NOTE

Species Specificity of Radioreceptor Assay and Radioimmunoassay for Rat FSH

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Abstract

Species specificity of the radioreceptor assay (RRA) for rat FSH, in which pregnant mare serum gonadotropin (PMSG)-treated immature rat ovary was employed as the receptor, was compared with that of NIAMDD rat FSH radioimmunoassay (RIA). In the RIA system, pituitary preparations from mammals only showed significant crossreaction. Their inhibition curves, however, were not always parallel to the standard curve. On the other hand, in the RRA system, the pituitary preparations from mammals, avians, lizard and amphibians competitively inhibited the binding of radioactive rat FSH to the ovarian receptor. Only the pituitary preparation from dog salmon failed to show any crossreaction in the RRA system. These results indicated that this RRA system would be useful for the measurement of FSH or gonadotropins of the pituitaries from mammals to amphibians.

Biological assay methods for FSH (Steelman Pohley, 1953; Igarashi and McCann, 1964) are not sensitive enough for the measurement of circulating FSH. In earlier reports (Wakabayashi et al., 1979, Minegishi et al., 1980), we have established an RRA system for FSH with pregnant mare serum gonadotropin (PMSG)-primed immature rat ovary, the sensitivity of which is comparable to that of RIA. If this system is applicable to many species of animals, it must be very useful also for comparative endocrinological studies. In the present report, we examined the crossreactivity of pituitary preparations from various species of animals to this RRA system.

Materials and Methods

Animals and treatments

Immature female rats of Wistar strain were purchased from Imai Experimental Animal Farm, Kodama, Saitama Prefecture, at 22-23 days of age, and kept in a temperature- and light-controlled room (24±2°C, light on from 500 h to 1900 h) with free access to water and food. They were subcutaneously injected with 50 IU per head of PMSG (Serotropin, Teikoku Hormone Mfg. Co., Tokyo) dissolved in physiological saline at 25 days of age and sacrificed 3 days after the injection.

Receptor preparation

Immediately after the animals were decapitated, their ovaries were removed and cleared of fat, oviducts and capsules. Then, all the ovaries were pooled, weighed and homogenized in a teflon-glass homogenizer with Tris-MgCl₂-BSA (0.04 M tris (hydroxymethyl)-aminomethane-HCl buffer pH 7.5 containing 5 mM of MgCl₂ and 1% bovine serum albumin) at a concentration of 200 mg tissue/ml. The ovarian homogenates were then filtered through a single layer of nylon gauze, and the filtrates were centrifuged at 3,000 rpm for 10 min in a refrigerated centrifuge. The precipitates were then suspended in Tris-MgCl₂-BSA at a concentration of 50 mg equivalent of ovarian wet weight per ml, and used as the receptor preparation.

Hormone preparations and antiserum

Rat FSH preparations used in the present experi-
ments were kindly supplied by Dr. A. F. Parlow of Rat Pituitary Hormone Program, NIAMDD, NIH, Bethesda, Md., U.S.A. as an RIA kit consisting of NIAMDD rat FSH I-3 (150 NIH-U/mg), NIAMDD rat FSH RP-1 (2.1 NIH-U/mg), and NIAMDD anti-rat FSH S-8. Radioiodination of FSH I-3 with $^{125}$I was carried out with lactoperoxidase (Boehringer Mannheim GmbH, Germany) according to the method of Miyachi et al. (1972) with minor modification.

Pituitary preparations

The pituitary glands were obtained from mammals including human, dog, cattle, goat, pig, and horse, and lower vertebrates including chicken, duck, Japanese guinea fowl, lizard (Gekko gekko), frog (Rana catesbeiana), newt (Cynops pyrrhogaster pyrrhogaster), and dog salmon (Onochynchus keta). The glands were homogenized with either distilled water or phosphate-buffered saline (0.01 M phosphate pH 7.5, 0.14 M NaCl containing 0.01% merthiolate), then frozen and thawed, and centrifuged at 12,000 rpm for 15 min. The clear supernatant fluid was stored at -70°C until use. Prior to assay, a part of the supernatant fluid was serially diluted with Tris-MgCl$_2$-BSA for RRA, or PBS containing 0.1% gelatin for RIA.

RRA for FSH

Incubation was carried out at 37°C for 3 h under constant shaking at 90 cycles/min with the following system in polystyrene or polypropylene tubes (12-10 x 75 mm): 100 µl of Tris-MgCl$_2$-BSA, 100 µl of receptor preparation, 100 µl of $^{125}$I-FSH. All the components were dissolved in Tris-MgCl$_2$-BSA. In order to estimate non-specific binding of $^{125}$I-FSH, 100 µl of Tris-MgCl$_2$-BSA, containing 50 IU of PMSG, was added instead of the standard hormone solution. After incubation, the mixture was chilled in ice water and 500 µl of ice-cold Tris-MgCl$_2$-BSA was added, then centrifuged at 3,000 rpm for 15 min. The supernatant fluid was removed by suction, and the precipitate was counted for radioactivity in an automatic gamma counter (Beckman, System 8000).

RIA for FSH

Double antibody RIA was carried out with the NIAMDD RIA kit mentioned above according to the method of Monroe et al. (1968) with minor modification. The assay system consisted of 400 µl of 0.1% gelatin-PBS, 200 µl of standard or sample dissolved in 0.1% gelatin-PBS, 100 µl of diluted antiserum (1:2,000, in 1% normal rabbit serum-0.05 M EDTA-PBS) and 100 µl of $^{125}$I-labelled hormone in 0.1% gelatin-PBS. A goat anti-rabbit gamma globulin serum, H-4, prepared in the Hormone Assay Center, was employed as the second antibody.

