their effect on the response to PHA (fig. 1) compared with that to PMA (fig. 2). The PHA-stimulated lymphocytes have still an 80% and 60% mitogenic response with ethyl etrinoate and 13-cis-retinoic acid respectively even at a concentration of 10 ng/ml of these substances. This concentration (10 µg/ml) is one that could only be achieved in vivo by high pharmacological doses (the physiological level of retinol in the blood is 0.2–0.4 µg/ml). The reason for the difference in their effect on PHA stimulation compared with PMA stimulation is not clear nor can any definite significance be attributed to it, although one would hope that the retinol analogues maintain an anti-tumor promoter activity against PMA stimulation with less effect on the mitogenic response of lymphocytes to PHA than might be expected in an immune reaction. It is generally accepted that the retinol analogues such as 13-cis-retinoic acid are less toxic than retinol in animals while maintaining full anti tumor promoting activity[9-11]. This difference in their inhibitory effect on lymphocyte stimulation with PHA is another factor to be considered in chemoprevention of cancer[14].

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Development of the hypothalamic-hypophysial-gonadotrophic activities in fetal rats

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Summary. Pituitary responsiveness to LHRH and anti-LHRH serum was investigated in fetal rats aged 18.5–22.5 days. Synthetic LHRH injection in utero into fetuses brought about a remarkable depletion of pituitary-LH with a corresponding increase of serum-LH on day 18.5. On the contrary, anti-LHRH serum administration to day-20.5 fetuses caused a significant augmentation of pituitary-LH 1 day later. These data indicate that LH-gonadotrophs respond to LHRH even on day 18.5, and that endogenous LHRH begins to affect LH-gonadotrophs on day 20.5.

Information on the appearance of hypothalamic gonadotropic hormone releasing hormone (LHRH)[3-7] and of gonadotrophs[8-10] is available in rat fetuses. However, the time of the first occurrence of their functional relations has still not been determined, although the existence of the hypothalamic-hypophysial-gonadal axis is well established in fetal rats[11]. Synthetic LHRH induces LH discharge in newborn rats in vivo[12] and fetal rats[13] and fetal mice[14] in vitro. Our previous immunohistochemical study has demonstrated in fetal rats that synthetic LHRH depletes immunoreactive LH from the pituitary, and the storage of pituitary LH follows an administration of anti-LHRH serum[15]. In the present study, we attempted to determine by radioimmunoassay when the fetal pituitary responds to exogenous LHRH and when endogenous LHRH begins to stimulate LH release.

Materials and methods. Female Sprague-Dawley rats were mated overnight, and the vaginal smear was examined the next morning. If spermatozoa were found in the smear, the animals were determined as day 0.5 of gestation. For examination of the response of gonadotrophs to LHRH, day-18.5 and -19.5 fetuses were used. The pregnant rats were anesthesized with ether between 10.00 and 11.00 h, and the uterine horns were exposed by laparotomy. 2 µg of synthetic LHRH (Peptide Institute, Osaka, Japan) dissolved in 10 µl saline was injected i.p. into the fetuses in one uterine horn through the uterine wall. The fetuses in another uterine horn were injected with 10 µl saline and served as controls. After replacement of the uterine horns, the abdominal wall was closed. 2 h later, the rat thus treated was killed by cervical dislocation and the fetuses were removed. The heads of the fetuses were cut and the trunk blood spurting out from the cervix was collected in an ice-cold 1-ml glass tube. The pituitaries removed were dropped into ice-cold 1-ml homogenizers containing 0.1 ml of phosphate buffered saline (0.01 M PO_4–0.15 M NaCl, pH 7.4) and homogenized. The homogenate was transferred into a 0.4-ml plastic tube and stored at −80 °C until the assay procedure was carried out. The blood was collected from 3–11 animals. The serum of each experimental group consisted of 2 blood samples. The serum samples were stored at −80 °C.

Serum LH and FSH concentrations in fetal rats receiving LHRH or saline

Age (day)	Treatments	Gonadotrophins	Concentrations (ng/ml) Sample 1	Sample 2
18.5	Saline	LH	0.13 (11)*	0.32 (8)
18.5	LHRH	LH	0.39 (8)	0.55 (9)
19.5	Saline	LH	0.17 (5)	0.31 (7)
19.5	LHRH	LH	0.90 (8)	1.40 (11)
19.5	Saline	FSH	30.0 67)	37.4 (5)
19.5	LHRH	FSH	41.3 (8)	63.4 (11)

* Number of animals in parentheses.

