

## Effect of Pinealectomy and of Bilateral Cervical Ganglionectomy on Serum LH Levels in Constant Estrous-Anovulatory Rats (39575)

G. P. TRENTINI, C. F. DE GAETANI, L. MARTINI, AND B. MESSI<sup>1, 2</sup>

*Institute of Morbid Anatomy, University of Modena, Italy; Department of Endocrinology, University of Milan, Italy; and Department of Anatomy, University Medical School, Pecs, Hungary*

It has been previously reported that pinealectomy and superior cervical ganglionectomy are able to reinitiate ovulation in constant estrous-anovulatory (CEA) rats bearing a frontal hypothalamic deafferentation (FHD) (1, 2). Repeated ovulations and pregnancies have been shown to occur in these animals (1, 2). However, the interval between two consecutive ovulations is not constant and the vaginal cycle is completely irregular (1, 2).

FHD has been reported to block the release but not the synthesis of pituitary LH (3). On the contrary, in normal rats pinealectomy has been shown to cause a marked rise in the synthesis as well as in the release of LH; the administration of the pineal principle melatonin exerts the opposite effects (4-8).

The present experiments have been designed to test whether pinealectomy and superior cervical ganglionectomy reinitiate ovulation in CEA rats bearing a FHD by activating the release of LH. In order to verify this hypothesis, serum levels of LH have been measured at different hours of the day in animals made CEA by FHD and subsequently submitted either to pinealectomy or to superior cervical ganglionectomy.

**Materials and Methods.** Female virgin rats of the Wistar strain were used. The animals were maintained throughout the experiment on a 14:10 hr light-dark cycle (lights on

from 6 AM to 8 PM), in a temperature and humidity controlled room ( $24 \pm 1^\circ$ ); they were fed a standard pellet diet; water was allowed *ad libitum*. At the age of 10 weeks, the animals were submitted to a frontal deafferentation (FHD) behind the optic chiasm using the technique of Halász and Pupp (9). The characteristics of the knife were as follows: rotational radius of the blade, 1.5 mm, height of the blade, 2.0 mm. The animals were allowed approximately 4 weeks to establish the CEA syndrome. After that interval, vaginal smears were recorded daily. Only CEA rats which showed persistent vaginal cornification for at least 1 month (about 50% of the rats subjected to FHD) were submitted to further surgical procedures. These CEA rats were divided randomly into four groups and were subjected to pinealectomy (30 rats), or to bilateral superior cervical ganglionectomy (30 rats), or to the corresponding sham-operations (15 rats). The surgical procedures were performed under barbital anesthesia (Evipan Natrium Bayer, Leverkusen), according to the techniques previously described (1, 2). As a consequence of anesthesia and of the operations, the vaginal smears of CEA animals acquired the characteristics of diestrus for a variable number of days (from 2 to 10). Subsequently, the vaginal smears of pinealectomized and of ganglionectomized rats showed the appearance of an irregular cyclicity. These phenomena have been previously reported (1, 2). On the day in which the vaginal smears of these animals showed for the second time the characteristics typical of proestrus (this day was named of "vaginal proestrus"), blood samples were collected four times to test whether the release of ovulatory amounts of LH had occurred. Blood collections were performed by puncture of the retroorbital plexus, under light ether anesthesia at 10

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<sup>2</sup> Requests for reprints should be addressed to Dr. G. P. Trentini, Institute of Morbid Anatomy, University of Modena, Via Berengario 4, 41100 Modena, Italy.

AM, and 2, 4, and 6 PM. These times were selected for sampling because, under the lighting conditions adopted and in the strain of rats used, the ovulatory surge of LH normally occurs between 2 and 4 PM. Ovulation was checked on the next morning by investigating the presence of ova in the oviducts. In accordance with previous findings (1), when ovulation occurred the number of tubal ova was within the range found in normal animals of the same strain. The localization and the completeness of the frontal deafferentation were carefully checked by histological verification.

LH was measured in the serum using the double-antibody radioimmunoassay procedure described by Niswender *et al.* (10). An antiserum against ovine LH, and pure ovine LH iodinated with  $^{125}\text{I}$  were used. Values have been expressed in the tables in terms of NIH-LH-S 17 (conversion factor to NIH-LH-S 1 = 1.01). The lowest amount of LH detectable with the procedure here adopted is of the order of 0.1 ng/ml.

**Results.** As shown in Table I, pinealectomy was able to induce ovulation in 65.5% of CEA rats; almost the same figure was obtained in CEA rats subjected to bilateral resection of the superior cervical ganglia.

