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Developmental Changes of Testicular Gonadotropin Receptors and Serum Gonadotropin Levels in Two Strains of Mice

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ABSTRACT—The relationship between testicular gonadotropin receptors and serum gonadotropin concentrations during sexual maturation was studied in two inbred mouse strains (BALB/cAJcl and ICR/Jcl strains). The specific binding of [^{125}I]iodo-FSH per unit testicular weight was the highest at 21 days of age in the BALB/cAJcl strain and 7 days in the ICR/Jcl strain, followed by a rapid decrease thereafter. The total FSH binding in the ICR/Jcl strain was significantly decreased at 35 days from the level at 28 days and remained low thereafter. However, such decrease was not observed in the BALB/cAJcl strain. Scatchard plot analyses showed that the changes in FSH binding were due to the changes in the number of binding sites and not in the affinity of binding. The specific binding of [^{125}I]iodo-LH per unit testicular weight reached a peak around puberty , and the total LH binding tended to remain constant after puberty in both strains. During early testicular developmental stages, the increase in the serum FSH levels was well correlated with the number of FSH receptors. Prepubertal increase in LH binding sites occurred after the rise in the serum FSH levels. To conclude, we found a strain difference in the regulation of the number of FSH receptors, indicating that down-regulation is not unique to the C57BL/6NCrj strain, but that it is not universal among strains of mice.

INTRODUCTION

The action of gonadotropins on the gonad is mediated by specific gonadotropin receptors localized in the plasma membrane of target cells. For example, follicle-stimulating hormone (FSH) first binds to the plasma membrane receptors of Sertoli cells [2, 8] and elicits various biochemical responses after stimulation of adenylyl cyclase [5, 12]. Therefore, the number of FSH receptors is one of the most important factors determining the sensitivity of Sertoli cells. Tsutsui [19] recently suggested that FSH and testosterone acted synergistically to induce FSH receptors during testicular development in the rat.

In immature and adult male rats, exogenous administration of large amount of FSH was effective in decreasing the number of FSH receptors in Sertoli cells [4, 10]. In male C57BL/6NCrj mice, endogeneous FSH was effective in decreasing its own receptors after puberty, and hypophysectomy when adult induced a significant increase in the number of FSH receptors in Sertoli cells [17]. This is the discovery showing that physiological level of FSH is effective in inducing down-regulation of FSH receptors. As the mechanism for this down-regulation of FSH receptors, internalization of FSH-FSH receptor complexes was claimed by autoradiographic and kinetic studies [13–15]. These findings in the mouse point out that the mouse testis serves as a suitable model for the study of down-regulation and the relationship between gonadotropin receptors and gonadotropin levels.

The present study was designed first to examine whether the down-regulation of FSH receptors found in male C57BL/6NCrj mice is unique to this strain or general phenomenon among various strains of mice, and secondly to afford fundamental knowledge on the developmental changes of gonadotropin receptors and serum gonadotropin concentrations in male mice of

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strains other than C57BL/6NCrj.

MATERIALS AND METHODS

Animals

Male mice of the BALB/cAJcl and ICR/Jcl strains maintained in this laboratory were used. They were housed in a temperature-controlled room(25 ± 0.5°C) under daily photoperiods of 12-hr light and 12-hr dark cycles (lights on at 0600 h), and were given laboratory chow and tap water *ad libitum*.

Preparation of serum and receptor samples

Intact mice of various ages during testicular development were sacrificed by decapitation between 1000–1200 h. Trunk blood was collected and allowed to clot at room temperature for one hour. After centrifugation at $1,800 \times g$ for 20 min, serum was stored at −20°C until radioimmunoassay (RIA) for FSH and LH. In order to secure sufficient volume of serum for assay in younger mice, serum from one to six animals was pooled. Immediately after the blood collection, the testes were taken out and weighed. The testes from one to six mice were pooled (the number of pooled testes differed according to the weight). The testes were homogenized in 0.04 M Tris-HCl buffer (pH 7.4) containing 0.005 M $MgSO_4$ and 0.1% BSA. The concentration of the homogenates was adjusted to contain 40 mg fresh testes per 100 μl in each age group. The homogenates were centrifuged at $11,000 \times g$ for 20 min at 4°C. The resulting pellets were resuspended in cold Tris-HCl buffer and adjusted to contain 20 mg original tissue/100 μl. The suspension was instantaneously frozen on dry ice-ethanol and stored at −70°C until the binding assay was performed. For the binding assay, the frozen samples were quickly thawed and diluted in cold Tris-HCl buffer. The final receptor preparations were adjusted to contain 5 mg equivalent original tissue/100 μl.

