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The Temporal Pattern of LH, FSH, and Ovulatory Responsiveness To Luteinizing Hormone Releasing Hormone in the Cyclic Hamster[1] (40195)

HAROLD I. SIEGEL[2] AND GILBERT S. GREENWALD[3]

Department of Physiology, Ralph L. Smith Research Center, Kansas City, Kansas 66103

Several previous studies have dealt with the effects of luteinizing hormone releasing hormone (LHRH) on the hamster pituitary-ovarian axis (1–4). However, these investigations have usually focused on a limited time span within the estrous cycle and they have not assessed the release of LH and FSH and whether ovulation is induced in the same group of animals.

The present study was designed to determine LH and FSH levels in hamsters serially bled over a period of several hours after the administration of LHRH on each day of the 4-day estrous cycle. In this study, Day 1 designates the morning of ovulation which in our colony occurs between 0100 and 0003 hr (5) and Day 4 corresponds to proestrus. The animals were sacrificed the day after LHRH treatment to ascertain whether they had ovulated.

Materials and methods. Sexually mature hamsters (85–135 g) were used after at least three consecutive 4-day cycles. The animals were maintained on a 14 hr light: 10 hr dark sequence with lights on from 0500 to 0900 hr (CST). The cycle was monitored by checking for the postovulatory vaginal discharge which occurs on Day 1.

At 1200 hr on each day of the cycle, a baseline blood sample (0.4–0.5 ml) was collected from unanesthetized hamsters by cardiac puncture; the details on the technique and the nontraumatic effects of bleeding have been previously documented (6, 7). Thereafter, at 1300 and/or 1500 hr, various amounts of a synthetic LHRH (Parke-Davis, Lot P) or saline (as a control) were injected SC and the unanesthetized animals were bled several times over the next few hours (specific times and doses of LHRH are indicated in Tables I–IV). The LHRH doses were chosen on the basis of their ability to induce ovulation in a pilot study. On Day 4, animals were injected at 1300 hr with phenobarbital (6.5 mg/100 g body wt, SC) to block ovulation and the endogenous surges of gonadotropins (6, 8).

The ability of LHRH to restore or to induce preovulatory surges of LH and FSH and ovulation 16–18 hr later was then tested by killing all animals the next morning at 0900–1000 hr. The ovaries were checked for newly formed corpora lutea and the oviducts were flushed to recover ova still embedded in granulosa cells.

Radioimmunoassays (RIA). General methodology for the double antibody RIA for FSH and LH was similar to that described for bovine and ovine LH (9) which has been subsequently modified and validated for use in the hamster (10). The ovine:ovine LH RIA system and the rat:rat FSH RIA were used. Two procedural modifications were made to facilitate the use of reduced volumes of serum: (1) the final incubation volume in an assay tube was 0.5 ml, instead of 1 ml, and was obtained by reducing the volume of each component in the final incubation solution by 50%; (2) NIAMDD-anti-rat FSH serum 6 was used in the FSH RIA. Anti-ovine LH serum (GDN-15) and an ovine LH for iodination (LER-1056-C2) were kindly provided by Drs. Gordon D. Niswender and Leo E. Reichert, respectively. Serum hormone levels are expressed in terms of NIAMMD-rat FSH-RP-1 (2.1 × NIH-FSH-S1) or NIAMMD-rat LH-RP-1 (0.03 × NIH-LH-S1). Statistical differences were determined by analyses of variance and multiple t tests and were considered significant at the $P < 0.05$ level (two-tailed).

Results. Injection of LHRH on Day 1 (Table I). No animals ovulated the morning after injecting LHRH which is understandable in

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[2] Present address: Institute of Animal Behavior, Rutgers University, Newark, New Jersey 07102.

[3] To whom reprint requests should be addressed.

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TABLE I. CHANGES IN SERUM LEVELS OF FSH AND LH AFTER INJECTING LHRH ON DAY 1 OF THE HAMSTER ESTROUS CYCLE.