Table II summarizes the values of serum LH found in pinealectomized FHD rats which had ova in the oviducts. For comparison, serum LH levels of normal control animals have also been included in the table. Out of 19 pinealectomized animals, 9 seem to show some sort of fluctuation in their serum LH levels. Three animals (Nos. 1, 5, and 11) presented LH peaks around the time at which these appear in normal animals of the strain used in the present series of experiments (2–4 PM). In three other animals (Nos. 6, 10, and 15), LH concentrations reached a maximum at 6 PM. Three

other rats (Nos. 7, 9, and 17) showed the highest levels of serum LH at 10 AM. In the remaining 10 animals (Nos. 2, 3, 4, 8, 12, 13, 14, 16, 18, and 19), the concentrations of LH remained constant during the period in which blood was sampled on the day of "vaginal proestrus," in spite of the presence of ova in the oviducts the following day.

The concentrations of LH in the sera of FHD rats, which ovulated after ganglionectomy are reported in Table III. Serum LH levels of normal controls are given also for comparison. Out of 16 ganglionectomized animals, 7 showed the presence of elevated levels of LH. Two animals (Nos. 6 and 8) showed the elevation around the time at which the LH surge occurs in normal animals. Two other rats (Nos. 1 and 7) exhibited the highest levels of serum LH at 10 AM. Finally, three other animals (Nos. 3, 10, and 14) had an irregular behavior of their serum LH titers with a tendency to reach the highest values in the late afternoon. The remaining rats (Nos. 2, 4, 5, 9, 11, 12, 13, 15, and 16) did not show significant variations of serum LH values.

Table IV summarizes the data obtained in animals bearing a FHD and subsequently submitted to pinealectomy and to superior cervical ganglionectomy and which did not ovulate. It is interesting that not one of the animals belonging to these groups showed increased release of LH during the day of "vaginal proestrus." Consequently, we have pooled these results. It is also interesting to note that in these two groups of animals (and especially in those submitted to ganglionectomy), serum LH levels were lower than in FHD-sham-operated animals. Since plasma LH levels in CEA rats do not change throughout the day, only a single blood collection was made in the last group of animals.

**Discussion.** The observation that 65% of the CEA rats ovulated following either pinealectomy or superior cervical ganglionectomy confirms previous reports (1, 2, 11). While 50% of the animals which ovulated following either operation showed elevated serum LH levels during the day of "vaginal proestrus," the time at which this elevation occurred was not identical in all animals. In one-third of these, LH increased at the time LH surges occur in normally cycling rats (2–

TABLE I. OVULATIONS INDUCED BY PINEALECTOMY AND SUPERIOR CERVICAL GANGLIONECTOMY IN CONSTANT ESTROUS-ANOVULATORY (CEA) RATS BEARING A FRONTAL HYPOTHALAMIC DEAFFERENTATION (FHD).

Treatment	Ovulating rats/operated rats	%
FHD + pinealectomy	19/29	65.5
FHD + ganglionectomy	16/25	64
FHD + sham operations	0/15	0

TABLE II. SERUM LH LEVELS (NANOGRAMS PER MILLILITER) OF OVULATING PINEALECTOMIZED FHD RATS AT DIFFERENT HOURS OF THE DAY OF "VAGINAL PROESTRUS."

	10:00 AM	2:00 PM	4:00 PM	6:00 PM
Pinealectomized-FHD rats				
Rat no. 1	8.50	14.00	14.50	9.00
2	4.25	5.25	5.50	7.25
3	4.75	2.80	3.00	4.80
4	3.31	0.84	1.07	0.95
5	0.18	11.80	1.00	0.47
6	1.04	1.12	6.48	11.13
7	10.00	2.00	0.75	2.25
8	1.46	1.24	1.18	0.63
9	8.00	8.50	2.25	5.00
10	0.73	0.19	10.69	47.10
11	4.00	17.75	15.00	13.00
12	3.00	3.00	3.20	2.00
13	0.13	1.06	0.14	0.32
14	0.28	0.37	0.63	0.74
15	0.35	0.47	0.32	36.68
16	4.63	0.66	1.03	3.51
17	8.50	6.00	0.75	0.50
18	2.20	1.24	1.18	0.63
19	0.64	1.06	0.59	1.63
Normal controls <sup>a</sup>	13.30 ± 1.60	28.70 ± 10.00	35.05 ± 6.78	17.75 ± 2.69

<sup>a</sup> Means ± SE of six animals.

TABLE III. SERUM LH LEVELS (NANOGRAMS PER MILLILITER) OF OVULATING GANGLIONECTOMIZED FHD RATS AT DIFFERENT HOURS OF THE DAY OF "VAGINAL PROESTRUS."

