PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **186**, 229–233 (1987)

GH Binding to Liver in Young and Old Female Rats: Relation to Somatomedin-C Secretion[1] (42608)

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Abstract. Age-related changes in binding of ^{125}I-bovine GH to liver membrane fractions were measured in female Long–Evans rats 2, 6, 12, and 20 months of age. Specific GH binding did not change between 2 and 6 months of age but increased significantly at 12 and 20 months of age. Scatchard analyses showed that the plots were curvilinear and consisted of high- and low-affinity binding sites. The age-related increases in binding sites were mainly due to an increase in number of low-affinity binding sites. Serum somatomedin-C (SM-C) levels in 20-month-old rats were about half those in the 6-month-old rats. Twice daily injections of ovine GH (2 mg/kg body wt) for 7 days depressed liver GH binding and increased serum SM-C levels in 19-month-old female rats, but had no effect on GH binding in 2-month-old female rats. These results suggest that the increase in liver GH binding sites and the decrease in SM-C secretion are associated with our previously reported decrease in GH secretion in old female rats.

Growth hormone (GH) is the most important protein anabolic agent in the body and is essential for protein synthesis throughout the lifespan. The protein-anabolic actions of GH are believed to be mediated mainly via somatomedins which are produced primarily by the liver in response to GH stimulation (1, 2). GH is normally secreted in pulses (3, 4) and has been shown to decline with age in male (5) and female (6) rats. Plasma levels of somatomedins, when measured by bioassay or radioreceptor assay, also decrease with age in the rat (7, 8), probably reflecting the decline in GH secretion during aging.

The ontogeny of liver GH binding in male and female rats has been studied from birth to sexual maturity (9), but there are no reports on possible changes in GH binding in the liver of old rats. It was of interest, therefore, to determine whether there were changes in liver GH receptors in female rats of different ages, and whether such changes may be related to somatomedin-C (SM-C) secretion. The effects of GH administration on liver GH receptors and SM-C secretion in old and young female rats were also investigated.

Materials and Methods. *Animals.* Long–Evans female rats were obtained from Harlan Sprague–Dawley, Inc. (Indianapolis, IN). They were housed in a temperature-controlled room (22°C) on a 14:10 light:dark cycle (lights on at 0700 h), and food and water were provided *ad libitum.* Daily vaginal smears were collected for at least 2 weeks prior to initiation of experiments. Young estrous and middle-aged and old constant estrous females were used in order to equalize possible effects of the estrous state on GH receptors and SM-C secretion by the liver.

GH receptor preparation. Preparation of GH receptors from liver was based on the method of Posner *et al.* (10). The animals were decapitated and the liver was quickly removed, weighed, and rinsed in ice-cold 0.3 *M* sucrose. After rinsing, the liver was minced and homogenized with a Teflon–glass homogenizer in 10 vol (w/v) of 0.3 *M* sucrose. The homogenate was centrifuged at a speed of 600*g* for 15 min and the resultant supernatant was centrifuged at 15,000*g* for 20 min. The supernatant was then ultracentrifuged at 100,000*g* for 60 min. The resultant pellet was suspended in

[1] The research reported here was aided in part by NIH Grant AG00416 from the National Institute on Aging. We thank Dr. Victoria Kingsbury for editorial assistance and typing of the manuscript, and Rhonda Brown for technical assistance.

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0037-9727/87 $1.50

Tris–Ca^{2+} buffer (25 m*M* Tris–Cl, 10 m*M* $CaCl_2$, pH 7.4). Protein was measured by the modified Lowry method of Hartree (11) using bovine serum albumin (BSA) as the reference standard.

Iodination of bGH. Bovine GH (NIADDK-I-1) was iodinated by a modified lactoperoxidase–glucoseoxidase method. The labeled hormone was purified on a 1 × 40-cm Sephadex G-75 column equilibrated with 0.01 *M* phosphate buffer (pH 7.4). Specific activity of labeled bGH was 91 μCi/μg.

