BINDING OF HUMAN GROWTH HORMONE TO RAT LIVER MEMBRANES: LACTOGENIC AND SOMATOTROPIC SITES

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1. Introduction

Binding sites for human growth hormone have recently been identified in various tissues from various species [1–6]. In certain cases, such as with cultured human lymphocytes [5,6] and rabbit liver membranes [1–3], these sites bind exclusively or predominantly growth hormones; in other cases, such as with rat liver membranes, they also bind hormones possessing lactogenic activity [3,4]. On the basis of these findings, the existence of two distinct types of hGH binding sites, 'somatotropic' and 'lactogenic', has been postulated.

Earlier studies [2–4] have shown that, in liver membranes of female rats, the hGH binding sites have almost exclusively 'lactogenic' specificity; it was also demonstrated that the hGH binding capacity increases in female rats after puberty. With liver membranes of male rats, there was much less binding than with those of female animals, and the specificity of the binding sites was not assessed.

In the present studies we demonstrate the existence, in rat liver membranes, of 'somatotropic' sites in addition to the 'lactogenic' sites. In membranes of male rats the somatotropic sites account for virtually all the binding sites for hGH, whereas in those of female animals, they represent only a small fraction of the hGH binding sites. The variation, with sex and age, of the somatotropic sites has been investigated using [^{125}I]bovine growth hormone as the tracer; unlike hGH, bGH does not possess lactogenic activity. In membranes of male rats, the number of somatotropic sites is the same before and after puberty, whereas in female rats, a 2-fold increase in the bGH binding capacity occurs after puberty.

Abbrevations: hGH, Human growth hormone; bGH, Bovine growth hormone; oPRL, Ovine prolactin; SEM, Standard error of the mean.

2. Materials and methods

Rats, male and female of various ages, were obtained from Charles River France. They were given laboratory chow ad libitum until sacrifice.

2.1. *Liver membranes preparation*

Liver membranes ('microsomal fraction') were prepared by differential centrifugation of rat liver homogenates in 0.25 M sucrose solution [7]. Protein concentration was determined by the method of Lowry [8] using bovine serum albumin as a standard.

2.2. *Hormones*

hGH (2 IU/mg) was purified at URIA, Institut Pasteur, Paris, France; bGH was a gift from Dr Martin Sonenberg, Sloan-Kettering Institute for Cancer Research, New York; oPRL (NIH-PS II) was kindly donated by the National Institute of Arthritis and Metabolic Diseases, National Institute of Health, Bethesda, Md., USA.

2.3. *Iodination of hormones*

[^{125}I]hGH was prepared by a modification of the method of Greenwood, Hunter and Glover as described

by Lesniak et al. [9,5]; its specific activity ranged from 50–120 μCi/μg hGH. The same procedure was used to prepare [^{125}I]bGH. [^{125}I]oPRL was prepared using lactoperoxidase (Calbiochem.) as described by Thorell and Johansson [10] and purified by gel filtration on a Sephadex G-75 column.

2.4. *Assay procedure for binding determinations*

[^{125}I]hGH was incubated with liver membranes in 200 or 250 μl of 50 mM phosphate buffer pH 7.4, containing 0.1% bovine serum albumin. Incubations, in duplicate or triplicate, were carried out at room temperature for 120 min. Bound and free hormone was separated by centrifugation as follows: an aliquot (160 μl) of each incubation mixture was added to 800 μl of cold phosphate buffer in conical plastic tubes. These were centrifuged for 10 min at 4°C; the supernatants were aspirated and the pellets were washed once. Tips of tubes were cut off and counted in a Packard model 548. Parallel incubations were performed in the presence of excess unlabeled hGH (0.5×10^{-6} M). Specific binding was the difference between the total radioactive uptake and the amount that was not displaced by the excess of native hormone.

3. Results and discussion

The specificity of the binding of [^{125}I]hGH to liver membranes from adult female and male rats is presented in fig.1. As shown in an earlier study [2], hGH, oPRL and to a lesser extent bGH inhibit the binding of [^{125}I]bGH to liver membranes of female rats; the trations required for 50% inhibition are 2×10^{-9} M for hGH, 3×10^{-9} M for oPRL and 2.5×10^{-7} M for bGH (fig.1A). It is of interest that, differing with earlier studies [2,4], the apparent affinity of oPRL for the hGH binding sites is slightly but consistently lower than the one of hGH; maximal inhibition achieved by oPRL is always inferior to the one obtained with hGH (fig.1A). This difference which does not occur when [^{125}I]oPRL is used as a ligand (fig.2), suggests that, besides binding to the 'lactogenic' sites, hGH interacts with additional, 'growth hormone' binding sites.