Hormone preparations

NIDDK-rat FSH(rFSH)-I-7 and NIDDK-rat LH(rLH)-I-7 were radioiodinated for the assay of FSH and LH receptors, respectively. Unlabeled ovine FSH (oFSH) and ovine LH (oLH) (Bio-Active Chem. Lab.,Tokyo) were used to correct for non-specific binding throughout the assays of FSH and LH receptors, respectively. NIDDK-rat FSH-RP-2 and NIDDK-rat LH-RP-3 were used as reference preparations in RIA of FSH and LH, respectively. These hormone preparations were the gifts from Dr. S. Raiti the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, NIH, and Dr. A. F. Parlow, Pituitary Hormone and Antisera Center, University of Maryland School of Medicine.

Binding assay

For the assays of FSH and LH receptors highly purified FSH (NIDDK-rFSH-I-7) and LH (NIDDK-rLH-I-7) were radioiodinated with ^{125}I in the presence of lactoperoxidase and hydrogen peroxide using the method described previously [16]. The specific activities of [^{125}I]iodo-FSH and [^{125}I]iodo-LH were 50 and 40 μCi/μg, respectively.

In radioreceptor assay (RRA) of FSH, receptor preparation and [^{125}I]iodo-FSH (1.0 ng) were incubated at 35°C for 2.5 hr with or without unlabeled oFSH. For RRA of LH, receptor preparation and [^{125}I]iodo-LH (1.0 ng) were incubated at 35°C for 2.5 hr with or without unlabeled oLH. At the end of incubation, the reaction tubes were centrifuged at $11,000 \times g$ for 20 min at 4°C and washed three times by adding 1 ml Tris-HCl buffer containing 0.1% BSA to each tube and centrifuged. Radioactivity of resulting pellets was counted in an auto-well gamma counter. Specific binding was calculated by total binding minus non-specific binding. In Scatchard plot analysis, saturation binding experiments were performed, where the receptor preparation (100 μl) and labeled ligand were incubated with or without different amount (0.2–256 ng) of unlabeled ligand. Final volume of reaction mixture for saturation-binding experiments was adjusted to 400 μl. The equilibrium constant of dissociation (Kd) was determined with the Scatchard plots, constructed from the data obtained from the saturation-binding experiments.

RIA of FSH and LH

Serum FSH and LH were measured by RIA

using a double antibody method [18]. Concentrations of FSH and LH (both being assayed in 100 μl serum) were expressed in terms of ng NIDDK-rat FSH-RP-2 and NIDDK-rat LH-RP-3 per ml serum, respectively.

Statistical analysis

Differences between testicular weights, FSH and LH bindings, and serum FSH and LH concentrations were analyzed by Student's *t*-test.

RESULTS

Changes in testicular weight

Male mice of the BALB/cAJcl strain were sacrificed at 14, 21, 28, 35, 42, 56 and 77 days of age. Fig. 1 shows the changes in the testicular weight. The testicular weight steadily increased from 14 to 42 days ($P<0.001$). After 42 days the testicular weight continued to increase much slowly (Fig. 1, upper panel).

In ICR/Jcl strain, mice were killed at 7, 14, 21, 28, 35, 42, 56 and 77 days. The testicular weight increased progressively from 7 to 42 days ($P<0.001$), and after that the weight of testes showed no significant increases (Fig. 1, lower panel).