Binding studies. All binding studies were carried out in triplicate by competitive binding techniques using polypropylene tubes (12 × 75 mm) prewashed with 5% BSA. The reaction mixture (500 μl) consisted of a 100 μl membrane preparation (300 μg of membrane protein), 100 μl of ^{125}I-labeled bGH (approximately 100,000 cpm), 100 μl of labeled bGH (500 ng, NIADDK-bGH-B1) when required, and 25 m*M* Tris–Cl (pH 7.4) containing 10 m*M* $CaCl_2$ and 0.1% BSA. Incubation was continued at room temperature (23°C) for 20 hr with constant shaking. The reaction was terminated by the addition of 3 ml of ice-cold incubation buffer. Bound and free hormones were separated by centrifugation at 2500 rpm for 30 min at 4°C, and the bound ^{125}I-bGH in the precipitate was counted by an autogamma counter (counting efficiency, 64%).

Specific binding was calculated from the difference between total binding and nonspecific binding. Nonspecific binding was determined by incubating labeled bGH with an excess (500 ng/tube) of unlabeled bGH. Specific binding was expressed as a percentage of the total radioactivity of labeled bGH added to the incubation mixture (300 μg membrane protein). In preliminary binding experiments it was found that specific binding ^{125}I-bGH to liver membrane preparations was dependent on time, temperature, pH, and concentrations of labeled hormone and membrane preparation. The maximal binding was reached by 16 hr and no further increase was observed by 42 hr at 23°C. The optimal pH was between 7.0 and 7.4. Hormone specificity was checked by adding various amounts of unlabeled bGH (NIADDK-bGH-B1), rat GH (NIADDK-rGH-I-5), and rat prolactin (NIADDK-rPRL-I-5). Displacement of ^{125}I-bGH binding occurred with 1 ng per tube of bGH and rGH, but no displacement occurred by addition of rat prolactin in the range of 1 to 500 ng per tube.

Scatchard analysis was based on the binding-inhibition experiments (12). Curvilinear plots were derived and were considered to consist of two binding sites (13). The affinity and number of binding sites were analyzed by the method of Rosenthal (14).

In one experiment, membrane preparations were treated with $MgCl_2$ to deplete endogenous ligands, using a modification of the method of Baxter *et al.* (15). Membrane preparations (4 mg protein in 1 ml Tris buffer, pH 7.4) were mixed with 4 ml Tris–buffer containing 3.8 M $MgCl_2$ (3.0 M final concentration). After 5 min incubation at room temperature, 15 ml ice-cold Tris–buffer containing 10 mM $CaCl_2$ and 0.1% BSA was added to the mixture and centrifuged at 1000*g* for 20 min at 4°C. The resultant precipitate was suspended in the incubation buffer.

Ovine GH effects on GH binding and serum SM-C levels. Ovine GH (oGH) (NIADDK-oGH-13) was dissolved in 0.01 *M* $NaHCO_3$ (pH 8.5) and injected sc twice daily (9:00 AM and 5:00 PM) for 7 days at a dose of 2 mg/kg body weight into 2-month-old estrous rats and 19-month-old constant estrous female rats. Sixteen hours after the last injection, the rats were killed and the liver and trunk blood were collected for assay of SM-C.

Radioimmunoassay (RIA) of SM-C. Serum SM-C levels were measured by RIA using antiserum (CH549/805C) and ^{125}I-SM-C kindly supplied by Drs. Underwood and Van Wyck of the University of North Carolina, and the National Hormone and Pituitary Program, NIH. The reference preparation used was ^{59}Thr-SM-C (AMGen Biologicals, Thousand Oaks, CA). Serum samples were extracted by an acid–ethanol method (16), slightly modified. Serum sample (50 μl) was added to 0.8 ml of a mixture of 87.5% ethanol and 12.5% 2 *N* HCl acid (vol/vol) in 12 × 75-mm polypropylene tubes, and 150 μl of 0.25% BSA. These were allowed to stand at room temperature for 30 min and centrifuged at 3000 rpm for 30 min. The supernatant (50 μl) was transferred to polypropylene tubes containing 700 μl assay buffer (0.05 *M* phosphate buffer, 0.05 *M* NaCl, 0.01 *M* EDTA, 0.002% NaN_3, 0.02% protamine sulfate, 0.25% BSA, pH 7.5).