	10:00 AM	2:00 PM	4:00 PM	6:00 PM
Ganglionectomized-FHD rats				
Rat no. 1	13.50	7.75	3.25	5.50
2	3.82	1.07	1.11	1.28
3	0.51	8.22	12.24	38.87
4	2.50	2.75	2.25	3.00
5	0.88	1.03	1.08	1.12
6	3.73	3.09	32.89	23.73
7	13.00	11.00	2.75	0.50
8	8.50	35.50	50.00	21.00
9	3.82	1.98	1.12	0.91
10	0.45	6.42	1.26	10.42
11	0.32	0.88	0.71	0.64
12	1.46	0.21	0.33	0.64
13	2.50	3.50	4.00	5.00
14	7.22	3.00	6.08	10.51
15	1.00	3.25	1.00	4.75
16	0.75	1.20	1.00	1.75
Normal controls <sup>a</sup>	13.30 ± 1.60	28.70 ± 10.00	35.05 ± 6.78	17.75 ± 2.69

<sup>a</sup> Means ± SE of six animals.

4 PM). In the second third, LH did not increase until 6 PM, whereas in the final third, serum LH was elevated at 10 AM. The remaining 50% of FHD rats which ovulated after pinealectomy or after superior cervical ganglionectomy did not exhibit any significant variation in serum LH during the hours of the day in which serum was collected. In FHD animals submitted to pinealectomy or to superior cervical ganglionectomy which

did not ovulate, serum LH levels remained unchanged.

In the majority of FHD rats which ovulated after pinealectomy or after superior cervical ganglionectomy and which exhibited an elevation of serum LH titers, this elevation did not approach the levels found in normally cycling animals. The only exception were rats Nos. 10 and 15 of Table II and rats Nos. 3, 6, and 8 of Table III. Three

TABLE IV. SERUM LH LEVELS (NANOGRAMS PER MILLILITER) OF NONOVULATING PINEALECTOMIZED AND GANGLIONECTOMIZED FHD RATS.

Treatment	No. of rats	10:00 AM	2:00 PM	4:00 PM	6:00 PM
FHD + pinealectomy	10	3.50 ± 0.74 <sup>a</sup>	3.26 ± 0.55	3.75 ± 0.69	4.73 ± 0.87
FHD + ganglionectomy	9	1.86 ± 0.48 <sup>b</sup>	2.25 ± 0.52 <sup>c</sup>	2.25 ± 0.55 <sup>c</sup>	3.16 ± 0.82
FHD + sham operations	15		4.36 ± 0.47		

<sup>a</sup> Means ± SE.

<sup>b</sup>  $P < 0.005$  vs FHD sham-operated animals.

<sup>c</sup>  $P < 0.01$  vs FHD sham-operated animals.

interpretations may be offered to explain these results. (A) It is possible that the moderate elevation of LH found in the majority of pinealectomized and ganglionectomized FHD rats is the consequence of the lower initial basal levels of LH observed by us and by Hayashi *et al.* (12). (B) The LH release which occurs after pinealectomy or ganglionectomy is anomalous in nature, and it is not due to a stimulus originating in the preoptic area. Considerable evidence has been presented which suggests that FHD brings about the CEA syndrome by disconnecting the preoptic input into the arcuate-median eminence region (13). Pinealectomy could induce ovulation through a depression of the activity of the serotonergic system of the brain stem (14-16), which normally inhibits the release of LH (7, 17-20). In support of such a hypothesis, the following data may be quoted: injections of serotonin or of melatonin are able to suppress the ovulation-inducing effect of pinealectomy in FHD rats (11, 14); the administration of melatonin increases the concentrations of serotonin in the hypothalamus and in the midbrain (21); the depression of serotonin brain levels induced by treatment with parachlorophenylalanine or by feeding with a tryptophan-poor diet brings about luteinization in CEA-FHD rats (15, 16). Finally, (C) it is possible that in some animals (particularly those in which LH levels were high at 10 AM or at 6 PM) the peak LH values were missed because of inadequate frequency of sampling of blood. Consequently, in these animals only the ascending or the descending portions of the LH surge, respectively, were measured.

The data also indicate that small elevations of serum LH are sufficient to induce

ovulation in CEA rats bearing a FHD. This is not surprising since it is well known that the serum LH concentrations which occur in normal rats on the afternoon of the day of proestrus far exceed the threshold amounts needed to induce ovulation (22-25).

*Summary.* In 65% of constant estrous anovulatory (CEA) rats bearing a frontal hypothalamic deafferentation (FHD), ovulation reappears after pinealectomy or bilateral superior cervical ganglionectomy. In nearly 50% of the FHD rats which ovulated after pinealectomy or ganglionectomy, serum LH showed an elevation on the day of "vaginal proestrus." In the remaining 50% of the animals, ovulation occurred without observable variations in serum LH concentrations. The LH peak was probably missed in these animals because insufficient numbers of blood samples were collected. The data suggest that pinealectomy and cervical sympathectomy may induce LH release. It is proposed that this occurs through an interference with the hypothalamic and brain stem serotonergic system which normally keeps LH under an inhibitory tone.

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