Group	LHRH (time injected)	FSH (ng/ml) at: 1200	1300	1400	1530	1600	LH (ng/ml) at: 1200	1300	1400	1530	1600	Number ovulating on Day 2/total
1	saline (1300)	616 ± 44	693 ± 117	556 ± 25	599 ± 75	596 ± 83	23 ± 1	28 ± 8	28 ± 8	30 ± 11	22 ± 9	0/4
2	1μg (1300)	693 ± 85	1336 ± 100[a]	1074 ± 141	682 ± 93	790 ± 144	39 ± 5	580 ± 54	353 ± 45	33 ± 5	25 ± 2	0/5
3	1 μg (1500)	898 ± 103	921 ± 197	762 ± 63	1772 ± 127	1789 ± 138	39 ± 7	25 ± 3	28 ± 8	703 ± 74	683 ± 141	0/5
4	1 μg (1300, 1500)	457 ± 27	1139 ± 129	1105 ± 155	1068 ± 175	1030 ± 153	39 ± 7	636 ± 64	268 ± 59	502 ± 195	295 ± 56	0/5
5	2 μg (1300)	702 ± 41	1290 ± 319	1146 ± 168	738 ± 101	794 ± 137	44 ± 13	499 ± 62	404 ± 162	47 ± 12	39 ± 18	0/5
6	2 μg (1500)	628 ± 65	561 ± 68	504 ± 56	804 ± 127	858 ± 104	27 ± 4	39 ± 9	29 ± 3	559 ± 74	413 ± 135	0/6
7	2 μg (1300, 1500)	610 ± 58	794 ± 56	954 ± 120	896 ± 92	958 ± 109	29 ± 7	516 ± 138	483 ± 95	301 ± 44	380 ± 55	0/6

[a] In Tables I-IV, underlined values are significantly different from the 1200 hr control bleeding ($P < 0.05$, two-tailed).

TABLE II. CHANGES IN SERUM LEVELS OF FSH AND LH AFTER INJECTING LHRH ON DAY 2 OF THE HAMSTER ESTROUS CYCLE.

Group	LHRH (time injected)	FSH (ng/ml) at: 1200	1300	1400	1530	1600	LH (ng/ml) at: 1200	1300	1400	1530	1600	Number ovulating on Day 3/total
1	saline (1300)	178 ± 24	159 ± 20	156 ± 16	172 ± 10	123 ± 18	11 ± 3	14 ± 4	11 ± 1	8 ± 1	7 ± 3	0/5
2	1 μg (1300)	213 ± 16	557 ± 54	486 ± 28	244 ± 20	225 ± 12	16 ± 2	546 ± 50	267 ± 49	17 ± 2	19 ± 6	0/5
3	1 μg (1500)	267 ± 19	245 ± 26	249 ± 21	618 ± 74	655 ± 50	13 ± 5	12 ± 3	5 ± 1	753 ± 78	732 ± 99	0/5
4	1 μg (1300, 1500)	266 ± 9	576 ± 58	667 ± 51	722 ± 58	732 ± 50	11 ± 4	719 ± 100	671 ± 98	483 ± 65	349 ± 58	0/7
5	2 μg (1300)	323 ± 16	669 ± 78	733 ± 78	484 ± 80	449 ± 34	4 ± 1	627 ± 40	628 ± 21	42 ± 25	13 ± 4	0/5
6	2 μg (1500)	329 ± 65	271 ± 22	260 ± 20	650 ± 74	835 ± 83	15 ± 3	14 ± 5	18 ± 5	1158 ± 80	1071 ± 101	0/6
7	2 μg (1300, 1500)	211 ± 24	445 ± 43	610 ± 59	537 ± 49	675 ± 81	26 ± 4	597 ± 42	535 ± 19	432 ± 48	606 ± 169	0/4

TABLE III. CHANGES IN SERUM LEVELS OF FSH AND LH AFTER INJECTING LHRH ON DAY 3 OF THE HAMSTER ESTROUS CYCLE.