In the SM-C assays, 100 μl of sample or SM-C standard, 100 μl of antiserum (final dilution, 1:7,000), 100 μl of ^{125}I-SM-C, and 400 μl of assay buffer were added to 12 × 75-mm polypropylene tubes. Incubation was carried out at 4°C for 42 hr. Bound and free hormones were separated with IgG Sorb (The Enzyme Center Inc., Malden, MA); 50% inhibition of binding was 1.1 pg/tube. Since it was necessary to kill the rats in order to remove the liver for binding assays, pulsatile GH secretion could not be measured in these rats.

Statistics. Data for age-related changes in GH binding (Table I) were analyzed by the Kruskal–Wallis method and the Mann–Whitney *U* test. In other experiments, individual differences were analyzed by the Mann–Whitney *U* test.

Results. *GH binding in liver and serum somatomedin-C levels.* Specific GH binding in liver membranes did not change between 2 and 6 months of age, but rose significantly in 12- and 20-month-old female rats (Table I; $P < 0.01$, 6 months vs 12 months). Serum SM-C values in 20-month-old female rats (324.0 ± 23.1 ng/ml, $n = 7$) were significantly lower than those in 6-month-old female rats (640.5 ± 102.2 ng/ml, $n = 6$, $P < 0.01$).

Scatchard plot analyses of specific binding of GH were carried out in 6- and 19- to 20-month-old rats (Fig. 1, Table II). Scatchard plots were curvilinear and consisted of two independent classes of binding sites. Resolution of these curves showed that the higher affinity sites were unchanged, but the number of low-affinity binding sites in the old female rats was significantly higher than that in young control rats ($P < 0.05$).

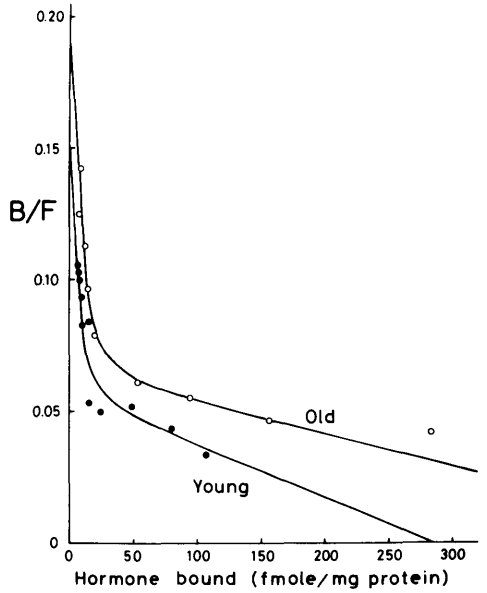

FIG. 1. Typical Scatchard plots of GH binding in liver membrane preparations of a young 6-month-old estrous female rat (●) and an old 20-month-old constant estrous female rat (○). Points represent the means of three determinations.

TABLE I. SPECIFIC BINDING OF ^{125}I-LABELED bGH TO LIVER MEMBRANE PREPARATIONS OF FEMALE RATS OF DIFFERENT AGES

Age (months)	Reproductive state	No. of rats	Specific binding (% of total)[a]
2	Estrus	5	8.13 ± 0.65[b]
6	Estrus	6	7.34 ± 0.69
12	Constant estrus	7	13.64 ± 0.64
20	Constant estrus	7	12.27 ± 0.25

[a] Significant age-related changes were observed in the 12- and 20-month-old as compared to the 2- and 6-month-old rats ($P < 0.001$, Kruskal–Wallis test).

[b] Mean ± SE.

Effect of oGH injections on liver GH binding sites and serum SM-C levels in young and old female rats. Injection of oGH twice daily for 7 days significantly decreased the specific binding of GH in the liver membranes of old females when compared with control values ($P < 0.05$, Table III), but had no effect on liver binding sites in young females. When $MgCl_2$ was added to the incubation mixture, it did not significantly increase the liver binding of GH from oGH-treated rats. Therefore, the decrease in GH binding in the liver of old oGH-treated rats was not due to occupation of binding sites by the exogenously administered oGH. The specific GH binding in liver of old control rats was higher than that in liver of young control rats ($P < 0.01$).

