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Lutropin Receptors from Male and Female Tissues: Different Responses to a Lutropin Receptor Binding Inhibitor (39450)

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We recently reported a lutropin receptor binding inhibitor (LH-RBI) which strongly inhibits the binding of ^{125}I-labeled ovine lutropin ([^{125}I]oLH) to its ovarian receptor site (1, 2). The LH-RBI was found in pregnant and pseudopregnant rat ovary, but was not found in either the ovary of nonpregnant mature rats and immature rats or in extraovarian tissues such as liver, oviduct, and testis. The LH-RBI was estimated to have a molecular weight of approximately 3800 as determined by Sephadex G-50 column chromatography and was heat stable and also protease sensitive. It had been shown by us (1) that LH-RBI did not appear to be due to endogenous lysosomal enzyme activities (20-min boiling of the LH-RBI did not destroy its inhibitory activity); also, there was no detectable quantity of [^{125}I]oLH bound to the LH-RBI (chromatographic data). In our present work, we investigate the effect of the LH-RBI on the binding of [^{125}I]oLH to testicular LH receptors. The results show that LH-RBI does not inhibit LH binding to testicular LH receptors, although the inhibition is highly significant in the ovary. Possible implications of the inhibition pattern for the mechanism through which the LH-RBI exerts its binding-inhibitory effect are discussed in the present paper.

Materials and methods. Ovine lutropin was prepared as described previously (3). Lutropin was labeled with ^{125}I using the chloramine-T method as described previously (4). Pseudopregnancy in the female Sprague–Dawley rat was induced at the age of 25 days by single subcutaneous injection with 50 IU of pregnant mare serum gonadotropin, followed 65 hr later by single subcutaneous injection of 25 IU of human chorionic gonadotropin (hCG) as described by Parlow (5). Five days after the injection of hCG, ovaries were obtained from rats sacrificed with CO_2 inhalation. The organs were trimmed free of fat and immediately frozen on dry ice. The frozen ovaries were stored at −20° until use.

The ovarian and testicular receptors were prepared as follows: Pseudopregnant ovaries were homogenized in 6 vol of Solution A (0.1 *M* Tris buffer, pH 7.4, containing 5m*M* $MgCl_2$ and 0.1 *M* Sucrose, plus 0.1% bovine serum albumin). The homogenization was done with a glass–Teflon tissue homogenizer with a loose-fitting pestle driven by motor. The homogenization was accomplished by 10 strokes exactly. Testes from adult male rats were decapsulated, weighed, and homogenized in 3 vol of Solution A. Homogenization was done in a Duall-type hand homogenizer with loose-fitting pestle using exactly 20 strokes. The ovarian and testicular homogenates were strained through two thin layers of cheesecloth, and filtrates separately centrifuged at 5000*g* for 15 min at 4°. The ovarian supernatant was centrifuged again at 30,000*g* for 15 min, and the 30,000*g* supernatant was used as the ovarian extract which contained the LH-RBI. The ovarian pellets were washed three times by centrifugation with 8 ml of Solution A per wash, resuspended in Solution A, and used as the ovarian receptor preparations for the incubation studies. In previous studies (6), it was found that three washes were adequate to remove the LH-RBI in the receptor preparation. Since the LH-RBI activity was not present in the rat testes (1), the testicular pellets were washed only once, resuspended in Solution A, and used as the testicular receptor preparation for the incubation studies.

In a standard binding assay, an aliquot (0.1 ml) of the 5000*g* pellets equivalent to 10 mg of ovary or testis, 0.1 ml of the ovarian extract (LH-RBI), and 10 μl containing 1 ng of [^{125}I]oLH were added in sequence in each tube (Falcon plastic tubes,

10 × 75 mm). In some of the tubes, 10 μg (10 μl) of unlabeled oLH was added to serve as the control to determine the nonspecific binding of [^{125}I]oLH. The final volume of each tube was 0.22 ml. The tubes were incubated for 1 hr at 37°, chilled, and immediately centrifuged at 12,000*g* for 10 min at 4°. The supernatants were withdrawn and the pellets were washed once with 0.5 ml of Solution A. The tubes were again centrifuged and the pellets obtained were counted in a well-type scintillation gamma counter (Nuclear-Chicago, Model 4224). The data from all of the LH binding experiments described in this paper represent the specific binding of [^{125}I]oLH to the ovarian or testicular receptors. Specific binding is defined as the total binding of [^{125}I]oLH minus the nonspecific binding of [^{125}I]oLH. Nonspecific binding is the uptake of labeled hormone in the presence of 1000-fold excess of unlabeled hormone and is usually 8–12% of the total binding. This amount of nonspecific binding was not further reducible by additional wash with Solution A. The significance, if any, of the nonspecific binding is unknown. Each assay point was accompanied by a parallel nonspecific binding determination.