To examine the presence of endogenous LHRH activity, 10 µl of an undiluted anti-LHRH serum[5] generated in rabbits was injected i.p. into the fetuses in one uterine horn by the procedure described above. The fetuses in the other uterine horn were injected with 10 µl normal rabbit serum and served as controls. The animals were killed on the following day and the pituitaries and blood samples from the fetuses were prepared as described above. Gonadotrophins in serum and pituitaries were assayed with the NIAMDD rat LH radioimmunoassay kit and NIAMDD rat FSH radioimmunoassay kit by the double antibody method[16] which was modified for micro-determination. Assay values of LH and FSH were expressed in terms of NIH-LH-S-1 and NIH-FSH-S-1 equivalents, respectively.

Results. Effects of LHRH. Figure 1 shows LH levels in the pituitary after the administration of LHRH. On days 18.5 and 19.5 of gestation, LHRH injected depletes LH from the pituitary by 67.9% and 61.6% of the control levels, respectively. The reverse occurs in the serum; serum LH concentrations increase in the LHRH-treated animals (table). FSH levels in the pituitary and serum are also measured on day 19.5 of gestation but not day 18.5 because FSH is barely detectable. On day 19.5 of gestation, a decrease of the pituitary content of FSH is found after the LHRH injection but is not significant (fig. 1). Serum level of FSH in the treated fetuses is higher than that of controls (table).

Effects of anti-LHRH serum. The effect of anti-LHRH serum is regarded as being immuno-neutralization of endogenous LHRH. Figure 2 shows LH levels in the pituitary treated by the administration of anti-LHRH serum. LH content is elevated in 21.5- and 22.5-day fetuses, in which anti-LHRH serum was injected 1 day before. In 20.5-day fetuses which received the antiserum injection on day 19.5, LH content does not increase in comparison to the controls. The LH concentration in the serum does not show any significant difference between the treated and control animals.

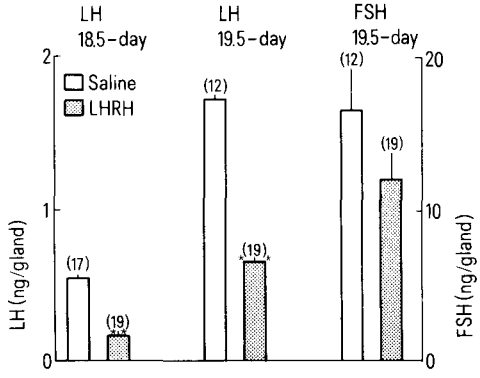

Figure 1. Effect of LHRH on gonadotrophin content in fetal pituitary. Vertical bars represent the SEM. Number of determinations in parentheses. ** $p < 0.01$ by Student's t-test.

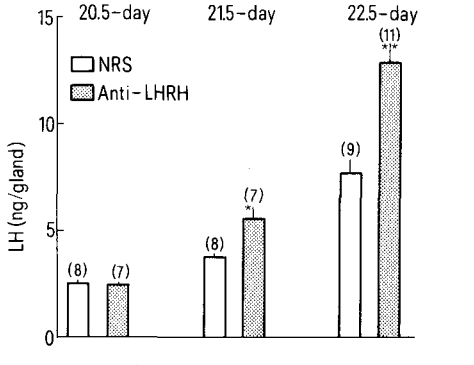

Figure 2. Effect of anti-LHRH serum on LH content in fetal pituitary. Vertical bars represent the SEM. Number of determinations in parentheses. NRS, Normal rabbit serum. * $p < 0.05$, ** $p < 0.01$ by Student's t-test.

Discussion. The present data demonstrate that in rats the fetal pituitary responds to synthetic LHRH by a reduction of the pituitary LH content with a corresponding rise in blood LH levels even on day 18.5 of gestation. Moreover, it is shown that immuno-neutralization of endogenous LHRH by the administration of anti-LHRH serum causes an increase of the LH content in the fetal pituitary after day 20.5 of gestation. This suggest that endogenous LHRH begins to stimulate LH-release on day 20.5 of gestation. Immunoreactive LH and FSH first occur on day 17.5 and 19.5 of gestation[8-10], respectively. LHRH-containing fiber terminals appeared in the external layer of the median eminence of rats on days 18.5 and 19.5 of gestation when observed by electron microscopy and light microscopy, respectively[5,6]. The present observations support the existence of a hypothalamic-hypophysial-gonadal system in fetal rats; this had previously been deduced from experiments involving fetal encephalectomy[17] and decapitation[18]. When the encephalectomy or decapitation was performed on day 16.5 of gestation, the ultrastructual appearance of Leydig cells is immature, especially in decapitated fetuses, on day 21.5 of gestation[19].

The present findings are consistent with our previous immunohistochemical observations in rats, in which LH-gonadotrophs disappeared after an injection of synthetic LHRH into fetuses on day 18.5 of gestation, and in which the immunoreactivity of LH-gonadotrophs was augmented following anti-LHRH serum administration on day 20.5[15]. It seems to be reasonable that the pituitary responsiveness to LHRH occurs previously to the appearance of hypothalamic regulation of LH-gonadotrophs.

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