Serum SM-C levels in old control rats were significantly lower than those in young control rats ($P < 0.05$). oGH treatment significantly increased serum SM-C levels in the old rats ($P < 0.05$), but had no effect in the young rats. Thus oGH injections reduced the number of GH binding sites in the liver, but at the same

TABLE II. ASSOCIATION CONSTANTS (K) AND CONCENTRATIONS (n) OF HIGH- AND LOW-AFFINITY GH BINDING SITES IN LIVER MEMBRANE PREPARATIONS IN YOUNG AND OLD FEMALE RATS

Age (months)	Reproductive state	No. of rats	High-affinity sites		Low-affinity sites	
			K_1 (nM^{-1})	n_1 (fmole/mg/protein)	K_2 (nM^{-1})	n_2 (fmole/mg/protein)
6	Estrus	5	8.56 ± 0.91[a]	26.2 ± 4.8	0.25 ± 0.03	253 ± 25
19–20	Constant estrus	4	9.98 ± 1.22	30.5 ± 4.5	0.30 ± 0.10	454 ± 102*

[a] Mean ± SE.

* $P < 0.05$, significantly different from 6-month-old rats.

time increased SM-C secretion in the old females.

Discussion. These results show that GH receptors in the liver membranes of untreated 12- and 20-month-old female rats were significantly greater than those in 2- or 6-month-old female rats, and this increase was mainly due to elevation in the number of low-affinity binding sites. The physiological significance of the differences in the two types of GH binding sites in the liver is unknown at present (13). We recently reported reduced pulsatile GH secretion in middle-aged and old as compared to young female rats (6), but could not measure pulsatile GH secretion in the present study since it was necessary to kill the rats for liver removal. In this study, oGH administration to old female rats reduced the number of liver GH receptors, indicating that an inverse relationship may exist between GH secretion and liver GH receptors in old female rats. It is possible that liver GH receptors increased in the old rats in order to enhance the sensitivity of the liver to the reduced levels of circulating GH (up-regulation).

Serum SM-C levels were lower in the 20-month-old constant estrous females than in the young females, in agreement with the report by Florini *et al.* (7, 8), who observed reduced levels of somatomedins in old as compared to young rats. The significant decrease in serum SM-C levels appears to be associated with the reduction in serum GH levels in the old rats. In elderly humans, a similar age-related decrease in both GH and SM-C secretion was reported (17).

Injections of oGH restored serum SM-C levels in the old female rats to the same levels present in young females, but produced no change in serum SM-C levels in the young females. This suggests that the production of SM-C may already have been maximally stimulated by the high circulating GH levels in the young rats, whereas SM-C secretion was

TABLE III. EFFECT OF OVINE (oGH) INJECTED TWICE DAILY FOR 7 DAYS ON LIVER GH RECEPTORS AND SERUM SM-C LEVELS IN FEMALE RATS

Age (cyclic state, months)	Treatment	No. of rats	% Specific binding (% of total)		Serum SM-C (ng/ml)
			Non-$MgCl_2$ treated	$MgCl_2$ treated	
2 (estrus)	Controls	5	6.72 ± 0.5[a]	7.37 ± 0.96	916.2 ± 56.7
	oGH[b]	5	5.29 ± 0.4	6.17 ± 1.76	902.4 ± 41.7
19 (constant estrus)	Controls	5	12.57 ± 1.4****	17.27 ± 2.55****	608.4 ± 16.7***
	oGH[b]	5	7.99 ± 0.90*	6.42 ± 1.58**	1267.8 ± 105.7**

[a] Mean ± SE.

[b] oGH (2 mg/kg) was given sc twice daily for 7 days.

* $P < 0.05$, ** $P < 0.01$; significantly different from controls of the same age.

*** $P < 0.05$, **** $P < 0.01$; significantly different from the corresponding group of young rats.

not maximally stimulated in the old females because of the low secretion of GH. Several reports have indicated that GH can regulate its own receptors; e.g., infusion of rat GH was reported to induce GH receptors in the liver of young rats (18). In the present study, the liver of young female rats did not respond to oGH injections for the reason already stated above, and perhaps also because of differences in the source of GH hormone, the different method of GH administration, and the influence of other hormones. Both insulin (19) and thyroxine (20) have been reported to alter the binding of GH to liver membranes, and these hormones may be involved in the age-related difference observed in GH receptors in the present study. Roth (21) has suggested that changes in hypothalamic function may be partly responsible for altered receptor levels in some tissues with aging.

