Sex differences in binding of human growth hormone to isolated rat hepatocytes

(hormone receptors/prolactin/estrogen effect)

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ABSTRACT Since liver is a target for growth hormone action, binding of 125I-labeled human growth hormone to enzymatically isolated rat hepatocytes was studied. Specific binding was shown with hepatocytes from both male and female animals. There was a single class of receptors for human growth hormone on cells from males (affinity constant, $K_a = 1.18 	imes 10^8$ liters/mole; sites per cell, $q = 6800$). In females, bovine growth hormone was almost as potent as human growth hormone in displacing bound 125I-labeled human growth hormone, while ovine prolactin was about 1000 times less potent.

Cells from female rats bound more 125I-labeled human growth hormone than cells from males. The cells from females contained at least two classes of receptors for human growth hormone. The receptor of highest affinity had the same affinity for human growth hormone as the single receptor found in males ($K_a = 0.96 	imes 10^8$ liters/mole). However, there were three to four times as many of these receptors per cell in females ($q = 21,000$). In females, bovine growth hormone and ovine prolactin were both about 20 times less potent than human growth hormone. Treatment of male rats with estrone produced cells that showed the same binding characteristics as females.

These results indicate that human growth hormone binds to a somatogenic receptor in hepatocytes from male rats. In females and estrogen-treated males, the receptors that bind human growth hormone recognize lactogenic as well as somatogenic properties. This suggests that the lactogenic and growth-promoting effects of human growth hormone in the rat are mediated by different receptors.

The effects of growth hormone on skeletal growth appear to be mediated by somatomedins (1). When 125I-labeled human growth hormone (hGH) is injected in rats in vivo, much of the activity is localized in the liver (2). Somatomedin is produced when rat liver is perfused with growth hormone (3, 4). These findings suggest that liver is a target organ for growth hormone. The first step in the action of polypeptide hormones is binding to specific receptors on the surface of target cells. Recently, binding of 125I-labeled hGH to cell membranes prepared from livers of rabbits, rats, and other species has been reported (5-8). Binding to rat liver membranes could only be demonstrated using female ovariectomized, estrogen-treated male animals. In these membrane preparations, ovine prolactin (oPRL) was identical to hGH in displacing bound labeled hGH (8). It was therefore concluded that hGH binds to a lactogenic receptor in rat liver. The present study was carried out to examine the binding characteristics of hGH to rat liver using enzymatically isolated rat hepatocytes. Differences in males and females and effects of estrone treatment in male animals on binding of 125I-labeled hGH were studied, leading to models for growth hormone receptors in the rat liver.

MATERIALS AND METHODS

Animals. Sprague-Dawley rats, obtained from Charles River, Inc. and weighing 150-250 g, were starved overnight prior to preparation of isolated hepatocytes. Estrone-treated male rats were given 50 μg of estrone diluted in corn oil (8) by subcutaneous injection for 10 days.

Hepatocytes. Suspensions of isolated hepatocytes were prepared by enzymatic digestion of perfused liver with collagenase (Worthington, crude bacterial collagenase, Type I) using the method of Berry and Friend (9) modified by Krebs et al. (10). The yield of cells from one 200-g rat was about 20 ml of 10-15% (vol/vol) suspension or about 200 x 10⁶ cells. The suspension consisted largely of single isolated cells with a few clumps of 2 to 10 cells. Viability, as judged by exclusion of 0.4% Trypan blue, was 90-95%. For radioreceptor studies the cells were centrifuged and resuspended in Tris-HCl buffer (pH 7.35), consisting of 25 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1.2 mM MgSO₄, 1 mM EDTA, 10 mM dextrose, 15 mM Na-acetate, and 0.1% bovine serum albumin.