In males, which bind much less hGH than females, bGH and hGH are almost equivalent competitors, whereas oPRL competes for binding only at very high

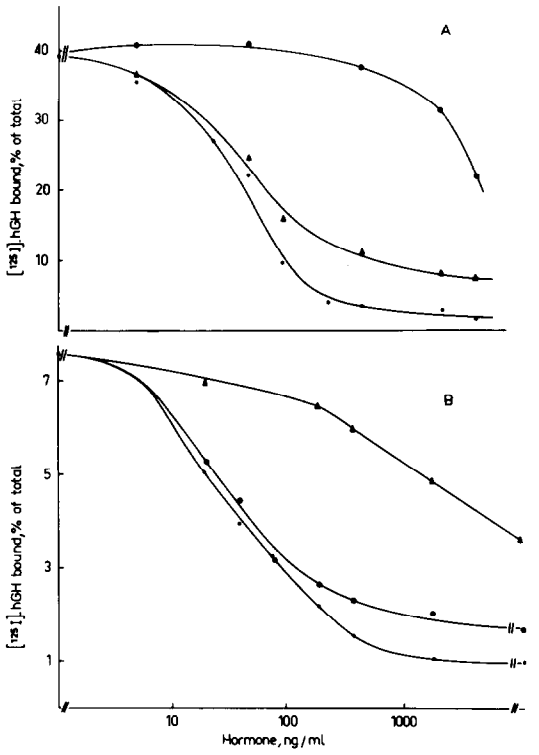

Fig.1. Effects of native hGH (•), oPRL (▲) and bGH (●) on the binding of [^{125}I]hGH to liver membranes from female (A) and from male (B) adult rats. Liver membranes of female (0.80 mg protein/ml) and male (1.39 mg protein/ml) rats were incubated with [^{125}I]hGH (0.88×10^{-10} M and 1.58×10^{-10} M respectively) and increasing concentration of native hormone. Binding is expressed as percent of the total radioactivity added per incubation.

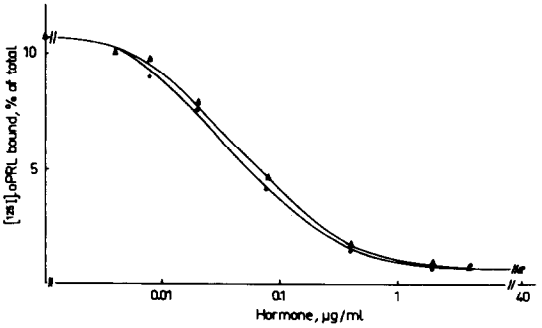

Fig.2. Specificity of [^{125}I]oPRL binding to adult female rat liver membranes. Membranes (0.99 mg protein/ml) were incubated with [^{125}I]oPRL (1.45×10^{-10} M) and increasing concentrations of oPRL (▲) and hGH (•). Binding is expressed as percent of the total radioactivity per incubation.

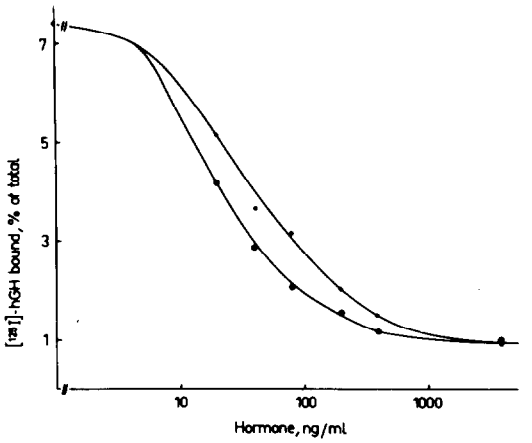

Fig.3. Specificity of [^{125}I]hGH binding to adult female rat liver membranes in presence of an excess oPRL. Membranes (1.48 mg protein/ml) were preincubated for 30 min with oPRL (1×10^{-6} M); at this concentration oPRL gave maximal displacement that could be achieved (see fig.1A). [^{125}I]hGH (1.2×10^{-10} M) was then added with increasing concentrations of hGH (•) and bGH (●). Incubation was continued for 90 min. Binding is expressed as percent of total radioactivity added per incubation.

