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Prolactin Receptors in Mouse Liver: Species Differences in Response to Estrogenic Stimulation[1] (40327)

STEPHEN MARSHALL, JOHN F. BRUNI, AND JOSEPH MEITES[2, 3]

Department of Physiology, Neuroendocrine Research Laboratory, Michigan State University, East Lansing, Michigan 48824

Specific prolactin (PRL) receptors have been demonstrated in the liver of many species, including rats and mice (1–3). Ovariectomy (OVX) decreased and estrogen replacement increased PRL binding sites. The inductive effects of estrogen on PRL binding in the liver was dose related in OVX rats, and anti-estrogens reduced PRL receptors in the liver of female rats (4). One mechanism whereby estrogen induced PRL receptors is by stimulation of pituitary PRL release, resulting in induction of hepatic PRL binding sites in the liver (5, 6). However, since very low doses of estrogen increased PRL binding in the liver without altering serum PRL levels (4), and since the PRL-inhibiting ergot drug CB-154 did not decrease the estrogen-induced increase in hepatic PRL binding sites (4), it is possible that estrogen may act directly on the liver to increase PRL receptors.

All of the above studies were performed in rats. To determine whether the estrogen effect on PRL receptors was observable in other species, we examined the effects of estrogen on PRL receptors in the liver of mice. The results indicate that estrogen inhibits induction of PRL receptors in the liver of female mice, in contrast to its stimulation of PRL receptors in the liver of male and female rats.

Materials and methods. Adult male and female Swiss–Webster mice were obtained from Spartan Research Animals, Haslett, Michigan. Mice were housed in a temperature-controlled (25 ± 1°) and artificially illuminated room (lights on from 0500 to 1900 hr daily) and received food and water *ad libitum.*

Experiment 1. Female mice were OVX on Day 1 and were injected sc daily with 2 μg of estradiol benzoate (EB) in 50 μl of corn oil on Days 8 through 14. On the 15th day all OVX were killed together with a group of intact females which were similarly injected daily on Days 8 through 14 with vehicle alone.

Experiment 2. Female mice, OVX 14 days prior to estrogen treatment, were given daily sc injections of either 1, 10, 20, or 50 μg of EB in 50 μl of corn oil. Mice were then killed after 12 days of treatment, together with groups of intact and OVX controls which were injected with vehicle alone. Additional treatment groups given daily injections of 20 μg of EB were killed after 6 or 9 days of treatment.

Experiment 3. Male mice were given a single 2-μg EB sc injection in 50 μl of corn oil and killed 7 days later. Controls were injected with vehicle alone.

At the end of each experiment the mice were anesthetized with ether and decapitated, and the blood obtained from the cervical wound was allowed to clot at 4°. The serum was separated by centrifugation and stored at −20° for later serum PRL measurements. Livers were removed and a microsomal membrane fraction was obtained by differential centrifugation as described previously (1). PRL was iodinated by a lactoperoxidase method (1) and the binding of [^{125}I]iodo-PRL to liver membranes was determined. Incubations with membrane protein and [^{125}I]iodo-PRL were performed at 4° for 60 hr, in the presence of excess (1 μg) unlabeled PRL and in its absence. Livers from female mice were assayed for PRL binding, using 300 μg of membrane protein per tube, whereas for male livers 1000 μg per tube was used. Specific binding refers to the difference in radioactiv-

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ity bound to membranes after incubations with and without unlabeled PRL, and for ease of representation is expressed as a percentage of the total counts added. PRL binding to liver membranes from mice has been shown to be both time and temperature dependent, and specific for lactogenic hormones (3). Mouse PRL was measured by a double antibody radioimmunoassay using the materials and methods of Sinha *et al.* (7). The biological potency of the mouse PRL standard was 25.0 IU/mg. The data in Expts 1 and 2 were treated by an analysis of variance for unequal sample size, followed by a Student–Neuman–Kuels test for comparison of means among groups. Student's t test was used to determine significance in Expt 3. $P < 0.05$ was considered to be significant.