Changes in FSH binding during testicular development

In BALB/cAJcl mice, the specific binding of [^{125}I]iodo-FSH /5 mg tissue equivalent was the greatest at 14 or 21 days (Fig. 2, upper panel). It rapidly decreased from 21 to 35 days, followed by a gradual decrease until 77 days of age. The specific binding of [^{125}I]iodo-FSH per two testes increased rapidly from 14 to 21 days ($P<0.001$), and then continued to increase slowly until 42 days of age. The levels at 56 and 77 days were almost the same as the level at 42 days (Fig. 2, lower panel).

In ICR/Jcl mice, the level of specific binding of [^{125}I]iodo-FSH/5 mg tissue was the highest at 7 days of age. A rapid decrease was observed between 7 and 21 days of age ($P<0.001$). The

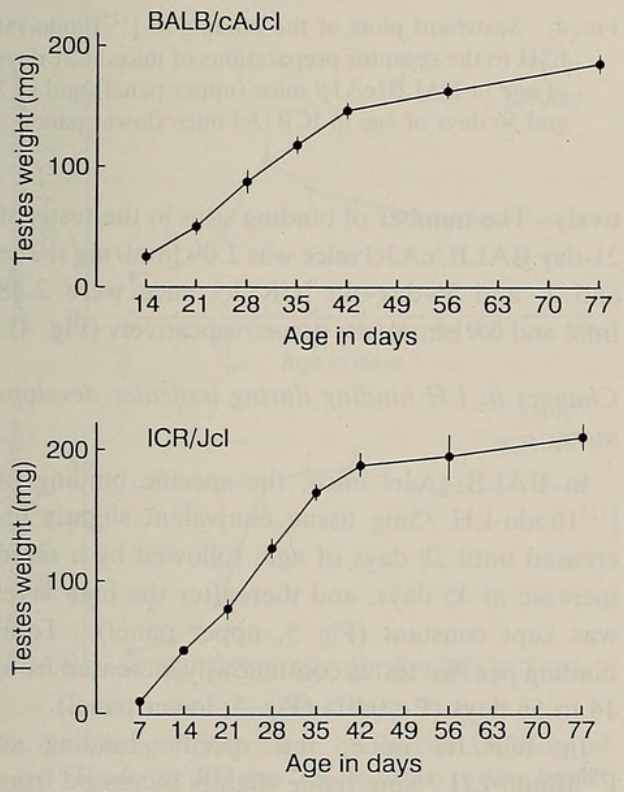

FIG. 1. Changes with age in the testicular weight in BALB/cAJcl (upper panel) and ICR/Jcl mice (lower panel). Each point represents the mean ± SEM (n =4).

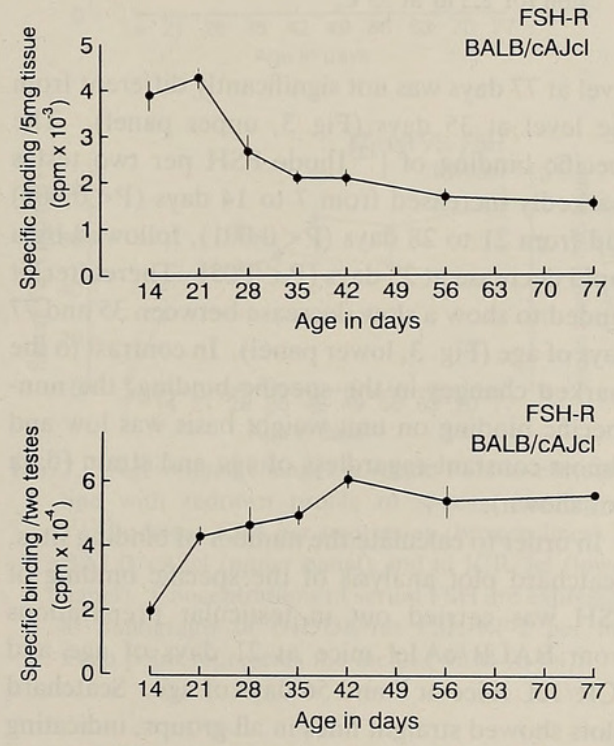

FIG. 2. Changes with age in specific binding of [^{125}I]iodo-rat FSH per 5 mg testicular tissue (upper panel) and specific binding of [^{125}I]iodo-rat FSH per two testes (lower panel) in BALB/cAJcl mice. Each point represents the mean ± SEM (n=4). Incubation of 2.5 hr at 35°C.