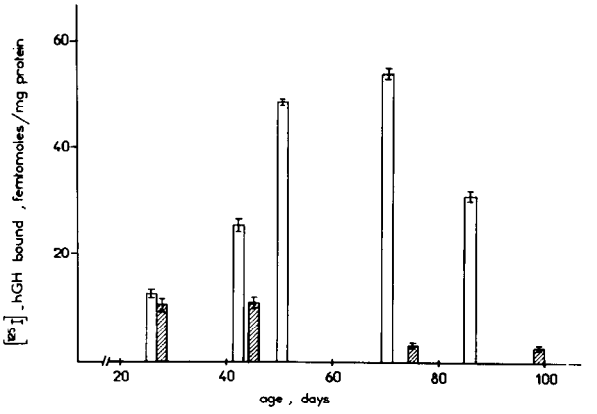

Fig.4. Specific binding of [^{125}I]hGH to liver membranes of male (▨) and female (□) rats as a function of age. Rat liver membranes were incubated with [^{125}I]hGH (1.6×10^{-10} M). Each bar represents specific binding expressed in moles of hormone bound per mg of membrane protein, and is the mean ± SEM (vertical line) of at least 2 determinations. The number of animals used for each liver membrane preparation varied between 3 and 6.

concentrations (fig.1B). These data indicate that, unlike in females, virtually all the binding sites in males have 'growth hormone' specificity. The same conclusions were suggested by our study of the interaction of hGH with isolated rat liver cells [11].

More convincing evidence for sites with growth hormone specificity in female rats are obtained by saturating the lactogenic hormone specific sites with excess native oPRL (fig.3). Under these conditions, the residual binding of [^{125}I]hGH to liver membranes is, as in males, equally inhibited by hGH and bGH. It is also of interest that this residual binding is higher (1.5 times) than the [^{125}I]hGH binding in males; a comparable difference is found in the membranes of the same animals when [^{125}I]bGH is used as the tracer (fig.5).

Variations of 'lactogenic' and 'somatotropic' sites with age and sex are shown in figs.4 and 5. In view of the identification of two types of binding sites in female rats, it was important to determine whether the increase in hGH binding capacity reported earlier reflected an increase only in lactogenic sites or in both 'lactogenic' and 'somatotropic' binding sites. This was investigated by the use of [^{125}I]hGH and [^{125}I]bGH as tracers; the latter was assumed to bind only to 'growth hormone' sites. These studies, in membranes of female rats, confirm that the hGH binding capacity increases by 4- to 6-fold after puberty and, in addition, they show a 2-fold increase in the bGH binding capacity. Thus the enhanced hGH binding capacity observed in postpubertal female rats reflects an increase in the number of both the 'lactogenic' and the 'growth

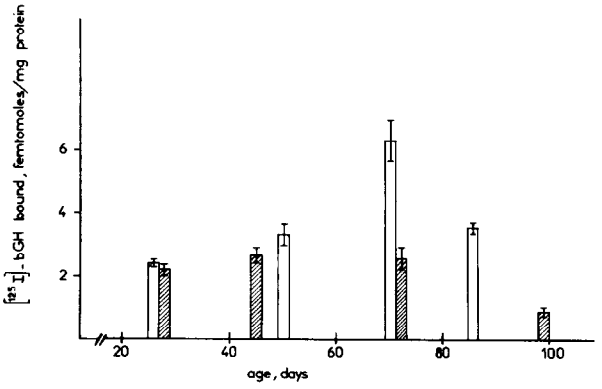

Fig.5. Specific binding of [^{125}I]bGH to liver membranes of male (▨) and female (□) rats as a function of age. Rat liver membranes were incubated with [^{125}I]bGH (1.6×10^{-10} M). For expression of the results see legend to fig.4.

hormone' binding sites. No increase with age in the number of 'growth hormone' binding sites is observed in males, the liver membranes of which bind the same amount of [^{125}I]bGH before and after puberty (fig.5). In both males and females, the number of growth hormone binding sites decreases after 3 months of age.

The correlation between the binding to one or the other site and a specific biological activity of hGH in this system has to be investigated. The physiological significance of the modulation in the number of hGH binding sites remains to be established.

Acknowledgements

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