Results. Figure 1 shows that OVX significantly increased ($P < 0.01$) [^{125}I]iodo-PRL binding to mouse liver membranes and that this enhanced binding could be decreased to intact control values by estrogen replacement. When this experiment was repeated (Fig. 2) with various doses of EB and longer treatment times, similar results were obtained. OVX increased ($P < 0.05$) specific [^{125}I]iodo-PRL binding from 14.48 ± 0.85% in the intact controls to 19.93 ± 0.60%. Replacement by injecting 1 and 10 μg of EB for 12 days reduced PRL binding to 11.84 ± 0.53 and 10.90 ± 0.81%, respectively, which were not significantly different from intact control values, whereas 20 and 50 μg of EB significantly reduced binding to below intact levels. Serum PRL was reduced from 12.0 ± 1.5 ng/ml (intact controls) to 8.09 ± 2.0 ng/ml in the OVX rats. All estrogen-treated groups had serum PRL values significantly higher than those in intact controls.

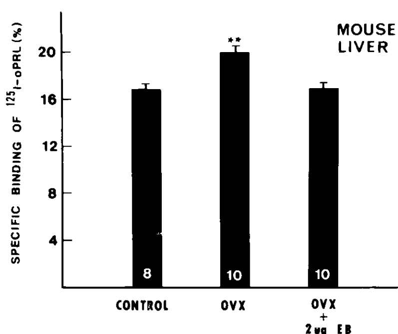

FIG. 1. Effects of OVX and OVX with EB replacement on specific [^{125}I]iodo-PRL binding to liver membrane preparations from female mice. For each tissue sample, incubations were performed in triplicate at 4° for 60 hr, using 300 μg of membrane protein per tube. The amount of [^{125}I]iodo-PRL per tube was 1.0×10^5 cpm. The line above each bar represents 1 SEM, and the numbers in white indicate the number of observations per group. $^{**}P < 0.01$ when compared to intact controls.

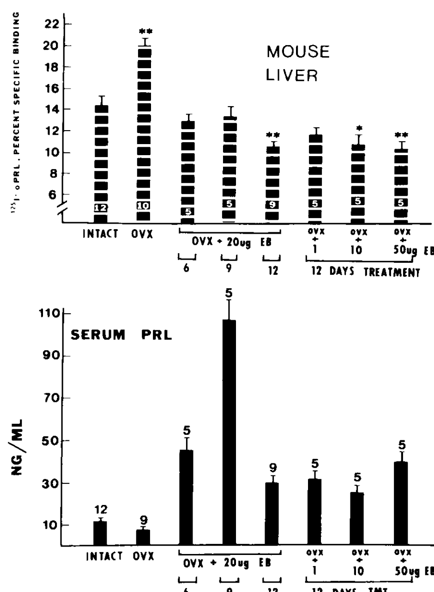

FIG. 2. Serum PRL levels and [^{125}I]iodo-PRL binding to liver membranes from intact and OVX mice and OVX mice given daily injections of different doses of EB. For each tissue sample, incubations were performed in triplicate at 4° for 60 hr, using 300 μg of membrane protein per tube. The amount of [^{125}I]iodo-PRL per tube was 1.0×10^5cpm. The line above each bar represents 1 SEM, and the numbers in white indicate the number of observations for each group. $^{*}P < 0.05$ as compared to intact controls. $^{**}P < 0.01$ as compared to intact controls.

Figure 3 demonstrates the effects of a single injection of 2 μg of EB on specific PRL binding sites in liver membranes obtained from male mice. PRL binding increased ($P < 0.01$) from 22.61 ± 1.16 to 33.72 ± 1.29% at 7 days postinjection. Since PRL binding sites on male liver membranes were measured using 1000 μg of membrane protein rather than 300 μg of membrane protein (as used in quantitating PRL receptors in the liver of females), specific binding is higher in the livers of females than in the livers of males when compared on a milligram of protein basis. This is in agreement with the data of Posner (3).

