Tissue-Specific Regulation of Growth Hormone Receptor and Growth Hormone Binding Protein Gene Expression during Pregnancy and Lactation in the Rat

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Materials and Methods

Total RNA was isolated from various tissues of rat on days 7, 14 and 20 during pregnancy and days 3, 8 and 21 during lactation, and the amounts of GH-R and GH-BP mRNAs in rat tissues were measured by RNase protection assay. An antisense RNA probe for rat GH-BP mRNA was synthesized by transcribing the 369 bp EcoRV fragment of rat GH-BP cDNA cloned in pCR™II plasmid vector (Invitrogen) with SP6 polymerase and [α-32P]CTP. The radiolabeled RNA probe was hybridized with 30 µg of total RNA and then digested with RNases A and T1. The 152 and 272 base fragments of the RNA probe were protected from RNase digestion by hybridizing with GH-R and GH-BP mRNAs, respectively. The protected fragments were separated by electrophoresis on a 7 M urea-6% polyacrylamide gel, and the radioactivity of the fragments were measured in a Fuji BAS1000 imaging analyzer. The serum GH concentration was measured by the standard double antibody radioimmunoassay (RIA) protocol. These data were analyzed with the Macintosh SuperANOVA program. The significance of differences among the values was determined by Dunnett's post-hoc procedure test after performing the Bartlett test.

Results

The serum concentration of GH continuously increased in pregnancy, reaching the levels 20-30 times higher than that of the control (nulliparous) rat, and immediately decreased after parturition (Table 1). Fig. 1 shows the relative expression levels of both GH-R and GH-BP mRNAs in various tissues during pregnancy and lactation. The expression of GH-R mRNA in the liver and ovary increased during pregnancy, being highest on day 14, and a...
transient increase in the expression also appeared on 8 day after delivery. In the intestines, the level of expression of GH-R mRNA rose continuously until late pregnancy then decreased gradually after parturition. In the kidney, the mRNA expression increased during pregnancy and the high expression levels were maintained during lactation. In the mammary gland, the level of expression of GH-R mRNA during pregnancy was maintained at the same level as that in the control rat, and immediately decreased after parturition. In the uterus, the level of expression was rather low.

Table 1. Serum GH concentration during pregnancy and lactation

<table>
<thead>
<tr>
<th>Day (Number)</th>
<th>Control (8W)</th>
<th>Pregnancy</th>
<th>Lactation</th>
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<tbody>
<tr>
<td></td>
<td>(9)</td>
<td>7 (7)</td>
<td>3 (7)</td>
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<td></td>
<td>1.49 ± 0.64</td>
<td>6.49 ± 2.17*</td>
<td>3.57 ± 1.52</td>
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*P<0.05 vs. control level, **P<0.01 vs. control level.
during pregnancy and gradually increased after delivery. No significant change in GH-R mRNA expression was observed in the cerebrum, heart or adrenal gland throughout pregnancy and lactation (data not shown). The levels of expression of GH-BP mRNA during pregnancy and lactation were almost the same as those of GH-R mRNA in all rat tissues examined.

Discussion

Levels of expression of GH-R and GH-BP mRNAs were examined by RNase protection assay in various rat tissues such as liver, ovary, intestine, kidney, mammary gland and uterus. As shown in Fig. 1, levels of GH-R and GH-BP mRNAs are synergistically changed during pregnancy and lactation in all the tissues, but the profiles of expression of both mRNAs vary among tissues, indicating that the synthenses of GH-R and GH-BP mRNAs in these tissues are differentially regulated. In rodents, GH-R and GH-BP mRNAs are known to be generated from a single precursor RNA by alternative splicing [4]. Both mRNAs are generated at nearly equal molar ratios in tissues except for liver where the amount of GH-BP mRNA is larger than that of GH-R mRNA, especially in late pregnancy. It has been reported that the serum GH-BP level increases during pregnancy [5, 6]. The level of expression of GH-BP mRNA in the liver during pregnancy is much higher than that in other tissues, suggesting that the liver is the major source of serum GH-BP.

The well known function of GH is promotion of postnatal body growth, mainly by stimulating the synthesis of insulin-like growth factor-I (IGF-I) in the liver, and also by stimulating the synthesis of IGF-I in other tissues, where IGF-I acts as an autocrine or paracrine factor. The serum GH level strikingly increases during pregnancy (Table 1), whereas the serum IGF-I level and its mRNA expression in the liver decrease during that stage [7]. On the other hand, the expression of GH-R mRNA increased not only in the liver but also in the ovary, intestine and kidney during pregnancy. The physiological function of GH in these tissues during pregnancy remains to be clarified.

References