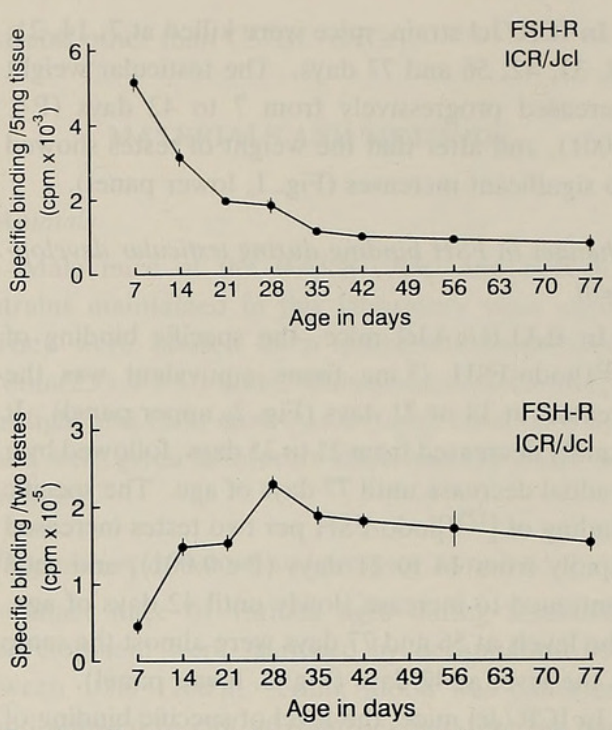

FIG. 3. Changes with age in specific binding of [^{125}I]iodo-rat FSH per 5 mg testicular tissue (upper panel) and specific binding of [^{125}I]iodo-rat FSH per two testes (lower panel) in ICR/Jcl mice. Each point represents the mean ± SEM (n=4). Incubation for 2.5 hr at 35°C.

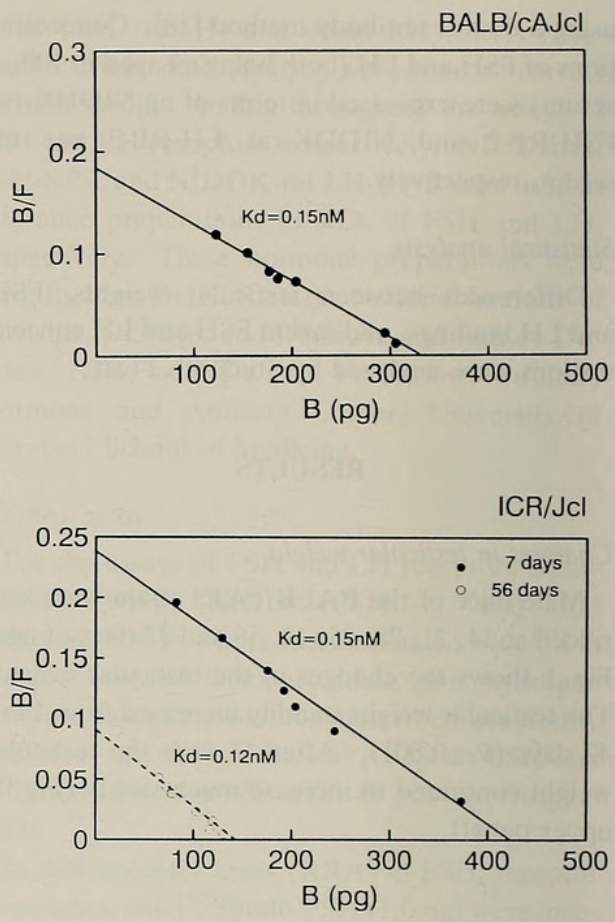

FIG. 4. Scatchard plots of the binding of [^{125}I]iodo-rat FSH to the receptor preparations of mice at 21 days of age in BALB/cAJcl mice (upper panel) and at 7 and 56 days of age in ICR/Jcl mice (lower panel).