1. Vassilopoulou-Sellin R, Phillips LS. Extraction of somatomedin activity from rat liver. Endocrinology **110**: 582–589, 1982.
2. Baxter RC, Zaltsman Z, Oliver JR, Willoughby JO. Pulsatility of immunoreactive somatomedin-C in chronically cannulated rats. Endocrinology **113**:729–734, 1983.
3. Tannenbaum GS, Martin JB. Evidence for an endogenous ultraradian rhythm governing growth hormone secretion in the rat. Endocrinology **98**:540–548, 1976.
4. Saunders A, Terry LC, Audet J, Brazeau P, Martin JB. Dynamic studies of growth hormone and prolactin secretion in the female rat. Neuroendocrinology **21**: 193–203, 1976.
5. Sonntag WE, Steger RW, Forman LJ, Meites J. Decreased pulsatile release of growth hormone in old male rats. Endocrinology **107**:1875–1879, 1980.
6. Takahashi S, Gottschall PE, Quigley KL, Goya RG, Meites J. Growth hormone secretory patterns in young, middle-aged, and old female rats. Neuroendocrinology **46**:137–142, 1987.
7. Florini JR, Roberts SB. Effect of rat age on blood levels of somatomedin-like growth factors. J Gerontol **35**:23–30, 1980.
8. Florini JR, Harned JA, Richamn RA, Weiss JP. Effect of rat age on serum levels of growth hormone and somatomedins: *Mech Aging Dev* **15**:165–176, 1981.
9. Maes M, De Hertogh R, Watrin-Granger P, Ketelslegers JM. Ontogeny of liver somatotropic and lactogenic binding sites in male and female rats. Endocrinology **113**:1325–1332, 1983.
10. Posner BI, Kelly PA, Shiu RPC, Friesen HG, Studies of insulin, growth hormone and prolactin binding: Tissue distribution, species variation and characterization. Endocrinology **95**:521–531, 1974.
11. Hartree EF. Determination of protein: A modification of the Lowry method that gives a linear photometric response. Anal Biochem **48**:422–427, 1972.
12. Scatchard G. The attractions of proteins for small molecules and ions. Ann NY Acad Sci **51**:660–672, 1949.
13. Hughes JP. Identification and characterization of high and low affinity binding sites for growth hormone in rabbit liver. Endocrinology **105**:414–420, 1979.
14. Rosenthal HE. A graphic method for the determination and presentation of binding parameters in a complex system. Anal Biochem **20**:525–532, 1967.
15. Baxter RC, Zaltsman Z, Turtle JR. Rat growth hormone (GH) but not prolactin (PRL) induces both GH and PRL receptors in female rat liver. Endocrinology **114**:1893–1901, 1984.
16. Daughaday WH, Mariz IK, Blethen SL. Inhibition of access of bound somatomedin to membrane receptor and immunobinding sites: a comparison of radioreceptor and radioimmunoassay of somatomedin in native and acid-ethanol-extracted serum. J Clin Endocrinol Metab **51**:781–788, 1980.
17. Florini JR, Prinz PN, Vitiello MV, Hintz RL. Somatomedin-C levels in healthy young and old men: Relationship to peak and 24-hour integrated levels of growth hormone. J Gerontol **40**:2–7, 1985.
18. Baxter RC, Zaltsman Z. Induction of hepatic receptors for growth hormone (GH) and prolactin by GH infusion is sex independent. Endocrinology **115**:2009–2014, 1984.
19. Baxter RC, Bryson JM, Turtle JR. Somatogenic receptors of rat liver: Regulation by insulin. Endocrinology **107**:1176–1181, 1980.
20. Chernausek SD, Underwood LE, Van Wyk JJ. Influence of hypothyroidism on growth hormone binding by rat liver. Endocrinology **111**:1534–1538, 1982.
21. Roth GS. Age-related changes in hormone action: The role of hormone receptors. In: Adelman RC, Roth GS, Eds. Endocrine and Neuroendocrine Mechanisms of Aging. Boca Raton, CRC Press, p51, 1982.

Received March 16, 1987. P.S.E.B.M. 1987, Vol. 186.
Accepted July 14, 1987.