Group	LHRH (time injected)	FSH (ng/ml) at: 1200	1300	1400	1530	1600	LH (ng/ml) at: 1200	1300	1400	1530	1600	Number ovulating on Day 4/total
1	saline (1300)	209 ± 19	194 ± 15	201 ± 16	227 ± 21	233 ± 17	26 ± 1	23 ± 3	22 ± 3	18 ± 1	22 ± 3	0/5
2a	0.5 μg (1300)	190	370	397	249	240	28	1252	344	52	29	2/5 { 2/2
2b	0.5 μg (1300)	175 ± 15	446 ± 67	408 ± 48	252 ± 25	221 ± 31	22 ± 3	974 ± 393	563 ± 174	36 ± 5	24 ± 2	0/3
3a	0.5 μg (1500)	156 ± 14	177 ± 15	156 ± 14	572 ± 124	601 ± 98	26 ± 3	22 ± 2	21 ± 2	841 ± 70	422 ± 34	3/6 { 3/3
3b	0.5 μg (1500)	150 ± 16	194 ± 11	165 ± 12	217 ± 87	236 ± 56	15 ± 3	25 ± 11	26 ± 10	83 ± 11	212 ± 40	0/3
4	0.5 μg (1300, 1500)	236 ± 10	450 ± 29	465 ± 52	530 ± 83	594 ± 108	31 ± 4	632 ± 118	495 ± 138	1117 ± 287	1038 ± 283	5/5
5a	1 μg (1300)	178 ± 15	404 ± 54	388 ± 44	257 ± 25	219 ± 20	25 ± 6	626 ± 91	620 ± 81	63 ± 23	48 ± 13	5/8 { 5/5
5b	1 μg (1300)	151 ± 13	343 ± 3	350 ± 35	250 ± 15	218 ± 27	17 ± 3	412 ± 51	281 ± 56	32 ± 4	31 ± 4	0/3
6	1 μg (1300)	204 ± 13	221 ± 17	204 ± 21	549 ± 43	583 ± 89	19 ± 2	24 ± 4	24 ± 3	850 ± 60	785 ± 64	5/5
7a	1 μg (1300, 1500)	250 ± 23	630 ± 68	611 ± 41	834 ± 118	750 ± 113	26 ± 2	813 ± 115	1024 ± 464	1067 ± 374	815 ± 141	5/6 { 5/5
7b	1 μg (1300, 1500)	217	544	546	738	899	32	573	423	683	719	0/1

TABLE IV. CHANGES IN SERUM FSH AND LH AFTER INJECTING LHRH ON DAY 4 OF THE HAMSTER ESTROUS CYCLE.

Group	LHRH (time injected)	FSH (ng/ml) at:					LH (ng/ml) at:					Number ovulating on Day 1/total
		1200	1330	1400	1530	1600	1200	1330	1400	1530	1600	
1	saline (1300)	197 ± 11	210 ± 15	214 ± 16	535 ± 66	773 ± 104	16 ± 2	22 ± 4	40 ± 9	1808 ± 439	2521 ± 438	18/18
2	Phen (1300)	157 ± 17	150 ± 14	135 ± 12	158 ± 22	139 ± 14	14 ± 2	8 ± 2	13 ± 6	9 ± 1	15 ± 2	0/6
3	Phen + 50 ng (1300)	135 ± 17	166 ± 31	176 ± 43	147 ± 14	144 ± 11	16 ± 2	192 ± 32	102 ± 37	12 ± 2	13 ± 2	0/5
4a	Phen + 50 ng (1500)	116 ± 22	114 ± 6	122 ± 16	179 ± 66	182 ± 16	25 ± 4	14 ± 4	23 ± 7	795 ± 218	409 ± 93	4/4
4b	Phen + 50 ng (1500)	139	127	118	158	212	17	9	12	455	314	4/6 0/2
5	Phen + 50 ng (1300, 1500)	124 ± 12	165 ± 24	211 ± 15	227 ± 25	233 ± 22	17 ± 2	396 ± 74	148 ± 44	321 ± 63	287 ± 37	5/5
6	Phen + 100 ng (1300)	190 ± 15	304 ± 35	293 ± 31	284 ± 24	261 ± 14	16 ± 3	1000 ± 207	1289 ± 305	33 ± 3	31 ± 7	10/10
7	Phen + 100 ng (1500)	149 ± 25	148 ± 21	161 ± 24	449 ± 144	218 ± 51	28 ± 4	23 ± 3	34 ± 6	637 ± 182	746 ± 118	4/4
8	Phen + 100 ng (1300, 1500)	167 ± 9	260 ± 23	289 ± 26	447 ± 40	612 ± 73	18 ± 4	1222 ± 287	1489 ± 404	2213 ± 488	1654 ± 341	7/7

