Induction of a Lactogenic Receptor in Rat Liver: Influence of Estrogen and the Pituitary

(growth hormone/prolactin/estrone/steroids)

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ABSTRACT A receptor exists in female rat liver with high specificity for lactogenic hormones. Previous work showed the receptor level increased at the time of puberty in female but not male animals. Pregnancy caused a further substantial increase. Here we show that estrone (50 μ g/day) administration to male rats induced a 10- to 30-fold increase in specific binding of ovine prolactin and human growth hormone after 8-12 days with a significant increase first seen after 4 but not 2 days of injection. In females, this regimen increased binding to pregnancy levels. In prepuberal (20-days-old) male and female rats, estrone was also markedly stimulatory. The binding sites for ovine prolactin and human growth hormone were of high affinity in liver membranes from both female and estrone-treated male rats ($K_a = 0.6$ to 1.4×10^9 M $^{-1}$).

Estrone and estradiol were equally effective in inducing the lactogenic receptor. Estradiol (50 μ g/day), progesterone (500 μ g/day), human placental lactogen (1 mg/day), and testosterone (100 μ g/day) were without influence. Hypophysectomy drastically decreased the levels of lactogenic receptor in mature female rats, and estrogen treatment failed to restore receptor levels to normal. Hypophysectomized male rats were also unresponsive to estrogen. Throughout these studies the specific binding of 125 I-labeled insulin remained relatively constant.

This work demonstrates estrogen induction of a lactogenic receptor. The pituitary gland appears to have a critical, though presently undefined, role in the induction process.

Previous work has established the presence of lactogenic receptors in female but not male rat liver membranes (1). The receptors are virtually absent in prepuberal animals, only increasing towards adult levels at the time of puberty. Pregnancy was found to produce substantial augmentation of these receptors (2). These observations raised the possibility that sex steroids might play a role in regulating the level of the hepatic lactogenic receptor. In this communication we demonstrate the induction by estrogen of hepatic lactogenic receptors in the male rat.

MATERIALS AND METHODS

Male and female adult, weanling, and hypophysectomized rats were obtained from the Fauna Breeding Co., Quebec. Adult animals weighed 180-200 g when studied. The methods

Abbreviations: hGH, mGH, dGH, bGH, oGH, and pGH are, respectively, human, monkey, dog, bovine, ovine (sheep), and porcine growth hormone; hPRL, oPRL, bPRL, and rPRL are, respectively, human, ovine, bovine, and rat prolactin; mPL and hPL are, respectively, monkey and human placental lactogen.

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used for tissue fractionation, membrane preparation, and iodination of the different polypeptide hormones have been described in detail elsewhere (1). Binding of 125 I-labeled hormone was determined by incubating labeled hormone (80 to 120×10^3 cpm) with 150 μ g of membrane protein in a final volume of 0.5 ml containing Tris-Ca $^{++}$ buffer (25 mM Tris-HCl, pH 7.4-10 mM CaCl $_2$), and 400 μ g of bovine-serum albumin. The incubation was in duplicate or triplicate at 24° with constant shaking for 60 min (insulin) or 120 min (hGH and oPRL), and was terminated by the addition of 3 ml of ice-cold 0.1% bovine-serum albumin-Tris-Ca $^{++}$ buffer. Bound and free 125 I-labeled hormone were separated by centrifugation or filtration through Millipore filters as described (1). Parallel incubations were performed in the presence of excess unlabeled hormone (insulin, 2.5 μ g; hGH or oPRL, 1 μ g). Specific binding was the difference between radioactivity bound to the membranes in the presence of excess unlabeled hormone and that bound in its absence.

The steroid hormones used in these studies were purchased from Sigma Chemical Co., St. Louis, Mo. They were dissolved in a small volume of ethanol, diluted in corn oil, and administered by subcutaneous injection. Crystalline porcine zinc insulin (24.4 units/mg) was a gift from the Connaught Research Laboratories, Toronto. The various growth hormone preparations as well as ovine, bovine, and rat prolactins were generously supplied through the Pituitary Distribution Program of NIAMDD. ACTH and glucagon were purchased from the Sigma Chemical Co. The partially pure placental lactogens were obtained by described procedures (3).

RESULTS

Fig. 1 illustrates the specificity characteristics of the binding of 125 I-labeled hGH to the rat-liver receptor. Primate GH and human, ovine, and bovine prolactin were equally efficacious in displacing 125 I-labeled hGH from binding sites. Rat prolactin and human and monkey placental lactogen were also effective in producing parallel displacement curves. In contrast, nonprimate growth hormones, as well as insulin, ACTH, and glucagon, were without significant competitive effect.