Statistical analysis

The inhibition curves obtained with samples and standard preparations were linearized by the method of least squares on logarithmic amounts of pituitary preparation versus logit B/Bo, and their slopes were compared by Student’s $t$-tests.

Results

Crossreactivity of pituitary preparations with RRA system

The pituitary preparations from the mammals, avians, lizard, and amphibians competitively inhibited the binding of radioactive rat FSH to the rat ovarian receptor (Fig. 1). Only that from dog salmon failed to show any crossreaction to the RRA system.

The correlation coefficients, slopes with their standard errors and confident limits are shown in Table 1. Statistical analyses indicated that among the animal species showing competitive inhibition only the pituitary preparation of Japanese quail showed a slope significantly different from that of the rat FSH standard preparation, RP-1.

Crossreactivity of pituitary preparations with the RIA system

In RIA, which was carried out simultaneously for the comparison, the pituitary preparations only from the mammals showed significant crossreaction, and their inhibition curves were not always parallel to the standard curve. Practically, the parallelism was observed only with human, dog, and horse pituitary preparations (Fig. 2).

Discussion

Bioassay systems, which are essential to the real hormonal information of biological materials, are very often insufficient in their sensitivity and precision, and cannot be applied to the measurement to circula-
Table 1. Inhibition curves of pituitary extracts from various species of animals

<table>
<thead>
<tr>
<th>Species</th>
<th>R</th>
<th>Slope*</th>
<th>S. E.</th>
<th>95% C.L.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower limit</td>
</tr>
<tr>
<td>RP-1 (1)</td>
<td>-0.993</td>
<td>-2.337</td>
<td>0.117</td>
<td>-2.622</td>
</tr>
<tr>
<td>Japanese quail</td>
<td>-0.993</td>
<td>-1.981**</td>
<td>0.099</td>
<td>-2.222</td>
</tr>
<tr>
<td>Guinea fowl</td>
<td>-0.993</td>
<td>-2.101</td>
<td>0.100</td>
<td>-2.346</td>
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<tr>
<td>Goat</td>
<td>-0.980</td>
<td>-2.710</td>
<td>0.276</td>
<td>-3.476</td>
</tr>
<tr>
<td>RP-1 (2)</td>
<td>-0.985</td>
<td>-2.067</td>
<td>0.147</td>
<td>-2.426</td>
</tr>
<tr>
<td>Duck</td>
<td>-0.994</td>
<td>-1.799</td>
<td>0.088</td>
<td>-2.026</td>
</tr>
<tr>
<td>Chicken</td>
<td>-0.992</td>
<td>-2.036</td>
<td>0.108</td>
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<tr>
<td>Pig</td>
<td>-0.973</td>
<td>-2.287</td>
<td>0.311</td>
<td>-3.276</td>
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<tr>
<td>Dog</td>
<td>-0.991</td>
<td>-2.241</td>
<td>0.132</td>
<td>-2.109</td>
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<tr>
<td>Cattle</td>
<td>-0.999</td>
<td>-2.263</td>
<td>0.045</td>
<td>-2.417</td>
</tr>
<tr>
<td>RP-1 (3)</td>
<td>-0.997</td>
<td>-2.619</td>
<td>0.138</td>
<td>-2.956</td>
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<tr>
<td>Horse</td>
<td>-0.994</td>
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<td>-3.797</td>
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<td>Newt</td>
<td>-0.982</td>
<td>-3.271</td>
<td>0.366</td>
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<tr>
<td>Human</td>
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<td>-2.696</td>
<td>0.110</td>
<td>-2.978</td>
</tr>
<tr>
<td>Frog</td>
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<td>-2.644</td>
<td>0.270</td>
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<tr>
<td>Lizard</td>
<td>-0.980</td>
<td>-1.973</td>
<td>0.285</td>
<td>-3.201</td>
</tr>
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</table>

* Slopes are from plots of logit % bound vs log of hormone concentration.
** This slope is significantly different from the slope of RP-1 (1). p<0.05

Fig. 2. The inhibition curves of the pituitary preparations from various species of animals in the NIAMDD rat FSH RIA system.
Fig. 1. The inhibition curves of the pituitary preparations from various species of animals in the RRA system. The curves were linearized by the method of least squares on logarithmic amounts of these preparations versus logit B/Bo.

As to gonadotropins, Leidenberger and Reichert (1973) described an RRA system for LH employing rat testicular homogenates and compared LH preparations from a variety of sources, and divided them into 4 groups according to the slopes of their inhibition curves. On the other hand, Reichert and Bhalla (1974) compared mammalian FSH by RRA employing the homogenates of rat seminiferous tubules as the receptor, and demonstrated that the slopes of the competition curves were common except for that obtained with horse pituitary.

Adachi and Ishii (1977), using avian testis as the receptor, showed that the
slopes of the competition curves were common among the pituitary extract of lower vertebrates except salmon.

In earlier reports we established an RRA system for rat FSH with the PMS-primed rat ovarian homogenates as the receptor, and showed that this RRA system was as sensitive as RIA. The present results with this RRA system indicated that it also allows wide latitude for the pituitary preparations from mammals to amphibians, and that our RRA system would be useful for the measurement of the FSH or gonadotropin in these animals. The slope of the competition curve with Japanese quail pituitary preparation was significantly different from that of the standard rat FSH RP-1. But even in this case this RRA system is applicable if a standard preparation obtained from quail pituitary is employed.

The sensitivity of this RRA system for hormone other than that rat origin was not examined in the present study, so its application to the measurement of the blood levels of hormone remains to be examined.

Acknowledgements

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References