Results and discussion. Evidence that LH-RBI does not inhibit labeled lutropin binding to ovarian lutropin receptors by a preferential binding of the hormone to the inhibiror (instead of the receptor) or by a selective damage of lutropin by LH-RBI is summarized in Table I, Experiment I. The [^{125}I]-oLH was first incubated with the LH-RBI for 1 hr at 37° before incubation with the ovarian LH receptors. The results indicate that preincubation of labeled lutropin with LH-RBI did not lead to greater inhibition of binding, a result consistent with earlier studies (1) indicating LH-RBI was not attributable to either degradative enzymes or soluble lutropin binding components (e.g., soluble receptors). Therefore, the inhibition seems to be due to one of two mechanisms: (i) competition between the LH-RBI and [^{125}I]oLH for the same binding site on the receptor; (ii) binding of LH-RBI and [^{125}I]oLH to different sites on the receptor, with the binding of the LH-RBI at the second site preventing the binding of [^{125}I]oLH at the lutropin binding site.

The effect of the LH-RBI on the binding of lutropin to the hormone receptors of ovary or testis was next compared. This comparison is summarized in Fig. 1. In view of the experimental results indicating that the LH-RBI does not inhibit the binding of [^{125}I]oLH to testicular LH receptors (Fig. 1), the possibility of the competition between the LH-RBI and [^{125}I]oLH for the same binding site becomes unlikely (*vide supra,* mechanism (i)). If the LH-RBI inhibited the [^{125}I]oLh binding through the mechanism of competition for the same binding site, the LH-RBI would have inhibited the binding of [^{125}I]oLH to the testicular LH receptors. By elimination of al-

TABLE I. Effects of Incubation Sequence on the Inhibition of [^{125}I]oLH Binding by LH-RBI to Ovarian or Testicular LH Receptors.[a]

Experiment	Incubation systems		Specific binding of [^{125}I]oLH (%)	Inhibition of binding (%)
	Preincubation mixture[b]	Additives after preincubation		
I	[^{125}I]oLH + Solution A[a]	Ovarian receptors	23.89	Control
	[^{125}I]oLH + LH-RBI	Ovarian receptors	9.85	59
	[^{125}I]oLH	LH-RBI + ovarian receptors	10.15	58
II	Ovarian receptors + Solution A	[^{125}I]oLH	15.50	Control
	Ovarian receptors + LH-RBI	[^{125}I]oLH	1.95	88
	Ovarian receptors	LH-RBI + [^{125}I]oLH	5.58	64
III	Testicular receptors + Solution A	[^{125}I]oLH	9.66	Control
	Testicular receptors + LH-RBI	[^{125}I]oLH	9.58	1
	Testicular receptors	LH-RBI + [^{125}I]oLH	9.65	0

[a] For details see text.

[b] The preincubation and the final incubation were carried out at 37° for 1 hr as described in Methods.

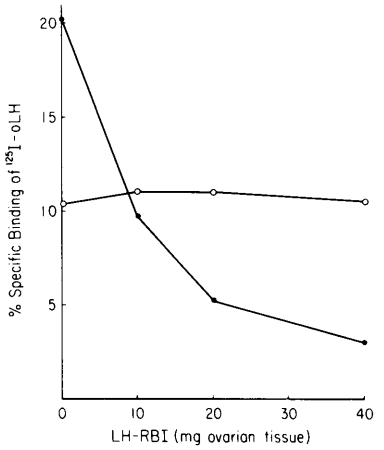

FIG. 1. Different effects of the LH-RBI on the specific binding of [^{125}I]oLH to the 5000*g* pellets (receptor preparations) of testicular (O-O-O) and ovarian (●—●—●) homogenates. The pellets were incubated with [^{125}I]oLH in the presence of various concentrations of the LH-RBI (pseudopregnant ovary extract, see methods section). LH-RBI concentration is expressed as milligram equivalents of ovarian tissue. The incubation was carried out at 37° for 1 hr as described in the methods section.