light of the absence of antral follicles in the hamster ovary on Day 1 of the cycle (11). These animals did ovulate on the morning of Day 1 but by the time of autopsy on Day 2, the ova were more than 24 hr old and were easily distinguishable from freshly released ova still surrounded by granulosa cells. Injection of LHRH led to significant increases in both serum FSH and LH (groups 2–7). The increase in FSH was especially dramatic although the normally high levels at 1200 hr in all groups and at the later sampling periods in the saline-injected group (group 1) should be noted.

Injection of LHRH on Day 2 (Table II). Again, no animals ovulated after administering any dose of LHRH. The saline-treated control group (group 1) showed a significant decline in both FSH and LH from the Day 1 values. LHRH (groups 2–9) induced a prompt increase in both gonadotropins but while the levels of LH were comparable to the Day 1 results, serum FSH levels were approximately one-half the values obtained on Day 1.

Injection of LHRH on Day 3 (Table III). For the first time during the cycle, ovulation (8–12 ova) was induced by injecting LHRH. The most effective treatment involved multiple injections of either 0.5 or 1.0 μg of LHRH at 1300 and 1500 hr (groups 4 and 7a) or single injections of 1.0 μg at 1300 or 1500 hr (groups 5a and 6). Single injections of 0.5 μg of LHRH (groups 2 and 3) resulted in ovulation in approximately half of the treated animals.

In control animals, serum FSH was about the same on the afternoons of Days 2 and 3 but serum LH was doubled (10–22 ng/ml) during the same time span (group 1). Injections of either 0.5 or 1.0 μg induced significant increases in serum FSH comparable to the results obtained on Day 2 and a potentiation action of a second LHRH injection was observed in group 7a. After a single injection of 0.5 μg LHRH at 1300 hr of Day 3 (group 3), serum levels of LH were similar to the values found on the previous days. However, a second injection of 0.5 μg at 1500 hr (group 4) caused an approximate doubling in serum LH from the previous hour.

Injection of LHRH on Day 4 (Table IV). The saline control group (group 1) showed

an increase in serum LH at 1400 hr whereas it was not until the 1530 hr sample that FSH rose above the baseline values of 200 ng/ml. Phenobarbital injected at 1300 hr (group 2) blocked the preovulatory surges of FSH and LH and the next morning prevented the anticipated ovulation.

Fifty ng of LHRH given at 1300 hr (group 3) was unable to reverse the blocking action of phenobarbital on ovulation whereas the same amount of LHRH at 1500 hr (group 4) induced ovulation in four of six animals. Multiple injections of 50 ng LHRH at 1300 hr and 1500 hr (group 5) restored ovulation to 100 per cent of the animals and 100 ng, either singly (groups 6 and 7) or as two injections (group 8) enabled all groups to ovulate by the next morning.

It is evident that the administration of LHRH on Day 4 produced a much more dramatic increase in serum levels of LH than of FSH. The regimen that best simulated the normal FSH pattern on the afternoon of Day 4 was obtained by multiple injections of 100 ng LHRH at 1300 and 1500 hr (group 8). Treatment with 50 ng singly (group 4a) or as multiple injections (group 5) led to ovulation and were associated with appreciable increases in serum LH but with only minor fluctuations in serum FSH.

There was a distinct dose-response relationship between the amount of LHRH injected and the resultant increase in serum LH; 100 ng of LHRH was able to restore serum LH to the maximal values normally observed during the preovulatory surge (group 1 vs 8) and multiple injections of 100 ng resulted in a potentiation of FSH at 1600 hr and of LH at 1530 and 1600 hr.