Hormones. Human growth hormone (NIH HS 1523D) was used for iodination. A preparation of hGH from Kabi/AS/Stockholm (lot no. 1620-58383) was used for unlabeled hormone. This preparation was shown to be equal in potency to the NIH preparation by radioimmunoassay as well as in a radioreceptor assay using cultured human lymphoid cells (11). Other hormones used were: bovine growth hormone (bGH) (NIH GH-B; 0.92 IU/mg; lactogenic activity not specified); oPRL (NIH PS 10; 25 IU/mg; growth hormone activity less than 0.01 IU/mg); porcine insulin (Lilly, lot no. 615-108253-1081); porcine glucagon (Lilly, lot no. 25802348-167-1); and bovine parathormone (Dr. Howard Rasmussen, PC 72 53-65). All dilutions were made in Tris-HCl buffer.

Iodination of hGH. 125I-Labeled hGH was prepared by a modification of the chloramine-T method of Hunter and Greenwood (12). The reaction mixture consisted of 1 mCi of carrier-free Na[125I] (Nuclear Chicago Corp.), 5 μg of hGH, 0.01 ml of 1.0 M Na₂PO₄ - buffer, pH 7.4, and 4 μg of chloramine T in a total volume of 0.034 ml. The reaction was stopped after 15 sec by addition of 8 μg of Na₂S₂O₅ and 0.2 ml of 5% bovine serum albumin solution. Labeled hGH was separated from free 125I by gel filtration on Sephadex G-75 (column size: 0.5 x 12 cm) using Tris-HCl buffer. The specific activity of the 125I-labeled hGH used was 120 μCi/μg. Further purification of the labeled hormone was achieved.

Abbreviations: hGH and bGH are, respectively, human and bovine growth hormone; oPRL is ovine prolactin; EDSA₀, dose at which displacement is half-maximal.
**RESULTS**

Optimization of assay conditions

As shown in Fig. 1, binding of $^{125}$I-labeled hGH to isolated rat hepatocytes was time and temperature dependent. Maximum specific binding at 15°, 22°, and 37° was reached by 120 min and remained stable for an additional 120 min. Specific binding was greatest at 22°. Little variation in binding was noted between pH 7.1 and 7.6. Specific binding of labeled hGH was directly proportional to the cell concentration over a range of 2 to 12 x $10^6$ cells per ml.

To test degradation of labeled hormone during incubation with hepatocytes, identical amounts of $^{125}$I-labeled hGH were incubated with 5 x $10^6$ hepatocytes and with equal volumes of buffer alone for 120 min at 22°. Aliquots of the supernatants were used for binding to fresh hepatocytes and an excess of anti-hGH antibody. After preincubation with hepatocytes, binding to fresh cells was 96% of the control and binding to anti-hGH antibody was 98% of the control.

Displacement of $^{125}$I-labeled hGH from hepatocytes by unlabelled hormones

Hepatocytes from males, females, and estrone-treated males demonstrated specific binding of $^{125}$I-labeled hGH. As shown in Fig. 2, the initial binding was similar in females and estrone-treated males, but higher than in males. There was significant displacement of bound $^{125}$I-labeled hGH by 2.9 ng/ml (1.47 x $10^{-10}$ M) of unlabeled hGH. Porcine insulin, porcine glucagon, and bovine parathormone, at concentrations up to 1.7 μg/ml, failed to displace bound labeled hGH.
The dose-response curves for hGH, bGH, and oPRL using hepatocytes from males, females, and estrogen-treated males are shown in Figs. 3, 4, and 5, respectively. Both bGH (30 μg/ml) and oPRL (177 μg/ml) would completely displace specifically bound 125I-labeled hGH in all three groups of animals. Computer analysis of these results using a logit-logarithm transformation showed that the curves for bGH and hGH were parallel in males, but were not parallel in females and estrogen-treated males. The curves for oPRL were not parallel to those for hGH in any of the three groups. This invalidated the use of parallel line potency estimates. Instead, potencies were expressed as dose levels resulting in half-maximal displacement of bound 125I-labeled hGH (ED₅₀). As shown in Table 1, the ED₅₀ for hGH was lower in males (22 ng/ml) than in females (40 ng/ml, P < 0.001). Males and females also differed in the potencies of bGH (48 compared to 990 ng/ml) and of oPRL (32,000 compared to 750 ng/ml). In estrone-treated males, the potencies of the three hormones were similar to those found in females.