level at 77 days was not significantly different from the level at 35 days (Fig. 3, upper panel). The specific binding of [^{125}I]iodo-FSH per two testes markedly increased from 7 to 14 days ($P<0.001$) and from 21 to 28 days ($P<0.001$), followed by a rapid decrease at 35 days ($P<0.05$). Thereafter, it tended to show a slow decrease between 35 and 77 days of age (Fig. 3, lower panel). In contrast to the marked changes in the specific binding, the non-specific binding on unit weight basis was low and almost constant regardless of age and strain (data not shown).

In order to calculate the number of binding sites, Scatchard plot analysis of the specific binding of FSH was carried out in testicular preparations from BALB/cAJcl mice at 21 days of age and ICR/JcL mice at 7 and 56 days of age. Scatchard plots showed straight lines in all groups, indicating the presence of one kind of binding sites. Kd calculated from the fitted lines of the plots in the testis from BALB/cAJcl mice at 21 days of age was 0.15 nM and those from ICR/Jcl mice at 7 and 56 days of age were 0.15 nM and 0.12 nM, respectively. The number of binding sites in the testis of 21-day BALB/cAJcl mice was 2.09 fmol/mg tissue and 7- and 56-day-old ICR/Jcl mice were 2.48 fmol and 0.91 fmol/mg tissue respectively (Fig. 4).

Changes in LH binding during testicular development

In BALB/cAJcl mice, the specific binding of [^{125}I]iodo-LH /5mg tissue equivalent slightly increased until 28 days of age, followed by a rapid increase at 35 days, and thereafter the high level was kept constant (Fig. 5, upper panel). Total binding per two testes continuously increased from 14 to 56 days ($P<0.01$) (Fig. 5, lower panel).

In ICR/Jcl mice, the specific binding of [^{125}I]iodo-LH /5 mg tissue slightly increased from 7 to 14 days, followed by a rapid increase between 14 to 28 days of age ($P<0.05$, 14 days vs. 28 days) (Fig. 6, upper panel). Then, the level tended to

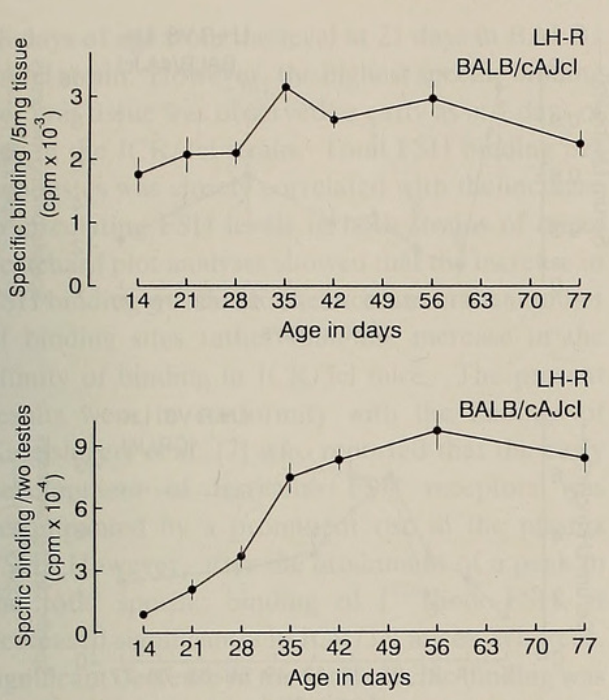

FIG. 5. Changes with age in specific binding of [^{125}I]iodo-rat LH per 5 mg testicular tissue (upper panel) and specific binding of [^{125}I]iodo-rat LH per two testes (lower panel) in BALB/cAJcl mice. Each point represents the mean ± SEM (n=4). Incubation for 2.5 hr at 35°C.