Fig. 2 (left panel) demonstrates that estrone administration, at a dose of 50 μ g per 200-g animal per day, induced a marked increase in the specific binding of both 125 I-labeled hGH and 125 I-labeled oPRL by male rat-liver membranes. There was no significant effect on the specific binding of 125 I-labeled

† 0.7-2.8 ng of human growth hormone (hGH) or ovine prolactin (oPRL), and 0.5-1.1 ng of insulin.

FIG. 1. Specificity of binding of ^{125}I -labeled hGH to female rat-liver membranes. The *abscissa* denotes the final concentration of unlabeled hormone in the incubation. The *ordinate* represents the amount of radioactivity bound to the membranes expressed as a percent of the control incubations in which no unlabeled hormone was present. Each point is the mean $\pm$ SEM of three or more determinations.

insulin. Estrone treatment effected an increase of specific binding of ^{125}I -labeled hGH from 0.9% to 33.0%, and of ^{125}I -labeled oPRL from 0.8% to 14.4%, whereas the specific binding of insulin was 8.1% and 11.7% in control and estrone-treated animals, respectively $\dagger$. It is also seen (*right panel*) that estrone administration to adult female rats increased the specific binding of lactogenic hormones by liver membrane preparations with no significant effect on the specific binding of ^{125}I -labeled insulin. Of interest is the marked diminution in specific binding of ^{125}I -labeled hGH and ^{125}I -labeled oPRL with only a modest decrease of ^{125}I -labeled insulin binding by liver membranes of hypophysectomized female rats. Fig. 3 illustrates that estrone is stimulatory in both male and female prepubertal animals. Female animals showed higher control values for the specific binding of hGH and oPRL and underwent a larger absolute increase in binding activity than did males. When considered from the viewpoint of degree of stimulation above control, both male and female rats showed about a 4.5-fold increase in binding activity.

Fig. 4 illustrates displacement dose-response curves of ^{125}I -labeled hGH binding. The conditions were those previously shown to yield a plateau of binding with a minimum of ^{125}I -labeled hGH degradation (2). The degree of specific binding in control male rat-liver membranes was so low as to preclude any meaningful analysis by Scatchard plot. The affinity constants for hGH binding were 1.4×10^9 and $0.9 \times 10^9 \text{ M}^{-1}$ for estrone-treated male and female rat-liver membranes, respectively. A similar analysis using ^{125}I -labeled oPRL gave values of 1.1×10^9 and $0.6 \times 10^9 \text{ M}^{-1}$ for the respective

$\dagger$ Data were analyzed by analysis of variance and Tukey's *w* procedure (4).

FIG. 2. Effect of estrone (E_1) administration to adult male and female rats, and of hypophysectomy of adult females on the specific binding of ^{125}I -labeled hGH, oPRL, and insulin by liver membranes. Estrone ($50 \mu\text{g}$ per animal) was administered by subcutaneous injection for 7 days. Control animals received daily injections of corn oil. On day 8, all animals were killed. The specific binding was determined for all three hormones simultaneously in all the membrane preparations. Hypophysectomy was performed 8 days before the animals were killed. Each value is the mean $\pm$ SEM of five animals. *Hypox*, hypophysectomized.

** $P < 0.01$ when compared to control.

preparations. Thus the affinity constants for hGH and oPRL are similar.

In Fig. 5 it is seen that only estrone and estradiol increased the specific binding of ^{125}I -labeled hGH. The administration of estriol, human placental lactogen (hPL), progesterone, or testosterone was ineffectual. Parallel changes in ^{125}I -labeled oPRL binding were also observed. A dose-response relationship was observed for both estrone and estradiol. Of interest was the failure of estrone administration to augment binding activity in liver membranes of hypophysectomized male rats. This has also been shown to be true for hypophysectomized female rats. We have also demonstrated that hPL or progesterone did not augment or diminish the stimulation by estrone.

The time course of the estrone-induced stimulation of both hGH and oPRL binding activity in male rat-liver membranes is depicted in Fig. 6. The daily administration of estrone produced a significant increase above control by 4 days, with maximum effect attained by 8–12 days.