**Scatchard plot analysis**

Shown in Fig. 6 are Scatchard plots (14) for hGH binding to hepatocytes from a male, a female, and an estrone-treated male rat. The plot is linear in the male, indicating the presence of a single class of receptors. In the females and estrone-treated males, curvature of the plots indicates the presence of two or more classes of receptors.

The Scatchard plots from each of the experiments in six males, eight females, and four estrone-treated males were calculated and analyzed using the computer program previously described (13). This program permits a choice, based on residual variance and objective F-test, among three models for the best fit of the data: a two-parameter model corresponding to a single class of noninteracting binding sites; a three-parameter model corresponding to one class of satura-

**Table 1. Relative potency of hGH, bGH, and oPRL in competing with 125I-labeled hGH for binding to isolated rat hepatocytes:** Dose at which displacement is half-maximal (ED₅₀)*

<table>
<thead>
<tr>
<th></th>
<th>hGH</th>
<th>bGH</th>
<th>oPRL</th>
</tr>
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<tbody>
<tr>
<td>Male</td>
<td>22.35†</td>
<td>48.1</td>
<td>32,000</td>
</tr>
<tr>
<td></td>
<td>(17.3–28.9)</td>
<td>(40.5–60.3)</td>
<td>(26,000; 40,000)</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(2)</td>
<td>(2)</td>
</tr>
<tr>
<td>Female</td>
<td>39.73†</td>
<td>990</td>
<td>750</td>
</tr>
<tr>
<td></td>
<td>(33.0–50.1)</td>
<td>(250–3880)</td>
<td>(170–3250)</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>Estrone</td>
<td>32.12†</td>
<td>206.7</td>
<td>2880</td>
</tr>
<tr>
<td>treated</td>
<td>(17.2–60.0)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>males</td>
<td>(4)</td>
<td>(1)</td>
<td>(1)</td>
</tr>
</tbody>
</table>

*Mean (ng/ml); in parentheses, 95% limits and number of experiments. Means and 95% confidence limits calculated from logarithms to approximate a normal distribution.
† For hGH: male compared to female, P < 0.001; male compared to estrogen-treated males, not significant; female compared to estrogen-treated males, not significant.
The binding of $^{125}$I-labeled hGH to isolated hepatocytes is compatible with the presence of physiologically important hormone receptors on the surface of liver cells. Dose-response curves for hGH in males, females, and estrone-treated males show significant displacement of $^{125}$I-labeled hGH at physiologic dose levels. The affinity constants and number of binding sites per cell in all three groups of animals are of the same order of magnitude found for other polypeptide hormone receptors (11, 15).

Hepatocytes prepared from male and female rats show differences in binding of $^{125}$I-labeled hGH. Linearity of the Scatchard plots of hGH binding to male hepatocytes indicates the presence of a single class of receptors. In rodent bioassays, hGH has been shown to have both growth-promoting (somatogenic) (16) and lactogenic (17) effects. Bovine growth hormone has somatogenic (17) but not lactogenic effects and oPRL has only lactogenic effects (18). Binding of bGH to hepatocytes from male rats is nearly identical to binding of hGH, as indicated by similar potency estimates and parallel dose-response curves for the two hormones. In contrast, oPRL is 1000-fold less potent than hGH. Displacement of $^{125}$I-labeled hGH by bGH but not oPRL at physiologic concentrations suggests that membrane receptors on hepatocytes from male rats recognize only the somatogenic properties of hGH.