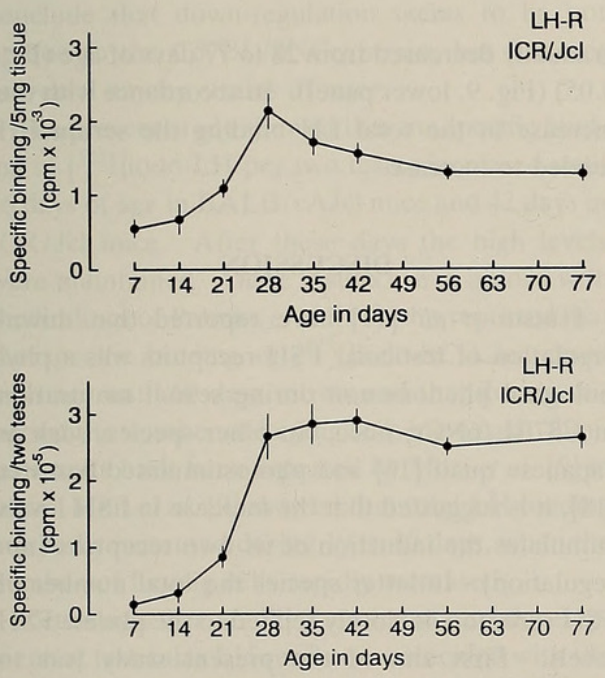

FIG. 6. Changes with age in specific binding of [^{125}I]iodo-rat LH per mg testicular tissue (upper panel) and specific binding of [^{125}I]iodo-rat LH per two testes (lower panel) in ICR/Jcl mice. Each point represents the mean ± SEM (n=4). Incubation for 2.5 hr at 35°C.

decrease until 77 days. The specific binding per two testes gradually increased from 7 to 21 days ($P < 0.01$), followed by a marked increase during 21 and 28 days ($P < 0.05$) (Fig. 6, lower panel). During 28 and 77 days the high level was maintained.

Changes in serum FSH level in male mice

In BALB/cAJcl mice, the serum FSH level increased rapidly until 28 days of age ($P < 0.05$, 7 days vs. 28 days). Thereafter, the serum FSH concentrations were almost constant (Fig. 7, upper panel). The period of rapid increase in the serum FSH level coincided well with the period of increase in the total binding shown as a broken line adopted from Fig. 2 (lower panel).

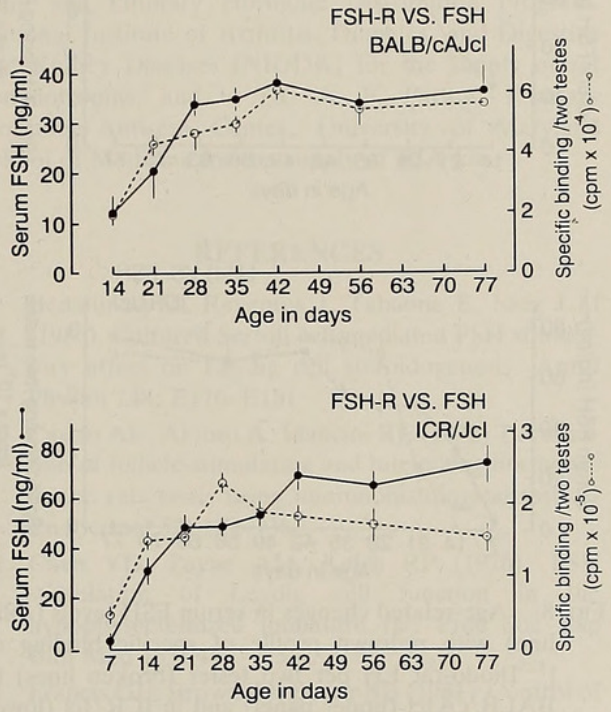

FIG. 7. Age-related changes in serum FSH levels (solid line with redrawn profile of specific binding of [^{125}I]iodo-rat FSH per two testes (broken lines) in BALB/cAJcl (upper panel) and in ICR/Jcl (lower panel). Concentrations of serum FSH are expressed as nanograms of NIDDK-rat FSH-RP-2 per ml. Each point represents the mean ± SEM (n=4).

