NOTE

Induction of Specific Human Growth Hormone Binding Sites in Rat Liver by Human Growth Hormone

KAZUE TAKANO, NAOMI HIZUKA, KOICHI KAWAI,
AND KAZUO SHIZUME

*Department of Medicine Tokyo Women's Medical College
Shinjuku-ku, Tokyo 162, Japan*

Synopsis

¹²⁵I-Labeled hGH was bound to liver plasma membranes which were obtained from female rats. The binding was displaced by hGH, hPRL, bPRL, rPRL and bGH but not by rGH. This result indicated that hGH was bound to lactogenic binding sites in rat livers. After hypophysectomy, the binding was markedly decreased. Treatment of hypophysectomized rats with hGH (80 µg/day) for 10 days increased the binding sites for hGH. These binding sites were different from those found in normal female rat livers because of their high affinity and specificity for hGH. These results indicate that hGH induces specific binding sites for hGH in rat livers.

The initial step in the action of polypeptide hormone is binding to specific receptors on the surface of target cells. Recently, the binding of ¹²⁵I-labeled hGH to cell membranes prepared from livers of rabbits, rats and other species has been reported (Tsushima and Friesen, 1973; Posner *et al.*, 1974; Kelly *et al.*, 1974; Carr and Friesen, 1976; Posner, 1976a). Binding to liver membranes could only be demonstrated by using female or estrogen-treated male rats. In these membrane preparations, ovine prolactin was identical with hGH in displacing labeled bound-hGH (Posner *et al.*, 1974). It has been reported that hGH binding to rat liver membranes was decreased by hypophysectomy and restored by renal implantation of pituitaries (Posner *et al.*, 1975; Posner, 1976b), but was not increased by treatment with bGH (Herington *et al.*, 1976a). Therefore, whether hGH played a role in inducing its own binding site in rat livers was investigated in the present study.

Materials and Methods

Normal male and female Sprague-Dawley rats were used and were divided into 3 groups: Group I consisted of 5 male and 5 female rats of 52 days old which were used as controls; Group II consisted of 4 male and 6 female rats which were hypophysectomized at 28 days of age and were sacrificed 24 days later; Group III consisted of 4 male and 6 female rats which were hypophysectomized at 28 days of age and to which hGH was administered ip 80 µg per day for 10 days beginning on the 40th day and which were sacrificed on the 52nd day. At the time of sacrifice, livers and kidneys were quickly removed and kept frozen (-70°C) until studied.

Rat livers or kidneys were homogenized in 0.25 mol per liter ice-cold sucrose and crude membranes were prepared by stepwise ultracentrifugation according to the method described by Cuatrecasas (1972). The membrane fraction sedimenting between 12,000×g and 40,000×g was used for the binding study.

Hormone binding was measured in triplicate in a reaction mixture consisting of 100 µl 0.05 mol per liter Tris-HCl buffer containing 1% human serum albumin with or without unlabeled hormone, 100 µl membrane preparation (250 µg protein), and 100 µl ¹²⁵I-hGH (20,000 cpm). In some experiment indicated in the results, pooled membranes were used. After 16 hr of incubation at 4°C, bound and free hormones were separated by centrifugation at 6,000×g for 15 min, and membrane-bound ¹²⁵I-hGH in the

pellet was counted in a well-type gamma spectrometer. The binding of labeled hGH was time- and temperature-dependent. At 4°C, the maximal binding was obtained after 16 hr when 12% of the total activity was bound.

Protein concentration was determined by the method of Lowry *et al.* (1951).

A preparation of hGH, Crescormone (R), from AB Kabi/Recip, Stockholm, Sweden, was used for injection and assay of hGH. This preparation contains 2IU per mg of hGH and is essentially free from ACTH, FSH, LH, TSH, vasopressin, oxytocin and polymers of GH. Other hormones used were: bovine GH (NIH-GH-B18); rGH (NIAMD-RAT GH-B-4); bPRL (PROLACTIN, BOVINE, NIH-P-B4); rPRL (NIAMDD-RAT PROLACTIN-B-1). Human GH (NIH-GH-HS 2160E) and hPRL (NIH-hPRL-VLS-4) were iodinated by the chloramine-T method (Hunter and Greenwood, 1962) with a specific activity of $100 \pm 50 \mu\text{Ci}/\mu\text{g}$ and $50 \pm 10 \mu\text{Ci}/\mu\text{g}$, respectively.