The interactions of hGH with hepatocytes from female rats are considerably more complex. Total binding of hGH is greater than to male hepatocytes. Scatchard plots of hGH binding are nonlinear. This indicates either the presence of two or more classes of receptors with different affinities for hGH or a negative cooperative interaction of the hormone with its receptor, such as has been shown for insulin binding to lymphocytes (19). If the former interpretation is correct, the higher affinity site has the same affinity constant for hGH as the single site in males. Estimates of the number of such receptors per cell are higher than for males. The number and affinity of the secondary class of receptors for hGH cannot be estimated from the present data.

With hepatocytes from female rats, bGH and oPRL are approximately equipotent in displacing bound $^{125}$I-labeled hGH. In contrast to results using male cells, the $ED_{50}$ of bGH is 20-fold greater than that of hGH and the displacement curves are not parallel. Ovine prolactin is much more potent in displacing $^{125}$I-labeled hGH from female than from male cells. However, the $ED_{50}$ of ovine prolactin is also 20-fold greater than that of hGH and the displacement curves are not parallel. Since physiologic concentrations of either bGH or oPRL give partial displacement of $^{125}$I-labeled hGH, the binding of hGH in females appears to combine both somatogenic and lactogenic properties.

The characteristics of $^{125}$I-labeled hGH binding to hepatocytes from female rats suggest the presence of two distinct classes of binding sites with differing affinities and specifici-

### DISCUSSION

The binding of $^{125}$I-labeled hGH to isolated hepatocytes is compatible with the presence of physiologically important hormone receptors on the surface of liver cells. Dose-response curves for hGH in males, females, and estrone-treated males show significant displacement of $^{125}$I-labeled hGH at physiologic dose levels. The affinity constants and number of binding sites per cell in all three groups of animals are of

<table>
<thead>
<tr>
<th>No. of expts.</th>
<th>$K \times 10^{-9}$ (liters/mol)</th>
<th>Sites/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>6</td>
<td>1.16† (0.75–1.78)</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>0.96† (0.64–1.46)</td>
</tr>
<tr>
<td>Estrone-treated males</td>
<td>4</td>
<td>1.45† (0.50–4.18)</td>
</tr>
</tbody>
</table>

* Mean and 95% limits are given. Mean and 95% confidence limits calculated from logarithms to approximate a normal distribution.
† Differences between groups, not significant.
‡ Males compared to females, $P < 0.005$; males compared to estrone-treated males, $P < 0.005$; females compared to estrone-treated males, not significant.

**Table 2. Affinity constants ($K$) and number of sites* for binding of hGH to isolated rat hepatocytes**
ties. In this model, the first site has a higher affinity for hGH, recognizes the somatogenic properties of hGH, and recognizes the lactogenic hormone, bGH. This site may be identical to the receptor found in males. The second site has a lower affinity for hGH, recognizes the lactogenic properties of hGH, and recognizes the lactogenic hormone, oPRL. This site is lacking in males but can be induced by estrogen treatment. The fact that oPRL at high concentrations completely displaces $^{125}$I-labeled hGH in females, as in males, suggests some weak interaction with the first site. This may represent trace contamination of the oPRL or weak intrinsic activity of the oPRL molecule. A similar weak interaction of bGH with the second site is suggested by the fact that bGH at high concentrations also completely displaces $^{125}$I-labeled hGH in females. While the two-site model is consistent with the experimental data, validation of the model and full characterization of the postulated second class of receptors will require experiments using labeled oPRL.

The postulated second site for hGH binding to hepatocytes from female and estrogen-treated male rats may be identical to the "lactogenic" receptor reported by Posner et al. (8) for rat liver membranes. However, liver membranes from untreated male rats did not bind $^{125}$I-labeled hGH and there was no evidence for a separate site with somatogenic specificity in liver membranes from female rats. The demonstration of "somatogenic" binding sites in rat hepatocytes is consistent with the hypothesis that the liver is a target organ for the growth promoting properties of growth hormones and suggests that the lactogenic and somatogenic effects of hGH in rats are mediated by different receptors.

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