In ICR/Jcl mice, the serum FSH concentrations rapidly increased from 7 to 21 days of age ($P < 0.001$) (Fig. 7, lower panel). Gradual increase was observed during 21 to 42 days. After that the serum FSH level was almost constant until 77 days

of age (Fig. 7, lower panel). The period of rapid increase in the serum FSH level fitted well with the period of increase in the total FSH binding (Fig. 7 lower panel). Increase in the serum FSH level was preceded by the increase in the total FSH binding at prepubertal period in ICR/Jcl strain, and the curve for the serum FSH level and that for FSH binding were roughly mirror-imaged between 21 and 35 days (Fig. 7, lower panel). Increase in the total LH binding occurred after the increase in the serum FSH at prepubertal period in both strains (Fig. 8).

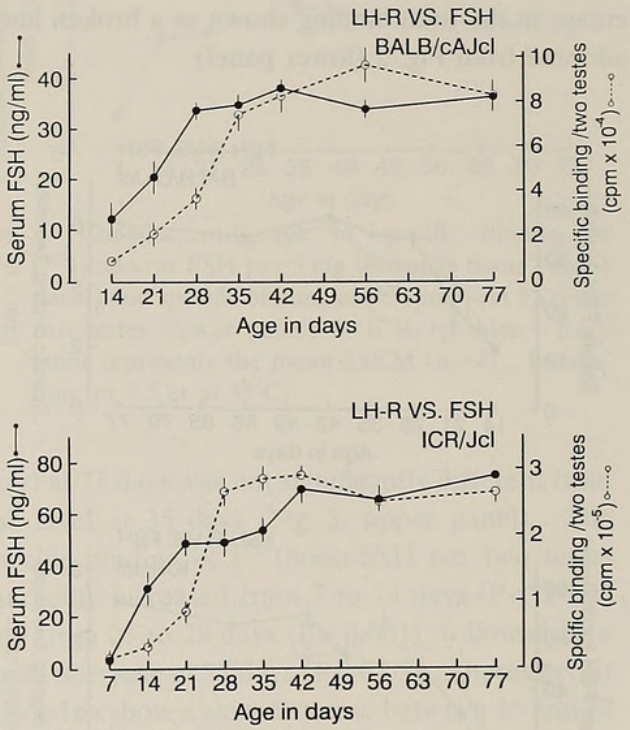

FIG. 8. Age-related changes in serum FSH levels (solid line) with redrawn profile of specific binding of [^{125}I]iodo-rat LH per two testes (broken lines) in BALB/cAJcl (upper panel) and in ICR/Jcl (lower panel). Concentrations of serum FSH are expressed as nanograms of NIDDK-rat FSH-RP-2 per ml. Each point represents the mean ± SEM (n=4).

Changes in serum LH level in male mice

The serum LH levels were generally variable in both strains. In BALB/cAJcl mice, the serum LH concentration was high at 14 days of age and suddenly declined at 21 days. Thereafter, it tended to increase until 56 days of age (Fig. 9, upper panel). In ICR/Jcl mice, the serum LH level steadily increased during 7 to 28 days of age, and