DISCUSSION

Primate growth hormones, such as hGH, can stimulate lactation as well as growth (5). Correspondingly it has been shown that hGH binds to two different sites, one with lactogenic and the other with growth hormone specificity (1). The specificity of hGH binding in rat-liver membranes has been examined in greater detail than previously (1). ^{125}I -labeled hGH was displaced by hormones capable of stimulating lactation (i.e., primate growth hormones, prolactins, placental lactogens).

FIG. 3. Effect of injecting weanling (20-day-old) male and female rats with estrone (E_1) for 7 days on specific binding of 125 I-labeled hGH, oPRL, and insulin by liver membranes. Each group consisted of six animals. Procedure was as in legend of Fig. 2.

** $P < 0.01$ when compared to control.

Nonprimate growth hormones, which possess no lactogenic activity (5), showed minimal ability to displace 125 I-labeled hGH. The binding of 125 I-labeled oPRL, though lower, always paralleled that of 125 I-labeled hGH. The binding constants for hGH and oPRL were similar and compatible with a capacity of these sites to bind physiologic levels of circulating hormone. In terms of both specificity and affinity, these sites possess the properties expected of biologically important receptors.

Prior observations raised the possibility that sex steroids might play a role in determining the presence of the lactogenic receptor in rat liver (2). In this study we have shown that estrone and estradiol are able to induce the appearance of this receptor in adult male rat liver. Immature animals are also responsive. Other compounds, including estriol and progesterone, had no effect. The equipotency of estrone and estradiol contrasts with the much greater uterotropic potency of the latter (6). Further work is required to establish that these estrogens mediate the physiological increase in receptor levels associated with puberty and pregnancy.

Whereas glucocorticoid-induced hepatic enzyme changes are apparent within hours of hormone administration (7), the increase in lactogenic receptor consequent to estrogen administration was not apparent at 48 hr. The maximum response took fully 8–12 days to become evident. This suggests that a more complex series of events may be involved in the induction of this receptor. Further support for this viewpoint is forthcoming from our observations on the hypophysectomized rat. Hypophysectomy of the female rat produces diminution to very low levels of the hepatic lactogenic receptor. Estrone administration to the hypophysectomized female did not repair this deficiency nor was estrone able to induce the appearance of

FIG. 4. Displacement of 125 I-labeled hGH from liver membranes of a female (●), male (Δ), and estrone-treated male (○) rat as a function of unlabeled hGH concentration. Each tube contained 125 I-labeled hGH at a concentration of 0.93×10^{-10} M (approximately 81000 cpm). Tubes were incubated for 24 hr at 4° . Inset: Scatchard plot of data obtained from female and estrone-treated male animals. Ordinate depicts ratio of bound/free hormone, and the abscissa the amount of hGH bound to the rat-liver membranes.

lactogenic receptors in hypophysectomized male rats. By contrast, the effect of hypophysectomy on steroid-induced hepatic enzyme changes has been shown to be minor (7). Concerning the role of the pituitary in the induction process, the distinction will have to be made between estrogen-induced alterations of pituitary function [namely, estrogen stimulation of prolactin secretion (8)] and direct hepatic effects of estrogen in the presence of a permissive level of pituitary function.

The physiologic significance of hepatic lactogenic receptors requires elucidation. That they may mediate effects of lacto-

FIG. 5. Effect of daily administration for 9 days of estrone (E_1), estradiol (E_2), estriol (E_3), hPL, progesterone (P), and testosterone (T) on the specific binding of 125 I-labeled hGH by male rat-liver membranes. The daily dose in μ g is noted on the abscissa. The procedure was otherwise as in the legend of Fig. 2. Each bar is the mean $\pm$ SEM of three animals. Hypox, hypophysectomized.

** $P < 0.01$ when compared to control.

§ We attribute this to a more damaged, less stable tracer obtained with oPRL.

FIG. 6. Time course of change in the specific binding activity for hGH and oPRL of rat-liver membranes as a function of duration of treatment with estrone. Estrone in oil (50 μ g) or oil alone (control) was administered by daily subcutaneous injection. Each point represents mean of three separate animals.

genic hormones on the liver is suggested by the increase in RNA synthesis in rat liver during pregnancy and lactation (9), and by the stimulation of mouse hepatic RNA synthesis by prolactin (10). The variation in the level of hepatic lactogenic receptors in defined physiologic circumstances (2) is consistent with the view that hormonal effects are modulated by the level of receptor as well as by the circulating level of hormone. In this regard the demonstration of reduced insulin receptors in liver and fat in insulin-resistant states is of interest (11, 12). The study of the regulation of receptor levels

could provide important information as to how the peripheral sensitivity to hormones is controlled.

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