Discussion. The presence of specific PRL

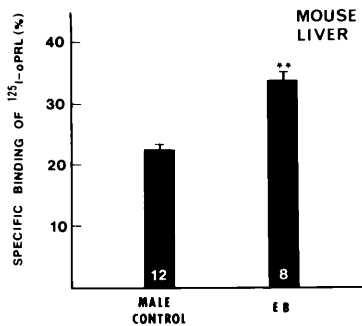

FIG. 3. Specific binding of [^{125}I]iodo-PRL to liver membranes from male mice 7 days after a single injection of EB. For each tissue sample, incubations were performed in triplicate at 4° for 60 hr, using 1000 μg of membrane protein per tube. The amount of [^{125}I]iodo-PRL per tube was 1.0×10^5 cpm. The line above each bar represents 1 SEM, and the number in white indicates the number of observations for each group. $^{**}P < 0.01$ as compared to male controls.

receptors in liver membranes of female mice agrees with the findings of other investigators (3, 8). However, our results indicate that OVX results in an increase of hepatic PRL receptors in female mice, whereas estrogen treatment over a large dose range reduced PRL binding to intact or below intact values. These data in female mice represent a striking contrast to the effects of OVX and estrogen replacement on PRL receptors in liver of female and male rats.

In female rats the effects of estrogen on increasing hepatic PRL receptors was convincingly demonstrated to be mediated through stimulation of pituitary PRL release (5, 9). However, other data suggest a direct effect of estrogen on the liver to modulate PRL binding sites (4). In the present study, all doses of estrogen significantly increased serum PRL levels in female mice. The increase in PRL, however, is not believed to have altered hepatic PRL receptors since other investigators have reported that neither the high levels of endogenous PRL during pregnancy, nor exogenous PRL injections to female mice, influenced PRL binding sites in the liver (3, 8). Therefore, a direct effect on the liver appears likely, although an indirect effect of estrogen cannot be excluded.

In male mice a single injection of 2 μg of EB was able to significantly increase PRL binding sites in the liver. Since estradiol valerate has been reported to stimulate PRL binding sites in the liver of male rats (5), it is apparent that both male rats and male mice respond similarly to the stimulatory action of estrogen on hepatic PRL receptors. This is in contrast to the opposite effects of estrogen on hepatic PRL binding sites of female rats and mice.

Although the physiological significance of these results is not known at this time, PRL has been shown to have numerous effects on liver function of various species. Thus, PRL was reported to regulate free fatty acid synthesis in dog (10) and rat (11) livers, stimulate hepatic RNA synthesis in dwarf mice (12), modulate ornithine decarboxylase activity in the liver of rats (13), and increase somatomedin release from rat livers (14). However, in order for PRL to exert an effect on a target cell, it must first bind to a stereospecific plasma membrane receptor to induce intracellular changes. Consequently, receptor modulation could provide a mechanism for altering the sensitivity of target organs to circulating PRL. Therefore, determining which hormones can alter PRL receptors and the direction of these changes are important for clarifying the physiological actions of PRL on liver function.

The present data clearly demonstrate an important species difference between female rats and mice in estrogenic control of hepatic PRL receptors and may have several implications. Thus, the use of the rat as a model for investigating factors modulating PRL receptors in the liver cannot be considered valid for other species. Moreover, the functions of PRL on liver function may be different between males and females of even the same species, since control of PRL receptors in liver of male and female mice are different. Our data indicate that estrogen inhibits PRL binding sites in the female, whereas in the male, binding is stimulated. Thus, the response of hepatic PRL receptors to estrogen is both species and sex dependent. The mechanisms of action by which these effects are mediated remain to be clarified. The differential findings in these two species need to be considered when designing and interpreting studies on the effects of PRL on liver function.

Summary. Serum PRL and hepatic PRL receptors were measured in intact and OVX

mice and OVX mice given several doses of EB. OVX significantly increased PRL binding in the liver of female mice, and EB reduced receptors to intact or below intact levels. It was concluded that estrogen decreases PRL receptors in the liver of female mice. This is a striking contrast to the stimulatory effect of estrogen on hepatic PRL receptors in male and female rats. EB elevated serum PRL in OVX mice, but since other investigators reported that PRL does not alter hepatic PRL receptors in female mice, it appears likely that estrogen reduced PRL binding sites by a direct effect on the liver. However, an indirect effect cannot be excluded. In male mice, estrogen increased PRL receptors in the liver as in male rats.

The present data demonstrate important species differences between female rats and female mice in estrogenic control of hepatic PRL receptors. Moreover, the inhibitory effect of estrogen in female mice, and its stimulatory action in male mice, suggest that the response of hepatic PRL receptors to estrogen may be sex dependent in different species. The mechanisms of action by which these effects are mediated remain to be clarified.

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