Results

The binding of ^{125}I -hGH to liver membrane was a saturable process with respect to the increased membrane concentration in the medium. Membranes prepared from normal female and male rats bound 10.8 ± 2.5 and $2.2 \pm 0.15\%$ (mean $\pm$ SEM) ^{125}I -hGH, respectively, when 1 mg/ml membrane protein was used. A significant displacement was obtained with $3.3 \mu\text{g}/\text{ml}$ of hGH in normal female rat livers but not in male rat livers indicating that the binding of ^{125}I -hGH to male rat liver was not specific. There were no specific binding sites for hGH to kidney membranes obtained from normal male and female rats.

After hypophysectomy (Group II), the binding of ^{125}I -hGH to liver membranes per mg protein of female rats decreased to $2.8 \pm 0.18\%$ which was not significantly displaced by $3.3 \mu\text{g}/\text{ml}$ of hGH. There was no change in the binding of ^{125}I -hGH to liver membranes obtained from hypophysectomized male rats. After hGH treatment for 10 days, the binding of ^{125}I -hGH to liver membranes per mg protein increased in livers obtained from both female and

male rats from 2.8 ± 0.18 and 2.6 ± 0.11 to 8.5 ± 1.6 and $11.8 \pm 2.1\%$, respectively. No significant difference for ^{125}I -hGH binding to liver membranes was observed between hGH-treated male and female rats. There was no specific binding of hGH to the kidney membranes obtained from Group II and III.

The ^{125}I -hGH bound to normal female rat livers was displaced by hGH. A linear logdose-response curve was obtained between 13 and $833 \text{ ng}/\text{ml}$ in normal female rat livers (Fig. 1). Human PRL, bPRL and rPRL in similar doses also displaced ^{125}I -hGH from its binding sites. Bovine GH in larger doses displaced ^{125}I -hGH from its binding sites; however, rGH did not displace ^{125}I -hGH even when a concentration of $167 \mu\text{g}/\text{ml}$ was used. The Scatchard plot (Scatchard, 1949) of the binding of hGH to liver membranes from normal female rats was concave. Assuming two classes of binding sites, the affinity constant and capacity of high affinity binding sites were $2.3 \pm 0.05 \times 10^8 \text{ M}^{-1}$ and $602.6 \pm 142 \text{ fmole}/\text{mg}$ protein, respectively,

The ^{125}I -hGH bound to liver membranes

Fig. 1. The effect of hGH, bGH, rGH, hPRL, bPRL and rPRL on the binding of ^{125}I -hGH to pooled liver membranes ($800 \mu\text{g}/\text{ml}$ membrane protein used) from normal female rats. Nonspecific binding was not subtracted. Each point shows the mean of the triplicate.

obtained from hGH-treated male and female rats was displaced by hGH and a linear logdose-response curve was obtained between 0.81 and 52 ng/ml. (Fig. 2). The dose of hGH required for 50% displacement of ^{125}I -hGH was 150 times less than that required in normal female rat livers. Human prolactin in high doses was able to displace ^{125}I -hGH from its binding sites; however, rGH, bGH, rPRL and bPRL did not displace ^{125}I -hGH even when concentrations as high as 33 $\mu\text{g}/\text{ml}$ were used as shown in Fig. 2. The Scatchard plot of the binding of hGH was concave also in the hGH-treated rats. Assuming two classes of binding sites, the calculated affinity constant of high-affinity binding sites in female and male rats were 1.07 ± 0.19 and $1.30 \pm 0.07 \times 10^9 \text{M}^{-1}$, respectively, and there were 111.2 ± 14.1 and 90.3 ± 16.4 fmoles of binding sites per mg of membrane protein, respectively. There was no significant difference in these parameters between female and male rats.

Fig. 2. The effect of hGH, bGH, rGH, hPRL, bPRL and rPRL on the binding of ^{125}I -hGH to pooled liver membranes (800 $\mu\text{g}/\text{ml}$ membrane protein used) from hGH-treated male rats. Nonspecific binding was not subtracted. The point shows the mean of the triplicate.

Discussion

In this study specific binding sites of hGH in liver membranes of normal female rats were demonstrated, which decreased after hypophysectomy. It was also shown that different binding sites were induced by the administration of hGH to hypophysectomized rats.

Human GH has a potency of both somatogenic and lactogenic effect. The binding of hGH to liver membranes from normal female rats was the result of binding to lactogenic sites, which was observed only in female rats and was similarly displaced by hGH, hPRL, bPRL and rPRL. These results confirmed earlier reports by others (Posner *et al.*, 1974; Herington *et al.*, 1976b). The binding of hGH was also displaced by bGH. However, higher doses of bGH were required in comparison with those of bPRL to get the same amount of displacement of ^{125}I -hGH. Therefore, we think the displacement of bGH resulted from bPRL which was contaminated in this bGH preparation.