ternatives, the data thus favors mechanism (ii) (*vide supra*). One may argue that the failure of the LH-RBI to show the inhibition in the testicular system could also be due to low binding affinity of the LH-RBI for the LH binding site in the testis. We examined this possibility using two criteria: (i) preincubating the testicular receptors with the LH-RBI for 1 hr at 37° before the addition of [^{125}I]oLH and a second incubation (see Table I, Experiment III) and (ii) increasing the quantity of the LH-RBI for the incubation from 10 mg tissue equivalent up to 40 mg tissue equivalent (Fig. 1). Again, however, no inhibition of the binding of [^{125}I]oLH to testicular LH receptors was observed in either of these two conditions (Table I and Fig. 1). Experiment II, Table I, was included to compare ovarian responses to lutropin and LH-RBI effects on ovarian tissue under the same incubation sequences applied to the testicular tissue (Experiment III). Although the inhibition difference for the sequence of addition (Experiment II, 88 vs 64%) could be interpreted by either mechanism (i) or (ii) (*vide supra*), these data, when considered with the data in Fig. 1 and Experiment III, Table I, are only consistent with mechanism (ii).

Our suggestion that the LH-RBI inhibits the LH binding through the mechanism other than competitive inhibition was also supported by the results of the binding kinetics study. The ovarian LH receptors were incubated with increased concentrations of [^{125}I]oLH in the absence or presence of a fixed concentration of the LH-RBI. The results were used to construct the Scatchard plot (7) in Fig. 2. The dissociation constant, i.e., K_d for the [^{125}I]oLH binding, was essentially the same in either the absence or presence of the LH-RBI. Since the association constant (the reciprocal of K_d) of the [^{125}I]oLH binding was not reduced by the presence of the inhibitor, this would suggest that the LH-RBI did not compete with [^{125}I]oLH for the same binding site on the receptor. On the other hand, the displacement of the curve obtained in the presence of the LH-RBI indicated the total number of available LH binding sites is significantly reduced (i.e., the *x*-axis intercept is proportional to the number of available LH binding sites).

The phenomenon of "allosteric" or "different site" inhibition as applied to the enzyme-substrate-inhibitor interaction has

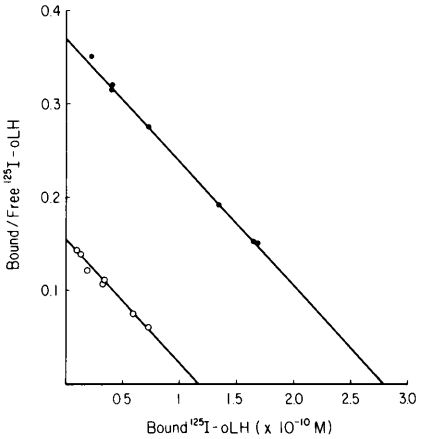

FIG. 2. Scatchard plot (7) of the specific binding of [^{125}I]oLH to the 5000*g* pellets of pseudopregnant ovarian homogenates in the presence (O-O-O) or absence (●-●-●) of the LH-RBI. The slope is equivalent to $1/K_d$; the K_d was calculated to be 7.56×10^{-10} *M* (O-O-O) or 7.52×10^{-10} *M* (●-●-●). Molecular weight of ovine LH was taken as 30,000.

been recognized in the field of enzymology. A similar control mechanism is also likely to occur in hormone–receptor interactions.

The present work suggests that the lutropin receptor of ovaries, but not of testes, has a specific LH-RBI binding site in addition to the lutropin binding site; and that the binding of the LH-RBI produced an "allosteric" type of inhibition to the binding of lutropin at the lutropin binding site. In this respect, a demonstration of the specific binding of the LH-RBI to the ovarian lutropin receptor would serve as direct evidence for supporting the existence of such a phenomenon. Since a preparation of a homogeneous LH-RBI material suitable for radioactive labeling has not been achieved in our laboratory, we are not able, at this stage, to provide the direct evidence clarifying this possibility.

Summary. An inhibitor for lutropin receptor site binding (LH-RBI), which strongly inhibited the binding of ^{125}I-labeled ovine lutropin ([^{125}I]oLH) to ovarian LH receptors, did not inhibit the [^{125}I]oLH binding to testicular LH receptors. Preincubation of the LH-RBI with [^{125}I]oLH did not affect the binding of preincubated [^{125}I]oLH to ovarian LH receptors. No inhibition of [^{125}I]oLH binding to testicular LH receptors was observed even when the concentration of LH-RBI was significantly increased or when the testicular LH receptors were first incubated with LH-RBI prior to the addition of [^{125}I]oLH and a second incubation. Scatchard analysis revealed that the dissociation constant of [^{125}I]oLH binding was essentially the same in the presence or absence of LH-RBI. The results suggest that: (i) the lutropin receptor of ovaries, but not of testes, has a specific LH-RBI binding site in addition to the lutropin binding site, and (ii) the binding of the LH-RBI produces an "allosteric" type of inhibition to the binding of lutropin at the lutropin binding site.

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