Gonadotrophin release induced by Gn-RH or progesterone in female rats maintained on high or low levels of feed intake

B. E. Howland

Department of Oral Biology, University of Manitoba, Winnipeg, Manitoba R3E 0W3, Canada

Underfeeding in the female rat results in ovarian atrophy (Piacsek & Meites, 1967; Howland, 1971a) which appears to be related to reduced basal serum levels of LH (Howland, 1971b, 1972; Gombe, Apgar & Hansel, 1973). The observation that ovariectomized underfed rats are capable of maintaining high serum levels of gonadotrophins (Howland, 1971c; Howland & Ibrahim, 1973) suggests that the hypothalamus, if not inhibited, can stimulate the pituitary to secrete gonadotrophins at rates higher than those necessary to maintain normal basal levels in the serum. It is still possible, however, that pituitary responsiveness to hypothalamic hormones is altered in underfed rats or that the ability of the hypothalamus to release sufficient amounts of Gn-RH to cause an ovulatory surge of LH is impaired.

The present study was therefore conducted to compare the effect of high or low feed levels on the response of intact rats to injected Gn-RH and of ovariectomized rats to Gn-RH or progesterone.

The rats, of the Long-Evans strain produced in our own colony, were individually caged in a light- (lights on 06.00–18.00 hours) and temperature- ($23 \pm 0.5^\circ\text{C}$) controlled room. Animals on the high feed level (HF) were allowed unrestricted intake of Purina Laboratory Chow, those on the low level (LF) were fed 50% of the normal daily feed intake as measured in a 3-day pre-experimental period.

Experiment 1

Rats were ovariectomized at 3 months of age and at 5 months they were maintained on HF or LF. On Day 19 of the feed treatment, a s.c. injection of 25 mg progesterone + 50 µg oestradiol benzoate in sesame oil was given. On Day 22, the rats were anaesthetized with ether and 5 ng Gn-RH/100 g body wt were injected into the tail vein. The Gn-RH (synthetic LH-RH/FSH-RH, amide form) was dissolved in physiological saline. A blood sample (1.2 ml) was obtained by heart puncture immediately before the Gn-RH injection. The rats were decapitated at 5, 10, 15 or 20 min after the injection and trunk blood was collected for determination of serum LH concentrations by the radioimmunoassay described by Niswender, Midgley, Monroe & Reichert (1968). Information on the assay sensitivity and variation in this laboratory is given by Howland (1975).

The serum concentration of LH before Gn-RH injection was higher ($P < 0.05$) in HF than in LF rats (164 ± 21 versus 94 ± 10 ng/ml). The results in Table 1 show that the LH response was maximal at 5 min after Gn-RH and did not change significantly over the next 15 min. Neither the effect of feed level nor the interaction of feed level with time were significant.

Table 1. Mean ($\pm$ S.E.M.) increases in serum LH concentrations (ng NIAMDD rat LH-RP1/ml) in rats on high and low feed intakes after i.v. injection of Gn-RH

	Time after Gn-RH (min)							
	High intake				Low intake			
	5	10	15	20	5	10	15	20
No. of rats	8	7	7	6	7	7	6	7
Δ LH conc.	1436 ± 168	1302 ± 123	1176 ± 111	1089 ± 251	1494 ± 355	1517 ± 137	1631 ± 292	1112 ± 97

Experiment 2

At the beginning of experimental feeding the rats were 3 months of age and half of the rats had been ovariectomized 3 weeks previously. Intact rats were anaesthetized with ether on the first morning they displayed a predominantly leucocytic vaginal smear after 20 days of the feeding regimen (20, 21 or 22 days). A blood sample (1.2 ml) was taken from the heart and 0.5 µg Gn-RH/100 g body wt was immediately injected into the tail vein. The animals were decapitated 20 min after the injection and trunk blood was collected for assay of LH concentrations. Ovariectomized rats were injected s.c. with 25 mg progesterone + 50 µg oestradiol benzoate in sesame oil on Day 17 of experimental feeding and 3 days later were injected with 5 ng Gn-RH/100 g body wt. Trunk blood was collected after decapitation 20 min later. The results are shown in Table 2. In spite of the low LH levels, some LF rats were still showing oestrous cycles, since 4 of the 13 rats exhibited oestrous or pro-oestrous vaginal smears after 20 days of feed treatment. In the ovariectomized rats, Gn-RH elevated LH levels to values much higher than those observed in intact Gn-RH-treated rats and the response of HF and LF rats was similar, suggesting that LF rats are capable of responding to Gn-RH with a release of LH which is at least as great as that in HF rats. Similarly, Root & Duckett (1973) have reported that fasted or fed male rats are equally responsive to Gn-RH.

