls was positively correlated (*r = 0.87, *P < 0.01; linear regression analysis).

Discussion

No increment in serum IGF-I concentrations after treatment with rhGH was observed in our study, which strongly suggests that serum circulating GH does not have an important role in the regulation of maternal IGF-I during pregnancy in rats. This finding demonstrates that an apparent GH resistance occurs in late pregnancy in rats, as indicated in previous reports (Gargosky et al. 1991, Travers et al. 1993, Spence et al. 1995). In contrast with our results, Chiang & Nicoll (1991) reported that GH treatment retained the ability to increase IGF-I in rat pregnancy. The reason for this discrepancy may be that different concentrations of GH were used in the experiments: the pregnant rats in our study were administered 100 µg/day human GH for 3 days, whereas Chiang & Nicoll administered 1 mg/day bovine GH or 5 mg/day ovine GH for 9 days. This hypothesis is supported by a report (Spence et al. 1995) that showed that circulating IGF-I concentrations in pregnant rats are significantly increased on day 21 after treatment with 5 IU/kg per day porcine GH from day 6 to day 21, but not after treatment with 1 IU/kg per day.

Nutritional influences are likely to have a major effect on circulating IGF-I concentrations under acquired GH resistance, because GH resistance is a commonly recognised feature of protein catabolic status (Donaghy & Baxter, 1999).
1996). Indeed, the association of circulating IGF-I with nutrient balance is observed in fasted volunteers (Clemmons et al. 1981) and malnourished patients (Clemmons et al. 1985). In our study, we have demonstrated, for the first time, a close relation between maternal IGF-I concentration and nitrogen balance during pregnancy in rats.

The mechanism of GH resistance during rat pregnancy remains unclear. Hepatic GH binding activity does not vary during pregnancy (Travers et al. 1993). Furthermore, a recent study has shown that hepatic GH receptor and GH binding protein gene expression are not suppressed by maternal malnutrition during rat pregnancy (Woodall et al. 1998). These reports suggest that a possible postreceptor defect in GH action may contribute to GH resistance observed in pregnant rats.

The reduction in circulating IGF-I in pregnant rats may also be associated with the effect of IGFBP, presumed to have an important role in determining the biological actions of IGF-I in various tissues (Jones & Clemmons 1995). The predominant IGFBP in blood is IGFBP-3, part of a ternary complex with IGF-I peptide and an acid labile subunit (Furlanetto 1980, Baxter 1988). This complex serves as the endocrine storage depot for IGF-I, which extends the half-lives of IGF-I peptides in the circulation (Zapf et al. 1986, Cascieri et al. 1988, Francis et al. 1988). In rats, IGFBP-3 concentrations are decreased in late pregnancy (Davenport et al. 1990a, Gargosky et al. 1990), in spite of unchanged hepatic expression of IGFBP-3 mRNA (Donovan et al. 1991). The reduction in IGFBP-3 concentrations is regulated by intrinsic proteases, such as serine proteases and matrix metalloproteinases (Davenport et al. 1992, Fowlkes et al. 1994). Although lower hepatic concentrations of IGF-I are observed in late pregnant rats than in non-pregnant rats (Davenport et al. 1990a), maternal IGF-I concentrations are likely to be reduced because of proteolysis of circulating IGFBP-3 (Davenport et al. 1992). According to Davies et al. (1991), malnutrition during severe illness in humans induces the activity of serum proteases specific for IGFBP-3. From these investigations, the reduction in circulating IGF-I in pregnant rats might be attributable to the proteolysis of IGFBP-3 in maternal serum under maternal malnutrition. Whether the reduction in circulating concentrations of IGF-I results from the suppression of IGF-I hepatic production or from the proteolysis of IGFBP-3 remains to be investigated.

IGF-I peptide acts by stimulating anabolic metabolism in tissues, as shown by the increase in nitrogen balance induced by IGF-I treatment in humans (Tomas et al. 1991). Restricting maternal protein intake reduces fetal growth retardation and maternal IGF-I concentrations (Davenport et al. 1990b; Muaku et al. 1995). In undernourished pregnant rats, increases in maternal IGF-I concentrations induced by treatment with high doses of ovine or bovine GH led to reductions in fetal growth, whereas maternal body weight increases (Chiang & Nicoll 1991). From these results and our own, we assume that the reduction in circulating IGF-I concentrations during late pregnancy in rats may be necessary for the inhibition of maternal anabolic metabolism and redistribution of maternal nutrients to support fetal growth.

In summary, we have provided evidence suggesting that there is an association between maternal nitrogen balance and serum IGF-I concentrations in pregnant rats. We are not aware of any study in pregnant women assessing the relationship between maternal nutritional status and serum IGF-I concentration. Further studies will be necessary to understand better how IGF-I is regulated in pregnant women with nutritional and metabolic disorders.

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References


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