The binding sites of hGH in liver membranes induced by hGH treatment in hypophysectomized rats were quite different from those of normal female rats in their specificity and affinity. The affinity constant was five times higher in comparison to that of the binding sites of hGH in normal female rats. They were not significantly displaced by bGH, bPRL, rGH and rPRL at the concentration used in this study. Human PRL interfered with this binding site, but were about 1000 times higher doses of hPRL were required to get the similar displacement compared with those of hGH. Furthermore, ^{125}I -hPRL did not bind to this binding site (data not shown). Therefore, the binding site was not a lactogenic site. The displacement of this binding site by hPRL might be because of the contamination of hGH in hPRL pre-

paration. However, the direct effect of hPRL on this binding sites was also probable.

We have concluded that high affinity, low capacity binding sites specific for hGH were induced by hGH and this binding site was only interfered by hPRL in higher concentration. Other pituitary hormones besides hGH, which were contained in this hGH preparation used in this study, might induce this specific binding sites. However, contamination with other pituitary hormones was negligible, thus the possibility was extremely unlikely.

Contamination of antibody against hGH in membrane fraction might produce similar findings. However such specific binding was observed only in liver membranes and not in kidney membranes which were obtained by the same procedures. This result ruled out the above possibility.

Binding sites for insulin in various tissues were observed to be decreased during chronic elevation of insulin levels (Kahn *et al.*, 1973; Gavin *et al.*, 1974). Similar results were obtained in the studies of the effect of hGH on lymphocyte hGH receptors (Lesniak *et al.*, 1974). However, Posner (1976b) reported the chronic elevation of prolactin-induced specific lactogenic-binding sites in rat livers. Herington *et al.* (1976a) reported that treatment of hypophysectomized rats with 500 μ g of bGH for 5 days did not result in the increased binding of 125 I-hGH to liver membranes. We did not find increased hGH-binding in livers of hypophysectomized rats treated with 500 μ g of rGH for 5 days (unpublished data). On the other hand, we have found that hGH induced specific hGH-binding sites in rat livers. Tsushima *et al.* observed that hGH, but not bGH or hPRL induced binding sites for hGH in rabbit livers (Tsushima, 1976, 1977). These data suggest that the induction of hGH binding sites is specific for hGH. However, the physiological role of this finding is yet to

be elucidated.

Acknowledgements

The authors thank Dr. A. Takagi of Dainabot Company for providing us the labeled hGH and hPRL.

This study was supported by a Research Grant from the Intractable Diseases Division, Public Health Bureau, Ministry of Health and Welfare, Japan.

References

- Carr, D. and H. G. Friesen (1976). *J. Clin. Endocrinol. Metab.* 42, 484.
- Cuatrecasas, P. (1972). *Proc. Natl. Acad. Sci. USA* 69, 318.
- Gavin, J. R., J. Roth, D. M. Neville, Jr., P. DeMeyts and D. N. Buell (1974). *ibid.* 71, 84.
- Herington, A. C., L. S. Phillips and W. H. Daughaday (1976a). *Metabolism* 25, 341.
- Herington, A. C., N. Veith and H. G. Burger (1976b). *Biochem. J.* 158, 61.
- Hunter, W. H. and F. C. Greenwood (1962). *Nature* 194, 495.
- Kahn, C. R., D. M. Neville, Jr. and J. Roth (1973). *J. Biol. Chem.* 248, 244.
- Kelly, P. A., B. I. Posner, T. Tsushima and H. G. Friesen (1974). *Endocrinology* 95, 532.
- Lesniak, M. A., A. R. Bianco, J. Roth and J. R. Gavin III (1974). *Clin. Res.* 22, 343 A.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall (1951). *J. Biol. Chem.* 193, 265.
- Posner, B. I. (1976a). *Endocrinology* 98, 645.
- Posner, B. I. (1976b). *ibid.* 99, 1168.
- Posner, B. I., P. A. Kelly and H. G. Friesen (1974). *Proc. Natl. Acad. Sci. USA* 71, 2407.
- Posner, B. I., P. A. Kelly and H. G. Friesen (1975). *Science* 188, 57.
- Posner, B. I., P. A. Kelly, R. P. C. Shiu and H. G. Friesen (1974). *Endocrinology* 95, 521.
- Scatchard, G. (1949). *Ann. N.Y. Acad. Sci.* 51, 660.
- Tsushima, T. and H. G. Friesen (1973). *J. Clin. Endocrinol. Metab.* 37, 334.
- Tsushima, T. (1976). *Folia. Endocrinol. Japon.* 52, 259, (Abstract, in Japanese).
- Tsushima, T. (1977). *Clin. Endocrinol. (Tokyo)* 25, 143, (In Japanese).