Table 2. The effects of high and low levels of feed intake on the mean ($\pm$ S.E.M.) body weight (g) and serum concentrations of FSH (ng NIAMDD rat FSH-RP1/ml) and LH (ng NIAMDD rat LH-RP1/ml) in rats

	High intake	Low intake	Significance
<i>Experiment 2</i>			
Intact rats			
No. of animals	13	13	
Body weight	273 $\pm$ 4	193 $\pm$ 4	$P < 0.01$
LH before Gn-RH	84 $\pm$ 15	22 $\pm$ 3	$P < 0.01$
LH after Gn-RH	385 $\pm$ 48	424 $\pm$ 38	N.S.
Ovariectomized rats			
No. of animals	10	11	
Body weight	305 $\pm$ 6	237 $\pm$ 4	$P < 0.01$
LH before Gn-RH	160 $\pm$ 16	183 $\pm$ 20	N.S.
LH after Gn-RH	1362 $\pm$ 127	1650 $\pm$ 110	N.S.
<i>Experiment 3</i>			
<i>Vehicle-vehicle</i>			
No. of animals	8	8	
Body weight	365 $\pm$ 10	263 $\pm$ 9	$P < 0.01$
LH	695 $\pm$ 61	773 $\pm$ 96	N.S.
FSH	1491 $\pm$ 81	1799 $\pm$ 56	$P < 0.01$
<i>Oestrogen-vehicle</i>			
No. of animals	7	8	
Body weight	348 $\pm$ 9	274 $\pm$ 9	$P < 0.01$
LH	443 $\pm$ 42	454 $\pm$ 114	N.S.
FSH	1371 $\pm$ 64	1228 $\pm$ 68	N.S.
<i>Oestrogen-progesterone</i>			
No. of animals	8	8	
Body weight	364 $\pm$ 8	274 $\pm$ 6	$P < 0.01$
LH	2277 $\pm$ 310	3215 $\pm$ 369	N.S.
FSH	1695 $\pm$ 95	2239 $\pm$ 163	$P < 0.05$

Experiment 3

After 17 days on HF or LF, the rats were allocated to 3 treatment groups: rats in one group received a s.c. injection of the corn oil vehicle, and those in the other groups received 7.5 µg oestradiol benzoate/100 g body wt in corn oil. Three days later (73 hr after first injection) at 10.00 hours the oil-

injected rats received a second injection of oil (vehicle-vehicle) as did those in one of the oestrogen-treated groups (oestrogen-vehicle). The third group received 0.7 mg progesterone/100 g body wt in sesame oil (oestrogen-progesterone). The rats were anaesthetized with ether and blood samples were obtained by heart puncture at approximately 15.00 hours (5 hr after the second injection). The oestrogen-progesterone treatment used was reported by Jackson (1972) to induce release of LH. Concentrations of LH and FSH were measured in the blood samples, the FSH by the NIAMDD radioimmunoassay kit (see Howland, 1975).

Oestrogen treatment depressed the serum LH levels, while oestrogen followed by progesterone resulted in a release of LH which produced very high serum LH levels (Table 2). A similar pattern of steroid effects was evident in the case of FSH although the magnitude of treatment group differences was not as great. Feed level did not significantly affect serum levels of LH although mean levels of LH tended to be higher in LF than in HF rats. Serum FSH levels were higher in LF rats than in HF rats in two of the treatment groups.

These results demonstrate that LF rats are equally or perhaps more responsive to the gonadotrophin-releasing action of progesterone than HF rats. The positive effect of progesterone is believed to play an essential role in inducing the ovulatory surge of gonadotrophins (Armstrong, 1970; Jackson, 1972). The responses to progesterone observed in the present study indicated that the cells involved in synthesis of the hypothalamic hormone(s) required for gonadotrophin release and the pituitary cells which respond to Gn-RH all seem to be functioning adequately in rats on a low level of feed intake. The eventual cessation of oestrous cycles, therefore, is probably explained by a lack of the ovarian steroids required for the positive feedback effects which trigger an ovulatory release of hormone. This lack of adequate steroid secretion may reflect a failure of hypothalamic mechanisms which govern basal LH secretion.

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