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Effect of Synthetic Luteinizing Hormone–Releasing Hormone in Newborn Rats[1] (38598)

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The production of hypospadias by maternal administration of antibodies to luteinizing (LH) and follicle stimulating (FSH) hormones in the fetal male rat has provided evidence of the functional integrity of the male fetal pituitary gonadal axis (1). This observation has provided direct support of the findings obtained from hypophysectomy of the fetal male rabbit and has clarified the ambiguous results obtained from fetal hypophysectomy in the male rat (2). Disruption of the programming of puberty and adult reproduction in female rats manifested by early onset of puberty, changes in vaginal cyclicity and in the formation of undersized offspring by maternal administration of antibodies to LH:FSH has suggested that this axis is also functional in the female rat fetus (3). Hypothalamic regulation of the secretion of pituitary LH and FSH is mediated by the decapeptide luteinizing hormone–releasing hormone (LH–RH) (4). Synthetic LH–RH stimulates the release of LH and FSH in prepubertal and adult humans and experimental animals (5–7). Dohler and Wuttke (8) have observed high levels of serum LH and FSH in one day old male and female rat pups, the values in the females being higher than those in males. In this report we demonstrate that both male and female rat pups between 10 and 20 hr of age are responsive to synthetic LH-RH *in vivo*.

Materials and Methods. Female rats with timed pregnancies were obtained from Charles River Co. and maintained under standard conditions until delivery. Between 10 and 20 hr after birth randomly selected male and female pups received either synthetic LH–RH (Beckman), 1 μg in 5 μl of normal saline subcutaneously, or normal saline alone. The animals were sacrificed by decapitation 20 min after injection. Trunk blood was collected, the red cells separated and the sera stored individually at −20°. After dissection the pituitaries were immediately frozen in individual plastic bags without weighing. Frozen sera and pituitaries were sent safely by air freight from Philadelphia (BHS, ASG) to St. Petersburg (AWR, GED). The pituitary glands were thawed and homogenized in phosphate (0.01 *M*)-saline (0.15 *M*) immediately prior to assay.

Pituitary LH and FSH contents were determined by radioimmunoassays previously described employing reagents provided by the Hormone Distribution Officer, NIAMDD (9). Serum LH concentrations were determined by radioimmunoassay employing 50 μl of serum. Serum from hypophysectomized adult male rats was included in the assay in order to correct for the nonspecific effects of serum. All determinations were performed in the same assay in order to eliminate interassay variability. The intra-assay coefficients of variation for LH and FSH radioimmunoassays were 3.5 and 5.1% respectively. Insufficient serum was available for FSH determinations.

Results. The pituitary contents of LH and FSH were significantly reduced in female rats who had received LH-RH when compared to control animals. Synthetic LH–RH appeared to lower the pituitary content of LH and FSH in male pups, although the differences were not statistically significant (Table I).

Serum LH concentrations of newborn female pups who had received saline were significantly higher ($P < 0.01$) than levels recorded in similarly prepared male rats.

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TABLE I. EFFECT OF SYNTHETIC LH-RH UPON PITUITARY GONADOTROPIN CONTENT IN NEWBORN RATS.

	Luteinizing hormone	Follicle stimulating hormone
Male		
Control	0.595 ± 0.364 (12)[a,b]	1.129 ± 0.580 (9)[a,b]
LH-RH	0.518 ± 0.135 (13)	0.833 ± 0.306 (12)
Female		
Control	0.725 ± 0.184 (8)	1.118 ± 0.326 (8)
LH-RH	0.527 ± 0.110 (9)[c]	0.746 ± 0.104 (7)[c]

[a] Mean ± 1 SD.
[b] Microgram NIAMDD reference preparation/pituitary.
[c] $P < 0.02$.

TABLE II. EFFECT OF SYNTHETIC LH-RH UPON SERUM CONCENTRATIONS OF LUTEINIZING HORMONE IN NEWBORN RATS.

Male	
Control	25.7 ± 18.3 (12)[a, b]
LH-RH	98.4 ± 23.3 (14)[c]
Female	
Control	62.0 ± 14.6 (9)
LH-RH	132.6 ± 14.8 (8)[d]

[a] Mean ± 1 SD.
[b] Nanogram NIAMDD reference preparation/ml.
[c] $P < 0.01$.
[d] $P < 0.02$.

Serum concentrations of LH were higher in male and female pups who had received LH-RH than in saline-treated animals (Table II).

Discussion. Present data demonstrate that male and female pups, 10–20 hr of age, respond to synthetic LH-RH with reduction of the pituitary content of LH and FSH together with a corresponding rise in the blood level of LH. These observations support the existence of a regulatory control mechanism for gonadotropin secretion in newborn rats which had been previously deduced from experiments involving fetal hypophysectomy or the maternal administration of antibodies to gonadotropins. It seems reasonable to conclude that in the male fetus and neonate, the hypothalamic–pituitary–testicular axis is intact and functioning. The present report is consistent with previous observations in the rat and other species showing that the female neonate has a relatively higher level of circulating LH and FSH than does the male (8, 10, 11) and suggests that hypothalamic control of gonadotropin production may also be operative in the female rat during fetal and neonatal life. This finding is somewhat surprising because the ovary does not become functional or steroidogenic until the animal is 10 days of age (12) and apparently does not play a role in the sex differentiation of the female (13). Pituitary responsiveness to LH-RH in the neonatal female rat is another indication of the activity of the short feedback loop and extends previous observations that antibodies to LH:FSH can disrupt the programming of the hypothalamic centers controlling the timing of the onset of puberty, vaginal cyclicity and fetal and placental weights in females (3).

Summary. Administration of synthetic LH-RH to male and female rats on the first day of life reduced pituitary content of FSH and LH and increased serum concentrations of LH.

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