Discussion. Using ovulation and LH and FSH release as endpoints, the results confirm other studies in hamsters (2) and rats (12–15) demonstrating a changing responsiveness of the pituitary to LHRH treatment as a function of stage of the estrous cycle. Ovulation of the normal complement of ova was induced in 100% of phenobarbital-blocked hamsters following the injection on Day 4 of 100 ng LHRH at either 1300 and/or 1500 hr or two injections of 50 ng at both 1300 and 1500 hr (Table IV). Females treated on Day 3 required 1 μg at either 1300 and/or 1500 hr or 0.5 μg injected at both 1300 and 1500 hr (Table III). Animals injected on Day 1 or 2 failed to ovulate within 20 hr after treatment with up to 2 μg LHRH at both injection times.

Although the release of FSH in response to LHRH appears to be higher on Day 1 than on Days 2 and 3 and the release of LH appears about equal on Days 1, 2, and 3, it should be noted that the 1200 hr pretreatment levels of LH and especially FSH on Day 1 are higher than those observed on Days 2 and 3. When the amount of change from pretreatment or posttreatment levels is examined at comparable doses and injection times, it is clear that the release of both LH and FSH was greater on Days 2 and 3 than on Day 1 with one exception (the Day 1 FSH level was higher than the Day 2 level for group 4 at 1330 hr).

Results from studies in rats show that one administration of LHRH potentiates the pituitary's response to a subsequent treatment (16, 17). Potentiation can be considered to have occurred when the level of gonadotropin following the second LHRH treatment increased significantly above the level following the first administration and also above the level found in animals treated only at the time of the second injection: 1500 hr in the present study. Using these criteria, a potentiation of LHRH-induced LH release occurred on Day 3 (Table III, group 4) and Day 4 (Table IV, group 8) and a potentiation of FSH release occurred on Day 3 (group 7a) and Day 4 (group 8 at 1600 hr). The failure to find similar effects on LH and FSH in group 5 on Day 4 may have been due to the lower dose (50 ng), route of administration, and/or the two hr interval between LHRH injections. Studies in the rat which have demonstrated potentiating effects have used either a 1 hr interval between IV injections or a continuous infusion (16, 17).

LHRH is incapable of augmenting the pituitary's response to a second LHRH treatment on Days 1 and 2 of the hamster cycle. This situation is similar to that found in rats and is due to the lack of sufficient concentrations of circulating estrogen on these days (16, 18). In the hamster, estradiol only begins to increase during the late afternoon of Day 2 and then increases further on Day 3 (19).

Tables III and IV permit partial compari-

sons of LH and FSH levels in groups in which some animals ovulated and some did not. The sample sizes are small but in all cases there were little if any differences in FSH levels in these subgroups. Although FSH alone can induce ovulation in the hamster (8), a proestrous surge is not essential for ovulation to occur (6). In contrast to FSH levels, LH levels in most cases were higher after LHRH in those animals that ovulated within the next 20 hr. For example, on Day 3 (Table III), ovulating hamsters had significantly higher LH responses to LHRH at 1530 (group 3a), 1400 (group 5a), and 1400 and 1530 hr (group 7a). However, in ovulating animals, the LH levels following LHRH administration often did not reach the concentrations found in the saline-treated Day 4 hamsters. The present data suggest that threshold levels of LH may be more critical in determining whether ovulation will occur and also that ovulation in the hamster as in the rat (20) need not be associated with normal control levels of LH.

Summary. LH, and FSH were measured by radioimmunoassays in hamsters injected SC with LHRH on each day of the estrous cycle. The ovulatory dose of LHRH required to reverse the phenobarbital-block in proestrous (Day 4) hamsters was 100 ng as a single injection or 50 ng as two injections 2 hr apart while a tenfold greater dose was required in Day 3 animals. Hamsters injected on Day 1 or 2 could not be induced to ovulate with up to a total of 4 μg LHRH. LHRH generally resulted in significant increases in LH and FSH on all days of the cycle including larger pre- to posttreatment rises on Days 2 and 3 than on Day 1 and, with smaller LHRH doses, potentiation of LH and FSH release on Days 3 and 4.

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