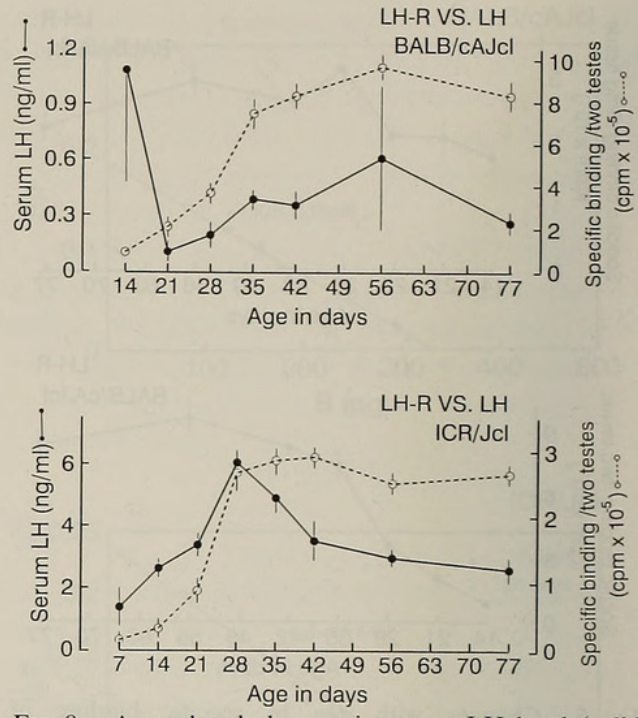

FIG. 9. Age-related changes in serum LH level (solid line) with redrawn profile of specific binding of [^{125}I]iodo-rat LH per two testes (broken line) in BALB/cAJcl (upper panel) and in ICR/Jcl (lower panel). Concentrations of serum LH are expressed as nanograms of NIDDK- rat LH-RP-3 per ml. Each point represents the mean ± SEM (n=4).

gradually decreased from 28 to 77 days of age ($P < 0.05$) (Fig. 9, lower panel). In accordance with the increase in the total LH binding the serum LH tended to increase.

DISCUSSION

Tsutsui *et al.* [17] have reported that down-regulation of testicular FSH receptors was a physiological phenomenon during sexual maturation in C57BL/6NCrj mice. In other species, such as Japanese quail [16] and photostimulated hamster [18], it is suggested that the increase in FSH levels stimulates the induction of its own receptors (up-regulation). In latter species the total number of FSH receptors is closely related to the plasma FSH levels. First aim of the present study was to examine whether the down-regulation of FSH receptors is unique to male C57BL/6NCrj mice or general phenomenon among various strains of mice. We found that the specific binding of [^{125}I]iodo-FSH per unit tissue weight decreased at

28 days of age from the level at 21 days in BALB/cAJcl strain. However, the highest specific binding per unit tissue was observed as early as at 7 days of age in the ICR/Jcl strain. Total FSH binding per two testes was closely correlated with the increase in circulating FSH levels in both strains of mice. Scatchard plot analyses showed that the increase in FSH binding was due to the increase in the number of binding sites rather than the increase in the affinity of binding in ICR/Jcl mice. The present results were in conformity with the findings of Ketelslegers *et al.* [7] who reported that the early development of testicular FSH receptors was accompanied by a prominent rise in the plasma FSH. However, after the attainment of a peak in the total specific binding of [^{125}I]iodo-FSH, it decreased significantly in ICR/Jcl mice. Whereas, significant decrease in the total specific binding was not observed in BALB/cAJcl mice. These results clearly show a difference in the two strains of mice. For the decrease from the pubertal level in the ICR/Jcl strain, down-regulatory effects of endogenous FSH might be a possible cause, because the serum level of FSH was much higher in the ICR/Jcl strain than BALB/cAJcl strain. We may conclude that down-regulation seems to be not unique to the C57BL/6NCrj strain, but it is not universal among strains of mice.

The present study showed that the specific binding of [^{125}I]iodo-LH per two testes increased until 56 days of age in BALB/cAJcl mice and 42 days in ICR/Jcl mice. After these days the high levels were maintained. These results are in accord with the findings of Pahnke *et al.* [11] who reported that the specific binding of [^{125}I]iodo-hCG increased with age until 60 days in rats and that during this period the number of Leydig cells increased. Similar results have been reported by Mori *et al.* [9] in mice. Mori *et al.* [9] showed that total LH binding markedly increased during 19 to 40 days, when the number of Leydig cells rapidly increased.

Ketelslegers *et al.* [7] found that the development of testicular LH receptors coincided with the phase of rise in the plasma FSH. In the present study, the specific binding of [^{125}I]iodo-LH per two testes increased rapidly in the prepubertal period in male mice in two strains, accompanied by an increase in the serum FSH levels. This finding indicates that the serum FSH is an important factor for the induction of LH receptors. In contrast to the serum FSH, the level of serum LH failed to show a good correlation with the number of LH receptors. In this conjunction, stimulatory effects of Sertoli cells to Leydig cell function have been well documented [1, 3, 6]. However, factors secreted from Sertoli cells by the stimulation of FSH and inducing LH receptors in Leydig cells have not yet been identified.

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